J. Med. Chem. **2004,** *47,* 5251−5257

Stereoselective Synthesis of Sugar-Modified Enyne Analogues of Adenosine and Uridine. Interaction with *S*-Adenosyl-L-homocysteine Hydrolase and Antiviral and Cytotoxic Effects

Stanislaw F. Wnuk,*,† Elzbieta Lewandowska,†,‡ Pablo R. Sacasa,† Leigh N. Crain,† Jinsong Zhang,§ Ronald T. Borchardt,§ and Erik De Clercq||

Department of Chemistry and Biochemistry, Florida International University, Miami, Florida 33199, Department of Pharmaceutical Chemistry, University of Kansas, Lawrence, Kansas 66047, and Rega Institute for Medical Research, Katholieke Universiteit Leuven, B-3000 Leuven, Belgium

Received February 27, 2004

Sonogashira coupling of (*E*)-6′-iodohomovinyl nucleosides **1** with (trimethylsilyl)acetylene gave the conjugated 8′-(trimethylsilyl)enyne derivatives of the adenosine **2a** and uridine **2b** with expected *E*-stereochemistry. Desilylation of **2a**,**b** with tetrabutylammonium fluoride followed by treatment with *N*-iodosuccinimide/$AgNO_3$ afforded 8′-iodoenynes **4a**,**b**. Analogous coupling of (*Z*)-6′-iodohomovinyl nucleosides **7a**,**b** produced (*Z*)-8′-(trimethylsilyl)enynes **8a**,**b**, which were deprotected with aqueous trifluoroacetic acid to give the *Z*-enynes **9a**,**b**. Stereoselective coupling of the adenosine 4′-acetylenic **11** and ethyl (Z*)*-3-bromoacrylate followed by deprotection gave the conjugated enyne system attached in the reverse orientation at C4′ **13**. Because of their diverse stereochemical attributes, deprotected enyne analogues **5a**, **6a**, **9a**, and **13** derived from adenosine require a different vicinity for binding with *S*-adenosyl-L-homocysteine (AdoHcy) hydrolase and/or addition of enzyme-bound water across the conjugated enyne system. Enyne **5a** and 8′-iodoenyne **6a** produced time-dependent and concentration-dependent inhibition of AdoHcy hydrolase (K_i, 0.55 and 118.5 μM, respectively). No reduction in NAD^+ content of the enzyme and no iodide ion released were observed upon incubation of **6a** with the enzyme, while incubation of **5a** produced 30% reduction in the NAD^+ content of the enzyme. No specific antiviral activity was noted for **5a**,**b**, **6a**,**b**, **9a**,**b**, and **13** against any of the viruses tested; the *E*-iodoenynes **6a** and **6b** inhibited HIV-1 virus (IC_{50}, 1.1 and 1.8 μM; selectivity index, 7 and 3, respectively). The *E*-enyne **5a** showed activity against cytomegalovirus at a concentration (EC_{50}, 30 μM) that was 3- to 10-fold lower than the cytotoxic concentration.

Introduction

The *S*-adenosyl-L-homocysteine (AdoHcy) hydrolase (EC 3.3.1.1) effects hydrolytic cleavage of AdoHcy to adenosine (Ado) and L-homocysteine (Hcy).[1] The cellular levels of AdoHcy and Hcy are critical since AdoHcy is a potent feedback inhibitor of crucial transmethylation enzymes,[1,2] and Hcy appears to be a risk factor for coronary artery diseases.[3] AdoHcy hydrolase is a target for antiviral therapy. A number of inhibitors, which function as substrates for the "3′-oxidative activity" of AdoHcy hydrolase and convert the enzyme from its active form (NAD^+) to its inactive form (NADH, type I inhibition), have been prepared.[1a]

Inhibitors which function as substrates for the "5′/6′-hydrolytic activity" include the vinyl fluorides [9-(5-deoxy-5-fluoro-*β*-D-*erythro*-pent-4-enofuranosyl)-adenine],[4] the homovinyl halides **A**[5] and **B**,[6] and the 4′-acetylenic[5,7] **E** (X = H) and 4′-(halo)acetylenic[8] **E** (X = I) and **F** (X = Br, Cl) adenosine derivatives, among others[1c] (Figures 1 and 2). Addition of an enzyme-

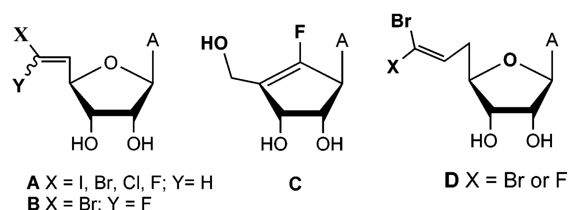


Figure 1. Selected inhibitors of AdoHcy hydrolase.

sequestered water across the 5′,6′-triple bond of 6′-bromo or 6′-chloro acetylenes **F** followed by tautomerization of the hydroxyvinyl intermediates was postulated to generate α-halomethyl ketone **G** (C5′ addition) and/or acyl halide **I** (C6′ addition) electrophiles at the enzyme active site without oxidation of the 3′-hydroxyl group (Figure 2). Subsequent nucleophilic attack by proximal amino acid functionalities caused type II (covalent binding) inhibition[8b] (similar reactive electrophiles of type **I** were generated during inhibition with dihalohomovinyl **B**).[6b] Contrary to these results, inactivation of the AdoHcy hydrolase by the 4′-acetylenic derivative **E** (X = H)[7a] and its 6′-iodo analogue **E** (X = I)[8b] involves 3′-oxidative activity in the "activation" of the inhibitor (**E** → **H**) which resulted in covalent modification of AdoHcy hydrolase by allenic 3′-ketones (**K** → **J**).

The X-ray crystal structures of AdoHcy hydrolase revealed an unusual dual role for a catalytic water

* To whom correspondence should be addressed at Florida International University. Tel: 305-348-6195. Fax: 305-348-3772. E-mail: wnuk@fiu.edu.
† Florida International University.
‡ Faculty leave from the Department of Chemistry, University of Agriculture, Poznan, Poland.
§ University of Kansas.
|| Katholieke Universiteit Leuven.

10.1021/jm040054+ CCC: $27.50 © 2004 American Chemical Society
Published on Web 09/08/2004

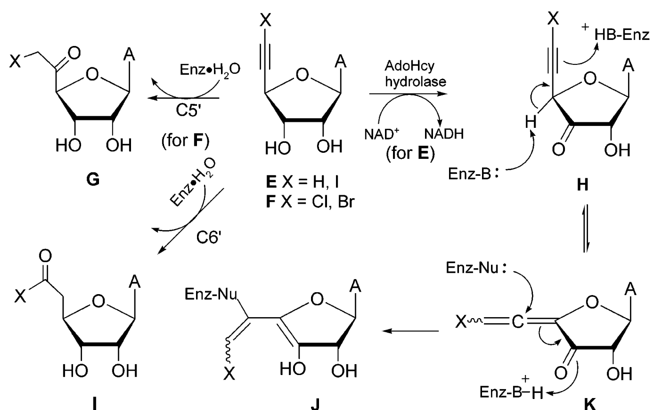


Figure 2. Mechanisms of inactivation of AdoHcy hydrolase by the acetylenic and (halo)acetylenic adenosine analogues **E** and **F**.

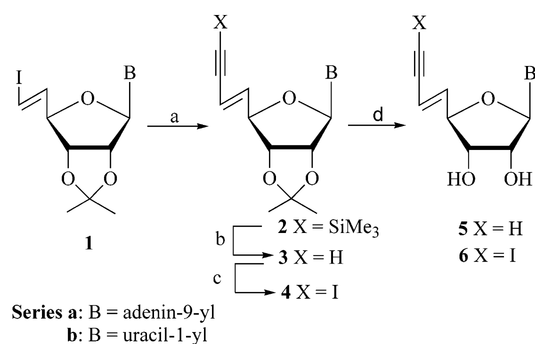
molecule at the active site.[9] Recent computational, kinetic, and crystallographic studies provide insight into the conformational changes of the enzyme required for substrate binding and catalysis.[10] Guillerm et al. reported that 5′-thioadenosine analogues substituted at sulfur with difluoromethyl[11a] and allenyl and propynyl groups[11b] were processed by "hydrolytic activity" of the enzyme causing type II inactivation. Jeong et al. recently showed that fluoroneplanocin A **C** is a potent irreversible inhibitor of the AdoHcy hydrolase and also covalently traps the enzyme.[12] Interestingly, the doubly homologated vinyl halides[13] **D**, with greater conformational flexibility at C5′ relative to vinylogous **A** and **B**, as well as 5′-diene derivatives[14] were not substrates for the hydrolytic activity of the AdoHcy hydrolase but were type I inhibitors.

We now describe the synthesis of the sugar-modified enyne analogues of uridine and adenosine; their antiviral and cytotoxic activities; and the interaction of adenosine analogues with AdoHcy hydrolase. The adenosine derivatives, with extended conjugation to the furanosyl ring, provide different stereochemical and conformational attributes for proper binding with AdoHcy hydrolase and/or 1,2- or 1,4-addition processes of enzyme-bound water across the conjugated enyne system. Base-induced isomerization of the enynes **5a**, **6a**, **9a**, and **13** via the abstraction of H4′ might provide access to various allenic nucleosides. Isomerization of enynes to reactive allenic intermediates at the active site of AdoHcy hydrolase will be more apparent after enzymatic oxidation of OH3′ and activation of H4′.

Chemistry

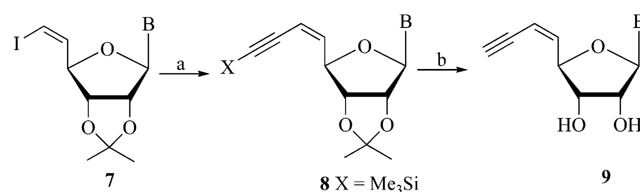
We have prepared the conjugated enyne analogues with defined stereochemistry employing Pd-catalyzed coupling reactions[15] of 6′-iodohomovinyl adenosine[5,16] and uridine[17] precursors. The nucleoside precursors were prepared from the readily available vinyl sulfones by stannylodesulfonylation followed by iododestannylation sequence. Thus, Sonogashira[18] coupling [CuI/$(PPh_3)_2PdCl_2/Et_2NH$][18a] of (*E*)-6′-iodohomovinyl adenosine[5] **1a** with (trimethylsilyl)acetylene gave the conjugated enyne **2a** (88%) with expected *E*-stereochemistry ($J_{5'-6'}$ = 15.9 Hz; Scheme 1). Treatment of **2a** with tetrabutylammonium fluoride (TBAF) effected desilylation to give **3a**, while reaction of **2a** with trifluoroacetic acid (TFA)/H_2O resulted in concomitant desilylation and

Scheme 1[a]



[a] Reagents: (a) TMS-acetylene/CuI/$(PPh_3)_2PdCl_2/Et_2NH$; (b) TBAF/THF; (c) NIS/$AgNO_3$/Me_2CO; (d) TFA/H_2O.

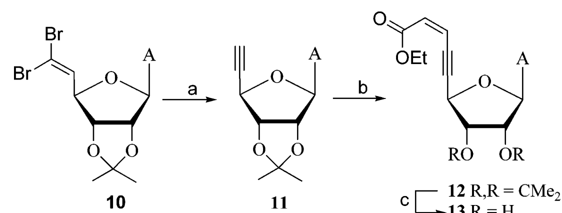
Scheme 2[a]



Series **a**: B = adenin-9-yl
b: B = uracil-1-yl

[a] Reagents: (a) TMS-acetylene/CuI/$(PPh_3)_2PdCl_2/Et_2NH$; (b) TFA/H_2O.

Scheme 3[a]



[a] Reagents: (a) BuLi/THF/−78 °C; (b) (Z)-ICH=CH_2CO_2Et/CuI/$(PPh_3)_2PdCl_2/Et_2NH$; (c) TFA/H_2O.

deacetonization to afford **5a** in 85% overall yield. Treatment of **3a** with *N*-iodosuccinimide (NIS)/$AgNO_3$[19] gave 8′-iodoenyne **4a** (84%). Deprotection and purification on a silica gel column yielded 8′-iodoenyne adenosine analogue **6a**. Subjection of the (*E*)-6′-iodohomovinyl uridine[17b] **1b** to the coupling, desilylation, and iodination sequence gave conjugated enyne **3b** and 8′-iodoenyne **4b** in good overall yields. Deprotection of **3b** and **4b** yielded **5b** and **6b**, respectively.

Sonogashira coupling of (*Z*)-6′-iodohomovinyl analogues **7a**[5] and **7b**[17b] produced (*Z*)-8′-(trimethylsilyl)-enynes of the purine **8a** (86%; $J_{5'-6'}$ = 11.0 Hz) and pyrimidine **8b** (92%) nucleosides (Scheme 2). Treatment with TFA/H_2O effected simultaneous desilylation and deacetonization to give conjugated enynes **9a** and **9b**. The adenosine *Z* isomer **9a** requires a different stereochemical vicinity for binding with AdoHcy hydrolase and/or addition of enzyme-bound water than the corresponding *E* isomers **5a** or **6a**.

Enyne analogue (e.g., **13**; Scheme 3) with a linear triple bond attached to C4′ was also synthesized. Stereoselective Pd-catalyzed coupling of terminal acetylenic **11** and ethyl (*Z*)-3-bromoacrylate gave **12** (86%) with the conjugated enyne system attached in the reverse orientation at C4′ (Scheme 3). Acetylenic **11** was con-

Table 1. Kinetic Constants for Inhibition of Recombinant Human Placental AdoHcy Hydrolase by **5a** and **6a**[a]

inhibitor	K_i (μM)	k_{inact} (min^{-1})	k_{inact}/K_i (μM^{-1} min^{-1})
5a	0.55	2.57	4.67
6a	118.5	1.49	0.01

[a] Various concentrations (0–200 μM) of the potential inhibitors were incubated with AdoHcy hydrolase for varying times (0–20 min). Remaining enzyme activity was then determined as described by Yang et al.[8b] The data were analyzed as described by Kitz and Wilson[20] affording K_i and k_{inact} values for each compound.

veniently prepared by double dehydrobromination of 5′-dibromomethylene derivative **10** with BuLi[7b,8a] or oxidative destannylation of 6′-(tributylstannyl)homovinyl adenosine with $Pb(OAc)_4$.[5] Deprotection of **12** produced the conjugated enyne **13** with 9′-carboxylate function which might approach the binding site for the carboxylate function of AdoHcy. Analogue **13** with the linear triple bond attached to C4′ would require a different vicinity for binding and/or addition of water across the enyne system, as compared to the analogues **5a**, **6a**, and **9a**.

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

Enynes **5a**, **6a**, **9a**, and **13** were initially evaluated for their ability to inhibit the activity of recombinant human placental AdoHcy hydrolase by incubating the enzyme with the compounds at 200 μM for 20 min at 37 °C. The AdoHcy hydrolase activity was determined by assaying the enzyme's ability to catalyze the conversion of Ado and Hcy to AdoHcy. Under these conditions, both **5a** and **6a** reduced the enzyme activity by >85%. In contrast, **9a** reduced the enzyme activity by only 15%. Because of the propensity of **13**, an ethyl ester, to undergo hydrolysis in the assay buffer, meaningful inhibitory data with this compound could not be obtained.

Compounds **5a** and **6a** produced time-dependent and concentration-dependent inhibition of AdoHcy hydrolase, and these data were then used to calculate K_i and k_{inact} values (Table 1).[20] When 200 μM **6a** was incubated with AdoHcy hydrolase at 37 °C for 20 min, no reduction in NAD^+ content of the enzyme was observed and no iodide ion was released into assay buffer (data not shown). When 200 μM **5a** was incubated with AdoHcy hydrolase at 37° for 20 min, a slight reduction (approximately 30%) in the NAD^+ content of the enzyme was observed.

It is interesting to compare the data reported here for **5a**, **6a**, and **9a** with the data reported previously for the 4′-acetylenic **E** (X = H)[5,7a] and 4′-(iodo)acetylenic **E** (X = I)[8] analogues. Enynes **5a** and **9a** are structurally related to the 4′-acetylenic analogue **E** (X = H). However each has an extended conjugation to the furanosyl ring. Obviously, the *E*-isomer **5a** of these conjugated analogues exhibits higher binding to AdoHcy hydrolase than the *Z*-isomer **9a**. If one compares the affinities of **5a** (K_i = 0.55 μM) and the 4′-acetylenic **E** (X = H, K_i = 0.68 μM),[5] they are nearly identical. However, **5a** has a k_{inact} value (2.57 min^{-1}) that was approximately 10 times higher than the k_{inact} value (0.25 min^{-1}) reported for the 4′-acetylenic analogue **E** (X = H).[5] While detailed mechanistic studies of the inhibition of AdoHcy hydrolase by **5a** have not yet been conducted, it is interesting to note that this compound did cause a partial reduction in the enzyme's NAD^+ content. This observation would be consistent with the mechanism reported by Parry et al. for the inhibition of AdoHcy hydrolase by **E** (X = H; i.e., the enzyme catalyzes the oxidation of the 3′-hydroxyl group of the nucleoside leading to the generation of a reactive intermediate that covalently binds to the enzyme as depicted in Figure 2).[7a]

If one compares the affinity of **6a** (K_i = 118.5 μM) with the affinity of the 4′-(iodo)acetylenic analogue **E** (X = I, K_i = 1.10 μM)[8a] to AdoHcy hydrolase, it is readily apparent that the extended conjugation to the furanosyl ring in **6a** has a detrimental effect on binding to the enzyme. On the other hand, this structural feature appears to have an enhancing effect on the rate at which the compound inactivates the enzyme [**6a**, k_{inact} = 1.49 min^{-1}; **E** (X = I), k_{inact} = 0.095 min^{-1}].[8a] Similar to the 4′-(iodo)acetylenic **E** (X = I),[8b] **6a** did not release iodide ion into the assay buffer upon incubation with AdoHcy hydrolase. In addition, **6a** did not reduce significantly the NAD^+ content of the enzyme. If we had seen a reduction in the NAD^+ content of the enzyme when incubated with **6a**, this would have suggested a mechanism of enzyme inactivation similar to that of the 4′-(iodo)acetylenic analogue **E** (X = I; i.e., the enzyme catalyzes the oxidation of the 3′-hydroxyl group of the nucleoside leading to generation of a reactive intermediate that covalently binds to the enzyme). It is also unlikely that the enzyme's hydrolytic activity adds water across the 7′–8′ triple bond. Such an addition would generate intermediates structurally related to **G** and **I** depicted in Figure 2. These intermediates would likely be reactive with proteins to release iodide ion. As noted above, no release of iodide ion was observed upon incubation of **6a** with AdoHcy hydrolase. However, we cannot rule out at this time the possibility that enzyme adds water across the 5′–6′ double bond of **6a**, which would yield either the 5′ or 6′ alcohol.

Antiviral and Cytotoxic Activities

Adenosine **5a**, **6a**, **9a**, and **13** as well as uridine **5b**, **6b**, and **9b** analogues were examined for their antiviral and cytotoxic activity in a broad variety of test systems. The *E*-iodoenynes **6a** and **6b** showed inhibition of viral replication when evaluated in an MT-4/MTT based assay against human immunodeficiency virus (HIV-1, III_B strain; HIV-2, ROD strain) at a 50% inhibitory concentration (IC_{50}) of 1.1 and 1.8 μM, respectively. Both compounds proved also to be cytotoxic with a selectivity index (CC_{50}/IC_{50}) equal to 7 (**6a**) and 3 (**6b**). However, the antiviral activity was lost on keeping the compounds in solution. No antiviral effects were noted with **5a**, **5b**, **9a**, **9b**, and **13** at subtoxic concentrations.

In human embryonic lung (HEL) cells, no activity was noted against herpes simplex virus (HSV-1, KOS and TK^- KOS ACV^r strains; HSV-2, G strain), vaccinia virus (VV), or vesicular stomatitis virus (VSV) at subtoxic concentrations. In HeLa cell cultures, the enynes showed no activity against vesicular stomatitis virus, Coxsackie B4 virus, or respiratory syncytial virus [minimum inhibitory concentration (MIC) >100 μM (**5a**, **6a**, **6b**, **9b**, and **13**) and >700 μM (**5b**, **9a**)]. In Vero cell cultures, again, no specific activity against parainfluenza virus type 3, reovirus type 1, Sindbis virus, Coxsackie B4

virus, or Punta Toro virus was noted at subtoxic concentrations (Table 2, Supporting Information).

The enynes (except for **5a**) showed no appreciable activity against human cytomegalovirus (CMV, strains AD-169 and Davis) and varicella-zoster virus (VZV, strains OKA and 07/1) in HEL cells. The minimum cytotoxic concentrations were $\geq$350 μM for **5a**,**b** and **9a**,**b**, $\geq$11 μM for **13**, and $\geq$50 μM for **6a**,**b** (Table 3, Supporting Information). The *E*-enyne **5a** showed activity against CMV [50% effective concentration (EC_{50}), 50 μM (AD-169 strain) and 30 μM (Davis strain)], at a concentration that was 3- to 10-fold lower than the cytotoxic concentration (CC_{50})].

Summary and Conclusions

The novel *E*- and *Z*-enyne **5** and **9** as well as 8′-(iodo)-enyne **6** analogues with the extended conjugation to the ribose ring at C4′ were prepared by Sonogashira cross coupling between the 6′-iodohomovinyl uridine and adenosine analogues and (trimethlysilyl)acetylene. Enyne analogue **13** with the linear triple bond attached to C4′ was synthesized by Pd-catalyzed coupling of terminal adenosine 4′-acetylenic and ethyl (*Z*)-3-bromoacrylate. The adenosine analogues **5a**, **6a**, **9a**, and **13** were found to be time-dependent and concentration-dependent inhibitors of AdoHcy hydrolase [**5a** (K_i, 0.55 μM) and **6a** (K_i, 118.5 μM)]. No reduction in NAD^+ content of the enzyme and no iodide ion release was observed upon incubation of 8′-iodoenyne **6a** with the enzyme, while incubation of enyne **5a** produced 30% reduction in the NAD^+ content of the enzyme. This observation for **5a** would be consistent with the mechanism reported by Parry et al.[7a] for the inhibition of AdoHcy hydrolase by **E** (X = H; i.e., the enzyme catalyzes the oxidation of the 3'-hydroxyl group of the nucleoside leading to the generation of a reactive intermediate that covalently binds to the enzyme). In contrast **6a** appears to inactivate the enzyme by a yet unknown mechanism that does not involve either the oxidative (not dependent on NAD) or the 7′−8′ hydrolytic (no release of iodide ion) activities of this enzyme. We cannot at this time rule out the possible involvement of the enzyme's 5′−6′ hydrolytic activity in the mechanism of inactivation by **6a**. As to their antiviral activity, the *E*-iodoenynes **6a** and **6b** showed activity against HIV-1 (IC_{50}, 1.1 and 1.8 μM) with a selectivity index of 7 and 3, respectively. The *E*-enyne **5a** proved active against cytomegalovirus at a concentration (EC_{50}, 30 μM) that was 3- to 10-fold lower than the cytotoxic concentration.

Experimental Section

Uncorrected melting points were determined with a capillary apparatus. UV spectra were measured with solutions in MeOH. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectra were determined with solutions in $CDCl_3$ unless otherwise noted. Mass spectra (MS) were obtained with an atmospheric pressure chemical ionization (APCI) technique. Merck kieselgel 60-F_{254} sheets were used for TLC, and products were detected with 254 nm light or by development of color with $Ce(SO_4)_2/(NH_4)_6Mo_7O_{24}\cdot 4H_2O/H_2SO_4/H_2O$. Merck kieselgel 60 (230−400 mesh) was used for column chromatography. Analytical RP-HPLC for enzymatic assays was performed with Vydac C18 (5 μm, 250 × 4.6 mm; Hesperia, CA) column. Elemental analyses were determined by Galbraith Laboratories, Knoxville, TN. Reagent grade chemicals were used, and solvents were dried by reflux over and distillation from CaH_2 (except THF/potassium) under argon.

9-[5,6,7,8-Tetradeoxy-2,3-*O*-isopropylidene-8-(trimethylsilyl)-*β*-D-*ribo*-oct-5(*E*)-en-7-ynofuranosyl]adenine (2a). Procedure A. (Trimethylsilyl)acetylene (0.026 mL, 18.3 mg, 0.186 mmol) was added to a solution of **1a**[5] (50 mg, 0.12 mmol) in diethylamine (2.5 mL) containing CuI (1.14 mg, 0.006 mmol) and dichlorobis(triphenylphoshine)palladium(II) (1.7 mg, 0.0024 mmol). The reaction mixture was heated at 50 °C for 30 min. TLC showed formation of less polar compound. The volatiles were evaporated, and the brown residue was partitioned (EtOAc/H_2O). The organic layer was washed (brine), dried (Na_2SO_4), and evaporated, and the residue was column chromatographed (EtOAc/hexane; 1:1) to give **2a** (41 mg, 88%): ^{1}H NMR δ 0.17 (s, 9, CH_3), 1.35 (s, 3, CH_3), 1.68 (s, 3, CH_3), 4.68 (ddd, *J* = 2.4, 3.5, 7.0 Hz, 1, H4′), 5.03 (dd, *J* = 3.5, 6.2 Hz, 1, H3′), 5.52 (dd, *J* = 1.9, 6.3 Hz, 1, H2′), 5.70 (dd, *J* = 1.2, 15.9 Hz, 1, H6′), 5.94 (s, 2, NH_2), 6.11 (d, *J* = 1.9 Hz, 1, H1′), 6.25 (dd, *J* = 7.0, 15.9 Hz, 1, H5′), 7.90 (s, 1, H8), 8.38 (s, 1, H2); MS *m*/*z* 400 (100, MH^+).

1-[5,6,7,8-Tetradeoxy-2,3-*O*-isopropylidene-8-(trimethylsilyl)-*β*-D-*ribo*-oct-5(*E*)-en-7-ynofuranosyl]uracil (2b). Treatment of **1b**[17b] (40 mg, 0.1 mmol) with TMS-acetylene (0.023 mL, 15.7 mg, 0.16 mmol) by procedure A [column chromatography (1 → 3% MeOH/$CHCl_3$)] gave **2b** (30 mg, 81%): ^{1}H NMR δ 0.21 (s, 9, CH_3), 1.30 (s, 3, CH_3), 1.61 (s, 3, CH_3), 4.55 (dd, *J* = 4.1, 7.2 Hz, 1, H4′), 4.80 (dd, *J* = 4.2, 6.3 Hz, 1, H3′), 5.05 (dd, *J* = 1.7, 6.4 Hz, 1, H2′), 5.62 (d, *J* = 1.6 Hz, 1, H1′), 5.77 (d, *J* = 15.8 Hz, 1, H6′), 5.82 (d, *J* = 8.0 Hz, 1, H5), 6.28 (dd, *J* = 7.3, 15.9 Hz, 1, H5′), 7.21 (d, *J* = 8.0 Hz, 1, H6), 9.0 (s, 1, NH); MS *m*/*z* 377 (100, MH^+).

9-[5,6,7,8-Tetradeoxy-2,3-*O*-isopropylidene-*β*-D-*ribo*-oct-5(*E*)-en-7-ynofuranosyl]adenine (3a). Procedure B. TBAF (0.1 mL, 1 M/THF, 0.1 mmol) was added to a stirred solution of **2a** (40 mg, 0.1 mmol) in THF (4 mL) at ambient temperature. The reaction mixture was stirred for 30 min, volatiles were evaporated, and the brown residue was partitioned ($CHCl_3$//H_2O/$NaHCO_3$). The organic layer was washed (brine), dried (Na_2SO_4), and evaporated, and the residue was column chromatographed ($CHCl_3$/MeOH; 98:2) to give **3a** (31 mg, 92%): ^{1}H NMR δ 1.35 (s, 3, CH_3), 1.68 (s, 3, CH_3), 2.90 (d, *J* = 1.8 Hz, 1, H8′), 4.70−4.76 (m, 1, H4′), 5.10 (dd, *J* = 3.5, 6.3 Hz, 1, H3′), 5.55 (dd, *J* = 1.8, 6.3 Hz, 1, H2′), 5.65 (dm, *J* = 15.9 Hz, 1, H6′), 5.79 (s, 2, NH_2), 6.12 (d, *J* = 1.8 Hz, 1, H1′), 6.31 (dd, *J* = 7.0, 15.9 Hz, 1, H5′), 7.88 (s, 1, H8), 8.38 (s, 1, H2); MS *m*/*z* 328 (100, MH^+).

1-[5,6,7,8-Tetradeoxy-2,3-*O*-isopropylidene-*β*-D-*ribo*-oct-5(*E*)-en-7-ynofuranosyl]uracil (3b). Treatment of **2b** (52 mg, 0.13 mmol) with TBAF (0.13 mL, 1 M/THF, 0.13 mmol) by procedure B gave **3b** (39 mg, 64%): ^{1}H NMR δ 1.34 (s, 3, CH_3), 1.67 (s, 3, CH_3), 2.97 (d, *J* = 2.2 Hz, 1, H8′), 4.56 (dd, *J* = 4.3, 6.4 Hz, 1, H4′), 4.84 (dd, *J* = 4.3, 6.3 Hz, 1, H3′), 5.09 (dd, *J* = 1.6, 6.4 Hz, 1, H2′), 5.61 (d, *J* = 1.6 Hz, 1, H1′) 5.75 (d, *J* = 16.0 Hz, 1, H6′), 5.78 (d, *J* = 8.0 Hz, 1, H5), 5.37 (dd, *J* = 7.2, 16.0 Hz, 1, H5′), 7.22 (d, *J* = 8.0 Hz, 1, H6); MS *m*/*z* 305 (100, MH^+).

9-[5,6,7,8-Tetradeoxy-8-iodo-2,3-*O*-isopropylidene-*β*-D-*ribo*-oct-5(*E*)-en-7-ynofuranosyl]adenine (4a). Procedure C. A solution of **3a** (19 mg, 0.058 mmol), NIS (15.7 mg, 0.07 mmol), and $AgNO_3$ (0.1 mg, 0.0058 mmol) in acetone (3 mL) was stirred for 3 h at ambient temperature. The volatiles were evaporated, the residue was partitioned ($CHCl_3$/H_2O), and the organic layer was dried (Na_2SO_4), evaporated, and column chromatographed ($CHCl_3$ → 2% MeOH) to give **4a** (22 mg, 84%): ^{1}H NMR δ 1.33 (s, 3, CH_3), 1.63 (s, 3, CH_3), 4.70 (ddd, *J* = 2.3, 3.4, 5.8 Hz, 1, H3′), 5.06 (dd, *J* = 3.4, 6.2 Hz, 1, H4′), 5.50 (dd, *J* = 1.7, 6.3 Hz, 1, H2′), 5.76 (dd, *J* = 1.3, 15.8 Hz, 1, H6′), 6.12 (d, *J* = 1.8 Hz, 1, H1′), 6.19 (s, 2, NH_2), 6.22 (dd, *J* = 6.9, 15.8 Hz, 1, H5′), 7.91 (s, 1, H8), 8.39 (s, 1, H2); MS *m*/*z* 454 (100, MH^+).

1-[5,6,7,8-Tetradeoxy-8-iodo-2,3-*O*-isopropylidene-*β*-D-*ribo*-oct-5(*E*)-en-7-ynofuranosyl]uracil (4b). Treatment of **3b** (30 mg, 0.10 mmol) with NIS (27 mg, 0.12 mmol) and $AgNO_3$ (1.7 mg, 0.01 mmol) in acetone (3 mL) by procedure C gave **4b** (29 mg, 68%): ^{1}H NMR δ 1.35 (s, 3, CH_3), 1.62 (s, 3, CH_3), 4.56 (dd, *J* = 4.2, 6.1 Hz, 1, H3′), 4.83 (dd, *J* = 4.1, 6.3

Hz, 1, H4′), 5.11 (dd, $J = 1.5$, 6.3 Hz, 1, H2′), 5.58 (d, $J = 1.4$ Hz, 1, H1′), 5.76 (d, $J = 8.1$ Hz, 1, H5), 5.86 (dd, $J = 1.2$, 15.9 Hz, 1, H6′), 6.29 (dd, $J = 7.2$, 15.9 Hz, 1, H5′), 7.22 (d, $J = 8.1$ Hz, 1, H6), 9.10 (s, 1, NH); MS m/z 431 (100, MH^+).

9-[5,6,7,8-Tetradeoxy-β-D-*ribo*-oct-5(*E*)-en-7-ynofuranosyl]adenine (5a). Procedure D. A solution of **2a** (32 mg, 0.08 mmol) in CF_3CO_2H/H_2O (9:1, 1.5 mL) was stirred at 0 °C for 1 h. The volatiles were evaporated, and the residue was coevaporated (3×) with toluene. The residue was column chromatographed ($CHCl_3$/MeOH; 95:5) to give **5a** (19 mg, 85%) as a white solid: mp 107−109 °C dec; UV max 260 nm (ϵ 15500), min 233 nm (ϵ 10800); ^1H NMR (MeOH-d_4) δ 3.36 (d, $J = 2.0$ Hz, 1, H8′), 4.31 ("t", $J = 5.2$ Hz, 1, H3′), 4.52 ("t", $J = 5.8$ Hz, 1, H4′), 4.73 ("t", $J = 4.7$ Hz, 1, H2′), 5.84 (dm, $J = 15.9$ Hz, 1, H6′), 6.06 (d, $J = 4.5$ Hz, 1, H1′), 6.47 (dd, $J = 6.0$, 15.9 Hz, 1, H5′), 7.99 (s, 1, H8), 8.31 (s, 1, H2); ^{13}C NMR (MeOH-d_4) δ 72.9 and 74.1 (C2′/C3′), 79.4 (C8′), 81.4 (C7′), 84.3 (C4′), 88.8 (C1′), 112.6 (C6′) 119.4 (C5), 139.6 (C5′), 142.4 (C8), 148.6 (C4), 153.2 (C2), 156.5 (C6); MS m/z 288 (100, MH^+). Anal. [$C_{13}H_{13}N_5O_3 \cdot CH_3OH$ (319.30)] C, H, N.

1-[5,6,7,8-Tetradeoxy-β-D-*ribo*-oct-5(*E*)-en-7-ynofuranosyl]uracil (5b). Treatment of **2b** (39 mg, 0.10 mmol) with CF_3CO_2H/H_2O (9:1, 1.5 mL) by procedure D gave **5b** (22 mg, 81%) as a white solid: mp 148−150 °C dec; UV max 262 nm (ϵ 9200), min 245 nm (ϵ 7000); ^1H NMR (MeOH-d_4) δ 3.38 (d, $J = 2{,}2$ Hz, 1, H8′), 3.99 ("t", $J = 5.9$ Hz, 1, H3′), 4.20 (dd, $J = 3.7$, 5.3 Hz, 1, H2′), 4.38 ("t", $J = 6.1$ Hz, 1, H4′), 5.65 (d, $J = 8.0$ Hz, 1, H5), 5.83 (d, $J = 3.7$ Hz, 1, H1′), 5.81 (dm, $J = 15.8$ Hz, 1, H6′), 6.37 (dd, $J = 6.9$, 15.9 Hz, 1, H5′), 7.64 (d, $J = 8.0$ Hz, 1, H6); ^{13}C NMR (MeOH-d_4) δ 73.4 and73.8 (C2′/C3′), 79.4 (C8′), 81.0 (C7′), 83.6 (C4′), 91.3 (C1′), 101.9 (C5), 112.1 (C6′), 141.3 (C5′), 141.6 (C6), 151.2 (C2), 165.3 (C4); MS m/z 265 (100, MH^+). Anal. [$C_{12}H_{12}N_2O_5 \cdot 0.5MeOH$ (280.56)] C, H, N.

9-[5,6,7,8-Tetradeoxy-8-iodo-β-D-*ribo*-oct-5(*E*)-en-7-ynofuranosyl]adenine (6a). Treatment of **4a** (22 mg, 0.049 mmol) with CF_3CO_2H/H_2O (9:1, 1.5 mL) by procedure D gave **6a** (14 mg, 72%): UV max 255 nm (ϵ 14200), min 225 nm (ϵ 8400); ^1H NMR (Me_2SO-d_6) δ 4.13 ("t", $J = 4.4$ Hz, 1, H3′), 4.37 (dd, $J = 4.3$, 7.1 Hz, 1, H4′), 4.66 ("t", $J = 5.0$ Hz, 1, H2′), 5.55 (br s, 1, OH), 5.65 (br s, 1, OH), 5.91 (d, $J = 5.3$ Hz, 1, H1′), 5.92 (dd, $J = 1.1$, 15.8 Hz, 1, H6′), 6.38 (dd, $J = 7.2$, 15.8 Hz, 1, H5′), 7.33 (s, 2, NH_2), 8.16 (s, 1, H8), 8.36 (s, 1, H2); ^{13}C NMR (Me_2SO-d_6) δ 70.6 (C8′), 73.5 and 74.7 (C2′/C3′), 84.4 (C4′), 88.3 (C7′), 91.7 (C1′), 112.9 (C6′) 120.0 (C5), 140.7 (C5′), 143.7 (C8), 150.3 (C4), 153.5 (C2), 156.9 (C6); MS m/z 414 (100, MH^+). Anal. [$C_{13}H_{12}IN_5O_3$ (413.17)] C, H, N.

1-[5,6,7,8-Tetradeoxy-8-iodo-β-D-*ribo*-oct-5(*E*)-en-7-ynofuranosyl]uracil (6b). Treatment of **4b** (19 mg, 0.044 mmol) with CF_3CO_2H/H_2O (9:1, 1.5 mL) by procedure D gave **6b** (13 mg, 74%): UV max 255 nm (ϵ 13500), min 224 nm (ϵ 8600); ^1H NMR (MeOH-d_4) δ 3.81 ("t", $J = 5.6$ Hz, 1, H3′), 4.20 (dd, $J = 3.8$, 5.4 Hz, 1, H2′), 4.37 ("t", $J = 6.5$ Hz, 1, H4′), 5.70 (d, $J = 8.1$ Hz, 1, H5), 5.79 (d, $J = 3.7$ Hz, 1, H1′), 5.96 (dd, $J = 1.2$, 15.8 Hz, 1, H6′), 6.28 (dd, $J = 6.9$, 15.8 Hz, 1, H5′), 7.59 (d, $J = 8.1$ Hz, 1, H6), 9.1 (s, 1, NH); ^{13}C NMR (MeOH-d_4) δ 73.9 (C8′), 74.0 and 74.5 (C2′/C3′), 83.5 (C4′), 90.9 (C7′), 91.2 (C1′), 101.9 (C5), 113.2 (C6′), 141.4 (C6), 141.5 (C5′), 151.2 (C2), 165.1 (C4); MS m/z 391 (100, MH^+). Anal. [$C_{12}H_{11}IN_2O_5$ (390.53)] C, H, N.

9-[5,6,7,8-Tetradeoxy-2,3-*O*-isopropylidene-8-(trimethylsilyl)-β-D-*ribo*-oct-5(*Z*)-en-7-ynofuranosyl]adenine (8a). Treatment of **7a**[5] (40 mg, 0.09 mmol) with TMS-acetylene (0.021 mL, 14.6 mg, 0.15 mmol) by procedure A gave **8a** (32 mg, 86%): ^1H NMR δ 0.23 (s, 9, CH_3), 1.35 (s, 3, CH_3), 1.68 (s, 3, CH_3), 5.14 (dd, $J = 3.3$, 6.2 Hz, 1, H3′), 5.32 (dd, $J = 2.4$, 8.5 Hz, 1, H4′), 5.56 (dd, $J = 1.6$, 6.2 Hz, 1, H2′), 5.77 (s, 2, NH_2), 5.65 (dd, $J = 1.0$, 11.0 Hz, 1, H6′), 5.97 (dd, $J = 8.7$, 11.0 Hz, 1, H5′), 6.13 (d, $J = 1.6$ Hz, 1, H1′), 7.90 (s, 1, H8), 8.38 (s, 1, H2); MS m/z 400 (100, MH^+).

1-[5,6,7,8-Tetradeoxy-2,3-*O*-isopropylidene-8-(trimethylsilyl)-β-D-*ribo*-oct-5(*Z*)-en-7-ynofuranosyl]uracil (8b). Treatment of **7b**[17b] (90 mg, 0.22 mmol) with TMS-acetylene (0.05 mL, 34.5 mg, 0.35 mmol) by procedure A gave [column chromatography (1 → 3% MeOH/$CHCl_3$)] **8b** (76 mg, 92%): ^1H NMR δ 0.14 (s, 9, CH_3), 1.25 (s, 3, CH_3), 1.58 (s, 3, CH_3), 4.75 (dd, $J = 4.0$, 6.5 Hz, 1, H3′), 5.09 (dd, $J = 1.5$, 6.5 Hz, 1, H2′), 5.21 (dd, $J = 4.0$, 8.5 Hz, 1, H4′), 5.58 (d, $J = 1.5$ Hz, 1, H1′), 5.73 (d, $J = 10.0$ Hz, 1, H6′), 5.82 (d, $J = 8.0$ Hz, 1, H5), 6.08 (dd, $J = 7.8$, 10.5 Hz, 1, H5′), 7.25 (d, $J = 8.0$ Hz, 1, H6), 9.2 (s, 1, NH); MS m/z 377 (100, MH^+).

9-[5,6,7,8-Tetradeoxy-β-D-*ribo*-oct-5(*Z*)-en-7-ynofuranosyl]adenine (9a). Treatment of **8a** (33 mg, 0.083 mmol) with CF_3CO_2H/H_2O (9:1, 1.5 mL) by procedure D gave **9a** (16 mg, 67%) as a white solid: UV max 259 nm (ϵ 15700), min 242 nm (ϵ 8500); ^1H NMR (MeOH-d_4) δ 3.67 (d, $J = 2.3$ Hz, 1, H8′), 4.30 ("t", $J = 4.4$ Hz, 1, H3′), 4.94 (H2′, collapsed with solvent signal), 5.09 (dd, $J = 4.0$, 9.0 Hz, 1, H4′), 5.76 (dm, $J = 11.0$ Hz, 1, H6′), 6.03 (d, $J = 5.4$ Hz, 1, H1′), 6.39 ("t", $J = 10.0$ Hz, 1, H5′), 8.23 (s, 1, H2), 8.28 (s, 1, H8); ^{13}C NMR (MeOH-d_4) δ 73.9, 75.3 (C2′/C3′), 78.9 (C7′), 82.8 (C8′), 84.4 (C4′), 89.3 (C1′), 111.7 (C6′) 119.6 (C5), 140.8 (C5′), 141.8 (C8), 149.6 (C4), 152.8 (C2), 156.3 (C6); MS m/z 288 (100, MH^+). Anal. Calcd for [$C_{13}H_{13}N_5O_3 \cdot 1.25H_2O$ (309.77)] C, H, N.

1-[5,6,7,8-Tetradeoxy-β-D-*ribo*-oct-5(*Z*)-en-7-ynofuranosyl]uracil (9b). Treatment of **8b** (27 mg, 0.07 mmol) with CF_3CO_2H/H_2O (9:1, 1.5 mL) by procedure D gave **9b** (12 mg, 65%): UV max 260 nm (ϵ 9200), min 243 nm (ϵ 6300); ^1H NMR (Me_2OH-d_4) δ 3.66 (d, $J = 2.3$ Hz, 1, H8′), 4.04 ("t", $J = 5.2$ Hz, 1, H3′), 4.28 ("t", $J = 5.1$ Hz, 1, H2′), 4.97 (dd, $J = 5.1$, 9.0 Hz H4′), 5.74 (d, $J = 8.1$ Hz, 1, H5), 5.78 (dm, $J = 10.9$ Hz, 1, H6′), 5.84 (d, $J = 4.5$ Hz, 1, H1′), 6.61 ("t", $J = 10.0$ Hz, 1, H5′), 7.65 (d, $J = 8.1$ Hz, 1, H6); ^{13}C NMR (MeOH-d_4) δ 73.9 and 74.7 (C2′/C3′), 78.8 (C7′), 81.7 (C8′), 84.4 (C4′), 91.1 (C1′), 101.9 (C5), 112.7 (C6′), 140.9 (C5′), 141.8 (C6), 151.2 (C2), 165.1 (C4); MS m/z 265 (100, MH^+). Anal. [$C_{12}H_{12}N_2O_5.H_2O$ (282.23)] C, H, N.

Ethyl 1-(Adenin-9-yl)-1,5,6,7,8-pentadeoxy-2,3-*O*-isopropylidene-β-D-*ribo*-oct-7(*Z*)-en-5-ynofuranuronate (12). Compound **11**[5,7b] (22 mg, 0.073 mmol) was added to a solution of ethyl 3(*Z*)-iodopropenoate (0.01 mL, 18.1 mg 0.08 mmol), containing CuI (0.76 mg, 0.004 mmol) and dichlorobis(triphenylphoshine)palladium(II) (1.0 mg, 0.0015 mmol). The reaction mixture was heated at 50 °C for 30 min, was purified as described in procedure A [column chromatography (1 → 3% MeOH/$CHCl_3$)], and gave **12** (25 mg, 86%): ^1H NMR δ 1.28 (t, $J = 7.0$ Hz, 3, CH_3), 1.43 (s, 3, CH_3), 1.62 (s, 3, CH_3), 4.17 (q, $J = 7.0$ Hz, 2, CH_2), 5.23 (d, $J = 5.8$ Hz, 1, H3′), 5.27 (s, 1, H4′), 5.72 (d, $J = 5.8$ Hz, 1, H2′), 5.91 (dd, $J = 2.0$, 11.5 Hz, 1, H7′), 5.96 (s, 2, NH_2), 6.10 (d, $J = 11.5$ Hz, 1, H8′), 6.30 (s, 1, H1′), 8.22 (s, 1, H8), 8.32 (s, 1, H2); MS m/z 400 (100, MH^+).

Ethyl 1-(Adenin-9-yl)-1,5,6,7,8-pentadeoxy-β-D-*ribo*-oct-7(Z)-en-5-ynofuranuronate (13). Treatment of **12** (24 mg, 0.06 mmol) with CF_3CO_2H/H_2O (9:1, 1.5 mL) by procedure D gave **13** (17 mg, 79%) as a white solid: UV max 259 nm (ϵ 20400), min 227 nm (ϵ 5600); ^1H NMR (MeOH-d_4) δ 1.31 (t, $J = 7.0$ Hz, 3, CH_3), 4.26 (q, $J = 7.0$ Hz, 2, CH_2), 4.43 (dd, $J = 2.6$, 4.2 Hz, 1, H3′), 4.92 (dd, $J = 2.3$, 4.6 Hz, 1, H4′), 4.98 (dd, $J = 4.3$, 6.0 Hz, 1, H2′), 6.21 (d, $J = 6.1$ Hz, 1, H1′), 6.30 (d, $J = 11.4$ Hz, 1, H8′), 6.36 (dd, $J = 2.3$, 11.4 Hz, 1, H7′), 8.23 (s, 1, H2), 8.59 (s, 1, H8); ^{13}C NMR (MeOH-d_4) δ 13.5 (CH_3), 60.7 (CH_2), 75.1 (C4′), 76.0 and 76.5 (C2′/C3′), 84.5 (C5′), 88.0 (C1′), 96.9 (C6′), 119.1 (C5), 122.2 (C8′), 130.0 (C7′), 140.1 (C8), 150.1 (C4), 153.0 (C2), 156.3 (C6), 165.1 (CO); MS m/z 360 (100, MH^+). Anal. [$C_{16}H_{17}N_5O_5$ (359.34)] C, H, N.

Inactivation of AdoHcy Hydrolase. Recombinant human placental AdoHcy hydrolase (2.5 μg) was incubated with 200 μM of **5a**, **6a**, **9a** and **13** in potassium-phosphate buffer (250 μL; 50 mM, pH 7.2 containing 1 mM EDTA) (buffer A) at 37 °C for 20 min. The remaining enzyme activity was assayed in the synthetic direction essentially as previously described.[8b] The enzyme was incubated with 1 mM Ado and 1 mM Hcy in buffer A at 37 °C for 1 min followed by addition of $HClO_4$ (10 μL, 5 M) to terminate the reaction. The reaction product, AdoHcy, was quantitatively measured using reversed-phase HPLC with the detector monitoring at 258 nm.[8b]

Various concentrations (0−200 μM) of **5a** and **6a** were incubated with AdoHcy hydrolase (2.5 μg) in buffer A at 37

°C for different times (0−20 min). Remaining enzyme activity was then determined as described above. These data were then used to calculate K_i and k_{inact} values by fitting the data to the following equation: $k_{obs} = k_{inact}[I]/(K_i + [I])$.[20]

Determination of the NAD^+ Content of AdoHcy Hydrolase after Incubation with Inhibitors 5a, 6a, and 9a. AdoHcy hydrolase (5.8 μM) was incubated with 200 μM inhibitor in buffer A at 37 °C for 20 min. NAD^+ was then released from the enzyme by the addition of $HClO_4$ (10 μL 5 M). The concentration of NAD^+ was analyzed by HPLC on a C_{18} reverse-phase column (Vydac C18, 5 mM, 250 × 4.5 mm, Hesperia, CA). The column was operated at a flow rate of 1 mL/min solvent A with a linear gradient of 2−98% solvent B over 25 min. Solvent A is 25 mM phosphate, pH 3.2, 10 mM heptane.

Antiviral Activity and Cytotoxicity Assays. The antiviral activity and cytotoxicity activity assays were performed according to well-established procedures.[21]

Acknowledgment. We thank American Heart Association, Florida/Puerto Rico Affiliate (S.F.W.), NIH S06 GM08205 (S.F.W.), and MBRS RISE (L.N.C., R25 GM61347) programs and the United States Public Health Service (R.T.B., Grant GM-29332) for financial support.

Supporting Information Available: Cytotoxicity and antiviral activity data for the different viruses (Tables 2 and 3). Elemental analysis for **5a**,**b**, **6a**,**b**, **9a**,**b**, and **13**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) 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. (b) Turner, M. A.; Yang, X.; Yin, D.; Kuczera, K.; Borchardt, R. T.; Howell, P. L. Structure and Function of *S*-Adenosylhomocysteine Hydrolase. *Cell Biochem. Biophys.* **2000**, *33*, 101−125. (c) Wnuk, S. F. Targeting "Hydrolytic" Activity of the *S*-Adenosyl-L-homocysteine Hydrolase. *Mini-Rev. Med. Chem.* **2001**, *1*, 307−316.

(2) (a) Chiang, P. K. Biological Effects of Inhibitors of *S*-Adenosylhomocysteine Hydrolase. *Pharmacol. Ther.* **1998**, *77*, 115−134. (b) Yuan, C.-S.; Saso, Y.; Lazarides, E.; Borchardt, R. T.; Robins, M. J. Recent Advances in *S*-Adenosyl-L-homocysteine Hydrolase Inhibitors and their Potential Clinical Applications. *Exp. Opin. Ther. Patents* **1999**, *9*, 1197−1206.

(3) (a) Refsum, H.; Ueland, P. M.; Nygard, O.; Vollset, S. E. Homocysteine and Cardiovascular Disease. *Annu. Rev. Med.* **1998**, *49*, 31−62. (b) Schneyder, G.; Roffi, M.; Pin, R.; Flammer, Y.; Lange, H.; Eberli, F. R.; Meier, B.; Turi, Z. G.; Hess, O. M. Decreased rate of coronary restenosis after lowering of plasma homocysteine levels. *N. Engl. J. Med.* **2001**, *345*, 1593−1600.

(4) (a) 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. (b) Yuan, C.-S.; Yeh, J.; Liu, S.; Borchardt, R. T. Mechanism of Inactivation of *S*-Adenosyl-L-homocysteine Hydrolase by Fluorine-containing Adenosine Analogues. *J. Biol. Chem.* **1993**, *268*, 17030−17037.

(5) 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.

(6) (a) Wnuk, S. F.; Mao, Y.; Yuan, C.-S.; Borchardt, R. T.; Andrei, J.; Balzarini, J.; De Clercq, E.; Robins, M. J. Discovery of Type II (Covalent) Inactivation of *S*-Adenosyl-L-homocysteine Hydrolase. Synthesis and Evaluation of Dihalohomovinyl Nucleoside Analogues Derived from Adenosine. *J. Med. Chem.* **1998**, *41,* 3078−3083. (b) 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.

(7) (a) 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. (b) 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.

(8) (a) Robins, M. J.; Wnuk, S. F.; Yang, X.; Yuan, C.-S.; Borchardt, R. T.; Balzarini, J.; De Clercq, E. 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). *J. Med. Chem.* **1998**, *41*, 3857−3864. (b) Yang, X.; Yin, D.; Wnuk, S. F.; Robins, M. J.; Borchardt, R. T. Mechanism of Inactivation of Human *S*-Adenosyl-L-homocysteine Hydrolase by 5′,5′,6′,6′-Tetrahydro-6′-Deoxy-6′-Halohomoadenosines. *Biochemistry* **2000**, *39*, 15234−15241.

(9) (a) Turner, M. A.; Yuan, C.-S.; Borchardt, R. T.; Hershfield, M. S.; Smith, G. D.; Howell, P. L. Structure Determination of Selenomethionyl *S*-Adenosylhomocysteine Hydrolase using data at a single wavelength. *Nat. Struct. Biol.* **1998**, *5*, 369−376. (b) Hu, Y.; Komoto, J.; Huang, Y.; Gomi, T.; Ogawa, H.; Takata, Y.; Fujioka, M.; Takusagawa, F. Crystal Structure of *S*-Adenosylhomocysteine Hydrolase from Rat Liver. *Biochemistry* **1999**, *38*, 8323−8333.

(10) (a) Yin, D.; Yang, X.; Hu, Y.; Kuczera, K.; Schowen, R. L.; Borchardt, R. T.; Squier, T. C. Substrate Binding Stabilizes *S*-Adenosylhomocysteine Hydrolase in a Closed Conformation. *Biochemistry* **2000**, *39*, 9811−9819. (b) Komoto, J.; Huang, Y.; Gomi, T.; Ogawa, H.; Takata, Y.; Fujioka, M.; Takusagawa, F. Effect of Site-directed Mutagenesis on Structure and Function of Recombinant Rat Liver *S*-Adenosylhomocysteine Hydrolase. *J. Biol. Chem.* **2000**, *275*, 32147−32156. (c) Hu, Y.; Yang, X.; Yin, D.; Mahadevan, J.; Kuczera, K.; Schowen, R. L.; Borchardt, R. T. Computational Characterization of Substrate Binding and Catalysis in *S*-Adenosylhomocysteine Hydrolase. *Biochemistry* **2001**, *40*, 15143−15152. (d) Elrod, P.; Zhang, J.; Yang, X.; Yin, D.; Hu, Y.; Borchardt, R. T.; Schowen, R. L. Contribution of Active Site Residues to the Partial and Overall Activities of Human *S*-Adenosylhomocysteine Hydrolase. *Biochemistry* **2002**, *41*, 8134−8142. (e) Huang, Y.; Komoto, J.; Takata, Y.; Powell, D. R.; Gomi, T.; Ogawa, H.; Fujioka, M.; Takusagawa, F. Inhibition of *S*-Adenosylhomocysteine Hydrolase by Acyclic Sugar Adenosine Analogue D-Eritadenine. *J. Biol. Chem.* **2002**, *277*, 7474−7482. (f) Yang, X.; Hu, Y.; Yin, D. H.; Turner, M. A.; Wang, M.; Borchardt, R. T.; Howell, P.; Kuczera, K.; Schowen, R. L. Catalytic strategy for *S*-Adenosylhomocysteine Hydrolase: Transition-State Stabilization and the Avoidance of Abortive Reactions. *Biochemistry* **2003**, *42*, 1900−1909.

(11) (a) Muzard. M.; Guillerm, D.; Vandenplas, C.; Guillerm, G. 5′-Deoxy-5′-difluoromethylthioadenosine. A potent enzyme-activated acylating agent of *S*-adenosyl-L-homocysteine hydrolase. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 1037−1040. (b) Guillerm, G.; Guillerm, D.; Vandenplas-Witkowki, C.; Rogniaux, H.; Carte, N.; Leize, E.; Van Dorsselaer, A.; De Clercq, E.; Lambert, C. Synthesis, Mechanism of Action, and Antiviral Activity of a New Series of Covalent Mechanism-Based Inhibitors of *S*-adenosyl-L-Homocysteine Hydrolase. *J. Med. Chem.* **2001**, *44*, 2743−2752.

(12) Jeong, L. S.; Yoo, S. J.; Lee, K. M.; Koo, M. J.; Choi, W. J.; Kim, H. O.; Moon, H. R.; Lee, M. Y.; Park, J. G.; Lee, S. K.; Chun, M. W. Design, Synthesis, and Biological Evaluation of Fluoroneplanocin A as the Novel Mechanism-Based Inhibitor of S-Adenosylhomocysteine Hydrolase. *J. Med. Chem.* **2003**, *46*, 201−203.

(13) Wnuk, S. F.; Valdez, C. A.; Khan, J.; Moutinho, P.; Robins, M. J.; Yang, X.; Borchardt, R. T.; Balzarini, J.; De Clercq, E. Doubly Homologated Dihalovinyl and Acetylene Analogues of Adenosine. Synthesis, Interaction with *S*-Adenosyl-L-homocysteine Hydrolase, and Antiviral and Cytostatic Effects. *J. Med. Chem.* **2000**, *43*, 1180−1186.

(14) Wnuk, S. F.; Ro, B.-O.; Valdez, C. A.; Lewandowska, E.; Valdez, N. X.; Sacasa, P. R.; Yin, D.; Zhang, J.; Borchardt, R. T.; De Clercq, E. Sugar-Modified Conjugated Diene Analogues of Adenosine and Uridine. Synthesis, Interaction with *S*-Adenosyl-L-homocysteine Hydrolase and Antiviral and Cytostatic Effects. *J. Med. Chem.* **2002**, *45*, 2651−2658.

(15) (a) Diederich, F., Stang, P. J., Eds. *Metal-Catalyzed Cross-Coupling Reactions;* Wiley-VCH: Weinheim, 1998. (b) Miyaura, N., Ed. *Topics in Current Chemistry: Cross-Coupling Reactions*; Springer-Verlag: Berlin, 2002; Vol. 219.

(16) Wnuk, S. F.; Robins, M. J. Nucleic Acid Related Compounds. 63. Syntheses of 5′-deoxy-5′-methyleneadenosine and related Wittig-extended nucleosides. *Can. J. Chem.* **1991**, *69*, 334−338.

(17) (a) Wnuk, S. F.; Robins, M. J. Nucleic Acid Related Compounds. 67. Synthesis of 5′-amino and 5′-methylthio chain-extended nucleosides from uridine. *Can. J. Chem.* **1991**, *69*, 2104−2111. (b) 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.

(18) (a) Sonogashira, K.; Tohda, Y.; Hagihara, N. A Convenient Synthesis of Acetylenes: Catalytic Substitutions of Acetylenic Hydrogen with Bromoalkenes, Iodoarenes, and Bromopyridines. *Tetrahedron Lett.* **1975**, 4467−4470. (b) Sonogashira, K. Cross-coupling Reactions to *sp* Carbon Atoms. In *Metal-Catalyzed Cross-Coupling Reactions*; Diederich, F., Stang, P. J., Eds; Wiley-VCH: Weinheim, 1998; Chapter 5, pp 203−229.

(19) 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.

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

(21) (a) De Clercq, E.; Descamps, J.; Verhelst, G.; Walker, R. T.; Jones, A. S.; Torrence, P. F.; Shugar, D. Comparative Efficacy of Different Antiherpes Drugs against Different Strains of Herpes Simplex Virus. *J. Infect. Dis.* **1980**, *141*, 563−574. (b) De Clercq, E. Antiviral and Antimetabolic Activities of Neplanocins. *Antimicrob. Agents Chemother.* **1985**, *28*, 309−321. (c) Pauwels, R.; Balzarini, J.; Baba, M.; Snoeck, R.; Schols, D.; Herdewijn, P.; Desmyter, J.; De Clercq, E. Rapid and Automated Tetrazolium-based Colorimetric Assays for the Detection of anti-HIV Compounds. *J. Virol. Methods* **1988**, *20*, 309−321.

JM040054+