Inactivation of *S*-Adenosyl-L-homocysteine Hydrolase by Amide and Ester Derivatives of Adenosine-5′-carboxylic Acid

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　　　　S-Adenosyl-L-homocysteine (AdoHcy) hydrolase has been shown to have (5′/6′) hydrolytic activity with vinyl (5′) or homovinyl (6′) halides derived from adenosine (Ado). This hydrolytic activity is independent of its 3′-oxidative activity. The vinyl (or homovinyl) halides are converted into 5′(or 6′)-carboxaldehydes by the hydrolytic activity of the enzyme, and inactivation occurs via the oxidative activity. Amide and ester derivatives of Ado-5′-carboxylic acid were prepared to further probe the hydrolytic capability of AdoHcy hydrolase. The oxidative activity (but not the hydrolytic activity) is involved in the mechanism of inhibition of the enzyme by the ester and amide derivatives of Ado-5′-carboxylic acid, in contrast to the inactivation of this enzyme by adenosine-derived vinyl or homovinyl halide analogues during which both activities are manifested.

Introduction

The cellular enzyme *S*-adenosyl-L-homocysteine (Ado-Hcy) hydrolase (EC 3.3.1.1) effects cleavage of AdoHcy to adenosine (Ado) and L-homocysteine (Hcy).[1] Since AdoHcy is a potent feedback inhibitor of crucial trans-methylation enzymes, inhibitors of AdoHcy hydrolase represent putative mechanism-based anticancer and antiviral agents.[2,3] Cellular accumulation of AdoHcy can cause inhibition of viral mRNA methyltransferases essential for viral replication.[4] It has been shown that direct correlations exist between antiviral potency of nucleosides and their ability to elevate cellular levels of AdoHcy,[5] and correlations between antiviral and cytostatic potencies of Ado analogues and their inhibitory effects on AdoHcy hydrolase have been demonstrated.[6] Inhibitors of AdoHcy hydrolase recently have been shown to inhibit replication of the Ebola virus, which causes hemorrhagic fever with mortalities of 40−90% in sporadic human outbreaks.[7]

The "adenosine-5′-carboxaldehyde" mixture (e.g., 4′*S*-isomer **A**; Figure 1) was shown to inactivate AdoHcy hydrolase by reduction of NAD$^+$ to NADH,[8] the so-called "cofactor depletion" or type I mechanism.[3a] NADH or apoforms of the enzyme converted (*Z*)-4′,5′-didehydro-5′-deoxy-5′-fluoroadenosine[9] (**B**, ZDDFA) into the Ado-5′-carboxaldehyde inhibitor(s).[10] It had been postulated[11a] that 5′-*S*-alkyl(or aryl)-5′-fluoro-5′-thioadenosines might function as mechanism-based inhibitors of AdoHcy hydrolase, and it was found[11b,c] that such α-fluoro thioethers (thioacetal analogues) undergo chemical hydrolysis in situ to give the Ado-5′-carboxaldehyde inhibitor(s). We recently have synthesized (*E*)-5′,6′-didehydro-6′-deoxy-6′-halohomoadenosine analogues (**C**, EDDHHAs) which inhibit AdoHcy hydrolase.[6b] Amazingly, homovinyl halides **C** are hydrolyzed at the active site of the enzyme to produce "homoadenosine-6′-carboxaldehyde" which decomposes spontaneously.[12,13] The enzyme catalyzes addition of water to the 5′,6′-double
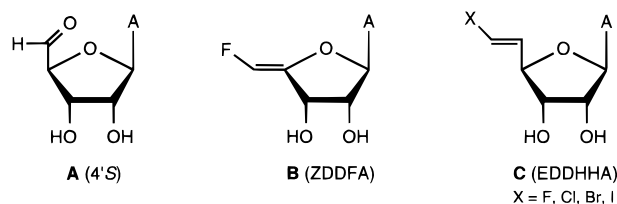

Figure 1.

bond (hydrolytic activity) independently of its 3′-oxidative activity rapidly with the homovinyl fluoride **C** (X = F).[13]

Other 5′-modified adenosine derivatives that inhibit AdoHcy hydrolase include 5′-azido-5′-deoxyadenosine,[14] 5′-cyano-5′-deoxyadenosine,[14] 5′-deoxy-5′-methynyladenosine[15] (the 4′-acetylenic derivative), and Ado-5′-carboxaldehyde oxime derivatives.[2b] We now report studies designed to further investigate the 5′-hydrolytic potential of AdoHcy hydrolase with 5′-modified analogues derived from Ado-5′-carboxylic acid. A number of studies on the binding of Ado-5′-carboxylic (uronic) acid ester[16,17] and amide[18−20] derivatives with adenosine receptors have been reported.[18−20]

Chemistry

Oxidation of 2′,3′-*O*-isopropylideneadenosine with potassium permanganate[21] gave more reliable yields of 2′,3′-*O*-isopropylideneadenosine-5′-carboxylic acid (**1a**; 83%) than recently described procedures.[22] Treatment of **1a** with diazomethane gave methyl ester **2a**[23] (92%), and treatment of **1a** and **2a** with aqueous trifluoroacetic acid (TFA) gave Ado-5′-carboxylic acid[23] (**1b**) and its methyl ester[16,17] **2b** (Scheme 1). Treatment of **1b** with thionyl chloride and the alcohol[16,17] gave butyl (**3b**), isobutyl (**4b**), benzyl (**5b**), and 2-chloroethyl (**6b**) esters. Conversion of **1b** to the acid chloride[16] **13b** and its treatment with isopropyl alcohol gave **7b**.

Ado-5′-carboxamide (**8a**) and *N*-substituted amides **9a**−**11a** were prepared by ammonolysis of **2a**. Dicyclohexylcarbodiimide (DCC) effected coupling of **1a** with aniline to give *N*-phenylcarboxamide **12a**. Treatment of acid chloride **13a**[16] with diethylamine gave **14a**.

Table 1. ^{13}C NMR Spectral Data^{a,b}

compd	C-2	C-4	C-5	C-6	C-8	C-1'	C-2'^{c}	C-3'^{c}	C-4'	C-5'
1b	152.84	149.55	118.93	156.13	139.54	87.35	73.93	73.44	82.80	172.09
2b^{d,e}	152.50	149.19	119.17	156.31	140.93	87.62	73.99	73.24	82.85	170.05
5b^f	152.30	148.90	118.30	155.50	138.40	86.40	73.10	72.70	81.90	169.70
8b^d	151.63	148.99	119.09	155.80	141.33	88.03	73.42	73.22	84.23	171.16
9b^g	152.85	148.95	119.52	156.13	141.08	88.03	73.11	72.15	84.75	170.36
10b^h	152.66	149.01	119.74	156.34	141.09	88.09	73.31	72.09	84.89	169.61
11b^i	152.51	148.68	119.64	156.19	141.19	88.33	73.29	71.95	84.98	169.95
12b^j	152.91	149.30	119.68	156.24	140.72	88.20	73.31	72.66	84.64	168.49
14b^k	153.06	149.98	118.72	156.03	139.38	87.18	74.89	73.06	80.70	168.67

^{a} δ (Me$_2$SO-d_6) at 50 MHz. ^{b} Proton-decoupled singlets. ^{c} Assignments might be reversed. ^{d} APT experiment. ^{e} δ 52.70 (Me). ^{f} δ 66.00 (CH$_2$), 127.60, 127.80, 128.00, 135.00 (Ph). ^{g} δ 25.56 (Me). ^{h} δ 13.90, 19.76, 31.43, 39.78 (Bu). ^{i} δ 42.27 (CH$_2$), 127.38, 127.62, 128.74, 138.98 (Ph). ^{j} δ 120.62, 124.68, 129.16, 137.88 (Ph). ^{k} δ 13.05, 14.71, 41.52 (Et's).

Scheme 1^a

Series **a** R,R = C(CH$_3$)$_2$
      b R = H
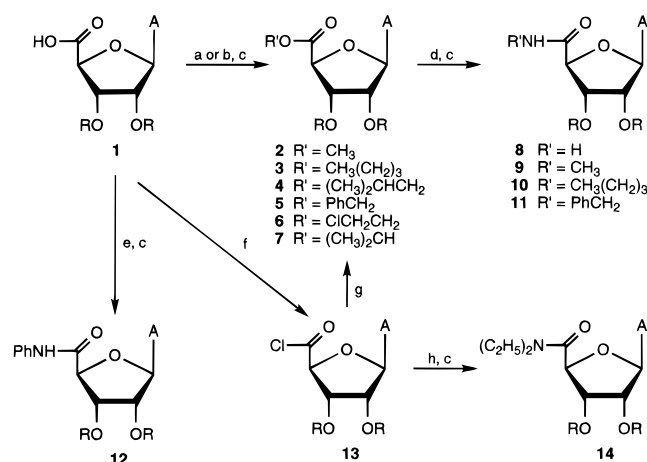
^{a} (a) CH$_2$N$_2$/MeOH/dioxane; (b) SOCl$_2$/ROH; (c) CF$_3$CO$_2$H/H$_2$O; (d) RNH$_2$; (e) PhNH$_2$/DCC; (f) SOCl$_2$/DMF; (g) i-PrOH; (h) (C$_2$H$_5$)$_2$NH.

Deprotection (TFA/H$_2$O) of **8a**−**12a** and **14a** and chromatography (short silica columns) and/or recrystallization gave amides **8b**−**12b** and **14b**. All known esters[16,17] and amides[18] had properties and ^{1}H and ^{13}C (Table 1) NMR spectral data consistent with those reported. Characterization data for **5b** and **14b** are given in the Experimental Section.

Table 2. Kinetic Constants for AdoHcy Hydrolase Inhibition Activity of Ado-5'-carboxylic Acid (**1b**) and Its Esters **2b**−**7b** and Amides **8b**−**12b** and **14b**^{a}

compd	k_2 (min^{-1})	K_I (μM)	k_2/K_I (M^{-1} min^{-1})
A (4'S)	0.65	0.039	1.7×10^7
1b	0.045	0.41	1.1×10^5
2b	0.78	1.7	4.6×10^5
3b	0.11	0.71	1.6×10^5
4b	0.077	0.57	1.4×10^5
5b	0.017	0.36	4.9×10^4
6b	0.35	0.32	1.1×10^6
7b	0.064	0.77	8.4×10^4
8b	0.1	2.6	3.8×10^4
9b	0.095	1.93	4.9×10^4
10b	0.03	19.8	1.5×10^3
11b	0.03	43.4	6.9×10^2
12b	0.028	48.5	5.8×10^2
14b	0.05	0.57	8.8×10^4

^{a} See the Experimental Section for details concerning the procedures for conducting enzyme inactivation studies and data analysis for determination of K_I and k_2 values.

Inhibition of AdoHcy Hydrolase

Ado-5'-carboxylic acid (**1b**), esters **2b**−**7b**, and amides **8b**−**12b** and **14b** were evaluated for inhibition of purified recombinant human placental AdoHcy hydrolase. Table 2 contains the dissociation constants (K_I), inactivation rate constants (k_2), and derived second-order rate constants (k_2/K_I). Figure 2 contains representative Kitz and Wilson plots for compounds **2b** and **8b** from which the kinetic constants were obtained.
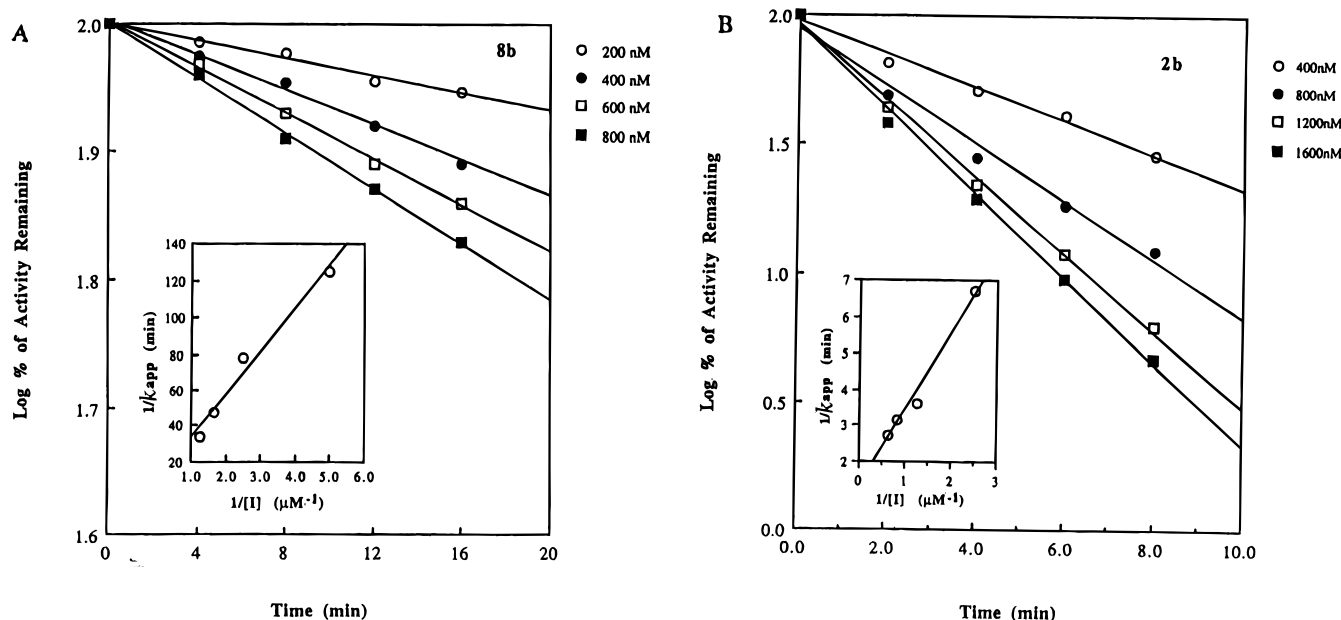
Figure 2. Kitz and Wilson plots of compounds **8b** (A) and **2b** (B).

It is apparent from Figure 2 and Table 2 that all of the compounds evaluated showed time- and inhibitor concentration-dependent inactivation of AdoHcy hydrolase with k_2/K_I values in the range of $(5.8 \times 10^2)-(1.1 \times 10^6)$ M^{-1} min^{-1}. Thus, Ado-5′-carboxylic acid (**1b**), its esters **2b**−**7b**, and amides **8b**−**12b** and **14b** are much weaker inhibitors of AdoHcy hydrolase than Ado-5′-carboxaldehyde (**A**), and amides **8b**−**12b** and **14b** are generally weaker inhibitors than their ester analogues **2b**−**7b** (k_2/K_I).

The time dependency of inactivation of AdoHcy hydrolase upon incubation with **1b**, **2b**−**7b**, **8b**−**12b** and **14b** is consient with a type I mechanism-based inhibition of AdoHcy hydrolase.[3a] This involves reduction of the enzyme-bound NAD$^+$ to NADH with concomitant oxidization of the 3′-hydroxyl group of the inhibitors to a ketone. The observation (UV spectral results not shown) that NAD$^+$ is converted to NADH upon incubation of **2b** or **8b** with the enzyme is in harmony with the type I mechanism-based enzyme inhibition mechanism.

Ado-5′-carboxaldehyde (**A**) was recently shown to be a potent AdoHcy hydrolase inhibitor ($k_2/K_I = 1.7 \times 10^7$ M^{-1} min^{-1}).[8,10] **A** also inactivates AdoHcy hydrolase by the type I mechanism (reduction of cofactor NAD$^+$), and ZDDFA[9] (**B**) was shown to be an enzyme-activated prodrug of Ado-5′-carboxaldehyde (**A**).[10] That mechanism involves rapid addition of water at the C-5′ position of ZDDFA, elimination of hydrogen fluoride to give Ado-5′-carboxaldehyde (**A**), and slower oxidation of **A** to give 3′-keto-Ado-5′-carboxaldehyde with concomitant reduction of E·NAD$^+$ to E·NADH. This mechanism indicates that AdoHcy hydrolase has (C-5′) hydrolytic activity that is independent of its (C-3′) oxidation capability.[10] This hydrolytic feature of AdoHcy hydrolase, functioning at 6′ as well as at C-5′ of the EDDHHAs (**C**), was recently shown to be involved in the mechanism by which they inactivate the enzyme.[6b,12,13] Current knowledge of the amino acid residues involved in the hydrolytic activity of AdoHcy hydrolase is minimal. If this activity involves nucleophilic catalysis, it is conceivable that a covalent bond might be formed linking the enzyme and inhibitor during hydrolysis of the esters **2b**−**7b** and amides **8b**−**12b** and **14b**. Unless this were merely a transitory intermediate, covalent type II mechanism-based inhibition of AdoHcy hydrolase[3a] should occur in addition to the cofactor depletion type I mechanism. The order of potency of the present compounds (esters > amides) as inhibitors of AdoHcy hydrolase is consistent with nucleophilic catalysis, so this possibility was examined by determinig if enzyme-facilitated hydrolysis of esters or amides to acid **1b** occurred. Compounds **2b** and **8b** underwent spontaneous hydrolysis to **1b** in phosphate buffer, pH 7.2. The rate constant for spontaneous hydrolysis of **2b** was 0.0053 min^{-1}, and spontaneous hydrolysis of **8b** was much slower. Incubation of **2b** or **8b** with AdoHcy hydrolase at 37 °C for 2 h did not increase the rate of formation of **1b** (data not shown), indicating that these compounds are not substrates for the hydrolytic activity of the enzyme. Thus, the ester and amide derivatives most likely inhibit AdoHcy hydrolase by the cofactor depletion type I mechanism without involvement of hydrolytic activity of the enzyme.

In summary, both ester (**2b**−**7b**) and amide (**8b**−**12b**, **14b**) derivatives of Ado-5′-carboxylic acid (**1b**) produced time-dependent enzyme inactivation. Observation of the reduction of NAD$^+$ to NADH indicates that they are type I mechanism-based inhibitors. In contrast with recent results with other mechanism-based inactivators, no involvement of the hydrolytic activity of AdoHcy hydrolase was detected. Thus, Ado-5′-carboxylic ester and amide derivatives do not appear to form type II covalent adducts with this enzyme.

Experimental Section

Uncorrected melting points were determined with a capillary apparatus. UV spectra were determined with solutions in MeOH. ^{1}H (200 MHz) and ^{13}C (50 MHz) NMR spectra were recorded with solutions in Me$_2$SO-d_6 unless otherwise noted. Low-resolution electron-impact (20 eV) (MS) and chemical ionization (CI, CH$_4$) mass spectra were obtained with direct probe techniques. Elemental analyses were determined by M-H-W Laboratories, Phoenix, AZ. Reagents and solvents were of reagent quality, and solvents were purified and dried before use. TLC was performed on Merck Kieselgel 60 F$_{254}$ sheets with S$_1$ (EtOAc/i-PrOH/H$_2$O, 4:1:2; upper layer), S$_2$ (i-PrOH/NH$_4$OH/H$_2$O, 7:1:2), or S$_3$ (MeOH/CHCl$_3$, 1:6) with sample observation under 254-nm light. Column chromatography was performed with Merck kieselgel 60 (230−400 mesh). Adenosine-5′-carboxylic acid[23] (**1b**) and its methyl ester **2b**[16,17] were prepared by deprotection (TFA/H$_2$O; as described for **8b**) of the known **1a**[21] and **2a**.[23]

Adenosine-5′-carboxylic Acid Benzyl Ester (5b). Thionyl chloride (0.25 mL) was added slowly to a suspension of Ado-5′-carboxylic acid[16] (**1b**; 70 mg, 0.25 mmol) in benzyl alcohol (3.5 mL) at 0 °C, and stirring was continued for 60 h. The reaction mixture was cooled to −10 °C, and Et$_2$O (40 mL) was added. The precipitate was filtered, washed (Et$_2$O), and dissolved in cold H$_2$O, and the solution was neutralized (saturated NaHCO$_3$/H$_2$O, to pH ∼9). The precipitate was filtered, washed with cold H$_2$O, and dried to give **5b** (16 mg, 17%): mp 135−137 °C; ^{1}H NMR δ 4.42 (m, 1, H3′), 4.54 (d, $J_{4'-3'}$ = 2.8 Hz, 1, H4′), 4.62 (m, 1, H2′), 5.21 (s, 2, CH$_2$), 5.66 (d, $J_{OH-3'}$ = 5.0 Hz, 1, OH3′), 5.84 (d, $J_{OH-2'}$ = 5.3 Hz, 1, OH2′), 6.05 (d, $J_{1'-2'}$ = 6.3 Hz, 1, Hl′), 7.30 (br s, 2, NH$_2$), 7.36 (m, 5, Ph), 8.14 (s, 1, H2), 8.34 (s, 1, H8); HRMS (FAB) m/z 372.1307 (MH$^+$ [$C_{17}H_{18}N_5O_5$] = 372.1308).

Other Esters. Butyl ester **3b** [13%; mp 169−171 °C (lit.[16] mp 145−148 °C); MS m/z 337 (M$^+$)], isobutyl ester **4b** [23%; mp 181−183 °C (lit.[17] mp 178−180 °C); MS m/z 337 (M$^+$)], and 2-chloroethyl ester **6b** [49%; mp 205−208 °C (lit.[16] mp 208−210 °C); MS m/z 343 (M$^+$, ^{35}Cl)] were prepared with the respective alcohols by the procedure for **5b**. Isopropyl ester **7b** was prepared as described[16] [mp ∼200 °C dec (lit.[16] mp 222−223 °C, lit.[17] mp 208 °C); MS (CI) m/z 324 (MH$^+$)].

Adenosine-5′-carboxamide (8b). (a) Ammonolysis: 2′,3′-O-Isopropylideneadenosine-5′-carboxylic acid methyl ester[23] (**2a**; 100 mg, 0.3 mmol) was stirred with NH$_3$/H$_2$O (d = 0.88 g/cm^3, 5 mL) at ambient temperature for 2 h. The reaction mixture [TLC (S$_1$) R_f ∼0.5; **2a** has R_f ∼0.8] was cooled (ice bath, 2 h), and the white solid was filtered and crystallized (MeOH) to give **8a** (84 mg, 87%): mp 223−226 °C (lit.[18] mp 220−222 °C).

(b) Deprotection: A solution of **8a** (58 mg, 0.18 mmol) in CF$_3$CO$_2$H/H$_2$O (9:1, 5 mL) was stirred at 0 °C for 2 h, evaporated, and coevaporated (EtOH), and the residue was crystallized (MeOH) to give **8b** (38 mg, 75%): mp 238−240 °C dec (lit.[18] mp 245−247 °C); MS (CI) m/z 281 (100, MH$^+$).

Adenosine-5′-(N-methylcarboxamide) (9b). Ester **2a** (100 mg, 0.3 mmol) was stirred with MeNH$_2$/H$_2$O (40%, 1.5 mL) at ambient temperature for 4 h. Undissolved **2a** (12 mg) was filtered, the mother liquor was deprotected (as described for **8b**), and the residue was purified on a short silica column

[EtOAc/MeOH (19:1) → S₁] and recrystallized (MeOH) to give **9b** (70 mg, 80%): mp 241−243 °C (lit.[18] mp 240−241 °C); MS *m/z* 294 (7, M⁺).

Adenosine-5′-(*N*-butylcarboxamide) (10b). Ester **2a** (100 mg, 0.3 mmol) was suspended in dioxane (5 mL), butylamine (1 mL) was added, and the mixture was stirred at ambient temperature for 2 h. TLC (S₁) showed a mixture of **10a/2a** (∼3:2). MeOH (2 mL) and H₂O (0.5 mmol) were added, the reaction mixture was gently refluxed for 2 h, and volatiles were evaporated. The residue was deprotected (as described for **8b**), chromatographed [EtOAc/MeOH (19:1) → S₁], and crystallized (MeOH/EtOAc, 2:1) to give **10b** (62 mg, 62%): mp 122−124 °C (lit.[18] mp 125 °C); MS *m/z* 336 (10, M⁺).

Adenosine-5′-(*N*-benzylcarboxamide) (11b). Ester **2a** (67 mg, 0.2 mmol) and benzylamine (0.22 mL, 214 mg, 2 mmol) in dioxane/MeOH (1:1, 10 mL) were refluxed for 4 h. Deprotection, purification, and crystallization (MeOH/H₂O, 1:1) (as described for **10b**) gave **11b** (53 mg, 72%): mp 133−134 °C (lit.[18] mp 130−133 °C); MS *m/z* 370 (20, M⁺).

Adenosine-5′-(*N*-phenylcarboxamide) (12b). A solution of **1a** (64 mg, 0.2 mmol) in MeOH/dioxane/H₂O (3:3:1, 10 mL) was added to a solution of aniline (45 µL, 46 mg, 0.5 mmol) and dicyclohexylcarbodiimide (82 mg, 0.4 mmol) in THF (3 mL), and stirring was continued overnight at ambient temperature. Volatiles were evaporated in vacuo, and the residue was chromatographed [EtOAc/MeOH (19:1) → S₁] to give **12a** (68 mg). This **12a** (68 mg) was deprotected (as described for **8b**) and crystallized (MeOH/EtOAc, 1:1) to give **12b** (58 mg, 81%): mp 246−248 °C (lit.[18] mp 252−254 °C); MS *m/z* 356 (22, M⁺).

Adenosine-5′-(*N,N*-diethylcarboxamide) (14b). Treatment of **1a** (160 mg, 0.5 mmol) with thionyl chloride (0.50 mL, 0.81 mg, 6.8 mmol) and DMF (2 drops) gave the acid chloride **13a** as described.[16] Et₂NH (1 mL) was added dropwise at 0 °C to this **13a** in THF (2 mL), and stirring was continued at ambient temperature for 2 h. The solution was evaporated, the residue was partitioned (HCl/H₂O/CHCl₃), and the organic layer was washed (NaHCO₃/H₂O, brine), dried (MgSO₄), and evaporated to give **14a** (yellow oil). Deprotection of this **14a** (as described for **8b**), chromatography [EtOAc/MeOH (19:1) → S₁], and crystallization (MeOH) gave **14b** (104 mg, 62% from **1a**): mp 244−245 °C; UV max 259 nm (ϵ 15 100), min 230 nm (ϵ 4100); ¹H NMR 1.07 and 1.12 (t and t, *J* = 7.0 Hz, 3 and 3, CH₃'s), 3.28−3.45 (m, 4, CH₂'s), 4.28 (ddd, $J_{3'-4'}$ = 2.9 Hz, $J_{OH-3'}$ = 5.6 Hz, $J_{3'-2'}$ = 4.4 Hz, 1, H3′), 4.54 (ddd, $J_{2'-1'}$ = 5.8 Hz, $J_{OH-2'}$ = 5.7 Hz, 1, H2′), 4.77 (d, 1, H4′), 5.65 (d, 1, OH3′), 5.68 (d, 1, OH2′), 6.07 (d, 1, H1′), 7.32 (br s, 2, NH₂), 8.18 (s, 1, H2), 8.59 (s, 1, H8); MS *m/z* 336 (20, M⁺). Anal. (C₁₄H₂₀N₆O₄) C,H,N.

Determination of AdoHcy Hydrolase Inhibition Constants. The purified recombinant human placental AdoHcy hydrolase was prepared from *Escherichia coli* carrying the expression vector (*p*PUCSAH) and grown in the presence of isopropyl β-D-thiogalactopyranoside essentially as described.[24] The enzyme activity was determined in the synthetic direction by incubating AdoHcy hydrolase with 0.2 mM Ado and 5 mM Hcy for 5 min at 37 °C in 50 mM potassium phosphate buffer (pH 7.2) containing 1 mM EDTA and assaying the reaction product, AdoHcy, by HPLC after quenching the reaction by addition of perchloric acid (final concentration: 0.5 M). An aliquot (100 µL) of the reaction mixture was injected into a HPLC column (Econosphere Alltech, 25 cm × 4.6 mm, C-18 reversed-phase column) at a flow rate of 1 mL/min. The elution gradient consisted of two sequential linear gradients: 6−15% B over 0−9 min and 15−50% B over 9−15 min, where the mobile phase B was acetonitrile and A was 50 mM sodium phosphate buffer (pH 3.2) containing 10 mM heptanesulfonic acid. The peak of AdoHcy was detected by UV at 254 nm. For the determination of inhibition constants, AdoHcy hydrolase was preincubated with various concentrations of the inhibitors for various time intervals and the remaining enzyme activity was measured. The pseudo-first-order rate constants (K_{app}) were obtained from the plot of log(% activity remaining) vs time, and K_I and k_2 values (Table 1) were estimated from the double-reciprocal plot of $1/K_{app}$ vs $1/[$inhibitor$]$ using the following equation:

$$1/K_{app} = 1/k_2 + K_I/k_2(1/[I])$$

Conversion of E·NAD⁺ to E·NADH. The inhibitor-induced E·NADH formation was determined by measuring the increase in absorbance at 320 nm at different time intervals after the enzyme was mixed with the inhibitor. To AdoHcy hydrolase (44 µM) in 1 mL of 50 mM phosphate buffer (pH 7.2) containing 1 mM EDTA (buffer A) was added 100 µL of Ado 5′-ester **2b** (22 mM), with mixing for 10 s. The UV spectrum (280−500 nm) was recorded periodically at 25 °C using a HP 8452 diode array spectrophotometer. The reference cell contained the same enzyme solution to which had been added 100 µL of water. Spectra were recorded until no increase in absorbance at 320 nm was observed (∼20 min).

Spontaneous Hydrolysis of the Ado 5′-Ester 2b and Ado 5′-Amide 8b. Hydrolyses of ester **2b** and amide **8b** were studied by incubation in buffer A (with and without enzyme) at 37 °C for various times. At each time point, an aliquot (30 µL) of the reaction solution was injected into a C-18 reversed-phase HPLC column (Econosphere Alltech, 250 × 4.6 mm). Chromatography was carried out with a linear gradient of 8−20% A in B for 0−15 min at a flow rate of 1 mL/min, where mobile phase A was acetonitrile and B was 50 mM sodium phosphate buffer (pH 3.2) containing 10 mM heptanesulfonic acid. Peak areas of the product and remaining reactant were monitored by UV at 258 nm. Kinetic data were fitted to a first-order reaction equation.

Supporting Information Available: Kitz and Wilson plots for AdoHcy hydrolase inhibition kinetic data in Table 2 and UV absorbance vs time plots for reduction of NAD⁺ to NADH upon incubation of **2b** with the enzyme (13 pages). Ordering information is given on any current masthead page.

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