1 H, H-6), 6.75 (d, 1 H, H-7), 3.94–4.25 (t, 2 H, 2 H-1′), 3.06–3.44 (m, 2 H, 2 H-4′), 1.13–1.94 (2 m, 4 H, 2 H-2′ and 2 H-3′). Anal. ($C_{10}H_{14}N_4O$) C, H, N.

(±)-3-Nitro-4-[(1,5-dihydroxy-2-pentyl)amino]pyridine (4). Compound 4 was prepared by heating to 130 °C a mixture of 4-ethoxy-3-nitropyridine (2 g, 0.012 mol) and crude 1,5-dihydroxy-2-aminopentane (6.7 g), which contained ~10–40% prolinol. This solution was heated until boiling stopped (~40 min) and then for an additional 10 min. The reaction solution was cooled and the resulting solid residue was dissolved in 75 mL of hot water. This solution was cooled, yielding yellow crystals which proved to be the prolinol condensation product with 3-nitropyridine. This byproduct was recrystallized from water and the mother liquor saved, the crystals were discarded. The combined filtrates were reduced in vacuo to 10 mL, acidified (pH 2) with 10% HCl, and loaded on a Dowex 50-W (H^+) column (~2.0 × 25 cm). The column was washed with 300 mL of water and then 400 mL of 2 N ammonia. The basic fractions containing primarily 4 as determined by TLC were collected, combined, and reduced to dryness to give 3.7 g of a yellow oil, which was used without further purification. The material showed two UV-positive and visibly yellow spots by TLC [~1:10 with the major spot being 4 and the minor spot being the prolinol derivative [R_f(major) 0.64, R_f(minor) 0.83, $CHCl_3$–MeOH (3:1), 250-μm silica gel GF, Analtech].

(±)-2-Chloro-3-amino-4-[(1,5-dihydroxy-2-pentyl)amino]pyridine (8). Compound 8 was obtained in the same manner as 5 with crude 4 as starting material. Dowex 50-W (H^+) ion-exchange column chromatography yielded an extremely hygroscopic oil in ~86% yield. The material showed the UV-positive spot by TLC, [R_f 0.49, $CHCl_3$–MeOH (3:1), 250-μm silica gel GF, Analtech] and it was used without further purification.

(±)-4-Chloro-1-(1,5-dihydroxy-2-pentyl)imidazo[4,5-c]pyridine (12). Compound 12 was prepared in the same manner as 9 with 8 as starting material. Recrystallization from water gave a white solid in 33% yield: mp 159–161 °C; ^1H NMR (Me_2SO-d_6) δ 8.45 (s, 1 H, H-2), 8.05 (d, 1 H, H-6), 7.7 (d, 1 H, H-7), 4.3–4.7 (m, 1 H, H-2′), 3.6–3.9 (t, 2 H, 2 H-1′), 3.15–3.50 (q, 2 H, 2 H-5′), 1.75–2.15 (q, 2 H, 2 H-3′), 0.95–1.6 (m, 2 H, 2 H-5′). Anal. ($C_{11}H_{14}ClN_3O_2 \cdot H_2O$) C, H, N.

(±)-4-Amino-1-(1,5-dihydroxy-2-pentyl)imidazo[4,5-c]pyridine (16). Compound 16 was reported in the same manner as 13 with 12 as starting material. The hydrochloride salt was obtained after recrystallization from ethanol–acetone in a 66% yield: mp 150–155 °C (decomposed at 160 °C); ^1H NMR (Me_2SO-d_6 plus two drops deuterium oxide) δ 8.5 (s, 1 H, H-8), 7.63 (d, 1 H, H-6), 7.25 (d, 1 H, H-7), 4.38–4.69 (m, 1 H, H-2′), 3.75 (d, 2 H, 2 H-1′), 3.36 (t, 2 H, 2 H-5′), 1.75–2.13 (m, 2 H, 2 H-3′), 1.06–1.50 (m, 2 H, 2 H-5′). Anal. ($C_{11}H_{16}N_4O_2 \cdot 2HCl \cdot EtOH$) C, H. N.

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Registry No. 1, 62194-80-3; 2, 94751-20-9; 3, 94751-21-0; 4, 94751-22-1; 5, 94751-23-2; 6, 94781-00-7; 7, 94751-24-3; 8, 94751-25-4; 9, 94751-26-5; 10, 94751-27-6; 11, 94751-28-7; 12, 94751-29-8; 13, 94751-30-1; 14, 94751-31-2; 15, 94751-32-3; 16, 94751-33-4; (±)-2-amino-1,5-pentanediol, 94840-93-4; glutamic acid, 56-86-0; glutamic acid dimethyl ester hydrochloride, 23150-65-4; 4-hydroxy-3-nitropyridine, 5435-54-1; 4-hydroxypyridine nitrate, 3454-03-3; 4-chloro-3-nitropyridine, 13091-23-1; 4-ethoxy-3-nitropyridine, 1796-84-5; ethanolamine, 141-43-5; (±)-2,3-dihydroxypropylamine, 13552-31-3; triethyl orthoformate, 122-51-0; AdoHcy hydrolase, 9025-54-1.

Potential Inhibitors of *S*-Adenosylmethionine-Dependent Methyltransferases. 9. 2′,3′-Dialdehyde Derivatives of Carbocyclic Purine Nucleosides as Inhibitors of *S*-Adenosylhomocysteine Hydrolase[1a]

D. Michael Houston,[†1b] E. K. Dolence,[†1c] Bradley T. Keller,[‡] Usha Patel-Thombre,[‡] and Ronald T. Borchardt*[†,‡]

Departments of Biochemistry and Medicinal Chemistry, Smissman Research Laboratories, The University of Kansas, Lawrence, Kansas 66044. *Received February 1, 1984*

A series of purine (e.g., adenine, N^6-methyladenine, 8-azaadenine, 3-deazaadenine) carbocyclic nucleosides, nucleoside 2′,3′-dialdehydes, and nucleoside 2′,3′-diols were synthesized as potential inhibitors of bovine liver *S*-adenosyl-L-homocysteine (AdoHcy) hydrolase (EC 3.3.1.1) and as potential inhibitors of vaccinia virus replication. The 2′,3′-dialdehydes were prepared by periodate oxidation of the corresponding carbocyclic nucleosides. Reduction of the intermediate dialdehydes with sodium borohydride afforded the corresponding 2′,3′-diols. Of the nucleosides tested, the most potent inhibitors of AdoHcy hydrolase were the adenine analogue (K_i = 110 ± 38 nM) and the 3-deazaadenine analogue (K_i = 4 ± 0.9 nM), which were reversible, competitive inhibitors. In contrast, the 2′,3′-dialdehydes produced irreversible inhibition of AdoHcy hydrolase, resulting in incorporation of two to four molecules of the dialdehyde per molecule (tetramer) of the enzyme. On the basis of an Ackermann–Potter analysis, the following "apparent" K_i values were determined for the 2′,3′-dialdehydes: adenine analogue, 61 nM; 8-azaadenine analogue, 57.5 nM; and 3-deazaadenine analogue, 32 nM. The nucleoside 2′,3′-diols were substantially less effective as inhibitors of AdoHcy hydrolase, requiring millimolar concentrations to achieve significant inhibition. When tested for their ability to inhibit vaccinia virus replication, several carbocyclic nucleosides (e.g., adenine and 3-deazaadenine analogues) and several nucleoside 2′,3′-dialdehydes (e.g., adenine, N^6-methyladenine, 8-azaadenine, and 3-deazaadenine analogues) exhibited good antiviral effects. A good correlation existed between a compound's inhibitory effects on AdoHcy hydrolase and its antiviral effects, suggesting that the inhibition of viral replication is caused by inhibition of a critical methylation reaction, e.g., methylation of the 5′-cap of viral mRNA.

In recent years, *S*-adenosyl-L-homocysteine (AdoHcy) hydrolase (EC 3.3.1.1) has emerged as a specific target for the design of potential chemotherapeutic agents.[2-4] Such an approach has been prompted by recognition of the important role that this enzyme plays in regulating biological methylation reactions (i.e., modulating the intracellular AdoHcy/*S*-adenosylmethionine (AdoMet) ratio). AdoHcy hydrolase catalyzes the reversible hydrolysis of

† Department of Medicinal Chemistry.
‡ Department of Biochemistry.

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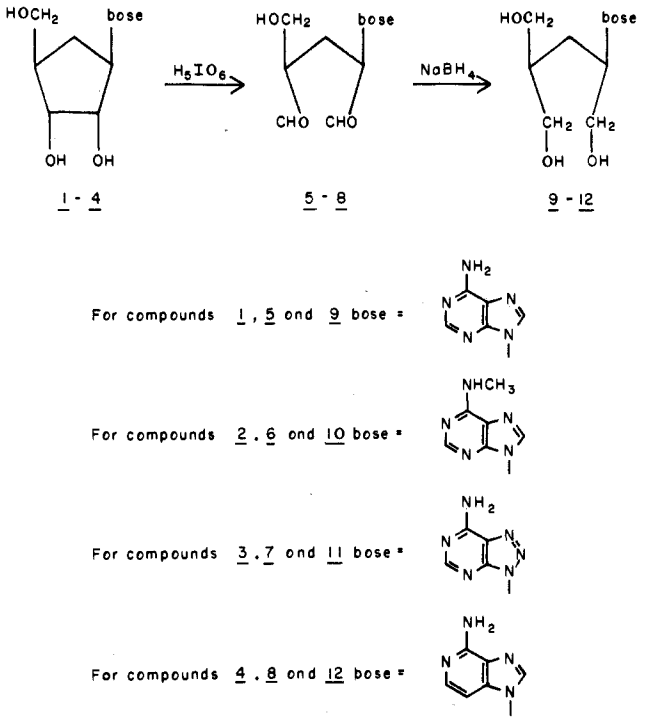
AdoHcy to adenosine and homocysteine. Although the equilibrium of the reaction favors synthesis, AdoHcy is efficiently hydrolyzed under physiological conditions because adenosine and homocysteine are simultaneously removed by several metabolic routes.[5] Inhibition of AdoHcy hydrolase in intact cellular systems results in the accumulation of AdoHcy, a product inhibitor of AdoMet-dependent methylation reactions.[6,7] As a consequence of inhibiting AdoHcy metabolism, cellular methylation reactions are perturbed, many of which are required for maintenance of the normal metabolic integrity of the cell.

An example of an essential methylation reaction is found in the maturation scheme of certain eukaryotic and viral messenger RNA molecules. It is known that, in many instances, these mRNA molecules must be both capped and methylated on their 5′-terminus (e.g., $m^7Gpppm^6A^mpAp^m$...) to promote active translation of the corresponding proteins.[8] Methylation of the 5′-cap structure has been demonstrated to enhance the efficiency of initiation of translation at the 5′-end of the mRNA.[9] Moreover, it has been shown that the vaccinia virus specific enzymes which catalyze these reactions for viral mRNAs (i.e., guanine-7-methyltransferase, 2′-*O*-nucleoside methyltransferase) are susceptible to inhibition by AdoHcy.[10,11] It is not surprising, therefore, that potential inhibitors of AdoHcy hydrolase such as 3-deazaadenosine,[12] 3-deazaaristeromycin,[13,14] Neplanocin A,[15] and adenosine dialdehyde,[16] elicit significant antiviral activity against viruses which require a methylated-5′-cap structure on their mRNAs.

Adenosine dialdehyde was first reported by Hoffman[17,18] as an inhibitor of AdoHcy hydrolase. Our laboratory has shown that adenosine dialdehyde is a tight-binding type inhibitor, which exhibits a K_i = 2.39 nM and a stoichiometry of one to two molecules of inhibitor bound per tetramer of enzyme.[19a,b] In an effort to determine the structural features of adenosine dialdehyde, which are necessary for binding to AdoHcy hydrolase, we have synthesized a series of carbocyclic purine nucleoside dialdehydes. These carbocyclic purine nucleoside dialdehydes, as well as the parent carbocyclic nucleosides, were evaluated as inhibitors of bovine liver AdoHcy hydrolase in vitro and as inhibitors of vaccinia virus replication in mouse L 929 cells.

Scheme I. General Route for the Synthesis of 2′,3′-Dialdehyde and 2′,3′-Diol Derivatives of Carbocyclic Purine Nucleosides



Results

Chemistry. The carbocyclic purine nucleoside dialdehydes, which were prepared as potential inhibitors of AdoHcy hydrolase, included the adenine (C-AdoDA, **5**), N^6-methyladenine (N^6-methyl-C-AdoDA, **6**), 8-azaadenine (8-aza-C-AdoDA, **7**), and 3-deazaadenine (3-deaza-C-AdoDA, **8**) analogues. These compounds were prepared by oxidation of the corresponding carbocyclic nucleosides [C-Ado (**1**), N^6-methyl-C-Ado (**2**), 8-aza-C-Ado (**3**), 3-deaza-C-Ado (**4**)] with use of periodic acid according to the procedures of Borchardt et al.[20] (Scheme I). The dialdehydes **5**–**8** were subsequently reduced with sodium borohydride to the corresponding carbocyclic purine nucleoside diols [C-AdoDO (**9**), N^6-methyl-C-AdoDO (**10**), 8-aza-C-Ado (**11**), 3-deaza-C-Ado (**12**)]. The dialdehydes **5**–**8** and the diols **9**–**12** were found to be quite unstable in aqueous solution; subsequently they were stored at −20 °C in a lyophilized form and solutions were prepared fresh for biological testing.

The prerequisite carbocyclic nucleosides [C-Ado (**1**),[21] N^6-methyl-C-Ado (**2**),[22] 8-aza-C-Ado (**3**)[23]] were prepared

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Table I. Inhibition of Bovine Liver AdoHcy Hydrolase by Carbocyclic Purine Nucleosides, Nucleoside 2′,3′-Dialdehydes, and Nucleoside 2′,3′-Diols[a]

compd	% inhibn		compd	% inhibn	
	I = 0.2 mM	I = 2.0 mM		I = 0.2 mM	I = 2.0 mM
C-Ado (1)	99	ND	8-aza-C-AdoDA (7)	100	ND
N^6-methyl-C-Ado (2)	22	ND	3-deaza-C-AdoDA (8)	100	ND
8-aza-C-Ado (3)	46	ND	C-AdoDO (9)	24	98
3-deaza-C-Ado (4)	97	ND	N^6-methyl-C-AdoDO (10)	0	82
C-AdoDA (5)	98	ND	8-aza-C-AdoDO (11)	0	94
N^6-methyl-C-AdoDA (6)	32	ND	3-deaza-C-AdoDO (12)	57	94

[a] AdoHcy hydrolase activity was determined by using the procedure of Richards et al.,[27] which involves measuring the hydrolysis of [2,8-^{3}H]AdoHcy to [2,8-^{3}H]adenosine and homocysteine. The assay mixture includes adenosine deaminase, which converts [2,8-^{3}H]-adenosine to [2,8-^{3}H]inosine and the [2,8-^{3}H]inosine is measured by scintillation spectrometry after separation on SP-Sephadex C-25. See Experimental Section for details.

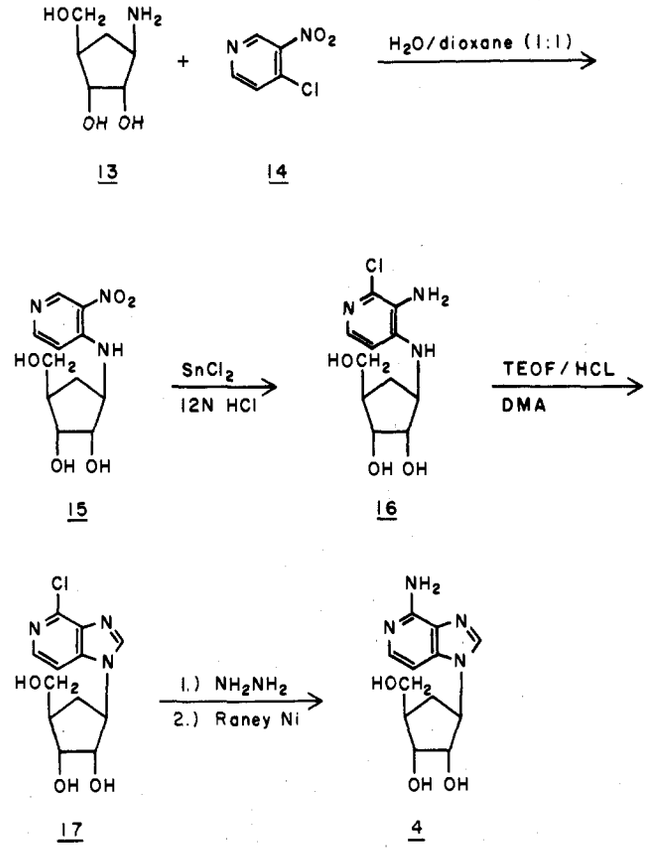
by literature procedures. Montgomery et al.[13] have previously reported the synthesis of 3-deaza-C-Ado (4) by (1) reaction of 2,4-dichloro-3-nitropyridine with (±)-4β-amino-2α,3α-dihydroxy-1β-cyclopentanemethanol, (2) reduction with H_2/Raney nickel to the diamino compound, (3) cyclization with triethyl orthoformate to the basic 3-deazapurine nucleus, and (4) incorporation of the 6-amino group by treatment with hydrazine followed by reduction with Raney nickel.

Our procedure for synthesis of 3-deaza-C-Ado (4) involved condensation of 4-chloro-3-nitropyridine[24] (14) with (±)-4β-amino-2α,3α-dihydroxy-1β-cyclopentanemethanol (13)[21] (Scheme II). The reaction product 15 was reductively chlorinated to 16 with stannous chloride in hot 12 N HCl following the general procedure of Koenig et al.[25] The ^{1}H NMR spectra showed a coupling constant between H-5 and H-6 equal to 6 Hz, which is consistent with the assigned structure. Koenig et al.[25] had reductively chlorinated 3-nitro-4-aminopyridine to give what was thought to be 3,4-diamino-6-chloropyridine, but the product was later shown by Mizuno et al.[26] to be 3,4-diamino-2-chloropyridine. Compound 15 was converted to 3-deaza-C-Ado (4) by cyclization with triethyl orthoformate to 17, followed by reaction with hydrazine and reduction with Raney nickel according to the procedures of Montgomery et al.[13] The spectral data (^{1}H NMR, mass spectra) for compound 4 are consistent with the assigned structure.

Biological Activity. In Vitro Studies with Bovine Liver AdoHcy Hydrolase. In preliminary experiments the carbocyclic purine nucleosides 1–4, the nucleoside 2′,3′-dialdehydes 5–8 and the nucleoside 2′,3′-diols 9–12 were screened for their inhibitory activity toward bovine liver AdoHcy hydrolase and the results are shown in Table I. The enzyme activity was determined in the hydrolytic direction with [2,8-^{3}H]AdoHcy as the substrate. This assay measures the appearance of [2,8-^{3}H]inosine, which is generated from [2,8-^{3}H]adenosine by the action of adenosine deaminase.

Of the carbocyclic purine nucleosides tested, the most potent inhibitors of AdoHcy hydrolase were C-Ado (1) and 3-deaza-C-Ado (4), which totally inhibited the enzyme at concentrations of 0.2 mM. At the same concentrations, N^6-methyl-C-Ado (2) and 8-aza-C-Ado (3) were substantially less effective as inhibitors (22% and 46% inhibition,

Scheme II. Route for the Synthesis of 3-Deaza-C-Ado



respectively). These carbocyclic purine nucleosides were shown to be competitive inhibitors of AdoHcy hydrolase when [2,8-^{3}H]AdoHcy was the variable substrate. The following inhibition constants were determined: C-Ado (1), K_i = 110 ± 38 nM; 8-aza-C-Ado (3), K_i = 22 ± 6 μM; and 3-deaza-C-Ado (4), K_i = 4.0 ± 0.9 nM. Guranowski et al.[29] have reported a K_i = 5 nM for C-Ado (1) and Montgomery et al.[13] have reported a K_i = 3 μM for 3-deaza-C-Ado. At this time we are unable to explain the large discrepancy between our observed K_i values for C-Ado (1) and 3-deaza-C-Ado (4) and those reported by Guranowski et al.[29] and Montgomery et al.[13]

The order of inhibitory activity of the carbocyclic purine nucleosides [N^6-methyl-C-Ado (2), 8-aza-C-Ado (3), 3-deaza-C-Ado (4)] parallels that reported for the corresponding ribose analogues (N^6-methyladenosine, 8-azaadenosine, and 3-deazaadenosine). Guranowski et al.[29] have reported K_i values of 190 μM for both 8-azaadenosine and N^6-methyladenosine and a K_i value of 4 μM for 3-

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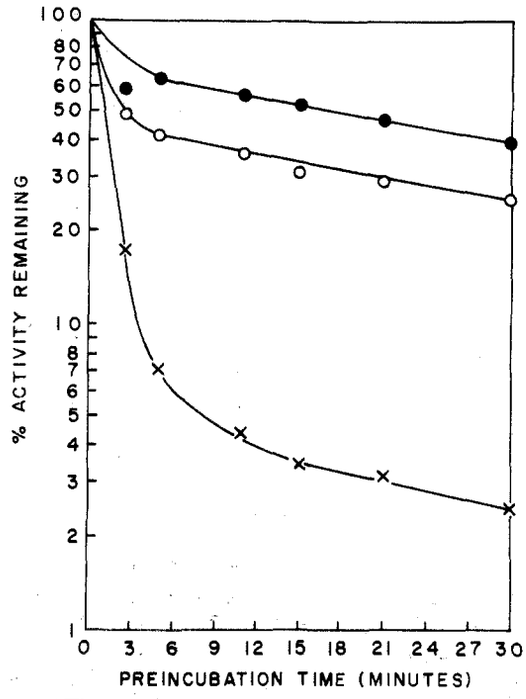

Figure 1. The effect of carbocyclic 3-deazaadenosine-2′,3′-dialdehyde (8) on the rate of inactivation of AdoHcy hydrolase. The preincubation mixture consisted of the inhibitor [(●) 550 nM, (○) 700 nM, (X) 800 nM)], 150 mM potassium phosphate buffer (pH 7.6), EDTA (1 mM), and purified AdoHcy hydrolase (188 mM). The mixtures were preincubated at 28 °C and samples were withdrawn at the indicated times and assayed for residual AdoHcy hydrolase activity as described in the Experimental Section.

deazaadenosine. Therefore, it appears that the structure–activity relationships for the base portion of both the carbocyclic and ribose series are similar.

Similar to adenosine dialdehyde,[19a,b] several of the carbocyclic purine nucleoside 2′,3′-dialdehydes were found to be potent inhibitors of AdoHcy hydrolase (Table I). In our preliminary screen the least effective inhibitor was N^6-methyl-C-AdoDA (**6**), which only produced 32% inhibition at a concentration of 0.2 mM, where as C-AdoDA (**5**), 8-aza-C-AdoDA (**7**), and 3-deaza-C-AdoDA (**8**) at the same concentrations all produced total inhibition of the enzyme. In contrast to the potent inhibitory effects of the 2′,3′-dialdehydes, the corresponding 2′,3′-diols [C-AdoDO (**9**), N^6-methyl-C-AdoDO (**10**), 8-aza-C-AdoDO (**11**), 3-deaza-C-AdoDO (**12**)] only produced significant inhibition at millimolar concentrations (Table I). These results suggest a crucial role for the aldehyde functionalities of **5**–**8** in the mechanism by which they inhibit the hydrolase.

When AdoHcy hydrolase was incubated with 3-deaza-C-AdoDA (**8**), we observed both concentration-dependent and time-dependent loss of enzyme activity (Figure 1). Similar results were obtained for 8-aza-C-AdoDA (**7**) and C-AdoDA (**5**). The inactivation produced by these 2′,3′-dialdehydes appears to be irreversible, since enzyme activity could not be recovered by dialysis (data not shown).

Since these 2′,3′-dialdehydes appear to act as irreversible inhibitors of AdoHcy hydrolase, we employed the Ackermann–Potter approach[30] to analyze the kinetics of inactivation of the enzyme. Varying amounts of the enzyme were incubated with several concentrations of the nucleoside 2′,3′-dialdehydes for 5 min at 37 °C, after which the residual hydrolase activity was determined by the

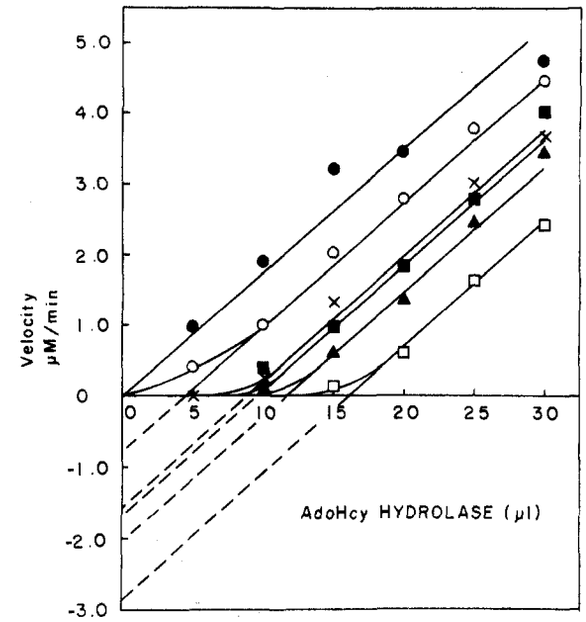

Figure 2. Carbocyclic 3-deazaadenosine-2′,3′-dialdehyde (8) inactivation of bovine liver AdoHcy hydrolase. Ackermann–Potter plot of enzyme velocity vs. enzyme concentration in the presence of varying concentrations of carbocyclic 3-deazaadenosine-2′,3′-dialdehyde. Various concentrations of AdoHcy hydrolase were preincubated with the indicated concentrations of carbocyclic 3-deazaadenosine-2′,3′-dialdehyde (●) 0 nM (○) 100 nM, (X) 150 nM, (■) 200 nM, (▲) 300 nM, (□) 400 nM)] in a total volume of 0.445 mL containing 150 mM potassium phosphate buffer (pH 7.6) and 1 mM EDTA. After preincubation for 5 min at 28 °C, the residual enzyme activity was determined by the addition of 100 μM [2,8-^{3}H]AdoHcy and 4 units of adenosine deaminase, and the mixture was further incubated at 37 °C for 5 min. The reaction was terminated with 100 μL of 5 N formic acid. The product, [2,8-^{3}H]inosine, was then isolated as described under the Materials and Methods section.

addition of [2,8-^{3}H]AdoHcy and adenosine deaminase. Figure 2 shows an Ackermann–Potter plot[30] of the data generated with 3-deaza-C-AdoDA (**8**). Similar results were obtained with 8-aza-C-AdoDA (**7**) and C-AdoDA (**5**) (data not shown). From these data, we calculated stoichiometries of two to four molecules of the dialdehydes bound per molecule (tetramer) of AdoHcy hydrolase (C-AdoDA (**5**), three; 8-aza-C-AdoDA (**7**), two; 3-deaza-C-AdoDA (**8**), four).

Although the information obtained from the Ackermann–Potter plot is useful, it cannot be used directly to estimate the inhibition constants (K_i's). However, the data can be utilized to determine the I_{50} values[31] (the total inhibitor concentration at which the enzyme reaction velocity is 50% of the uninhibited reaction) as seen in Figure 3A. A plot of these I_{50} values vs. enzyme concentration should yield a straight line with an I_{50} intercept, which represents the K_i value.[31] However, when the data for 3-deaza-C-AdoDA (**8**) were analyzed in this manner, a sigmoidal relationship (Figure 3B) was observed. Similar results were obtained with 8-aza-C-AdoDA (**7**) and C-AdoDA (**5**). The fact that a nonlinear relationship was observed may be due to the fact that these 2′,3′-dialdehydes form 2:1, 3:1, or 4:1 type complexes with the hydrolase rather than the 1:1 complexes traditionally seen with tight-binding inhibitors.[31] However, if one extrapolates the linear portion of the data at low enzyme concentrations to the I_{50} axis, the following "apparent" K_i

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(31) S. Cha, R. P. Agarwal, and R. E. Parks, *Biochem. Pharmacol.*, **24**, 2187 (1975).

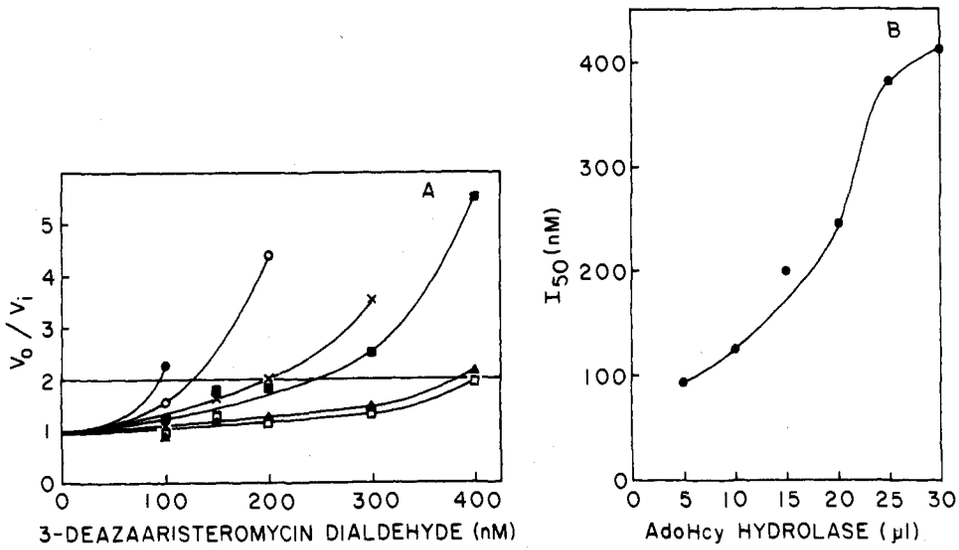

Figure 3. Determination of K_i for carbocyclic 3-deazaadenosine-2′,3′-dialdehyde inhibition of AdoHcy hydrolase. (A) A plot of V_0/V_i vs. carbocyclic 3-deazaadenosine-2′,3′-dialdehyde concentration where V_0 is the velocity in the absence of inhibitor and V_i is the velocity in the presence of various amounts of the inhibitor (from Figure 2). The V_0/V_i values for different amounts of AdoHcy hydrolase [(●) 5 μL, (○) 10 μL, (X) 15 μL, (■) 20 μL, (▲) 25 μL, (□) 30 μL)] were plotted vs. the appropriate inhibitor concentrations. (b) Plot of I_{50} values (from Figure 3A vs. the amount of AdoHcy hydrolase.

values were observed: C-AdoDA (**5**), 61 nM, 8-aza-C-AdoDA (**7**), 32 nM; and 3-deaza-C-AdoDA (**8**), 57.5 nM. For comparative purposes, adenosine dialdehyde[19b] and Neplanocin A[15] have K_i values of 2.39 and 8.39 nM, respectively.

Antiviral Activity. Previous work from this laboratory,[15,16a,32] as well as other laboratories,[13,14] have shown that inhibitors of AdoHcy hydrolase often possess antiviral activity. The potent inhibitory activity of C-Ado (**1**), 3-deaza-C-Ado (**4**), C-AdoDA (**5**), 8-aza-C-AdoDA (**7**), and 3-deaza-C-AdoDA (**8**) toward AdoHcy hydrolase prompted us to test these analogues, as well as N^6-methyl-C-Ado (**2**), 8-aza-C-Ado (**3**), N^6-methyl-C-AdoDA (**6**) and the nucleoside 2′,3′-diols **9–12**, for their antiviral effects. To evaluate their antiviral effects, the compounds were tested for their ability to inhibit vaccinia virus plaque formation in monolayer cultures of mouse L cells. As shown in Table II, significant inhibition of plaque formation was observed with C-Ado (**1**), 3-deaza-C-Ado (**4**), C-AdoDA (**5**), N^6-methyl-C-AdoDA (**6**), 8-aza-C-AdoDA (**7**), and 3-deaza-C-AdoDA (**8**). Little or no activity was observed with N^6-methyl-C-Ado (**2**), 8-aza-C-Ado (**3**), C-AdoDO (**9**), N^6-methyl-C-AdoDO (**10**), 8-aza-C-AdoDO (**11**), and 3-deaza-C-AdoDO (**12**). There appears to be a good correlation between a compound's inhibitory effects on AdoHcy hydrolase and its antiviral effects with the exception of N^6-methyl-C-AdoDA (**6**). These results suggest the possibility that the inhibition of viral replication is caused by inhibition of a critical AdoMet-dependent methylation, e.g., methylation of the 5′-cap of viral mRNA resulting from increases in intracellular levels of AdoHcy. A similar mechanism appears to be responsible for the antiviral effects of adenosine dialdehyde[16a] and Neplanocin A.[15]

In evaluating the specificity of a potential antiviral agent, an important consideration is the effect of the compound of the host cell system. To examine the aspect of cellular toxicity, [^{3}H]thymidine incorporation into DNA of uninfected L-cell cultures was measured after 36- and 72-h exposures to the carbocyclic purine nucleosides 1–4 and nucleoside 2′,3′-dialdehydes **5–8**. When cells were exposed to C-Ado (**1**), N^6-methyl-C-Ado (**2**), 8-aza-C-Ado (**3**), 3-deaza-C-Ado (**4**), 8-aza-C-AdoDA (**7**), and 3-deaza-C-AdoDA (**8**) for 36 h, little or no inhibition of [^{3}H]thymidine was observed. However, significant toxicity (30–40%) was observed with C-AdoDA (**5**) and N^6-methyl-C-AdoDA (**6**). After the cells were exposed to the compounds for 72 h, a stimulation of DNA synthesis was observed. The largest effect (246%) was observed with 3-deaza-C-Ado (**4**). Similar results were observed with adenosine dialdehyde[16a] and Neplanocin A.[15] In the case of adenosine dialdehyde[16a] and Neplanocin A,[15] we also studied the growth curves of L cells treated with and without the drugs. The results indicated that between 12 and 24 h cell growth was suppressed. However, between 48 and 72 h the rates of increase in cell number for the drug-treated and untreated cells became parallel, indicating a similar growth rate for both sets of cultures. These data suggest that adenosine dialdehyde,[16a] Neplanocin A,[15] the carbocyclic purine nucleosides 1–4, and the nucleoside 2′,3′-dialdehydes **5–8** are not toxic to L cells but rather they

Table II. Effects of Carbocyclic Purine Nucleosides, Nucleoside 2′,3′-Dialdehydes, and Nucleoside 2′,3′-Diols on Vaccinia Virus Replication and DNA Synthesis in Mouse L 929 Cells[a]

compd (10 μM)	% inhibn of plaque formation (72 h)	% of control incorporation of [^{3}H]thymidine	
		36 h	72 h
C-Ado (1)	65	81	134
N^6-methyl-C-Ado (2)	19	95	137
8-aza-C-Ado (3)	7	89	146
3-deaza-C-Ado (4)	95	86	246
C-AdoDA (5)	75	67	130
N^6-methyl-C-AdoDA (6)	60	63	106
8-aza-C-AdoDA (7)	63	91	81
3-deaza-C-AdoDA (8)	91	107	133
C-AdoDO (9)	13	ND[b]	ND
N^6-methyl-C-AdoDO (10)	7	ND	ND
8-aza-C-AdoDO (11)	11	ND	ND
3-deaza-C-AdoDO (12)	9	ND	ND

[a] Monolayer cultures of mouse L cells, 1.5 × 10^6 cells/35-mm dish for virus plaque assays and 2.5 × 10^6 cells/60-mm dish for [^{3}H]thymidine incorporation studies, were treated as described in the text. Results for both sets of experiments are expressed as a percent of the untreated control cultures. [b] ND, not determined.

(32) D. M. Houston, E. K. Dolence, B. T. Keller, U. Patel-Thombre, and R. T. Borchardt, previous article in this issue.

act as cytostatic agents causing temporary growth inhibition.

Experimental Section

Biological Methods. AdoHcy hydrolase activity was determined in the direction of hydrolysis with use of [2,8-^{3}H]AdoHcy (19 μCi/μmol) according to the procedures of Richards et al.[27] A typical incubation mixture (0.5 mL) contained 150 mM potassium phosphate (pH 7.6), appropriate concentration of inhibitor (2.0 mM or 0.2 mM), 1.0 mM EDTA, [2,8-^{3}H]AdoHcy (100 μM; 19 μCi/μmol), and 4 units of intestinal adenosine deaminase at 37 °C. (Adenosine deaminase unit is defined by the supplier as the following: 1 unit will deaminate 1.0 μmol of adenosine to inosine per minute at pH 7.5 at 25 °C.) The reaction was started by the addition of purified bovine liver AdoHcy hydrolase. After 5 min, the reaction was stopped by the addition of 100 μL of 5 N formic acid and the reaction mixture was then poured onto a column (1.2 × 4 cm) of SP-sephadex-C-25, which was previously equilibrated with 0.1 N formic acid. Each test tube was rinsed with 0.5 mL of 0.1 N formic acid. [2,8-^{3}H]Inosine, the product of the deamination of [2,8-^{3}H]adenosine formed by the hydrolysis of [2,8-^{3}H]AdoHcy, was subsequently eluted from the column by addition of 8 mL of 0.1 N formic acid. A 1-mL aliquot of the elute was added to 10 mL of 3a70 scintillation fluid and the radioactivity determined. The results were corrected with the blank, which contained everything except AdoHcy hydrolase.

Vaccinia virus plaque assays were carried out in 35-mm culture dishes containing 1.5 × 10^6 cells/dish. The medium was removed by aspiration and fresh serum-free KU-1 medium[32] containing vaccinia virus (WR) was added. The plates were incubated for 60 min, the viral inoculum was removed, and fresh medium (3 mL) containing the indicated concentrations of the potential inhibitor was added. The infected cultures were then incubated for 72 h (37 °C), after which the medium was discarded, the cells stained with crystal violet, and the plaques counted. All samples were carried out in quadruplicate.

For [^{3}H]thymidine incorporation studies, L cells (2.5 × 10^6 cells/60-mm tissue culture dish) were treated with or without the indicated drugs (10 μM) in serum-free KU-1 medium[32] for 36 and 72 h. At these times the medium was aspirated, 1.5 mL of fresh medium (without the drugs) containing 0.5 μCi [^{3}H]thymidine was added, and the dishes were incubated for 2 h at 37 °C. Following incubation the cells were removed from the dishes by trypsinization, suspended in 10% TCA, and kept at 4 °C for several hours. The acid precipitates were collected on Whatman GF/C filters and digested with 0.5 mL of NCS tissue solubilizer (New England Nuclear, Boston, MA) and the radioactivity quantitated by liquid scintillation counting in 10 mL of Bray's solution (New England Nuclear, Boston, MA). All samples were done in duplicate.

Chemistry. Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Varian FT-80A (80 MHz for proton nuclei) spectrophotometer. Chemical shifts are reported in δ from the internal standard tetramethylsilane (Me_4Si), δ_{Me_4Si} = 0.00. Mass spectra were recorded on a Ribermag R10-10 quadrupole mass spectrometer. Peak matching was performed by a Varian MAT CH5 magnetic deflection mass spectrometer. Elemental analyses were performed on an F and M Model 185 C, H, N analyzer in the Department of Medicinal Chemistry, University of Kansas. Where analyses are indicated by symbols of the elements, analytical results obtained for those elements were within ±0.04% of the theoretical values. Ion-exchange column chromatography was performed with a strong cation-exchange resin Dowex 50-W (H^+ form), 4% cross-linked, 100–200 dry mesh. Thin-layer chromatography was carried out on silica gel GF (Analtech, 0.25 mm) commercial glass backed plates and cellulose (Avicel F, Analtech) glass backed plates. Preparative-layer chromatography was carried out on cellulose (Avicel F, Analtech, 1.00 mm) glass backed plates. R_f values are shown in Table III.

(±)-3-Nitro-4-[[2α,3α-dihydroxy-4β-(hydroxymethyl)-1β-cyclopentyl]amino]pyridine (15). Compound 15 was prepared by reaction of (±)-4β-amino-2α,3α-dihydroxy-1β-cyclopentanemethanol[21] (13: 175 mg, 1.2 mmol) and 4-chloro-3-nitropyridine[24,28] (14: 200 mg, 1.3 mmol) in H_2O–dioxane (8:3) for 30 min at ambient temperature. The solution was cooled in an ice bath and 1 mL of triethylamine was added. This solution was stirred for 30 min at ice-bath temperature and then at ambient temperature for 15 h. The solution was reduced in vacuo to dryness and the residue dissolved in 5 mL of H_2O. After neutralization with 10% HCl, the solution was loaded onto a Dowex 50-W (H^+) column (1.5 × 25 cm) and the column was eluted with 150 mL of H_2O and then 200 mL of 2 N NH_3. The alkaline fractions which contained mostly 15 were combined, reduced in vacuo to 5 mL, and cooled to give 78 mg of product: mp 179–182 °C; ^{1}H NMR (Me_2SO-d_6) δ 9.0 (s, 1 H, H-2), 8.05 (d, 1 H, H-6), 7.05 (d, 1 H, H-5), 3.6–4.05 (several overlapping m, 3 H, H-1′, H-2′, and H-3′), 3.25–3.55 (m, 2 H, 2 H-5′), 1.75–2.5 (2 overlapping m, 2 H, H-4′ and H-6′α), 1.1–1.5 (m, 1 H, H-6′β). Anal. ($C_{11}H_{15}N_3O_5$) C, H, N.

(±)-2-Chloro-3-amino-4-[[2α,3α-dihydroxy-4β-(hydroxymethyl)-1β-cyclopentyl]amino]pyridine (16). Compound 16 was prepared by using a modified procedure of Koenig et al.[25] A stirred solution of 15 (100 mg, 0.37 mmol) in 12 N HCl (0.88 mL) was heated to 90 °C. To this hot solution was added $SnCl_2$ (412 mg, 1.84 mmol) in five portions over a 60-s period. This solution was stirred at 90 °C for 30 min, cooled to 5 °C, diluted with 10 mL of water, and reduced in vacuo to dryness. The residue was dissolved in 5 mL of H_2O and stirred in an ice bath while 2 N NH_3 was added dropwise till a precipitate formed. An excess of 1 mL of 2 N NH_3 was added to the cooled stirred mixture after which the solution was kept at 2 °C for 15 h. The cloudy mixture was filtered through a Celite pad and the pad was washed with H_2O (3 × 10 mL). The combined filtrate and washings were reduced in vacuo to 5 mL. This solution was acidified (pH 3) with 10% HCl and loaded on to a Dowex 50-W (H^+) column (1.0 × 18 cm). The column was washed with 100 mL of water and then 250 mL of 2 N NH_3. The alkaline eluant was reduced in vacuo to 2 mL and cooled to give 74 mg of a white solid (73% yield): mp 204–207 °C; ^{1}H NMR (Me_2SO-d_6) δ 7.35 (d, 1 H, J = 6 Hz, H-6), 6.45 (d, 1 H, J = 6 Hz, H-5). These signals are consistent with the basic 2-chloro-3-amino-4-substituted-pyridine structure. Anal. ($C_{11}H_{16}ClN_3O_3$) C, H, N.

(±)-4-Chloro-1-[2α,3α-dihydroxy-4β-(hydroxymethyl)-1β-cyclopentyl]imidazo[4,5-c]pyridine (17). Compound 17 was prepared by using a modified procedure of Montgometry et al.[13] A solution of 16 (294 mg, 1.08 mmol) in triethyl orthoformate (6.9 mL), N,N-dimethylacetamide (3.5 mL), and 12 N HCl (0.12 mL) was stirred at ambient temperature for 12 h under N_2. The solution was reduced in vacuo to dryness and the residue dissolved in 10 mL of 2% HCl and warmed at 40 °C for 30 min. The solution was partially neutralized (pH 4) with 2 N NH_3. This solution was loaded on a Dowex 50-W (H^+) column (1.5 × 25 cm). The column was washed with 100 mL of water and 250 mL of 2 N NH_3. The alkaline eluant was reduced in vacuo to 10 mL and cooled to give 250 mg of a white solid (82% yield): 217–219 °C (lit.[13] mp 219–221 °C); ^{1}H NMR (Me_2SO-d_6) δ 8.55 (s, 1 H, H-8), 8.11 (d, 1 H, J = 6 Hz, H-2), 7.76 (d, 1 H, J = 6 Hz, H-3).

Table III. R_f Values for Carbocyclic Purine Nucleosides, Nucleoside 2′,3′-Dialdehydes, and Nucleoside 2′,3′-Diols

compd	R_f values in chromatography systems[a]				
	A	B	C	D	E
C-Ado (1)[b]	0.18	0.43	0.62	0.46	0.51
N^6-methyl-C-Ado (2)	0.31	0.53	0.71	0.61	0.60
8-aza-C-Ado (3)[b]	0.31	0.43	0.46	0.63	0.75
3-deaza-C-Ado (4)[b]	0.10	0.32	0.53	0.27	0.41
C-AdoDA (5)	0.43	0.66	0.67	0.52	0.73
N^6-methyl-C-AdoDA (6)	0.54	0.74	0.79	0.64	0.78
8-aza-C-AdoDA (7)	0.58	0.66	0.54	0.67	0.87
3-deaza-C-AdoDA (8)	0.23	0.50	0.58	0.21	0.44
C-AdoDO (9)	0.18	0.45	0.65	0.64	0.65
N^6-methyl-C-AdoDO (10)	0.27	0.54	0.79	0.77	0.73
8-aza-C-AdoDO (11)	0.29	0.47	0.54	0.84	0.83
3-deaza-C-AdoDO (12)	0.09	0.33	0.62	0.28	0.53

[a] The chromatography systems had the following composition: system A, $CHCl_3$–MeOH (3:1) on silica gel GF; system B, n-BuOH–H_2O–HOAc (10:6:3) on silica gel GF; system C, 5% Na_2HPO_4 on silica gel GF; system D, H_2O on Avicel F; system E, EtOH–H_2O (2:1) on Avicel F. [b] Dissolved in 0.1 N HOAc.

These signals are consistent with the basic 4-chloro-1-substituted-imidazo[4,5-c]pyridine structure. Anal. ($C_{12}H_{14}ClN_3O_3$) C, H, N.

(±)-4-Amino-1-[2α,3α-dihydroxy-4β-(hydroxymethyl)-1β-cyclopentyl]imidazo[4,5-c]pyridine (3-Deaza-C-Ado, 4). 3-Deaza-C-Ado (4) was prepared by using a modification of the procedure of Montgomery et al.[13] A solution of 17 (246 mg, 0.87 mmol) in hydrazine (97+%, 7.4 mL) was heated at 95 °C for 2 h under N_2. The solution was reduced in vacuo to dryness and 8 mL of a slurry of freshly prepared Raney nickel in H_2O was added. [The Raney nickel slurry was prepared by the slow (1 h) dropwise addition of 20% NaOH (20 mL) to a stirred slurry of 1.5 g of nickel–aluminum alloy (Ventron Division of Alfa, Ni–Al, 50/50). The slurry was stirred 12 h after the addition was complete and then allowed to settle. The H_2O was decanted and the solid was resuspended in 20 mL of H_2O for 10 min, allowed to settle, and decanted. This was repeated (~7x) until the H_2O was neutral (pH 7).] The slurry obtained after adding the Raney nickel was heated with stirring at 90 °C until effervescence stopped (5–15 min). This slurry was filtered while still hot. The filter pad of Raney nickel was quickly washed with boiling H_2O (5 × 15 mL). The combined washes and filtrate were reduced in vacuo to 5 mL and cooled to give 101 mg of white crystals (44% yield): mp 238–240 °C (lit.[13] mp 236–238 °C); for R_f values, see Table III. Anal. ($C_{12}H_{16}N_4O_3$) C, H, N.

General Procedure for Oxidation of Carbocyclic Purine Nucleosides to the 2′,3′-Dialdehyde Derivatives. The carbocyclic purine nucleosides [C-Ado (1),[21] N^6-methyl-C-Ado (2),[22] 8-aza-C-Ado (3),[23] 3-deaza-C-Ado (4)] were oxidized with periodic acid to the corresponding 2′,3′-dialdehydes [C-AdoDA (5), N^6-methyl-C-AdoDA (6), 8-aza-C-AdoDA (7), 3-deaza-C-AdoDA (8)] by using a general procedure previously described by Borchardt et al.[20] A suspension of the nucleoside (0.2 mmol) in H_2O (2 mL) was stirred with ice bath cooling and periodic acid (45.6 mg, 0.2 mmol), previously dried in vacuo at 35 °C for 12 h, was added in one portion. The reaction mixture was protected from light and stirred at ambient temperature for 4 h. The solution was extracted with Et_2O (4 × 5 mL), diluted with H_2O (2 mL), and neutralized (pH 6.0) with lead carbonate. After filtration through a cellulose column (0.5 × 2.0 cm), the filtrate was lyophilized. The carbocyclic nucleoside dialdehydes were shown to be pure (single spot) by thin-layer chromatography (Table III). The ^1H NMR and mass spectra were consistent with the assigned structures. Reduction of the dialdehydes to the corresponding carbocyclic nucleoside diols provided further evidence to support the assigned structures. Attempts to crystallize the carbocyclic nucleoside dialdehydes for elemental analyses resulted in rapid degradation. The lyophilized samples were stored at −20 °C and solutions were freshly prepared for biological testing.

Carbocyclic adenosine-2′,3′-dialdehyde (C-AdoDA, 5): yield 81%; 200 °C dec; for R_f values, see Table III; ^1H NMR (Me_2SO-d_6) δ 9.72 (2 s, 2 H, H-2′ and H-3′), 8.31–7.94 (2 br s, 2 H, H-2 and H-8), 5.31–4.94 (m, 1 H, H-1′), 4.94–2.63 (several overlapping m, 3 H, 2 H-5′ and H-4′), 2.63–1.50 (m, 2 H, 2 H-6′); mass spectrum (D-EI, methanol), m/e 263 (M), 245 (M − H_2O), 234 (M − CHO), 216 [M − (H_2O + CHO)], 188 (adenine $C_4H_6^+$), 177 (adenine − $CH_2CH_2CH_3$), 162 (adenine $C_2H_4^+$), 148 (adenine CH_2^+), 136 (adenine H^+). Anal. ($C_{11}H_{13}N_5O_3 \cdot {}^3/_2H_2O$) C, H, N.

Carbocyclic N^6-methyladenosine-2′,3′-dialdehyde (N^6-methyl-C-AdoDA, 6): yield 100%; mp 115–130 °C dec; for R_f values, see Table III; ^1H NMR (Me_2SO-d_6) δ 9.69 (2 s, 2 H, H-2′ and H-3′), 8.12–7.88 (2 br s, 2 H, H-2 and H-8), 7.69–7.31 (m, 1 H, N*H*CH_3), 5.25–4.81 (m, 1 H, H-1′), 4.81–2.63 (several overlapping m, 3 H, 2 H-5′ and H-4′), 2.94 (d, 3 H, NHC*H*$_3$), 2.63–1.5 (m, 2 H, 2 H-6′); mass spectrum (D-EI, methanol), m/e 277 (M), 248 (M − CHO), 191 (N^6-methyladenine $CH_2CH_2CH_3$), 176 (N^6-methyladenine $C_2H_4^+$), 162 (N^6-methyladenine CH_2^+), 150 (N^6-methyladenine H^+).

Carbocyclic 8-azaadenosine-2′,3′-dialdehyde (8-aza-C-AdoDA, 7): yield 92%; mp 155–170 °C dec; for R_f values, see Table III; ^1H NMR (Me_2SO-d_6) δ 9.75 (s, 2 H, H-2′ and H-3′), 8.25 (s, 1 H, H-2), 5.38–4.94 (m, 1 H, H-1′), 4.94–2.69 (several overlapping m, 3 H, 2 H-5 and H-4′), 2.69–1.75 (m, 2 H, 2 H-6′); mass spectrum (D-EI, methanol), m/e 265 (MH^+), 247 (MH^+ − H_2O), 236 (MH^+ − CHO), 217 [M − (H_2O + CHO)], 207 (MH^+ − 2 CHO), 161 (8-azaadenine $C_2H_2^+$), 149 (8-azaadenine CH_2^+), 137 (8-azaadenine H^+).

Carbocyclic 3-deazaadenosine-2′,3′-dialdehyde (3-deaza-C-AdoDA, 8): yield 77% mp >130 °C (slowly dec); for R_f values, see Table III; ^1H NMR (Me_2SO-d_6) δ 9.69 (2 s, 2 H, H-2′ and H-3′), 8.44 (s, 1 H, H-8), 7.63 (d, 1 H, H-2), 7.13 (d, 1 H, H-3), 6.94–4.69 (m, 1 H, H-1′), 4.69–3.75 (2 m, 3 H, H-4′ and 2 H-5′), 3.00–2.06 (2 m, 2 H, 2 H-6′); mass spectrum (D-EI, methanol), m/e 262 (M), 244 (M − H_2O), 215 [M − (H_2O + CHO)], 188 (3-deazaadenine C_4H_7), 176 (3-deazaadenine $CH_2CH_2CH_3$), 161 (3-deazaadenine $C_2H_4^+$), 149 (3-deazaadenine CH_4^+), 134 (3-deazaadenine).

General Procedure for Reduction of Carbocyclic Purine Nucleosides 2′,3′-Dialdehydes to the 2′,3′-Diol Derivatives. The carbocyclic purine nucleoside dialdehydes [C-AdoDA (5), N^6-methyl-C-Ado-DA (6), 8-aza-C-AdoDA (7), 3-deaza-C-AdoDA (8)] were reduced with $NaBH_4$ to the corresponding 2′,3′-diols [C-AdoDO (9), N^6-methyl-C-AdoDO (10), 8-aza-C-AdoDO (11), 3-deaza-C-AdoDO (12)]. To a stirred solution of the carbocyclic purine nucleoside 2′,3′-dialdehydes (0.10 mmol) in 0.1 M phosphate buffer, pH 8.4 (0.5 mL), at ambient temperature was added slowly $NaBH_4$ (15 mg, 0.40 mmol). The reaction mixture was stirred at ambient temperature for 4 h, after which the solution was adjusted to pH 4.0 with 5% HCl and then readjusted to pH 7 with 0.2 N NaOH. The 2′,3′-diols were purified on a Dowex 50-W (H^+) and shown to be pure (single spot) by thin-layer chromatography (see Table III). The ^1H NMR and mass spectra were consistent with the assigned structures. Attempts to crystallize the 2′,3′-diols for elemental analyses resulted in rapid degradation. The lyophilized samples were stored at −20 °C and solutions were freshly prepared for biological testing.

Carbocyclic adenosine-2′,3′-diol (C-AdoDO, 9): yield 95%; for R_f values see Table III; ^1H NMR (Me_2SO-d_6) δ 8.07 (s, 2 H, H-8 and H-2), 4.60 (m, 1 H, H-1′), 4.30 (m, 2 H, and CH-5′), 3.72 (m, 2 H, 2 H-2′), 3.30 (several overlapping m, 4 H, 2 H-3′ and 2 H-5′), 1.86 (m, 2 H, 2H-6′), 1.15 (m, 1 H, H-4′); mass spectrum (D-EI, methanol), m/e 267 (M) peak match Δ = 0.0013, 250 (MH^+ − H_2O), 236 (M − CH_2OH), 179 (adenine − CH_2CH_2OH), 162 (adenine $C_2H_4^+$), 136 (adenine H^+).

Carbocyclic N^6-methyladenosine-2′,3′-diol (N^6-methyl-C-AdoDO, 10): yield 96%; for R_f values, see Table III; ^1H NMR (Me_2SO-d_6) δ 8.10 (2 s, 2 H, H-2 and H-8), 7.50 (br m, $NHCH_3$), 4.60 (m, 1 H, H-1′), 3.70 (m, 2 H, 2 H-2′), 3.26 (several overlapping m, 4 H, 2 H-3′, 2 H-5′), 2.97 (d, 3 H, $NHCH_3$), 1.85 (m, 2 H, 2 H-6′), 1.15 (m, 1 H, H-4′); mass spectrum (D-EI, methanol), m/e 281 (M) peak match Δ = 0.0014, 264 (MH^+ − H_2O), 250 (M − CH_2OH), 193 (N^6-methyladenine CH_2CH_2OH), 176 (N^6-methyladenine $C_2H_4^+$), 150 (N^6-methyladenine H^+).

Carbocyclic 8-azaadenosine-2′,3′-diol (8-aza-C-AdoDO, 11): yield 75%; for R_f values, see Table III; ^1H NMR (Me_2SO-d_6) δ 8.20 (s, 1 H, H-2), 4.87 (m, H, H-1′), 3.80 (m, 2 H, 2 H-2′), 3.30 (several overlapping m, 4 H, 2 H-5′, 2 H-3′), 1.90 (m, 2 H, 2 H-6′), 1.12 (m, 1 H, H-4′); mass spectrum (D-EI, methanol), m/e 268 (M) peak match Δ = 0.0001, 251 (MH^+ − H_2O), 237 (M − CH_2OH), 210 (8-azaadenine − $C_2H_4^+$).

Carbocyclic 3-deazaadenosine-2′,3′-diol (3-deaza-C-AdoDO, 12): yield 94%; for R_f values, see Table III; ^1H NMR (Me_2SO-d_6) δ 8.03 (s, 1 H, H-8), 7.58 (d, 1 H, H-2), 6.73 (d, 1 H, H-3), 4.35 (m, 3 H, H-1′, OH-3 and -OH-5′), 3.67 (m, 2 H, 2 H-2′), 3.32 (several overlapping m, 4 H, 2 H-5′, 2 H-3′), 1.80 (m, 2 H, 2 H-6′), 1.20 (m, 1 H, H-4′); mass spectrum (D-EI, methanol), m/e 266 (M) peak match Δ = 0.0003, 235 (M − CH_2OH), 161 (3-deazaadenine $C_2H_4^+$), 135 (3-deazaadenine H^+).

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