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[1]

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Treatment of the 5′-carboxaldehyde derived by Moffatt oxidation of 6-*N*-benzoyl-2′,3′-*O*-isopropylideneadenosine (**1**) with the "(bromofluoromethylene)triphenylphosphorane" reagent and deprotection gave 9-(6-bromo-5,6-dideoxy-6-fluoro-*β*-D-*ribo*-hex-5-enofuranosyl)adenine (**4**). Parallel treatment with a "dibromomethylene Wittig reagent" and deprotection gave 9-(6,6-dibromo-5,6-dideoxy-*β*-D-*ribo*-hex-5-enofuranosyl)adenine (**7**), which also was prepared by successive bromination and dehydrobromination of the 6′-bromohomovinyl nucleoside **8**. Bromination−dehydrobromination of the 5′-bromohomovinyl analogue **11** and deprotection gave (*E*)-9-(5,6-dibromo-5,6-dideoxy-*β*-D-*ribo*-hex-5-enofuranosyl)adenine (**15**). Compounds **4**, **7**, and **15** were designed as putative substrates of the "hydrolytic activity" of *S*-adenosyl-L-homocysteine (AdoHcy) hydrolase. Enzyme-mediated addition of water across the 5,6-double bond could generate electrophilic acyl halide or α-halo ketone species that could undergo nucleophilic attack by proximal groups on the enzyme. Such type II (covalent) mechanism-based inactivation is supported by protein labeling with 8-[^{3}H]-**4** and concomitant release of bromide and fluoride ions. Incubation of AdoHcy hydrolase with **7** or **15** resulted in irreversible inactivation and release of bromide ion. In contrast with type I mechanism-based inactivation, reduction of enzyme-bound NAD^+ to NADH was not observed. Compounds **4**, **7**, and **15** were not inhibitory to a variety of viruses in cell culture, and weak cytotoxicity was observed only for CEM cells.

Introduction

The cellular enzyme *S*-adenosyl-L-homocysteine (AdoHcy) hydrolase (EC 3.3.1.1) effects cleavage of AdoHcy to adenosine and L-homocysteine. Since AdoHcy is a potent feedback inhibitor of crucial transmethylation enzymes, the design of inhibitors of AdoHcy hydrolase represents a rational approach for anticancer and antiviral chemotherapy.[2] The *Z*-isomer of 4′,5′-didehydro-5′-deoxy-5′-fluoroadenosine[3] (ZDDFA) and its chloro analogue[4] are potent mechanism-based inactivators of AdoHcy hydrolase. The mechanism of inactivation by ZDDFA involves addition of water at C5′ of ZDDFA, elimination of hydrogen fluoride to give the 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 indicated that AdoHcy hydrolase has "hydrolytic activity" (addition of water at C5′) that functions independently of its C3′-oxidation activity.[5b]

Our homologated halovinyl analogues[6] [(*E*)-5′,6′-didehydro-6′-deoxy-6′-halohomoadenosines, EDDHHAs] inhibit AdoHcy hydrolase and are enzymatically hydrolyzed to produce "homoadenosine-6′-carboxaldehyde" which undergoes spontaneous decomposition.[7] The hydrolytic (C5′/C6′) and oxidative (C3′) activities of AdoHcy hydrolase were differentiated most effectively with the 6′-fluoro analogue (EDDFHA)[7b] which was employed in a study that implicated Lys-426 as a crucial residue for the hydrolytic activity of the enzyme.[8] We now report the design, synthesis, and bioactivity of the geminal and vicinal (dihalohomovinyl)adenosines **4**, **7**, and **15**. These analogues were designed as putative new substrates for the hydrolytic activity of AdoHcy hydrolase that might cause type II (covalent[2b]) mechanism-based inhibition. Addition of enzyme-sequestered water across the 5′,6′-double bond[7] of such dihalohomovinyl analogues followed by loss of hydrogen halide might produce an electrophilic α-halomethyl ketone or acyl halide (Figure 1). Nucleophilic attack by proximal amino acid functionalities (e.g., an NH_2 group on Lys-426 or Arg-196) might cause type II inactivation of the enzyme.

Homologated 5′,5′-dibromomethylene-5′-deoxyuridine[9] and adenosine[10] analogues have been synthesized from nucleoside 5′-carboxaldehydes (Corey−Fuchs procedure,[11] $CBr_4/PPh_3/Zn$) and have been converted into 5′-deoxy-5′-methynyl nucleosides (4′-acetylenic derivatives).[9,10] The acetylenic analogue derived from adenosine is a potent type II inhibitor of AdoHcy hydrolase[6,12] with

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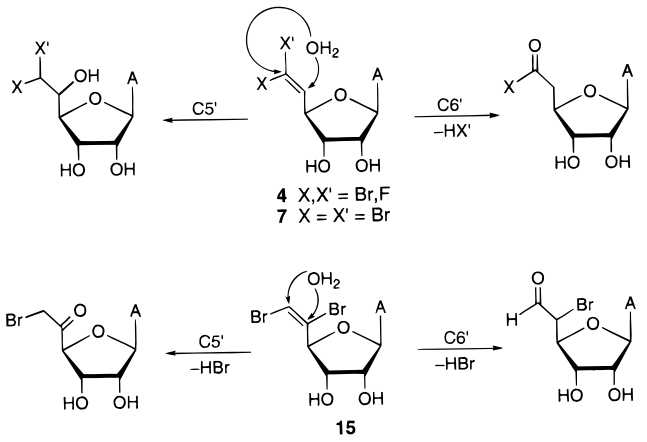
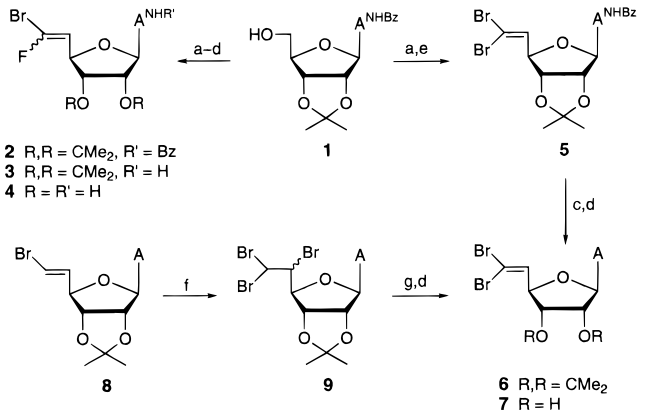


Figure 1. Possible generation of active intermediates from 5′,6′- and 6′,6′-dihalohomovinyl nucleoside analogues by the "hydrolytic activity" of AdoHcy hydrolase.

Scheme 1[a]



[a] (a) DMSO/DCC/Cl_2CHCO_2H; (b) $CBr_3F/Ph_3P/Zn/CH_2Cl_2$; (c) NH_3/MeOH; (d) TFA/H_2O; (e) $CBr_4/Ph_3P/Zn/CH_2Cl_2$; (f) Br_2/CH_2Cl_2; (g) DBU/THF.

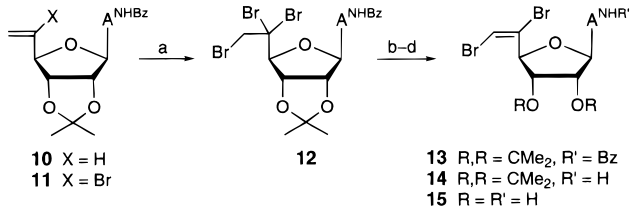
antiviral[6,10] and cytostatic activity.[6] We also have prepared 4′-acetylenic derivatives by oxidative destannylation (lead tetraacetate) of vinyl 6′-stannanes.[6]

Chemistry

Moffatt oxidation[13] of 6-*N*-benzoyl-2′,3′-*O*-isopropylideneadenosine (**1**) and treatment of the 5′-carboxaldehyde (crude) with (bromofluoromethylene)triphenylphosphorane (generated[11] in situ with CBr_3F/Ph_3P/Zn) gave the bromofluorovinyl diastereomers **2** (*E*/*Z*, ∼3:2, 30%; Scheme 1). Stereochemistry was assigned from $J_{F-H5'}$ coupling constants [29.2 Hz (*E*) and 11.8 Hz (*Z*)]. Analogous treatment of the purified 5′-carboxaldehyde[13] gave **2** in similar yields. Debenzoylation of **2** (NH_3/MeOH) gave **3** which was deprotected (TFA/H_2O) to give **4** (21% overall from **1**). RP-HPLC purification of **4** gave fractions enriched in *E*- or *Z*-isomers (71:29 or 44:56).

Oxidation[13] of **1** and treatment of the 5′-carboxaldehyde (crude or purified) with (dibromomethylene)triphenylphosphorane ($CBr_4/Ph_3P/Zn$)[11] gave the 6′,6′-dibromohomovinyl analogue **5** (55−64%) which was debenzoylated to give **6**. Treatment of the bromohomovinyl derivative **8**[6] (prepared by halodestannylation of the vinyl 6′-stannanes[6,14]) with bromine gave the somewhat unstable 5′,6′,6′-tribromo diasteromers **9** (46% after column chromatography). Treatment of **9** with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) gave **6** (H6′ of **9** more acidic than H5′) which was deprotected to give the 6′,6′-dibromohomovinyl product **7** (73%).

Scheme 2[a]



[a] (a) Br_2/CH_2Cl_2; (b) DBU/CH_2Cl_2; (c) NH_3/MeOH; (d) TFA/H_2O.

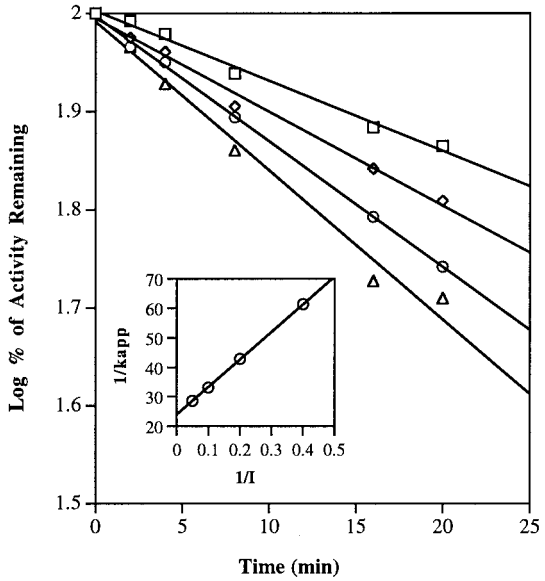


Figure 2. Time-dependent inactivation of AdoHcy hydrolase with **4**. AdoHcy hydrolase (20 nM) was incubated with **4** (□, 2.5 μM; ◇, 5 μM; ○, 10 μM; △, 20 μM) in buffer A at 37 °C. At the indicated time points, residual enzyme activity was determined in the synthetic direction[5b] as described in the Experimental Section. Inset: Plot of $(k_{app})^{-1}$ versus $[I]^{-1}$ from which the K_i and k_{inact} values were calculated.[17] Data are the average of duplicate measurements.

Bromination of homovinyl derivative **10**[6,15] gave 5′,6′-dibromo diastereomers which were dehydrobrominated (DBU) to give 5′-bromovinyl compound **11**[6] (Scheme 2). A second addition of bromine (with **11**) gave 5′,5′,6′-tribromo derivative **12** which decomposed during column chromatography. One-flask treatment of **11** with bromine followed by dehydrobromination (DBU) gave 5′,6′-dibromohomovinyl derivative **13** (64%) as a single diastereomer. The *E* configuration is expected from an E2 (anti elimination) process involving the most stable conformations of **12** with one bromine atom on C5′ anti to the bromine on C6′. Debenzoylation of **13** and deacetonization of **14** gave the crystalline 5′,6′-dibromohomovinyl target **15** (64%).

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

Recombinant human placental AdoHcy hydrolase was irreversibly inactivated by **4**, **7**, and **15**. Inactivation with **4** was shown to be both concentration- and time-dependent (Figure 2) and resulted in covalent linkage of the enzyme and inhibitor with concomitant release of halide ions (F^- and Br^-). K_i values for **4**, **7**, and **15**

Table 1. Kinetic Constants for Inhibition of AdoHcy Hydrolase by **4**, **7**, and **15**

compd	K_i (μM)	k_{inact} (min^{-1})	k_{inact}/K_i (min^{-1} M^{-1})
4	3.9	0.040	1.02×10^4
7	3.1	0.036	1.16×10^4
15	3.6	0.081	2.25×10^4

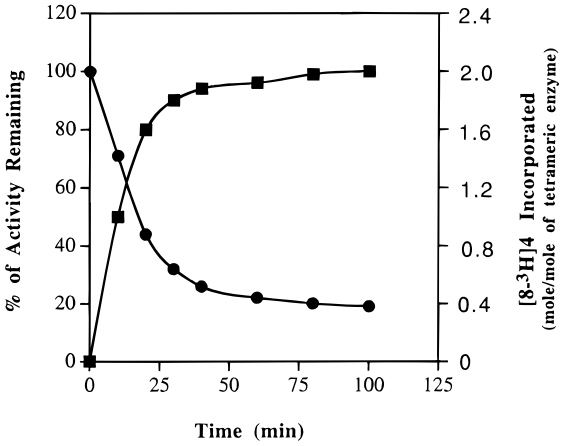


Figure 3. Stoichiometry of the covalent binding of **4** with AdoHcy hydrolase. AdoHcy hydrolase (0.5 mg) was incubated with 8-[^{3}H]-**4** (500 μM, 187 Ci/mol) in buffer A (250 μL) at 37 °C. At each time point, the reaction mixture was analyzed as described in the Experimental Section (●, residual enzyme activity; ■, covalently bound 8-[^{3}H]-**4**).

were in the range of 3−4 μM, and the k_{inact} value for **15** was about twice those for **4** and **7** (Table 1). Enzyme-mediated addition of water at C6′ of **4** or **7** followed by elimination of hydrogen halide could generate an electrophilic acyl halide, whereas addition of water at C5′ of **15** could generate an α-halo ketone (Figure 1). Nucleophilic attack by proximal group(s) on the enzyme could result in type II mechanism-based enzyme inactivation. Such type II inhibitors that were specifically activated by the "hydrolytic activity" of AdoHcy hydrolase (i.e., without prior oxidation at C3′) would be unique.

Incubation of **4** with the enzyme resulted in release of Br^- and F^- ions and adenine (Ade). Approximately 2 mol equiv of **4** was covalently incorporated per mole of the tetrameric enzyme at ∼80% inactivation (Figure 3). The rate constant for Br^- release was greater than those for release of F^- and Ade (data not shown), and the stoichiometry for release of Br^-/F^-/Ade was approximately 1:1:0.5 per inactivated enzyme subunit. The mechanism for enzyme inactivation with 8-[^{3}H]-**4** is proposed[16] to involve enzyme-mediated addition of water at C6′ followed by elimination of HBr to give the homoAdo-6′-carboxylic acid fluoride (HACF). HACF is partitioned by (a) attack by a protein nucleophile and expulsion of F^- to form a covalent bond or (b) depurination[7b] to release Ade and sugar byproducts that release further fluoride ion. The partition ratio of pathways a/b was approximately 1:1, and two enzymatic turnovers result in one lethal event (enzyme inactivation). In contrast with type I mechanism-based inhibition ("cofactor depletion" in which enzyme-bound NAD^+ is reduced to NADH), inactivation of AdoHcy hydrolase with **4**, **7**, and **15** did not result in changes in the initial NAD^+/NADH ratios (data not shown).

Antiviral and Cytotoxicity Evaluation

Compounds **4**, **7**, and **15** were evaluated for their inhibitory activity against a variety of viruses. These included herpes simplex virus type 1 (HSV-1) (strains KOS, F, and McIntyre), HSV-2 (strains G, 196, and Lyons), thymidine kinase-deficient HSV-1/TK^- (strains B2006 and VMW 1837), vaccinia virus, and vesicular stomatitis virus in E_6SM cell cultures; varicella-zoster virus (strains YS and Oka) and cytomegalovirus (strains Davis and AD 196) in HEL cell cultures; Coxsackie virus B4, parainfluenza virus-3, Sindbis virus, reovirus-1, and Punta Toro virus in Vero cell cultures; and human immunodeficiency virus type 1 (HIV-1) (strain III_a) and HIV-2 (strain ROD) in CEM cell cultures. No inhibitory effects were observed with **4**, **7**, or **15** in any of the virus cultures at subtoxic concentrations (EC_{50} >100−500 μM). These compounds had weak cytostatic activity only against CEM cells (IC_{50} 75−160 μM).

Summary and Conclusions

We have designed and synthesized the dihalohomovinyl nucleoside analogues **4**, **7**, and **15** (from adenosine or hexofuranose precursors) that demonstrate type II (covalent) mechanism-based inactivation of *S*-adenosylhomocysteine hydrolase. These are the first examples of type II inhibitors that are activated by the "hydrolytic activity" of the enzyme without prior oxidation at C3′ (i.e., reduction of enzyme-bound NAD^+ to NADH was not observed). It is noteworthy that only moderate cytotoxicity in one cell line (CEM) was observed with **4**, **7**, and **15**. This is in harmony with our goal to minimize cellular toxicity with these 5′-modified adenosine analogues (which do not contain a 5′-hydroxyl group that could be phosphorylated and undergo further metabolic processing) that become inactivators only after mechanism-based interaction with the targeted enzyme. The present compounds appear to be activated exclusively by the 5′/6′-hydrolytic activity of AdoHcy hydrolase to generate electrophilic species that undergo nucleophilic attack by protein residues to give half of the sites covalent binding, and the resulting functional tetrameric subunit complexes retained both oxidative and hydrolytic activity.[16] Further treatment of such partially inactivated enzymes with a type I inhibitor caused complete inactivation. Studies are in progress to further probe these fascinating observations and utilize them in the design of selective inhibitors for human versus parasite AdoHcy hydrolases.

Experimental Section

Uncorrected melting points were determined with a capillary apparatus. UV spectra were measured with solutions in MeOH. ^{1}H (200 or 500 MHz), ^{13}C (50 or 125 MHz), and ^{19}F [470.3 MHz (CCl_3F)] NMR spectra were determined with solutions in $CDCl_3$ unless otherwise specified. Mass spectra (MS and HRMS) were obtained with electron impact (20 eV), chemical ionization (CI, isobutane), or fast atom bombardment (FAB, thioglycerol matrix) techniques. Merck Kieselgel 60-F_{254} sheets were used for TLC, and products were detected with 254-nm light. Merck Kieselgel 60 (230−400 mesh) was used for column chromatography. Preparative reversed-phase (RP)-HPLC was performed with a Dynamax C_{18} column with a Spectra Physics SP 8800 ternary pump system (gradient solvent systems are noted). Elemental analyses were determined by M-H-W Laboratories, Phoenix, AZ. Reagent grade chemicals were used, and solvents were dried by reflux over

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

6-*N*-Benzoyl-9-(6-bromo-5,6-dideoxy-6-fluoro-2,3-*O*-isopropylidene-*β*-D-*ribo*-hex-5-enofuranosyl)adenine (2). A solution of **1**[13] (1.23 g, 3 mmol) and *N*,*N*′-dicyclohexylcarbodiimide (DCC; 1.84 g, 9 mmol) in dried Me_2SO (7.5 mL) was cooled (∼10 °C) under argon, Cl_2CHCO_2H (0.12 mL, 193 mg, 1.5 mmol) was added, and stirring was continued for 90 min at ambient temperature. The red-brown solution of 5′-carboxaldehyde was injected (syringe) into a mixture containing (bromofluoromethylene)triphenylphosphorane [generated in situ by stirring CBr_3F (0.53 mL, 1.42 g, 5.25 mmol), Ph_3P (1.37 g, 5.25 mmol), and activated Zn (dust; 343 mg, 5.25 mmol) in dried CH_2Cl_2 (20 mL) for 5 h at ambient temperature under Ar; sonication was applied intermittently for a total of 30 min], stirring was continued for 16 h, and oxalic acid dihydrate (756 mg, 6 mmol) in MeOH (15 mL) was added. After 20 min the reaction mixture was concentrated (to ∼1/3 volume), the dicyclohexylurea was filtered and washed with cold MeOH, and the combined filtrates were evaporated (in vacuo). The residue was partitioned ($NaHCO_3/H_2O$//$CHCl_3$), the organic layer was washed (H_2O, brine) and dried ($MgSO_4$), and volatiles were evaporated. Column chromatography of the residue (EtOAc → 1% MeOH/EtOAc) gave **2**. Repeated chromatography of this material gave **2** (*E*/*Z*, ∼3:2; 360 mg, 30%): ^{1}H NMR δ 1.39 and 1.67 (2 × s, 2 × 3, 2 × Me), 4.91 (ddd, $J_{4'-5'} = 9.2$ Hz, $J_{4'-3'} = 2.2$ Hz, $J_{4'-F} = 2.2$ Hz, 0.4, H4′*Z*), 5.09−5.19 (m, 1.6, H3′, H4′*E*), 5.40 (dd, $J_{5'-4'} = 9.2$ Hz, $J_{5'-F} = 29.2$ Hz, 0.6, H5′*E*), 5.58−5.67 (m, 1, H2′), 5.83 (dd, $J_{5'-F} = 11.8$ Hz, 0.4, H5′*Z*), 6.12 (s, 1, H1′), 7.44−8.12 (m, 6, Arom, NH), 8.84 (s, 1, H2), 9.03 (s, 1, H8); ^{19}F NMR δ −68.11 (d, $J_{F-5'} = 29.2$ Hz, 0.6, F6′*E*), −63.62 (d, $J_{F-5'} = 11.8$ Hz, 0.4, F6′*Z*).

Analogous treatment (24 h, ambient temperature, under Ar; inverse Wittig reagent addition via a double-ended needle) of the purified 6-*N*-benzoyl-2′,3′-*O*-isopropylideneadenosine-5′-carboxaldehyde [prepared from the aldehyde hydrate (200 mg, 0.468 mmol) as described[13]] in dried CH_2Cl_2 (25 mL) with (bromofluoromethylene)triphenylphosphorane [prepared from CBr_3F (0.092 mL, 254 mg, 0.937 mmol), Ph_3P (245 mg, 0.937 mmol), and activated Zn (dust; 61 mg, 0.937 mmol) in dried CH_2Cl_2 (5 mL)], evaporation, and chromatography (CH_2Cl_2 → 3% EtOH/CH_2Cl_2) gave **2** (71 mg, 30%) plus recovered aldehyde hydrate (85 mg, 43%).

9-(6-Bromo-5,6-dideoxy-6-fluoro-2,3-*O*-isopropylidene-*β*-D-*ribo*-hex-5-enofuranosyl)adenine (3). A solution of **2** (71 mg, 0.14 mmol) in NH_3/MeOH (10 mL) in a sealed flask was stirred at ambient temperature for 24 h. Volatiles were evaporated, and the residue was flash-chromatographed (CH_2Cl_2 → 8% EtOH/CH_2Cl_2) to give **3** (57 mg, quantitative) as a white powder: ^{1}H NMR δ 1.39 and 1.63 (2 × s, 2 × 3, 2 × Me), 4.87 (ddd, $J_{4'-5'} = 9.2$ Hz, $J_{4'-3'} = 2.5$ Hz, $J_{4'-F} = 2.5$ Hz, 0.4, H4′*Z*), 5.07−5.19 (m, 1.6, H3′, H4′*E*), 5.46 (dd, $J_{5'-4'} = 9.2$ Hz, $J_{5'-F} = 29.7$ Hz, 0.6, H5′*E*), 5.54−5.67 (m, 1, H2′), 5.85 (br s, 2, NH_2), 5.89 (dd, $J_{5'-F} = 11.8$ Hz, 0.4, H5′*Z*), 6.05 (s, 1, H1′), 7.84 (s, 1, H2), 8.37 (s, 1, H8); ^{19}F NMR δ −69.07 (d, $J_{F-5'} = 29.2$ Hz, 0.6, F6′*E*), −64.52 (d, $J_{F-5'} = 11.8$ Hz, 0.4, F6′*Z*); HRMS (CI) *m*/*z* 402.0396 (96, MH^+ [$C_{14}H_{16}{}^{81}BrFN_5O_3$] = 402.0400), 400.0414 (100, MH^+ [^{79}Br] = 400.0421).

9-(6-Bromo-5,6-dideoxy-6-fluoro-*β*-D-*ribo*-hex-5-enofuranosyl)adenine (4). A solution of **3** (57 mg, 0.14 mmol) in CF_3CO_2H/H_2O (9:1, 5 mL) was stirred at ambient temperature for 1 h. Volatiles were evaporated, and the slightly yellow residue was purified by preparative RP-HPLC (30% CH_3CN/H_2O for 30 min followed by a gradient of 30% → 70% for 25 min at flow rate of 2.8 mL/min) to give **4** (36 mg, 70%; early and late fractions had *E*/*Z* ratios of 71:29 and 44:56, respectively) as an off-white solid. "Diffusion crystallization"[17] (EtOAc/hexane) gave **4** (18 mg, 35%) as white crystals: mp 100 °C dec; UV max 261 nm (ϵ 14 500), min 228 nm (ϵ 2200); ^{1}H NMR (CD_3OD) δ 4.28−4.39 (m, 1, H3′), 4.63 (ddd, $J_{4'-5'} = 9.2$ Hz, $J_{4'-3'} = 4.5$ Hz, $J_{4'-F} = 2.1$ Hz, 0.4, H4′*Z*), 4.76−4.90 (m, 1.6, H2′, H4′*E*), 5.71 (dd, $J_{5'-4'} = 9.4$ Hz, $J_{5'-F} = 30.4$ Hz, 0.6, H5′*E*), 5.98−6.04 (m, 1, H1′), 6.14 (dd, $J_{5'-F} = 12.4$ Hz, 0.4, H5′*Z*), 8.26 (s, 1, H2), 8.33 (s, 1, H8); ^{13}C NMR (Me_2SO-d_6) δ 72.75 and 72.85 (C3′), 73.89 and 74.06 (C2′), 77.72 and 81.34 (d, $J_{C4'-F} = 7.7$ Hz) (C4′), 87.66 and 87.72 (C1′), 110.53 (d, $J_{C5'-F} = 16.0$ Hz) and 112.41 (d, $J_{C5'-F} = 7.6$ Hz) (C5′), 119.23 (C5), 134.29 (d, $J_{C6'-F} = 322.0$ Hz) and 137.53 (d, $J_{C6'-F} = 315.9$ Hz) (C6′), 139.89 (C8), 149.23 (C4), 152.58 (C2), 156.06 (C6); ^{19}F NMR (Me_2SO-d_6/CCl_3F) δ −71.08 (d, $J_{F-H5'} = 32.3$ Hz, F6′*E*), −66.26 (d, $J_{F-H5'} = 12.9$ Hz, F6′*Z*); HRMS *m*/*z* 361.0013 (94, M^+ [$C_{11}H_{11}{}^{81}BrFN_5O_3$] = 361.0009), 359.0034 (100, M^+ [^{79}Br] = 359.029). Anal. [$C_{11}H_{11}BrFN_5O_3 \cdot 0.5H_2O$ (369.2)] C, H, N.

The preparation of 8-[^{3}H]-**4** was modeled by heating a suspension of **4** (5 mg) in D_2O (0.7 mL) at 85 °C for 6 h. The resulting 8-[^{2}H]-**4** had ^{1}H NMR (Me_2SO-d_6) δ 8.13 (s, 1, H2) [but no peak at δ 8.37 (^{2}H8)]; MS (FAB) *m*/*z* 363 (95, MH^+ [^{81}Br, ^{2}H8]), 361 (100, MH^+ [^{79}Br,^{2}H8]).

6-*N*-Benzoyl-9-(6,6-dibromo-5,6-dideoxy-2,3-*O*-isopropylidene-*β*-D-*ribo*-hex-5-enofuranosyl)adenine (5). Compound **1** (1.23 g, 3 mmol) was oxidized[13] and the resulting (crude) 5′-carboxaldehyde was treated with (dibromomethylene)triphenylphosphorane [generated in situ by stirring CBr_4 (1.74 g, 5.25 mmol), Ph_3P (1.37 g, 5.25 mmol), and activated Zn (dust; 343 mg, 5.25 mmol) in dried CH_2Cl_2 (20 mL) for 5 h at ambient temperature under Ar; sonication was applied intermittently for a total of 15 min] for 4 h, as described for the synthesis of **2**. Analogous workup and purification gave **5**[10] (1.09 g, 64%) as a light-tan solid: ^{1}H NMR δ 1.40 and 1.55 (2 × s, 2 × 3, 2 × Me), 4.94 (dd, $J_{4'-5'} = 8.1$ Hz, $J_{4'-3'} = 3.0$ Hz, 1, H4′), 5.16 (dd, $J_{3'-2'} = 6.0$ Hz, 1, H3′), 5.56 (dd, $J_{2'-1'} = 1.5$ Hz, 1, H2′), 6.11 (d, 1, H1′), 6.59 (d, 1, H5′), 7.39−8.14 (m, 6, Arom, NH), 7.97 (s, 1, H2), 8.74 (s, 1, H8); HRMS (CI) *m*/*z* 567.9854 (48, MH^+ [$C_{21}H_{20}{}^{81}Br_2N_5O_4$] = 567.9841), 565.9875 (100, MH^+ [$^{81/79}Br_2$] = 565.9862), 563.9883 (56, MH^+ [$^{79}Br_2$] = 563.9882).

Analogous treatment of the 6-*N*-benzoyl-2′,3′-*O*-isopropylideneadenosine-5′-carboxaldehyde [prepared from the purified aldehyde hydrate[13] (200 mg, 0.47 mmol)] with (dibromomethylene)triphenylphosphorane [prepared from CBr_4/Ph_3P/Zn (0.94 mmol)] gave **5** (145 mg, 55%).

9-(6,6-Dibromo-5,6-dideoxy-2,3-*O*-isopropylidene-*β*-D-*ribo*-hex-5-enofuranosyl)adenine (6). Method A. DBU (40 μL, 41 mg, 0.27 mmol) was added to a solution of **9** (50 mg, 0.092 mmol) in dried THF (25 mL) under argon at ∼0 °C. Stirring was continued at ∼0 °C for 30 min and then at ambient temperature for 2 h. Volatiles were evaporated, and the residue was chromatographed (CH_2Cl_2 → 8% EtOH/CH_2Cl_2) to give **6** (42 mg, quantitative) as a white powder: ^{1}H NMR δ 1.44 and 1.65 (2 × s, 2 × 3, 2 × Me), 4.95 (dd, $J_{4'-5'} = 8.2$ Hz, $J_{4'-3'} = 2.7$ Hz, 1, H4′), 5.14 (dd, $J_{3'-2'} = 6.1$ Hz, 1, H3′), 5.54 (dd, $J_{2'-1'} = 1.5$ Hz 1, H2′), 6.08 (d, 1, H1′), 6.64 (d, 1, H5′), 6.79 (br s, 2, NH_2), 7.97 (s, 1, H2), 8.36 (s, 1, H8).

Method B. A solution of **5** (145 mg, 0.26 mmol) in saturated NH_3/MeOH (50 mL) in a sealed flask was stirred at ambient temperature for 24 h. Evaporation of volatiles and flash chromatography of the residue (CH_2Cl_2 → 8% EtOH/CH_2Cl_2) gave **6**[10] (118 mg, 100%) as a white powder with data identical to that in method A.

9-(6,6-Dibromo-5,6-dideoxy-*β*-D-*ribo*-hex-5-enofuranosyl)adenine (7). A solution of **6** (44 mg, 0.095 mmol) in CF_3CO_2H/H_2O (9:1, 7 mL) was stirred at ambient temperature for 1 h. Volatiles were evaporated immediately in vacuo, and the slightly yellow residue was purified by preparative RP-HPLC (20% CH_3CN/H_2O for 20 min, gradient of 20% → 50% for 25 min, flow rate 2.8 mL/min) to give **7** (35 mg, 90%) as an off-white solid. Recrystallization (H_2O) gave **7**[10] (28 mg, 73%) as white crystals: mp 105 °C dec; UV max 258 nm (ϵ 14 500), min 237 nm (ϵ 7000); ^{1}H NMR (Me_2SO-d_6) δ 4.23 (dd, $J_{3'-4'} = 3.9$ Hz, $J_{3'-2'} = 5.0$ Hz, 1, H3′), 4.56 (dd, $J_{4'-5'} = 8.6$ Hz, 1, H4′), 4.70 (dd, $J_{2'-1'} = 5.5$ Hz, 1, H2′), 5.75 (br s, 2, OH2′,3′), 5.98 (d, 1, H1′), 7.11 (d, 1, H5′), 8.55 (br s, 2, NH_2), 8.41 (s, 1, H2), 8.63 (s, 1, H8); ^{13}C NMR (Me_2SO-d_6) δ 73.06 (C3′), 74.00 (C2′), 84.58 (C4′), 88.20 (C1′), 93.45 (C6′), 119.16 (C5), 137.39 (C5′), 140.90 (C8), 149.64 (C4), 153.16 (C2), 156.26 (C6); MS

m/z 424 (MH^+ [$^{81}Br_2$]), 422 (MH^+ [$^{81/79}Br_2$]), 420 (MH^+ [$^{79}Br_2$]). Anal. [$C_{11}H_{11}Br_2N_5O_3 \cdot 0.5H_2O$ (430.1)] C, H, N.

9-(5,6,6-Tribromo-5,6-dideoxy-2,3-*O*-isopropylidene-β-D-*ribo*-hexofuranosyl)adenine (9). Br_2 (35 mg, 0.22 mmol) in CH_2Cl_2 (2.5 mL) was added dropwise during 1 h to a solution of **8**[6] (78 mg, 0.20 mmol) in CH_2Cl_2 (10 mL) cooled to −10 °C, and stirring was continued at ∼0 °C for 3.5 h. The solution was washed [H_2O (10 mL), $NaHCO_3/H_2O$ (2 × 10 mL)] and dried ($MgSO_4$), and volatiles were evaporated. The residue was chromatographed (EtOAc) to give **9** (50 mg, 46%) as an off-white powder: ^{1}H NMR δ 1.42 and 1.65 (2 × s, 2 × 3, 2 × CH_3), 4.51 (d, $J_{4'-5'}$ = 10.2 Hz, 1, H4′), 5.17 (d, 1, H5′), 5.35 (d, $J_{3'-2'}$ = 6.2 Hz, 1, H3′), 5.42 (d, 1, H2′), 5.82 (s, 1, H6′), 6.09 (s, 1, H1′), 6.26 (br s, 2, NH_2), 7.87 (s, 1, H2), 8.36 (s, 1, H8).

(*E*)-6-*N*-Benzoyl-9-(5,6-dibromo-5,6-dideoxy-2,3-*O*-isopropylidene-β-D-*ribo*-hex-5-enofuranosyl)adenine (13). Br_2 (60 mg, 0.38 mmol) in CH_2Cl_2 (4.3 mL) was added dropwise during 1 h to a solution of **11**[6] (180 mg, 0.37 mmol) in CH_2Cl_2 (15 mL) cooled at −10 °C. A slight orange color persisted, and stirring was continued at −5 °C for 5 h. DBU (0.165 mL, 167 mg, 1.1 mmol) was added to crude **12**, and stirring was continued at ambient temperature for 10 h. Volatiles were evaporated, and chromatography of the residue (CH_2Cl_2 → 3% EtOH/CH_2Cl_2) gave **13** (133 mg, 64%) as a white powder: ^{1}H NMR δ 1.41 and 1.70 (2 × s, 2 × 3, 2 × Me), 5.07 (dd, $J_{3'-4'}$ = 4.1 Hz, $J_{3'-2'}$ = 6.2 Hz, 1, H3′), 5.34 (dd, $J_{2'-1'}$ = 2.8 Hz, 1, H2′), 5.41 (d, 1, H4′), 6.31 (d, 1, H1′), 6.78 (s, 1, H6′), 7.60−8.10 (m, 5, Arom), 8.30 (s, 1, H2), 8.81 (s, 1, H8), 9.28 (s, 1, NH).

(*E*)-9-(5,6-Dibromo-5,6-dideoxy-2,3-*O*-isopropylidene-β-D-*ribo*-hex-5-enofuranosyl)adenine (14). A solution of **13** (133 mg, 0.24 mmol) in saturated NH_3/MeOH (50 mL) in a sealed flask was stirred at ambient temperature for 24 h. Volatiles were evaporated, and the residue was flash-chromatographed (CH_2Cl_2 → 8% EtOH/CH_2Cl_2) to give **14** (106 mg, 98%) as a white powder: ^{1}H NMR δ 1.39 and 1.69 (2 × s, 2 × 3, 2 × Me), 5.04 (dd, $J_{3'-4'}$ = 4.1 Hz, $J_{3'-2'}$ = 6.2 Hz, 1, H3′), 5.29 (dd, $J_{2'-1'}$ = 3.0 Hz, 1, H2′), 5.36 (d, 1, H4′), 5.77 (br s, 2, NH_2), 6.23 (d, 1, H1′), 6.77 (s, 1, H6′), 8.06 (s, 1, H2), 8.37 (s, 1, H8).

(*E*)-9-(5,6-Dibromo-5,6-dideoxy-β-D-*ribo*-hex-5-enofuranosyl)adenine (15). A solution of **14** (30 mg, 0.065 mmol) in CF_3CO_2H/H_2O (9:1, 7 mL) was stirred at ambient temperature for 1 h. Volatiles were evaporated in vacuo, and the slightly yellow residue was purified by preparative RP-HPLC (20% CH_3CN/H_2O for 40 min, gradient of 20% → 50% for 30 min, flow rate of 2.8 mL/min) to give **15** (23 mg, 85%) as an off-white solid. "Diffusion crystallization"[17] (MeOH/EtOAc) gave **15** (18 mg, 66%) as white crystals: mp 115−118 °C, 125 °C dec; UV max 259 nm (ε 15 700), min 233 nm (ε 5300); ^{1}H NMR (Me_2SO-d_6) δ 4.48 (dd, $J_{3'-4'}$ = 6.2 Hz, $J_{3'-2'}$ = 5.5 Hz, 1, H3′), 4.70 (dd, $J_{2'-1'}$ = 3.5 Hz, 1, H2′), 5.03 (d, 1, H4′), 5.50−5.90 (br s, 2, OH2′,3′), 5.99 (d, 1, H1′), 7.26 (s, 1, H6′), 7.58 (br s, 2, NH_2), 8.23 (s, 1, H2), 8.40 (s, 1, H8); ^{13}C NMR (Me_2SO-d_6) δ 72.99 (C2′,3′), 81.38 (C4′), 87.98 (C1′), 109.21 (C6′), 118.88 (C5), 124.19 (C5′), 139.93 (C8), 149.45 (C4), 152.21 (C2), 156.78 (C6); MS *m/z* 424 (MH^+ [$^{81}Br_2$]), 422 (MH^+ [$^{81/79}Br_2$]), 420 (MH^+ [$^{79}Br_2$]). Anal. [$C_{11}H_{11}Br_2N_5O_3$ (421.1)] C, H, N.

Time-Dependent Inactivation of *S*-Adenosyl-L-homocysteine Hydrolase with 4, 7, and 15. AdoHcy hydrolase [20 nM in 50 mM potassium phosphate buffer (pH 7.2) containing 1 mM EDTA (buffer A)] was incubated with various concentrations of inhibitors (2.5−20 μM) for different times (0−20 min). The remaining enzyme activity at each time point was determined in the synthetic direction as described previously.[5b] The pseudo-first-order rate constant (k_{app}) was obtained by plotting the logarithm of the remaining activity versus time, and the K_i and k_{inact} values were obtained by the method of Kitz and Wilson.[18]

The stoichiometry of labeling of the enzyme (0.5 mg) with 8-[^{3}H]-**4** (500 μM, 187 Ci/mol) was determined by incubation in buffer A (250 μL) at 37 °C for 2 h. The reaction mixture was then applied to a Sephadex G-25 spin column to remove nonbound **4**. Fractions containing the enzyme−**4** complex were combined and assayed for remaining hydrolase activity and then denatured with 8 M urea. Gel filtration with the spin column was repeated to remove noncovalently bound 8-[^{3}H]-**4**. The protein concentration and radioactivity were determined, and the ratio of bound 8-[^{3}H]-**4** (mole) to inactivated enzyme (mole of subunit) was calculated.

Antiviral Assays. These assays were based on inhibition of virus-induced cytopathicity in E_6SM, Vero, and HEL or CEM cell cultures following previously established procedures.[19−21] Briefly, for all the viruses except HIV, confluent E_6SM, Vero, or HEL 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 at 37 °C in a CO_2-controlled, humidified incubator in the presence of varying concentrations (400, 200, 80, 40, ... μg/mL) of **4**, **7**, or **15**. Viral cytopathicity was recorded microscopically as soon as it reached completion in the control virus-infected cell cultures. CEM cells were seeded in microtiter trays at 50 000 cells/200-μL well and infected with 100 $CCID_{50}$ of HIV-1 or HIV-2 in the presence of different concentrations of **4**, **7**, or **15**. After 4 days of incubation, virus-induced giant cell formation was recorded microscopically.

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