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

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Received March 18, 1998

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-NADH to E-NADH. Compounds 3a, 3b, and 3c were inhibitory to replication of vaccinia virus, vesicular stomatitis virus, parafluenza-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. ZDDFA (the Z-isomer of 4′,5′-didehydro-5′-deoxy-5′-fluoroadenosine) and its chloro analogue 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 (nonlethal event), and their oxidation to 3′-keto derivatives (lethal event) with concomitant reduction of E-NAD+ to E-NADH ("cofactor depletion" or type I inhibition). This indicates that AdoHcy hydrolase has hydrolytic activity (addition of water at C5′) that functions independently of its C3′-oxidation activity.

Our homologated analogues (E)-5′,6′-didehydro-6′-deoxy-6′-halohomoadenosines, EDDHHAs inhibit AdoHcy hydrolase and are enzymatically hydrolyzed to give "homoadenosine 6′-carboxaldehyde" which undergoes decomposition. The hydrolytic (C5′/C6′) and oxidative (C3′) activities of AdoHcy hydrolase were differentiated effectively with the 6′-fluoro analogue (EDDFHA). Lys-426 was identified as an important residue for the hydrolytic activity. We recently found that geminal and vicinal (dihalohomovinyl)adenosine analogues are new putative hydrolytic substrates for mechanism-based inhibition of AdoHcy hydrolase. 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) inhibition of the enzyme.

Homologated 5′,5′-dibromomethylene-5′-deoxyuridine and adenosine analogues had been synthesized from nucleoside 5′-carboxaldehydes (with CBr3/PPh3/Zn12) and converted into 5′-deoxy-5′-methylfluoroadenosines (the 4′-acetylenic derivatives). We had prepared such 4′-acetylenic derivatives by oxidative destannylation of 6′-stannanes. The corresponding adenosine analogue is a type II inhibitor of AdoHcy hydrolase with antiviral and cytostatic activity. Examples of 2′-15 and 3′-ethynyl nucleoside derivatives have been synthesized, and 1-(3-C-ethynyl-β-D-ribo-pentofuranosyl)cytosine has antitumor activity. An acetylenic linkage has been substituted for the phosphodiester moiety in an oligonucleotide mimic and a 2′,3′-dideoxyuridine analogue with ethynyl groups at C3′ and C4′ has been reported.

We now describe syntheses of the first (6′-halo)-acetylenic adenosine nucleosides (from adenosine or glucosamine), their inhibitory effects on AdoHcy hydrolase, and...
their antiviral activities. Addition of enzyme-seques-
tered water at C5′/C6′ followed by tautomeration 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.\textsuperscript{1,9}

Chemistry

Moffatt oxidation\textsuperscript{19} of 6-N-benzoyl-2′,3′-O-isoprop-
ylideneadenosine and treatment of the crude 5′-carbox-
aldehyde with “diphenylmethylene”triphenylphosph-
phorane (CBr\textsubscript{4}/Ph\textsubscript{3}P/Zn\textsubscript{12}) gave the dibromovinyl ana-
logue 1\textsuperscript{1,11} (74%, Scheme 1). Treatment of 1 with excess
BuLi gave the acetylenic derivative 2 (53%) plus byprod-
tuct(s), as noted by others.\textsuperscript{11} Treatment of 2 with
N-iodosuccinimide (NIS) and AgNO\textsubscript{3}\textsuperscript{20,21} (catalytic or
chboroacetylenes are known to be somewhat unstable,
did not proceed cleanly, in contrast with iodination
2\textsuperscript{1} and chlorination 2\textsuperscript{2} did not proceed cleanly, in contrast with iodination
and testing results must be considered tentative (al-
though testing data were repeatable). All attempts to
make the fluoroacetylenic analogue failed.

As anticipated,\textsuperscript{20} treatment of 6 or 7 with NCS/AgNO\textsubscript{3}
failed to effect 6-chlorination. However, treatment of
6 with an aqueous sodium hypochlorite solution\textsuperscript{21a}
(commercial laundry bleach) at ambient temperature
gave the chloroacetylene derivative 8\textsuperscript{a} (87%, purifi-
cation by chromatography on silica gel). Benzoylation of 8\textsuperscript{a},
hydrolisis of the acetal, and acetylation gave the
anomeric acetates 10\textsuperscript{a}. Coupling of 10\textsuperscript{a} with 6-N-
benzoyladenine, deacylation, and chromatography gave
the chloroacetylene nucleoside 3\textsuperscript{a} (20% from 9\textsuperscript{b}).

Inactivation of S-Adenosyl-L-homocysteine Hy-
drolase. Recombinant human placental AdoHcy hy-
drolase 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 \(\mu\)M)
was approximately 55% of the original enzyme activity
with the chloro 3a and bromo 3b analogues. In con-
trast, the 6′-ido analogue 3c caused complete inactiva-
tion of AdoHcy hydrolase at a concentration of 100 \(\mu\)M.

Kinetic analysis of the inactivation processes by the Kitz
and Wilson method gave the \(k_i\) values in Table 1.

from 9b (TFA/H\textsubscript{2}O) and the product was acetylated to
give the anomeric acetates 10. Coupling (SnCl\textsubscript{4}/CH\textsubscript{3}CN\textsuperscript{23}) of 10
and 6-N-benzoyladenine followed by deacy-
lation (NH\textsubscript{3}/MeOH) gave the crystalline bromoacetylene
homonucleoside 3\textsuperscript{a} (36% from 9a).

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

\textsuperscript{1} (a) H\textsubscript{5}IO\textsubscript{6}/EtOAc. (b) Ph\textsubscript{3}P/CBr\textsubscript{4}/CH\textsubscript{2}Cl\textsubscript{2}. (c) BuLi/THF/\textdegree \textsubscript{78} C. (d) BzCl/pyridine. (e) NaOCl/H\textsubscript{2}O. (f) NBS/AgNO\textsubscript{3}/Me\textsubscript{2}CO. (g) i,
TFA/H\textsubscript{2}O; ii, Ac\textsubscript{2}O/pyridine/DMAP. (h) 6-N-Benzoyladenine/SnCl\textsubscript{4}/
CH\textsubscript{3}CN. (i) NH\textsubscript{3}/MeOH.

\textsuperscript{2} (a) BuLi/THF/\textdegree \textsubscript{78} C. (b) AgNO\textsubscript{3}/NIS/Me\textsubscript{2}CO. (c) NH\textsubscript{3}/MeOH.
(d) TFA/H\textsubscript{2}O.
Table 1. Inhibition of AdoHcy Hydrolase with 3a–c

<table>
<thead>
<tr>
<th>concn (μM)</th>
<th>3a</th>
<th>3b</th>
<th>3c</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>89.3 ± 2.9</td>
<td>91 ± 1.5</td>
<td>80.0 ± 0.8</td>
</tr>
<tr>
<td>1.0</td>
<td>64.0 ± 0.6</td>
<td>73.2 ± 3.2</td>
<td>44.6 ± 1.0</td>
</tr>
<tr>
<td>10</td>
<td>58.9 ± 1.3</td>
<td>62.9 ± 0.7</td>
<td>8.8 ± 0.2</td>
</tr>
<tr>
<td>100</td>
<td>55.0 ± 0.6</td>
<td>56.0 ± 3.1</td>
<td>&lt;2.0</td>
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</table>

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. Data are the averages of duplicate determinations.

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

Results observed with the chloro 3a and bromo 3b analogues are different from those with 6'-iodo analogue 3c. Partial (~50%) reduction of E-NAD+ to E-NADH was observed with 3a or 3b with concomitant partial loss (~50%) of enzyme activity. Incubation of 3a or 3b with AdoHcy hydrolase resulted in release of adenine (3b > 3a) and halide ions (3b > 3a). These results suggest that 3a and 3b are substrates for both the 3'-oxidative activity (partial reduction of E-NAD+ to E-NADH) and the 5'/6'-hydrolytic activity (release of Ade and halide ions) of AdoHcy hydrolase. More specifically, the release of Ade suggests 6'-hydrolytic activity, whereas release of halide ions could result from either the 5'- or 6'-hydrolytic activity of the enzyme.7 Since halide ions are released from the chloro 3a and bromo 3b analogues upon incubation with AdoHcy hydrolase, it is plausible that these inhibitors are converted into acyl halides and/or α-halomethyl ketones (Figure 1) and subsequently react with protein nucleophiles. However, 3a and/or 3b also might inactive the enzyme by type I (cofactor depletion) and/or type II (covalent binding via an allenic ketone24) mechanisms. Studies to characterize the molecular mechanism(s) involved in these inactivation processes are in progress.

Antiviral Activity. Haloacylenes 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.22 The order of increasing antiviral activity was 3a < 3b < 3c, irrespective of the virus (VV, VSV, parainfluenza, or...
Table 4. Antiviral Evaluation of Compounds 3a-c

<table>
<thead>
<tr>
<th>Virus/Host</th>
<th>EC_{50} (μM)</th>
<th>Compd E 6SM</th>
<th>Minimal inhibitory concentration required to elicit a microscopically visible alteration of cell morphology.a</th>
<th>Effective concentration required to inhibit virus-induced cytopathicity by 50%.b</th>
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</thead>
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<tr>
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<td>&gt;140</td>
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<td>&gt;140</td>
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<tr>
<td>Coxsackie virus B4 (HeLa)</td>
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<td>&gt;140</td>
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<tr>
<td>HSV-1 (IC50)</td>
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<td>&gt;140</td>
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<td>HSV-2 (G)</td>
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<tr>
<td>Placental virus</td>
<td>&gt;140</td>
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</table>

a Minimal inhibitory concentration required to elicit a microscopically visible alteration of cell morphology. Effective concentration required to inhibit virus-induced cytopathicity by 50%.

b Effective concentration required to inhibit virus-induced cytopathicity by 50%.

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

Experimental Section

Uncorrected melting points were determined with a capillary apparatus. UV spectra were measured with a UV spectrophotometer. 1H (200 or 500 MHz) and 13C (125 MHz) NMR spectra were determined. Mass spectra (MS and HRMS) were obtained by electron impact (20 eV), chemical ionization (CI, isobutane), or fast atom bombardment (FAB, thioglycolic acid). Merck kieselgel 60-F254 sheets were used for TLC (detection at 254 nm, or by color with I2 in a sealed chamber). Merck kieselgel 60 (230–400 mesh) was used for column chromatography. Preparative reversed-phase (RP)-HPLC was performed with a Spectronex C18 column and a Spectronex SP 8000 ternary pump system (gradient solvent systems are noted). Elemental analyses were determined by M-H-W Laboratories, Phoenix, AZ. AdoHcy, Ado, Hcy, and calf intestinal Ado deaminase (EC 3.5.4.4) were obtained from Sigma. Standard Cl-, Br-, and I- ions were obtained from P. J. Cobert Association, Inc. (St. Louis, MO). Recombinant human placental AdoHcy hydrolase was overexpressed and purified as described. Reagent grade chemicals were used and solvents were dried by reflux over...
and distillation from CaCO3 (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-(5,6-di-bromo-5,6-dideoxy-2,3-O-isopropylidene-β-D-ribo-hex-5-enofuranosyl)adenine (1) (289 mg, 0.5 mmol) in THF (20 mL) at −78 °C under Ar, and stirring was continued for 2 h (TLC indicated conversion to a more polar product and minor fluorescent byproducts). The mixture was neutralized (HOAc, pH ~6) and evaporated, and the residue was partitioned (NaHCO3/H2O/CHCl3). The organic layer was washed (brine), dried (MgSO4), and evaporated. Chromatography (hexanes/EtOAc, 1:4) of the residue gave 2 (280 mg, 87%) as an amorphous solid. 1H NMR (CD3OD/CDCl3, 500 MHz) δ 1.32 and 1.48 (2 × 3H, J = 6.5 Hz, 1H2), 5.11 (d, J = 6.0 Hz, 1H1), 5.91 (d, J = 5.5 Hz, 1H4), 7.16 (s, 1H1), 7.20 (s, 1H1), 7.31 (d, J = 5.8 Hz, 1H2), 7.48 (t, J = 8.6 Hz, 1H3), 8.49 (d, J = 4.0 Hz, 1H3); HRMS (CI) m/z 387.9915 (100, MH+ [C9H1237ClO4] = 387.9917). The aqueous layer was extracted (CHCl3, 2×), and the organic phase was washed (brine) and dried (MgSO4), and volatiles were evaporated. The residue was chromatographed (CHCl3 → 2% MeOH/EtOAc) to give 2a (289 mg, 87%) as a syrup: 1H NMR (CD3OD/CDCl3, 500 MHz) δ 1.52 and 1.64 (2 × 3H, J = 6.0 Hz, 1H2), 5.71 (d, J = 5.5 Hz, 1H1), 6.16 (s, 1H1), 6.80 (s, 1H2), 7.24 (s, 1H1), 8.01 (s, 1H1); HRMS (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 ~0 °C (ice bath) for 1 h, and volatiles were evaporated. EtOH was added and evaporated, and the residue was chromatographed (EtOAc → 10% MeOH/EtOAc) and crystallized (MeOH) to give 2c (32 mg, 44%: 31% from 2). The mother liquor was purified by RP-HPLC (pre-packing C18; 150 × 4.6 mm, 5 μm, 15% CH3CN in 0.05% MeOH, 0.1% TFA in H2O, 1.0 ml/min, followed by a gradient of 15 → 40% for 30 min at 2 mL/min; tR = 53 min) to give additional 2c (10 mg, 16%; 42% total from 2): mp 180–183 °C (dec); UV max 259 (ε 14 200), min 228 nm (ε 2800); 1H NMR (MeSO4-d6) δ 4.32 (q, J = 6.8 Hz, 1H3), 4.65 (s, J = 6.5 Hz, 1H2), 6.81 (d, J = 6.2 Hz, 1H1), 7.03 (d, J = 6.0 Hz, 1H2), 8.24 (d, J = 8.8 Hz, 1H1); 13C NMR (MeSO4-d6) δ 35.38, 37.57, 50.23, 126.90, 129.56, 141.01, 153.32, 158.02, 180.13. Anal. C23H21ClN5O2 [387.9907] C, 57.26; H, 3.39; N, 15.83; O, 23.50. 

10a and 10b, respectively.
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 × 2, 2 × 3, 2 × Me), 4.89–5.06 (m, 3, H2,3,4), 5.92 (d, J = 3.4 Hz, H1), 7.42–8.10 (m, 5, Arom); HRMS (CI) m/z 369.0154 (10, MH⁺ [C₉H₁₀₆O₆BrO₃⁺] = 369.0161), 369.0180 (10, MH⁺ [Br⁻] = 367.0181).

9-(6-Chloro-5,6-dideoxy-β-D-ribo-hex-5-ynofuranosyl)-adenine (3a). (a) Hydrolysis and Acetylation. A solution of 3a (250 mg, 0.77 mmol) in TFA/H₂O (9:1, 10 mL) was stirred at −9 °C (ice bath) for 1 h. Volatiles were evaporated, traces were coevaporated from the residue (toluene 2 ×, pyridine). Pyridine (8 mL), Ac₂O (1 mL), and 4-dimethylaminopyridine (DMAP, 5 mg) were added, the mixture was stirred at −20 °C for 2 h and at 0–15 °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, H₂O, and brine) and dried (Na₂SO₄). Volatiles were evaporated, and the residue was chromatographed (20 → 30% EtOAc/hexanes) to give 1,2,3-Diacetyl-3-O-benzoyl-6-chloro-5,6-dideoxy-β-D-ribo-hex-5-ynofuranose (10a, 0.17 g, 74%, 245 mg, 87%). ¹H NMR δ 1.99 and 2.14 (2 × 2, 2 × 0.2, 2 × Ac), 2.02 and 2.15 (2 × 2, 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, 5.2, 0.8, H3), 5.69 (t, J = 4.6 Hz, 0.8, H3), 6.26 (d, J = 2.0 Hz, 0.81 H1), 6.30 (d, J = 4.4 Hz, 0.2, H1), 7.40–8.10 (m, 5, Arom); HRMS (FAB) m/z 391.0374 [C₇H₅N₃O₆ Na⁺] = 391.0375, 389.0396 (100, Mn⁺ [Na⁺] = 389.0404).

(b) Coupling. SnCl₂ (0.18 mL, 310 mg, 0.75 mmol) with 6-[C₁₇H₁₅₃₇ClNaO₇] (0.18 mL, 391 mg, 1.5 mmol) was evaporated, the residue was partitioned (NaHCO₃/H₂O, NaHCO₃/H₂O, H₂O, and brine) and dried (Na₂SO₄). 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⁺ [C₁₉H₂₁N₃O₇BrO₃⁺] = 548.1151), 546.1162 (23, MH⁺ [Cl⁺] = 546.1180).

(c) Deprotection. A solution of the product from step b (274 mg, 0.5 mmol) in NH₄H₂O (20 mL) was stirred for 24 h at −5 °C. Volatiles were evaporated, and the residue was flash chromatographed (EtOAc − 15% MeOH/EtOAc). “Dif- fusion crystallization”²⁸–³⁰ (MeOH/EtOAc) of the residue gave 3a (52 mg, 2 crops) as a white crystalline solvate (variable). RP-HPLC: purification on preparative C₁₈ column, gradient 15%–45% CH₃CN/H₂O for 80 min at 2 mL/min, t₀ = 64 min) of the mother liquor gave addition 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 (Me₅SO-d₅) δ 4.36–4.44 (m, 1, H₃), 4.59 (d, J = 4.4 Hz, 1, H₄), 4.83–4.92 (m, 2, H₁, H₂), 5.27 (d, J = 5.6 Hz, OH), 5.59 (t, 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 (Me₅SO-d₅) δ 66.43, 67.32, 73.19, 73.68, 75.25, 87.94, 119.29, 140.15, 149.82, 152.33, 156.16; HRMS (FAB) m/z 298.0503 (38, MH⁺ [C₁₇H₁₃N₃O₇BrO₃⁺] = 298.0521), 296.0539 (100, MH⁺ [Cl⁺] = 296.0550).

9-(6-Bromo-5,6-dideoxy-β-D-ribo-hex-5-ynofuranosyl)-adenine (3b). Hydrolysis and acetylation (as with 3a) of 3b (300 mg, 0.9 mmol) gave anomer 10b (0.17 g, −1.3; 310 mg, 84%); ¹H NMR δ 1.99 and 2.11 (2 × 2, 2 × 0.2, 2 × Me), 2.02 and 2.12 (2 × 2, s × 0.75, 2 × Me), 4.97 (d, J = 4.4 Hz, 0.75, H₄), 5.03 (d, J = 1.6 Hz, 0.25, H₄), 5.48 (t, J = 5.3 Hz, 0.25, H₂), 5.58 (d, J = 1.6, 4.6 Hz, 0.75, H₂), 5.69 (d, J = 1.8, 6.0 Hz, 0.75, H₂), 5.73 (t, J = 4.7 Hz, 0.75, H₂), 7.42–8.10 (m, 5, Arom); HRMS (EI) m/z 411.9984 (15, MH⁺ [C₁₉H₁₃N₃O₇BrO₃⁺] = 411.9981), 409.9993 (15, M⁺ [Br⁻] = 410.0001). Coupling of 10b (310 mg, 0.75 mmol) with 6-N-benzoyladenine and purification (as with 3a) gave 9-(2-O-acetyl-3-O-benzoyl-6-bromo-5,6-dideoxy-β-D-ribo-hex-5-ynofuranosyl)-6-N-benzoyla-
Inactivation of S-Adenosyl-L-homocysteine Hydrolase

μL). The precipitate was removed by centrifugation, and the supernatant was analyzed for Ade (HPLC with a reverse-phase C18 column as described [9]). The HPLC peak assigned to Ade was verified (co-injection with authentic Ade) and confirmed by mass spectral analysis (CI/NI).

**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.[9] AdoHcy hydrolase (10 μM) in buffer A (0.6 ml) was incubated with 3a, 3b, or 3c (300 μM) at 37°C for 20 min. NADH formation was monitored at 340 nm with a HP 8452 diode-array UV spectrophotometer and quantified using a standard curve.

**Antiviral Evaluation.** Antiviral assays were based on inhibition of virus-induced cytopathicity in E(2)SM, HeLa, or vero cell cultures following established procedures.[9,30] Briefly, confluent cell cultures in microtiter trays were inoculated with 100 CTCDD of virus (1 CTCDD 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 concentrations (400, 200, 100, ... μg/ml) of the test compounds. Viral cytopathicity was recorded as soon as it reached completion in the control virus-infected cell cultures.

**Acknowledgment.** We thank the American Cancer Society (Grant DHP-34), Brigham Young University development funds, the United States Public Health Service (Grant GM-29332), the Belgian Fonds voor Geneeskundig Wetenschappelijk Onderzoek (Project 3.0026.91), the Belgian Geconcerteerde Onderzoeksacties (Conventie 95/5), and the Biomedical Research Program of the European Commission for support. We thank Anita Van Lierde and Frieda De Meyer for excellent technical assistance and Mrs. Jeanne K. Gordon for assistance with the manuscript.

**References**


J M 980163M