Potential Inhibitors of *S*-Adenosylmethionine-Dependent Methyltransferases. 8. Molecular Dissections of Carbocyclic 3-Deazaadenosine 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 9-(hydroxyalkyl)-3-deazaadenines, which are analogues of the carbocyclic derivative of 3-deazaadenosine (3-deaza-C-Ado), were synthesized. The analogues were tested as inhibitors of bovine liver *S*-adenosyl-L-homocysteine (AdoHcy) hydrolase (EC 3.3.1.1) and as inhibitors of vaccinia virus (WR) replication in clone 929 mouse L cells and the results were compared to those observed for the parent compound, 3-deaza-C-Ado. 4-Amino-1-(2,3-dihydroxy-1-propyl)imidazo[4,5-*c*]pyridine (**14**), the analogue which included the 1′-, 2′-, and 3′-carbons of 3-deaza-C-Ado, was the most active inhibitor toward purified AdoHcy hydrolase. The inhibitory effect of **14** (K_i = 768 nM) on AdoHcy hydrolase was significantly less than that observed for 3-deaza-C-Ado (K_i = 4 nM). Analogue **14** also exhibited inhibitory activity against vaccinia virus replication, but the activity was less than that observed with 3-deaza-C-Ado. 4-Amino-1-(4-hydroxy-1-butyl)imidazo[4,5-*c*]pyridine (**15**) showed little or no inhibitory activity toward AdoHcy hydrolase, but it did exhibit antiviral effects comparable to **14**. These results suggest that 3-deaza-C-Ado and analogue **14** may be producing their antiviral effects by altering a critical viral methylation (e.g., methylation of the 5′-cap of viral mRNA), whereas analogue **15** may be acting through an alternative mechanism.

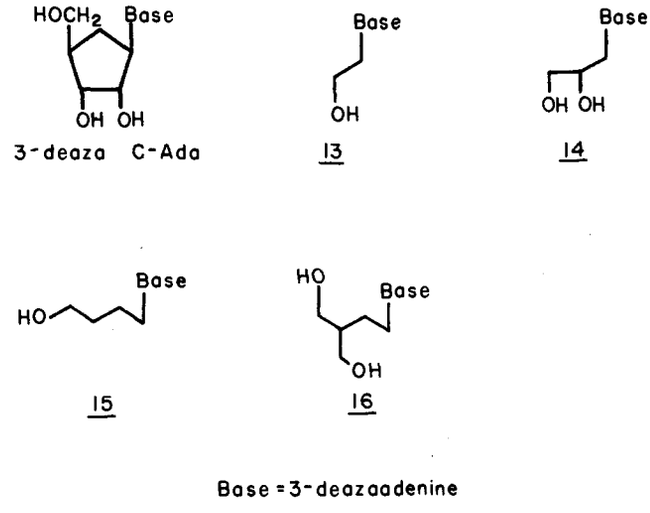
S-Adenosylmethionine (AdoMet) dependent methyltransferases are sensitive to inhibition by *S*-adenosyl-L-homocysteine (AdoHcy), a product of these reaction.[2-4] Therefore, AdoHcy hydrolase (EC 3.3.1.1), which in mammals catalyzes the hydrolysis of AdoHcy to homocysteine and adenosine,[5] is a key enzyme in regulating AdoMet-dependent methylations.[6] Because of its regulatory role, AdoHcy hydrolase has become an attractive target for the design of potential therapeutic agents.[2,6,7] Various inhibitors of AdoHcy hydrolase have been identified, including 3-deazaadenosine,[8] adenine arabinoside,[9] aristeromycin,[10] 2′-deoxyadenosine,[11] 9(*S*)-(2,3-dihydroxypropyl)adenine,[12] adenosine dialdehyde,[14,15] and related purine nucleosides.[7] Recently, Montgomery et al.[16] reported the synthesis of (±)-4-amino-1-[(1α,2β,3β,4α)-2,3-dihydroxy-4-(hydroxymethyl)cyclopentyl]imidazo[4,5-*c*]pyridine (3-deaza-C-Ado), the carbocyclic analogue of 3-deazaadenosine. 3-Deaza-C-Ado was shown to be a potent inhibitor of AdoHcy hydrolase and found to have antiviral activity in cell culture against herpes simplex virus type 1, vaccinia virus, and HL-23 C-type virus.[16]

On the basis of reported inhibitory activities of 3-deaza-C-Ado[16] and the 9-(hydroxyalkyl)adenine analogues[12,13] toward AdoHcy hydrolase, we undertook the synthesis of the 9-(hydroxyalkyl)-3-deazaadenine analogues **13–16** (Chart I). These analogues should be useful in elucidating those structure features of the trihydroxy carbocyclic portion of 3-deaza-C-Ado that are necessary for binding to AdoHcy hydrolase.

Chemistry. Compounds **13–16** were prepared by condensing 4-ethoxy-3-nitropyridine with the appropriate amino alcohol followed by base elaboration (Scheme I). Three of the necessary amino alcohols, ethanolamine, 2,3-dihydroxy-1-aminopropane, and 4-hydroxy-1-aminobutane, were commercially available. 1,5-Dihydroxy-2-aminopentane, the amino alcohol used in the preparation of **16**, was obtained by reduction of the dimethyl ester of glutamic acid with $LiAlH_4$. 4-Ethoxy-3-nitropyridine was prepared from 4-chloro-3-nitropyridine or its hydrochloride salt.[17]

The 3-nitro-4-[(hydroxyalkyl)amino]pyridines **1–4** were reductively chlorinated to 2-chloro-3-amino-4-[(hydroxyalkyl)amino]pyridines **5–8** with use of stannous chloride in hot 12 N HCl following the procedure of Koenig et al.,[18]

Chart I. 3-Deaza-C-Ado and Its Molecular Dissections **13–16**



† Department of Medicinal Chemistry.
‡ Department of Biochemistry.

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Scheme I. Preparation of 9-(Hydroxyalkyl)-3-deazaadenines

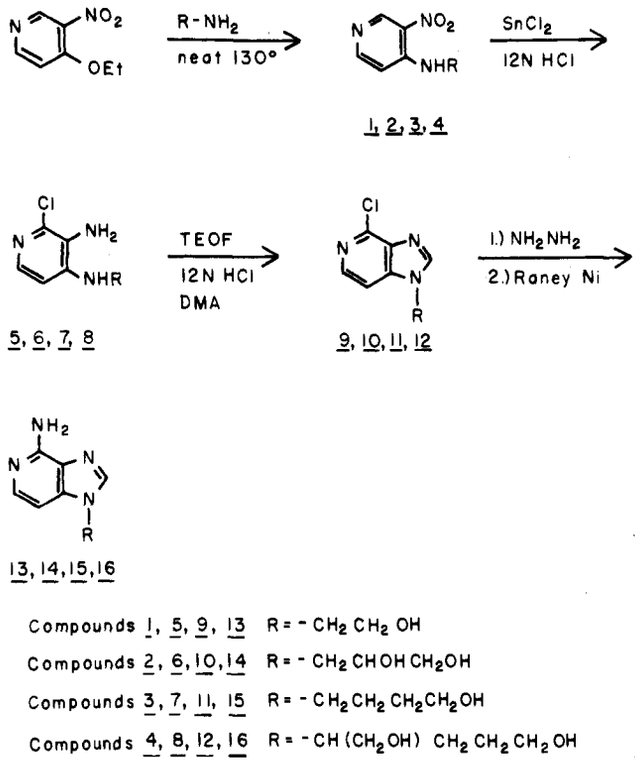

Table I. Inhibition of Bovine Liver AdoHcy Hydrolase by 9-(Hydroxyalkyl)-3-deazaadenines[a]

compd	% inhibition	
	I = 0.2 mM	I = 2.0 mM
3-deaza-C-Ado	100	ND
13	12	27
14	84	95
15	11	12
16	12	50

[a] AdoHcy hydrolase activity was determined by using the procedure of Richards et al.,[5] which involves measuring the hydrolysis of [2,8-^{3}H]AdoHcy to [2,8-^{3}H]adenosine and homocysteine. The assay 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.

which was later modified by Mizuno et al.[19] The 2-chloro-3-amino-4-[(hydroxyalkyl)amino]pyridines **5–8** were treated with triethyl orthoformate to yield the 4-chloro-1-(hydroxyalkyl)imidazo[4,5-*c*]pyridines **9–12**, which were then converted to the 9-(hydroxyalkyl)-3-deazaadenine analogues **13–16** by reaction with hydrazine followed by Raney nickel reduction.[16]

Table II. Effects of 9-(Hydroxyalkyl)-3-deazaadenines on Vaccinia Virus Replication and DNA Synthesis in L-929 Cells[a]

compd (10 μM)	% inhibn of plaque formation (72 h)	% of control incorp of [^{3}H]thymidine	
		36 h	72 h
3-deaza-C-Ado	95	86	246
13	33	95	113
14	53	88	138
15	51	102	110
16	27	91	140

[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.

Results

Biological Activity. Inhibitory Effects on AdoHcy Hydrolase. Montgomery et al.[16] have previously reported that 3-deaza-C-Ado is a potent inhibitor of bovine liver (K_i = 3 μM) and hamster liver (K_i = 1 nM) AdoHcy hydrolase. We have also found 3-deaza-C-Ado to be a potent competitive inhibitor of the bovine liver enzyme with a K_i = 4 nM. We are unable to explain the discrepancy between our observed K_i value for 3-deaza-C-Ado and that reported by Montgomery et al.[16] against the bovine liver enzyme.

A comparison of the inhibitory effects of the 9-(hydroxyalkyl)-3-deazaadenine analogues **13–16** and 3-deaza-C-Ado is shown in Table I. The only analogue which showed significant inhibitory activity toward bovine liver AdoHcy hydrolase was the 2,3-dihydroxy-1-propyl analogue **14**. Analogue **14** can be viewed as a molecular dissection of 3-deaza-C-Ado which includes the 1′-, 2′-, and 3′-carbons (Chart I). Analogue **13**, which contains the equivalent of the 1′- and 2′-carbons, and analogue **15**, which contains the equivalent of the 1′-, 4′-, and 5′- (or 3′-) carbons, and analogue **16**, which contains the equivalent of the 1′-, 2′-, 4′-, and 5′- (or 3′-) carbons were all substantially less effective as inhibitors of the hydrolase, even at concentrations of up to 2 mM.

Analogue **14** was shown to be a competitive inhibitor (K_i = 768 nM) of AdoHcy hydrolase when AdoHcy was the variable substrate and the enzyme activity was measured in the hydrolytic direction. Analogue **14** is a less effective inhibitor than 3-deaza-C-Ado (K_i = 4 nM) yet sufficiently active to suggest that 9-(hydroxyalkyl)-3-deazaadenine analogues may represent a new lead in the efforts to design more effective inhibitors of AdoHcy hydrolase.

Antiviral Activity. Previous work from this laboratory,[22-26] as well as other laboratories,[16,27] indicates that inhibitors of AdoHcy hydrolase often possess antiviral activity. Our findings that (±)-4-amino-1-(2,3-dihydroxy-1-propyl)imidazo[4,5-*c*]pyridine (**14**) is a potent inhibitor of AdoHcy hydrolase prompted us to test this

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analogue as well as the other 9-(hydroxyalkyl)adenine analogues **13**, **15**, and **16** for their antiviral effects. The ability of 3-deaza-C-Ado and the analogues **13–16** to inhibit vaccinia virus plaque formation after 72 h in monolayer cultures of mouse L cells was examined. As shown in Table II, significant inhibition of plaque formation was observed with 3-deaza-C-Ado, analogue **14**, and analogue **15**. For 3-deaza-C-Ado and analogue **14** there appears to be a correlation between the AdoHcy hydrolase inhibitory effects (Table I) and the antiviral effects (Table II). These results support a mechanism involving inhibition of a critical viral methylation (e.g., methylation of the 5′-cap of viral mRNA) due to the intracellular inhibition of AdoHcy hydrolase and the resulting increase in AdoHcy levels. This correlation does not appear to hold for analogue **15**, which was a poor inhibitor of AdoHcy hydrolase, but showed antiviral effects comparable to analogue **14**.

In evaluating the specificity of a potential antiviral agent, an important consideration is the effect of the compound on 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 3-deaza-C-Ado or the analogues **13–16**. When cells were exposed to the compounds (10 μM) for 36 h, little or no inhibition of [^{3}H]thymidine was observed. However, after 72 h of treatment with 3-deaza-C-Ado or the analogues **13–16**, stimulation of DNA synthesis was observed which was compared to untreated controls. The largest effect (246%) was observed with 3-deaza-C-Ado. These results are similar to those we have observed for adenosine dialdehyde and Neplanocin A,[24] which were shown to be 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.[5] A typical incubation mixture (0.5 mL) contained 150 mM potassium phosphate, pH 7.6, an appropriate concentration of the inhibitor (0.2 or 2.0 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. (A unit of adenosine deaminase 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 1.0 × 10^6 mouse L 929 cells/dish. The medium was removed by aspiration and fresh serum-free KU-1 medium 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 3-deaza-C-Ado or the analogues **13–16** were added. The infected cultures were then incubated for 72 h (37 °C), after which the medium was discarded. The cells were stained with 0.1% 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[20] 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.

Table III. R_f Values of 9-(Hydroxyalkyl)-3-deazaadenines

compd	chromatography system[a] A	B	C	D	E
13[b]	0.19	0.34	0.49	0.28	0.54
14[b]	0.13	0.34	0.53	0.17	0.49
15[b]	0.26	0.36	0.32	0.35	0.72
16[c]	0.09	0.36	0.45	0.28	0.69
3-deaza-C-Ado[b]	0.10	0.32	0.53	0.27	0.41

[a] (A) $CHCl_3$–MeOH (3:1, 250-μm silica gel GF, Analtech); (B) *n*-BuOH–H_2O–HOAc (10:6:3, 250-μm silica gel GF, Analtech); (C) 5% Na_2HPO_4 (250-μm silica gel GF, Analtech); (D) H_2O (250-μm Avicel F, Analtech); (E) EtOH–H_2O (2:1, Avicel F, Analtech). [b] Dissolved in 0.1 N HOAc. [c] Prepared as the hydrochloride salt and dissolved in 2 N ammonia.

Synthesis. 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. Elemental analyses were performed on a F and M Model 185 C, H, N analyzer in the Department of Medicinal Chemistry, University of Kansas. Where analyses are indicted by symbols of the elements, analytical results obtained for those elements were within ±0.4% 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 plates and cellulose (Avicel F, Analtech, 0.25 mm) glass plates. R_f values for the 9-(hydroxyalkyl)-3-deazaadenine analogues **13–16** and 3-deaza-C-Ado are shown in Table III.

(±)-2-Amino-1,5-pentanediol. To a mixture of dry glutamic acid (35 g) in 400 mL of absolute MeOH cooled in an ice bath with stirring under N_2 was bubbled HCl gas for 90 min. The HCl bubbler was removed and the resulting solution was then stirred for 12 h at room temperature under N_2. The solution was reduced in vacuo to dryness, leaving the HCl salt of the dimethyl ester of glutamic acid as a white solid. This solid was dissolved in a minimum of ice-cold water and stirred in an ice bath while neutralization was carried out with 20% NaOH (pH 8). This solution was quickly extracted with Et_2O (4 × 100 mL). The combined Et_2O extracts were dried (Na_2SO_4) and reduced in vacuo, leaving 39 g of a clear colorless oil. This oil was dissolved in freshly dried THF (300 mL) and to this solution was added, by a dropping funnel, a solution of $LiAlH_4$ (9.33 g) in 175 mL of THF with cooling as required. After the addition was complete, the reaction mixture was stirred at room temperature for 15 h. THF (300 mL) was added with stirring and this mixture was then carefully quenched by the dropwise addition of water (20 mL) (1 μL of water/mg of $LiAlH_4$). This solution was allowed to stir for an additional 10 min after which a solution of 10% NaOH (40 mL) was slowly added dropwise with vigorous stirring. The reaction mixture was stirred an additional 10 min. Water (60 mL) was again added slowly and the mixture was allowed to stir for 1 h with ice-bath cooling. This mixture was filtered, leaving a fluffy white solid and a clear colorless filtrate. The salts were washed with 200 mL of Et_2O. The combined filtrates were dried (Na_2SO_4) and reduced in vacuo to give a slightly yellow viscous oil, which was generally satisfactory for subsequent reactions.

4-Hydroxy-3-nitropyridine. 4-Hydroxy-3-nitropyridine was prepared by a modification of the procedure of Kruger and Mann.[21] The nitrate salt of 4-hydroxypyridine (18.7 g, 0.118 mol) was added to a solution of fuming nitric acid (21 g, density 1.5) and 20% oleum (21 g). After the initial reaction subsided, the

solution was refluxed under nitrogen for 1 h. The resulting red solution was cooled and poured onto 150 mL of ice. This mixture was neutralized with 150 mL of concentrated ammonia (pH 7–8). The resulting solution was cooled for 15 h. Filtration gave a light yellow solid, which was recrystallized from water to give 7.45 g (45% yield): mp 278–279 °C (lit.[21] mp 278–279 °C).

4-Chloro-3-nitropyridine. 4-Chloro-3-nitropyridine was prepared from 4-hydroxy-3-nitropyridine by a modification of the procedure of Wright.[22] To 4-hydroxy-3-nitropyridine (2.0 g, 14.3 mmol) was added $POCl_3$ (5 mL). This mixture was stirred under nitrogen while warmed to 45 °C. PCl_5 (2.36 g) was added to the mixture, which was then heated to reflux (~125 °C). This solution was refluxed with stirring under nitrogen for 3 h. The solution was reduced in vacuo to an oil and cooled in an ice bath and stirred vigorously with 3 mL of water and 6 mL of $CHCl_3$. The cold aqueous layer was extracted with $CHCl_3$ (5 × 5 mL), and the combined $CHCl_3$ extracts were dried ($MgSO_4$) and reduced in vacuo to dryness to give 2.13 g of a lemon yellow crystalline solid (94% yield). This moisture-sensitive solid was stored under nitrogen at 2 °C; mp 43–45 °C (lit.[22] mp 45 °C).

4-Ethoxy-3-nitropyridine. A mixture of 4-chloro-3-nitropyridine in absolute ethanol was stirred overnight under nitrogen. This solution was neutralized (pH 8) with 2 N ammonia and reduced in vacuo to dryness. The residue was triturated with three portions of Et_2O and filtered. The ethereal filtrate was reduced in vacuo to dryness. This solid was recrystallied from 95% ethanol with chilling in an ice-salt bath, followed by rapid filtration to give the product in 60.4% yield: mp 46.5–48 °C (lit.[17] mp 46.5–48.5 °C).

3-Nitro-4-[(2-hydroxyethyl)amino]pyridine (1). Compound 1 was prepared following the general procedure of Bremer.[23] A mixture of 4-ethoxy-3-nitropyridine (1.0 g, 5.9 mmol) and ethanolamine (0.74 g, 12.1 mmol) was heated at 120 °C for 2 min and cooled. Recrystallization of the resulting yellow solid from hot water gave 1.02 g of 1 (73.4% yield): mp 141–142.5 °C (lit.[22] mp 144 °C); ^1H NMR (Me_2SO-d_6) δ 9.0 (2 s, 2 H, H-2), 8.19 (d, 1 H, H-6), 7.0 (d, 1 H, H-5), 3.31–3.75 (2 m, 4 H, 2 H-1′ and 2 H-2′). Anal. ($C_7H_9N_3O_3$) C, H, N.

2-Chloro-3-amino-4-[(2-hydroxyethyl)amino]pyridine (5). Compound 5 was prepared by using a modification of the procedure of Koenig et al.[18] Koenig et al.[18] prepared what was thought to be 6-chloro-3,4-diaminopyridine from 3-nitro-4-aminopyridine with use of stannous chloride ($SnCl_2$) and 12 N HCl. However, Mizuno et al.[19] later showed that the product was 2-chloro-3,4-diaminopyridine. A stirred solution of 1 (68 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 water and stirred in an ice bath while 2 N ammonia was added dropwise till a precipitate formed. An excess of 1 mL of 2 N ammonia was added to the cooled stirred mixture and then the solution was stored for 15 h at 2 °C. The cloudy mixture was filtered through a Celite pad and the pad washed with water (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 ammonia. The basic eluant was reduced in vacuo to 2 mL and cooled to give a white solid. Recrystallization from hot water gave light brown needles in 72% yield: mp 142–144 °C; ^1H NMR (Me_2SO-d_6) δ 7.34 (d, 1 H, H-6), 6.38 (d, 1 H, H-5), 3.4–3.7 (m, 2 H, H-2′), 3.0–3.3 (m, 2 H, 2 H-1′). Anal. ($C_7H_{10}ClN_3O$) C, H, N.

4-Chloro-1-(2-hydroxyethyl)imidazo[4,5-*c*]pyridine (9). Compound 9 was prepared from 5 by using a modification of the procedure of Montgomery et al.[16] A solution of 5 (203 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 room temperature for 12 h under nitrogen. 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 ammonia. 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 ammonia. The basic eluant was reduced in vacuo to 10 mL and cooled to give a white solid in 65% yield: mp 183–185 °C; ^1H NMR (Me_2SO-d_6) δ 8.31 (s, 1 H, H-2), 8.06 (d, 1 H, H-6), 7.63 (d, 1 H, H-7), 4.19–4.38 (t, 2 H, 2 H-1′), 3.56–3.81 (m, 2 H, 2 H-2′). Anal. ($C_8H_8ClN_3O$) C, H, N.

4-Amino-1-(2-hydroxyethyl)imidazo[4,5-*c*]pyridine (13). Compound 13 was prepared from 9 using a modified procedure of Montgomery et al.[16] A solution of 9 (173 mg, 0.87 mmol) in hyrazine (97+%, 7.4 mL) was heated at 95 °C for 2 h under nitrogen. The solution was reduced in vacuo to dryness and 8 mL of a slurry of freshly prepared Raney nickel in water 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). After the addition was complete, the slurry was stirred for 12 h and then allowed to settle. The liquid was decanted and the solid was resuspended in 20 mL of water. This process was repeated (~7×) until the water washes were neutral (pH 7).] The mixture obtained after adding the Raney nickel/water slurry was heated with stirring at 90 °C until effervescence stopped (5–15 min). This slurry was filtered while still hot and the filter pad of Raney nickel was quickly washed with boiling water (5 × 15 mL). The combined washes and filtrate were reduced in vacuo to 5 mL and cooled to give white crystals in 54% yield: mp 227–229 °C; ^1H NMR (Me_2SO-d_6) δ 7.94 (s, 1 H, H-2), 4.06–4.25 (t, 2 H, 2 H-1′), 3.5–3.75 (q, 2 H, 2 H-2′). Anal. ($C_8H_{10}N_4O$) C, H. N.

(±)-3-Nitro-4-[(2,3-dihydroxy-1-propyl)amino]pyridine (2). Compound 2 was prepared in the same manner as 1. Recrystallization from hot water gave yellow crystals in 72% yield: mp 132–134 °C; ^1H NMR (Me_2SO-d_6) δ 9.0 (s, 1 H, H-2), 8.19 (d, 1 H, H-6), 7.0 (d, 1 H, H-5), 3.13–3.88 (several overlapping m, 5H, 2 H-1′, H-2′, and 2 H-3′). Anal. ($C_8H_{11}N_3O_4$) C, H, N.

(±)-2-Chloro-3-amino-4-[(2,3-dihydroxy-1-propyl)amino]pyridine (6). Compound 6 was prepared in the same manner as 5. Recrystallization from water gave white crystals in 78% yield: 191–193 °C; ^1H NMR (Me_2SO-d_6) δ 7.37 (d, 1 H, H-6), 6.38 (d, 1 H, H-5), 2.75–3.81 (several overlapping m, 5 H, 2 H-1′, H-2′, and 2 H-3′). Anal. ($C_8H_{12}ClN_3O_2$) C, H, N.

(±)-4-Chloro-1-(2,3-dihydroxy-1-propyl)imidazo[4,5-*c*]pyridine (10). Compound 10 was prepared in the same way as 9. Dowex 50 W (H^+) ion-exchange column chromatography gave in quantitative yield the product as a hygroscopic oil. The material showed one UV positive spot on TLC [R_f 0.39, $CHCl_3$–MeOH (3:1), 250-μm silica gel GF, Analtech] and it was satisfactory for subsequent reactions.

(±)-4-Amino-1-(2,3-dihydroxy-1-propyl)imidazo[4,5-*c*]pyridine (14). Compound 14 was prepared in the same manner as 13. Recrystallization from water gave white crystals in 45.6% yield: mp 181–183 °C; ^1H NMR (Me_2SO-d_6) δ 7.88 (s, 1 H, H-2), 7.56 (d, 1 H, H-6), 6.69 (d, 1 H, H-7), 3.06–4.19 (several overlapping m, 5 H, 2 H-1′, H-2′, and 2 H-3′). Anal. ($C_9H_{12}N_4O_2 \cdot {}^5/_4H_2O$) C, H, N.

3-Nitro-4-[(4-hydroxy-1-butyl)amino]pyridine (3). Compound 3 was prepared in the same manner as 1. Recrystallization from hot water gave yellow crystals in quantitative yield: mp 46–48 °C; ^1H NMR (Me_2SO-d_6) δ 8.94 (s, 1 H, H-2), 8.19 (d, 1 H, H-6), 6.94 (d, 1 H, H-5), 3.25–3.56 (2 overlapping m, 4 H, 2 H-2′, and 2 H-3′), 1.31–1.75 (2 overlapping m, 2 H-1′ and 2 H-4′). Anal. ($C_9H_{13}N_3O_3$) C, H, N.

2-Chloro-3-amino-4-[(4-hydroxy-1-butyl)amino]pyridine (7). Compound 7 was prepared in the same way as 5. Recrystallization from hot water gave white crystals in 81% yield: mp 63–65 °C; ^1H NMR (Me_2SO-d_6) δ 7.31 (d, 1 H, H-6), 6.31 (d, 1 H, H-5), 2.95–3.56 (2 m, 4 H, 2 H-1′ and 2 H-4′), 1.44–1.63 (2 overlapping m, 4 H, 2 H-2′ and 2 H-3′). Anal. ($C_9H_{14}ClN_3O \cdot {}^1/_8H_2O$) C, H, N.

4-Chloro-1-(4-hydroxy-1-butyl)imidazo[4,5-*c*]pyridine (11). Compound 11 was prepared in the same manner as 9. Dowex 50-W (H^+) ion-exchange column chromatography gave in quantitative yield a hygroscopic oil. The material showed one UV positive spot by TLC [R_f = 0.50, $CHCl_3$–MeOH (3:1), 250-μm silica gel GF, Analtech] and it was satisfactory for subsequent reactions.

4-Amino-1-(4-hydroxy-1-butyl)imidazo[4,5-*c*]pyridine (15). Compound 15 was prepared in the same manner as 13. Recrystallization from hot water gave white crystals in 30% yield: mp 130–132 °C; ^1H NMR (Me_2SO-d_6) δ 8.0 (s, 1 H, H-2), 7.56 (d,

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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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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