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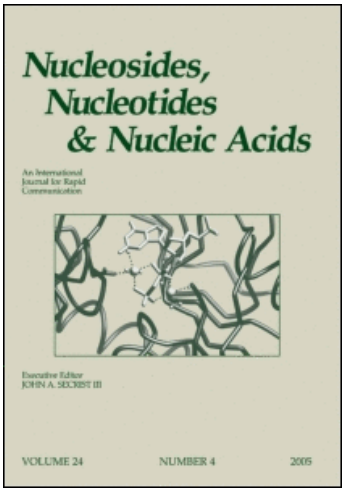

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Synthesis of Homologated Halovinyl Derivatives from Aristeromycin and Their Inhibition of Human Placental *S*-Adenosyl-L-homocysteine Hydrolase

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NUCLEOSIDES & NUCLEOTIDES, 17(1-3), 99-113 (1998)

SYNTHESIS OF HOMOLOGATED HALOVINYL DERIVATIVES FROM ARISTEROMYCIN AND THEIR INHIBITION OF HUMAN PLACENTAL *S*-ADENOSYL-L-HOMOCYSTEINE HYDROLASE[1,¶]

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ABSTRACT: Moffatt oxidation of 2',3'-*O*-isopropylidenearisteromycin (**1a**) and treatment of the 5'-carboxaldehyde with [(*p*-tolylsulfonyl)methylene]triphenylphosphorane gave the homologated vinylsulfone **2**. Treatment of **2** with tributylstannane/AIBN gave the (*E*/*Z*)-vinylstannanes which were converted into the *E* and *Z* fluoro- and iodovinyl analogs. Chain extension via the 5'-cyano-5'-deoxy derivative **10a** gave the 6'-carboxaldehyde of homoaristeromycin. *S*-Adenosyl-L-homocysteine hydrolase was strongly inhibited by the fluorovinyl, **5b**, and iodovinyl, **4b** and **7b**, compounds, and time-dependent kinetics were observed [1–2 μM (K_i) and 0.1–0.2 min^{-1} (k_{inact})]. The mechanism of inactivation was shown to involve addition of water at the vinyl 5' or 6' carbons with elimination of halide.

Introduction

The cellular enzyme *S*-adenosyl-L-homocysteine (AdoHcy) hydrolase (EC 3.3.1.1) effects cleavage of AdoHcy to adenosine (Ado) and L-homocysteine (Hcy).[2] Since AdoHcy is a potent feedback inhibitor of crucial transmethylation enzymes,[3] the design of inhibitors of AdoHcy hydrolase represents a rational approach for anticancer and antiviral chemotherapy.[2] Direct correlations between antiviral[4] and cytostatic[5] potencies of Ado analogs and their inhibitory effects on AdoHcy hydrolase have been demonstrated. AdoHcy hydrolase has been shown to be inactivated by various Ado-derived nucleoside

¶This paper is dedicated to the late Professor T. Hata.

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derivatives via the "cofactor depletion" or type I mechanism.[2b] During this process, the type I inhibitors are oxidized stoichiometrically to the corresponding 3'-ketonucleosides by enzyme-bound NAD^+, and this essential cofactor is depleted as tightly bound NADH.

The fluorovinyl nucleoside **A** [(*Z*)-4',5'-didehydro-5'-deoxy-5'-fluoroadenosine, ZDDFA; Figure 1) was shown to be a potent inhibitor of AdoHcy hydrolase.[6] ZDDFA was converted into the inhibitory "adenosine 5'-carboxaldehydes"[7,8] by enzyme-catalyzed addition of water to the 4',5'-double bond (hydrolytic activity), and this hydration occurred independently of the 3'-oxidative activity of AdoHcy hydrolase.[7] We also have shown that 5'-(α-fluoro thioethers)[9] **B**, 5'-carboxaldehyde oximes,[2c,10] and the 5'-methoxyvinyl analog[9c,10b] **C** derived from adenosine are "pro-inhibitors" of AdoHcy hydrolase which are converted into Ado-5'-carboxaldehydes at the active site of the enzyme. Intriguingly, the homologated vinyl halides **D** [(*E*)-5',6'-didehydro-6'-deoxy-6'-halohomoadenosines, EDDHHAs] also undergo hydrolysis at the active site (with halide ion release) to produce "homoadenosine 6'-carboxaldehyde" which decomposes spontaneously with release of adenine.[5,11] However, "hydrolytic activity" was not involved during inhibition of AdoHcy hydrolase by amide and ester derivatives of Ado-5'-carboxylic acid[12] or with the acetylenic compound 9-(5,6-dideoxy-β-D-*ribo*-hex-5-ynofuranosyl)adenine.[5,13]

The naturally occurring "carbocyclic adenosine analogs" aristeromycin[14a] (Ari, **1b**; Scheme 1) and neplanocin A[14b] are potent inhibitors of AdoHcy hydrolase[2c,14] whose chemistry[15a] and structure-activity relationships[15b] have been reviewed. Strong inhibition was observed with the fluorovinyl analog[16] **E**, but no fluoride release was detected and no conversion of **E** into Ari-5'-carboxaldehyde was observed.[16b,17] Different mechanisms of inhibition for the ribofuranosyl (**A**) and cyclopentanyl (**E**) fluoromethylene analogs were attributed to electronic and hydrogen bonding differences.[17] Ari-5'-carboxaldehyde was found to be an inhibitor of AdoHcy hydrolase,[17] but a cyclopropane-fused neplanocin C analog was devoid of activity.[18] Diverse biological activities have been reported[19,20] for several 4'-modified Ari and neplanocin analogs, including homologated derivatives.[19]

Spontaneous decomposition of HomoAdo-6'-carboxaldehyde presumably occurs by β-elimination (H5' and the ring oxygen O4') with accompanying expulsion of adenine upon AdoHcy hydrolase-catalyzed hydration of EDDHHAs.[5,11] Formal replacement of the furanosyl ring oxygen with a methylene unit would be expected to provide analogs that could not suffer cyclopentanyl ring cleavage by β-elimination. We now report syntheses of the iodo (**4b**) and fluoro (**5b**) homoaristeromycin analogs of **D** and evaluation of their inhibitory effects on AdoHcy hydrolase. Homologation of Ari via a 5'-cyano-5'-deoxy derivative gave the HomoAri-6'-carboxaldehyde for comparison.

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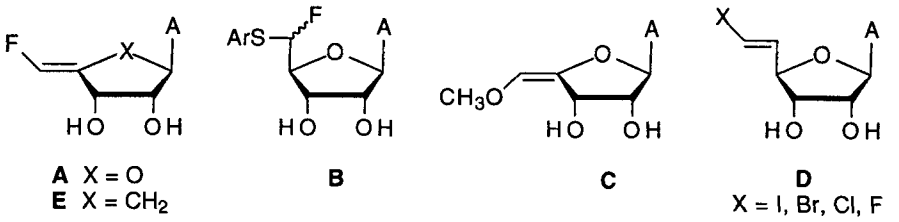
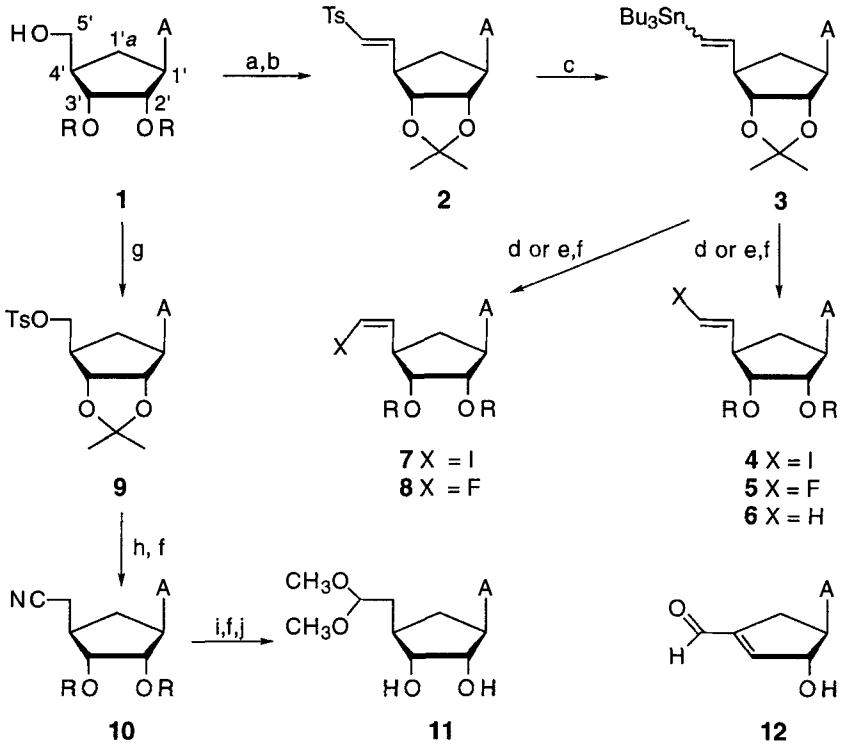


FIG. 1. Structures of some inhibitors of AdoHcy hydrolase.



Series: **a** R,R = CMe_2 Ts = (4)$CH_3C_6H_4SO_2$
b R = H

[a] (a) DMSO/DCC/Cl_2CHCO_2H. (b) Ph_3P=CHTs. (c) Bu_3SnH/AIBN/toluene/Δ. (d) NIS/CH_2Cl_2/CCl_4. (e) XeF_2/AgOTf/THF. (f) TFA/H_2O. (g) TsCl/pyridine. (h) KCN/ 18-crown-6/dioxane/DMF. (i) DIBALH/CH_2Cl_2. (j) MeOH.

Scheme 1[a]

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Chemistry

The conventional nucleoside numbering system has been modified by assignment of "1'*a*" as the "carba" locant for the ring atom corresponding to O4' in ribonucleosides.[15b] Moffatt oxidation[21] of 2',3'-*O*-isopropylidenearisteromycin[16a] (**1a**) and treatment of the 5'-carboxaldehyde with [(*p*-tolylsulfonyl)methylene]triphenylphosphorane[22] gave the (*E*)-homovinyl sulfone **2** ($J_{5'-6'}$ = 15.1 Hz; 43% after chromatography and crystallization). The structure of a dehydrated byproduct (~7%), with an endocyclic double bond isomeric[23] to that in neplanocin A, was tentatively assigned as **12** (NMR, MS).

Stannodesulfonylation[5,24] (Bu_3SnH/AIBN) of **2** gave a partially separated mixture of vinylstannanes **3** (*E/Z*, ~9:1; 67%). Quantitative iododestannylation[5,25] (NIS) of **3**(*E*) gave the protected (*E*)-6'-iodohomovinyl analog **4a**. Deprotection (TFA/H_2O) of **4a** and crystallization gave **4b** (74% from **3**). Treatment of **3** (*E/Z*, ~7:3) with NIS, deprotection, and preparative RP-HPLC gave **4b** and **7b**(*Z*). Fluorodestannylation (XeF_2/AgOTf)[5,26] of **3** (*E/Z*, ~9:1), deprotection, and preparative RP-HPLC gave the (*E*)-6'-fluorohomovinyl isomer **5b** (38% from **3**) plus a mixture of the 5'-deoxy-5'-methylene compound **6b**[20a] and the *Z*-fluoro isomer **8b** (~3:1; ~18%).

Tosylation of **1a** and treatment of the tosylate **9** with KCN/18-crown-6[27] gave the 5'-cyano derivative **10a**. Deprotection of **10a** gave 5'-cyano-5'-deoxyAri (**10b**) whose ribosyl analog is a potent inhibitor of AdoHcy hydrolase.[28] Treatment of **10a** with diisobutylaluminum hydride[29] (DIBALH) followed by TFA/H_2O (hydrolysis of imine intermediates, removal of the isopropylidene group, and formation of the 6'-acetal upon coevaporation with methanol) gave the HomoAri-6'-carboxaldehyde dimethyl acetal **11**. *In situ* generation of HomoAri-6'-carboxaldehyde occurred upon treatment of **11** with dilute sulfuric acid.

Inhibition of *S*-Adenosyl-L-homocysteine Hydrolase

Incubation of human placental AdoHcy hydrolase with the fluorovinyl, **5b**, and iodovinyl, **4b** and **7b**, compounds resulted in irreversible inactivation of the enzyme. The inhibitory activity was shown to be both time- and inhibitor concentration-dependent, as shown in Table 1 and Fig. 2. At concentrations of 10 μM, the enzyme was completely inactivated in 10 min by **4b**, whereas **5b** (90%) and **7b** (70%) had weaker effects. Compound **4b** had a lower K_i value than did **5b** and **7b**, whereas **5b** had a larger k_{inact} value than those of **4b** and **7b** (Table 2). The inhibitory specificity of these compounds toward AdoHcy hydrolase was in the order of **4b** > **5b** > **7b** (Table 2).

Inactivation of AdoHcy hydrolase by **5b** was investigated in more detail and found to occur concomitantly with reduction of the enzyme-bound NAD^+ to NADH, release of

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TABLE 1. Concentration-dependent Inactivation of AdoHcy Hydrolase with **4b**, **5b**, and **7b**.

Inhibitors	Inhibitor concentration (μM)				
	0.1	1.0	10	50	100
	Enzyme activity (% of control)				
4b	101	64.4	*a*	*a*	*a*
5b	89.2	48.7	12.2	3.4	2.5
7b	104	89.3	31.3	2.5	0.5

[a] No activity detected.

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fluoride, and formation of hydrolysis products. Three pathways were identified: (a) attack of water at the 6'-position and loss of fluoride resulting in the formation of HomoAri-6'-carboxaldehyde (**13**); (b) attack of water at the 5'-position resulting in the formation of the 6'-deoxy-6'-fluoro-5'-hydroxyHomoAri derivative **14**; and (c) oxidation at C3' resulting in formation of the inactivated NADH form of the enzyme and the 3'-keto intermediate **15**, which could undergo enzyme-catalyzed hydration by attack of water at either C5' or C6' (Scheme 2). Partition ratios among the three pathways were determined to be $k_{3'}/k_{5'}/k_{6'}$ = 1:0.6:1.7, which indicated that one enzyme inactivation event occurred for every 2.3 nonlethal turnovers. Evidence in support of these pathways includes the observation that incubation of **5b** with AdoHcy hydrolase (NAD^+ form) generated 1.7 equivalents of fluoride ion (^{19}F NMR) and 2.3 combined equivalents of **13** and **14** (HPLC, mixture). Chemical ionization mass spectra had peaks at *m/z* 278 (**13**, MH^+) and 298 (**14**, MH^+).

Results from this study provide further evidence in support of the two functionally independent catalytic activities of AdoHcy hydrolase. The hydrolytic activity can operate without the oxidative activity, as clearly demonstrated by the formation of **13** and **14**. It is amazing that this enzyme has the hydrolytic power to effect addition of water to the isolated double bond of a vinyl halide (the 5',6'-bond of these halovinyl-substituted cyclopentane systems). Investigations of new inhibitors that specifically target the hydrolytic activity of AdoHcy hydrolase are currently underway in our laboratories.

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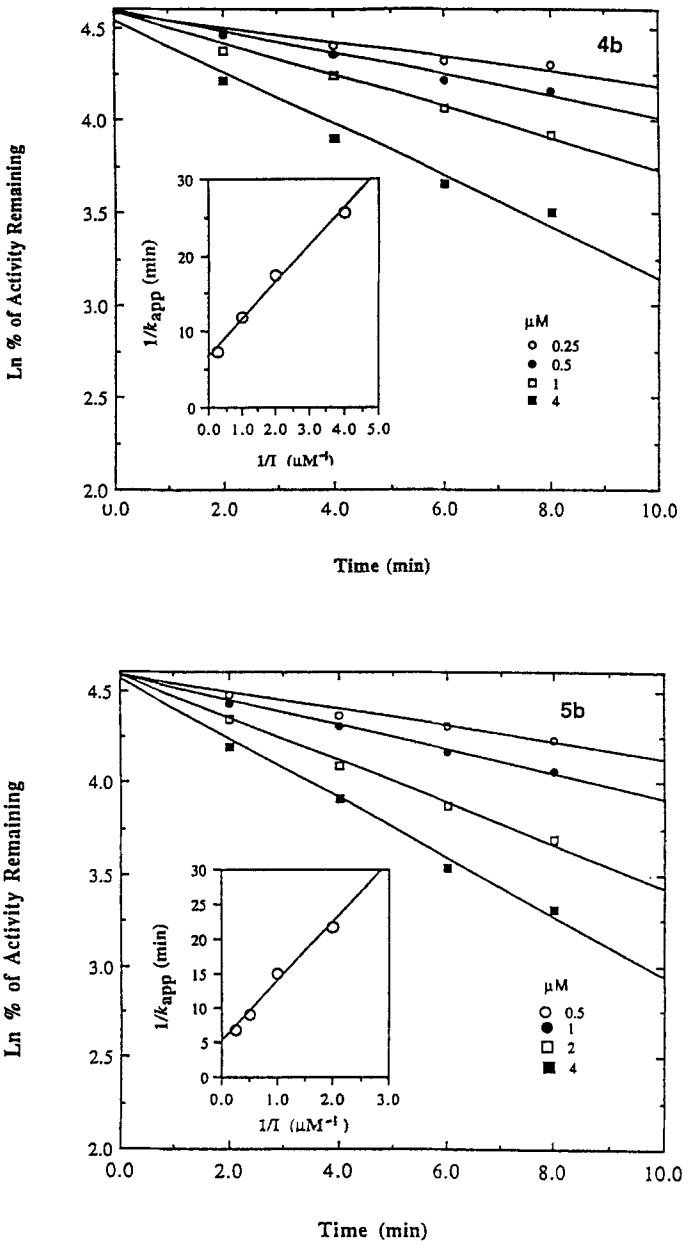
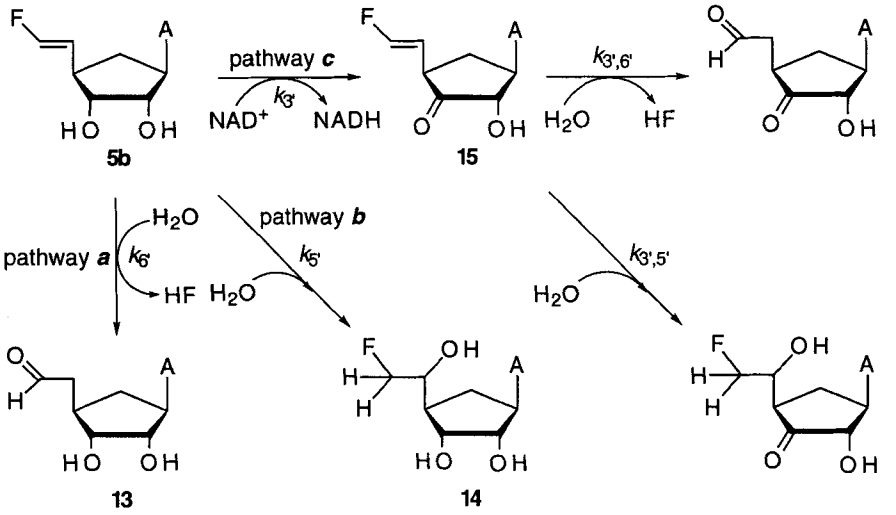


FIG. 2. Kitz and Wilson plots for compounds **4b** and **5b**.

TABLE 2. Kinetic Constants for Inactivation of AdoHcy Hydrolase with **4b**, **5b**, and **7b**.

Inhibitors	K_i (μM)	k_{inact} (min^{-1})	k_{inact}/K_i (M^{-1} min^{-1})
4b	0.72	0.15	2.1×10^5
5b	1.70	0.20	1.2×10^5
7b	1.40	0.11	0.8×10^5



[a] Possible catalytic modes by which AdoHcy hydrolase modifies **5b**.

Scheme 2[a]

Experimental Section

Uncorrected melting points were determined with a capillary apparatus. UV spectra were determined with solutions in MeOH. ^1H (200 MHz), ^{13}C (50 MHz) and ^{19}F (CCl_3F, 476 MHz) NMR spectra were recorded with solutions in Me_2SO-d_6 unless otherwise noted. Mass spectra (MS and HRMS) were obtained with electron impact (20eV), chemical ionization (CI, CH_4), or fast atom bombardment (FAB, thioglycerol matrix) 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

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use. Column chromatography was performed with Merck kieselgel 60 (230-400 mesh). TLC was performed on Merck Kieselgel 60 F_{254} sheets with: S_1 (EtOAc/*i*-PrOH/H_2O, 4:1:2; upper layer), S_2 (MeOH/EtOAc, 1:10), or S_3 (MeOH/$CHCl_3$, 1:6) with sample observation under 254-nm light. Analytical and preparative reversed-phase HPLC were performed with a Spectra Physics SP 8800 pump system and Dynamax C_{18} columns.

9-{(1*R*,2*S*,3*R*,4*R*)-2,3-Isopropylidenedioxy-4-[(*E*)-2-(*p*-tolylsulfonyl)ethenyl]cyclopentan-1-yl}adenine (2). A solution of **1a**[16a] (915 mg, 3 mmol) and dicyclohexylcarbodiimide (DCC; 2.16 g, 10.5 mmol) in anhydrous Me_2SO (10 mL) was stirred with cooling (ice bath) while Cl_2CHCO_2H (0.123 mL, 193 mg; 1.5 mmol) was added. The ice bath was removed after 10 min, stirring was continued for 2.5 h, and [(tosyl)methylene]triphenylphosphorane[22] (1.42 g, 3.3 mmol) was added. After 18 h, oxalic acid dihydrate (675 mg, 7.5 mmoL) in MeOH (15 mL) was added and stirring was continued for 30 min. Dicyclohexylurea was filtered, the filtrate was evaporated *in vacuo*, and the residue was partitioned ($CHCl_3$/$NaHCO_3$). The organic layer was washed [H_2O, 3 × 20 mL; brine], dried ($MgSO_4$), and evaporated. Chromatography of the yellow foam ($CHCl_3$ → 3% MeOH/$CHCl_3$) and crystallization (EtOAc/hexanes) gave **2a** (590 mg, 43%): mp 188-189 °C (dec); UV max 237, 259 nm (ε 21 800, 16 700), min 224, 252 nm (ε 14 900, 15 700); ^{1}H NMR ($CDCl_3$) δ 1.28 & 1.52 (2 × s, 2 × 3, Me_2C), 2.44 (s, 3, Me), 2.44–2.77 (m, 2, H1'*a*,1"*a*), 2.85–3.01 (m, 1, H4'), 4.69–4.80 (m, 2, H1',3'), 5.11 (dd, $J_{2'\text{-}1'}$ = 7.3 Hz, $J_{2'\text{-}3'}$ = 4.9 Hz, 1, H2'), 5.88 (br s 2, NH_2), 6.47 (dd, $J_{6'\text{-}5'}$ = 15.1 Hz, $J_{6'\text{-}4'}$ = 1.3 Hz, 1, H6'), 7.07 (dd, $J_{5'\text{-}4'}$ = 6.7 Hz, 1, H5'), 7.33 & 7.78 (2 × d, *J* = 8.5 Hz, 2 × 2, arom), 7.80 (s, 1, H2), 8.30 (s, 1, H8); ^{13}C NMR ($CDCl_3$) δ 22.12 (Me), 25.55 & 27.89 (CMe_2), 36.31 (C1'*a*), 46.74 (C4'), 61.74 (C1'), 83.40, 83.79 (C2',3'), 114.96 (CMe_2), 120.99 (C5), 132.57 (C5'), 140.31 (C8), 144.37 (C6'), 150.31 (C4), 153.35 (C2), 156.13 (C6), 128.34, 130.46, 137.64, 145.01 (arom); MS (CI) *m/z* 456 (100, MH^+). Anal. Calcd. for $C_{22}H_{25}N_5O_4S$ (455.5): C, 58.01; H, 5.53; N, 15.37. Found: C, 57.86; H, 5.65; N, 15.26.

Further elution (3 → 8% MeOH/$CHCl_3$) gave the 2',3'-*O*-isopropylideneAri-5'-carboxaldehyde [~90 mg, 10%; purity ~85% (^{1}H NMR)] and a byproduct (~100 mg) which was repurified ($CHCl_3$ → 8% MeOH/$CHCl_3$) and crystallized (MeOH) to give the presumed 9-[(1*R*,2*R*)-2-hydroxy-4-formylcyclopent-3-en-1-yl]adenine (**12**; 51 mg, 7% from **1a**): mp >250 °C (dec); UV max 260 nm (ε 14 500), min 229 nm (ε 7800); ^{1}H NMR δ 2.95 (dm, *J* = 8.1 Hz, 2, H1'*a*,1"*a*), 4.83 (q, *J* = 7.4 Hz, 1, H1'), 5.43 (tm, *J* = 7.1, 1.8 Hz, 1, H2'), 5.98 (d, *J* = 6.3 Hz, 1, OH2'), 7.05 (d, $J_{3'\text{-}2'}$ = 1.7 Hz, 1, H3'), 7.30 (br s, 2, NH_2), 8.11 (s, 1, H2), 8.22 (s, 1, H8), 9.79 (s, 1, CHO); ^{13}C NMR δ

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32.22 (C1'*a*), 63.61 (C1'), 79.03 (C2'), 119.48 (C5), 141.01 (C8), 142.66 (C3'/4'), 149.64 (C4), 151.66 (C4'/3'), 152.44 (C2), 156.08 (C6), 191.23 (C5'); MS (CI) *m/z* 246 (100, MH^+); HRMS *m/z* 245.0905 (10; $M^+[C_{11}H_{11}N_5O_2]$ = 245.0913).

9-{(1*R*,2*S*,3*R*,4*R*)-4-[2-(Tributylstannyl)ethenyl]-2,3-isopropylidenedioxycyclopentan-1-yl}adenine (3). A suspension of **2** (228 mg, 0.5 mmol) in toluene (8 mL) was deoxygenated (Ar, 1 h), Bu_3SnH (0.47 mL, 507 mg, 1.75 mmol) and AIBN (25 mg, 0.15 mmol) were added, and the solution was refluxed under Ar for 2.5 h [TLC (S_3) showed ~50% conversion to less polar material]. Bu_3SnH (0.20 mL, 218 mg; 0.75 mmol) and AIBN (16 mg, 0.1 mmol) were added, reflux was continued for 2.5 h, and volatiles were evaporated. The residue was partitioned ($NaHCO_3/H_2O//CHCl_3$) and the organic layer was washed (brine), dried ($MgSO_4$), and evaporated. Chromatography [40% pentanes/EtOAc (extensive washing to remove tin derivatives) → EtOAc → 3% MeOH/EtOAc] gave **3**(*E*) (124 mg, 42%) and **3** (*E/Z*, ~7:3; 74 mg, 25%) as viscous oils, and recovered **1a** (23 mg, 10%). Compound **3**(*E*): ^1H NMR ($CDCl_3$) δ 0.85–1.62 (m, 33), 2.41–2.61 (m, 2, H1'*a*,1"*a*), 2.75–2.92 (m, 1, H4'), 4.65 (dd, $J_{3'\text{-}2'}$ = 7.3 Hz, $J_{3'\text{-}4'}$ = 6.2 Hz, 1, H3'), 4.75 (ddd, $J_{1'\text{-}2'}$ = 5.4 Hz, $J_{1'\text{-}1'a}$ = 7.5 Hz, $J_{1'\text{-}1"a}$ = 11.1 Hz, 1, H1'), 5.10 (dd, 1, H2') 6.03 (br s, 2, NH_2), 6.07–6.10 (m, 2, H5',6'), 7.81 (s, 1, H2), 8.34 (s, 1, H8); ^{13}C NMR ($CDCl_3$) δ 9.94, 14.19, 27.74, 29.54 (Bu), 25.56, 27.93, 114.46 (CMe_2), 37.08, 51.40, 62.13, 83.90, 84.33 (cyclopentyl), 130.42, 147.63 (C6'/5'), 120.81, 140.25, 150.31, 153.25, 156.08 (adenine); MS (CI) *m/z* 592 (100, MH^+, ^{120}Sn), 590 (72, MH^+, ^{118}Sn), 588 (36, MH^+, ^{116}Sn). ^1H NMR shifts for the **3**(*Z*) isomer were similar, except δ 5.98 (d, $J_{6'\text{-}5'}$ = 12.2 Hz, 0.3, H6'), 6.51 (dd, $J_{5'\text{-}4'}$ = 8.1 Hz, 0.3, H5') [determined with the **3** (*E/Z*, ~7:3) mixture].

9-{(1*R*,2*S*,3*R*,4*R*)-2,3-Dihydroxy-4-[(*E*)-2-iodoethenyl]cyclopentan-1-yl}adenine [4b(*E*)]. (A) *Iododestannylation.* A solution of NIS (68 mg, 0.3 mmol) in CH_2Cl_2/CCl_4 (10 mL, 1:1) was added dropwise to a stirred solution of **3**(*E*) (130 mg, 0.22 mmol) in CH_2Cl_2/CCl_4 (10 mL, 1:1) at –20 °C. After 30 min, [only the more polar **4a**(*E*) was visible, TLC (S_2)] the pink mixture was washed [$NaHCO_3/H_2O$, $NaHSO_3/H_2O$ (dilute), brine], dried ($MgSO_4$), and evaporated to give crude **4a**(*E*) (~120 mg) as a white solid: ^1H NMR ($CDCl_3$) δ 0.77–1.63 (m, Bu_3SnX), 1.28 & 1.50 (2 × s, 2 × 3, CMe_2), 2.31–2.81 (m, 3, H1*a*',1"*a*,4'), 4.57–4.75 (m, 2, Hl',3'), 5.03 (dd, $J_{2'\text{-}1'}$ = 7.3 Hz, $J_{2'\text{-}3'}$ = 4.8 Hz, l, H2'), 5.95 (br s, 2, NH_2), 6.21 (d, $J_{6'\text{-}5'}$ = 14.5 Hz, l, H6'), 6.60 (dd, $J_{5'\text{-}4'}$ = 7.2 Hz, l, H5'), 7.74 (s, l, H2), 8.28 (s, l, H8); MS *m/z* 427 (15, M^+), 412 (28), 369 (100), 300 (70), 242 (92), 135 (78). (B) *Deprotection.* The crude **4a**(*E*) (~120 mg) was dissolved in TFA/H_2O (9:1, 5 mL), the solution was stirred at ~0 °C (ice bath) for 1 h, and

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volatiles were evaporated. The residue was coevaporated (EtOH), chromatographed (EtOAc → 15% S_1/EtOAc), and crystallized (EtOAc/MeOH) to give **4b**(*E*) (63 mg, 74% from **3**): mp 216–217 °C (dec); UV max 260 nm (ε 15 200), min 235 nm (ε 3000); ^{1}H NMR δ 1.86–1.93 (m, 1, H1"a) 2.24–2.30 (m, 1, H1'a), 2.58–2.64 (m, 1, H4'), 3.82 (q, J = 5.3 Hz, 1, H3'), 4.25 (q, J = 5.9 Hz, l, H2'), 4.65 (q, J = 8.3 Hz, 1, H1'), 4.95 (d, J = 5.3 Hz, 1, OH3'), 5.04 (d, J = 5.9 Hz, l, OH2'), 6.35 (d, $J_{6'\text{-}5'}$ = 14.6 Hz, 1, H6'), 6.70 (dd, $J_{5'\text{-}4'}$ = 7.8 Hz, 1, H5'), 7.21 (br s, 2, NH_2), 8.11 (s, 1, H2), 8.22 (s, 1, H8); MS *m/z* 387 (10, M^+), 260 (100), 135 (92). Anal. Calcd. for $C_{12}H_{14}IN_5O_2$ (387.2): C, 37.23; H, 3.64; N, 18.09. Found: C, 37.35; H, 3.65; N, 17.97.

9-{(1*R*,2*S*,3*R*,4*R*)-2,3-Dihydroxy-4-[(*Z*)-2-iodoethenyl]cyclopentan-1-yl}adenine [7b(*Z*)]. Treatment of **3** (*E/Z*, ~7:3; 145 mg, 0.25 mmol) with NIS, and deprotection (TFA/H_2O) [as described for **4b**(*E*)], gave a white solid that was purified (preparative RP-HPLC; program: 20% CH_3CN/H_2O for 30 min, 20 → 50% gradient for 60 min at 2.7 mL/min) to give **6b**[20a] (~4 mg, 6%; t_R = 48 min), **7b**(*Z*) (21 mg, 22%; t_R = 68 min), and **4b**(*E*) (52 mg, 54%; t_R = 75 min). Compound **7b**(*Z*): mp 217–219 °C (EtOAc/MeOH); UV max 260 nm (ε 15 300), min 232 (ε 4500); ^{1}H NMR δ 1.77–1.92 (m, 1, H1"*a*), 2.33–2.48 (m, 1, H1'*a*), 2.77–2.90 (m, 1, H4'), 3.90–3.96 (m, 1, H3'), 4.38–4.46 (m, 1, H2'), 4.74 (q, J = 8.8 Hz, 1, Hl'), 5.02 (d, J = 4.4 Hz, 1, OH3'), 5.13 (d J = 4.7 Hz, 1, OH2'), 6.41–6.59 (m, 2, H5',6'), 7.23 (br s, 2, NH_2), 8.13 (s, 1, H2), 8.21 (s, 1, H8); MS (CI) *m/z* 388 (100, MH^+). Anal. Calcd. for $C_{12}H_{14}IN_5O_2$ (387.2): C, 37.23; H, 3.64; N, 18.09. Found: C, 37.25; H, 3.70; N, 17.91.

9-{(1*R*,2*S*,3*R*,4*R*)-4-[(*E*)-2-Fluoroethenyl]-2,3-dihydroxycyclopentan-1-yl}adenine [5b(*E*)]. (A) *Fluorodestannylation.* A solution of **3** (*E/Z*, ~9:1; 177 mg, 0.3 mmol) in dried CH_2Cl_2 (2 mL) was injected into a stirred suspension of silver triflate (AgOTf; 154 mg, 0.6 mmol) in dried CH_2Cl_2 (5 mL) under Ar at ambient temperature in a flame-dried flask with a rubber septum. XeF_2 (111 mg, 0.66 mmol) in dried CH_2Cl_2 (5 mL) was transferred immediately into the mixture by cannula. The flask was covered with aluminum foil, stirring was continued for 30 min, and the mixture was partitioned ($NaHCO_3/H_2O//CHCl_3$). The aqueous layer was extracted [2 × $CHCl_3$, 2 × EtOAc, 2 × EtOAc/MeOH (3:1), 2 × EtOAc] and the combined organic phase was dried ($MgSO_4$) and evaporated. Chromatography ($CHCl_3$ → 4% MeOH/$CHCl_3$) gave **5a**(*E*)/**6a**/**8a**(*Z*) (~9:2:1; 66 mg, ~69%): ^{19}F NMR ($CDCl_3$) δ −127.79 [dd, $J_{F\text{-}H6'}$ = 83.6 Hz, $J_{F\text{-}H5'}$ = 17.8 Hz, 0.9, F6'; **5a**(*E*)], −127.20 [dd, $J_{F\text{-}H6'}$ = 83.6 Hz, $J_{F\text{-}H5'}$ = 41.8 Hz, 0.1, F6'; **8a**(*Z*)]; MS (CI) *m/z* 320 (100, MH^+). (B) *Deprotection.* Treatment of this mixture (59 mg) with TFA/H_2O [as described for **4b**(*E*)] gave an oily residue that was

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purified by preparative RP-HPLC (program: 10% CH_3CN/H_2O for 15 min, 10 → 45% gradient for 60 min at 2.8 mL/min) to give **6b/8b**(*Z*) (~3:1; 14 mg, ~18% from **3**; t_R = 61 min) and **5b**(*E*) (32 mg, 38% from **3**; t_R = 65 min). Compound **5b**(*E*): mp 228–229 °C ($MeOH/H_2O/CH_3CN$); UV max 261 nm (ε 14 700), min 230 nm (ε 3700); ^1H NMR (DMSO-d_6/D_2O) δ 1.77–1.94 (m, 1, H1"a), 2.22–2.36 (m, 1, H1'a), 2.43–2.56 (m, 1, H4'), 3.83 (t, J = 5.7 Hz, 1, H3'), 4.31 (t, J = 6.4 Hz, 1, H2'), 4.63–4.75 (m, 1, H1'), 5.65 (ddd, $J_{5'-4'}$ = 9.1 Hz, $J_{5'-6'}$ = 11.0 Hz, $J_{5'-F}$ = 19.9 Hz, 1, H5'), 6.88 (dd, $J_{6'-F}$ = 86.1 Hz, 1, H6'), 8.15 (s, 1, H2), 8.23 (s, 1, H8); ^{19}F NMR δ −130.46 (dd, $J_{F-6'}$ = 86.1 Hz, $J_{F-5'}$ = 19.7 Hz, 1, 6'F); MS (CI) *m/z* 280 (100, MH^+). Anal. Cacld. for $C_{12}H_{14}FN_5O_2$ (279.3): C, 51.61; H, 5.05; N, 25.08. Found: 51.43; H, 5.25; N, 24.89.

No further attempts were made to separate the **6b/8b**(*Z*) mixture (~3:1), but its ^1H NMR spectrum contained distinct signals for **6b**.[20a] The fluorovinyl fragment of **8b**(*Z*) had: ^1H NMR δ 5.13 (ddd, $J_{5'-6'}$ = 4.5 Hz, $J_{5'-4'}$ = 9.3 Hz, $J_{5'-F}$ = 44.5 Hz, 0.25, H5'), 6.74 (dd, $J_{6'-F}$ = 86.1 Hz, 0.25, H6'); ^{19}F NMR δ −130.13 (dd, $J_{F-6'}$ = 85.7 Hz, $J_{F-5'}$ = 44.7 Hz, 1, 6'F).

9-[(1*R*,2*S*,3*R*,4*R*)-4-(Cyano)methyl-2,3-(isopropylidenedioxy)-cyclopentan-1-yl]adenine (10a). TsCl (358 mg, 1.87 mmol) was added to a solution of **1a**[16a] (440 mg, 1.44 mmol) in pyridine (7 mL) at −20 °C, stirring was continued for 2 h, volatiles were evaporated, and the residue was partitioned ($NaHCO_3/H_2O$//$CHCl_3$). The organic layer was washed (HCl/H_2O, $NaHCO_3/H_2O$, brine), dried ($MgSO_4$), evaporated, and chromatographed to give **9** (530 mg, 80%) as a white powder: mp 289-292 °C (dec); HRMS (FAB) *m/z* 460.1657 (100, $MH^+[C_{21}H_{26}N_5O_5S]$ = 460.1655).

Dried KCN (260 mg, 4 mmol), **9** (459 mg, 1 mmol), and 18-crown-6 (132 mg, 0.5 mmol) were suspended in dried dioxane/DMF (15 mL, 2:1) and stirring was continued for 18 h at ambient temperature. Volatiles were evaporated (*in vacuo*) from the dark-brown mixture [TLC (S_3) indicated formation of a less polar product and baseline byproduct(s), in addition to unreacted **9**] and the residue was chromatographed (1 → 4% $MeOH/CHCl_3$) to give **10a** (144 mg, 46%): mp 191–193 °C; ^1H NMR 1.41 & 1.59 (2 × s, 2 × 3, Me_2C), 2.41–2.82 (m, 5, H1'*a*,1"*a*,4',5',5"), 4.64 (dd, $J_{3'-4'}$ = 5.6 Hz, $J_{3'-2'}$ = 7.1 Hz, 1, H3'), 4.72–4.83 (m, 1, H1'), 5.13 (dd, $J_{2'-1'}$ = 4.9 Hz, 1, H2'), 5.80 (br s, 2, NH_2), 7.82 (s, 1, H2), 8.32 (s, 1, H8); HRMS (FAB) *m/z* 315.1576 (100, $MH^+[C_{15}H_{19}N_6O_2]$ = 315.1569).

9-[(1*R*,2*S*,3*R*,4*R*)-4-(Cyanomethyl)-2,3-dihydroxycyclopentan-1-yl]adenine (10b). A solution of **10a** (63 mg, 0.2 mmol) in TFA/H_2O (9:1, 3 mL) was stirred at ~0 °C (ice bath) for 1 h and volatiles were evaporated. The residue was

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coevaporated (EtOH) and chromatographed (EtOAc → 20% S_1/EtOAc) to give **10b** (39 mg, 71%): mp 120–122 °C (softening); ^{1}H NMR (DMSO-d_6/D_2O) δ 1.69–1.85 (m, 1, H1"*a*), 2.18–2.45 (m, 2, H1'*a*,4'), 2.75 ("dd", *J* = 3.3, 7.0 Hz, 2, H5',5"), 3.83 (t, *J* = 5.1 Hz, 1, H3'), 4.36 (dd, $J_{2'-3'}$ = 5.8 Hz, $J_{2'-1'}$ = 7.7 Hz, 1, H2'), 4.73 (q, *J* = 8.9 Hz, 1, H1'), 8.25 (s, 1, H2), 8.36 (s, 1, H8); HRMS *m/z* 274.1169 (15, $M^+[C_{12}H_{14}N_6O_2]$ = 274.1218), 257 (14), 234 (88), 136 (100, BH_2).

9-[(1*R*,2*S*,3*R*,4*R*)-2,3-Dihydroxy-4-(2,2-dimethoxyethyl)-cyclopentan-1-yl]adenine (11). DIBALH/hexane (1M; 0.25 mL, 0.25 mmol) was added to a solution of **10a** (31 mg, 0.1 mmol) in CH_2Cl_2 (4 mL) at −78 °C and stirring was continued for 5 min. The cooling bath was removed and stirring was continued for 45 min while the solution was allowed to warm to ambient temperature [TLC (S_3) indicated polar (imino) intermediate(s)]. Volatiles were evaporated and the residue was dissolved in TFA/H_2O (9:1, 3 mL) and stirred at ~0 °C for 30 min and at ambient temperature for 30 min. Volatiles were evaporated and the residue was coevaporated (MeOH, toluene). Preparative RP-HPLC (program: 10 → 50% CH_3CN/H_2O for 60 min at 2.8 mL/min) gave **11** (18 mg, 56%;t_R = 42 min): ^{1}H NMR δ 1.59–2.34 (m, 5, H1'*a*,1"*a*,4',5',5"), 3.24 (s, 6, 2 × OMe), 3.76 (q, *J* = 5.1 Hz, 1, H3'), 4.32 (q, *J* = 6.6 Hz, 1, H2'), 4.43 (t, *J* = 5.3 Hz, 1, H6'), 4.64 (q, *J* = 9.0 Hz, 1, H1'), 4.73 (d, *J* = 5.0 Hz, 1, OH3'), 4.95 (d, *J* = 6.1 Hz, 1, OH2'), 6.60 (br s, 2, NH_2), 8.17 (s, 1, H2), 8.22 (s, 1, H8); HRMS (FAB) *m/z* 324.1660 (100, $MH^+[C_{14}H_{22}N_5O_4]$ = 324.1627).

***In Situ* Generation of the Homoaristeromycin 6'-Carboxaldehyde.** A solution of **11** (~2 mg) in H_2SO_4/H_2O (0.5 M, 0.5 mL) was stirred for 1 h at ambient temperature and then was *cautiously* neutralized (to pH ~7) with solid $NaHCO_3$. TLC (S_3) indicated complete conversion of **11** to a more polar product: HRMS *m/z* 277.1154 (10, $M^+[C_{12}H_{15}N_5O_3]$ = 277.1175).

Evaluation of Concentration-Dependent Inhibitory Activity of Inhibitors Against AdoHcy Hydrolase. Different concentrations of inhibitors (0.1, 1, 10, 50, 100 μM) were preincubated with 20 nM AdoHcy hydrolase in a total volume of 500 μL at 37 °C for 10 min, and then 4 units of calf intestinal Ado deaminase and 100 μM [2,8-^{3}H]AdoHcy were added and incubation was continued for 5 min. Aqueous formic acid (5 M, 100 μL) was added and the reaction mixture was applied to a column (1 × 4 cm) of SP Sephadex C-25 equilibrated in 0.1 M formic acid. The [2,8-^{3}H]inosine product of deamination of [2,8-^{3}H]adenosine (formed by hydrolysis of AdoHcy) was eluted with 8 mL of 0.1 M formic acid. The eluate was collected and radioactivity was determined with 1 mL of eluate mixed with 10 mL of scintillation cocktail. Data are expressed as % of the control which contained no inhibitor in the reaction mixture.

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Determination of Kinetic Constants for the Inhibition of AdoHcy Hydrolase. The inactivation constants, K_i and k_{inact}, were determined by the method previously described.[20a] AdoHcy hydrolase was preincubated with various concentrations of inhibitors for various times and residual enzyme activity was measured in the synthetic direction (HPLC). The pseudo-first order rate of inactivation (k_{app}) was determined from a plot of the residual activity versus preincubation time. K_i and k_{inact} values were obtained from a plot of $1/k_{app}$ versus 1/[inhibitor] using the equation:

$$1/k_{app} = 1/k_{inact} + (K_i/k_{inact})/[I]$$

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