

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/15265822>

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 Antica...

ARTICLE in JOURNAL OF MEDICINAL CHEMISTRY · OCTOBER 1994

Impact Factor: 5.45 · DOI: 10.1021/jm00047a015 · Source: PubMed

CITATIONS

55

READS

4

6 AUTHORS, INCLUDING:



Stanislaw F Wnuk

Florida International University

162 PUBLICATIONS 2,126 CITATIONS

SEE PROFILE



Chung-Shin Yuan

National Sun Yat-sen University

172 PUBLICATIONS 4,896 CITATIONS

SEE PROFILE

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 Inhibition¹

Stanislaw F. Wnuk,[†] Chong-Sheng Yuan,[‡] Ronald T. Borchardt,[‡] Jan Balzarini,[§] Erik De Clercq,[§] and Morris J. Robins^{*,†}

Department of Chemistry and Biochemistry, Brigham Young University, Provo, Utah 84602, Departments of Biochemistry and Medicinal Chemistry, University of Kansas, Lawrence, Kansas 66045, and Rega Institute for Medical Research, Katholieke Universiteit of Leuven, Leuven, Belgium

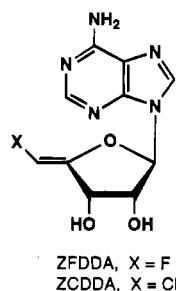
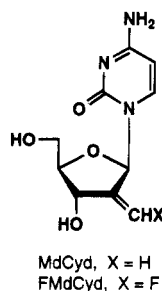
Received May 11, 1994[®]

Treatment of 9-[6-(*E*)-(tributylstannyl)-5,6-dideoxy-2,3-*O*-isopropylidene- β -D-ribo-hex-5-enofuranosyl]adenine [**2b**(*E*)] or the 6-*N*-benzoyl derivative **2a**(*E*) with iodine (or *N*-iodosuccinimide) or bromine (or *N*-bromosuccinimide) gave virtually quantitative and stereospecific conversions to the 6'-(*E*)-(halohomovinyl)nucleoside analogues. Analogous treatment of the 6'-(*Z*)-vinylstannanes gave the 6'-(*Z*)-halo compounds. Treatment of **2a** or **2b** with chlorine or xenon difluoride/silver triflate gave *E* and *Z* mixtures of the respective 6'-chloro- or 6'-fluorohomovinyl products. Deprotection gave the 9-[6-(*E* and *Z*)-halo-5,6-dideoxy- β -D-ribo-hex-5-enofuranosyl]adenines [(*E* and *Z*)-5',6'-didehydro-6'-deoxy-6'-halohomoadenosines, EDDHHAs and ZDDHHAs, **4c**–**7c**(*E* and *Z*)]. The acetylenic 5',5',6',6'-tetrahydro-6'-deoxyhomoadenosine (**3c**) and the 5'-bromo-5'-deoxy-5'-methyleneadenosine (**10c**) regioisomer of EDDBHA [**5c**(*E*)] also were obtained from **2**. Concentration- and time-dependent inactivations of *S*-adenosyl-L-homocysteine (AdoHcy) hydrolase were observed with **3c** and the 6'-(halohomovinyl)adenosine analogues. The order of inhibitory potency was I > Br > Cl > F and *E* > *Z* for the geometric isomers. AdoHcy hydrolase effected "hydrolysis" of the 6'-halogen from the (halohomovinyl)Ado compounds (to give the putative 6'-carboxaldehyde which underwent spontaneous decomposition) independently of its oxidative activity. Partition ratios for these hydrolytic turnovers/lethal inhibitory events were in the order F > Cl > Br > I. Biological activities were evaluated with several viruses and cancer cell lines, and potencies were generally in the order I > Br > Cl > F and *E* > *Z* isomers. This represents the first observation of a direct correlation of cytostatic activity with inhibition of AdoHcy hydrolase and highlights the potential of this enzyme as a viable target for chemotherapeutic intervention in anticancer as well as antiviral drug design.

Introduction

Methylene-sugar nucleoside analogues with anticancer and antiviral activity have been synthesized by several groups and shown to be mechanism-based inhibitors of key enzymes in the nucleic acid manifold.^{2–9} The first example, 3'-deoxy-3'-methyleneadenosine, was prepared by Tronchet and Tronchet by condensation of a 3-methylene- α -D-erythro-pentofuranose derivative with the chloromercury salt of 6-*N*-benzoyladenine.¹⁰ Ueda and co-workers transformed nucleosides into the anticancer agent² 2'-deoxy-2'-methylenecytidine (MdCyd), whose 5'-diphosphate functions as a potent time-dependent inactivator of ribonucleoside diphosphate reductase (RDPR).⁴ The 5'-fluoro- and 5'-chloro-4',5'-didehydro-5'-deoxyadenosine (ZFDDA³ and ZCDDA,⁹ respectively) derivatives were designed as mechanism-based inhibitors of *S*-adenosyl-L-homocysteine hydrolase (AdoHcy hydrolase) and, indeed, function as potent inactivators of that target enzyme.^{3,9} Likewise, the 2'-fluoromethylene (FMdCyd)⁶ (5'-diphosphate) analogue of MdCyd is an efficient inactivator of RDPR. Other 2'(or 3')-deoxy-2'(or 3')-methylenenucleosides in the

pyrimidine and purine series have been prepared,^{2,5,7,8} and some, as well as bis-methylene analogues of adenosine,⁷ have inhibitor activity.



Wittig chemistry has been employed to prepare 5'-deoxy-5'-methyleneadenosine¹¹ (**8c**), its neplanocin A analogue,¹² and acetylenic derivatives of adenosine¹³ **3c** and uridine,¹⁴ and an acetylenic acyclic adenine nucleoside analogue has been reported.¹⁵ Compound **3c** is a novel mechanism-based inactivator of AdoHcy hydrolase.^{13a} Vinyl derivatives of the carbocyclic antibiotic aristeromycin have been prepared from vinylcyclopentanyl precursors and shown to be potent type I inhibitors of AdoHcy hydrolase.¹⁶ The stereocontrolled synthesis of 6'-(*E* and *Z*)-halohomovinyl derivatives of uridine from the complementary vinyl 6'-sulfones¹⁷ via vinyltin intermediates has recently been reported.¹⁸

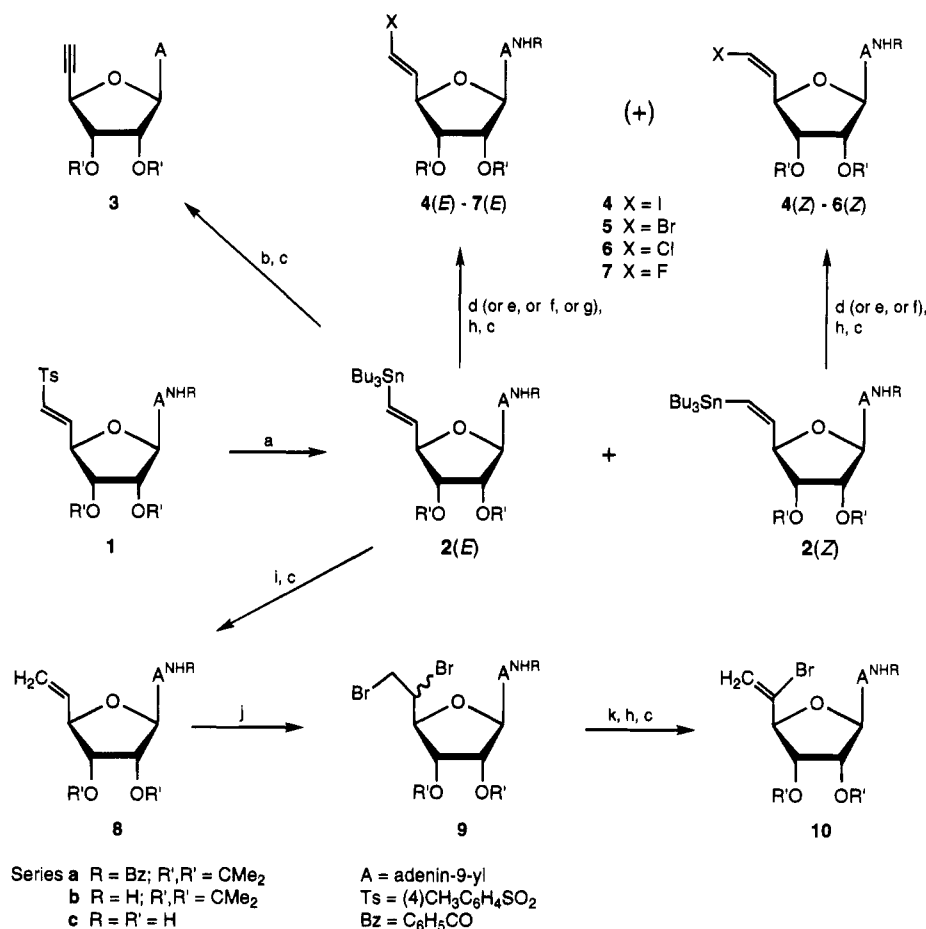
* Author to whom correspondence should be addressed at Brigham Young University.

[†] Brigham Young University.

[‡] University of Kansas.

[§] Rega Institute for Medical Research.

[®] Abstract published in *Advance ACS Abstracts*, September 1, 1994.

Scheme 1^a

^a (a) Bu₃SnH/AIBN/PhCH₃/Δ; (b) Pb(OAc)₄/CH₃CN; (c) CF₃CO₂H/H₂O; (d) I₂ or NIS; (e) Br₂ or NBS; (f) Cl₂; (g) XeF₂/AgOTf/THF; (h) NH₃/MeOH; (i) NH₄F/EtOH/Δ; (j) Br₂/CCl₄; (k) DBU/THF.

Since the 5'-halo-4',5'-unsaturated adenosine derivatives ZFDDA and ZCDDA had potent inhibitory activity against AdoHcy hydrolase,^{3,9} we extended studies to the 6'-halo-5',6'-unsaturated homoadenosines (5',6'-didehydro-6'-deoxy-6'-halohomoadenosines, DDHHAs, **4c–7c**). Inhibitors of AdoHcy hydrolase are promising potential antiviral agents,^{19,20} whose potency has been correlated with enzyme inhibitory activity.²⁰ We now report stereocontrolled syntheses of 6'-(*E*)-halohomovinyl (EDDHHA) and 6'-(*Z*)-halohomovinyl (ZDDHHA) analogues of adenosine **4c–7c** (*E* and *Z*) via vinyltin intermediates, their inhibitory effects on AdoHcy hydrolase, and correlated cytostatic and antiviral activities. This represents the first direct correlation between anticancer potency and AdoHcy hydrolase inhibitory activity.

Chemistry

Moffatt oxidation of 2',3'-*O*-isopropylideneadenosine or its 6-*N*-benzoyl derivative and Wittig treatment of the derived 5'-aldehydes with [*p*-tolylsulfonyl]methylene]triphenylphosphorane gave the 6'-(*E*)-vinyl sulfone homonucleosides **1**.¹¹ Stannyldesulfonation (Bu₃SnH/AIBN/toluene/Δ)²¹ of the 6'-(*E*)-sulfone **1b** gave separable mixtures of the vinyl 6'-stannanes **2b** (*E/Z*, ~4.2:1; ~61%). Treatment of the 6-*N*-benzoyl derivative **1a** under identical conditions gave the vinyl 6'-stannanes **2a** more stereoselectively (*E/Z*, ~6:1) but in lower yield (41%) and with more byproduct formation. Addition of Bu₃SnH to the 5'-alkene, double-bond reduction, and 8,5'-cyclonucleoside formation (tosyl group present) were

indicated (¹H NMR). Virtually quantitative and stereospecific halodestannylation²² of **2b** (*E* and *Z*) [Br₂ or *N*-bromosuccinimide (NBS) and I₂ or *N*-iodosuccinimide (NIS)] occurred to provide the Wittig-type 6'-[bromo(or iodo)homovinyl]adenosines **5c** and **4c** after deprotection. Treatment of **2a** with *N*-chlorosuccinimide did not give the 6'-chloro derivative **6a**. Elemental chlorine converted **2a** (*E*) to **6a** (*E*), but minor quantities of **6a** (*Z*) also were formed. Treatment of **2a** (*E/Z*, ~6:1) with chlorine and sequential removal of the 6-*N*-benzoyl (NH₃/MeOH) and isopropylidene (CF₃CO₂H/H₂O) groups afforded **6c** (*E/Z*, ~2:1; 66%). These geometric isomers were readily separated by preparative reversed-phase HPLC.

Attempted fluorodestannylation of **2a** with xenon difluoride or *N*-fluoropyridinium triflate resulted in hydrodestannylation to give the 5'-methylene compound **8a**. Four recent reports have described syntheses of vinyl fluorides from vinylstannanes.^{23,24} The procedure with XeF₂ and silver triflate^{23b} gave rapid conversion of **2a** (*E/Z*, ~6:1) to a mixture of the protected vinyl 6'-fluorides **7a** and the protiodestannylated **8a** (~3:1) in good yield. The **7a/8a** mixture was deprotected and separated (RP-HPLC) to give EDDFHA [**7c** (*E*)]. ¹H NMR (Table 1) coupling constants (³J_{5'-6'} ≥ 11.0 Hz) were in harmony with stereochemical assignments for the *E* isomers. In addition, the ³J_{H5'-F} = 17.5 Hz for **7c** (*E*) was in agreement with literature values²⁵ for fluoro olefins [³J_{H-F}(*cis*) ≈ 20 Hz, ³J_{H-F}(*trans*) ≈ 52 Hz]. Differences in the ¹³C NMR chemical shifts for C6' of the EDDHHAs [e.g., δ_{C6'} 82.61 for EDDIHA [**4c** (*E*)],

Table 1. ^1H NMR Spectral Data^{a,b}

compd	H1' ^c ($J_{1'-2'}$)	H2' ^d ($J_{2'-3'}$)	H3' ^d ($J_{3'-4'}$)	H4' ^d ($J_{4'-5'}$)	H5' ^d ($J_{5'-6'}$)	H6' ^c ($J_{6'-4'}$)	H2 ^e	H8 ^e	NH ₂ ^f	others ^e
2b(E) ^g	6.13 (2.0)	5.55 (6.3)	5.02 (3.1)	4.70 ^h (6.0)	6.01 (19.1)	6.25 ^d (1.0)	7.90	8.35	5.85	0.77–1.50 ⁱ (Bu ₃ Sn) 1.41, 1.63 (CH ₃ s)
2b(Z) ^g	6.08 (2.0)	5.50 (6.3)	4.97 (3.4)	4.47 (8.9)	6.55 (12.8)	6.20	7.91	8.37	5.90	0.78–1.52 ⁱ (Bu ₃ Sn) 1.40, 1.61 (CH ₃ s)
2a(E) ^g	6.19 (2.3)	5.57 (6.2)	5.04 (3.2)	4.72 ^h (6.1)	6.02 (19.1)	6.28 ^d (1.0)	8.10	8.81	9.01 ^j	0.79–1.51 ⁱ (Bu ₃ Sn) 1.41, 1.62 (CH ₃ s) 7.48–7.62 ⁱ , 8.03 ^c (Bz)
2a(Z) ^{g,k}	6.15 (2.1)	5.54 (6.1)	5.00 (3.3)	4.52 (9.01)	6.54 (12.6)	6.24	8.11	8.82	9.01 ^j	0.79–1.51 ⁱ (Bu ₃ Sn) 1.41, 1.62 (CH ₃ s) 7.48–7.62 ⁱ , 8.03 ^c (Bz)
3b ^g	6.24 ^e	5.73 ^c (5.7)	5.14 (1.1)	5.07 ⁱ		2.49 (2.4)	8.39	8.40	6.72	1.41, 1.62 (CH ₃ s)
4b(E) ^g	6.08 (1.8)	5.57 (6.3)	5.11 (3.3)	4.63 ^h (6.4)	6.69 (14.7)	6.35 ^d (1.0)	7.86	8.38	5.69	1.40, 1.61 (CH ₃ s)
4b(Z) ^g	6.10 (1.7)	5.64 (6.1)	5.13 (2.8)	5.02 (7.3)	6.41 (7.7)	6.49	7.88	8.37	5.80	1.42, 1.66 (CH ₃ s)
5b(E) ^g	6.07 (1.8)	5.56 (6.3)	5.11 (3.3)	4.65 (5.9)	6.26–6.44 ⁱ	6.26–6.44 ⁱ	7.85	8.37	5.78	1.40, 1.62 (CH ₃ s)
5b(Z) ^g	6.09 (1.5)	5.65 (6.1)	5.12 (2.7)	5.20 (7.3)	6.26 (7.5)	6.33	7.86	8.35	5.98	1.41, 1.65 (CH ₃ s)
6a(E) ^{g,l}	6.13 (1.6)	5.56 (6.3)	5.10 (3.4)	4.71 (7.4)	6.07 (13.2)	6.23	8.08	8.81	9.20 ^j	1.40, 1.62 (CH ₃ s) 7.47–7.61 ⁱ , 8.02 ^c (Bz)
6a(Z) ^{g,l}	6.17 (1.5)	5.65 (6.1)	5.13 (2.8)	5.31 (8.0)	5.86 (7.8)	6.19	8.09	8.80	9.25 ^j	1.40, 1.62 (CH ₃ s) 7.48–7.63 ⁱ , 8.03 ^c (Bz)
9a ^{g,m}	6.32 (4.0)	5.22 (6.4)	5.29 (1.5)	4.55–4.60 ⁱ	4.55–4.60 ⁱ	3.61–3.82 ⁱ	8.10	8.80	8.96	1.40, 1.41, 1.61, 1.63 (CH ₃ s) 7.48–7.63 ⁱ , 8.01 ^c (Bz)
	6.19 (2.1)	5.44 (6.4)	4.97 (3.4)	4.72 (2.3)	4.40–4.50 ⁱ		8.36			
10a ⁿ	6.30 (2.4)	5.47 (6.2)	5.22 (3.0)	4.77 ^c		5.45 (2.2 ^o)	8.20	8.78	9.20	1.41, 1.62 (CH ₃ s) 7.43–7.61 ⁱ , 8.02 ^c (Bz)
3c	5.95 (6.2)	4.80 ^h (4.6)	4.38 ^h (4.5)	4.57		3.77 (1.6)	8.18	8.31	7.40	5.73 ^c (5.5 ^p OH3') 5.76 ^c (5.7 ^p OH2')
4c(E)	5.90 (5.1)	4.67 ^h (4.7)	4.19 ^h (4.7)	4.35 (7.3)	6.87 (14.3)	6.69	8.17	8.36	7.58	5.53 ^c (5.3 ^p OH3') 5.64 ^c (5.4 ^p OH2')
4c(Z)	5.93 (6.1)	4.84 ^h (4.9)	4.17 ^h (3.4)	4.56 (6.9)	6.73 ^q	6.73 ^q	8.19	8.39	7.35	5.51 ^c (5.4 ^p OH3') 5.58 ^c (5.9 ^p OH2')
5c(E)	5.92 (5.0)	4.73 ^h (5.1)	4.21 ^h (4.5)	4.38 (7.6)	6.58 (13.4)	6.73	8.18	8.40	7.38	5.49 ^c (5.4 ^p OH3') 5.61 ^c (5.6 ^p OH2')
5c(Z)	5.93 (6.0)	4.85 ^h (4.9)	4.16 ^h (3.2)	4.75 (6.9)	6.65–6.76 ⁱ	6.65–6.76 ⁱ	8.18	8.39	7.35	5.54 ^c (5.5 ^p OH3') 5.60 ^c (5.7 ^p OH2')
6c(E)	5.92 (5.1)	4.72 ^h (5.0)	4.20 ^h (4.4)	4.41 (8.1)	6.33 (13.2)	6.62	8.18	8.38	7.35	5.48 ^c (5.3 ^p OH3') 5.61 ^c (5.5 ^p OH2')
6c(Z)	5.93 (5.8)	4.80–4.86 ⁱ (4.5)	4.16 ^h (3.8)	4.80–4.86 ⁱ (8.4)	6.39 (7.1)	6.54	8.17	8.39	7.32	5.56 ^c (5.5 ^p OH3') 5.60 ^c (6.0 ^p OH2')
7c(E) ^r	5.91 (5.1)	4.71 ^h (4.7)	4.16 ^h (4.7)	4.34 (9.8)	5.83 ^h (11.0, 17.5 ^s)	7.07 ^d (83.5 ^t)	8.19	8.38	7.32	5.41 ^c (5.5 ^p OH3') 5.58 ^c (5.7 ^p OH2')
10c ^u	5.98 (5.2)	4.77 ^h (4.8)	4.34 ^h (5.4)	4.45 ^c		5.74 (2.0 ^o)	8.18	8.38	7.32	5.63 ^c (5.6 ^p OH3') 5.68 ^c (5.7 ^p OH2')

^a Chemical shifts (δ) in Me₂SO-*d*₆ at 200 MHz (unless otherwise noted). ^b "Apparent" first-order coupling constants (Hz, in parentheses). ^c Doublet (unless otherwise noted). ^d Doublet of doublets (unless otherwise noted). ^e Singlet (unless otherwise noted). ^f Broad singlet. ^g In CDCl₃. ^h Doublet of doublets of doublets. ⁱ Multiplet. ^j NH. ^k Assigned from a spectrum of both isomers by comparison with that of **2a(E)**. ^l Assigned from a spectrum of both isomers on the basis of integration. ^m 3.61–3.82ⁱ (H6''). ⁿ 5.67ⁱ (H6''). ^o (² $J_{\text{H8}-\text{H6''}}$). ^p (³ $J_{\text{OH}-\text{CH}}$). ^q Signal for H5' and H6' collapsed into one triplet ($J = 7.2$ Hz). ^r At 500 MHz. ^s (³ $J_{\text{H5'}-\text{F}}$). ^t (² $J_{\text{H6'}-\text{F}}$). ^u 6.19ⁱ (H6'').

110.24 for EDDbHA [**5c(E)**], 121.74 for EDDCHA [**6c(E)**], and 152.57 for EDDFHA [**7c(E)**] (Table 2)] generally parallel electronegativities of the 6'-halogen substituents. Other trends among ¹³C and ¹H chemical shifts of the 5' and 6' carbons and protons and H5'–H6' coupling constants of the isomers are apparent in Tables 1 and 2.

Treatment of **2b(E)** with lead tetraacetate in acetonitrile resulted in oxidative destannylation²⁶ to give the acetylenic compound **3c** after deprotection. Destannylation of **2a** with ammonium fluoride²⁷ in ethanol at reflux gave the 5'-deoxy-5'-methylenenucleoside **8a**, but some **8b** was produced by partial cleavage of the 6-*N*-benzoyl group. Treatment of **8a** with bromine gave the 5',6'-dibromo diastereomers **9a** which were dehydrobrominated with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU)

and deprotected to give 5'-bromo-5'-deoxy-5'-methyleneadenosine (**10c**).

Inhibition of AdoHcy Hydrolase

EDDHAs **4c(E)**–**7c(E)**, ZDDHAs **4c(Z)**–**6c(Z)**, the acetylene derivative **3c**,^{13a} and the regioisomer **10c** were evaluated as potential inhibitors of purified recombinant human placental AdoHcy hydrolase. All of these compounds produced concentration-dependent inhibition of the enzyme, and the more potent agents were examined further and found to be time-dependent inactivators. As seen in Table 3, the inhibitory potencies of the DDHAs toward AdoHcy hydrolase were *E* > *Z* isomers and **4c** > **5c** > **6c** > **7c**. The kinetic data for **3c** and the EDDHAs are collected in Table 4. Mechanisms of interaction of the EDDHAs with AdoHcy hydrolase have been studied recently²⁸ and shown to involve reduction of enzyme-bound NAD⁺ to NADH, release of

Table 2. ^{13}C NMR Spectral Data^{a,b}

compd	C2	C4	C5	C6	C8	C1'	C2' ^c	C3' ^c	C4'	C5'	C6'
1c ^d	152.84	149.38	119.51	156.23	141.06	88.49	73.30	71.84	81.87	142.79	131.94
2b (E) ^e	152.42	149.09	119.39	156.42	140.46	89.90 ^c	84.55	83.55	89.13 ^c	145.79	131.49
2a (E) ^f	150.86	143.93	115.90	151.81	133.03	89.85 ^c	84.39	83.59	89.23 ^c	145.42	131.66
3c	153.06	149.74	119.03	156.00	139.61	87.51	73.58	73.24	81.23	78.88	75.26
4c (E)	150.05	149.14	119.38	153.89	141.33	87.94	73.25	72.92	86.14	143.66	82.61
4c (Z)	152.94	149.61	119.50	156.15	140.57	87.05 ^c	74.29	73.03	86.73 ^c	139.52	88.05 ^c
5c (E)	152.95	149.47	119.24	156.04	140.39	87.87	73.50	72.68	83.91	136.10	110.24
5c (Z)	152.89	149.63	119.68	156.39	140.60	87.98	74.54	73.01	82.47	133.89	111.41
6c (E)	152.93	149.59	119.49	156.37	140.24	87.91	73.94	72.96	82.56	132.47	121.74
6c (Z)	152.90	149.62	119.65	156.37	140.57	87.95	74.60	73.01	79.99	130.90	121.27
7c (E)	152.97	149.51	119.24	156.06	140.23	87.82	74.14	72.91	79.53 ^g	111.38 ^h	152.57 ⁱ
8c	153.10	149.73	119.23	156.18	140.19	87.65	73.82	72.76	84.89	136.74	117.54
10c	153.09	149.75	119.05	156.11	140.03	87.49	72.76	72.01	86.59	130.53	120.96

^a Chemical shifts (δ) in $\text{Me}_2\text{SO}-d_6$ at 50 MHz. ^b Proton-decoupled singlets. ^c Assignments might be reversed. ^d Peaks also at δ 21.03, 127.60, 130.43, 137.22, 144.93 (CH_3Ph). ^e Peaks also at δ 9.23, 13.78, 26.77, 28.70 (Bu_3Sn); 25.45, 27.13, 113.35 (CMe_2). ^f Peaks also at δ 9.22, 13.76, 26.78, 28.70 (Bu_3Sn); 25.47, 27.11, 113.47 (CMe_2); 125.95, 128.69, 128.77, 132.72, 165.75 (PhCO). ^g (d, $^3J_{4'-\text{F}}$ = 14.5 Hz). ^h (d, $^2J_{5'-\text{F}}$ = 10.7 Hz). ⁱ (d, $^1J_{6'-\text{F}}$ = 256.5 Hz).

Table 3. Inhibition of S-Adenosyl-L-homocysteine Hydrolase by Homoadenosine Derivatives

conc (μM)	enzyme activity remaining (%)							
	4c (E)	5c (E)	6c (E)	7c (E)	4c (Z)	5c (Z)	6c (Z)	3c
0.01	96.3	95.8	96.8	101.1	99.6	97.7	97.0	98.4
0.1	91.0	91.1	94.0	98.8	96.0	95.5	95.1	85.0
1	70.0	76.9	88.8	93.1	87.8	93.7	94.3	24.0
10	22.2	43.9	66.1	89.2	50.3	76.9	79.2	1.7
100	2.9	20.3	45.3	79.3	23.3	54.4	60.4	0

Table 4. Kinetic Constants for Inhibition of Recombinant Human Placental S-Adenosyl-L-homocysteine Hydrolase by Homoadenosine Derivatives

inhibitor	K_i (nM)	k_{inact} (min^{-1})	k_{inact}/K_i ($\mu\text{M}^{-1} \text{min}^{-1}$)	$t_{1/2}$ (min) ^a
4c (E)	96	0.058	0.60	25.7
5c (E)	134	0.028	0.21	53.3
6c (E)	110	0.014	0.13	77.2
7c (E)	1300	0.010	0.008	ND ^b
3c	681	0.25	0.37	21.8

^a Half-time for enzyme inactivation at 100 nM inhibitor concentration. ^b Not determined; see the discussion section on Inhibition of AdoHcy Hydrolase.

halide ion, and formation of adenine (Ade). Enzyme-catalyzed release of halide ion apparently results in formation of homoAdo-6'-carboxaldehyde, which is chemically unstable and spontaneously decomposes to Ade and a sugar moiety. The more rapid the halide ion release ($\text{F}^- > \text{Cl}^- > \text{Br}^- > \text{I}^-$) or the greater the partition ratios (nonlethal turnovers/lethal events) the lower the enzyme inactivation efficiency. Partition ratios for **4c**(E), **5c**(E), and **6c**(E) were determined to be 6.5, 11.5, and 28.2, respectively.^{28a} The partition ratio for the fluoro analogue **7c**(E) was 108, which makes this compound a valuable new tool for the selective evaluation of the hydrolytic activity of AdoHcy hydrolase independent of its oxidative function.^{28b} In contrast, bromide was not detected upon incubation of the regioisomer **10c** with the enzyme.

The iodo, bromo, and chloro EDDHHAs had similar K_i values (96–134 nM) but different k_{inact} values (0.058–0.014 min^{-1}), whereas **3c** had both greater K_i (681 nM) and k_{inact} (0.25 min^{-1}) values than those of the EDDHHAs. The half-time ($t_{1/2}$) for **3c**-induced loss of activity at 100 nM concentration was 21.8 min. That concentration of **4c**(E), **5c**(E), and **6c**(E) gave half-times of 25.7, 53.3, and 77.2 min, respectively (Table 4). The half-time for **7c**(E) at 100 nM was not determined since

Table 5. Cytostatic Activities of Homoadenosine Derivatives against Murine and Human Tumor Cell Lines *in Vitro*

compd	IC_{50}^a (μM)			
	L1210	FM3A	Molt-4 (clone 8)	CEM
1c	54 ± 14	208 ± 0.0	32 ± 1.3	20 ± 7.4
3c	1.2 ± 0.25	20 ± 9.9	10 ± 1.8	5.7 ± 3.2
4c (E)	15 ± 6.8	15 ± 6.0	18 ± 1.9	26 ± 9.9
4c (Z)	43 ± 26	43 ± 20	21 ± 7.8	32 ± 7.6
5c (E)	42 ± 28	60 ± 22	56 ± 3.2	56 ± 17
5c (Z)	104 ± 48	134 ± 66	93 ± 22	65 ± 9.4
6c (E)	139 ± 42	160 ± 7	80 ± 25	99 ± 33
6c (Z)	143 ± 19	164 ± 39	117 ± 20	86 ± 37
7c (E)	301 ± 55	287 ± 11	266 ± 5.0	147 ± 85
10c	25 ± 1.3	47 ± 9.2	ND ^b	13 ± 4.0

^a Concentration of compound that reduced the number of viable cells by 50%. Results are the means of at least two to three determinations. ^b Not determined.

higher concentrations of this agent were required for inactivation of the enzyme and the inhibition data with **7c**(E) were biphasic (nonlinear).^{28b} The acetylenic derivative **3c** appeared to be a better inhibitor of the enzyme in terms of its large k_{inact} value. This might reflect the fact that nonlethal turnovers and partitioning do not occur with **3c** during inactivation of AdoHcy hydrolase. Thus, in contrast with the EDDHHAs, every catalytic event with **3c** leads to inactivation of the enzyme. Irreversible inactivation of AdoHcy hydrolase by **3c** and the EDDHHAs was indicated by failure to regain catalytic activity after gel filtration to remove excess inhibitor or prolonged dialysis.

Cytostatic Activity

Cytostatic activities of the test compounds were determined against murine leukemia L1210, murine mammary carcinoma FM3A, and human lymphoblast Molt-4 (clone 8) and CEM cells (Table 5). With the exception of compound **3c** which was markedly more cytostatic to L1210 cells and compound **1c** which was markedly less cytostatic to FM3A cells than to the other

Table 6. Activities of Homoadenosine Derivatives against Viruses in Different Cell Systems

compd	minimum cytotoxic concentration ^a (μg/mL)			minimum inhibitory concentration ^b (MIC) (μg/mL)										
				human E ₆ SM cells				human HeLa cells			simian Vero cells			
	E ₆ SM	HeLa	Vero	HSV-1 (KOS)	HSV-2 (G)	VV	VSV	VSV	Coxsackie B4	polio-1	parainfluenza-3 virus	reovirus-1	Sindbis virus	Semliki forest virus
1c	200	>400	>400	20	20	20	>100	>400	>400	>400	>400	>400	>400	>400
4c(E)	≥200	200	≥200	>100	>100	7	2	7	>100	>100	>200	>200	>200	>200
4c(Z)	>200	>200	>200	>200	>200	20	7	7	>200	>200	>200	100	>200	>200
5c(E)	≥200	≥200	≥200	150	>100	20	7	7	>100	>100	>200	>100	>100	>100
5c(Z)	>200	>200	≥200	70	>200	150	150	150	>200	>200	150	>100	20	150
6c(E)	400	≥200	≥200	>200	>200	70	70	>200	>200	>200	>200	>100	>100	>100
6c(Z)	≥200	≥200	≥100	70	>100	20	20	70	>100	>100	>100	70	20	70
7c(E)	>200	>200	>200	>200	>200	150	70	100	>100	>100	150	150	70	>200

^a Required to cause a microscopically observable alteration of normal cell morphology. ^b Required to reduce virus-induced cytopathicity by 50%. Viral cytopathicity was recorded as soon as it reached completion in the control virus-infected cell cultures. The multiplicity of infection (MOI) invariably was $100 \times \text{CCID}_{50}$ (100 times the virus dose required to infect 50% of the cell cultures).

tumor cell lines, the present homoadenosine analogues showed comparable activities against the four different tumor cell lines.

The *E* isomers of **4c** and **5c** were slightly more cytostatic than their *Z* counterparts. The (iodovinyl)-homoadenosine derivatives **4c(E)** and **5c(E)** were 2–4-fold more inhibitory to tumor cell proliferation than bromovinyl derivatives **5c(E)** and **5c(Z)**, 3–10-fold more inhibitory than chlorovinyl derivatives **6c(E)** and **6c(Z)**, and up to 20-fold more cytostatic than (fluorovinyl)homoadenosine **7c(E)** (Table 5). The acetylene derivative **3c** was a more potent cytostatic agent than any of the other homoadenosine derivatives tested. Interestingly, the 5'-bromovinyl compound **10c** was slightly more cytostatic (1.5–4-fold) than its 6'-bromo regioisomer, **5c(E)**.

Antiviral Activity

The homoadenosine derivatives were evaluated for inhibitory effects on several viruses in different cell systems. The most pronounced antiviral activity was found for compounds **4c(E)** and **5c(E)**, which were inhibitory to vaccinia virus (VV) and vesicular stomatitis virus (VSV) at minimum inhibitory concentrations (MIC) of 2–20 and 7–20 $\mu\text{g/mL}$, respectively (Table 6).

Compound **6c(Z)** was active against VV (MIC: 20 $\mu\text{g/mL}$) and VSV (MIC: 20–70 $\mu\text{g/mL}$). This compound also showed moderate activity against Sindbis virus (MIC: 20 $\mu\text{g/mL}$), reovirus-1, and Semliki forest virus (MIC: 70 $\mu\text{g/mL}$). Compound **6c(E)** was clearly less inhibitory to these viruses than its *Z* isomer. Marginal antiviral activity (MIC: 70–150 $\mu\text{g/mL}$) was noted for compounds **5c(Z)** and **7c(E)** against several viruses in this study. The test compounds were not inhibitory to HIV-1(III_B)- and HIV-2(ROD)-induced giant cell formation at subtoxic concentrations. At 200 $\mu\text{g/mL}$, most of the test compounds were slightly cytotoxic to E₆SM, HeLa, and Vero cell cultures, as measured by a microscopically detectable alteration of normal cell morphology.

Correlation between Inhibition of S-Adenosyl-L-Homocysteine Hydrolase and Antiviral/Cytostatic Activities

A good correlation was observed between the inhibition of AdoHcy hydrolase by the homoadenosine derivatives and their cytostatic potencies. Thus, the most potent inhibitors of AdoHcy hydrolase (i.e., **3c** and **4c**) also showed the most potent cytostatic activities. This

trend also was apparent in the antiviral activities against VSV and VV, two viruses that are known to be highly sensitive to AdoHcy hydrolase inhibitors. Replacement of the iodo substituent at C6' of the DDHHAs by bromo, chloro, and fluoro resulted in progressive loss of cytostatic activities and parallel diminished inhibitory effects on AdoHcy hydrolase. The *E* isomers were more inhibitory to AdoHcy hydrolase than their *Z* counterparts, and this generally correlated with the more pronounced antiviral activity of the *E* isomers. Interestingly, **3c** which had the highest k_{inact} for AdoHcy hydrolase also was the most potent cytostatic agent.

Experimental Section

Uncorrected melting points were determined on a Hoover capillary apparatus. UV spectra of solutions in MeOH were recorded on a Hewlett Packard 8951A spectrophotometer. ¹H (200-MHz) and ¹³C (50-MHz) NMR spectra were recorded on a Varian Gemini-200 spectrometer in Me₂SO-*d*₆ unless otherwise noted. Low-resolution electron-impact (20-eV) (MS) and chemical ionization (CI, CH₄) mass spectra were obtained with a Finnigan MAT 8430 instrument ("BH" = adenine and "BH₂" = adenine + H⁺ in the MS designations). Elemental analyses were determined by M-H-W Laboratories, Phoenix, AZ. Reagents and solvents were of reagent quality, and solvents were purified and dried before use. "Diffusion crystallization" was performed with the noted solvent combinations as described.²⁹ TLC was performed on Merck kieselgel 60 F₂₅₄ sheets with S₁ (EtOAc/*i*-PrOH/H₂O, 4:1:2; upper layer), S₂ (MeOH/EtOAc, 1:20), or S₃ (MeOH/CHCl₃, 1:6) with sample observation under 254-nm light. Column chromatography was performed with Merck kieselgel 60 (230–400 mesh). Preparative and analytical RP-HPLC were performed with a Spectra Physics SP 8800 ternary pump system and Dynamax C₁₈ columns.

9-[6-(E/Z)-(Tributylstannyl)-5,6-dideoxy-2,3-O-isopropylidene- β -D-ribo-hex-5-enofuranosyl]adenine [2b(E/Z)]. A suspension of **1b**¹¹ (915 mg, 2 mmol) in toluene (30 mL) was deoxygenated (Ar, 30 min), and Bu₃SnH (2.04 g, 1.88 mL, 7 mmol) was added. Deoxygenation was continued for 15 min, and AIBN (41 mg, 0.25 mmol) was added. The solution was refluxed for 3 h [TLC (S₂) showed less polar products] and evaporated, and the residue was column chromatographed. Slow elution [hexanes/EtOAc (1:4) \rightarrow EtOAc \rightarrow MeOH/EtOAc (1:40)] and careful analysis of fractions gave viscous oils of **2b(E)** (403 mg, 34%), **2b(E/Z)** (225 mg, 19%), and **2b(Z)** (95 mg, 8%; contaminated with byproducts). Further elution gave recovered **1b** (166 mg, 18%; contaminated with byproducts). **2b(E)**: MS (CI) m/z 594 (89, MH⁺, ¹²⁰Sn), 592 (63, MH⁺, ¹¹⁸Sn), 590 (33, MH⁺, ¹¹⁶Sn), 536 (100, M - 57, ¹²⁰Sn), 534 (78, M - 57, ¹¹⁸Sn), 532 (42, M - 57, ¹¹⁶Sn). **2b(Z)**: MS (CI) m/z 594 (54, MH⁺, ¹²⁰Sn), 592 (43, MH⁺, ¹¹⁸Sn), 590 (22, MH⁺, ¹¹⁶Sn), 536 (100, M - 57, ¹²⁰Sn), 534 (77, M - 57, ¹¹⁸Sn), 532 (43, M - 57, ¹¹⁶Sn). Analogous treatment of **1b** in benzene (8 h, reflux) gave **2b(E/Z)** (~6:1; 37%) and recovered **1b** (53%) with less byproduct formation.

6-*N*-Benzoyl-9-[6-(*E/Z*)-(tributylstannyl)-5,6-dideoxy-2,3-*O*-isopropylidene- β -D-ribo-hex-5-enofuranosyl]adenine [2a(*E/Z*)]. Treatment of **1a**¹¹ (1.12 g, 2 mmol) with Bu₃SnH (3 equiv)/AIBN (0.13 equiv) in toluene (25 mL) at reflux (3 h) [as described above for **2b**(*E/Z*)] gave **2a**(*E*) (167 mg, 12%) [MS *m/z* 640 (100, M - 57, ¹²⁰Sn), 638 (71, M - 57, ¹¹⁸Sn), 636 (39, M - 57, ¹¹⁶Sn), 408 (81)] and **2a**(*E/Z*, ~4:1; 404 mg, 29%). Further elution gave recovered **1a**, debenzoylated starting material **1b**, and byproducts. Analogous treatment of **1a** in xylene (2 h, reflux) gave **2a**(*E/Z*, ~8:1; 35%).

9-[5,6-Dideoxy-2,3-*O*-isopropylidene- β -D-ribo-hex-5-enofuranosyl]adenine (3b**)**. A deoxygenated solution of **2b**(*E*) (118 mg, 0.2 mmol) in anhydrous CH₃CN (8 mL) under Ar was treated with Pb(OAc)₄ (111 mg, 0.25 mmol) and stirred at ~0 °C (ice bath) for 4 h. TLC (S₂) showed a mixture of **2b**(*E*) and the more polar **3b** (~1:1). Additional Pb(OAc)₄ (33 mg, 0.075 mmol) was added, and stirring was continued for 1 h at ~0 °C and then for 2 h at ambient temperature. The mixture was evaporated, the residue was partitioned (NaHCO₃/H₂O/CHCl₃), and the organic phase was washed (NaHCO₃/H₂O and brine), dried (MgSO₄), and evaporated. Column chromatography (EtOAc) of the residue gave **2b**(*E*) (23 mg, 19%) and **3b** (43 mg, 71%): mp 190–192 °C (white powder); MS *m/z* 301 (13, M⁺), 286 (16), 243 (45), 186 (65), 164 (100).

9-[5,6-Dideoxy- β -D-ribo-hex-5-enofuranosyl]adenine (3c**)**. A solution of **3b** (40 mg, 0.13 mmol) in CF₃CO₂H/H₂O (9:1, 5 mL) was stirred at 0 °C for 1 h, evaporated, and coevaporated (EtOH). The residue was dissolved (H₂O/MeOH, 4:1, 5 mL), the solution was cooled (ice bath), and H₂S was gently bubbled through for 30 s. The mixture was filtered through Celite, the filtrate was evaporated, and the residue was column chromatographed (EtOAc → S₁) and crystallized (MeOH) to give **3c** (25 mg, 74%): mp 204–205 °C dec (lit.^{13a} mp 208–210 °C); UV max 259 nm (ϵ 14 100), min 228 (ϵ 2400); MS (CI) *m/z* 262 (56, MH⁺), 136 (100, BH₂).

9-[5,6-Dideoxy-6-(*E*)-iodo-2,3-*O*-isopropylidene- β -D-ribo-hex-5-enofuranosyl]adenine [4b(*E*)]. A solution of NIS (56 mg, 0.25 mmol) in CH₂Cl₂ (10 mL) was added dropwise to a stirred solution of **2b**(*E*) (120 mg, 0.20 mmol) in CH₂Cl₂/CCl₄ (15 mL, 1:1) at ~-20 °C. After 1.5 h, the slightly pink mixture was poured into saturated NaHCO₃/H₂O and extracted (CHCl₃). The combined organic phase was washed with very dilute NaHSO₃/H₂O (to effect decolorization) and brine, dried (MgSO₄), and evaporated to give **4b**(*E*) as a white solid (86 mg, quantitative). Column chromatography [EtOAc → MeOH/EtOAc (1:20)] and diffusion crystallization (EtOAc/hexane) gave shiny crystals of **4b**(*E*) (78 mg, 91%): mp 197–198 °C; UV max 259 nm (ϵ 14 300), min 236 (ϵ 6100); MS *m/z* 429 (50, M⁺), 302 (100), 135 (95, BH). Anal. (C₁₄H₁₆IN₅O₃) C, H, N.

9-[5,6-Dideoxy-6-(*E*)-iodo- β -D-ribo-hex-5-enofuranosyl]adenine [4c(*E*)]. A solution of **4b**(*E*) (70 mg, 0.16 mmol) in CF₃CO₂H/H₂O (9:1, 5 mL) was stirred at ~0 °C for 1 h, evaporated, and coevaporated (EtOH). The colorless solid was crystallized (MeOH) to give **4c**(*E*) (54 mg, 86%): mp 133–138 °C (softening), 218–220 °C dec; UV max 259 nm (ϵ 14 300), min 236 (ϵ 5400); MS *m/z* 389 (19, M⁺), 262 (100), 136 (84, BH₂). Anal. (C₁₁H₁₂IN₅O₃) C, H, N.

9-[5,6-Dideoxy-6-(*Z*)-iodo- β -D-ribo-hex-5-enofuranosyl]adenine [4b(*Z*)]. Treatment of **2b**(*Z*) (59 mg, 0.1 mmol) with NIS (27 mg, 0.12 mmol) [as described for **4b**(*E*)] gave amorphous **4b**(*Z*) (40 mg, 93%): MS *m/z* 429 (31, M⁺), 371 (100), 302 (96), 164 (92), 135 (57, BH). Identical treatment of **2b**(*Z*) (*R*_f 0.72 in S₂; contaminated with byproducts) with NIS gave **4b**(*Z*) (*R*_f 0.50 in S₂; unchanged byproducts, *R*_f 0.70). Treatment of **2b**(*E/Z*) with NIS gave **4b**(*E/Z*), which could be partially separated on a silica gel column [EtOAc → MeOH/EtOAc (1:20); **4b**(*E*), *R*_f 0.55 in S₂] or easily after deprotection as described under **4c**(*Z*). Analogous treatment of **2a**(*E/Z*) with iodine (1.15 equiv) at ~-15 °C for 1.5 h gave **4a**(*E/Z*) (95%).

9-[5,6-Dideoxy-6-(*Z*)-iodo- β -D-ribo-hex-5-enofuranosyl]adenine [4c(*Z*)]. Deprotection of **4b**(*Z*) (38 mg, 0.09 mmol) [as described for **4c**(*E*)] gave an oily residue which was purified by RP-HPLC (preparative column; program: 18% CH₃CN/H₂O for 40 min followed by a gradient of 18–25% for 100 min at 2.7 mL/min) and crystallized (MeOH) to give **4c**(*Z*) (26 mg,

75%): mp 206–208 °C dec; UV max 259 nm (ϵ 14 800), min 236 nm (ϵ 4800); MS (CI) *m/z* 390 (100, MH⁺). Anal. (C₁₁H₁₂IN₅O₃) C, H, N. Attempts to purify the crude **4c**(*Z*) (19 mg, 0.05 mmol) on Dowex 1 × 2 (OH⁻) resin with slow elution [H₂O → H₂O/MeOH (1:4)] resulted in elimination to give **3c** (9 mg, 70%); no **4c**(*Z*) was detected in the eluate. Deprotection of **4b**(*E/Z*) mixtures and RP-HPLC separation as described gave **4c**(*Z*) (*t*_R = 75 min) and **4c**(*E*) (*t*_R = 95 min).

9-[6-(*E*)-Bromo-5,6-dideoxy-2,3-*O*-isopropylidene- β -D-ribo-hex-5-enofuranosyl]adenine [5b(*E*)]. A solution of NBS (59 mg, 0.33 mmol) in CH₂Cl₂/CCl₄ (1:1, 10 mL) was added dropwise to a stirred solution of **2b**(*E*) (177 mg, 0.3 mmol) in CH₂Cl₂/CCl₄ (1:1, 6 mL) at ~-30 °C. After 30 min, the mixture was poured into saturated NaHCO₃/H₂O and extracted (CHCl₃). The combined organic phase was washed (brine), dried (MgSO₄), and evaporated, and the residue was column chromatographed [EtOAc → MeOH/EtOAc (1:20)] to afford colorless amorphous **5b**(*E*) (109 mg, 95%): MS (CI) *m/z* 384 (96, MH⁺, ⁸¹Br), 382 (100, MH⁺, ⁷⁹Br). Analogous treatment of **2b**(*E*) with Br₂ (1.1 equiv; ~-45 °C, 5 min) and workup as described gave **5b**(*E*) (91%).

9-[6-(*E*)-Bromo-5,6-dideoxy- β -D-ribo-hex-5-enofuranosyl]adenine [5c(*E*)]. Deprotection of **5b**(*E*) (77 mg, 0.2 mmol) [as described for **4c**(*E*)] gave a colorless solid that was chromatographed on a column of Dowex 1 × 2 (OH⁻) (~3 g of resin). The product was rapidly eluted (H₂O → MeOH), the eluate was evaporated, and the white solid was crystallized (MeOH) to give **5c**(*E*) (57 mg, 83%): mp 127–135 °C (softening), 202–204 °C dec; MS (CI) *m/z* 344 (98, MH⁺, ⁸¹Br), 342 (100, MH⁺, ⁷⁹Br); UV max 259 nm (ϵ 14 200), min 228 nm (ϵ 2400). Anal. (C₁₁H₁₂BrN₅O₃) C, H, N.

9-[6-(*Z*)-Bromo-5,6-dideoxy-2,3-*O*-isopropylidene- β -D-ribo-hex-5-enofuranosyl]adenine [5b(*Z*)]. Treatment of **2b**(*E/Z*, ~1:4; 177 mg, 0.3 mmol) with NBS [as described for **5b**(*E*)] gave **5b**(*E*) (13 mg, 11%), **5b**(*E/Z*, ~1:2; 24 mg, 21%), and **5b**(*Z*) (69 mg, 60%): MS (CI) *m/z* 384 (98, MH⁺, ⁸¹Br), 382 (100, MH⁺, ⁷⁹Br).

9-[6-(*Z*)-Bromo-5,6-dideoxy- β -D-ribo-hex-5-enofuranosyl]adenine [5c(*Z*)]. Deprotection of **5b**(*Z*) (57 mg, 0.15 mmol) [as described for **4c**(*E*)] gave a slightly yellow residue that was purified by RP-HPLC [as described for **4c**(*Z*)] and crystallized (MeOH) to give **5c**(*Z*) (45 mg, 88%, *t*_R = 90 min): mp 226–228 °C dec; UV max 259 nm (ϵ 14 200), min 229 nm (ϵ 2200); MS *m/z* 343 (24, M⁺, ⁸¹Br), 341 (25, M⁺, ⁷⁹Br), 262 (100), 136 (92, BH₂). Anal. (C₁₁H₁₂BrN₅O₃) C, H, N. Deprotection of various **5b**(*E/Z*) mixtures and RP-HPLC separation as described gave **5c**(*Z*) (*t*_R = 90 min) and **5c**(*E*) (*t*_R = 110 min).

9-[6-(*E*)-Chloro-5,6-dideoxy- β -D-ribo-hex-5-enofuranosyl]adenine [6c(*E*)] and 9-[6-(*Z*)-Chloro-5,6-dideoxy- β -D-ribo-hex-5-enofuranosyl]adenine [6c(*Z*)]. (A) Chlorodestannylation: Cl₂ was gently bubbled through a solution of **2a**(*E/Z*, ~6:1; 348 mg, 0.5 mmol) in CCl₄/CH₂Cl₂ (1:1, 5 mL) at ~-50 °C, and stirring was continued for 5 min. TLC (S₂) showed a single spot of less polar products. The solution was carefully washed (NaHCO₃/H₂O, dilute NaHSO₃/H₂O, and brine), dried (MgSO₄), and evaporated. Column chromatography of the white foam [EtOAc → MeOH/EtOAc (1:30)] gave **6a**(*E/Z*, ~2:1; 182 mg, 82%) with earlier fractions enriched in the *E* isomer and later fractions enriched in the *Z* isomer. **6a**(*E/Z*, ~1:2): MS *m/z* 443 (22, M⁺, ³⁷Cl), 441 (66, M⁺, ³⁵Cl), 414 (40), 412 (100), 406 (52).

(B) Deprotection: (a) Removal of the 6-*N*-Benzoyl Group. A solution of **6a**(*E/Z*, ~2:1; 160 mg, 0.36 mmol) in saturated NH₃/MeOH (15 mL) was stirred at ambient temperature overnight. TLC (S₁ and S₃) showed a single new polar spot. Evaporation of the solution gave crude **6b**(*E/Z*) (165 mg) which was used directly in the next reaction. (b) Removal of the 2',3'-*O*-Isopropylidene Group. A solution of crude **6b**(*E/Z*) (165 mg) in CF₃CO₂H/H₂O (9:1, 5 mL) was stirred at ~0 °C for 1 h, evaporated, and coevaporated (EtOH). The slightly yellow residue was purified on a short silica gel column (EtOAc → S₁) and separated by RP-HPLC (preparative column; program: 17% CH₃CN/H₂O for 30 min followed by a gradient of 17–30% for 90 min at 2.8 mL/min) to give **6c**(*Z*) (28 mg, 26%, *t*_R = 90 min) and **6c**(*E*) (59 mg, 55%, *t*_R = 115 min). **6c**(*Z*): mp 195–202 °C dec (diffusion crystallization, MeOH/EtOAc);

UV max 259 nm (ϵ 15 100), min 227 nm (ϵ 3900); MS m/z 299 (12, M^+ , ^{37}Cl), 297 (34, M^+ , ^{35}Cl), 262 (75, $M - \text{Cl}$), 178 (33), 164 (60), 135 (100, BH). Anal. ($\text{C}_{11}\text{H}_{12}\text{ClN}_5\text{O}_3$) C, H, N. **6c(E)**: mp 121–123 °C (softening), 200–203 °C dec (from MeOH); UV max 259 nm (ϵ 14 200), min 227 nm (ϵ 1900); MS (CI) m/z 300 (35, $M\text{H}^+$, ^{37}Cl), 298 (100, $M\text{H}^+$, ^{35}Cl). Anal. ($\text{C}_{11}\text{H}_{12}\text{ClN}_5\text{O}_3$) C, H, N.

9-[5,6-Dideoxy-6-(E)-fluoro- β -D-ribo-hex-5-enofuranosyl]-adenine [7c(E)]. (A) Fluorodestannylation: A solution of **2a (E/Z, ~6:1; 278 mg, 0.4 mmol) in anhydrous CH_2Cl_2 (3 mL) was injected into a stirred suspension of AgOTf (129 mg, 0.5 mmol) in anhydrous CH_2Cl_2 (1 mL) under Ar at ambient temperature in a flame-dried flask with a rubber septum. XeF_2 (93 mg, 0.55 mmol) in anhydrous CH_2Cl_2 was transferred immediately via cannula into the mixture. The flask was covered with aluminum foil, stirring was continued for 15 min, and the mixture was partitioned ($\text{NaHCO}_3/\text{H}_2\text{O}/\text{CHCl}_3$). The H_2O layer was extracted (CHCl_3), and the combined organic phase was washed ($\text{NaHCO}_3/\text{H}_2\text{O}$ and brine), dried (MgSO_4), concentrated, and column chromatographed (MeOH/ CHCl_3 , 1:30) to give **7a(E)/8a** (~3:1, ^1H NMR; 131 mg, 78%) as a slightly yellow foam: MS (CI) m/z 426 (26, $M\text{H}^+$), 397 (81), 105 (100).**

(B) Deprotection: (a) Treatment of **7a(E)/8a** (~3:1; 131 mg) with NH_3/MeOH (15 mL) [as described for **6c(E/Z)**] gave crude **7b(E)/8b** (135 mg). (b) A solution of crude **7b(E)/8b** (135 mg) in $\text{CF}_3\text{CO}_2\text{H}/\text{H}_2\text{O}$ (9:1, 5 mL) was stirred at ~0 °C for 1 h followed by evaporation and coevaporation (EtOH). The slightly yellow residue [TLC (S_1) showed the presence of adenine (~15%)] was purified on a short silica gel column (EtOAc \rightarrow S_1) and separated by RP-HPLC (preparative column; program: 12% $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ for 40 min followed by a gradient of 12–40% for 50 min at 3.0 mL/min) to give **8c** (22 mg, 21% from **2a**; t_R = 65 min) and **7c(E)** (45 mg, 40% from **2a**; t_R = 73 min). **7c(E)**: mp 205–207 °C dec (from MeOH); UV max 259 nm (ϵ 15 000), min 227 nm (ϵ 3400); ^{19}F NMR ($\text{DMSO}-d_6/\text{CCl}_3\text{F}$) δ -124.45 (dd, $^2J_{\text{F-H6}} = 83.7$ Hz, $^3J_{\text{F-H5}} = 17.8$ Hz, F6'); MS (CI) m/z 282 (20, $M\text{H}^+$), 164 (80), 135 (100, BH). Anal. ($\text{C}_{11}\text{H}_{12}\text{FN}_5\text{O}_3$) C, H, N.

6-N-Benzoyl-9-(5,6-dideoxy-2,3-O-isopropylidene- β -D-ribo-hex-5-enofuranosyl)adenine (8a). A solution of **2a** (E/Z, ~6:1; 348 mg, 0.5 mmol) and NH_4F (165 mg, 5 mmol) in anhydrous EtOH (25 mL) was refluxed for 14 h and evaporated. The white foam was partitioned ($\text{NaHCO}_3/\text{H}_2\text{O}/\text{CHCl}_3$), and the organic layer was washed (brine), dried (MgSO_4), and concentrated. Column chromatography [hexanes/EtOAc (1:5) \rightarrow EtOAc \rightarrow MeOH/EtOAc (1:20)] gave in order of elution: recovered **2a** (31 mg, 9%), **8a** (122 mg, 60%), and **8b** (36 mg, 24%). Compounds **8a,b** had spectral data identical to those reported.¹¹ Treatment of **2b** (E/Z, ~4:1; 120 mg, 0.2 mmol) with NH_4F (111 mg, 3 mmol) in anhydrous MeOH (reflux, 24 h) gave recovered **2b** (48 mg, 40%) and **8b** (33 mg, 55%), whereas $\text{NH}_4\text{F}/\text{EtOH}$ (anhydrous) at reflux (16 h) gave **8b** (55 mg, 91%). Deprotection of **8a** [as described for **6c(E/Z)**] gave **8c** (82%, recrystallized from MeOH) with data as reported.¹¹

6-N-Benzoyl-9-(5,6-dibromo-5,6-dideoxy-2,3-O-isopropylidene- β -D-ribo-hexofuranosyl)adenine (9a). Br_2 (70 mg, 0.44 mmol) in CCl_4 (5 mL) was added dropwise to a stirred solution of **8a** (163 mg, 0.40 mmol) in CCl_4 (10 mL) at ~-10 °C until a slightly yellow color persisted. After 2 h, a pale yellow precipitate (150 mg, mp 159–163 °C) was filtered and washed (CCl_4 , 5 mL). The concentrated mother liquor (~5 mL) was again treated with bromine (20 mg, 0.12 mmol) to afford additional crude **9a** (38 mg). The combined product was flash chromatographed (MeOH/ CHCl_3 , 1:19) to give colorless amorphous **9a** (138 mg, 61%): MS m/z 569 (10, M^+ , $^{81}\text{Br}_2$), 567 (21, M^+ , ^{81}Br , ^{79}Br), 565 (11, M^+ , $^{79}\text{Br}_2$), 540 (60, $^{81}\text{Br}_2$), 538 (100, ^{81}Br , ^{79}Br), 536 (50, $^{79}\text{Br}_2$), 322 (52).

6-N-Benzoyl-9-(5-bromo-5,6-dideoxy-2,3-O-isopropylidene- β -D-ribo-hex-5-enofuranosyl)adenine (10a). DBU (0.099 mL, 100 mg, 0.66 mmol) was injected into a stirred solution of purified **9a** (125 mg, 0.22 mmol) in anhydrous THF (5 mL) at ~0 °C. Stirring was continued at ~0 °C for 30 min and at ambient temperature for 5 h. DBU hydrobromide was filtered, and the mother liquor was evaporated. The residue was partitioned (0.1 M $\text{HCl}/\text{H}_2\text{O}/\text{CHCl}_3$), and the organic phase

was washed ($\text{NaHCO}_3/\text{H}_2\text{O}$ and brine), dried (MgSO_4), and evaporated. The residue was column chromatographed (MeOH/ CHCl_3 , 1:19) to give colorless amorphous **10a** (97 mg, 91%): MS m/z 487 (10, M^+ , ^{81}Br), 485 (10, M^+ , ^{79}Br), 458 (38, ^{81}Br), 456 (38, ^{79}Br), 406 (100, $M - \text{Br}$). [Analogous treatment of unpurified **9a** (68 mg) with DBU gave **10a** (39 mg, 59% from **8a**).]

9-(5-Bromo-5,6-dideoxy- β -D-ribo-hex-5-enofuranosyl)-adenine (10c). Deprotection of **10a** (97 mg, 0.2 mmol) with (a) NH_3/MeOH and (b) $\text{CF}_3\text{CO}_2\text{H}/\text{H}_2\text{O}$ [as described for **6c(E/Z)**] gave a polar compound [TLC (S_1 and S_3)] which was column chromatographed (EtOAc \rightarrow S_1) and crystallized (MeOH) to afford colorless crystalline **10c** (97 mg, 69%): mp 113–116 °C (softening), 162–165 °C dec; UV max 258 nm (ϵ 14 500), min 228 (ϵ 4000); MS (CI) m/z 344 (95, $M\text{H}^+$, ^{81}Br), 342 (98, $M\text{H}^+$, ^{79}Br), 262 (32), 164 (34), 136 (100, BH₂). Anal. ($\text{C}_{11}\text{H}_{12}\text{BrN}_5\text{O}_3$) C, H, N.

Purification of AdoHcy Hydrolase and Evaluation of the Effectiveness of Potential Inhibitors. Recombinant human placental AdoHcy hydrolase was purified from cell-free extracts of *Escherichia coli* transformed with the plasmid pPROKcd 20 and grown in the presence of isopropyl β -thiogalactopyranoside as previously described.³⁰ To evaluate the inhibitory potential of the compounds, different concentrations (0.01–100 μM) were preincubated with 20 nM enzyme at 37 °C for 10 min at pH 7.2 in 50 mM potassium phosphate buffer containing 1 mM EDTA. The mixture was then incubated with 100 μM [2,8- ^3H]AdoHcy (7.1 mCi/mmol) in the presence of 4 units of calf intestinal Ado deaminase for 5 min. This reaction was terminated by addition of 100 μL of 5 M formic acid, and the mixture was applied to a column (1 \times 4 cm) of SP Sephadex C-25 equilibrated in 0.1 N formic acid. The [2,8- ^3H]Ino that was formed by deamination of [2,8- ^3H]Ado (from hydrolysis of AdoHcy) was eluted with 8 mL of 0.1 N formic acid. The eluate was collected, and its radioactivity was determined with 1 mL of eluate mixed with 10 mL of scintillation cocktail (3a70; Research Products International) in a scintillation counter.

Determination of Kinetic Constants for Inhibition of AdoHcy Hydrolase. To determine the kinetic constants (K_i and k_{inact}) of enzyme inactivation, various concentrations of the inhibitors were preincubated with purified recombinant human placental AdoHcy hydrolase (10 nM) at 37 °C for various times at pH 7.2 in 0.5 mL of 50 mM potassium phosphate buffer containing 1 mM EDTA. Residual enzyme activity was determined in the synthetic direction by adding 10 μL of 10 mM Ado and 40 μL of 68.7 mM homocysteine to the mixture and continuing the incubation for 5 min. The reaction was terminated by adding 25 μL of 5 N perchloric acid, and the AdoHcy formed was analyzed by HPLC on a C-18 reversed-phase column (Econosphere, Alltech; 250 \times 4.6 mm). Elution was performed with two sequential linear gradients: 6–15% A in B over 0–9 min and 15–50% A in B over 9–15 min, where mobile phase A was acetonitrile and mobile phase B was 50 mM sodium phosphate buffer (pH 3.2) containing 10 mM heptanesulfonate. Quantitation of AdoHcy was monitored (UV) at 258 nm.

Pseudo-first-order constants (K_{app}) were obtained from plots of log percent of remaining activity vs preincubation time at each concentration of the inhibitor. K_i and k_{inact} values were obtained from plots of $1/K_{\text{app}}$ vs $1/[I]$ using the equation

$$1/K_{\text{app}} = 1/k_{\text{inact}} + K_i/k_{\text{inact}}[I]$$

Cytostatic Assays. Cytostatic assays were performed as previously described.³¹ Briefly, 100- μL aliquots of cell suspensions of 5×10^5 murine leukemia L1210 or murine mammary carcinoma FM3A cells/mL or 7.5×10^5 human T-lymphocyte Molt-4 (clone 8) or CEM cells/mL were added to the wells of a microtiter plate containing 100 μL of varying concentrations of the test compounds. After a 2-day (L1210 and FM3A) or 3-day [Molt-4 (clone 8) and CEM] incubation period at 37 °C in a humidified CO_2 -controlled incubator, the number of viable cells was determined using a Coulter Counter. Cytostatic activity is expressed as the concentration of compound that reduces the number of viable cells by 50% (IC_{50}).

Antiviral Assays. Antiviral assays were based on inhibition of virus-induced cytopathicity in E₆SM, HeLa, or Vero cell cultures following previously established procedures.^{32,33} Briefly, confluent cell cultures in microtiter trays were inoculated with 100 CCID₅₀ of virus, 1 CCID₅₀ being 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 varying 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. Effects of the test compounds on HIV-1(III_B)- and HIV-2(ROD)-induced giant cell formation was performed in CEM cell cultures as previously described.³⁴

Acknowledgment. We thank the American Cancer Society (Grant No. DHP-34), Brigham Young University development funds, the United States Public Health Service (Grant No. GM-29332), the Belgian Fonds voor Geneeskundig Wetenschappelijk Onderzoek (Project No. 3.0026.91), the Belgian Geconcerteerde Onderzoeksacties (Conventie No. 90/94-2), and the Biomedical Research Programme of the European Community for generous support. We thank Anita Van Lierde, Frieda de Meyer, Anita Camps, Lizette van Berckelaer, and Ann Absillis for excellent technical assistance and Mrs. Hazel Dunsmore for assistance with the manuscript.

References

- (1) For the previous paper in this series, see: Samano, V.; Robins, M. J. Synthesis of 3'-Deoxyadenosine-3'-spirocyclopropane, 3'-Deoxyuridine-3'-spirocyclopropane, and 5'-Deoxy-4',5'-methanoadenosine. *Tetrahedron Lett.* **1994**, *35*, 3445-3448.
- (2) (a) Takenuki, K.; Matsuda, A.; Ueda, T.; Sasaki, T.; Fujii, A.; Yamagami, K. Design, Synthesis, and Antineoplastic Activity of 2'-Deoxy-2'-methylidenecytidine. *J. Med. Chem.* **1988**, *31*, 1063-1064. (b) Matsuda, A.; Takenuki, K.; Tanaka, M.; Sasaki, T.; Ueda, T. Nucleosides and Nucleotides. 97. Synthesis of New Broad Spectrum Antineoplastic Nucleosides, 2'-Deoxy-2'-methylidenecytidine (DMDC) and Its Derivatives. *J. Med. Chem.* **1991**, *34*, 812-819.
- (3) (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) Jarvi, E. T.; McCarthy, J. R.; Mehdi, S.; Matthews, D. P.; Edwards, M. L.; Prakash, N. J.; Bowlin, T. L.; Sunkara, P. S.; Bey, P. 4',5'-Unsaturated 5'-Halogenated Nucleosides. Mechanism-Based and Competitive Inhibitors of S-Adenosyl-L-homocysteine Hydrolase. *J. Med. Chem.* **1991**, *34*, 647-656.
- (4) Baker, C. H.; Banzon, J.; Bollinger, J. M.; Stubbe, J.; Samano, V.; Robins, M. J.; Lippert, B.; Jarvi, E.; Resvick, R. 2'-Deoxy-2'-methylidenecytidine and 2'-Deoxy-2',2'-difluorocytidine 5'-Diphosphates: Potent Mechanism-Based Inhibitors of Ribonucleotide Reductase. *J. Med. Chem.* **1991**, *34*, 1879-1884.
- (5) Lin, T.-S.; Luo, M.-Z.; Liu, M.-C.; Clarke-Katzenburg, R. H.; Cheng, Y.-C.; Prusoff, W. H.; Mancini, W. R.; Birnbaum, G. I.; Gabe, E. J.; Giziewicz, J. Synthesis and Anticancer and Antiviral Activities of Various 2'- and 3'-Methylidene-Substituted Nucleoside Analogues and Crystal Structure of 2'-Deoxy-2'-methylidenecytidine Hydrochloride. *J. Med. Chem.* **1991**, *34*, 2607-2615.
- (6) McCarthy, J. R.; Matthews, D. P.; Stemerick, D. M.; Huber, E. W.; Bey, P.; Lippert, B. J.; Snyder, R. D.; Sunkara, P. S. Stereospecific Method to E and Z Terminal Fluoro Olefins and Its Application to the Synthesis of 2'-Deoxy-2'-fluoromethylene Nucleosides as Potential Inhibitors of Ribonucleoside Diphosphate Reductase. *J. Am. Chem. Soc.* **1991**, *113*, 7439-7440.
- (7) Samano, V.; Robins, M. J. Nucleic Acid Related Compounds. 70. Synthesis of 2'(and 3')-Deoxy-2'(and 3')-methyleneadenosines and Bis(methylene)furan 4',5'-Didehydro-5'-deoxy-2'(and 3')-methyleneadenosines. Inhibitors of S-Adenosyl-L-homocysteine Hydrolase and Ribonucleotide Reductase. *J. Org. Chem.* **1991**, *56*, 7108-7113.
- (8) (a) Samano, V.; Robins, M. J. Stereoselective Addition of a Wittig Reagent To Give a Single Nucleoside Oxaphosphetane Diastereoisomer. Synthesis of 2'(and 3')-Deoxy-2'(and 3')-methyleneuridine (and cytidine) Derivatives from Uridine Ketone nucleosides. *Synthesis* **1991**, 283-288. (b) Robins, M. J.; Samano, V.; Zhang, W.; Balzarini, J.; De Clercq, E.; Borchardt, R. T.; Lee, Y.; Yuan, C.-S. Nucleic Acid Related Compounds. 74. Synthesis and Biological Activity of 2'(and 3')-Deoxy-2'(and 3')-methylene nucleoside Analogues That Function as Mechanism-Based Inhibitors of S-Adenosyl-L-homocysteine Hydrolase and/or Ribonucleotide Reductase. *J. Med. Chem.* **1992**, *35*, 2283-2293.
- (9) Wnuk, S. F.; Dalley, N. K.; Robins, M. J. Nucleic Acid Related Compounds. 76. Synthesis of 5'(E and Z)-Chloro-4',5'-didehydro-5'-deoxyadenosines via Chlorination and Thermolysis of Adenosine 5'-Sulfoxides. Mechanism-Based Inhibition of S-Adenosyl-L-homocysteine Hydrolase. *J. Org. Chem.* **1993**, *58*, 111-117.
- (10) Tronchet, J. M. J.; Tronchet, J. F. A Novel Example of Unsaturated Branched-Chain Sugar Nucleosides: 3'-Deoxy-3'-methylidenadenosine. *Helv. Chim. Acta* **1981**, *64*, 425-429.
- (11) Wnuk, S. F.; Robins, M. J. Nucleic Acid Related Compounds. 63. Synthesis of 5'-Deoxy-5'-methyleneadenosine and Related Wittig-Extended Nucleosides. *Can. J. Chem.* **1991**, *69*, 334-338.
- (12) Shuto, S.; Obara, T.; Toriya, M.; Hosoya, M.; Snoeck, R.; Andrei, G.; Balzarini, J.; De Clercq, E. New Neplanocin Analogues. 1. Synthesis of 6'-Modified Neplanocin A Derivatives as Broad-Spectrum Antiviral Agents. *J. Med. Chem.* **1992**, *35*, 324-331.
- (13) (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) Edwards, M. L.; Prakash, N. J.; McCarthy, J. R. Novel Acetylenic, Cyano and Allenic Aristeromycin/Adenosine Derivatives. Eur. Pat. Appl. EP 334,361, 1989; *Chem. Abstr.* **1990**, *112*, 99145s.
- (14) Sharma, R. A.; Bobek, M. Acetylenic Nucleosides. 1. Synthesis of 1-(5,6-Dideoxy-β-D-ribo-hex-5'-ynofuranosyl)uracil and 1-(2,5,6-Trideoxy-β-D-erythro-hex-5'-ynofuranosyl)-5-methyluracil. *J. Org. Chem.* **1978**, *43*, 367-369.
- (15) Hasan, A.; Srivastava, P. C. Synthesis and Biological Studies of Unsaturated Acyclonucleoside Analogues of S-Adenosyl-L-homocysteine Hydrolase Inhibitors. *J. Med. Chem.* **1992**, *35*, 1435-1439.
- (16) Wolfe, M. S.; Lee, Y.; Bartlett, W. J.; Borcharding, D. R.; Borchardt, R. T. 4'-Modified Analogues of Aristeromycin and Neplanocin A: Synthesis and Inhibitory Activity toward S-Adenosyl-L-homocysteine Hydrolase. *J. Med. Chem.* **1992**, *35*, 1782-1791.
- (17) Wnuk, S. F.; Dalley, N. K.; 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.
- (18) 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.
- (19) (a) Ueland, P. M. Pharmacological and Biochemical Aspects of S-Adenosylhomocysteine and S-Adenosylhomocysteine Hydrolase. *Pharmacol. Rev.* **1982**, *34*, 223-253. (b) Wolfe, M. S.; Borchardt, R. T. S-Adenosyl-L-homocysteine Hydrolase as a Target for Antiviral Chemotherapy. *J. Med. Chem.* **1991**, *34*, 1521-1530. (c) Liu, S.; Wolfe, M. S.; Borchardt, R. T. Rational Approaches to the Design of Antiviral Agents Based on S-Adenosyl-L-homocysteine Hydrolase as a Molecular Target. *Antiviral Res.* **1992**, *19*, 247-265.
- (20) (a) De Clercq, E.; Cools, M. Antiviral Potency of Adenosine Analogues: Correlation with Inhibition of S-Adenosylhomocysteine Hydrolase. *Biochem. Biophys. Res. Commun.* **1985**, *129*, 306-311. (b) De Clercq, E. S-Adenosylhomocysteine Hydrolase Inhibitors as Broad-Spectrum Antiviral Agents. *Biochem. Pharmacol.* **1987**, *36*, 2567-2575. (c) De Clercq, E.; Cools, M.; Balzarini, J. Homocysteine Potentiates the Antiviral and Cytostatic Activity of Those Nucleoside Analogues That are Targeted at S-Adenosylhomocysteine Hydrolase. *Biochem. Pharmacol.* **1989**, *38*, 1771-1778.
- (21) Watanabe, Y.; Ueno, Y.; Araki, T.; Endo, T.; Okawara, M. A Novel Homolytic Substitution on Vinyl Carbon. A New Route to Vinyl Stannanes. *Tetrahedron Lett.* **1986**, *27*, 215-218.
- (22) Pereyre, M.; Quintard, J.-P.; Rahm, A. *Tin in Organic Synthesis*; Butterworths: London, 1987.
- (23) (a) Tius, M. A.; Kawakami, J. K. Vinyl fluorides from vinyl stannanes. *Synth. Commun.* **1992**, *22*, 1461-1471. (b) Tius, M. A.; Kawakami, J. K. Rapid Fluorination of Alkenyl Stannanes with Silver Triflate and Xenon Difluoride. *Synlett* **1993**, 207-208.
- (24) (a) Hodson, H. F.; Madge, D. J.; Widdowson, D. A. Regioselective Electrophilic Fluorination of Alkenyl and Related Stannanes Using Caesium Fluoroxysulfate. *Synlett* **1992**, 831-832. (b) Matthews, D. P.; Miller, S. C.; Jarvi, E. T.; Sabol, J. S.; McCarthy, J. R. A New Method for the Electrophilic Fluorination of Vinyl Stannanes. *Tetrahedron Lett.* **1993**, *34*, 3029-3032.
- (25) Bovey, F. A.; Jelinski, L.; Mirau, P. A. *Nuclear Magnetic Resonance Spectroscopy*; Academic Press Inc.: London, 1988.
- (26) Corey, E. J.; Wollenberg, R. H. A Nucleophilic Ethynyl Group Equivalent and its use in Conjugate Addition to α,β-Enones. *J. Am. Chem. Soc.* **1974**, *96*, 5581-5583.

- (27) Zhang, W.; Robins, M. J. Removal of Silyl Protecting Groups from Hydroxyl Functions with Ammonium Fluoride in Methanol. *Tetrahedron Lett.* **1992**, *33*, 1177–1180.
- (28) (a) Yuan, C.-S.; Liu, S.; Wnuk, S. F.; Robins, M. J.; Borchardt, R. T. Mechanism of Inactivation of *S*-Adenosylhomocysteine Hydrolase by (*E*)-5',6'-Didehydro-6'-deoxy-6'-halohomoadenosines. *Biochemistry* **1994**, *33*, 3758–3765. (b) Yuan, C.-S.; Wnuk, S. F.; Liu, S.; Robins, M. J.; Borchardt, R. T. (*E*)-5',6'-Didehydro-6'-deoxy-6'-fluoro-homoadenosine: A Substrate That Measures the Hydrolytic Activity of *S*-Adenosylhomocysteine Hydrolase. *Biochemistry*, in press.
- (29) Robins, M. J.; Mengel, R.; Jones, R. A.; Fouron, Y. Nucleic Acid Related Compounds. 22. Transformation of Ribonucleoside 2',3'-*O*-Ortho Esters into Halo, Deoxy, and Epoxy Sugar Nucleosides Using Acyl Halides. Mechanism and Structure of Products. *J. Am. Chem. Soc.* **1976**, *98*, 8204–8213.
- (30) Yuan, C.-S.; Yeh, J.; Liu, S.; Borchardt, R. T. Mechanism of Inactivation of *S*-Adenosylhomocysteine Hydrolase by (*Z*)-4',5'-Didehydro-5'-deoxy-5'-fluoroadenosine. *J. Biol. Chem.* **1993**, *268*, 17030–17037.
- (31) De Clercq, E.; Balzarini, J.; Torrence, P. F.; Mertes, M. P.; Schmidt, C. L.; Shugar, D.; Barr, P. J.; Jones, A. S.; Verhelst, G.; Walker, R. T. Thymidylate Synthetase as Target Enzyme for the Inhibitory Activity of 5-Substituted 2'-Deoxyuridines on Mouse Leukemia L-1210 Cell Growth. *Mol. Pharmacol.* **1981**, *19*, 321–330.
- (32) 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.
- (33) De Clercq, E.; Holy, A.; Rosenberg, I.; Sakuma, T.; Balzarini, J.; Maudgal, P. C. A Novel Selective Broad-Spectrum Anti-DNA Virus Agent. *Nature* **1986**, *323*, 464–467.
- (34) Balzarini, J.; Naesens, L.; Slachmuylders, J.; Niphuis, H.; Rosenberg, I.; Holy, A.; Schellekens, H.; De Clercq, E. 9-(2-Phosphonylmethoxyethyl)adenine (PMEA) Effectively Inhibits Retrovirus Replication *In Vitro* and Simian Immunodeficiency Virus Infection in Rhesus Monkeys. *AIDS* **1991**, *5*, 21–28.