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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 ZDDFA³ (the *Z*-isomer of 4',5'-didehydro-5'-deoxy-5'-fluoroadenosine) and its chloro analogue⁴ 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 analogues⁶ [(*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 oxi-

dative (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'-deoxyuridine¹⁰ and adenosine^{1,11} analogues had been synthesized from nucleoside 5'-carboxaldehydes (with CBr₄/PPh₃/Zn¹²) and converted into 5'-deoxy-5'-methynynucleosides (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'-ethynyl¹⁶ 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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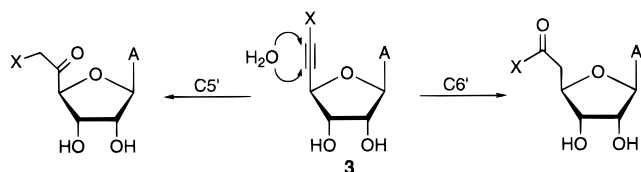
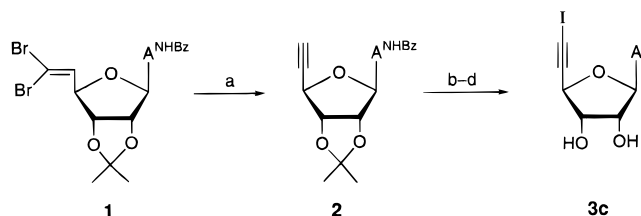


Figure 1. Possible generation of active intermediates from the 6'-halo acetylenic nucleoside analogues **3** by the "hydrolytic activity" of AdoHcy hydrolase.

Scheme 1^a



^a (a) BuLi/THF/ -78°C . (b) $\text{AgNO}_3/\text{NIS}/\text{Me}_2\text{CO}$. (c) NH_3/MeOH . (d) TFA/ H_2O .

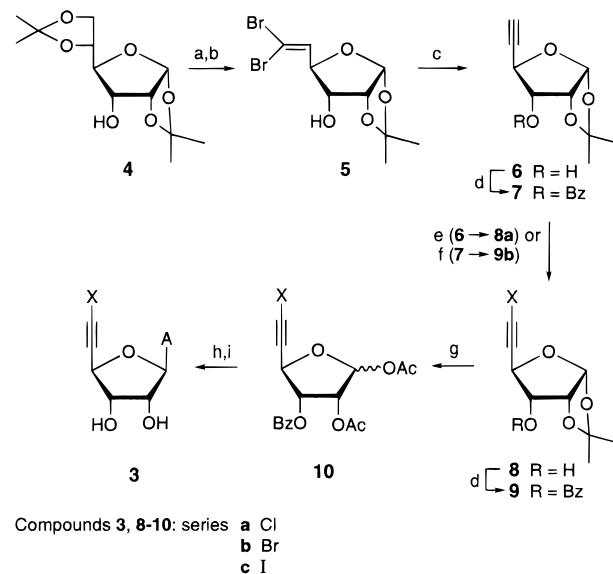
their antiviral activities. Addition of enzyme-sequestered water at C5'/C6' followed by tautomerization of hydroxyvinyl intermediates might generate acyl halides and/or α -halomethyl ketones (Figure 1). Attack of protein nucleophiles might cause type II (covalent) inhibition of AdoHcy hydrolase.^{1,9}

Chemistry

Moffatt oxidation¹⁹ of 6-*N*-benzoyl-2',3'-*O*-isopropylideneadenosine and treatment of the crude 5'-carboxaldehyde with "(dibromomethylene)triphenylphosphorane" ($\text{CBr}_4/\text{Ph}_3\text{P}/\text{Zn}^{12}$) gave the dibromovinyl analogue **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 [$\text{CF}_3\text{CO}_2\text{H}$ (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-diazabicyclo[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 acid²³ 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/THF²⁴ failed to produce the acetylenic derivative, but BuLi¹² effected its conversion to **6** (81%). Benzoylation of **6** and treatment of the product **7** with NBS/ AgNO_3 (catalytic²⁰) gave the bromoacetylene sugar **9b** (79% from **6**). The isopropylidene group was removed

Scheme 2^a



Compounds **3**, **8-10**: series **a** Cl
b Br
c I

^a (a) $\text{H}_5\text{IO}_6/\text{EtOAc}$. (b) $\text{Ph}_3\text{P}/\text{CBr}_4/\text{CH}_2\text{Cl}_2$. (c) BuLi/THF/ -78°C . (d) BzCl/pyridine. (e) NaOCl/ H_2O . (f) NBS/ $\text{AgNO}_3/\text{Me}_2\text{CO}$. (g) i, TFA/ H_2O ; ii, $\text{Ac}_2\text{O}/\text{pyridine}/\text{DMAP}$. (h) 6-*N*-Benzoyladenine/ $\text{SnCl}_4/\text{CH}_3\text{CN}$. (i) NH_3/MeOH .

from **9b** (TFA/ H_2O) and the product was acetylated to give the anomeric acetates **10**. Coupling ($\text{SnCl}_4/\text{CH}_3\text{CN}$ 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 (^1H 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 ($100\ \mu\text{M}$) 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 $100\ \mu\text{M}$. Kinetic analysis of the inactivation processes by the Kitz and Wilson method gave the k_2 and K_1 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**

concn (μM)	enzyme activity remaining ^{a,b} (%)		
	3a	3b	3c
0.1	89.3 \pm 2.8	91 \pm 1.5	80.0 \pm 0.8
1.0	64.0 \pm 0.6	73.2 \pm 3.2	44.6 \pm 1.0
10	58.9 \pm 1.3	62.9 \pm 0.7	8.8 \pm 0.2
100	55.0 \pm 0.6	56.0 \pm 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 ($\text{M}^{-1} \text{min}^{-1}$)
3a	0.48 \pm 0.05	1.7 \pm 0.2	2.82×10^5
3b	0.49 \pm 0.03	3.8 \pm 0.3	1.29×10^5
3c	0.095 \pm 0.004	1.10 \pm 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 \pm 3	18 \pm 2	28 \pm 2	53 \pm 2
3b	49 \pm 3	28 \pm 2	70 \pm 15	49 \pm 2
3c	1.4 \pm 0.2	5.7 \pm 0.5	0	96 \pm 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 inactivate the enzyme by type I (cofactor depletion) and/or type II (covalent binding via an allenic ketone¹⁴) 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 E₆SM 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 E₆SM 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 [EC₅₀ values for **3c** of 0.08 μM against VSV in E₆SM cells, 0.26 μM against VV in E₆SM 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 E₆SM cells. Also, **3a** and **3b** were less active against VSV in HeLa than in E₆SM cells. Since **3c** was more toxic to E₆SM than HeLa cells, differences in the antiviral potencies against VSV in E₆SM 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

and distillation from CaH₂ (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 \sim 6) and evaporated, and the residue was partitioned (NaHCO₃/H₂O/CHCl₃). The organic layer was washed (brine), dried (MgSO₄), and evaporated. Chromatography (hexanes/EtOAc, 1:4) of the residue gave **2**¹⁴ (107 mg, 53%): ¹H NMR δ 1.42 and 1.62 (2 \times s, 2 \times 3, 2 \times 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⁺ [C₂₁H₂₀N₅O₄] = 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 Me₂CO (7 mL) was added dropwise to a stirred solution of AgNO₃ (65 mg, 0.38 mmol) in H₂O (3 mL, to which "1 drop" of diluted NH₃/H₂O 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 \sim 5 min and then became turbid again), and volatiles were evaporated. The residue was partitioned (NaHCO₃/H₂O/CHCl₃), and the organic layer was washed (H₂O) and dried (MgSO₄). Volatiles were evaporated to give the protected iodoalkyne (\sim 90%) [MS (CI) m/z 532 (10, MH⁺), 406 (100), 240 (45), 135 (50)]. This material was stirred overnight with NH₃/MeOH (20 mL) at ambient temperature, and volatiles were evaporated. Chromatography of the residue (EtOAc \rightarrow 2% MeOH/EtOAc) gave the 2',3'-*O*-isopropylidene compound (76 mg, 71% from **2**): ¹H NMR (CD₃OD/CDCl₃, \sim 4:1) δ 1.32 and 1.48 (2 \times s, 2 \times 3, 2 \times 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/H₂O (9:1, 3 mL) was stirred at \sim 0 $^{\circ}\text{C}$ (ice bath) for 1 h, and volatiles were evaporated. EtOH was added and evaporated, and the residue was chromatographed (EtOAc \rightarrow 10% MeOH/EtOAc) and crystallized (MeOH) to give **3c** (27 mg, 44%; 31% from **2**). The mother liquor was purified by RP-HPLC (preparative C₁₈ column; program 15% CH₃CN/H₂O for 30 min followed by a gradient of 15 \rightarrow 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 $^{\circ}\text{C}$ dec; UV max 259 (ϵ 14 200), min 228 nm (ϵ 2800); ¹H NMR (Me₂SO-*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, NH₂), 8.14 (s, 1, H2), 8.24 (s, 1, H8); ¹³C NMR (Me₂SO-*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⁺ [C₁₁H₁₁IN₅O₃] = 387.9907). Anal. [C₁₁H₁₀IN₅O₃·0.25H₂O·0.5 EtOAc (435.7)] C, H, N. The presence of 0.5 equiv of EtOAc was verified by integration of the ¹H NMR solvent peaks.

Treatment of **2** with catalytic AgNO₃ (0.125 equiv; as described for **9b** with NIS instead of NBS), and deprotection gave **3c** in similar yield. The sugar/coupling route **7** \rightarrow **9c** \rightarrow **10c** \rightarrow **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). H₅IO₆ (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 CH₂Cl₂ (200 mL), and the solution was cooled to \sim 0 $^{\circ}\text{C}$ (ice bath). CBr₄ (16.6 g, 50 mmol) and Ph₃P (26 g, 100 mmol) were added, and the brown solution was

stirred at \sim 0 $^{\circ}\text{C}$ for 2 h and then at ambient temperature overnight. The solution was washed (NaHCO₃/H₂O, brine) and dried (MgSO₄), and volatiles were evaporated. Chromatography (EtOAc/hexanes, 2:3) of the residue gave **5** (4.99 g, 58%) as a solidified syrup: ¹H NMR δ 1.38 and 1.60 (2 \times s, 2 \times 3, 2 \times 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⁺ [C₉H₁₃⁸¹Br₂O₄] = 346.9140), 344.9153 (6, MH⁺ [^{81/79}Br₂] = 344.9160), 342.9162 (3, MH⁺ [⁷⁹Br₂] = 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 \sim 6.5), concentrated (\sim 10 mL, $<25^{\circ}\text{C}$), and partitioned (NaHCO₃/H₂O/CHCl₃). The aqueous layer was extracted (CHCl₃, 2 \times), the combined organic phase was washed (brine) and dried (MgSO₄), and volatiles were evaporated. The residue was chromatographed (CHCl₃ \rightarrow 2% MeOH/CHCl₃) to give **6** (1.49, 81%) as a white solid: ¹H NMR δ 1.36 and 1.57 (2 \times s, 2 \times 3, 2 \times 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⁺ [C₉H₁₃O₄] = 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 \sim 0 $^{\circ}\text{C}$ for 2 h and 30 min at ambient temperature, and chromatography (as described for **9a**) gave **7** (380 mg, 88%) as an amorphous solid: ¹H NMR δ 1.30 and 1.57 (2 \times s, 2 \times 3, 2 \times 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⁺ [C₁₆H₁₇O₅] = 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/H₂O (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 (NaHCO₃/H₂O/CHCl₃), the organic layer was washed (H₂O, brine) and dried (MgSO₄), and volatiles were evaporated. The residue was chromatographed (CHCl₃ \rightarrow 1.5% MeOH/CHCl₃) to give **8a** (289 mg, 87%) as a syrup: ¹H NMR δ 1.37 and 1.58 (2 \times s, 2 \times 3, 2 \times 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⁺ [C₉H₁₂³⁷ClO₄] = 221.0395), 219.0421 (36, MH⁺ [³⁵Cl] = 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 \sim 0 $^{\circ}\text{C}$ (ice bath) under N₂, and stirring was continued for 4 h. NaHCO₃/H₂O (2 mL) was added, stirring was continued for 10 min, and volatiles were evaporated. The residue was partitioned (H₂O/CHCl₃), the organic layer was washed (HCl/H₂O, NaHCO₃/H₂O, brine), dried (MgSO₄), and volatiles were evaporated. The residue was chromatographed (EtOAc/hexane, 1:4) to give **9a** (264 mg, 82%) as a solidified syrup: ¹H NMR δ 1.31 and 1.53 (2 \times s, 2 \times 3, 2 \times 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⁺ [C₁₆H₁₆³⁷ClO₅] = 325.0657), 323.0703 (100, MH⁺ [³⁵Cl] = 323.0686).

3-*O*-Benzoyl-6-bromo-5,6-dideoxy-1,2-*O*-isopropylidene- α -*D*-ribo-hex-5-ynofuranose (9b). AgNO₃ (25 mg, 0.15 mmol) was added to a solution of **7** (288 mg, 1 mmol) and NBS (205 mg, 1.15 mmol) in Me₂CO (10 mL) at ambient temperature, and stirring was continued for 30 min. Volatiles were evaporated, and the residue was partitioned (H₂O/CHCl₃). The aqueous layer was extracted (CHCl₃), the combined organic

phase was washed (NaHCO₃/H₂O, brine) and dried (MgSO₄), and volatiles were evaporated to give **9b** (351 mg, 96%) as an amorphous solid: ¹H 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⁺ [C₁₆H₁₆-⁸¹BrO₅] = 369.0161), 367.0180 (10, MH⁺ [⁷⁹Br] = 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/H₂O (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), Ac₂O (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/H₂O, NaHCO₃/H₂O, brine) and dried (Na₂SO₄). 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%): ¹H 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⁺ [C₁₇H₁₅³⁷ClN₅O₇] = 391.0375), 389.0396 (100, MNa⁺ [³⁵Cl] = 389.0404).

(b) **Coupling.** SnCl₄ (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 CH₃CN (15 mL), and stirring was continued for 16 h at ambient temperature. Volatiles were evaporated, the residue was partitioned (NaHCO₃/H₂O//CHCl₃), and the organic layer was washed (brine) and dried (MgSO₄). Volatiles were evaporated, and the residue was chromatographed (CHCl₃ → 2% MeOH/CHCl₃) 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⁺ [C₂₇H₂₁³⁷ClN₅O₆] = 548.1151), 546.1162 (23, MH⁺ [³⁵Cl] = 546.1180).

(c) **Deprotection.** A solution of the product from step b (274 mg, 0.5 mmol) in NH₃/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 C₁₈ column, gradient 15 → 45% CH₃CN/H₂O 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); ¹H NMR (Me₂SO-*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, NH₂), 8.16 (s, 1, H₂), 8.31 (s, 1, H₈); ¹³C NMR (Me₂SO-*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⁺ [C₁₁H₁₁³⁷ClN₅O₃] = 298.0521), 296.0539 (100, MH⁺ [³⁵Cl] = 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%): ¹H 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⁺ [C₁₇H₁₅⁸¹BrO₇] = 411.9981), 409.9993 (15, M⁺ [⁷⁹Br] = 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*-benzoyl-

adenine (252 mg, 57%): HRMS (FAB) *m/z* 592.0668 (96, MH⁺ [C₂₇H₂₁⁸¹BrN₅O₆] = 592.0655), 590.0671 (100, MH⁺ [⁷⁹Br] = 590.0675). Deprotection (NH₃/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); ¹H NMR (Me₂SO-*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, NH₂), 8.14 (s, 1, H₂), 8.28 (s, 1, H₈); ¹³C NMR (Me₂SO-*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⁺ [C₁₁H₁₁⁸¹BrN₅O₃] = 342.0025), 340.0039 (25, MH⁺ [⁷⁹Br] = 340.0045). Anal. [C₁₁H₁₀BrN₅O₃·0.5 H₂O (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 HClO₄/H₂O (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 C₁₈ 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 Wilson²⁷ 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 described²⁸ [C₁₈ reversed-phase column (ODS Hypersil 250 × 4.6 mm, Alltech, Deerfield, IL), 0.5 mM tetrabutylammonium hydroxide/5% CH₃OH (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 HClO₄/H₂O (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 ($10\ \mu\text{M}$) in buffer A ($0.8\ \text{mL}$) was incubated with **3a**, **3b**, or **3c** ($100\ \mu\text{M}$) at $37\ ^\circ\text{C}$ for 20 min. Inhibitor-induced NADH formation was monitored at $340\ \text{nm}$ 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 CCID₅₀ of virus (1 CCID₅₀ 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, ... $\mu\text{g}/\text{mL}$) 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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