at 20-min intervals. If the compound inhibited twitch height by at least 50%, then concentration–effect curves were determined in a minimum of six tissues.

Spontaneously Beating Isolated Guinea Pig Atria. Male guinea pigs were killed by cervical dislocation and the hearts rapidly removed and placed in oxygenated Krebs Hensebit solution of the following composition (mM): NaCl, 118; KCl, 4.7; $CaCl_2$, 2–52; $MgSO_4$, 1.64; $NaHCO_3$, 24.88; KH_2PO_4, 1.18; glucose, 5.55, bubbled with 5% CO_2 and 95% O_2. The test compounds were added to the bath, and the amplitude of the contractions was measured after 30 s of incubation, after which the compounds were washed off by overflow. In this way dose response curves were constructed for each compound with three to five preparations at each concentration. From these curves IC_{30} values were obtained with extrapolation, and the percentage maximum response obtained noted (acetylcholine and carbacol give a maximum 100% inhibition of the contractions).

Isolated Guinea Pig Ileum. Male Hartley albino guinea pigs (250–600 g) were sacrificed by cervical dislocation. The ileum was removed from each animal and cut into 2–3-cm sections. The segments of ileum were attached with thread to a glass rod and placed in isolated tissue baths containing Krebs' bicarbonate solution aerated with 95% O_2 and 5% CO_2, maintained at 37 °C. The tissues were then attached to a force–displacement transducer (Grass FT 03). All recordings were made with a Beckman dynograph recorder. After an equilibration period, noncumulative concentration–response curves were obtained in each tissue with the agonists.

Acknowledgment. We thank Dr. Peter G. Nielsen for GC–MS analysis and S. Bonde for typing this manuscript.

Registry No. **2**, 131988-63-1; **2** 2-hydroxy, 17604-74-9; **3**, 131986-28-2; **4a**, 131987-61-6; **4b**, 131987-62-7; **4c**, 131987-63-8; **4d**, 131987-64-9; **4e**, 131987-66-1; **4f**, 131987-69-4; **4g**, 141064-09-7; **4h**, 131987-78-5; **4i**, 141064-10-0; **4j**, 141064-11-1; **4k**, 141064-12-2; **4l**, 141064-13-3; **5a**, 131986-29-3; **5a** oxalate, 141064-18-8; **5b**, 131986-31-7; **5b** oxalate, 141064-19-9; **5c**, 131986-33-9; **5c** oxalate, 141064-20-2; **5d**, 131986-35-1; **5d** oxalate, 141064-21-3; **5e**, 131986-39-5; **5e** oxalate, 141064-22-4; **5f**, 131986-45-3; **5f** oxalate, 141064-23-5; **5g**, 131986-66-8; **5g** oxalate, 141064-24-6; **5h**, 131986-74-8; **5h** oxalate, 141064-25-7; **5i**, 141064-14-4; **5i** maleate, 141064-26-8; **5j**, 141064-15-5; **5j** fumarate, 141064-27-9; **5k**, 141064-16-6; **5k** fumarate, 141064-28-0; **5l**, 141064-17-7; **5l** fumarate, 141064-29-1; **6a**, 131988-00-6; **6b**, 131988-09-5; **6c**, 131987-98-9; **6d**, 131987-99-0; **6e**, 141064-30-4; **6f**, 131988-10-8; **6g**, 141064-31-5; **6h**, 131988-08-4; **7a**, 131987-18-3; **7a** oxalate, 141064-32-6; **7b**, 131987-50-3; **7b** oxalate, 141064-33-7; **7c**, 131987-14-9; **7c** oxalate, 141064-34-8; **7d**, 131987-16-1; **7d** oxalate, 141064-35-9; **7e**, 131987-52-5; **7e** oxalate, 141064-36-0; **7f**, 131987-54-7; **7f** oxalate, 141064-37-1; **7g**, 141064-38-2; **7g** oxalate, 141064-39-3; **7h**, 131987-48-9; **7h** oxalate, 141064-40-6; **8**, 131986-59-9; **8** oxalate, 141064-41-7; **9c**, 131987-46-7; **9c** oxalate, 141064-42-8; **9e**, 131987-38-7; **9e** oxalate, 141064-43-9; **9g**, 131987-34-3; **9g** oxalate, 141064-44-0; **9h**, 131987-40-1; **9h** oxalate, 141064-45-1; **9m**, 141064-46-2; **9m** oxalate, 141064-47-3; **9n**, 141064-48-4; **9n** oxalate, 141064-49-5; **10**, 131987-88-7; **11**, 131986-94-2; **11** oxalate, 141064-50-8; **12f**, 131987-97-8; **13f**, 131987-12-7; **13f** oxalate, 141088-19-9; **14**, 6443-85-2; **15**, 67936-83-8; **15**·Na, 141064-51-9; **16**, 131988-01-7; **17**, 131988-04-0; **18a**, 131988-06-2; **18b**, 131988-05-1; **19a**, 131987-30-9; **19a** oxalate, 141064-52-0; **19b**, 131987-28-5; **19b** oxalate, 141064-53-1; MeOH, 67-56-1; EtOH, 64-17-5; PrOH, 71-23-8; BuOH, 71-36-3; $CH_3(CH_2)_4OH$, 71-41-0; $CH_3(CH_2)_5OH$, 111-27-3; $CH_3(CH_2)_6OH$, 111-70-6; $CH_3(CH_2)_7OH$, 111-87-5; $CH_3CH(CH_3)(CH_2)_3OH$, 626-89-1; $CH_3CH_2CH(CH_3)(CH_2)_2OH$, 589-35-5; $CH_3(CH_2)_2CH(CH_3)CH_2OH$, 105-30-6; $CH_3(CH_2)_3CH(CH_3)OH$, 626-93-7; PrMgBr, 927-77-5; $CH_3(CH_2)_4MgBr$, 693-25-4; $CH_3(CH_2)_6MgBr$, 13125-66-1; $CH_3(CH_2)_7MgBr$, 17049-49-9; $CH_3(CH_2)_5NH_2$, 111-26-2; 3-pyridinecarboxaldehyde, 500-22-1; α-oximido-3-pyridylacetamidoxime, 131988-62-0.

Nucleic Acid Related Compounds. 74. Synthesis and Biological Activity of 2′(and 3′)-Deoxy-2′(and 3′)-methylenenucleoside Analogues That Function as Mechanism-Based Inhibitors of *S*-Adenosyl-L-homocysteine Hydrolase and/or Ribonucleotide Reductase[1]

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Treatment of 2-amino-6-chloro-9-(β-D-ribofuranosyl)purine (**21**) with TBDMS chloride/imidazole/DMF gave a separable mixture of 5′-*O*, 2′,5′-bis-*O* (**22**), 3′,5′-bis-*O* (**23**), and 2′,3′,5′-tris-*O*-TBDMS derivatives. Oxidation of **22** and **23** with CrO_3/pyridine/Ac_2O, treatment of the respective ketonucleosides with methylenetriphenylphosphorane, and deprotection gave 2-amino-6-chloro-9-[3(and 2)-deoxy-3(and 2)-methylene-β-D-*erythro*-pentofuranosyl]purines (**28** and **37**) that were converted into other 2-amino-6-substituted-purine analogues. Tubercidin was converted into 2′-deoxy-2′-methylenetubercidin (**49**) by an analogous route. Inactivation of *S*-adenosyl-L-homocysteine hydrolase by 2′- and 3′-methyleneadenosine analogues was investigated. Mechanism-based inhibition of *S*-adenosyl-L-homocysteine hydrolase and anticancer and antiviral activities of 2′(and 3′)-deoxy-2′(and 3′)-methylenenucleoside analogues are discussed.

Of the plethora of compounds investigated as anticancer and antiviral agents, significant activity has been demonstrated with a remarkable number of analogues and derivatives of nucleic acid components. Recent reviews of

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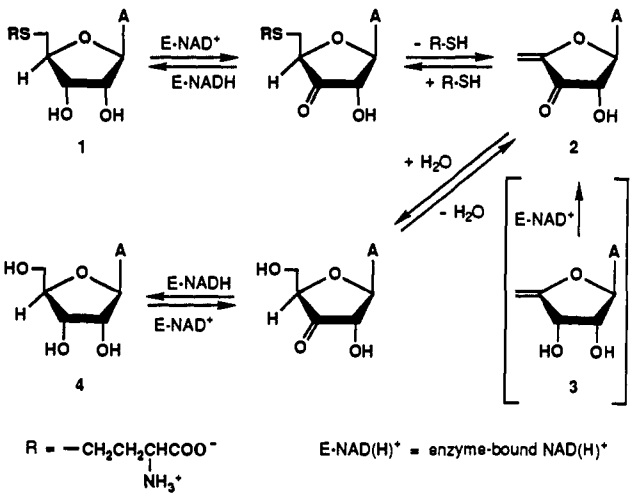
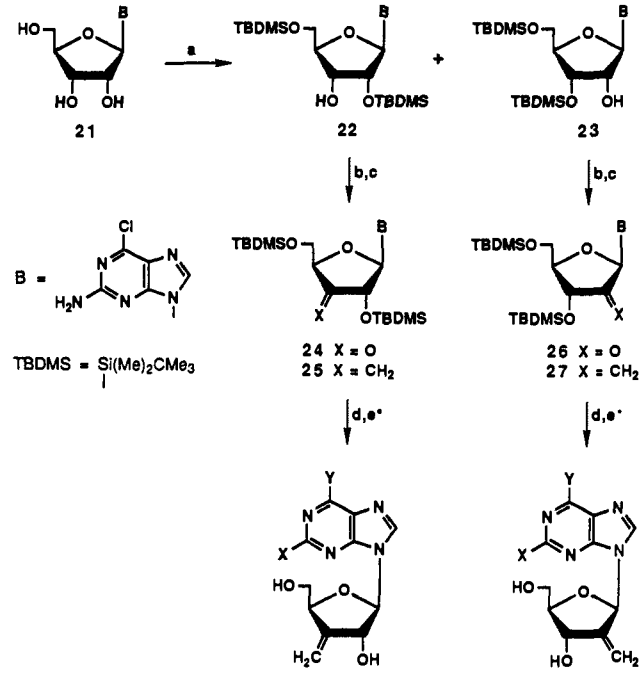


Figure 1. Proposed mechanism for *S*-adenosyl-L-homocysteine hydrolase.

the pyrimidine[2a] and purine[2b] related compounds that are in clinical use as cancer chemotherapeutic agents are available. The current renaissance in the chemistry and biology of nucleosides, nucleotides, and analogues was initiated by the discovery of anti-herpes-viral activity of acyclovir[3] and antiretroviral activity of 3′-azido-3′-deoxythymidine (AZT) and 2′,3′-dideoxynucleosides.[4]

Mechanism-based inhibition of enzymes that are crucial to metabolic pathways involved in cell division is an attractive concept for the design of rational chemotherapeutic agents.[5,6a] Two such enzymes in the nucleic acid manifold are *S*-adenosyl-L-homocysteine hydrolase (AdoHcy hydrolase) (EC 3.3.1.1) and ribonucleoside diphosphate reductase (RDPR) (EC 1.17.4.1). The byproduct of vital enzymatic transmethylation reactions involving the biological methyl donor *S*-adenosylmethionine is *S*-adenosylhomocysteine (AdoHcy), which acts as a potent feedback inhibitor of these transmethylases.[6]

Scheme I[a]



e		X	Y	
·····	28	NH_2	Cl	37
NH_3/MeOH	29	NH_2	NH_2	38
Adenosine deaminase	30	NH_2	OH	39
$NHMe_2$	31	NH_2	NMe_2	40
NaOMe	32	NH_2	OMe	41
H_2S/NaOMe	33	NH_2	SH	42
NaSMe	34	NH_2	SMe	43
·····	35	H	NH_2	44
·····	36	H	OH	45

[a] (a) TBDMSCl/imidazole/DMF; (b) CrO_3/pyridine/Ac_2O/CH_2Cl_2; (c) Ph_3PCH_3Br/NaOC(Me)$_2$Et/benzene/Et_2O; (d) Bu_4NF/THF; (e) see column for conditions.

Therefore, AdoHcy hydrolase modulates continuing biosynthesis by cleavage of the feedback inhibitor to the adenosine and L-homocysteine metabolites.[7] Ribonucleoside diphosphate reductases in mammalian systems provide the only de novo pathway to the 2′-deoxynucleotide components of DNA.[8] Inhibition of this enzyme obstructs that route to replication of the genetic material for cancer cell division and viral genome biosynthesis.

Figure 1 contains the proposed mechanistic scheme for the reversible enzymatic conversion of AdoHcy (1) to adenosine (4) and L-homocysteine by AdoHcy hydrolase,[9] and includes the alternative substrate oxidation[9b,c] of

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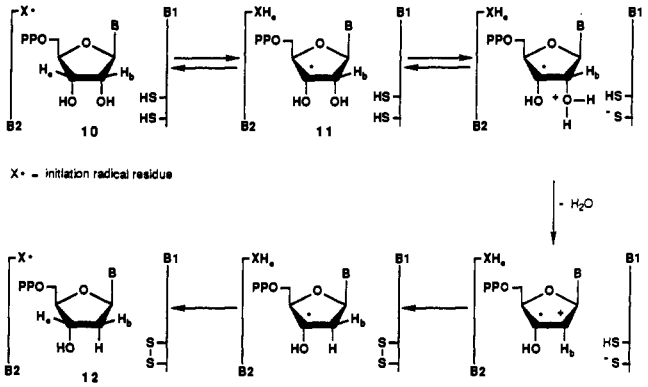
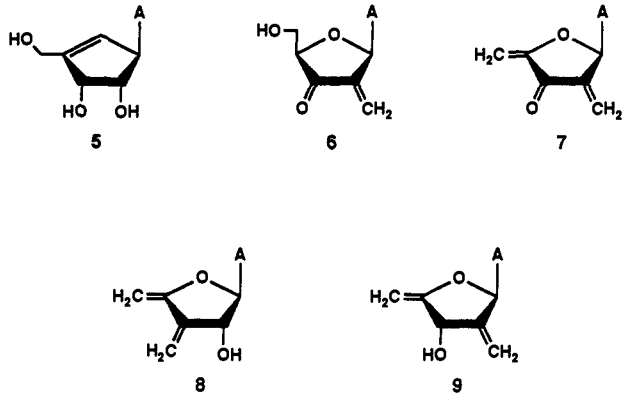


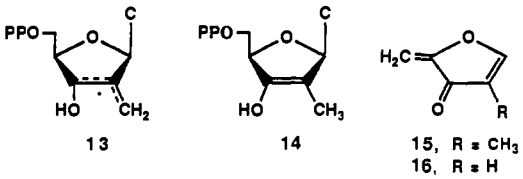
Figure 2. Proposed mechanism for ribonucleoside diphosphate reductase.

4′,5′-didehydro-5′-deoxyadenosine (**3**) to the 3′-keto-4′-methylene intermediate **2**. AdoHcy hydrolase has been shown to accept the cyclopentenyl antibiotic neplanocin A (**5**) as an alternative substrate and executes oxidation



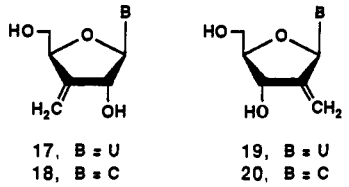
of the allylic 3′-hydroxyl group.[10,11] We reasoned[12] that analogous enzymatic oxidation of the allylic 3′-hydroxyl function of 2′-deoxy-2′-methyleneadenosine (MdAdo; **44**, Scheme I) would produce an exocyclic enone **6** that might function as a Michael-accepting mechanism-based inactivator of AdoHcy hydrolase, or that oxidation of **44** might result in direct inactivation of the enzyme by the "cofactor-depletion" mechanism.[6a] If further enzymatic processing of **6** by elimination of water occurred, the bis-methylene–one **7** would be produced. We prepared the 3′,5′- and 2′,5′-bis-methylene analogues **8** and **9** to further probe binding/inactivation with AdoHcy hydrolase.[12] The 3′,5′-bis-methylene compound **8** is seen to be a crude "isosteric" analogue of intermediate **2** in the biotransformation (Figure 1) with a CH_2 group in place of a carbonyl oxygen at the sp^2 hybridized C3′. Compound **9** would be converted to the same bis-methylene–one **7** upon oxidation at C3′ that would result from 4′,5′-dehydration of **6**. We now report that MdAdo (**44**) does cause time-dependent inactivation of AdoHcy hydrolase, as predicted. Further results of inhibition studies with the adenosine derivatives **8**, **9**, **35**, and **44** also are discussed.

Figure 2 contains the mechanistic scheme of Stubbe and co-workers for the reduction of ribonucleoside diphosphates (**10**) to their 2′-deoxy counterparts (**12**).[13] We reasoned[12,14] that enzymatic removal of the H3′ atom by the radical initiating function (that gives intermediate **11** with natural substrates) would produce an allylic radical intermediate **13** that might capture a hydrogen atom at



the exocyclic terminus to produce enol **14**. Conjugate elimination of the enol proton and the nucleoside base (followed by H4′ and inorganic pyrophosphate) from **14** would produce 4-methyl-2-methylene-3(2*H*)-furanone (**15**), the methyl analogue of **16**, which causes irreversible inactivation of ribonucleotide reductases.[13,15]

It is noteworthy that both 2′-deoxy-2′-methylenecytidine (MdCyd, **20**) 5′-diphosphate and 2′-deoxy-2′-methylene-



uridine (MdUrd, **19**) 5′-diphosphate function as time-dependent irreversible inactivators of ribonucleoside diphosphate reductase from *Escherichia coli*.[16] In addition, MdCyd (**20**) has been shown to be a potent anticancer agent against leukemia and solid tumor systems in vitro and in vivo.[17] The present biological studies were in

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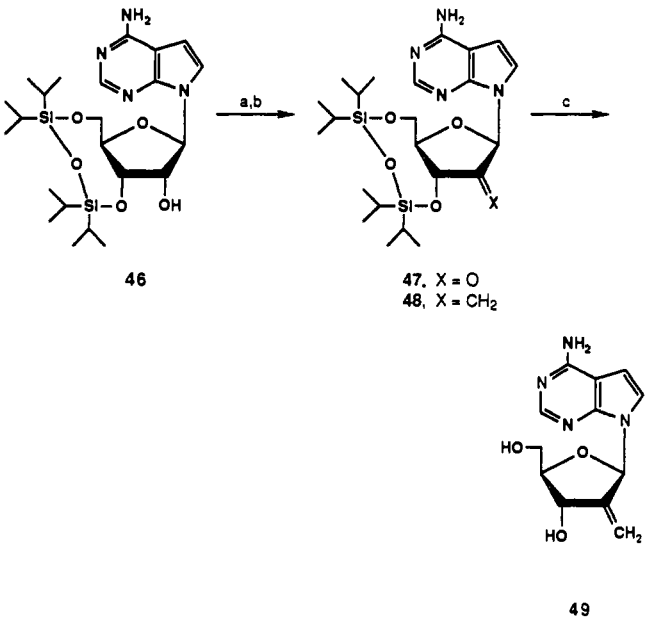
progress when the results of Ueda and co-workers appeared[17a] and confirm their results. Our recent discovery of the mechanism-based inhibition of ribonucleotide reductase[16] represents a more likely mechanism of drug action than incorporation of **20** into DNA followed by allylic elimination with DNA strand cleavage as previously suggested.[17b] We now report the preparation of 2′-deoxy-2′-methyleneguanosine (**39**), other new 2-amino-6-substituted-9-[2(and 3)-deoxy-2(and 3)-methylene-β-D-*erythro*-pentofuranosyl]purines, and 2′-deoxy-2′-methylenetubercidine (**49**); anticancer and antiviral activities of these and other purine[12] and pyrimidine[14] congeners; and inhibition studies with the methyleneadenosine analogues and AdoHcy hydrolase.

Chemistry

Protection of 2-amino-6-chloro-9-(β-D-ribofuranosyl)-purine (**21**)[18] with *tert*-butyldimethylsilyl (TBDMS) chloride/imidazole/dimethylformamide (DMF)[19] gave a mixture of 2′,3′,5′-tris-*O*-TBDMS (8%), 2′,5′-bis-*O*-TBDMS (**22**, 36%), 3′,5′-bis-*O*-TBDMS (**23**, 42%), and 5′-*O*-TBDMS (5%) derivatives of **21**. Oxidation[20] of **22** and **23** with chromium trioxide/pyridine/acetic anhydride gave the 3′- (**24**) and 2′-keto (**26**) derivatives, respectively. Compound **24** was purified by flash chromatography, but the 2′-keto compound **26** underwent anomerization on silica gel or in the presence of pyridine. The procedure described in the Experimental Section gave reliable results with **26**. Treatment of **24** and **26** with methylenetriphenylphosphorane (generated from methyltriphenylphosphonium bromide and sodium 2-methyl-2-butoxide in diethyl ether/benzene)[12,14] afforded the corresponding 3′- (**25**) and 2′-deoxy-2′-methylene (**27**) compounds. The 3′-methylene analogue was obtained without difficulty, but minor amounts of the α-anomer of **27** were obtained unless adequate care was exercised during the preparation and rapid workup of **26** and its treatment with the Wittig reagent. Deprotection of **25** and **27** with tetrabutylammonium fluoride in tetrahydrofuran (TBAF/THF), flash silica column chromatography, and crystallization

Scheme II[a]



[a] (a) CrO_3/pyridine/Ac_2O/CH_2Cl_2; (b) Ph_3PCH_3Br/NaOC(Me)$_2$Et/benzene/Et_2O; (c) Bu_4NF/THF.

provided clean samples of 2-amino-6-chloro-9-(3-deoxy-3-methylene-β-D-*erythro*-pentofuranosyl)purine (**28**, 49% overall from **22**) and 2-amino-6-chloro-9-(2-deoxy-2-methylene-β-D-*erythro*-pentofuranosyl)purine (**37**, 32% from **23**). The β-configuration of **37** was confirmed by its hydrolytic dechlorination with calf intestinal adenosine deaminase (adenosine aminohydrolase, EC 3.5.4.4) whereas its α-anomer was not an alternative substrate for this enzyme.[21]

The new 2-amino-6-substituted derivatives **29–34** and **38–43**, respectively, were obtained from **28** and **37** by nucleophilic displacements[22] of the 6-chloro group. Treatment of **28** and **37** with methanolic ammonia afforded the 2,6-diamino compounds **29** (90%) and **38** (87%) (plus small quantities of the respective 2-amino-6-methoxy byproducts **32** and **41**). Treatment of **29** and **38** with adenosine deaminase gave 3′- (**30**, 86%) and 2′-deoxy-2′-methyleneguanosine (**39**, 73%), again confirming the β-anomeric configuration[21] of the derived 2′-methylene analogues. Aqueous dimethylamine converted **28** and **37** into the 2-amino-6-(*N*,*N*-dimethylamino) nucleosides **31** (92%) and **40** (91%). Treatment of **28** and **37** with methanolic sodium methoxide gave the 2-amino-6-methoxy compounds **32** (82%) and **41** (80%). Conversion of **28** and **37** into the corresponding 2-amino 6-thione (6-thioguanine) (**33**, 82%; **42**, 82%) and 2-amino-6-(methylthio) (**34**, 82%; **43**, 58%) nucleosides was effected by treatment with hydrogen sulfide/sodium methoxide/methanol[23] and sodium thiomethoxide/2-propanol, respectively.

Since 2′-deoxy-2′-methyleneadenosine (**44**) was found to function as a time-dependent inactivator of AdoHcy

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Table I. ^{1}H NMR Spectral Data[a]

no.	H1′[b] ($J_{1'2'}$)	H2′[c] (J)	H3′ ($J_{3'4'}$)	H4′	H5′ ($J_{5'5''}$)	H5″ ($J_{5''4'}$)	CH_AH_B[d] (J)	CH_AH_B[d] (J)	H8′[e]	NH_2[e]	OH3′/2′[b] ($J_{OH3'/2'}$)	OH5′[f] ($J_{OH5'5''}$)	others
22	5.97 (5.1)	4.51[f] (5.0)	4.24[f,g] (4.5)	4.19[h]	3.96[i] (11.5)	3.82[i] (2.2)			8.17	5.05	2.68 (4.0)		0.0–0.11 (SiMe) 0.83, 0.92 ($SiCMe_3$)
23	5.91 (4.3)	4.50[g,i] (5.2)	4.42[g,i] (4.2)	4.06[h]	3.91[i] (11.5)	3.74[i] (2.6)			8.05	5.11	2.93 (6.0)		0.07–0.13 (SiMe) 0.89, 0.93 ($SiCMe_3$)
24	6.03 (8.2)	4.68[b]		4.27[h]	3.93[b]	3.93[b] (2.0)			8.16	5.09			0.0–0.05 (SiMe) 0.72, 0.88 ($SiCMe_3$)
25	5.76 (6.6)	4.91[j] (1.6)		4.69[h]	3.93[i] (11.0)	3.74[i] (3.0)	5.17[i] (2.2)	5.25[i] (2.2)	8.18	5.13			0.0–0.15 (SiMe) 0.80, 0.90 ($SiCMe_3$)
26	5.72[k]		5.05[b] (8.5)	3.99[h]	4.10[i] (12.0)	3.85[i] (2.6)			7.76	5.03			0.0–0.20 (SiMe) 0.81, 0.93 ($SiCMe_3$)
27	6.58[j] (1.4)[d]		4.95[k] (6.0)	3.83[h]	3.89[i] (11.0)	3.77[i] (2.0)	5.31[f] (2.0)	5.42[f] (2.0)	7.90	5.09			0.0–0.2(SiMe) 0.85, 0.92 ($SiCMe_3$)
28	5.60 (7.04)	5.03[h] (1.8)[g,j]		4.61[l]	3.53[g,i] (11.4)	3.62[g,i] (3.8)	5.20[f] (2.1)	5.25[f] (1.8)	8.40	7.0	5.97 (6.02)	5.06[h]	
29	5.50 (7.4)	5.06[h] (1.8)[g,j]		4.58[l]	3.65[m] (11.6)	3.53[m] (3.4)	5.18[l]	5.21[l]	7.94	5.73	5.91 (6.2)	5.45[i] (7.2, 4.4)	6.82 (NH_2)
30	5.47 (7.0)	4.91[h] (1.8)[g,j]		4.55[l]	3.55[g,i] (11.5)	3.65[g,i] (4.0)	5.17[f] (1.8)	5.22[f] (1.8)	7.99	6.53	5.94 (6.0)	5.02 (6.0)	10.75 (NH)
31	5.55 (7.3)	5.04[h] (1.8)[g,j]		4.58[l]	3.65[g,i] (11.8)	3.51[g,i] (3.8)	5.18[l]	5.22[l]	7.96	5.79	5.91 (6.3)	5.38 (4.5)	3.35 (NMe_2)
32	5.58 (7.0)	5.06[h] (1.8)[g,j]		4.58[l]	3.61[g,i] (12.0)	3.54[g,i] (3.6)	5.19[l]	5.23[l]	8.13	6.47	5.94 (5.9)	5.12 (5.5)	3.96 (OMe)
33	5.49 (7.0)	4.96[h] (1.8)[g,j]		4.58[l]	3.52[g,i] (11.4)	3.58[g,i] (4.0)	5.19[l]	5.23[l]	8.16	6.82	5.95 (5.9)	5.07[l]	11.98 (NH)
34	5.58 (7.0)	5.04[h]		4.58[l]	3.59[g,i] (11.4)	3.59[g,i] (3.3)	5.19[l]	5.23[l]	8.20	6.54	5.93[l]	6.05[l]	2.57 (SMe)
37	6.49[j] (1.4)[d]		4.70[l] (7.0)[g,k]	3.72[h]	3.69[g,i] (12.1)	3.56[g,i] (5.0)	5.28[l]	5.42[l]	8.16	6.99	5.7 (6.4)	4.91 (5.5)	
38	6.40[l]		4.68[l] (6.3)[g,k]	3.70[h]	3.65[f]	3.55[f]	5.16[l]	5.37[l]	7.72	5.78	5.66 (6.2)	5.00 (5.3)	6.73 (NH_2)
39	6.36[l]		4.64[l] (5.0)[g,k]	3.75[h]	3.70[h]	3.60[h]	5.20[l]	5.38[l]	7.72	6.47	5.67 (6.5)	4.91 (4.7)	10.63 (NH)
40	6.45[j] (1.5)[d]		4.68[l] (7.0)[g,k]	3.70[h]	3.60[h]	3.50[h]	5.14[f] (2.0)	5.38[f] (2.0)	7.73	5.85	5.70 (6.1)	5.00 (5.3)	3.36 (NMe_2)
41	6.47[j] (1.3)[d]		4.69[l] (5.7)[g,k]	3.72[h]	3.60[h]	3.52[h]	5.20[l]	5.39[l]	7.91	6.42	5.7 (6.2)	4.94 (5.5)	3.94 (OMe)
42	6.38[e]		4.67[f] (5.9)	3.70[h]	3.60[h]	3.52[h]	5.24[f] (1.9)	5.40[f] (1.5)	7.91	6.84	5.7[l]	4.94 (4.9)	11.50 (NH)
43	6.48[k]		4.69[l] (6.4)[g,k]	3.71[h]	3.60[h]	3.55[h]	5.22[l]	5.40[l]	7.96	6.55	5.7 (6.2)	4.91 (5.2)	2.56 (SMe)
48	6.83[j] (1.6)		5.11[g,k] (8.4)	3.75[n] (3.4)	4.04[b,o]		5.30[j] (2.8, 1.6)	5.45[f] (2.2)		5.10			8.32[e] (H2), 6.96[b] (H6), 6.34[b] (H5), 1.10[h] (*i*-Pr's)
49	6.74[j] (1.5)		4.60[l] (6.6)[g,k]	3.65[h]	3.70[h]	3.52[h]	5.03[f] (2.1)	5.33[f] (1.9)		7.03	5.66 (4.7)	4.94 (5.5)	8.08[e] (H2), 7.10[b] (H6), 6.57[b] (H5)

[a] Chemical shifts (δ, Me_4Si internal); coupling constants (Hz, in parentheses). Solvents: $CDCl_3$ for 22–27, 48; Me_2SO-d_6 for 28–34, 37–43, 49. [b] Doublet (unless noted otherwise). [c] Compounds 22, 23, $J_{2'3'}$; 25, 28–34, J "apparent" from incompletely resolved peaks. [d] J "apparent" from incompletely resolved peaks. [e] Singlet. [f] Triplet unless noted otherwise. [g] After exchange with D_2O. [h] Multiplet. [i] Doublet of doublets. [j] "Apparent" quartet. [k] Doublet of multiplets. [l] Broad singlet. [m] Doublet of doublets of doublets. [n] Doublet of triplets. [o] H5′,5″ collapsed.

hydrolase but had little biological activity in cell cultures, we sought an analogue that would not undergo deamination to an inosine congener that would be irrelevant to the inhibition of AdoHcy hydrolase. Tubercidin, an adenosine deaminase-resistant antibiotic, was converted into the known[24] 3′,5′-*O*-(1,1,3,3-tetraisopropyl-1,3-disiloxanediyl)tubercidin (**46**). As shown in Scheme II, oxidation[20] of **46**, Wittig treatment of the resulting 2′-keto derivative **47**, to give **48**, and deprotection gave 4-amino-7-(2-deoxy-2-methylene-β-D-*erythro*-pentofuranosyl)pyrrolo[2,3-*d*]-pyrimidine (2′-deoxy-2′-methylenetubercidin, **49**, 16% from **46**). As expected, **49** was completely resistant to deamination in the presence of a large excess of adenosine deaminase.

The ^{1}H and ^{13}C NMR data are organized for comparison in Tables I and II. The anomeric proton (H1′) peaks for the 3′-methylene compounds **28–34** were shifted upfield to $\delta \sim 5.5$, whereas those of the 2′-methylene compounds **37–43** were shifted downfield to $\delta \sim 6.4$. Analogous shift effects on H4′ were observed with peaks for the 2′ isomers at $\delta \sim 3.7$ and those for the 3′ isomers at $\delta \sim 4.6$. The geminal methylene proton peaks for the 3′ isomers had shift differences of $\Delta\delta \leq 0.05$ ppm whereas those for the 2′ isomers had $\Delta\delta \geq 0.14$ ppm. The ^{13}C resonance ranges for the C1′, C4′, and C5′ peaks in the two series were remarkably constant at $\delta \sim 87.1$, ~ 81.2, and ~ 64.4 for the 3′ isomers **28–34** and $\delta \sim 85.3$, ~ 82.1, and ~ 61.1 for the 2′ isomers **37–43**, respectively.

Biological

A number of purine and pyrimidine 3′-deoxy-3′-methylenenucleoside analogues (compounds **17**, **18**, **28–36**) and 2′-deoxy-2′-methylene congeners (compounds **19**, **20**, **37–45**) were evaluated for cytostatic activity against murine (L1210, FM3A) and human (Molt/4F, MT-4) tumor cell lines (Table III).

The 2′-methylene analogues generally were much more cytostatic than their 3′-methylene counterparts. The latter compounds were inhibitory to tumor cell proliferation only at concentrations >100 μM. Compound **33** was markedly

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Table II. ^{13}C NMR Spectral Data[a]

no.	C2	C4	C5	C6	C8	C1′	C2′	C3′	C4′	C5′	CH_2	other
22	154.19	151.88	125.92	159.62	140.84	88.02	77.47	71.74	85.66	63.53		
23	154.01	151.98	126.20	159.61	141.13	88.77	75.41	71.53	85.43	62.36		
24	154.48	152.29	125.77	159.82	140.17	85.07	78.52	209.13	82.74	63.00		
25	154.53	151.88	125.82	159.65	140.97	87.92	77.87	147.83	81.84	66.51	109.03	
26	154.01	152.37	125.57	159.63	141.68	82.51	207.57	71.64	80.40	62.38		
27	154.20	151.85	125.84	159.76	140.97	85.55	149.15	70.79	83.04	61.33	113.56	
28	154.60	149.95	123.71	160.21	141.61	87.11	73.90	148.33	81.34	64.29	108.32	
29	156.48	151.75	113.63	160.31	136.81	87.71	73.37	148.81	81.20	64.61	107.93	
30	154.08	151.85	116.98	157.09	136.00	86.79	74.02	148.84	81.13	64.48	108.05	
31	155.12	152.67	114.25	159.44	135.46	87.61	73.38	148.66	81.16	64.55	108.08	37.73 (NMe_2)
32	160.12	154.53	114.24	161.18	138.49	87.10	73.67	148.60	81.14	64.38	108.19	53.38 (OMe)
33	160.20	153.47	128.61	175.60	133.50	86.90	73.90	148.54	81.29	64.37	108.25	
34	159.62	150.96	124.05	160.31	138.85	86.61	73.50	148.37	80.89	64.13	107.81	10.41 (SMe)
37	153.98	153.98	123.62	160.14	139.19	85.66	149.73	70.31	82.42	61.03	112.78	
38	156.25	151.59	112.94	160.50	136.20	85.04	150.24	70.16	82.14	61.05	111.92	
39	154.14	151.35	116.69	157.09	135.65	85.30	150.48	70.24	82.00	61.11	111.82	
40	155.05	152.70	113.67	159.85	135.00	85.04	150.41	70.25	82.00	61.02	111.80	37.70 (NMe_2)
41	160.19	154.01	113.90	161.11	138.20	85.21	150.11	70.21	82.20	61.05	112.09	53.40 (OMe)
42	153.56	150.20	128.47	175.66	138.60	85.52	147.91	70.20	82.17	61.10	112.13	
43	159.89	150.65	124.30	160.66	138.97	85.32	150.04	70.22	82.10	61.05	112.32	10.73 (SMe)
49	152.08	157.63	100.35	122.08		84.38	150.78	70.35	82.48	61.01	111.46	102.66 (C4a)
												150.17 (C7a)

[a] Chemical shifts (δ, Me_4Si internal) of proton-decoupled singlets. Solvents: $CDCl_3$ for 22–27, Me_2SO-d_6 for 28–34, 37–43, 49.

Table III. Cytostatic Activity of Nucleoside Analogues in Vitro

	IC_{50}[a] (μM)			
compd	L1210	FM3A	Molt/4F	MT-4
17	>500	>500	>500	450
18	378	>500	172	121
28	441	469	≥500	>500
29	>200	>200	>200	>200
30	>200	>200	>200	>200
31	>500	>500	>500	>500
32	446	439	>500	>500
33	207	495	276	12
34	>500	>500	>500	>500
35	228	466	>500	43
36	>200	>200	>200	>200
19	>500	>500	>500	>500
20	2.0	11	0.11	0.19
37	10	50	22	176
38	8.2	67	22	≥500
39	8.5	102	22	150
40	454	>500	335	>500
41	10	41	23	180
42	20	26	24	0.48
43	>500	>500	>500	>500
44	65	232	>500	131
45	60	113	>200	>200

[a] Inhibitory concentration (50%), or concentration of compound required to inhibit the proliferation of murine leukemia L1210, murine mammary carcinoma FM3A, human T-lymphoblast Molt/4F, and human T-lymphocyte MT-4 cells by 50%. Data are the mean of a least two to four independent experiments.

cytostatic against MT-4 cells (IC_{50} = 12 μM) but not against other tumor cell lines evaluated. The 2′-methylene analogues except **19**, **40**, **43–45**, and **49** were cytostatic at concentrations well below 100 μM. Since 2′-deoxy-2′-methyleneinosine (**45**) demonstrated no activity of interest and 2′-deoxy-2′-methyleneadenosine (**44**) was efficiently converted to **45** by adenosine deaminase, it was not surprising that **44** also showed no significant activity. However, **44** was a time-dependent inactivator of purified AdoHcy hydrolase. Disappointingly, the deaminase-resistant 2′-deoxy-2′-methylenetubercidin (**49**) did not inhibit AdoHcy hydrolase in vitro nor exhibit biological activity in cell cultures, in contrast to the potent inhibition of RNA methylation exhibited by *S*-tubercidinyl-L-homocysteine.[6c] Interestingly, 2′-deoxy-2′-methylene-6-thioguanosine (**42**) was 50-fold more cytostatic against MT-4 cells than the other tumor lines.

Table IV. Cytostatic Activity of 18 and 20 against Raji and CEM Cells

	IC_{50}[a] (μM)				
compd	Raji/0	Raji/TK^-	Raji/dCK^-	CEM/0	CEM/dCK^-
18	33	52	>500	63	>500
20	0.025	0.035	31	0.046	60

[a] Concentration of compound required to inhibit human B-lymphoblast Raji/0, (d)Thd kinase-deficient (TK^-) Raji, dCyd kinase-deficient (dCK^-) Raji, human T-lymphocyte CEM/0, and dCK^- CEM cell proliferation by 50%. Data are the mean of at least two to four independent experiments.

Table V. Effect of Addition of Exogenous Nucleosides on the Cytostatic Activity of 20 against L1210 Cells

	IC_{50}[a] (μM)			
		upon addition[b] of		
compd	drug only	dUrd (125 μg/mL)	(d)Thd (5 μg/mL)	dCyd (500 μg/mL)
20	1.96	1.47	1.18	475

[a] Concentration of compound required to inhibit L1210 cell proliferation by 50%. [b] The nucleosides dUrd, (d)Thd, and dCyd were added at subtoxic concentrations. Data are the mean of at least two to four independent experiments.

MdCyd (2′-deoxy-2′-methylenecytidine, **20**) was a potent cytostatic agent and markedly more inhibitory to human tumor cell proliferation than murine tumor cells [IC_{50} (μM): 0.109, 0.193, 0.025, and 0.046 for Molt/4F, MT-4, Raji/0, and CEM/0, respectively, compared to 2.0 and 11 μM for L1210 and FM3A cells (Tables III and IV)]. Its MdUrd counterpart **19** was devoid of cytostatic activity even at 500 μM.

MdCyd (**20**) was evaluated against a number of Raji and CEM cell lines deficient in pyrimidine nucleoside kinase activity, and found to be as inhibitory to the thymidine kinase-deficient Raji/TK^- cells as to the wild-type Raji/0 cells. However, **20** was 1000-1500-fold less inhibitory to the proliferation of dCyd kinase-deficient Raji/dCK^- and CEM/dCK^- cells than wild-type Raji/0 and CEM/0 cells. The 3′-deoxy-3′-methylenecytidine (**18**) was 1000–1500-fold less cytostatic against Raji/0 and CEM/0 cells than MdCyd (**20**). Interestingly, **18** was equally inhibitory to Raji/TK^- or Raji/0 cells, but it had no inhibitory effect on Raji/dCK^- or CEM/dCK^- cell proliferation (Table IV).

Table VI. Cytostatic Activity of 29, 30, 38, and 39 against CEM Cells

	IC_{50}[a] (μM)			
compd	CEM/0	CEM/ AK^-	CEM/ dCK^-	CEM/ AK^-/dCK^-
29	>500	>500	>500	>500
30	>500	>500	>500	>500
38	7.7	38	196	194
39	9.4	36	221	201

[a] Concentration of compound required to inhibit CEM/0, Ado kinase-deficient (AK^-) CEM, dCyd kinase-deficient (dCK^-) CEM, and AK^-/dCK^- CEM cell proliferation by 50%. Data are the mean of at least two to four independent experiments.

The addition of exogenous dUrd or (d)Thd did not alter the cytostatic activity of **20** against L1210/0 cells (Table V), but the addition of dCyd diminished the cytostatic activity of **20** 250-fold. Our findings that MdCyd (**20**) is markedly less cytostatic to dCK-deficient tumor cell lines and has diminished cytostatic activity in the presence of exogenous dCyd implicate dCyd kinase in the activation (phosphorylation) of **20**.

Among the purine 2′-deoxy-2′-methylenenucleoside analogues, the 2-amino-6-chloro (**37**), 2,6-diamino (**38**), guanine (**39**), 2-amino-6-methoxy (**41**), and 6-thioguanine (**42**) derivatives were markedly cytostatic against L1210 and Molt/4F cells (IC_{50} = 8–24 μM) and, to a lesser extent, FM3A and MT-4 cells (IC_{50} = 26 to ≥500 μM; 0.48 μM for **42** against MT-4) (Table III). The 2-amino-6-(dimethylamino)- (**40**) and 2-amino-6-(methylthio)purine (**43**) derivatives were virtually devoid of cytostatic activity (Table III). These results are in harmony with prodrug activation of the responsive analogues **37–39** and **41** by adenosine deaminase to give the active guanine congener **39** (with possible accompanying base-cleavage and/or intrinsic activity for the 6-thioguanine analogue **42**). It is tempting to speculate that ribonucleotide reductase is a likely target[16] for the activity of **39** (and its prodrug precursors).

To probe for enzymes responsible for further activation (phosphorylation) of the purine 2′-deoxy-2′-methylene analogues, compounds **38** and **39** were evaluated for their cytostatic activity against Ado kinase (AK) deficient and dCK deficient CEM cell lines (Table VI). Both **38** and **39** were 4–5-fold less inhibitory to CEM/AK^-, and 25-fold less inhibitory to CEM/dCK^- cells than wild-type CEM/0 cells. These results suggest a relatively greater role for dCK than AK in the activation of **38** and **39**. When examined against CEM/dCK^-/AK^- cells (deficient in both AK and dCK) no further decrease in the inhibitory effect was noted (Table VI). This suggests either involvement of a third enzyme in the phosphorylation of these compounds or inhibition by their direct interaction with an unidentified target. It should be noted, however, that the residual cytostatic activity of **38** and **39** against the double mutant CEM/AK^-/dCK^- cells is rather poor and elucidation of this cytostatic mechanism might not be important.

To gain further insight into the nature of the phosphorylating enzymes, the cytostatic activity of compounds **38** and **39** against L1210 and CEM cells was monitored in the presence of high concentrations of exogenous dCyd, Ado, or Guo (Table VII). In CEM cells, the cytostatic activity of **38** and **39** was diminished 8–9-fold upon addition of dCyd, but not Ado. Combined addition of dCyd and Ado did not lower the cytostatic activity of **38** and **39** further. These observations suggest the involvement of dCK in the activation of **38** and **39** in CEM cells. In L1210 cells Ado and Guo, but not dCyd, were able to partially reverse the cytostatic activity of **38** and **39** (Table VII).

Table VII. Cytostatic Activity of 38 and 39 against L1210 and CEM Cells upon Addition of Exogenous Nucleosides

			IC_{50}[a] (μM)			
			upon addition[b] of			
compd	cell line	drug only	dCyd (1000 μM)	Ado (1000 μM)	dCyd + Ado (1000 μM)	Guo (50 μM)
38	L1210	10	16	48	41	44
	CEM	17	133	21	95	
39	L1210	10	17	55	75	51
	CEM	15	136	25	136	

[a] Concentration of compound required to inhibit L1210 and CEM cell proliferation by 50%. [b] The nucleosides dCyd, Ado, and Guo were added at subtoxic concentrations. Data are the mean of at least two to four independent experiments.

Table VIII. Antitumor Activity of 20, 38, and 39 in L1210 Tumor-Bearing Mice

compound	dose (mg/kg/day)	number of mice	mean survival time (days)
20	100	8	15.6 ± 2.9
	50	8	12.0 ± 1.9
38	100	8	2.0
	50	8	3.0 ± 0.5
	25	15	8.7 ± 1.4
	12.5	7	9.1 ± 0.7
39	100	8	2.0
	50	8	5.3 ± 3.0
	25	8	7.5 ± 0.5
	12.5	8	7.5 ± 0.5
none (control)	0	15	8.2 ± 1.0

Thus, different metabolic pathways may be involved in the activation of purine 2′-deoxy-2′-methylenenucleoside analogues, depending on the nature of the cell line. Kinetic studies would be required to determine their affinities for the phosphorylating enzymes AK and dCK.

Marked antitumor activity was observed upon treatment of L1210-bearing mice with MdCyd (**20**) for 5 days subsequent to tumor inoculation. No toxicity was apparent at a dose of 100 mg/kg per day, but the life span of the mice was doubled relative to controls (mean survival time, 15.6 and 8.2 days, respectively) (Table VIII). At a dose of 50 mg/kg, the mean life span was increased by 50%. In contrast, the 2,6-diaminopurine and guanine derivatives **38** and **39** were highly toxic to the mice at dosages of 100 and 50 mg/kg per day (Table VIII). At a dose of 12.5 mg/kg per day compound **38** prolonged the life span of tumor-bearing mice by ~10%, but no antitumor effect was observed with mice treated with **39** at this level (Table VIII). Thus, compound **38** afforded a slight in vivo antitumor effect at subtoxic doses whereas MdCyd (**20**) was very efficacious in prolonging the life span of L1210 tumor bearing mice.

Compounds **17**, **19**, **20**, **29**, **30**, **35**, **38**, **39**, and **44** were evaluated for inhibition of replication of herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2), vaccinia virus (VV), and vesicular stomatitis virus (VSV) in primary rabbit kidney (PRK) cells; parainfluenza-3, reovirus-1, Sindbis virus, Coxsackie virus B4, and Semliki forest virus in Vero cells; and VSV, Coxsackie virus B4, and poliovirus-1 in HeLa cells. The adenosine analogues **35** and **44** inhibited VV replication at 20 μg/mL, and MdCyd (**20**) and MdGuo (**39**) were inhibitory to HSV-1, HSV-2, and VV replication in PRK cells. None of the other compounds were inhibitory to virus replication at 200 or 400 μg/mL.

MdCyd (**20**) had an IC_{50} of 2–7 μg/mL against HSV-1 strains (KOS, F, and McIntyre) and thymidine kinase deficient (TK^-) HSV-1 strains (B2006, ACV^r, and $BVDU^r$), an IC_{50} of 7 μg/mL against HSV-2 strains (G, 196, and Lyons), and an IC_{50} of 2 μg/mL against vaccinia virus. The observation that HSV-1 wild-type and TK^- (deficient)

Table IX. Inhibition of S-Adenosylhomocysteine Hydrolase by Nucleoside Analogues

conc (μM)	% enzyme activity remaining				
	44	35	9	8	49
0.01	108	108	99	102	100
0.1	97	106	98	106	100
1	52	102	95	99	99
10	6	85	94	87	101
100	2	ND[a]	84	56	101

[a] Not determined.

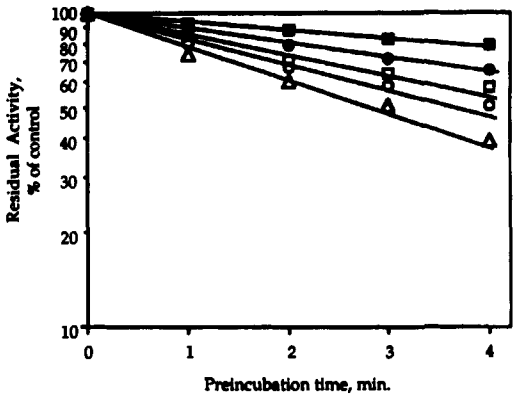

Figure 3. Time- and concentration-dependent inactivation of AdoHcy hydrolase by 44. 20 nM bovine liver AdoHcy hydrolase was preincubated for the indicated time at 37 °C with different concentrations of 44 (■, 2 μM; ●, 4 μM; □, 6 μM; ○, 8 μM; △, 12 μM).

strains are equally sensitive to 20 suggests that TK is not involved in the phosphorylation of MdCyd. Examination of the role of HSV-1- and HSV-2-specified ribonucleotide reductases as targets[16] for the antiviral activity of MdCyd (20) would be enlightening. Compounds 38 and MdGuo (39) were inhibitory against HSV-1 at 20 and 70 μg/mL, against HSV-2 at 150 and >200 μg/mL, and against VV at 7 and 20 μg/mL, respectively. None of the compounds in Table III were inhibitory to HIV-1 or HIV-2 replication in MT-4 cells at subtoxic concentrations (data not shown).

Inhibition of AdoHcy Hydrolase

The methyleneadenosine analogues 8, 9, 35, 44, and 49 were evaluated with purified bovine liver AdoHcy hydrolase (Table IX). Of these, 2′-deoxy-2′-methyleneadenosine (44) caused time-dependent inactivation with K_I = 13.1 μM and k_2 = 0.195 min^{-1}. The kinetic data for 44 are shown in Figures 3 and 4. Such time-dependent inactivation is compatible with a type I mechanism-based inhibitor of AdoHcy hydrolase[6a] which inactivates the enzyme by reduction of the enzyme-bound NAD^+ to NADH with simultaneous oxidation of 44 to enone 6. Alternatively, these time-dependent-inactivation results also are consistent with a type II mechanism-based inhibitor of AdoHcy hydrolase which inactivates the enzyme by reduction of the enzyme-bound NAD^+ to NADH followed by covalent attachment of the resulting 3′-ketonucleoside (e.g. Michael alkylation of enone 6) to the enzyme.[6a] More detailed mechanistic studies will be necessary in order to differentiate which mechanism is operative with 44. The 7-deaza analogue (2′-deoxy-2′-methylenetubercidin, 49) was completely inactive. Interestingly, 3′-deoxy-3′-methyleneadenosine (35) and its 4′,5′-didehydro-5′-deoxy analogue (8) exhibited weak apparent binding to the enzyme at high concentrations (10–100 μM), whereas the 2′,4′-bis-methylene analogue 9 did not bind to a significant extent. Thus, weak "isosteric" binding of the non-isopolar 3′-methylene group might occur in the region normally

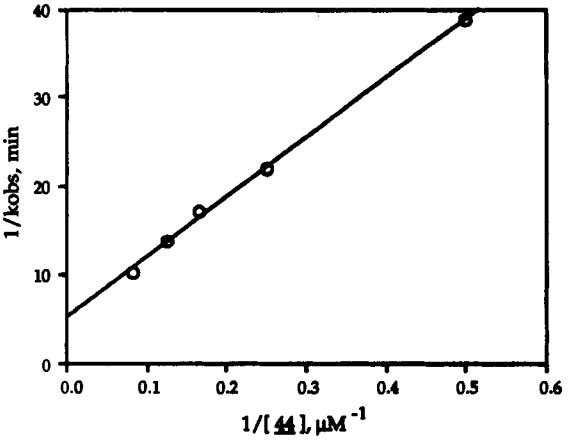

Figure 4. Plot of $1/k_{obs}$ vs 1/[44]. k_{obs} values were determined from the slopes of the lines shown in Figure 3.

occupied by the carbonyl group after oxidation of the secondary 3′ alcohol function. The lack of binding of 9 by the enzyme would preclude its oxidation to the putative cross-conjugated dienone intermediate 7 and inactivation of AdoHcy hydrolase.

In summary, reliable methods for the synthesis of 2′-(3′)-deoxy-2′(3′)-methyleneguanosine and 2-amino-6-substituted-purine nucleoside analogues were developed. MdAdo (2′-deoxy-2′-methyleneadenosine, 44) caused time-dependent inactivation of AdoHcy hydrolase. No adenosine- or uridine-derived analogues or 3′-deoxy-3′-methylene compounds exhibited potent biological activity in cells. MdCyd (2′-deoxy-2′-methylenecytidine, 20) was a potent antitumor agent in cells and had significant activity in vivo. MdGuo (2′-deoxy-2′-methyleneguanosine, 39) and its 2-amino-6-substituted analogues that are reasonably good alternative substrates of adenosine deaminase showed significant antitumor activity in cells, but were toxic in vivo. None of these analogues exhibited an antiviral potency of significant interest.

Experimental Section

Chemistry General Procedures. Uncorrected melting points were obtained with a capillary melting tube apparatus. Ultraviolet (UV) spectra were determined with a Hewlett-Packard 8451A spectrophotometer with MeOH solutions unless specified otherwise. ^1H and ^{13}C NMR spectra were obtained with a Varian Gemini-200 spectrometer at 200 and 50 MHz, respectively. Low-resolution electron-impact mass spectra (MS) were determined with a Finnigan MAT 8430 spectrometer at 20 eV. A rotary evaporator with water aspirator or mechanical oil pump vacuum was used for flash evaporations at <35 °C. Preparative HPLC was performed on a Waters Prep LC/System 500A with Prep-PAK-500/silica cartridges. TLC was performed on E. Merck 60-F_{254} sheets. Flash chromatography employed E. Merck Kieselgel 60, 230–400 mesh. Unless specified otherwise, the solvent for all chromatography was hexane/EtOAc (7:3, v/v). Reagent-grade solvents and reagents were redistilled prior to use. Pyridine and benzene were dried by refluxing with and distillation from CaH_2. Et_2O was distilled from sodium benzophenone ketyl. DMF was distilled from P_4O_{10}. *tert*-Butyldimethylsilyl chloride (TBDMSCl) and sodium thiomethoxide were purchased from Aldrich Chemical Co. Elemental analyses were determined by M-H-W Laboratories.

Silylation of 2-Amino-6-chloro-9-(β-D-ribofuranosyl)-purine.[19] TBDMSCl (14.9 g, 99.5 mmol) was added to a stirred solution of 21[18] (12.0 g, 39.8 mmol) and imidazole (13.5 g, 198.9 mmol) in DMF (190 mL) and the mixture was stirred at ambient temperature for 50 min. Solvent was removed in vacuo and the residue was dissolved in Et_2O (150 mL). The solution was washed with H_2O (10 mL) and the aqueous phase was extracted with Et_2O (2 × 10 mL). The combined organic phase was washed with ice-cold 0.01 N HCl/H_2O, saturated $NaHCO_3$/H_2O, and brine,

dried (Na_2SO_4), and evaporated to give a pale yellow oil. This mixture was separated by HPLC to give the 2',3',5'-tris-*O*-TBDMS (2.04 g, 8%; R_f ~0.74), 2',5'-bis-*O*-TBDMS (**22**; 7.60 g, 36%; R_f ~0.32), 3',5'-bis-*O*-TBDMS (**23**; 8.84 g, 42%; R_f ~0.21), and 5'-*O*-TBDMS (0.82 g, 5%; R_f ~0.01) derivatives of 2-amino-6-chloro-9-(β-D-ribofuranosyl)purine (**21**). Data for **22**: colorless solid (CH_2Cl_2/hexane); mp 159–160 °C; UV max 310, 248 nm (ε 8500, 7600); MS *m/z* 472 (100, M – CMe_3), 326 (98), 261 (29). Anal. ($C_{22}H_{40}ClN_5O_4Si_2$) C, H, N. Data for **23**: colorless solid (CH_2Cl_2/hexane); mp 165–166 °C; UV max 309, 248 nm (ε 8600, 7500); MS *m/z* 472 (100, M – CMe_3), 326 (80), 261 (41). Anal. ($C_{22}H_{40}ClN_5O_4Si_2$) C, H, N.

2-Amino-6-chloro-9-(2,5-bis-*O*-TBDMS-β-D-*erythro*-pentofuran-3-ulosyl)purine (24). Procedure A.[20] Ac_2O (3.26 mL, 3.52 g, 34.56 mmol) and pyridine (5.59 mL, 5.46 g, 69.12 mmol) were added to an ice-cold suspension of CrO_3 (3.46 g, 34.56 mmol) in CH_2Cl_2 (55 mL). The mixture was stirred at ambient temperature until homogeneous (15 min) and **22** (6.1 g, 11.52 mmol) was added. The mixture was stirred for 1 h and poured into cold EtOAc (1.5 L), stirred for 10 min, and filtered through a glass-microfiber filter GF/A. The filtrate was concentrated (<25 °C) and subjected to flash chromatography to give **24** (6.04 g) as a yellow solid foam that was used directly in the Wittig reaction: MS *m/z* 470 (10, M – CMe_3), 301 (100, M – BH – CMe_3), 169 (29, B).

2-Amino-6-chloro-9-(3,5-bis-*O*-TBDMS-β-D-*erythro*-pentofuran-2-ulosyl)purine (26). Procedure A was applied to **23** (7.50 g, 14.1 mmol) except after filtration through glass-microfiber GF/A, the filtrate was washed immediately with ice-cold HCl/H_2O (0.05 M, five times), $NaHCO_3/H_2O$, and brine, dried (Na_2SO_4) for 1 h, and evaporated to give **26** (7.50 g) as a yellow solid foam: MS *m/z* 470 (60, M – CMe_3), 301 (100, M – BH – CMe_3), 169 (38, B).

2-Amino-6-chloro-9-(2,5-bis-*O*-TBDMS-3-deoxy-3-methylene-β-D-*erythro*-pentofuranosyl)purine (25). Procedure B.[12,14] Sodium 2-methyl-2-butoxide (1.54 M in benzene; 15.3 mL, 24.0 mmol) was added to a stirred suspension of methyltriphenylphosphonium bromide (8.97 g, 25.0 mmol) in dry Et_2O (430 mL). The bright yellow mixture was stirred under N_2 for 2 h and cooled to –78 °C, and **24** (4.14 g, 7.8 mmol) was added. The mixture was allowed to warm to –10 °C over 1 h, kept at ~4 °C for 36 h, and then quenched with saturated NH_4Cl/H_2O. The aqueous phase was extracted with Et_2O (2 × 20 mL) and the combined organic phase was washed with brine, dried (Na_2SO_4), and evaporated. The residue was subjected to flash chromatography to give **25** (2.63 g) as a yellow glass that was used directly in the next step: MS *m/z* 468 (100, M – CMe_3), 357 (12, M – B).

2-Amino-6-chloro-9-(3,5-bis-*O*-TBDMS-2-deoxy-2-methylene-β-D-*erythro*-pentofuranosyl)purine (27). Crude **26** (4.0 g) was subjected to procedure B to give **27** (2.67 g) as an orange oil that was used directly in the next step: MS *m/z* 468 (100, M – CMe_3), 328 (35, M – BCHO).

2-Amino-6-chloro-9-(3-deoxy-3-methylene-β-D-*erythro*-pentofuranosyl)purine (28). Procedure C. Bu_4NF/THF (1 M; 9.6 mL, 9.6 mmol) was added to a stirred solution of crude **25** (2.51 g, 4.78 mmol) in THF (30 mL). After 1 h at ambient temperature the solvent was evaporated and the residue partitioned (Et_2O/H_2O) The aqueous phase was washed with Et_2O and concentrated (<35 °C), and the residue purified by flash chromatography (EtOAc/Me_2CO, 5:3). Crystallization (MeOH/H_2O) afforded **28** (1.1 g, 49% overall from **22**) as a colorless solid: mp 154 °C dec; UV max 310, 248 nm (ε 8300, 7600); MS *m/z* 297 (23, M^+), 169 (100, B), 134 (38, B – Cl). Anal. ($C_{11}H_{12}ClN_5O_3$) C, H, N.

2-Amino-6-chloro-9-(2-deoxy-2-methylene-β-D-*erythro*-pentofuranosyl)purine (37). Procedure C was applied to **27** (2.67 g) to give crystalline (MeOH/H_2O) **37** (0.71 g, 32% from **23**): mp 163 °C dec; UV max 310, 248 nm (ε 8100, 7500); MS *m/z* 297 (14, M^+), 169 (100, B). Anal. ($C_{11}H_{12}ClN_5O_3$) C, H, N.

2,6-Diamino-9-(3-deoxy-3-methylene-β-D-*erythro*-pentofuranosyl)purine (29). A mixture of **28** (0.25 g, 0.84 mmol) and NH_3/MeOH (saturated; 15 mL) was sealed in a steel bomb with a Teflon liner, heated at 80 °C for 24 h, and evaporated. The residue was purified by flash chromatography (CH_2Cl_2/MeOH, 9:1) to give **32** (19 mg, 8%) and **29** (0.21 g, 90%) as colorless needles (MeOH/acetone): mp 166–167 °C; UV max 257, 281 nm (ε 10 000, 11 000); MS *m/z* 278 (70, M^+), 248 (20, M – CH_2O), 179 (50, BHCHO), 150 (100, BH). Anal. ($C_{11}H_{14}N_6O_3$) C, H, N.

2,6-Diamino-9-(2-deoxy-2-methylene-β-D-*erythro*-pentofuranosyl)purine (38). Compound **37** (0.11 g, 0.37 mmol) was treated as described for the preparation of **29** to give **41** (8 mg, 8%) and **38** (89 mg, 87%) as colorless needles (MeOH/Me_2CO): mp 112–114 °C; UV max 257, 281 nm (ε 9000, 10 000); MS *m/z* 278 (60, M^+), 261 (10, M – OH), 150 (100, BH). Anal. ($C_{11}H_{14}N_6O_3$·0.75 MeOH) C, H, N.

9-(3-Deoxy-3-methylene-β-D-*erythro*-pentofuranosyl)guanine (3'-Deoxy-3'-methyleneguanosine) (30). Adenosine deaminase (200 mg, Sigma Type II) was added to **29** (0.22 g, 0.80 mmol) in aqueous phosphate buffer (0.05 M, pH 7.5; 40 mL), and the mixture was stirred at ambient temperature for 18 h and concentrated to 10 mL. The precipitated solid was filtered and washed with H_2O. Recrystallization (MeOH/H_2O) gave **30** (190 mg, 86%) as a colorless solid: mp 245 °C dec; UV max 255, 274 nm (ε 14 500, 9500); MS *m/z* 279 (20, M^+), 151 (100, BH). Anal. ($C_{11}H_{13}N_5O_4$·0.25H_2O) C, H, N.

9-(2-Deoxy-2-methylene-β-D-*erythro*-pentofuranosyl)guanine (2'-Deoxy-2'-methyleneguanosine) (39). Compound **38** (75 mg, 0.26 mmol) was treated as described for the preparation of **30** to give **39** (55 mg, 73%) as a colorless solid: mp 215 °C dec; UV max 255, 274 nm (ε 15 000, 10 000); MS *m/z* 261 (60, M – H_2O), 151 (100, BH). Anal. ($C_{11}H_{13}N_5O_4$·0.5H_2O) C, H, N.

2-Amino-9-(3-deoxy-3-methylene-β-D-*erythro*-pentofuranosyl)-6-(*N*,*N*-dimethylamino)purine (31). A solution of **28** (0.25 g, 0.84 mmol) in 40% $NHMe_2/H_2O$ (40 mL, 0.84 mmol) was stirred at ambient temperature for 20 min and evaporated, first with a water aspirator to remove excess $NHMe_2$ and then in vacuo. The residue was purified on a silica column (EtOH/$CHCl_3$, 1:19) to afford **31** (0.236 g, 92%) as a colorless solid (MeCN): mp 169–171 °C; UV max 284, 264, 230 nm (ε 15 100, 11 300, 21 200); MS *m/z* 306 (90, M^+), 178 (100, BH), 149 (87). Anal. ($C_{13}H_{18}N_6O_3$) C, H, N.

2-Amino-9-(2-deoxy-2-methylene-β-D-*erythro*-pentofuranosyl)-6-(*N*,*N*-dimethylamino)purine (40). Compound **37** (0.325 g, 1.093 mmol) was treated as described for the preparation of **31** to give **40** (0.304 g, 91%) as a colorless solid foam: UV max 285, 265, 230 nm (ε 14 400, 10 500, 20 500); MS *m/z* 306 (82, M^+), 178 (100, BH), 149 (40). Anal. ($C_{13}H_{18}N_6O_3$) C, H, N.

2-Amino-9-(3-deoxy-3-methylene-β-D-*erythro*-pentofuranosyl)-6-methoxypurine (32). Na metal (80 mg, 3.48 mmol) was dissolved in absolute MeOH (50 mL), and **28** (300 mg, 1.0 mmol) was added. The resulting solution was refluxed for 50 min and cooled to ambient temperature, silica (3 g) was added, and the mixture was evaporated. The impregnated powder was applied to a silica column (1 × 4 cm) that was eluted with EtOH/$CHCl_3$ (1:3). Evaporation of appropriate fractions and crystallization of the residue (MeOH/H_2O, 1:9) gave colorless **32** (0.242 g, 82%): mp 125–128 °C; UV max 281, 250 nm (ε 8500, 9600); MS *m/z* 293 (100, M^+), 263 (40, M – CH_2O), 165 (80, BH). Anal. ($C_{12}H_{15}N_5O_4$·0.5H_2O) C, H, N.

2-Amino-9-(2-deoxy-2-methylene-β-D-*erythro*-pentofuranosyl)-6-methoxypurine (41). Na metal (139 mg, 6.0 mmol) was dissolved in MeOH (20 mL), **37** (0.3 g, 1.0 mmol) was added, and the mixture was refluxed for 15 min and treated as described for the preparation of **32** to give colorless crystals (MeOH/H_2O) of **41** (236 mg, 80%): mp 178–180 °C; UV max 282, 248 nm (ε 9200, 9500); MS *m/z* 293 (30, M^+), 263 (17, M – CH_2O), 165 (100, BH). Anal. ($C_{12}H_{15}N_5O_4$) C, H, N.

2-Amino-9-(3-deoxy-3-methylene-β-D-*erythro*-pentofuranosyl)purine-6-thione (33). A suspension of **28** (0.3 g, 1.01 mmol) in anhydrous MeOH (20 mL) was saturated with H_2S (0.5 h at ambient temperature) and heated to reflux in a three-neck flask fitted with a gas inlet tube and a condenser protected with a drying tube. A slow influx of H_2S was maintained while NaSH/MeOH (1 M NaOMe/MeOH; 3.03 mL, 3.03 mmol; presaturated with H_2S for 0.5 h) was added to the solution.[23] Heating was continued for 2 h, H_2S addition was terminated, and heating was continued for 15 min. The solution was neutralized (pH ~6) with AcOH, concentrated, and passed through a silica column (1 × 4 cm; EtOH/$CHCl_3$, 1:4). Appropriate fractions were concentrated (~50 mL), H_2O (5 mL) was added, and evaporation continued until precipitation of crystals occurred. The flask was stored at 4 °C overnight; the crystals were filtered, washed with

cold H_2O, and dried in vacuo (65 °C, 48 h) to give **33** (0.243 g, 82%) as a pale green solid: mp 238 °C dec; UV max 345, 271, 259 nm (ε 26 700, 6900, 7100); MS *m/z* 295 (20, M^+), 167 (100, BH). Anal. ($C_{11}H_{13}N_5O_3S$), C, H, N.

2-Amino-9-(2-deoxy-2-methylene-β-D-*erythro*-pentofuranosyl)purine-6-thione (42). Compound **37** (0.3 g, 1.01 mmol) was treated as described for the preparation of **33** to give **42** (0.243 g, 82%): mp 184 °C dec; UV max 345, 270, 259 nm (ε 25 600, 6650, 7000); MS *m/z* 277 (66, M − H_2O), 244 (74, M − H_2O − HS), 167 (100, BH). Anal. ($C_{11}H_{13}N_5O_3S$·0.50H_2O) C, H, N.

2-Amino-9-(3-deoxy-3-methylene-β-D-*erythro*-pentofuranosyl)-6-methylthiopurine (34). A mixture of **28** (0.3 g, 1.01 mmol) and NaSMe (0.212 g, 3.03 mmol) in dry *i*-PrOH (70 mL) was refluxed for 30 min and neutralized with AcOH. Silica (3.0 g) was added, the mixture evaporated, and the impregnated powder applied to a silica column (1 × 10 cm). Elution (EtOH/$CHCl_3$, 1:9) and evaporation of appropriate fractions gave a yellow solid foam (0.334 g) that was dissolved (EtOAc) and precipitated (hexane) to afford a colorless powder (0.256 g, 82%) that was crystallized (CH_2Cl_2/hexane) to give **34**: mp 104–105 °C; UV max 310, 246 nm (ε 11 400, 15 200); MS *m/z* 309 (100, M^+), 210 (28, BHCHO), 181 (80, BH). Anal. ($C_{12}H_{15}N_5O_3S$) C, H, N.

2-Amino-9-(2-deoxy-2-methylene-β-D-*erythro*-pentofuranosyl)-6-methylthiopurine (43). To a solution of NaSMe (35.3 mg, 0.504 mmol) in dry *i*-PrOH (10 mL) was added 4-Å molecular sieves (1.0 g) and a solution of **37** (50 mg, 0.168 mmol) in *i*-PrOH (40 mL). The mixture was stirred at ambient temperature for 5 h and filtered, and H_2O (10 mL) was added to the filtrate. This mixture was concentrated and applied to a column of Dowex 1 × 2 (OH^-). Elution with H_2O (80 mL) and MeOH/H_2O (1:3, 50 mL) and evaporation of appropriate fractions gave **43** (30 mg, 58%) as a colorless solid: mp 181–182 °C dec; UV max 310, 246 nm (ε 12 200, 16 200); MS *m/z* 309 (82, M^+), 181 (100, BH), 127 (30, M − BH_2). Anal. ($C_{12}H_{15}N_5O_3S$), C, H, N.

4-Amino-7-(3,5-*O*-TPDS-β-D-*erythro*-pentofuran-2-ulosyl)pyrrolo[2,3-*d*]pyrimidine (47). Procedure A was applied to 3′,5′-*O*-(1,1,3,3-tetraisopropyl-1,3-disiloxanediyl)tubercidin[24] (**46**) (1.7 g, 3.34 mmol) to give **47** (1.1 g) as a light green solid foam: MS *m/z* 506 (10, M^+), 463 (45, M − $CHMe_2$), 345 (30, M − BCHO), 134 (100, BH_2).

4-Amino-7-(2-deoxy-3,5-*O*-TPDS-2-methylene-β-D-*erythro*-pentofuranosyl)pyrrolo[2,3-*d*]pyrimidine (48). Crude **47** (1.0 g) was subjected to procedure B to give **48** (0.70 g) as an orange solid that was used directly in the next step: MS *m/z* 504 (100, M^+), 461 (60, M − $CHMe_2$), 371 (30, M − BH), 343 (40, M − BCHO).

4-Amino-7-(2-deoxy-2-methylene-β-D-*erythro*-pentofuranosyl)pyrrolo[2,3-*d*]pyrimidine (2′-Deoxy-2′-methylenetubercidin, 49). Procedure C was applied to **48** (0.60 g) to give **49** (0.11 g, 16% from **46**) as a colorless powder (MeOH/Et_2O): mp 215–216 °C; UV (H_2O, pH 7 buffer) max 270 nm (ε 11 600); MS *m/z* 262 (30, M^+), 245 (14, M − OH), 231 (36, M − CH_2OH), 134 (100, BH). Anal. ($C_{12}H_{14}N_4O_3$·0.75H_2O) C, H, N.

In Vitro Cytostatic Assays. Murine leukemia L1210 and mammary carcinoma FM3A cells were grown in Eagle's minimal essential medium and human B-lymphoblast Raji, T-lymphoblast Molt/4F, T-lymphocyte MT-4, and T-lymphocyte CEM cells were grown in RPMI-1640 medium supplemented with 10% (v/v) inactivated fetal calf serum (Gibco, Glasgow, Scotland), 2 mM L-glutamine (Flow Laboratories, Irvine, Scotland), and 0.075% (v/v) $NaHCO_3$ (Flow Laboratories). The thymidine kinase-deficient Raji/TK^- cells, the dCyd kinase-deficient Raji/dCK^- and CEM/dCK^- cells, the Ado kinase-deficient CEM/AK^-, and the double mutant CEM cell line deficient in both dCK and AK (CEM/dCK^-/AK^-) were grown in essentially the same culture medium. The cytostatic assays were performed as previously described.[25,26] Briefly, 100-μL aliquots of the cell suspensions (5 × 10^5 L1210 or FM3A cells/mL, or 7.5 × 10^5 Raji, Molt/4F, or MT-4 cells/mL) were added to the wells of a microtiter tray containing a 50-μL solution of varying concentrations of the test compounds (500, 200, 100, 20, 4, 0.8, 0.16, 0.032, 0.006, 0.001 μM) and a 50-μL solution of thymidine [(d)Thd], dUrd, dCyd, Ado, Guo, or medium (control) at the concentrations indicated in the tables. After a 2-day (L1210 and FM3A) or 3-day (Raji, Molt/4F, MT-4 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 the test compound required to reduce the number of viable cells by 50% (IC_{50}). All values in Tables III–VII are means of at least two to four independent experiments.

In Vivo Antitumor Assay. DBA/2 mice weighing 20 g were injected intraperitoneally (ip) with 10^6 murine leukemia L1210 cells. Compounds **20**, **38**, and **39** were injected ip during 5 successive days starting 1 day after tumor cell inoculation. The test compounds were dissolved in Eagle's Minimal Essential Medium containing 2.5% Tween. The injection volume was 250 μL.

Viruses. The virus origins and preparations of the virus stocks have been described. An overview is given in ref 27. The following viruses were evaluated: herpes simplex virus type 1 (strains KOS, F, and McIntyre), herpes simplex virus type 2 (strains G, 196, and Lyons), vaccinia virus (VV), vesicular stomatitis virus (VSV), Coxsakie virus type B4, poliovirus type 1, parainfluenza virus type 3, reovirus type 1, Sindbis virus, Semliki forest virus, and human immunodeficiency virus type 1 (strain HTLV-III_B) and type 2 (strain ROD).

Antiviral Activity. Inhibition of virus-induced cytopathogenicity was measured following established procedures.[28,29] The virus inoculum was 100 $CCID_{50}$ (1 $CCID_{50}$ corresponds to the virus stock dilution that proved infective for 50% of the cell cultures) per microtiter well in all antiviral assays.

Purification of AdoHcy Hydrolase and Evaluation of the Effectiveness of Potential Inhibitors. AdoHcy hydrolase was purified from bovine liver as described,[30] except Q Sepharose (Pharmacia) was used instead of DE-52 cellulose and the CM-Sephadex step was omitted. The AdoHcy hydrolase activity was determined by the method of Richards et al.[31] which involves measuring the hydrolysis of [2,8-^{3}H]AdoHcy to [2,8-^{3}H]adenosine and homocysteine. The incubation medium contained 150 mM potassium phosphate (pH 7.6) and 1 mM EDTA, and all incubations were performed at 37 °C. Different concentrations of potential inhibitors were preincubated with 20 nM AdoHcy hydrolase for 10 min. The preincubation mixtures were then incubated for 5 min with 4 units of calf intestinal adenosine deaminase and 100 μM [2,8-^{3}H]AdoHcy. The reaction was stopped by addition of 100 μL of 5 N formic acid and the reaction mixture was applied to a column (1 × 4 cm) of SP Sephadex C-25 equilibrated in 0.1 N formic acid. The [2,8-^{3}H]inosine product of deamination of [2,8-^{3}H]adenosine (formed by the hydrolysis of AdoHcy) was eluted with 8 mL of 0.1 N formic acid. The eluate

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was collected and the radioactivity determined with 1 mL of eluate mixed with 10 mL of scintillation cocktail (3a70, Research Products International) in a scintillation counter. Data obtained for the methylene nucleoside analogues are presented in Table IX.

Determination of Kinetic Constants for Inactivation of AdoHcy Hydrolase. The inactivation constants, K_I and k_2 were determined by the method previously described.[32] For these determinations, AdoHcy hydrolase was preincubated with various concentrations of inhibitors for various amounts of time and the residual enzyme activity was measured. The enzyme activity was determined in the direction of synthesis of AdoHcy from adenosine and homocysteine by incubating 20 nM bovine liver AdoHcy hydrolase with 0.2 mM adenosine and 5 mM homocysteine for 5 min at 37 °C in 150 mM potassium phosphate buffer (pH 7.6) containing 1 mM EDTA (total reaction volume 0.5 mL). The amount of AdoHcy formed was measured by HPLC after the reaction was stopped by addition of perchloric acid (final concentration 0.25 M). An aliquot (100 μL) of the supernatant obtained by centrifugation of the reaction mixture was injected into an HPLC column (C-18 reverse phase column, Econosphere, Alltech, 25 cm × 4.6 mm) and analyzed with a gradient program at a flow rate of 1 mL/min [solvent A, acetonitrile; solvent B, 50 mM sodium phosphate buffer (pH 3.2) containing 10 mM heptanesulfonic acid; program, 8–15% A for 10 min, 50% A for 5 min]. The peak area was monitored at 254 nm to quantitate the AdoHcy.

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The pseudo-first-order rate of inactivation (k_{obs}) was determined from a plot of the residual activity versus preincubation time. K_I and k_2 were obtained from a plot of $1/k_{obs}$ versus 1/[inhibitor] ([I]) using the equation

$$\frac{1}{k_{obs}} = \frac{K_I}{k_2[I]} + \frac{1}{k_2}$$

The data for 2′-deoxy-2′-methyleneadenosine (**44**) are shown in Figures 3 and 4. For **44**, K_I and k_2 values of 13.1 μM and 0.195 min^{-1}, respectively, were calculated.

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Synthesis and Evaluation of Analogues of (*Z*)-1-(4-Methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)ethene as Potential Cytotoxic and Antimitotic Agents

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A series of stilbenes has been prepared and tested for cytotoxicity in the five human cancer cell lines A-549 non-small cell lung, MCF-7 breast, HT-29 colon, SKMEL-5 melanoma, and MLM melanoma. The cis stilbenes **6a–f** proved to be cytotoxic in all five cell lines, with potencies comparable to that of combretastatin A-4. These cytotoxic compounds were all potent inhibitors of tubulin polymerization. The corresponding trans stilbenes **7b–f** were inactive as tubulin polymerization inhibitors and were significantly less cytotoxic in the five cancer cell lines. In the dihydro series, **8b**, **8c**, and **8f** were inactive as tubulin polymerization inhibitors, while **8a**, **8d**, and **8e** were less active than the corresponding cis compounds **6a**, **6d**, and **6e**. The lack of tubulin polymerization inhibitory activity and cytotoxicity displayed by the phenanthrene **23b**, which was synthesized as a conformationally rigid analogue of the lead compound **1**, indicates that the activity of the stilbenes is not due to a totally planar conformation. Similarly, inactivity of the conformationally restricted analogue **26** suggests that the biologically active conformation of **1a** resembles that of the cis alkene **1**. Additional inactive compounds prepared include the benzylisoquinoline series **28–32** as well as the protoberberines **38** and **39**. Shortening the two-carbon bridge of **1a** to a one-carbon bridge in the diphenylmethane **20** resulted in a decrease in cytotoxicity and tubulin polymerization inhibitory activity. Although the corresponding benzophenone **18** was as active as **1a** as a tubulin polymerization inhibitor, it was less cytotoxic than **1a**, and the benzhydrol **19** was essentially inactive. With the exception of the amide **15c**, which displayed low antitubulin activity, all of the phenylcinnamic acid derivatives **14a–c** and **15a–f** were inactive in the tubulin polymerization inhibition assay. The acid **14b** and the ester **15a** were cytotoxic in several of the cancer cell cultures in spite of their inactivity as tubulin polymerization inhibitors.

The design of inhibitors of tubulin polymerization is an attractive strategy for the development of compounds useful in cancer chemotherapy. Ligands binding in the colchicine binding site of tubulin represent an array of antimitotic agents that inhibit cancer cell proliferation. Such compounds, including colchicine,[1-6] podophyllo-

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