Inactivation of *S*-Adenosyl-L-homocysteine Hydrolase and Antiviral Activity with 5′,5′,6′,6′-Tetradehydro-6′-deoxy-6′-halohomoadenosine Analogues (4′-Haloacetylene Analogues Derived from Adenosine)

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Treatment of a protected 9-(5,6-dideoxy-β-D-*ribo*-hex-5-ynofuranosyl)adenine derivative with silver nitrate and *N*-iodosuccinimide (NIS) and deprotection gave the 6′-iodo acetylenic nucleoside analogue **3c**. Halogenation of 3-*O*-benzoyl-5,6-dideoxy-1,2-*O*-isopropylidene-α-D-*ribo*-hex-5-enofuranose gave 6-halo acetylenic sugars that were converted to anomeric 1,2-di-*O*-acetyl derivatives and coupled with 6-*N*-benzoyladenine. These intermediates were deprotected to give the 6′-chloro **3a**, 6′-bromo **3b**, and 6′-iodo **3c** acetylenic nucleoside analogues. Iodo compound **3c** appears to inactivate *S*-adenosyl-L-homocysteine hydrolase by a type I ("cofactor depletion") mechanism since complete reduction of enzyme-bound NAD^+ to NADH was observed and no release of adenine or iodide ion was detected. In contrast, incubation of the enzyme with the chloro **3a** or bromo **3b** analogues resulted in release of Cl^- or Br^- and Ade, as well as partial reduction of E-NAD^+ to E-NADH. Compounds **3a**, **3b**, and **3c** were inhibitory to replication of vaccinia virus, vesicular stomatitis virus, parainfluenza-3 virus, and reovirus-1 (**3a** < **3b** < **3c**, in order of increasing activity). The antiviral effects appear to correlate with type I mechanism-based inhibition of *S*-adenosyl-L-homocysteine hydrolase. Mechanistic considerations are discussed.

Introduction

The cellular enzyme *S*-adenosyl-L-homocysteine (AdoHcy) hydrolase (EC 3.3.1.1) effects cleavage of AdoHcy to adenosine and L-homocysteine. AdoHcy is a potent feedback inhibitor of crucial transmethylation enzymes so the design of inhibitors of AdoHcy hydrolase represents a rational approach for anticancer and antiviral chemotherapy.[2] ZDDFA[3] (the *Z*-isomer of 4′,5′-didehydro-5′-deoxy-5′-fluoroadenosine) and its chloro analogue[4] are mechanism-based inactivators of AdoHcy hydrolase. Inactivation by ZDDFA involves addition of water at C5′, elimination of hydrogen fluoride to give adenosine 5′-carboxaldehydes[5a] (nonlethal event), and their oxidation to 3′-keto derivatives (lethal event) with concomitant reduction of E-NAD^+ to E-NADH[5b] ("cofactor depletion" or type I inhibition[2b]). This indicates that AdoHcy hydrolase has hydrolytic activity (addition of water at C5′) that functions independently of its C3′-oxidation activity.[5b]

Our homologated analogues[6] [(*E*)-5′,6′-didehydro-6′-deoxy-6′-halohomoadenosines, EDDHHAs] inhibit AdoHcy hydrolase and are enzymatically hydrolyzed to give "homoadenosine 6′-carboxaldehyde" which undergoes decomposition.[7] The hydrolytic (C5′/C6′) and oxidative (C3′) activities of AdoHcy hydrolase were differentiated effectively with the 6′-fluoro analogue (EDDFHA).[7b] Lys-426 was identified as an important residue for the hydrolytic activity.[8] We recently found that geminal and vicinal (dihalohomovinyl)adenosine analogues are new putative hydrolytic substrates for mechanism-based inhibition of AdoHcy hydrolase.[1,9] Electrophilic acyl halides and/or α-halomethyl ketones might result from addition of enzyme-sequestered water at C5′/C6′ followed by loss of hydrogen halide. Attack by amino acid functionalities (e.g., an amino group on Lys-426 or Arg-196) might cause type II (covalent binding[2b]) inhibition of the enzyme.[1,9]

Homologated 5′,5′-dibromomethylene-5′-deoxyuridine[10] and adenosine[1,11] analogues had been synthesized from nucleoside 5′-carboxaldehydes (with $CBr_4/PPh_3/Zn$[12]) and converted into 5′-deoxy-5′-methynylnucleosides (the 4′-acetylenic derivatives).[10,11] We had prepared such 4′-acetylenic derivatives by oxidative destannylation of vinyl 6′-stannanes.[6,13] The corresponding adenosine analogue is a type II inhibitor of AdoHcy hydrolase[6,14] with antiviral[6,11] and cytostatic activity.[6] Examples of 2′-[15] and 3′-ethynyl[16] nucleoside derivatives have been synthesized, and 1-(3-*C*-ethynyl-β-D-*ribo*-pentofuranosyl)cytosine has antitumor activity.[16b] An acetylenic linkage has been substituted for the phosphodiester moiety in an oligonucleotide mimic,[17] and a 2′,3′-dideoxyuridine analogue with ethynyl groups at C3′ and C4′ has been reported.[18]

We now describe syntheses of the first (6′-halo)-acetylenic adenine nucleosides (from adenosine or glucose), their inhibitory effects on AdoHcy hydrolase, and

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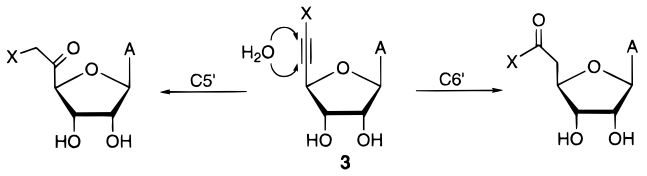
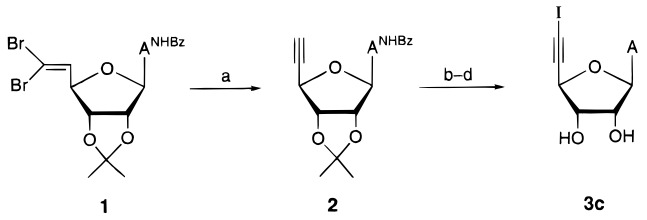


Figure 1. Possible generation of active intermediates from the 6′-halo acetylenic nucleoside analogues **3** by the "hydrolytic activity" of AdoHcy hydrolase.

Scheme 1[a]



[a] (a) BuLi/THF/−78 °C. (b) $AgNO_3$/NIS/Me_2CO. (c) NH_3/MeOH. (d) TFA/H_2O.

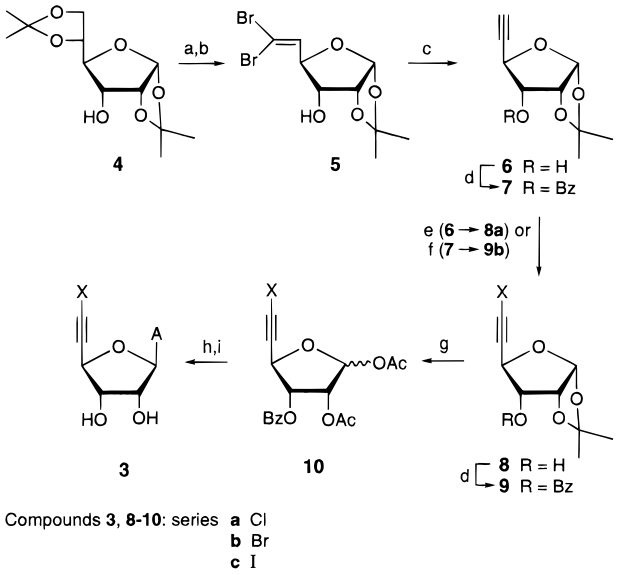
their antiviral activities. Addition of enzyme-sequestered water at C5′/C6′ followed by tautomerization of hydroxyvinyl intermediates might generate acyl halides and/or α-halomethyl ketones (Figure 1). Attack of protein nucleophiles might cause type II (covalent) inhibition of AdoHcy hydrolase.[1,9]

Chemistry

Moffatt oxidation[19] of 6-*N*-benzoyl-2′,3′-*O*-isopropylideneadenosine and treatment of the crude 5′-carboxaldehyde with "(dibromomethylene)triphenylphosphorane" (CBr_4/Ph_3P/Zn[12]) gave the dibromovinyl analogue **1**[1,11] (74%, Scheme 1). Treatment of **1** with excess BuLi gave the acetylenic derivative **2** (53%) plus byproduct(s), as noted by others.[11] Treatment of **2** with *N*-iodosuccinimide (NIS) and $AgNO_3$[20,21] (catalytic or equimolar) resulted in efficient 6′-iodination. Sequential removal of the 6-*N*-benzoyl (NH_3/MeOH) and isopropylidene [CF_3CO_2H (TFA)/H_2O] groups gave the iodoacetylene derivative **3c** (42% from **2**).

The bromo-, **3b**, and chloro-, **3a**, acetylene analogues were prepared from sugar precursors (Scheme 2). Attempted bromination (NBS) and especially chlorination of **2** did not proceed cleanly, in contrast with iodination (NIS). Attempted base-promoted dehydrobromination of **1** [BuLi, tetrabutylammonium fluoride[17a] (TBAF), or 1,8-dizazbicyclo[5.4.0]undec-7-ene (DBU)] also was problematic. Oxidation of 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose and stereoselective reduction of the 3-keto intermediate gave the α-D-allofuranose derivative **4** as reported.[22] Sequential (one-flask) selective hydrolysis of the 5,6-*O*-isopropylidene acetal and oxidative cleavage of the exposed glycol with periodic acid[23] gave the dehomologated 5-carboxaldehyde. Wittig-type olefination with the dibromomethylene reagent (generated in situ[24]) gave the (dibromohomovinyl)ribose **5** [58% overall; analogous treatment of the 3-*O*-benzoyl derivative of **4**[22c] gave 3-*O*-benzoyl-**5** (71%)]. Treatment of **5** with Mg/THF[24] failed to produce the acetylenic derivative, but BuLi[12] effected its conversion to **6** (81%). Benzoylation of **6** and treatment of the product **7** with NBS/$AgNO_3$ (catalytic[20]) gave the bromoacetylene sugar **9b** (79% from **6**). The isopropylidene group was removed

Scheme 2[a]



[a] (a) H_5IO_6/EtOAc. (b) Ph_3P/CBr_4/CH_2Cl_2. (c) BuLi/THF/−78 °C. (d) BzCl/pyridine. (e) NaOCl/H_2O. (f) NBS/$AgNO_3$/Me_2CO. (g) i, TFA/H_2O; ii, Ac_2O/pyridine/DMAP. (h) 6-*N*-Benzoyladenine/$SnCl_4$/CH_3CN. (i) NH_3/MeOH.

from **9b** (TFA/H_2O) and the product was acetylated to give the anomeric acetates **10**. Coupling ($SnCl_4$/CH_3CN[25]) of **10** and 6-*N*-benzoyladenine followed by deacylation (NH_3/MeOH) gave the crystalline bromoacetylene homonucleoside **3b** (20% from **9b**).

As anticipated,[20] treatment of **6** or **7** with NCS/$AgNO_3$ failed to effect 6-chlorination. However, treatment of **6** with an aqueous sodium hypochlorite solution[21a] (commercial laundry bleach) at ambient temperature gave the chloroacetylene derivative **8a** (87%, purification by chromatography on silica gel). Benzoylation of **8a**, hydrolysis of the acetal, and acetylation gave the anomeric acetates **10a**. Coupling of **10a** with 6-*N*-benzoyladenine, deacylation, and chromatography gave the chloroacetylene nucleoside **3a** (36% from **9a**). Compound **3a** was unstable upon heating and underwent decomposition upon attempted removal of solvent molecules (^{1}H NMR) for elemental analysis. The crystalline solvate was stored at 0 °C and checked for chromatographic homogeneity before biological testing. Terminal chloroacetylenes are known to be somewhat unstable, and testing results must be considered tentative (although testing data were repeatable). All attempts to make the fluoroacetylenic analogue failed.

Inactivation of *S*-Adenosyl-L-homocysteine Hydrolase. Recombinant human placental AdoHcy hydrolase was inactivated upon incubation with **3a**, **3b**, or **3c** by concentration-dependent (Table 1) and time-dependent (data not shown) processes. The maximal inactivation at the highest concentration used (100 μM) was approximately 55% of the original enzyme activity with the chloro **3a** and bromo **3b** analogues. In contrast, the 6′-iodo analogue **3c** caused complete inactivation of AdoHcy hydrolase at a concentration of 100 μM. Kinetic analysis of the inactivation processes by the Kitz and Wilson method gave the k_2 and K_i values in Table 2. The chloro **3a** and bromo **3b** analogues appear to have similar kinetic properties. In contrast, the 6′-iodo analogue **3c** inactivated the enzyme at a substantially

Table 1. Inhibition of AdoHcy Hydrolase with **3a**−**c**

	enzyme activity remaining[a,b] (%)		
concn (μM)	**3a**	**3b**	**3c**
0.1	89.3 ± 2.8	91 ± 1.5	80.0 ± 0.8
1.0	64.0 ± 0.6	73.2 ± 3.2	44.6 ± 1.0
10	58.9 ± 1.3	62.9 ± 0.7	8.8 ± 0.2
100	55.0 ± 0.6	56.0 ± 3.1	<2.0

[a] AdoHcy hydrolase (25 nM) was incubated with **3a**−**c** in buffer A at 37 °C for 20 min, and the remaining activity was assayed as described in the Experimental Section. [b] Data are the averages of duplicate determinations.

Table 2. Kinetic Constants for Inhibition of AdoHcy Hydrolase with **3a**−**c**[a]

compd	k_2 (min^{-1})	K_i (μM)	k_2/K_i (M^{-1} min^{-1})
3a	0.48 ± 0.05	1.7 ± 0.2	2.82×10^5
3b	0.49 ± 0.03	3.8 ± 0.3	1.29×10^5
3c	0.095 ± 0.004	1.10 ± 0.04	8.64×10^4

[a] Kinetic constants k_2 and K_i were calculated from the pseudo-first-order rate constants (k_{app}) for enzyme inactivation with various concentrations of **3a**−**c** as described in the Experimental Section. See ref 2c (p 54) for kinetic data with some type I inhibitors.

Table 3. Effects of **3a**−**c** on AdoHcy Hydrolase Activity and NADH Content, and Enzyme-Mediated Release of Ade and Halide Ions from **3a**−**c**[a]

compd	enzyme activity remaining (%)	Ade release (%)	halide ion release (%)	NADH content (% of total coenzyme)
3a	47 ± 3	18 ± 2	28 ± 2	53 ± 2
3b	49 ± 3	28 ± 2	70 ± 15	49 ± 2
3c	1.4 ± 0.2	5.7 ± 0.5	0	96 ± 3

[a] AdoHcy hydrolase was incubated with **3a**−**c** (100 μM) in buffer A at 37 °C for 20 min. Enzyme activity remaining, amounts of Ade and halide ions released, and NADH content were assayed as described in the Experimental Section.

slower rate than **3a** or **3b**. Kinetic data for some "pure type I" inhibitors are given in ref 2c (p 54).

We also determined the effects of **3a**−**c** on the NADH content of the enzyme as well as the enzyme-mediated release of halide ions and/or Ade from the inhibitors (Table 3). It can be determined whether the inactivation process involves a type I (cofactor depletion) mechanism of inactivation by monitoring the NADH content of the enzyme[2b] and whether inhibitors are substrates for the 5′/6′-hydrolytic activity of AdoHcy hydrolase by monitoring the release of Ade and/or halide ions.[7] The data in Table 3 show that the 6′-iodo analogue **3c** causes complete conversion of the enzyme from its E-NAD$^+$ to E-NADH form (with loss of enzyme activity). This indicates that **3c** is a substrate for the 3′-oxidative activity of AdoHcy hydrolase. Since Ade or I$^-$ is not released upon incubation of **3c** with the enzyme, it appears that this compound inactivates AdoHcy hydrolase by a type I process. The present results with **3c** are in marked contrast with those reported by Parry et al. for the nonhalogenated acetylenic analogue.[14] In that case, complete inactivation of AdoHcy hydrolase was observed but only partial (~50%) conversion of E-NAD$^+$ to E-NADH occurred. The remaining 2 equiv of NAD$^+$ was "lost" from the tetramer. It also was reported that 4 equiv of the inhibitor was bound to the tetramer upon inactivation and that 2 equiv of the inhibitor was "more tightly" bound.[14] This suggests partial covalent modification of the enzyme (type II mechanism).

We presently cannot rule out the possibility of covalent modification of AdoHcy hydrolase by **3c**, but it could not have occurred by an activation process involving the 5′/6′-hydrolytic activity (as illustrated in Figure 1) since Ade or I$^-$ release was not detected. However, it is possible that covalent modification of the enzyme occurred via oxidation of **3c** to its 3′-keto derivative followed by isomerization to give allenic ketone(s) and attack by protein nucleophile(s) on such electrophilic species analogous to the mechanism proposed by Parry et al. for the nonhalogenated analogue.[14] Mechanisms by which **3c** inactivate AdoHcy hydrolase are currently under investigation.

Results observed with the chloro **3a** and bromo **3b** analogues are different from those with 6′-iodo analogue **3c**. Partial (~50%) reduction of E-NAD$^+$ to E-NADH was observed with **3a** or **3b** with concomitant partial loss (~50%) of enzyme activity. Incubation of **3a** or **3b** with AdoHcy hydrolase resulted in release of adenine (**3b** > **3a**) and halide ions (**3b** > **3a**). These results suggest that **3a** and **3b** are substrates for both the 3′-oxidative activity (partial reduction of E-NAD$^+$ to E-NADH) and the 5′/6′-hydrolytic activity (release of Ade and halide ions) of AdoHcy hydrolase. More specifically, the release of Ade suggests 6′-hydrolytic activity, whereas release of halide ions could result from either the 5′- or 6′-hydrolytic activity of the enzyme.[7] Since halide ions are released from the chloro **3a** and bromo **3b** analogues upon incubation with AdoHcy hydrolase, it is plausible that these inhibitors are converted into acyl halides and/or α-halomethyl ketones (Figure 1) and subsequently react with protein nucleophiles. However, **3a** and/or **3b** also might inactive the enzyme by type I (cofactor depletion) and/or type II (covalent binding via an allenic ketone[14]) mechanisms. Studies to characterize the molecular mechanism(s) involved in these inactivation processes are in progress.

Antiviral Activity. Haloacetylenes **3a**, **3b**, and **3c** were evaluated for antiviral activity in cell culture (Table 4). They were inactive against HSV-1 and HSV-2 in E_6SM cells, Coxsackie virus B4 in HeLa and Vero cells, and Sindbis and Punta Toro viruses in Vero cells at subtoxic concentrations. Chloro derivative **3a** was marginally effective against vaccinia virus (VV) and vesicular stomatitis virus (VSV) in E_6SM cells and parainfluenza virus type 3 in Vero cells. However, the bromo **3b** and especially the iodo **3c** derivatives were markedly inhibitory to these viruses [EC_{50} values for **3c** of 0.08 μM against VSV in E_6SM cells, 0.26 μM against VV in E_6SM cells, 0.78 μM against reovirus-1, and 2.6 μM against parainfluenza-3 virus in Vero cells (Table 4)]. Surprisingly, **3c** was much less inhibitory to VSV in HeLa than in E_6SM cells. Also, **3a** and **3b** were less active against VSV in HeLa than in E_6SM cells. Since **3c** was more toxic to E_6SM than HeLa cells, differences in the antiviral potencies against VSV in E_6-SM and HeLa cell cultures might result from differences in metabolism of the compounds in these two cell lines.

The inhibitory effects of **3a**, **3b**, and **3c** against vaccinia virus, vesicular stomatitis virus, parainfluenza virus, and reoviruses are in overall harmony with the activity spectrum of AdoHcy hydrolase inhibitors.[2c] The order of increasing antiviral activity was **3a** < **3b** < **3c**, irrespective of the virus (VV, VSV, parainfluenza, or

Table 4. Antiviral Evaluation of **3a**−**c**

	MIC[a] (μM)			EC$_{50}$[b] (μM)										
				HSV-1 (KOS)	HSV-2 (G)	HSV-1/TK$^-$ B2006 or VMW1837	vaccinia virus	VSV		Coxsackie virus B4		RSV	parainfluenza-3 virus	Sindbis virus	reovirus-1	Punta Toro virus
compd	E$_6$SM	HeLa	Vero	(E$_6$SM)	(E$_6$SM)	(E$_6$SM)	(E$_6$SM)	(E$_6$SM)	(HeLa)	(HeLa)	(Vero)	(HeLa)	(Vero)	(Vero)	(Vero)	(Vero)
3a	≥340	≥340	≥140	>140	>140	>140	140	70	>140	>140	>35	>140	>35	>35	>35	>35
3b	≥300	≥300	≥120	>120	>120	>120	6	6	45	>300	>30	210	6	>30	3	>30
3c	26	>1040	≥180	>10	>10	>10	0.26	0.08	260	≥260	>104	≥260	2.6	>104	0.78	>104
c		>2000	>1600						400	>2000	>2000		1200	>2000	240	>2000
d	>1600	≥1600	>1600	960	960	128	192	192	192	320	>1600		64	64	64	64

[a] Minimal inhibitory concentration required to elicit a microscopically visible alteration of cell morphology. [b] Effective concentration required to inhibit virus-induced cytopathicity by 50%. [c] (*S*)-2,3-Dihydroxypropyladenine [(*S*)-DHPA]. [d] Ribavirin.

reoviruses). However, inactivation of purified human placental AdoHcy hydrolase was approximately the same with **3a** and **3b**, which both were considerably less potent than **3c**. The modes of action also appear to be different with **3a** and **3b** (mixed type I and type II inactivation) relative to that of **3c** (type I inactivation).

Summary and Conclusions

The 6′-chloro **3a**, 6′-bromo **3b**, and 6′-iodo **3c** acetylenic homonucleosides were prepared from adenosine or hexofuranose precursors. Incubation of AdoHcy hydrolase with **3a** or **3b** resulted in partial (∼50%) reduction of E-NAD$^+$ to E-NADH (3′-oxidative activity) and parallel loss of enzyme activity. In contrast, incubation with **3c** resulted in complete reduction of E-NAD$^+$ to E-NADH and complete loss of enzyme activity. Release of Ade plus Cl$^-$ or Br$^-$ (5′/6′-hydrolytic activity) was observed with **3a** or **3b**, but no Ade or I$^-$ release was detected with **3c**. These results are consistent with type I (cofactor depletion) inactivation for **3c** and mixed type I and type II (covalent binding) inactivation for **3a** and **3b**, although other mechanistic interpretations have not been excluded. It is remarkable that such apparent changes in mechanisms occur with the bromo **3b** versus iodo **3c** congeners. Greater antiviral potency was observed with **3c** relative to **3b** and **3a**. These observations are compatible with results on our dihalohomovinyl analogues[1,9] which appear to be activated by the 5′/6′-hydrolytic activity of AdoHcy hydrolase to generate electrophiles that undergo nucleophilic attack by protein residues. Half of the site's covalent binding was indicated, and the resulting functional tetrameric-subunit complexes retained both oxidative and hydrolytic activity. Treatment of such partially inactivated enzymes with a type I inhibitor caused complete inactivation. The dihalohomovinyl analogues had no significant antiviral activity and were not cytotoxic to certain cell cultures.[1,9] It is possible that antiviral activity was not observed owing to insufficient perturbation of cellular AdoMet/AdoHcy ratios. Reduction of E-NAD$^+$ to NADH with iodo analogue **3c** caused loss of AdoHcy hydrolase activity, and potent antiviral activity was observed. In contrast, approximately 50% of the E-NAD$^+$ is reduced to E-NADH with **3a** or **3b**, and lower antiviral effects are observed with these chloro and bromo congeners.

Experimental Section

Uncorrected melting points were determined with a capillary apparatus. UV spectra were measured with MeOH solutions. ^{1}H (200 or 500 MHz) and ^{13}C (125 MHz) NMR spectra were determined with $CDCl_3$ solutions unless specified. Mass spectra (MS and HRMS) were obtained by electron impact (20 eV), chemical ionization (CI, isobutane), or fast atom bombardment (FAB, thioglycerol matrix). Merck kieselgel 60-F_{254} sheets were used for TLC (detection at 254 nm, or by color with I_2 in a sealed chamber). Merck kieselgel 60 (230−400 mesh) was used for column chromatography. Preparative reversed-phase (RP)-HPLC was performed with a Dynamax C_{18} column and a Spectra Physics SP 8800 ternary pump system (gradient solvent systems are noted). Elemental analyses were determined by M-H-W Laboratories, Phoenix, AZ. AdoHcy, Ado, Hcy, and calf intestinal Ado deaminase (EC 3.5.4.4) were obtained from Sigma. Standard Cl$^-$, Br$^-$, and I$^-$ ions were obtained from P. J. Cobert Association, Inc. (St. Louis, MO). Recombinant human placental AdoHcy hydrolase was overexpressed and purified as described.[7a] Reagent grade chemicals were used and solvents were dried by reflux over

and distillation from CaH_2 (except THF/potassium) under argon. Sonication was performed with a 300 Ultrasonik unit.

6-*N*-Benzoyl-9-(5,6-dideoxy-2,3-*O*-isopropylidene-β-D-*ribo*-hex-5-ynofuranosyl)adenine (2). BuLi/hexane (1.6 M; 1.25 mL, 2 mmol) was added dropwise to a solution of 6-*N*-benzoyl-9-(6,6-dibromo-5,6-dideoxy-2,3-*O*-isopropylidene-β-D-*ribo*-hex-5-enofuranosyl)adenine[1,11] (**1**; 282 mg, 0.5 mmol) in THF (20 mL) at −78 °C under Ar, and stirring was continued for 2 h (TLC indicated conversion to a more polar product and minor fluorescent byproducts). The mixture was neutralized (HOAc, pH ∼6) and evaporated, and the residue was partitioned ($NaHCO_3/H_2O$//$CHCl_3$). The organic layer was washed (brine), dried ($MgSO_4$), and evaporated. Chromatography (hexanes/EtOAc, 1:4) of the residue gave **2**[14] (107 mg, 53%): ^{1}H NMR δ 1.42 and 1.62 (2 × s, 2 × 3, 2 × Me), 2.48 (d, J = 2.0 Hz, 1, H6′), 5.10 (br s, 1, H4′), 5.16 (d, J = 5.9 Hz, 1, H3′), 5.77 (d, J = 5.9 Hz, 1, H2′), 6.31 (s, 1, H1′), 7.48−8.10 (m, 5, Arom), 8.35 (s, 1, H2), 8.84 (s, 1, H8), 9.30 (s, 1, NH); HRMS (CI) m/z 406.1519 (50, MH^+ [$C_{21}H_{20}N_5O_4$] = 406.1515).

9-(5,6-Dideoxy-6-iodo-β-D-*ribo*-hex-5-ynofuranosyl)-adenine (3c). A solution of **2** (102 mg, 0.25 mmol) in Me_2CO (7 mL) was added dropwise to a stirred solution of $AgNO_3$ (65 mg, 0.38 mmol) in H_2O (3 mL, to which "1 drop" of diluted NH_3/H_2O had been added). Separation of the silver acetylide salt began almost immediately, and after 5 min NIS (90 mg, 0.4 mmol) was added. Stirring was continued for 30 min (the reaction mixture became homogeneous after ∼5 min and then became turbid again), and volatiles were evaporated. The residue was partitioned ($NaHCO_3/H_2O$//$CHCl_3$), and the organic layer was washed (H_2O) and dried ($MgSO_4$). Volatiles were evaporated to give the protected iodoalkyne (∼90%) [MS (CI) m/z 532 (10, MH^+), 406 (100), 240 (45), 135 (50)]. This material was stirred overnight with NH_3/MeOH (20 mL) at ambient temperature, and volatiles were evaporated. Chromatography of the residue (EtOAc → 2% MeOH/EtOAc) gave the 2′,3′-*O*-isopropylidene compound (76 mg, 71% from **2**): ^{1}H NMR ($CD_3OD/CDCl_3$, ∼4:1) δ 1.32 and 1.48 (2 × s, 2 × 3, 2 × Me), 5.02 (s, 1, H4′), 5.08 (d, J = 5.5 Hz, 1, H3′), 5.71 (d, J = 5.5 Hz, 1, H2′), 6.16 (s, 1, H1′), 8.08 (s, 1, H2), 8.24 (s, 1, H8); MS (CI) m/z 428 (80, MH^+), 302 (60), 242 (70), 213 (75), 135 (100). A solution of this material (68 mg, 0.16 mmol) in TFA/H_2O (9:1, 3 mL) was stirred at ∼0 °C (ice bath) for 1 h, and volatiles were evaporated. EtOH was added and evaporated, and the residue was chromatographed (EtOAc → 10% MeOH/EtOAc) and crystallized (MeOH) to give **3c** (27 mg, 44%; 31% from **2**). The mother liquor was purified by RP-HPLC (preparative C_{18} column; program 15% CH_3CN/H_2O for 30 min followed by a gradient of 15 → 40% for 30 min at 2.8 mL/min; t_R = 53 min) to give additional **3c** (10 mg, 16%; 42% total from **2**): mp 180−183 °C dec; UV max 259 (ε 14 200), min 228 nm (ε 2800); ^{1}H NMR (Me_2SO-d_6) δ 4.32 (q, J = 4.6 Hz, 1, H3′), 4.62 (d, J = 4.6 Hz, 1, H4′), 4.77 (q, J = 5.1 Hz, 1 H2′), 5.66 (d, J = 6.2 Hz, 1, OH3′), 5.69 (d, J = 6.0 Hz, 1, OH2′), 5.87 (d, J = 4.8 Hz, 1, H1′), 7.35 (br s, 2, NH_2), 8.14 (s, 1, H2), 8.24 (s, 1, H8); ^{13}C NMR (Me_2SO-d_6) δ 15.95, 73.36, 74.68, 75.30, 87.68, 90.45, 119.45, 139.75, 149.69, 153.09, 156.09; HRMS (CI) m/z 387.9915 (100, MH^+ [$C_{11}H_{11}IN_5O_3$] = 387.9907). Anal. [$C_{11}H_{10}IN_5O_3$·0.25H_2O·0.5 EtOAc (435.7)] C, H, N. The presence of 0.5 equiv of EtOAc was verified by integration of the ^{1}H NMR solvent peaks.

Treatment of **2** with catalytic $AgNO_3$ (0.125 equiv; as described for **9b** with NIS instead of NBS), and deprotection gave **3c** in similar yield. The sugar/coupling route **7** → **9c** → **10c** → **3c** (as described for **3b** with NIS instead of NBS) also gave **3c** (28% overall).

6,6-Dibromo-5,6-dideoxy-1,2-*O*-isopropylidene-α-D-*ribo*-hex-5-enofuranose (5). H_5IO_6 (6.84 g, 30 mmol) was added to a solution of **4**[22b] (6.5 g, 25 mmol) in dried EtOAc (200 mL) at ambient temperature, and stirring was continued for 2 h. The mixture was filtered, the filter cake was washed (EtOAc), and the combined filtrate was evaporated. The residue was dissolved in dried CH_2Cl_2 (200 mL), and the solution was cooled to ∼0 °C (ice bath). CBr_4 (16.6 g, 50 mmol) and Ph_3P (26 g, 100 mmol) were added, and the brown solution was stirred at ∼0 °C for 2 h and then at ambient temperature overnight. The solution was washed ($NaHCO_3/H_2O$, brine) and dried ($MgSO_4$), and volatiles were evaporated. Chromatography (EtOAc/hexanes, 2:3) of the residue gave **5** (4.99 g, 58%) as a solidified syrup: ^{1}H NMR δ 1.38 and 1.60 (2 × s, 2 × 3, 2 × Me), 2.47 (d, J = 11.0 Hz, 1, OH3), 3.75−3.88 (m, 1, H3), 4.45 (t, J = 8.6 Hz, 1, H4), 4.56 (t, J = 4.4 Hz, 1, H2), 5.79 (d, J = 3.6 Hz, 1, H1), 6.42 (d, J = 8.4 Hz, 1, H5); HRMS (CI) m/z 346.9135 (3, MH^+ [$C_9H_{13}{}^{81}Br_2O_4$] = 346.9140), 344.9153 (6, MH^+ [$^{81/79}Br_2$] = 344.9160), 342.9162 (3, MH^+ [$^{79}Br_2$] = 342.9181).

Analogous treatment of the 3-*O*-benzoyl derivative of **4**[22c] gave the 3-*O*-benzoyl derivative of **5** (71%).

5,6-Dideoxy-1,2-*O*-isopropylidene-α-D-*ribo*-hex-5-ynofuranose (6). BuLi/hexane (2.5 M; 28 mL, 70 mmol) was added dropwise to a solution of **5** (3.44 g, 10 mmoL) in dried THF (50 mL) at −78 °C, and stirring was continued for 3 h. The mixture was neutralized (AcOH, pH ∼6.5), concentrated (∼10 mL, <25 °C), and partitioned ($NaHCO_3/H_2O$//$CHCl_3$). The aqueous layer was extracted ($CHCl_3$, 2×), the combined organic phase was washed (brine) and dried ($MgSO_4$), and volatiles were evaporated. The residue was chromatographed ($CHCl_3$ → 2% MeOH/$CHCl_3$) to give **6** (1.49, 81%) as a white solid: ^{1}H NMR δ 1.36 and 1.57 (2 × s, 2 × 3, 2 × Me), 2.51 (d, J = 2.0 Hz, 1, H6), 2.60 (d, J = 9.2 Hz, 1, OH3), 3.98−4.10 (m, 1, H3), 4.36 (dd, J = 1.6, 8.6 Hz, 1, H4), 4.59 (t, J = 4.4 Hz, 1, H2), 5.84 (d, J = 4.0 Hz, 1, H1); HRMS (CI) m/z 185.0815 (100, MH^+ [$C_9H_{13}O_4$] = 185.0814).

3-*O*-Benzoyl-5,6-dideoxy-1,2-*O*-isopropylidene-α-D-*ribo*-hex-5-ynofuranose (7). Treatment of **6** (276 mg, 1.5 mmol) with BzCl (0.3 mL, 363 mg, 2.58 mmol) at ∼0 °C for 2 h and 30 min at ambient temperature, and chromatography (as described for **9a**) gave **7** (380 mg, 88%) as an amorphous solid: ^{1}H NMR δ 1.30 and 1.57 (2 × s, 2 × 3, 2 × Me), 2.54 (d, J = 1.8 Hz, 1, H6), 4.88 (dd, J = 1.6, 9.0 Hz, 1, H4), 4.94−5.07 (m, 2, H2,3), 5.92 (d, J = 3.8 Hz, 1, H1), 7.40−8.10 (m, 5, Arom); HRMS (CI) m/z 289.1069 (100, MH^+ [$C_{16}H_{17}O_5$] = 289.1076).

6-Chloro-5,6-dideoxy-1,2-*O*-isopropylidene-α-D-*ribo*-hex-5-ynofuranose (8a). A suspension of **6** (276 mg, 1.5 mmol) in NaOCl/H_2O (5.25% commercial laundry bleach, 50 mL) was stirred at ambient temperature for 16 h (the reaction mixture became homogeneous within 30 min). The solution was partitioned ($NaHCO_3/H_2O$//$CHCl_3$), the organic layer was washed (H_2O, brine) and dried ($MgSO_4$), and volatiles were evaporated. The residue was chromatographed ($CHCl_3$ → 1.5% MeOH/$CHCl_3$) to give **8a** (289 mg, 87%) as a syrup: ^{1}H NMR δ 1.37 and 1.58 (2 × s, 2 × 3, 2 × Me), 2.57 (d, J = 10.6 Hz, 1, OH3), 3.95−4.07 (m, 1, H3), 4.36 (d, J = 8.6 Hz, 1, H4), 4.57 (t, J = 4.4 Hz, 1, H2), 5.82 (d, J = 4.0 Hz, 1, H1); HRMS (CI) m/z 221.0390 (10, MH^+ [$C_9H_{12}{}^{37}ClO_4$] = 221.0395), 219.0421 (36, MH^+ [^{35}Cl] = 219.0424).

3-*O*-Benzoyl-6-chloro-5,6-dideoxy-1,2-*O*-isopropylidene-α-D-*ribo*-hex-5-ynofuranose (9a). BzCl (0.2 mL, 239 mg, 1.7 mmol) was added via syringe into a solution of **8a** (219 mg, 1 mmol) in dried pyridine (5 mL) at ∼0 °C (ice bath) under N_2, and stirring was continued for 4 h. $NaHCO_3/H_2O$ (2 mL) was added, stirring was continued for 10 min, and volatiles were evaporated. The residue was partitioned (H_2O/$CHCl_3$), the organic layer was washed (HCl/H_2O, $NaHCO_3/H_2O$, brine), dried ($MgSO_4$), and volatiles were evaporated. The residue was chromatographed (EtOAc/hexane, 1:4) to give **9a** (264 mg, 82%) as a solidified syrup: ^{1}H NMR δ 1.31 and 1.53 (2 × s, 2 × 3, 2 × Me), 4.87−5.04 (m, 3, H2,3,4), 5.91 (d, J = 3.6 Hz, 1, H1), 7.42−8.10 (m, 5, Arom); HRMS (CI) m/z 325.0677 (40, MH^+ [$C_{16}H_{16}{}^{37}ClO_5$] = 325.0657), 323.0703 (100, MH^+ [^{35}Cl] = 323.0686).

3-*O*-Benzoyl-6-bromo-5,6-dideoxy-1,2-*O*-isopropylidene-α-D-*ribo*-hex-5-ynofuranose (9b). $AgNO_3$ (25 mg, 0.15 mmol) was added to a solution of **7** (288 mg, 1 mmol) and NBS (205 mg, 1.15 mmol) in Me_2CO (10 mL) at ambient temperature, and stirring was continued for 30 min. Volatiles were evaporated, and the residue was partitioned (H_2O/$CHCl_3$). The aqueous layer was extracted ($CHCl_3$), the combined organic

phase was washed ($NaHCO_3/H_2O$, brine) and dried ($MgSO_4$), and volatiles were evaporated to give **9b** (351 mg, 96%) as an amorphous solid: ^{1}H NMR δ 1.35 and 1.57 (2 × s, 2 × 3, 2 × Me), 4.89−5.06 (m, 3, H2,3,4), 5.92 (d, J = 3.4 Hz, 1, H1), 7.42−8.10 (m, 5, Arom); HRMS (CI) m/z 369.0154 (10, MH^+ [$C_{16}H_{16}{}^{81}BrO_5$] = 369.0161), 367.0180 (10, MH^+ [^{79}Br] = 367.0181).

9-(6-Chloro-5,6-dideoxy-β-D-*ribo*-hex-5-ynofuranosyl)-adenine (3a). (a) Hydrolysis and Acetylation. A solution of **9a** (250 mg, 0.77 mmol) in TFA/H_2O (9:1, 10 mL) was stirred at ∼0 °C (ice bath) for 1 h. Volatiles were evaporated, and traces were coevaporated from the residue (toluene 2 ×, pyridine). Pyridine (8 mL), Ac_2O (1 mL), and 4-(dimethylamino)pyridine (DMAP, 5 mg) were added, the mixture was stirred at ∼0 °C for 2 h and at 10−15 °C for 2 h, and MeOH (5 mL) was added. Stirring was continued for 20 min, volatiles were evaporated, the residue was dissolved (EtOAc), and the solution was washed (HCl/H_2O, $NaHCO_3/H_2O$, brine) and dried (Na_2SO_4). Volatiles were evaporated, and the residue was chromatographed (20 → 30% EtOAc/hexanes) to give 1,2-di-*O*-acetyl-3-*O*-benzoyl-6-chloro-5,6-dideoxy-α,β-D-*ribo*-hex-5-ynofuranose (**10a**, α/β, ∼1:4; 245 mg, 87%): ^{1}H NMR δ 1.99 and 2.14 (2 × s, 2 × 0.2, 2 × Ac), 2.02 and 2.15 (2 × s, 2 × 0.8, 2 × Ac), 4.96 (d, J = 4.6 Hz, 0.8, H4), 5.01 (d, J = 1.6 Hz, 0.2, H4), 5.48 (t, J = 5.1 Hz, 0.2, H2), 5.57 (dd, J = 1.8, 4.6 Hz, 0.8, H2), 5.67 (dd, J = 1.8, 5.8, 0.2, H3), 5.69 (t, J = 4.6 Hz, 0.8, H3), 6.26 (d, J = 2.0 Hz, 0.8 H1), 6.50 (d, J = 4.4 Hz, 0.2, H1), 7.40−8.10 (m, 5, Arom); HRMS (FAB) m/z 391.0374 (34, MNa^+ [$C_{17}H_{15}{}^{37}ClNaO_7$] = 391.0375), 389.0396 (100, MNa^+ [^{35}Cl] = 389.0404).

(b) Coupling. $SnCl_4$ (0.18 mL, 391 mg, 1.5 mmol) was added dropwise to a suspension of 6-*N*-benzoyladenine (240 mg, 1 mmol) and the anomeric sugar [from step (a): 245 mg, 0.67 mmol] in dried CH_3CN (15 mL), and stirring was continued for 16 h at ambient temperature. Volatiles were evaporated, the residue was partitioned ($NaHCO_3/H_2O$// $CHCl_3$), and the organic layer was washed (brine) and dried ($MgSO_4$). Volatiles were evaporated, and the residue was chromatographed ($CHCl_3$ → 2% MeOH/$CHCl_3$) to give 9-(2-*O*-acetyl-3-*O*-benzoyl-6-chloro-5,6-dideoxy-β-D-*ribo*-hex-5-ynofuranosyl)-6-*N*-benzoyladenine (274 mg, 75%): HRMS (FAB) m/z 548.1144 (7, MH^+ [$C_{27}H_{21}{}^{37}ClN_5O_6$] = 548.1151), 546.1162 (23, MH^+ [^{35}Cl] = 546.1180).

(c) Deprotection. A solution of the product from step b (274 mg, 0.5 mmol) in NH_3/MeOH (20 mL) was stirred for 24 h at ∼5 °C. Volatiles were evaporated, and the residue was flash chromatographed (EtOAc → 15% MeOH/EtOAc). "Diffusion crystallization"[26] (MeOH/EtOAc) of the residue gave **3a** (52 mg, 2 crops) as a white crystalline solvate (variable). RP-HPLC purification (preparative C_{18} column, gradient 15 → 45% CH_3CN/H_2O for 80 min at 2 mL/min; t_R = 64 min) of the mother liquor gave additional **3a** (29 mg; ∼36% total from **9a**): mp ∼160−185 °C dec; UV max 259 nm (ϵ 13 800), min 228 nm (ϵ 3100); ^{1}H NMR (Me_2SO-d_6) δ 4.36−4.44 (m, 1, H3′), 4.59 (d, J = 4.4 Hz, 1, H4′), 4.83−4.92 (m, 1, H2′), 5.72 (d, J = 5.6 Hz, 1, OH3′), 5.79 (d, J = 5.4 Hz, 1 OH2′), 5.91 (d, J = 5.0 Hz, 1, H1′), 7.30 (br s, 2, NH_2), 8.16 (s, 1, H2), 8.31 (s, 1, H8); ^{13}C NMR (Me_2SO-d_6) δ 66.43, 67.32, 73.19, 73.68, 75.25, 87.94, 119.29, 140.15, 149.82, 153.23, 156.16; HRMS (FAB) m/z 298.0503 (38, MH^+ [$C_{11}H_{11}{}^{37}ClN_5O_3$] = 298.0521), 296.0539 (100, MH^+ [^{35}Cl] = 296.0550).

9-(6-Bromo-5,6-dideoxy-β-D-*ribo*-hex-5-ynofuranosyl)-adenine (3b). Hydrolysis and acetylation (as with **3a**) of **9b** (330 mg, 0.9 mmol) gave anomeric **10b** (α/β, ∼1:3; 310 mg, 84%): ^{1}H NMR δ 1.99 and 2.11 (2 × s, 2 × 0.25, 2 × Me), 2.02 and 2.12 (2 × s, 2 × 0.75, 2 × Me), 4.97 (d, J = 4.4 Hz, 0.75, H4), 5.03 (d, J = 1.6 Hz, 0.25, H4), 5.49 (t, J = 5.3 Hz, 0.25, H2), 5.58 (dd, J = 1.6, 4.6 Hz, 0.75, H2), 5.69 (dd, J = 1.8, 6.0 Hz, 0.25, H3), 5.73 (t, J = 4.7 Hz, 0.75, H3), 6.26 (d, J = 1.6 Hz, 0.75, H1), 6.51 (d, J = 4.6 Hz, 0.25, H1), 7.41−8.11 (m, 5, Arom); HRMS (EI) m/z 411.9964 (15, M^+ [$C_{17}H_{15}{}^{81}BrO_7$] = 411.9981), 409.9993 (15, M^+ [^{79}Br] = 410.0001). Coupling of **10b** (310 mg, 0.75 mmol) with 6-*N*-benzoyladenine and purification (as with **3a**) gave 9-(2-*O*-acetyl-3-*O*-benzoyl-6-bromo-5,6-dideoxy-β-D-*ribo*-hex-5-ynofuranosyl)-6-*N*-benzoyladenine (252 mg, 57%): HRMS (FAB) m/z 592.0668 (96, MH^+ [$C_{27}H_{21}{}^{81}BrN_5O_6$] = 592.0655), 590.0671 (100, MH^+ [^{79}Br] = 590.0675). Deprotection (NH_3/MeOH), chromatography, and "diffusion crystallization"[26] (as with **3a**) gave **3b** (65 mg, 2 crops; 20% from **9b**): mp 120−130 °C (transition), >160 °C dec; UV max 259 nm (ϵ 13 800), min 228 (ϵ 2900); ^{1}H NMR (Me_2SO-d_6) δ 4.36 (q, J = 4.8 Hz, H3′), 4.56 (d, J = 4.2 Hz, H4′), 4.82 (q, J = 5.2 Hz, H2′), 5.69 (d, J = 5.8 Hz, 1, OH3′), 5.75 (d, J = 5.6 Hz, 1, OH2′), 5.88 (d, J = 4.8 Hz, 1, H1′), 7.32 (br s 2, NH_2), 8.14 (s, 1, H2), 8.28 (s, 1, H8); ^{13}C NMR (Me_2SO-d_6) δ 50.89, 73.45, 74.37, 75.41, 77.83, 88.09, 119.48, 140.20, 150.011, 153.40, 156.41; HRMS (FAB) m/z 342.0038 (25, MH^+ [$C_{11}H_{11}{}^{81}BrN_5O_3$] = 342.0025), 340.0039 (25, MH^+ [^{79}Br] = 340.0045). Anal. [$C_{11}H_{10}BrN_5O_3$·0.5 H_2O (349.2)] C, H, N.

Compound **3b** also was obtained (∼9% overall) by the nucleoside route **1** → **2** → **3b**, as described for **3c**, with NBS instead of NIS. Treatment of **1** with TBAF/THF,[17a] DBU/THF, or BuLi/THF (1−2 equiv) did not give clean elimination of HBr.

AdoHcy Hydrolase Activity. Activity was assayed in the synthetic direction by measuring rates of formation of AdoHcy from Ado and Hcy. The enzyme was incubated with Ado (1 mM) and Hcy (5 mM) in potassium phosphate buffer [500 μL; 50 mM, pH 7.2, containing EDTA (1 mM)] (buffer A) at 37 °C for 5 min. Reaction was terminated by addition of $HClO_4/H_2O$ (5 M, 25 μL), the reaction mixture was cooled in ice−water for 15 min, and the clear supernatant was collected and analyzed for AdoHcy by HPLC using a C_{18} reverse-phase column (Econosphere C18, 5 μm, 250 × 4.6 mm, Alltech, Deerfield, IL) as described.[5b]

Inactivation of AdoHcy Hydrolase. Incubation of AdoHcy hydrolase (25 nM) with various concentrations of **3a**−**c** (0−100 μM) in buffer A at 37 °C for 20 min gave data shown in Table 1, and that with various concentrations of **3a**−**c** for different times (0−5 min) gave data shown in Table 2. The remaining enzyme activity was assayed in the synthetic direction as described in the previous experimental procedure. Time-dependent inactivation data were used to calculate the pseudo-first-order rate constants (k_{app}) by plotting the logarithm of the remaining activity versus time. The kinetic constants k_2 and K_i were calculated from the k_{app} values and inhibitor concentrations ([I]) by the method of Kitz and Wilson[27] with eq 1.

$$1/k_{app} = 1/k_2 + (K_i/k_2)/[I] \quad (1)$$

Analysis of Halide Ions Released from 3a−c. The Cl^- or Br^- ions released upon incubation of AdoHcy hydrolase with **3a** or **3b** were analyzed by ion-exchange chromatography. The enzyme (10 μM) was incubated with **3a** or **3b** (100 μM) in buffer A at 37 °C for 20 min. An aliquot of the reaction mixture was applied to an Amicon Centricon-3 microconcentrator (3000 Mr cutoff). The filtrate was applied to an ion-exchange column (Anion/R, 250 × 4.1 mm, 10 μm, Alltech, Deerfield, IL) in a HPLC system equipped with a conductivity detector (690 Ion Chromatograph, Omega, Metrohm, Ltd., Herisau, Switzerland) for halide ion analysis. The ions were eluted isocratically [1.5 mM *p*-hydroxybenzoic acid/2% methanol (pH 8.5, LiOH)] at a flow rate of 2 mL/min. Comparison of ion peak areas with those from known quantities of standard ions with standardized curves allowed quantitation of Cl^- and Br^- ions.

Iodide ions released by incubation of AdoHcy hydrolase with **3c** were detected as described[28] [C_{18} reversed-phase column (ODS Hypersil 250 × 4.6 mm, Alltech, Deerfield, IL), 0.5 mM tetrabutylammonium hydroxide/5% CH_3OH (pH 7.1 adjusted with 0.1 M potassium hydrogen phthalate), flow rate of 1.5 mL/min, detection at 260 nm]. Quantitation of I^- employed comparison of peak areas with those of known quantities of standard I^- with a standardized curve.

Analysis of Adenine. AdoHcy hydrolase and **3a**, **3b**, or **3c** were incubated in buffer A under the conditions described for the halide ion determinations except at each time point the reaction was stopped by addition of 5 M $HClO_4/H_2O$ (10

μL). The precipitate was removed by centrifugation, and the supernatant was analyzed for Ade (HPLC with a reverse-phase C_{18} column as described[7a]). The HPLC peak assigned to Ade was verified (co-injection with authentic Ade) and confirmed by mass spectral analysis (CI/NH_3).

Analysis of E-NAD$^+$ and E-NADH. The extent of conversion of the NAD^+ to the NADH form of the enzyme was analyzed by UV spectroscopy as described.[7a] AdoHcy hydrolase (10 μM) in buffer A (0.8 mL) was incubated with **3a**, **3b**, or **3c** (100 μM) at 37 °C for 20 min. Inhibitor-induced NADH formation was monitored at 340 nm with a HP 8452 diode-array UV spectrophotometer and quantified using a standard curve.

Antiviral Evaluation. Antiviral assays were based on inhibition of virus-induced cytopathicity in E_6SM, HeLa, or Vero cell cultures following established procedures.[29,30] Briefly, confluent cell cultures in microtiter trays were inoculated with 100 $CCID_{50}$ of virus (1 $CCID_{50}$ is the virus dose required to infect 50% of the cell cultures). After a 1 h virus adsorption period, residual virus was removed and the cell cultures were incubated in the presence of various concentrations (400, 200, 100, ... μg/mL) of the test compounds. Viral cytopathicity was recorded as soon as it reached completion in the control virus-infected cell cultures.

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References

(1) Nucleic Acid Related Compounds. 103. Paper 102: Wnuk, S. F.; Mao, Y.; Yuan, C.-S.; Borchardt, R. T.; Andrei, G.; Balzarini, J.; De Clercq, E.; Robins, M. J. Discovery of Type II (Covalent) Inactivation of *S*-Adenosyl-L-homocysteine Hydrolase Involving Its "Hydrolytic Activity." Synthesis and Evaluation of Dihalohomovinyl Nucleoside Analogues Derived from Adenosine. *J. Med. Chem.* **1998**, *41*, 3078−3083.

(2) (a) Ueland, P. M. Pharmacological and Biochemical Aspects of *S*-Adenosylhomocysteine and *S*-Adenosylhomocysteine Hydrolase. *Pharmacol. Rev.* **1982**, *34*, 223−253. (b) Wolfe, M. S.; Borchardt, R. T. *S*-Adenosyl-L-homocysteine Hydrolase as a Target for Antiviral Chemotherapy. *J. Med. Chem.* **1991**, *34*, 1521−1530. (c) Yuan, C.-S.; Liu, S.; Wnuk, S. F.; Robins, M. J.; Borchardt, R. T. Design and Synthesis of *S*-Adenosylhomocysteine Hydrolase Inhibitors as Broad-Spectrum Antiviral Agents. In *Advances in Antiviral Drug Design*; De Clercq, E., Ed; JAI Press: Greenwich, 1996; Vol. 2, pp 41−88.

(3) McCarthy, J. R.; Jarvi, E. T.; Matthews, D. P.; Edwards, M. L.; Prakash, N. J.; Bowlin, T. L.; Mehdi, S.; Sunkara, P. S.; Bey, P. 4′,5′-Unsaturated 5′-Fluoroadenosine Nucleosides: Potent Mechanism-Based Inhibitors of *S*-Adenosyl-L-homocysteine Hydrolase. *J. Am. Chem. Soc.* **1989**, *111*, 1127−1128.

(4) Wnuk, S. F.; Dalley, N. K.; Robins, M. J. Nucleic Acid Related Compounds. 76. Synthesis of 5′-(*E* and *Z*)-Chloro-4′,5′-didehydro-5′-deoxyadenosines via Chlorination and Thermolysis of Adenosine 5′-Sulfoxides. Mechanism-Based Inhibition of *S*-Adenosyl-L-homocysteine Hydrolase. *J. Org. Chem.* **1993**, *58*, 111−117.

(5) (a) Liu, S.; Wnuk, S. F.; Yuan, C.; Robins, M. J.; Borchardt, R. T. Adenosine-5′-carboxaldehyde: A Potent Inhibitor of *S*-Adenosyl-L-homocysteine Hydrolase. *J. Med. Chem.* **1993**, *36*, 883−887. (b) Yuan, C.-S.; Yeh, J.; Liu, S.; Borchardt, R. T. Mechanism of Inactivation of *S*-Adenosylhomocysteine Hydrolase by (*Z*)-4′,5′-Didehydro-5′-deoxy-5′-fluoroadenosine. *J. Biol. Chem.* **1993**, *268*, 17030−17037.

(6) Wnuk, S. F.; Yuan, C.-S.; Borchardt, R. T.; Balzarini, J.; De Clercq, E.; Robins, M. J. Nucleic Acid Related Compounds. 84. Synthesis of 6′-(*E* and *Z*)-Halohomovinyl Derivatives of Adenosine, Inactivation of *S*-Adenosyl-L-homocysteine Hydrolase, and Correlation of Anticancer and Antiviral Potencies with Enzyme Inhibition. *J. Med. Chem.* **1994**, *37*, 3579−3587.

(7) (a) Yuan, C.-S.; Liu, S.; Wnuk, S. F.; Robins, M. J.; Borchardt, R. T. Mechanism of Inactivation of *S*-Adenosylhomocysteine Hydrolase by (*E*)-5′,6′-Didehydro-6′-Deoxy-6′-Halohomoadenosines. *Biochemistry* **1994**, *33*, 3758−3765. (b) Yuan, C.-S.; Wnuk, S. F.; Liu, S.; Robins, M. J.; Borchardt, R. T. (*E*)-5′,6′-Didehydro-6′-Deoxy-6′-Fluorohomoadenosine: A Substrate That Measures The Hydrolytic Activity of *S*-Adenosylhomocysteine Hydrolase. *Biochemistry* **1994**, *33*, 12305−12311.

(8) Ault-Riché, D. B.; Yuan, C.-S.; Borchardt, R. T. A Single Mutation at Lysine 426 of Human Placental *S*-Adeonosylhomocysteine Hydrolase Inactivates the Enzyme. *J. Biol. Chem.* **1994**, *269*, 31472−31478.

(9) Yuan, C.-S.; Wnuk, S. F.; Robins, M. J.; Borchardt, R. T. A Novel Mechanism-based Inhibitor (6′-Bromo-5′,6′-didehydro-6′-deoxy-6′-fluorohomoadenosine) That Covalently Modifies Human Placental *S*-Adenosylhomocysteine Hydrolase. *J. Biol. Chem.* **1998**, *273*, 18191−18197.

(10) Sharma, R. A.; Bobek, M. Acetylenic Nucleosides. 1. Synthesis of 1-(5,6-Dideoxy-β-D-*ribo*-hex-5-ynofuranosyl)uracil and 1-(2,5,6-Trideoxy-β-D-*erythro*-hex-5-ynofuranosyl)-5-methyluracil. *J. Org. Chem.* **1978**, *43*, 367−369.

(11) Matsuda, A.; Kosaki, H.; Yoshimura, Y.; Shuto, S.; Ashida, N.; Konno, K.; Shigeta, S. Nucleosides and Nucleotides. 142. An Alternative Synthesis of 9-(5,6-Dideoxy-β-D-*ribo*-hex-5-ynofuranosyl)adenine and its Antiviral Activity. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 1685−1688.

(12) Corey, E. J.; Fuchs, P. L. A Synthetic Method for Formyl → Ethynyl Conversion (RCHO → RC≡CH or RC≡CR′). *Tetrahedron Lett.* **1972**, 3769−3772.

(13) Wnuk, S. F.; Robins, M. J. Nucleic Acid Related Compounds. 78. Stereocontrolled Syntheses of 6′-(*E* and *Z*)-Halovinyl Analogues from Uridine-derived Vinylsulfones via Vinyltin Intermediates. *Can. J. Chem.* **1993**, *71*, 192−198.

(14) Parry, R. J.; Muscate, A.; Askonas, L. J. 9-(5′,6′-Dideoxy-β-D-*ribo*-hex-5′-ynofuranosyl)adenine, a Novel Irreversible Inhibitor of *S*-Adenosylhomocysteine Hydrolase. *Biochemistry* **1991**, *30*, 9988−9997.

(15) Yoshimura, Y.; Iino, T.; Matsuda, A. Nucleosides and Nucleotides. 102. Stereoselective Radical Deoxygenation of *tert*-Propargyl Alcohols in Sugar Moiety of Pyrimidine Nucleosides: Synthesis of 2′-*C*-Alkynyl-2′-deoxy-1-β-D-arabinofuranosylpyrimidines. *Tetrahedron Lett.* **1991**, *32*, 6003−6006.

(16) (a) Jung, P. M. J.; Burger, A.; Biellmann, J.-F. Rapid and Efficient Stereocontrolled Synthesis of C-3′-Ethynyl Ribo and Xylonucleosides by Organocerium Additions to 3′-Ketonucleosides. *Tetrahedron Lett.* **1995**, *36*, 1031−1034. (b) Hattori, H.; Tanaka, M.; Fukushima, M.; Sasaki, T.; Matsuda, A. Nucleosides and Nucleotides. 158. 1-(3-*C*-Ethynyl-β-D-*ribo*-pentofuranosyl)-cytosine, 1-(3-*C*-Ethynyl-β-D-*ribo*-pentofuranosyl)uracil, and Their Nucleobase Analogues as New Potential Multifunctional Antitumor Nucleosides with a Broad Spectrum of Activity. *J. Med. Chem.* **1996**, *39*, 5005−5011.

(17) (a) Lebreton, J.; De Mesmaecker, A.; Waldner, A. Synthesis of Carbon Chain Internucleosides Thymidine Dimers. *Synlett* **1994**, 54−56. (b) Wendeborn, S.; Jouanno, C.; Wolf, R. M.; De Mesmaecker, A. Replacement of the Phosphodiester Linkage in Oligonucleotides by an Acetylenic Bond: Comparison between Carbon-, Sulfur-, and Oxygen-Containing Analogs. *Tetrahedron Lett.* **1996**, *37*, 5511−5514.

(18) Amin, M. A.; Stoeckli-Evans, H.; Gossauer, A. 141. 3′,4′-Diethynyl-2′, 3′,5′-trideoxy-5′-noruridine: A New Self-polymerizable 2′-Deoxyribonucleoside Analogue. *Helv. Chim. Acta* **1995**, *78*, 1879−1887.

(19) Ranganathan, R. S.; Jones, G. H.; Moffatt, J. G. Novel Analogs of Nucleoside 3′,5′-Cyclic Phosphates. I. 5′-Mono- and Dimethyl Analogs of Adenosine 3′,5′-Cyclic Phosphate. *J. Org. Chem.* **1974**, *39*, 290−298.

(20) Hofmeister, H.; Annen, K.; Laurent, H.; Wiechert, R. A Novel Entry to 17α-Bromo- and 17α-Iodoethynyl Steroids. *Angew. Chem., Int. Ed. Engl.* **1984**, *23*, 727−729.

(21) (a) Stang, P. J.; Zhdankin, V. V. Alkynyl Halides and Chalcogenides. In *Comprehensive Organic Functional Group Transformations*; Katritzky, A. R., Meth-Cohn, O., Rees, C. W., Eds.; Pergamon: Oxford, 1995; Vol. 2, pp 1011−1038. (b) Jäger, V.; Viehe, H. G. Methods for the Preparation and Transformation of Alkynes. In *Houben-Weyl*; Georg Thieme Verlag: Stuttgart, 1977; Vol 5/2a, pp 1−912.

(22) (a) Garegg, P. J.; Samuelsson, B. Oxidation of Primary and Secondary Alcohols in Partially Protected Sugars with the Chromium Trioxide-pyridine Complex in the Presence of Acetic Anhydride. *Carbohydr. Res.* **1978**, *67*, 267−270. (b) Sowa, W.; Thomas, G. H. S. The Oxidation of 1,2:5,6-Di-*O*-Isopropylidene-D-Glucose by Dimethyl Sulfoxide-Acetic Anhydride. *Can. J. Chem.* **1966**, *44*, 836−838. (c) David, S.; De Sennyey, G. Synthesis of 1-(5-Deoxy-β-D-*ribo*-hexofuranosyl)cytosine and

1-(2,5-Dideoxy-*β*-D-*erythro*-hexofuranosyl)cytosine, and Their Phosphates. Contribution to the Study of the Specificity of a Mammalian (Rat) Ribonucleotide Reductase. *Carbohydr. Res.* **1979**, *77*, 79−97.

(23) Xie, M.; Berges, D. A.; Robins, M. J. Efficient "Dehomologation" of Di-*O*-isopropylidenehexofuranose Derivatives to Give *O*-Isopropylidenepentofuranoses by Sequential Treatment with Periodic Acid in Ethyl Acetate and Sodium Borohydride. *J. Org. Chem.* **1996**, *61*, 5178−5179.

(24) Van Hijfte, L.; Kolb, M.; Witz, P. A Practical Procedure for the Conversion of Aldehydes to Terminal Alkynes by a One Carbon Homologation. *Tetrahedron Lett.* **1989**, *30*, 3655−3656.

(25) Saneyoshi, M.; Satoh, E. Synthetic Nucleosides and Nucleotides. XIII. Stannic Chloride Catalyzed Ribosylation of Several 6-Substituted Purines. *Chem. Pharm. Bull.* **1979**, *27*, 2518−2521.

(26) Robins, M. J.; Mengel, R.; Jones, R. A.; Fouron, Y. Nucleic Acid Related Compounds. 22. Transformation of Ribonucleoside 2′,3′-*O*-Ortho Esters into Halo, Deoxy, and Epoxy Sugar Nucleosides Using Acyl Halides. Mechanism and Structure of Products. *J. Am. Chem. Soc.* **1976**, *98*, 8204−8213.

(27) Kitz, R.; Wilson, I. B. Esters of Methanesulfonic Acid as Irreversible Inhibitors of Acetylcholinesterse. *J. Biol. Chem.* **1962**, *237*, 3245−3249.

(28) Bradfield, E. G.; Cooke, D. T. Determination of Inorganic Anions in Water Extracts of Plants and Soil by Ion Chromatography. *Analyst* **1985**, *110*, 1409−1410.

(29) De Clercq, E.; Descamps, J.; Verhelst, G.; Walker, R. T.; Jones, A. S.; Torrence, P. F.; Shugar, D. Comparative Efficacy of Antiherpes Drugs against Different Strains of Herpes Simplex Virus. *J. Infect. Dis.* **1980**, *141*, 563−574.

(30) De Clercq, E.; Holý, A.; Rosenberg, I.; Sakuma, T.; Balzarini, J.; Maudgal, P. C. A Novel Selective Broad-Spectrum Anti-DNA Virus Agent. *Nature* **1986**, *323*, 464−467.

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