# Aristeromycin-5′-carboxaldehyde: A Potent Inhibitor of *S*-Adenosyl-L-homocysteine Hydrolase

Siming Liu, Chong-sheng Yuan, and Ronald T. Borchardt*

*Departments of Medicinal Chemistry and Biochemistry, The University of Kansas, Lawrence, Kansas 66045*

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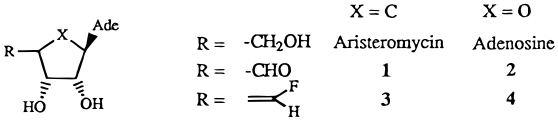
In an earlier study, Liu et al. (*Bioorg. Med. Chem. Lett.* **1992**, *2*, 1741−1744) showed that both the *E* and *Z* isomers of 4′,5′-didehydro-5′-fluoroaristeromycin were very potent irreversible inhibitors of *S*-adenosylhomocysteine (AdoHcy) hydrolase. However, it was unclear from a mechanistic standpoint whether these vinyl fluorides were themselves type-I mechanism-based inhibitors causing reduction of enzyme-bound $NAD^+$ or whether they were prodrug for aristeromycin-5′-carboxaldehyde, which was the ultimate type-I inhibitor. To elucidate this mechanism of enzyme inhibition, (4′*S*)- and (4′*R*)-aristeromycin-5′-carboxaldehydes (**1a**,**b)** were synthesized in this study and shown to be potent type-I mechanism-based inhibitors of AdoHcy hydrolase with $k_2/K_i$ values of $4.4 \times 10^6$ and $8.2 \times 10^4$ $M^{-1}$ $min^{-1}$, respectively. However, Using $^{19}F$ NMR and HPLC, it was shown that (4′S)-4′,5′-didehydro-5′-fluoroaristeromycin in the presence of AdoHcy hydrolase did not release fluoride ion or generate aristeromycin-5′-carboxaldehyde (**1a**,**b**). These results suggest that the *E* and *Z* isomers of 4′,5′-didehydro-5′-fluoroaristeromycin are inactivating AdoHcy hydrolase by directly reducing $NAD^+$ to NADH and not using the hydrolytic activity of the enzyme to generate aristeromycin-5′-carboxaldehyde.

## Introduction

The cellular enzyme *S*-adenosyl-L-homocysteine (AdoHcy) hydrolase (EC 3.3.1.1) catalyzes the reversible hydrolysis of AdoHcy to adenosine (Ado) and homocysteine (Hcy). Inhibition of AdoHcy hydrolase results in cellular accumulation of AdoHcy, which is a product inhibitor of adenosylmethionine (AdoMet)-dependent methyltransferases. It has been shown that viral mRNA methyltransferases, which are essential for viral replication, are very sensitive to elevated cellular levels of AdoHcy.[1−4] For example, Ransohoff and co-workers[2] have shown that undermethylation of the viral mRNA-capped structure induced by the inhibition of AdoHcy hydrolase can be correlated with the inhibition of influenza viral replication. In addition, Cools and De Clercq[5] have shown that a close correlation exists between the antiviral potency of Ado analogs and their inhibitory effects on AdoHcy hydrolase. Furthermore, Hasobe and co-workers[6] and Cools and De Clercq[7] have shown that a close correlation exists between the antiviral potency of the nucleosides and their ability to elevate cellular levels of AdoHcy. Inhibitors of AdoHcy hydrolase have been shown to exhibit broad-spectrum antiviral activity.[5] A more recent study showed that inhibitors of AdoHcy hydrolase also inhibit the replication of Ebola virus, which causes the viral hemorrhagic fevers with mortalities of 40−90% in sporadic human outbreaks.[8] Thus, development of novel and potent inhibitors of this enzyme would provide an attractive approach to the search for potentially clinically effective broad-spectrum antiviral agents.

Various Ado analogs and adenine carbocyclic nucleosides have been shown to be potent inhibitors of AdoHcy hydrolase.[1,3,4,9] Many of these compounds are type-I mechanism-based inhibitors of AdoHcy hydrolase,[1] which inactivate the enzyme by reducing the enzyme-bound $NAD^+$ to NADH. Ado-5′-carboxaldehydes (**2**, both 4′*S* and 4′*R* isomers, Figure 1), representatives of this type of inhibitor, have been recently shown to be very potent inhibitors of recombinant rat liver AdoHcy hydrolase with $k_2/K_i$ values of $1.7 \times 10^7$ and $5.1 \times 10^6$ $M^{-1}$ $min^{-1}$, respectively. In the process of inactivation, the inhibitors are oxidized stoichiometrically to the corresponding 3′-keto nucleosides (Scheme 1, **6**).[1,10] Recently, several laboratories have attempted to develop $k_{cat}$ inhibitors in which the enzyme would be inactivated through the covalent interaction with the inhibitor to give **9**, so-called type-II mechanism-based inhibitors.[1,9] The 4′,5′-didehydro-5′-fluoro analogs **4** of Ado (Figure 1) were first synthesized and reported to have potent AdoHcy hydrolase inhibitory effects as type-II mechanism-based inhibitors by McCarthy et al.[11] and Mehdi et al.[12] However, the fact that the vinyl fluorides **4** rapidly react with the NADH form or the apo ($NAD^+$-depleted) form, as well as the $NAD^+$ form, of AdoHcy hydrolase to release fluoride ion suggested that these vinyl fluorides upon binding to the active site of the $NAD^+$ form of the enzyme are initially hydrolyzed to yield the 5′-carboxaldehyde **2** (pathway a, Scheme 1).[13] The 5′-carboxaldehyde **2**, while bound to the enzyme active site, is then oxidized to the 3′-keto-5′-carboxaldehyde **6**, resulting in enzyme inactivation by a type-I mechanism.[1,10] This mechanism also indicates that AdoHcy hydrolase possesses C-5′ hydrolytic activity, which is independent of C-3′ oxidation reaction, and that the Ado vinyl fluorides are simply "prodrugs" of the Ado-5′-carboxaldehydes (**2**).




| | | X = C | X = O |
|---|---|---|---|
| R = | -$CH_2OH$ | Aristeromycin | Adenosine |
| R = | -CHO | **1** | **2** |
| R = | | **3** | **4** |

**Figure 1.** Structures of inhibitors of AdoHcy hydrolase.

Aristeromycin (Figure 1), a naturally occurring carbocyclic analog of Ado, is one of the most potent

* Correspondence should be sent to: Dr. Ronald T. Borchardt, Department of Pharmaceutical Chemistry, 2065 Constant Ave., The University of Kansas, Lawrence, KS 66047. Tel: 913-864-3427. Fax: 913-864-5736.

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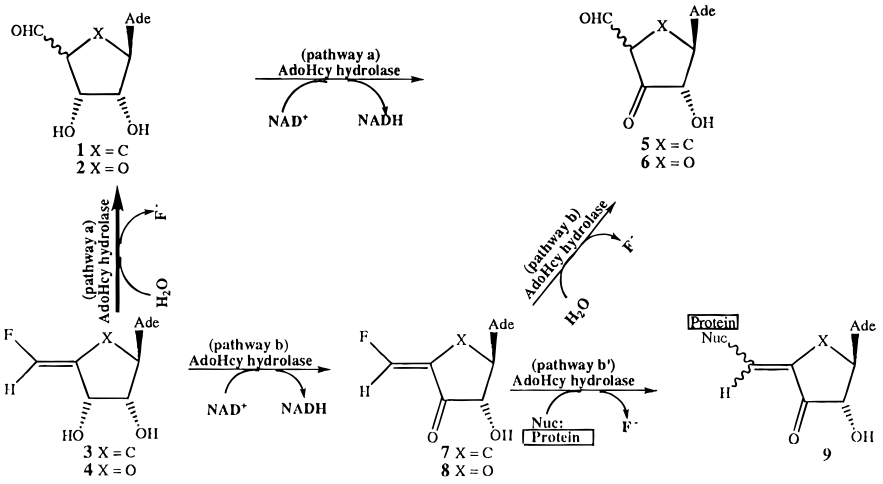
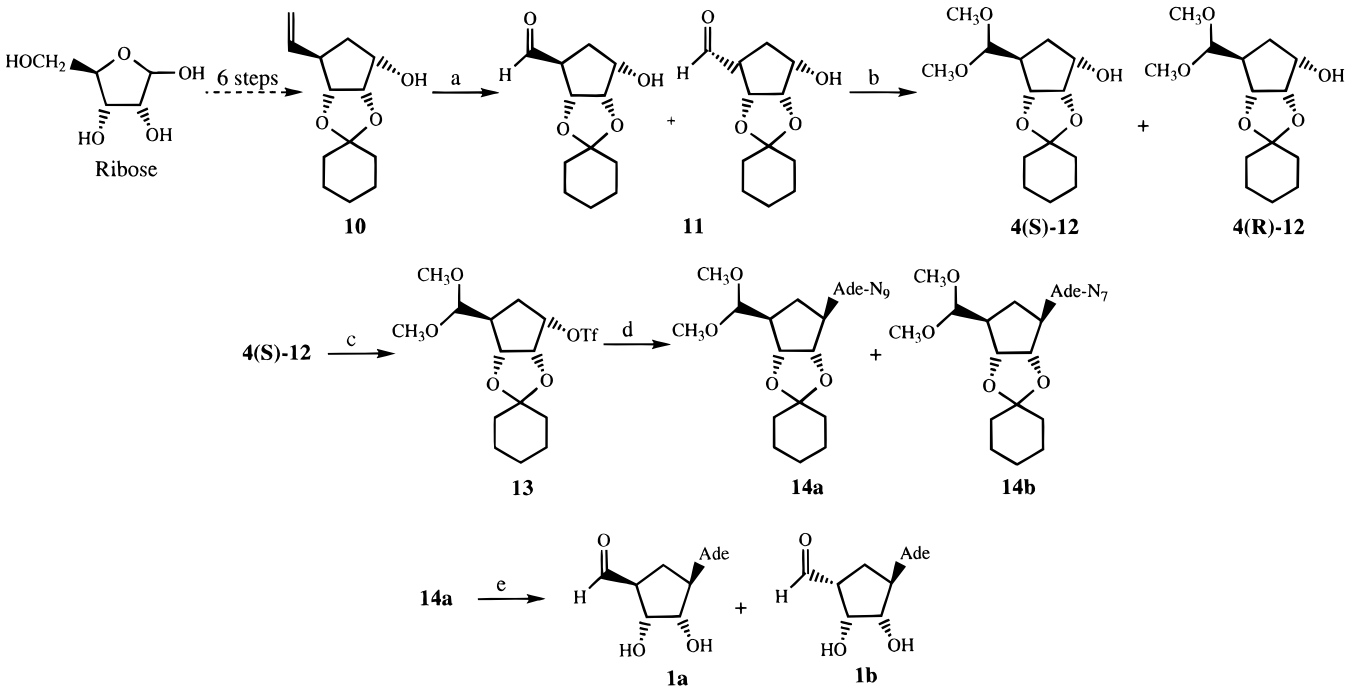
**Scheme 1.** Proposed Mechanism by which Aldehydes **1** and **2** and Vinyl Fluorides **3** and **4** Inactivate Hydrolase




**Scheme 2**[a]




[a] (a) (1) $O_3$/MeOH, −78 °C, (2) $(CH_3)_2S$; (b) trimethyl orthoformate, $NH_4Cl$/MeOH, rt, 24 h; (c) trifluoromethanesulfonic anhydride/pyridine/$CH_2Cl_2$, 0 °C, 1 H; (d) NaH/adenine/DMF, rt, 15 h; (e) 1% HCl, 12 h.

inhibitors of AdoHcy hydrolase.[14,15] Carbocyclic vinyl fluoride analogs of aristeromycin, 4′,5′-didehydro-5′-fluoroaristeromycin (**3**, both *E* and *Z* isomers, Figure 1), also showed very strong AdoHcy hydrolase inhibition activities with $k_2/K_i$ values of $2.77 \times 10^5$ and $5.75 \times 10^5$ $M^{-1}$ $min^{-1}$, respectively.[16] However, the question remained as to whether **3** also acts as "prodrugs" that could be hydrolyzed in the enzyme active site to the corresponding aristeromycin-5′-carboxaldehyde (**1**). To further elucidate the mechanism of inhibition of **3**, the possible product (**1**) of enzyme-catalyzed hydrolysis of **3** was synthesized and its inhibitory effects on AdoHcy hydrolase were determined in this study.

## Results and Discussion

**Chemistry.** The synthesis of aristeromycin-5′-carboxaldehyde (**1**) is shown in Scheme 2. Starting from ribose, (1*S*,2*S*,3*R*,4*R*)-2,3-(cyclohexylidenedioxy)-4-vinylcyclopentanol (**10**) was prepared through a six-step synthesis following literature procedures.[17] Ozonolysis of **10** in methanol solution at −78 °C followed by reduction with dimethyl sulfide gave an 84% yield of a mixture of 4*S* and 4*R* diastereoisomers **11** in a ratio of 3:2. Without separation, both isomers of **11** were reacted with trimethyl orthoformate in the presence of $NH_4Cl$ for 24 h at room temperature to give a 57% yield of a mixture of the (4*S*)- and (4*R*)-acetals **12** in a ratio of 5:1, respectively. The (4*S*)-**12** and (4*R*)-**12** acetals were then separated and purified by silica gel chromatography. The stereochemistry of the 4*S* isomer was characterized using $^1H$ NMR by analogy with the 5′-deoxyaristeromycin analog whose stereochemistry has been conclusively proven.[18] Evidence included the very small proton NMR coupling constant between $H_3$ and $H_4$ (1.7 Hz), which was consistent with expectations drawn from molecular models and the Karplus equation (3 Hz).

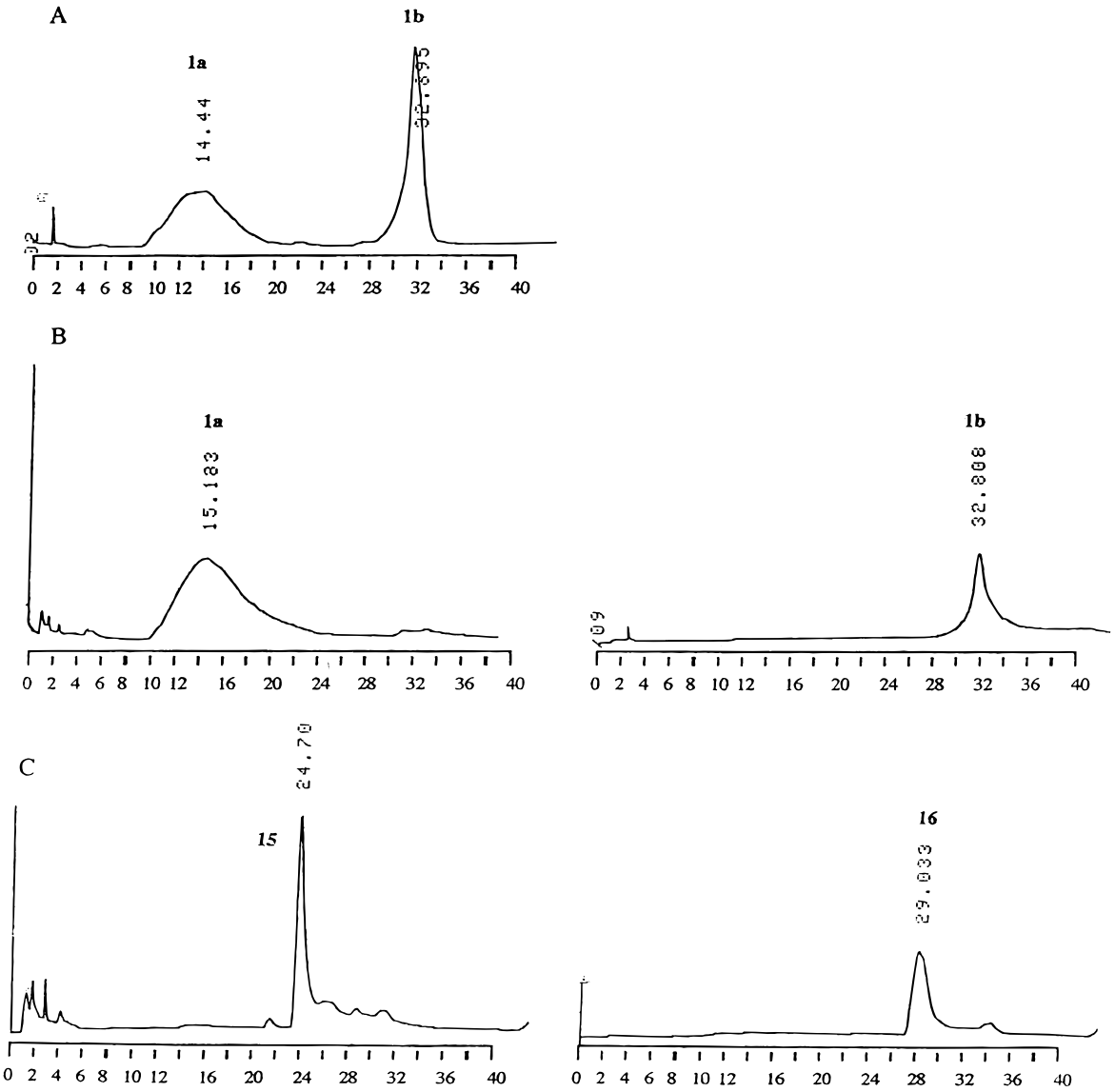



**Figure 2.** HPLC chromatograms of aristeromycin-5′-carboxaldehydes (**1a**,**b**) and their $NaBH_4$ reduction products. HPLC was performed on a Shimadzu SLC-6A chromatography system using an Econosil C-18 reversed-phase column and eluting with $CH_3CN$ (A) in $H_2O$ (B) (program, a gradient of 1−6% A in B from 0 to 30 min, 6% A in B from 30 to 40 min). Absorbance was monitored at 258 nm. Panel A, chromatograms of a mixture of **1a**,**b** generated from hydrolysis of **14**. Panel B, chromatograms of purified **1a**,**b**. Panel C, products derived from $NaBH_4$ reduction of **1a**,**b**. The peak labeled **15** was identified as aristeromycin by coinjection with authentic sample.

Reaction of (4*S*)-**12** with trifluoromethanesulfonic anhydride at 0 °C for 1 h offered the 1-triflate **13** in 69% yield. Without further purification, 1-triflate **13** was reacted with adenine to give a mixture of (1′*R*,2′*S*,3′*R*,4′*S*)-9- (**14a**) and -7- (**14b**) [2′,3′-(cyclohexylidenedioxy)-4′-(dimethoxymethinyl)cyclopentan-1′-yl]-adenine at a ratio of 8:1, respectively. The pure desired product **14a** was obtained in 7% yield by preparative thin layer chromatography . The low yield is probably due to the relatively poor nucleophilicity of the adenine nitrogen. In addition, there was a substantial loss of the compound during four times of preparative TLC separations.

The desired compound, aristeromycin-5′-carboxaldehyde (**1**), was obtained by hydrolysis of **14a** in 1% aqueous HCl. HPLC analysis of the hydrolysis solution showed that it contained a mixture of two compounds, possibly two 4′-isomers of **1** in a ratio of ca. 1:1 (Figure 2, panel A). Using HPLC, the individual isomers **1a**,**b** were obtained in pure form in a water−acetonitrile solution (Figure 2, panel B). However, attempts to lyophilize the solution were not successful. A black tar was obtained after lyophilization. Characterization of **1a**,**b** was performed by using NMR spectrometry and chemical transformation. $^1H$ NMR spectra of **1a** showed that it existed mainly in the hydrate form in aqueous solution. As shown in Figure 2 (panel C), $NaBH_4$ reduction of **1a** (retention time = 14.4 min) afforded a product which coeluted on HPLC with aristeromycin (retention time = 24.7 min). Compound **1b** (retention time = 32.9 min) was also reacted in the $NaBH_4$ solution to give a product with a retention time of 29.0 min. However, the standard of the possible reduction product (4*S*)-aristeromycin was not available to give the final confirmation of the structure.

**Biological**. The enzyme inhibitory studies of aristeromycin-5′-carboxaldehyde **1** (both **1a**,**b**) were conducted using purified recombinant human placental AdoHcy hydrolase. The evaluation of **1a**,**b** as inhibitors of AdoHcy hydrolase followed the method of Kitz and

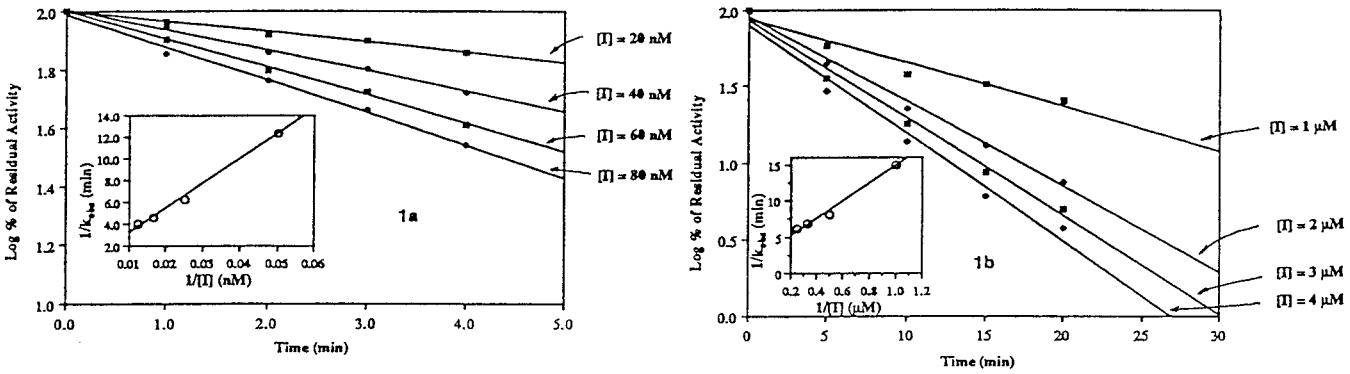
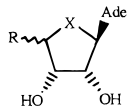



**Figure 3.** Kitz and Wilson plot of compounds **1a**,**b**. See the Experimental Section for detailed procedures.

**Table 1.** Inhibitory Activities of Compounds **1** and **2** on Recombinant AdoHcy Hydrolase




**1a** R = 4′(S)-CHO, X=C
**1b** R = 4′(R)-CHO, X=C
**2a** R = 4′(S)-CHO, X=O
**2b** R = 4′(R)-CHO, X=O

| compd | $k_2$ (min$^{-1}$) | $K_i$ (nM) | $k_2/K_i$ (M$^{-1}$ min$^{-1}$) |
|---|---|---|---|
| **1a** | 1.2 | 273 | $4.4 \times 10^6$ |
| **1b** | 0.37 | 4500 | $8.2 \times 10^4$ |
| **2a** | 0.65 | 39 | $1.7 \times 10^7$ |
| **2b** | 0.22 | 43 | $5.1 \times 10^6$ |

Wilson.[19] Both **1a**,**b** showed concentration- and time-dependent inhibition that could be analyzed using eqs 1−3.

$$E + I \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} E{\cdot}I \xrightarrow{k_2} E{-}I \quad (1)$$

$$K_i = \frac{k_{-1} + k_2}{k_1} \quad (2)$$

$$\frac{1}{k_{obs}} = \frac{K_i}{k_2}\frac{1}{[I]} + \frac{1}{k_2} \quad (3)$$

Plots of log percent of residual activity vs time were linear and gave the slopes $k_{obs}$. Replots of the reciprocal of these slopes vs $[I]^{-1}$ were used with eq 3 to determine the apparent binding constant ($K_i$), the apparent inactivation rate constant ($k_2$), and the apparent second-order rate constant for AdoHcy hydrolase inactivation ($k_2/K_i$). The Kitz and Wilson plots of **1a**,**b** are shown in Figure 3. The kinetic constants thus obtained are listed in Table 1. From Table 1, it can be seen that, similar to Ado-5′-carboxaldehydes (**2**),[10] both **1a**,**b** showed very potent enzyme inhibition activities with $k_2/K_i$ values of $4.4 \times 10^6$ and $8.2 \times 10^4$ M$^{-1}$ min$^{-1}$, respectively. However, by comparing the individual kinetic constants $k_2$, $K_i$, and $k_2/K_i$, the difference in $k_2/K_i$ between 5-carboxaldehyde analogs of aristeromycin **1** and **2** comes mainly from the apparent binding constants $K_i$.

As has been mentioned previously, the Ado vinyl fluoride analogs **4** are simple "prodrugs" of Ado-5′-carboxaldehyde (**2**), which is a potent type-I mechanism-based inhibitor of AdoHcy hydrolase.[10,13] Similar to the Ado vinyl fluoride analogs **4**, the corresponding carbocyclic vinyl fluoride analogs **3** (both *E* and *Z* isoforms) have also been shown to be potent AdoHcy hydrolase inhibitors with $k_2/K_i$ values of $2.77 \times 10^5$ and $5.75 \times 10^5$ M$^{-1}$ min$^{-1}$, respectively.[16] Because of the structural similarities between Ado vinyl fluoride analogs **4** and their corresponding carbocyclic vinyl fluoride analogs **3**, we were interested in whether they have the same mechanism of inhibition. Specifically, we were interested in studying whether the carbocyclic vinyl fluoride analogs **3** are also simple "prodrugs" of the corresponding carbocyclic aldehyde **1**. The mechanistic study of the enzyme inhibition pathway by **3** was carried out by monitoring $NAD^+$ to NADH conversion and the formation of possible 5′-hydrolytic products aristeromycin-5′-carboxaldehyde (**1**) and/or aristeromycin 3′-keto-5′-carboxaldehyde (**5**) and fluoride ion. Using UV spectroscopy, the $NAD^+$ to NADH conversion was studied. The aristeromycin vinyl fluoride **3** (60 $\mu$M) was incubated with recombinant AdoHcy hydrolase (22 $\mu$M) in 50 mM potassium phosphate buffer (pH = 7.2) containing 1 mM EDTA at 25 °C. At different time intervals, the UV absorption of NADH at 316 nm was recorded (Figure 4). From Figure 4, it can be seen that the aristeromycin vinyl fluoride **3** induced the conversion of $NAD^+$ to NADH during a 10 min incubation. The possible formation of intermediates and products of this reaction was monitored using HPLC techniques. However, unlike the inhibition reaction of AdoHcy hydrolase with **4**,[17] neither aristeromycin-5′-carboxaldehyde (**1**) nor aristeromycin 3′-keto-5′-carboxaldehyde (**5**) (data not shown) were formed during reaction of the enzyme with **3** (Figure 5), i.e., the carbocyclic vinyl fluoride **3** could not be hydrolyzed to the corresponding aldehyde **1** in the active site of the enzyme. This result was further supported by $^{19}$F NMR spectroscopy designed

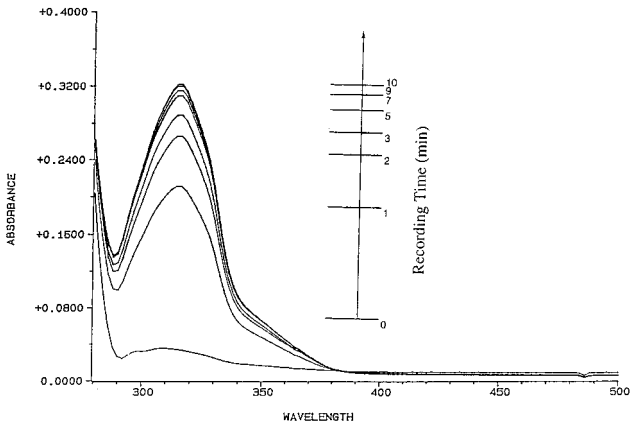



**Figure 4.** Spectra of a mixture of AdoHcy hydrolase and (*Z*)-aristeromycin vinyl fluoride **3**. AdoHcy hydrolase (22 $\mu$M) in buffer B was mixed with **3** (60 $\mu$M) in a UV cuvette and incubated at 25 °C for various times. At each time point (0, 1, 2, 3, 5, 6, 8, and 10 min), the spectra were recorded.

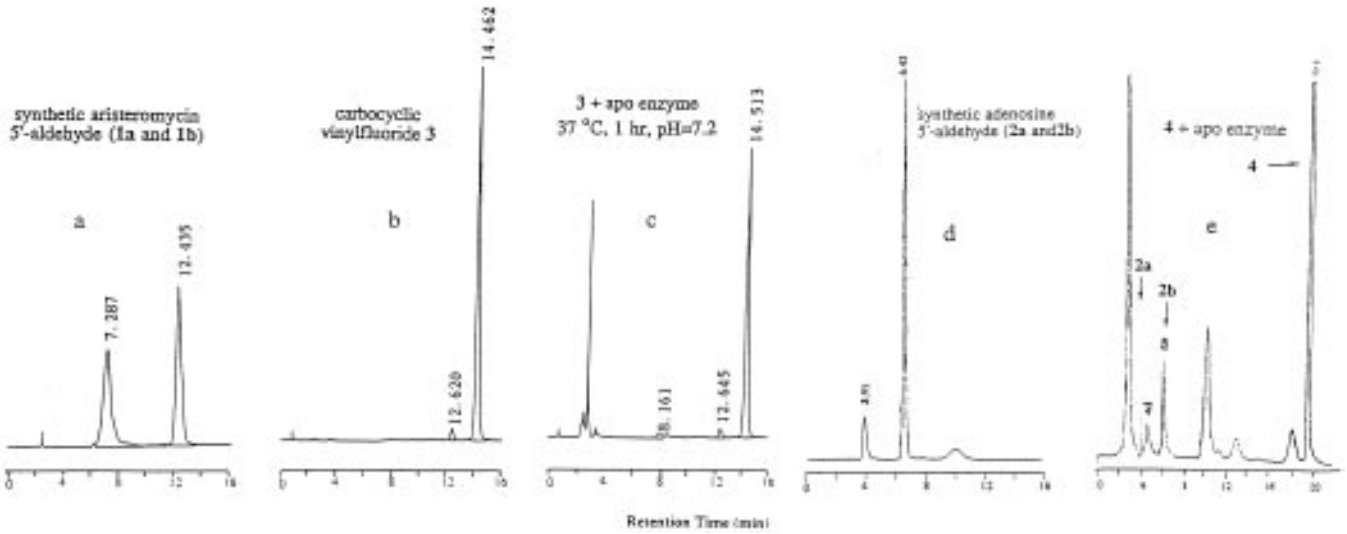



**Figure 5.** HPLC chromatogram of the reaction mixture of AdoHcy hydrolase (apoform) and aristeromycin vinyl fluoride **3**: (a) aristeromycin-5′-aldehydes (**1a**,**b**), (b) **3** alone, (c) **3** plus apoenzyme, (d) adenosine-5′-aldehydes (**2a**,**b**), and (e) **4** plus apoenzyme. The reaction and HPLC conditions are described in the Experimental Section.

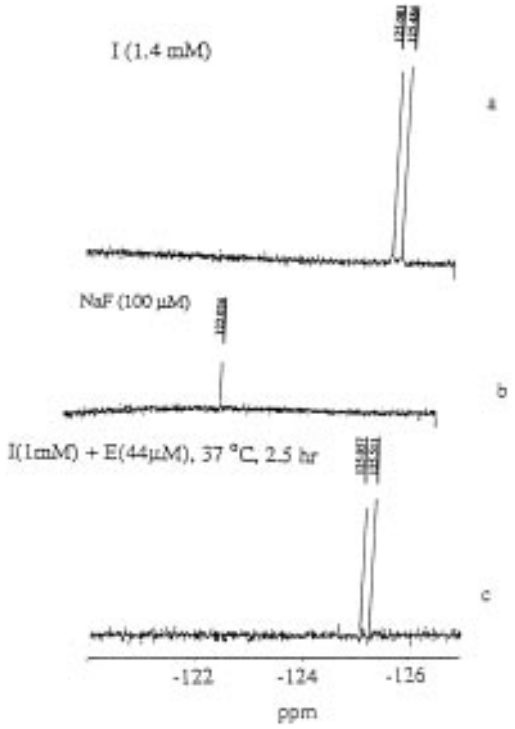



**Figure 6.** $^{19}F$ NMR spectra of aristeromycin vinyl fluoride **3** incubated with AdoHcy hydrolase ($NAD^+$ form). Enzyme (44 $\mu$M) was incubated with **3** (1 mM) in buffer B for 2.5 h at 37 °C. The sample was then treated with 97% ethanol, centrifuged, and lyophilized as described in the Experimental Section. $^{19}F$ NMR spectra were recorded in $D_2O$ in a 500 MHz Bruker AM spectrometer: (a) **3** (1.4 mM) alone, (b) NaF (0.1 mM), and (c) **3** plus apoenzyme.

to detect release of fluoride ion. Incubation of **3** with recombinant AdoHcy hydrolase at 37 °C for 2.5 h did not cause any release of $F^-$ as determined by $^{19}F$ NMR spectroscopic analysis (Figure 6).

The above-mentioned studies led us to the conclusion that, unlike the Ado vinyl fluoride analogs **4**, the carbocyclic vinyl fluoride analogs **3** inhibited the enzyme simply through converting the enzyme-bound $NAD^+$ to NADH (from **3** to **7**, Scheme 1) without going through the 5′-C hydrolysis. Therefore, carbocyclic vinyl fluoride analogs of Ado vinyl fluorides **4** in which the sugar moiety is replaced with a cyclopentyl analog apparently inhibited the enzyme through a very different pathway from that of the ribosyl analog. Specifically, they showed very different activities toward the enzyme-catalyzed 5′-hydrolysis. After simple energy minimization, it was shown that conformations of these two series of compounds (**3** and **4**) were very similar (results not shown). This suggests that the electronic difference caused by rings O and C could be the major contribution to both the binding and the 5′-hydrolytic activity differences. Alternatively, potential hydrogen bonding involving ring O of the ribose of Ado analogs **4** could help to orient the ribosyl unit in a way that favors the hydrolysis catalyzed by the enzyme, while the lack of this hydrogen bond could result in a slightly different orientation of the carbocyclic analogs **3** that is unfavorable for them to undergo the enzyme-catalyzed hydrolysis at the 5′-position.

In conclusion, the data presented in this manuscript clearly show that the aristeromycin-5′-carboxaldehydes (**1**) are potent type-I mechanism-based inhibitors of AdoHcy hydrolase. By comparing the kinetic constants of enzyme inhibition of carbocyclic and ribosyl 5′-carboxaldehydes (Table 1), it can be clearly seen that the difference in enzyme inhibitory activities ($k_2/K_i$) comes mainly from the difference of apparent binding constants $K_i$. Using **1** as a standard compound, it was demonstrated that unlike the Ado vinyl fluoride analogs **4**, the carbocyclic vinyl fluoride analogs **3** inhibit the enzyme simply through the conversion of $NAD^+$ to NADH (from **3** to **7**, Scheme 1) without being enzymatically hydrolyzed to the corresponding 5′-aldehydes (**1** or **5**, Scheme 1). However, it is not readily clear why these two series of compounds would have distinctly different propensities of being hydrolyzed at the 5′-position by the enzyme. Electronic or hydrogen bond differences caused by the replacement of ring O in ribosyl analogs to ring C in carbocyclic analogs could play a role in affecting their mechanisms of inhibition.

## Experimental Section

Solvents and reagents were purchased from commercial sources and used without further purification except as noted below. Melting points were determined on a Thomas-Hoover

melting point apparatus and are uncorrected. NMR spectra were obtained on either a Varian XL-300 or Bruker AM-500 spectrophotometer. All $^1$H chemical shifts are reported in ppm relative to the internal standard tetramethylsilane (TMS, $\delta$ 0.00) or $H_2O$ in $D_2O$ ($\delta$ 4.70). $^{13}$C chemical shifts are reported in ppm relative to $CDCl_3$ (center of triplet, $\delta$ 77.0). Mass spectra were recorded on a Ribermag R10-10 quadruple spectrometer. Elemental analyses were conducted at The University of Kansas and are within ±0.4% of the theoretical values. The purity of compounds **1a**,**b** was established by HPLC using an Econosil C-18 reversed-phase column and elution with $CH_3CN$ (A) in $H_2O$ (B) with a gradient of 1−6% A in B from 0 to 30 min, 6% A in B from 30 to 40 min. Detection was at 258 nm. Column chromatography was accomplished with flash silica gel (Aldrich Chemical Co.).

**(1*S*,2*S*,3*R*)-2,3-(Cyclohexylidenedioxy)-4-formylcyclopentanol (11).** (1*S*,2*S*,3*R*,4*R*)-2,3-(Cyclohexylidenedioxy)-4-vinylcyclopentanol (**10**) (4.5 g, 20 mmol) was dissolved in methanol (200 mL), and the solution was cooled to −70 °C in a dry ice−acetone bath. Ozone was then bubbled through the solution until the solution became dark blue. After bubbling $N_2$ through the solution until it became colorless again, dimethyl sulfide ($Me_2S$; 2.2 mL) was added and the solution was stirred at −70 °C for 1 h, warmed to −10 °C, and stirred for another 1 h and then warmed to room temperature. The solvent was evaporated, and the residue was purified through silica gel chromatography (EtOAc:hexane = 3:1). The product (3.8 g, 84%) was obtained as a mixture of two 1-epimers (A:B = 3:2): $^1$H NMR ($CDCl_3$) epimer A, $\delta$ 9.66 (s, 1 H, C*H*O), 4.83 (d, 1 H, $J$ = 5.9 Hz, H-2), 4.4 (dd, 1 H, $J_1$ = 5.4 Hz, $J_2$ = 5.8 Hz, H-3), 3.81 (m, 1 H, H-1), 2.96 (d, 1 H, $J$ = 6.3 Hz, H-4), 2.61 (b, 1 H, -OH), 1.85−2.15 (m, 2 H, H-5), 1.3−1.7 (m, 10 H, cyclohexyl-H), epimer B, $\delta$ 9.69 (b, 1 H, C*H*O), 4.86 (t, 1 H, $J$ = 5.4 Hz, H-2), 4.46 (t, 1 H, $J$ = 5.4 Hz, H-3), 3.81 (m, 1 H, H-1), 2.61 (b, 1 H, -OH), 2.52 (m, 1 H, H-4), 1.85−2.15 (m, 2 H, H-5), 1.3−1.7 (m, 10 H, cyclohexyl-H); $^{13}$C NMR ($CDCl_3$) epimer A, $\delta$ 200.9, 112.2, 78.3, 77.8, 71.0, 54.3, 35.7, 33.6, 30.9, 24.9, 23.9, 23.4, epimer B, $\delta$ 200.2, 112.4, 78.6, 78.4, 71.9, 51.0, 35.1, 33.5, 30.3, 24.9, 23.8, 23.4; HRMS (EI, *m*/*e*) 226.1200 ($M^+$, calcd 226.1205); MS (EI, *m*/*e*) 226 ($M^+$, 4.4), 197 (9.9), 183 (38.1), 111 (17.2), 84 (100).

**(1*S*,2*S*,3*R*)-2,3-(Cyclohexylidenedioxy)-4-(dimethoxymethinyl)cyclopentanol (12).** Aldehyde **11** (2.2 g, 9.7 mmol) was dissolved in dried methanol (10 mL), and then trimethyl orthoformate (3.3 mL) and ammonium chloride (39 mg) were added. After the solution was stirred at room temperature for 24 h, $NaHCO_3$ (100 mg) was added and then the reaction solution was stirred at room temperature overnight. The reaction solution was then filtered, and the solution was evaporated. The residue was dissolved in $CHCl_3$, washed with saturated $NaHCO_3$, and dried over $Na_2SO_4$. After filtration and evaporation, the residue was chromatographed on silica gel (EtOAc:hexane = 1:1). Two 4′-epimers (A, 1.25 g, 47%; B, 0.25 g, about 10%) of the product were separated: $^1$H NMR ($CDCl_3$) epimer A, $\delta$ 4.55 (dd, 1 H, $J_1$ = 6.1 Hz, $J_2$ = 1.7 Hz, H-3), 4.43 (t, 1 H, $J$ = 5.8 Hz, H-2), 4.16 (d, $J$ = 5.4 Hz, 1 H, $(CH_3O)_2CH$-), 4.13 (m, 1 H, H-1), 3.38 (s, 3 H, -$OCH_3$), 3.36 (s, 3 H, -$OCH_3$), 2.51 (b, 1 H, -OH), 2.41 (m, 1 H, H-4), 1.85 (m, 2 H, H-5), 1.3−1.7 (m, 10 H, cyclohexyl-H), epimer B, $\delta$ 4.35−4.55 (m, 4 H, $(CH_3O)_2CH$-, H-1, H-2, H-3), 3.4 (s, 3 H, -$OCH_3$), 3.3 (s, 3 H, -$OCH_3$), 2.5 (m, 1 H, H-4), 1.85−2.15 (m, 3 H, -OH, H-5), 1.3−1.7 (m, 10 H, cyclohexyl-H); $^{13}$C NMR ($CDCl_3$) epimer A, $\delta$ 112.2, 106, 80.7, 79.3, 71.3, 55.0, 54.3, 45.0, 35.9, 33.7, 33.6, 25.1, 24.0, 23.5, epimer B, $\delta$ 112.6, 104.7, 78.8, 78.2, 72.1, 54.4, 53.0, 41.5, 35.4, 33.6, 32.5, 25.2, 24.0, 23.6; HRMS ($FAB^+$ *m*/*e*) (epimer A) 273.1689 ($MH^+$, calcd 273.1702); MS (EI, *m*/*e*) 272 ($M^+$, 4), 125 (17), 75 (100).

**(1*S*,2*S*,3*R*,4*S*)-2,3-(Cyclohexylidenedioxy)-4-(dimethoxymethinyl)cyclopentanyl Trifluoromethanesulfonate (13).** To a solution of (1*S*,2*S*,3*R*,4*S*)-2,3-(cyclohexylidenedioxy)-4-(dimethoxymethinyl)cyclopentanol (**12**) (1.1 g, 4.2 mmol) in anhydrous $CH_2Cl_2$ (35 mL) was added anhydrous pyridine (382 $\mu$L, 4.65 mmol) at 0 °C followed by the addition of trifluoromethanesulfonic anhydride (711 $\mu$L, 4.23 mmol). After stirring at 0 °C for 1 h, ice−water (13 mL) was added slowly and the mixture was stirred at 0 °C for 5 min. $CH_2Cl_2$ (30 mL) was added, and the organic phase was separated, washed with cold water (2 × 45 mL), and then dried over $Na_2SO_4$. After filtering and evaporation, the red oil residue obtained (1.17 g, 69%) was used immediately without further purification: $^1$H NMR ($CDCl_3$) $\delta$ 5.15 (dd, 1 H, $J_1$ = 12.1 Hz, $J_2$ = 7.3 Hz, H-1), 4.56 (m, 2 H, H-2, H-3), 4.2 (d, $J$= 3.8 Hz, 1 H, $(CH_3O)_2CH$-), 3.4 (s, 6 H, -$OCH_3$), 2.49 (m, 1 H, H-4), 2.1−2.3 (m, 2 H, H-5), 1.3−1.8 (m, 10 H, cyclohexyl-H); $^{13}$C NMR ($CDCl_3$) $\delta$ 169.8, 113.2, 106.1, 86.9, 80.1, 78.1, 55.8, 44.7, 35.7, 34.0, 30.0, 25.1, 23.9, 23.6.

**(1′*R*,2′*S*,3′*R*,4′*S*)-9-[2′,3′-(Cyclohexylidenedioxy)-4′-(dimethoxymethinyl)cyclopentan-1′-yl]adenine (14a).** Sodium hydride (60% in oil mixture, 511 mg, 12.7 mmol) was washed with hexane (5 mL × 3) and mixed with dried DMF (40 mL). After adenine (1.87 g, 12.7 mmol) and 18-crown-6 (550 mg, 2.11 mmol) were added, the solution was heated at 70 °C for 4 h and then cooled to room temperature. Compound **13** (1.17 g, 2.79 mmol) in DMF (10 mL) was slowly added, and the reaction solution was stirred at room temperature for 20 h. It was filtered and washed with DMF (5 mL) and $CH_2Cl_2$ (120 mL). The filtrate was washed with saturated NaCl (45 mL × 3) and dried over $Na_2SO_4$. After filtration and evaporation, an oil residue was purified through silica gel chromatography eluted with $CH_2Cl_2$ and then preparative TLC ($CH_2Cl_2$:EtOH = 9:1, four times). The desired product **14a** (81 mg, 7%) was obtained: $^1$H NMR ($CDCl_3$) $\delta$ 8.25 (s, 1 H, H-8), 7.82 (s, 1 H, H-2), 6.16 (b, 2 H, -$NH_2$), 4.96 (m, 1 H, H-2′), 4.66 (m, 2 H, H-1′, H-3′), 4.37 (d, 1 H, $J$ = 6.4 Hz, $(CH_3O)_2CH$-), 3.36 (s, 3 H, -$OCH_3$), 3.33 (s, 3 H, -$OCH_3$), 2.23−2.56 (m, 3 H, H-4′, H-5′), 1.2−1.8 (m, 10 H, cyclohexyl-H); $^{13}$C NMR ($CDCl_3$) $\delta$ 155.6, 152.5, 150.0, 139.7, 120.0, 114.3, 105.5, 83.1, 80.0, 62.0, 54.1, 54.0, 46.0, 37.4, 34.5, 32.6, 27.1, 24.0, 23.5; HRMS (EI, *m*/*e*) 389.2040 ($M^+$, calcd 389.2063); MS (EI, *m*/*e*) 390 ($MH^+$, 3.5), 374 (11), 346 (26), 276 (14), 276 (14), 244 (12), 216 (99), 135 (12), 75 (100).

The isomer **14b** was also obtained. **14b**: $^1$H-NMR 8.27 (s, 1 H, H-8), 7.71 (s, 1 H, H-2), 6.05 (b, 2 H, $NH_2$), 5.20 (t, 1 H, H-2′), 5.13 (d, 1 H, $J$ = 5.5 Hz, H-1′), 4.75 (d, 1 H, $J$ = 6.8 Hz, H-3′), 3.48 (d, 1 H, $J$ = 8.3 Hz, $(CH_3O)_2CH$), 3.21 (s, 3 H, -$OCH_3$), 3.16 (s, 3 H, -$OCH_3$), 3.03 (m, 1 H, H-5′), 2.29 (m, 1 H, H-4′), 2.07 (m, 1 H, H-5′), 1.4−1.9 (m, 10 H, cyclohexyl-H); $^{13}$C NMR 155.3, 152.2, 142.5, 110.9, 108.6, 103.8, 84.3, 80.8, 64.1, 54.0, 52.0, 43.5, 42.0, 36.1, 34.3, 33.2, 27.0, 25.2, 25.0; HRMS 399.2063 (calcd), 389.2044 (found).

**(1′*R*,2′*S*,3′*R*,4′*S*)-9-(2′,3′-Dioxy-4′-formylcyclopentan-1′-yl)adenine Hydrate (1).** Acetal **14a** (15 mg, 0.038 mmol) was mixed with DCl solution in $D_2O$ (2%, 1 mL) and stirred at room temperature overnight. $CDCl_3$ (1 mL × 3) was used to extract the water solution. HPLC showed that the remaining $D_2O$ solution contained a mixture of **1a**,**b**, and the NMR spectra were taken directly in this $D_2O$ solution: $^1$H NMR ($D_2O$) $\delta$ 8.33 (s, 1 H, 8-H, isomer A), 8.30 (s, 1 H, 8-H, isomer B), 8.25 (s, 1 H, 2-H, isomer A), 8.23 (s, 1 H, 2-H, isomer B), 5.02 (d, 1 H, $J$ = 5.7 Hz, $(HO)_2CH$-, isomer A), 4.9 (m, 1 H, $J$ = 5.7 Hz, $(HO)_2CH$-, isomer B), 4.4−4.76 (m, 1 H, 1′-H, isomers A and B), 4.35 (dd, 1 H, $J_1$ = 6 Hz, $J_2$ = 4.7 Hz, 3′-H, isomers A and B), 4.0 (m, 1 H, 2′-H, isomers A and B), 1.8−2.4 (m, 3 H, 4′-H, 5′-H, isomers A and B).

Separation of **1a**,**b** was accomplished by HPLC using an Econosil C-18 reversed-phase column and eluting elution with $CH_3CN$ (A) in $H_2O$ (B) with a gradient of 1−6% A in B from 0 to 30 min, 6% A in B from 30 to 40 min. Compound **1a** eluted at 14.4 min, and compound **1b** eluted at 32.9 min.

**Reduction of 1a,b.** To an aqueous solution of **1a** or **1b** (0.5 mM, 0.5 mL) was added an aqueous solution of $NaBH_4$ (22.6 mM, 11 $\mu$L), which was then stirred at room temperature for 24 h. The solution was analyzed using HPLC on an Econosil C-18 reversed-phase column and eluted with $CH_3CN$ (A) in $H_2O$ (B) with a gradient of 1−6% A in B from 0 to 30 min, 6% A in B from 30 to 40 min at a flow rate of 1.5 mL/min. The new peak from the reduction of **1a**, which appeared with a retention time of 24.1 min, was characterized by coinjection with a standard solution of aristeromycin. The new peak appearing from the reduction of **1b** had a retention time of 29.0 min.

**Determination of AdoHcy Hydrolase Inhibition Constants.** Enzyme inhibition studies were conducted using a purified recombinant human placental AdoHcy hydrolase. The enzyme activity was determined in the synthetic direction using Ado (0.2 mM) and Hcy (5 mM) as substrates. The reaction was carried out in 50 mM phosphate buffer (pH 7.2) containing 1 mM EDTA (buffer B) at 37 °C for 5 min. The reaction product AdoHcy was assayed by HPLC after the reaction was stopped by addition of $HClO_4$. An aliquot (100 $\mu$L) of the reaction mixture was analyzed by HPLC (Econosphere Alltech 250 × 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% A in C over 0−9 min and 15−50% A in C over 9−15 min, where mobile phase A was $CH_3CN$ and C was 50 mM sodium phosphate buffer (pH 3.2) containing 10 mM heptanesulfonic acid. The peak area of AdoHcy was monitored by a UV detector at 258 nm to quantitate the AdoHcy. For the determination of inhibition constants, AdoHcy hydrolase was preincubated with various concentrations of inhibitors for varying amounts of time, and the remaining enzyme activity was measured. $K_i$ and $k_2$ values were obtained according to the Kitz and Wilson equations[19] as described in the text.

**Conversion of E·NAD$^+$ to E·NADH.** The inhibitor-induced E·NADH formation was determined by measuring the increase in absorbance at 316 nm at different time intervals after the enzyme was mixed with the inhibitor. To AdoHcy hydrolase (1.0 mg) in 1 mL of buffer B was added 20 $\mu$L of (*Z*)-aristeromycin vinyl fluoride **3** (3 mM) with mixing for 10 s. The UV spectrum (280−500 nm) was recorded periodically at 25 °C using an HP 8452 diode array spectrophotometer (Hewlett-Packard Co., Palo Alto, CA). The instrument was referenced against the enzyme solution with the addition of 20 $\mu$L of water instead of the inhibitor. The spectrum was recorded until no additional increase in the absorbance at 316 nm was observed (10 min).

**Determination of 5′-Hydrolytic Product Formation.** AdoHcy hydrolase (90 $\mu$g of apoenzyme or NAD$^+$ form) in 0.5 mL of buffer B was incubated with aristeromycin vinyl fluoride **3** (20 $\mu$ M) at 37 °C for 1 h, and the reaction was stopped by the addition of 10 $\mu$L of 5 N $HClO_4$. The precipitate was removed by centrifugation, and the supernatant was analyzed by HPLC using a reversed-phase C-18 column (Econosphere, Altech, 250 × 4.6 mm, 5 $\mu$m; Deerfield, IL). For the apoenzyme, aristeromycin-5′-aldehydes (**1a**,**b**) were directly used as standards, while for NAD$^+$ form of the enzyme, **1b** was incubated with the enzyme in the same conditions as described above and the 3′-keto product formed was used as a standard. HPLC was run in a linear gradient of 6−25% A in C over 15 min. The chromatography was monitored at 258 nm. The apoenzyme was prepared from the NAD$^+$ form of AdoHcy hydrolase by removal of the enzyme-bound NAD$^+$ with acidic $(NH_3)_2SO_4$ as described previously.[13]

**$^{19}$F NMR Studies.** Fluoride ion was determined by $^{19}$F NMR spectroscopy recorded at 500 MHz on a Bruker AM spectrometer interfaced to an Aspect 300 computer. AdoHcy hydrolase (NAD$^+$ form) was incubated with aristeromycin vinyl fluoride **3** (enzyme, 44.4 $\mu$M; inhibitor, 1 mM) in buffer B at 37 °C for 2.5 h. The reaction mixture was mixed with 3 vol of 97% EtOH. After centrifugation, the supernatant was collected and the precipitate was washed once with 50% EtOH; the combined supernatant and washing were lyophilized. The residue was dissolved in 0.5 mL of $D_2O$ for the $^{19}$F NMR spectrum. A total of 2048 transients were collected (acquisition time = 0.524 s, no pulse delay) using a spectral width of 62 500 Hz. NaF was used as a standard for the chemical shift of fluoride ion.

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