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<sub>2</sub>, 2–52; MgSO<sub>4</sub>, 1.64; NaHCO<sub>3</sub>, 24.88; KH<sub>2</sub>PO<sub>4</sub>, 1.18; glucose, 5.55, bubbled with 5% CO<sub>2</sub> and 95% O<sub>2</sub>. 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 constructed for each compound with three to five preparations at each concentration. From these curves IC<sub>30</sub> 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.

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**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;

41, 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<sub>3</sub>(C-H<sub>2</sub>)<sub>4</sub>OH, 71-41-0; CH<sub>3</sub>(CH<sub>2</sub>)<sub>5</sub>OH, 111-27-3; CH<sub>3</sub>(CH<sub>2</sub>)<sub>6</sub>OH, 111-70-6; CH<sub>3</sub>(CH<sub>2</sub>)<sub>7</sub>OH, 111-87-5; CH<sub>3</sub>CH(CH<sub>3</sub>)(CH<sub>2</sub>)<sub>3</sub>OH, 626-89-1; CH<sub>3</sub>CH<sub>2</sub>CH(CH<sub>3</sub>)(CH<sub>2</sub>)<sub>2</sub>OH, 589-35-5; CH<sub>3</sub>(CH<sub>2</sub>)<sub>2</sub>CH(CH<sub>3</sub>)C-H<sub>2</sub>OH, 105-30-6; CH<sub>3</sub>(CH<sub>2</sub>)<sub>3</sub>CH(CH<sub>3</sub>)OH, 626-93-7; PrMgBr, 927-77-5; CH<sub>3</sub>(CH<sub>2</sub>)<sub>4</sub>MgBr, 693-25-4; CH<sub>3</sub>(CH<sub>2</sub>)<sub>6</sub>MgBr, 13125-66-1; CH<sub>3</sub>(CH<sub>2</sub>)7MgBr, 17049-49-9; CH<sub>3</sub>(CH<sub>2</sub>)5NH<sub>2</sub>, 111-26-2; 3pyridinecarboxaldehyde, 500-22-1;  $\alpha$ -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<sup>1</sup>

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Treatment of 2-amino-6-chloro-9- $(\beta$ -D-ribofuranosyl)purine (21) with TBDMS chloride/imidazole/DMF gave a separable mixture of 5'-0, 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<sub>3</sub>/pyridine/Ac<sub>2</sub>O, treatment of the respective ketonucleosides with methylenetriphenylphosphorane, and deprotection gave 2-amino-6-chloro-9-[3(and 2)-deoxy-3(and 2)-methylene- $\beta$ -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 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



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

the pyrimidine<sup>2a</sup> and purine<sup>2b</sup> 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<sup>3</sup> and antiretroviral activity of 3'-azido-3'-deoxythymidine (AZT) and 2',3'-dideoxynucleosides.<sup>4</sup>

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.<sup>5,6a</sup> 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.<sup>6</sup>

- The previous paper in this series: Robins, M. J.; Mullah, K. B.; Wnuk, S. F.; Dalley, N. K. Nucleic Acid Related Compounds. 73. Fluorination of Uridine 2'-Thioethers with Xenon Difluoride or (Diethylamino)sulfur Trifluoride. Synthesis of Stable 2'-[Alkyl(or Aryl)sulfonyl]-2'-deoxy-2'-fluorouridines. J. Org. Chem. 1992, 57, 2357-2364.
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<sup>a</sup> (a) TBDMSCl/imidazole/DMF; (b)  $CrO_3/pyridine/Ac_2O/CH_2Cl_2$ ; (c)  $Ph_3PCH_3Br/NaOC(Me)_2Et/benzene/Et_2O$ ; (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.<sup>7</sup> Ribonucleoside diphosphate reductases in mammalian systems provide the only de novo pathway to the 2'-deoxynucleotide components of DNA.<sup>8</sup> 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,<sup>9</sup> and includes the alternative substrate oxidation<sup>9b,c</sup> 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.<sup>10,11</sup> We reasoned<sup>12</sup> 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.<sup>6a</sup> If further enzymatic processing of 6 by elimination of water occurred, the bismethylene-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.<sup>12</sup> The 3',5'-bis-methylene compound 8 is seen to be a crude "isosteric" analogue of intermediate 2 in the biotransfor-

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mation (Figure 1) with a  $CH_2$  group in place of a carbonyl oxygen at the sp<sup>2</sup> 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).<sup>13</sup> We reasoned<sup>12,14</sup> 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(2H)-furanone (15), the methyl analogue of 16, which causes irreversible in-activation of ribonucleotide reductases.<sup>13,15</sup>

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*.<sup>16</sup> In addition, MdCyd (20) has been shown to be a potent anticancer agent against leukemia and solid tumor systems in vitro and in vivo.<sup>17</sup> The present biological studies were in

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progress when the results of Ueda and co-workers appeared<sup>17a</sup> and confirm their results. Our recent discovery of the mechanism-based inhibition of ribonucleotide reductase<sup>16</sup> 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.<sup>17b</sup> 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- $\beta$ -D-erythro-pentofuranosyl]purines, and 2'-deoxy-2'-methylenetubercidine (49); anticancer and antiviral activities of these and other purine<sup>12</sup> and pyrimidine<sup>14</sup> congeners; and inhibition studies with the methyleneadenosine analogues and AdoHcy hydrolase.

# Chemistry

Protection of 2-amino-6-chloro-9-(8-D-ribofuranosvl)purine (21)<sup>18</sup> with tert-butyldimethylsilyl (TBDMS) chloride/imidazole/dimethylformamide (DMF)<sup>19</sup> 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<sup>20</sup> 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)<sup>12,14</sup> 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  $\alpha$ -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

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<sup>a</sup> (a)  $CrO_3/pyridine/Ac_2O/CH_2Cl_2$ ; (b)  $Ph_3PCH_3Br/NaOC-(Me)_2Et/benzene/Et_2O$ ; (c)  $Bu_4NF/THF$ .

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

The new 2-amino-6-substituted derivatives 29-34 and 38-43, respectively, were obtained from 28 and 37 by nucleophilic displacements<sup>22</sup> 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  $\beta$ -anomeric configuration<sup>21</sup> 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<sup>23</sup> 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. <sup>1</sup>H NMR Spectral Data<sup>a</sup>

	H1′ <sup>b</sup>	H2' °	H3′		H5′	H5″	CH <sub>A</sub> H <sub>B</sub> <sup>d</sup>	CH <sub>A</sub> H <sub>B</sub> <sup>d</sup>			OH3'/2'b	OH5' /	
no.	$(J_{1'-2'})$	(J)	$(J_{3'-4'})$	H4′	$(J_{5'-5''})$	$(J_{5''-4'})$	(J)	(J)	H8′ *	NH2 <sup>e</sup>	$(J_{\rm OH-8'/2'})$	$(J_{\mathrm{OH-5'5''}})$	others
22	5.97	4.51	4.24	4.19 <sup>h</sup>	3.96 <sup>i</sup>	3.82 <sup>i</sup>			8.17	5.05	2.68		0.0-0.11 (SiMe)
	(5.1)	(5.0)	(4.5)		(11.5)	(2.2)					(4.0)		0.83, 0.92 (SiCMe <sub>3</sub> )
23	5.91	4.50 <sup>g,i</sup>	4.42 <sup>g,i</sup>	4.06 <sup>h</sup>	3.91 <sup>i</sup>	3.74 <sup>i</sup>			8.05	5.11	2.93		0.07-0.13 (SiMe)
	(4.3)	(5.2)	(4.2)		(11.5)	(2.6)					(6.0)		0.89, 0.93 (SiCMe <sub>3</sub> )
24	6.03	4.68 <sup>b</sup>		$4.27^{h}$	3.93 <sup>6</sup>	3.93°			8.16	5.09			0.0-0.05 (SiMe)
	(8.2)					(2.0)							0.72, 0.88 (SiCMe <sub>3</sub> )
25	5.76	4.91 <sup>j</sup>		4.69 <sup>h</sup>	3.93 <sup>i</sup>	3.74 <sup>i</sup>	$5.17^{i}$	5.25 <sup>i</sup>	8.18	5.13			0.0-0.15 (SiMe)
	(6.6)	(1.6)			(11.0)	(3.0)	(2.2)	(2.2)					0.80, 0.90 (SiCMe <sub>3</sub> )
26	5.72 <sup>k</sup>		5.05 <sup>b</sup>	3. <b>99</b> <sup>h</sup>	4.10	3.85 <sup>i</sup>			7.76	5.03			0.0-0.20 (SiMe)
			(8.5)		(12.0)	(2.6)							0.81, 0.93 (SiCMe <sub>3</sub> )
27	6.58 <sup>/</sup>		4.95 <sup>*</sup>	3.83 <sup>h</sup>	3.89	3.77 <sup>i</sup>	5.31/	5.42	7.90	5.0 <del>9</del>			0.0-0.2(SiMe)
	$(1.4)^{d}$		(6.0)		(11.0)	(2.0)	(2.0)	(2.0)					0.85, 0.92 (SiCMe <sub>3</sub> )
28	5.60	5.03 <sup>h</sup>		<b>4.6</b> 1 <sup><i>i</i></sup>	3.53 <sup>8,4</sup>	3.62 <sup>g,i</sup>	5.20	5.25'	8.40	7.0	5.97	$5.06^{h}$	
	(7.04)	(1.8) <sup>gj</sup>			(11.4)	(3.8)	(2.1)	(1.8)			(6.02)		
29	5.50	5.06 <sup>h</sup>		4.58 <sup>/</sup>	$3.65^{m}$	$3.53^{m}$	5.18'	5.21'	7.94	5.73	5.91	5.45'	6.82 (NH <sub>2</sub> )
	(7.4)	(1.8) <sup>s</sup> J			(11.6)	(3.4)					(6.2)	(7.2, 4.4)	
30	5.47	4.91"		4.55 <sup>4</sup>	3.55**	3.65 <sup>g, ,</sup>	5.17	5.22'	7.99	6.53	5.94	5.02	10.75 (NH)
	(7.0)	(1.8) <sup>g</sup>			(11.5)	(4.0)	(1.8)	(1.8)			(6.0)	(6.0)	
31	5.55	5.04 <sup>h</sup>		4.58	3.65**	3.51*+	5.18'	5.22'	7.96	5.79	5.91	5.38	3.35 (NMe <sub>2</sub> )
	(7.3)	(1.8) <sup>g</sup>			(11.8)	(3.8)					(6.3)	(4.5)	
32	5.58	5.06"		4.58'	3.61**	3.54	5.19	5.23'	8.13	6.47	5.94	5.12	3.96 (OMe)
	(7.0)	$(1.8)^{g_J}$		1	(12.0)	(3.6)					(5.9)	(5.5)	
33	5.49	4.96"		4.58	3.52**	3.58**	5.19'	5.23	8.16	6.82	5.95	5.07	11.98 (NH)
<b>.</b> .	(7.0)	(1.8)		01	(11.4)	(4.0)		<b>-</b> aal			(5.9)	0.071	
34	5.58	5.04"		4.58	3.59	3.59**	5.19	5.23	8.20	6.54	5.93	6.05'	2.57 (SMe)
	(7.0)		4 50	0.00	(11.4)	(3.3)	r ool	F ADI	0.10	a aa		4.01	
37	6.49		4.70	3.72"	3.69""	3.00**	5.28	<b>ə.4</b> 2'	8.16	6.99	<b>D.</b> 7	4.91	
	(1.4)		(7.0) <sup>er</sup>	0 704	(12.1)	(0.0)	E 1 Cl	E orl	7 70	E 70	(0.4)	(0.0)	C 79 (NUL )
38	6.40		4.00	3.70*	3.00	3.00	9.10	5.37	1.12	9.19	0.00	0.00	$6.73 (INH_2)$
	a ad		(0.3)	o arh	o toh	a cak	F 901	E nol	7 70	0 47	(0.2)	(0.3)	10 69 (NILI)
39	0.30		4.04	3.70	3.70	3.00	0.20	0.30	1.12	0.47	0.07	4.91	10.03 (INH)
40	e ari		(0.0) <sup>a</sup>	9 70h	2 con	2 504	5 1 1	5 29f	7 79	5 95	(0.5)	(4.7)	2 26 (NMa)
40	0.40° (1 5)d		4.00 (7 Ak	3.70	3.00	0.00	(2.0)	(2.0)	1.10	0.00	(6 1)	(5.2)	3.30 (INIVIE2)
41	(1.0)° 6 47j		1 601	2 704	2 604	a sah	5 201	5 20	7 01	6 49	57	4 94	2.94 (OMa)
41	(1 2)d		4.05 (5.7)#*	0.72	0.00	0.02	0.20	0.03	1.51	0.42	(6.2)	(5 5)	0.04 (OME)
49	6 3 94		A 67/	3 704	3 60h	2 59h	5 9 <i>41</i>	5 401	7 91	6 84	571	4 94	11 50 (NH)
44	0.00		(5.9)	0.10	5.00	0.02	(1.9)	(1.5)	1.51	0.04	0.1	(4 9)	11.00 (111)
12	6 184		4 69 <sup>1</sup>	3 714	3 604	3 55 <sup>h</sup>	5 99	5.40	7 96	6 55	57	4 91	2 56 (SMa)
40	0.40		4.03 (6 A)8,k	0.71	5.00	0.00	0.22	0.10	1.30	0.00	(6.2)	(5.2)	2.50 (SME)
48	6 83		5 118,	3 754	4 040,0		5 30	5 45/		5 10	(0.2)	(0.2)	8 32° (H2) 6 96° (H6)
-20	(1.6)		(8.4)	(3.4)	7.07		(2.8.1.6)	(2,2)		0.10			$6.34^{\circ}$ (H5) 1 10 <sup>4</sup> ( <i>i</i> -Pr'a)
49	6.74		4.60	3.65 <sup>h</sup>	3.70*	$3.52^{h}$	5.03/	5.33/		7.03	5.66	4.94	8.08° (H2), 7.10 <sup>b</sup> (H6)
	(1.5)		(6.6)8,	0.00	0.10	0.02	(2.1)	(1.9)			(4.7)	(5.5)	6.57 <sup>b</sup> (H5)
			····,				·/	()			····	·/	

<sup>a</sup> Chemical shifts ( $\delta$ , Me<sub>4</sub>Si internal); coupling constants (Hz, in parentheses). Solvents: CDCl<sub>3</sub> for 22–27, 48; Me<sub>2</sub>SO-d<sub>6</sub> for 28–34, 37–43, 49. <sup>b</sup> Doublet (unless noted otherwise). <sup>c</sup> Compounds 22, 23, J<sub>2:3</sub>; 25, 28–34, J "apparent" from incompletely resolved peaks. <sup>d</sup> J "apparent" from incompletely resolved peaks. <sup>c</sup> Singlet. <sup>f</sup> Triplet unless noted otherwise. <sup>d</sup> After exchange with D<sub>2</sub>O. <sup>h</sup> Multiplet. <sup>i</sup> Doublet of doublets. <sup>j</sup> "Apparent" quartet. <sup>k</sup> Doublet of multiplets. <sup>l</sup> Broad singlet. <sup>m</sup> Doublet of doublets of doublets. <sup>n</sup> Doublet of triplets. <sup>o</sup> 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<sup>24</sup> 3',5'-O-(1,1,3,3-tetraisopropyl-1,3-disiloxanediyl)tubercidin (46). As shown in Scheme II, oxidation<sup>20</sup> of 46, Wittig treatment of the resulting 2'-keto derivative 47, to give 48, and deprotection gave 4-amino-7-(2-deoxy-2-methylene- $\beta$ -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 <sup>1</sup>H and <sup>13</sup>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 <sup>13</sup>C resonance ranges for the C1', C4', and C5' peaks in the two series were remarkably constant at  $\delta \sim 87.1$ ,  $\sim 81.2$ , and  $\sim 64.4$  for the 3' isomers 28-34 and  $\delta \sim 85.3$ ,  $\sim 82.1$ , and  $\sim 61.1$  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  $\mu$ M. Compound 33 was markedly

<sup>(24)</sup> Robins, M. J.; Wilson, J. S.; Hansske, F. Nucleic Acid Related Compounds. 42. A General Procedure for the Efficient Deoxygenation of Secondary Alcohols. Regiospecific and Stereoselective Conversion of Ribonucleosides to 2'-Deoxynucleosides. J. Am. Chem. Soc. 1983, 105, 4059-4063.

Table II. <sup>13</sup>C NMR Spectral Data<sup>a</sup>

_	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. <b>64</b>	80.40	62.38		
	27	154.20	151.85	125.84	159.76	140.97	85.55	149.15	70.7 <del>9</del>	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. <del>9</del> 3	
	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 <sub>2</sub> )
	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. <del>9</del> 0	148.54	81.29	64.37	108.25	
	34	159.62	150. <del>96</del>	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 <sub>2</sub> )
	41	160.19	154.01	113. <b>9</b> 0	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	1 <b>47.91</b>	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)

<sup>a</sup> Chemical shifts (δ, Me<sub>3</sub>Si internal) of proton-decoupled singlets. Solvents: CDCl<sub>3</sub> for 22-27, Me<sub>2</sub>SO-d<sub>6</sub> for 28-34, 37-43, 49.

Table III. Cytostatic Activity of Nucleoside Analogues in Vitro

	$\mathrm{IC}_{50}^{a}$ ( $\mu \mathbf{M}$ )					
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		

<sup>a</sup> 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<sub>50</sub> = 12  $\mu$ M) 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  $\mu$ M. 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.<sup>6c</sup> 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

	$IC_{50}^{a}$ ( $\mu$ <b>M</b> )								
compd	Raji/0	Raji/TK <sup>-</sup>	Raji/dCK <sup>-</sup>	CEM/0	CEM/ dCK <sup>-</sup>				
18	33	52	>500	63	>500				
20	0.025	0.035	31	0.046	60				

<sup>a</sup> Concentration of compound required to inhibit human B-lymphoblast Raji/0, (d)Thd kinase-deficient (TK<sup>-</sup>) Raji, dCyd kinase-deficient (dCK<sup>-</sup>) Raji, human T-lymphocyte CEM/0, and dCK<sup>-</sup> 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 <sub>50</sub> <sup>4</sup>	$\mathrm{IC}_{50}^{a}$ ( $\mu$ M)				
		upon addition <sup>b</sup> 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			

<sup>a</sup>Concentration of compound required to inhibit L1210 cell proliferation by 50%. <sup>b</sup>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<sub>50</sub> ( $\mu$ M): 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  $\mu$ M for L1210 and FM3A cells (Tables III and IV)]. Its MdUrd counterpart 19 was devoid of cytostatic activity even at 500  $\mu$ M.

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<sup>-</sup> 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<sup>-</sup> and CEM/dCK<sup>-</sup> 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<sup>-</sup> or Raji/0 cells, but it had no inhibitory effect on Raji/dCK<sup>-</sup> or CEM/dCK<sup>-</sup> cell proliferation (Table IV).

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

	$\mathrm{IC}_{50}^{a}$ ( $\mu \mathbf{M}$ )					
compd	CEM/0	CEM/ AK <sup>-</sup>	CEM/ dCK <sup>-</sup>	CEM/ AK <sup>-</sup> /dCK <sup>-</sup>		
29	>500	>500	>500	>500		
30	>500	>500	>500	>500		
38	7.7	38	196	194		
39	9.4	36	221	201		

<sup>a</sup>Concentration of compound required to inhibit CEM/0, Ado kinase-deficient (AK<sup>-</sup>) CEM, dCyd kinase-deficient (dCK<sup>-</sup>) CEM, and AK<sup>-</sup>/dCK<sup>-</sup> 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<sub>50</sub> = 8-24  $\mu$ M) and, to a lesser extent, FM3A and MT-4 cells (IC<sub>50</sub> = 26 to  $\geq$ 500  $\mu$ M; 0.48  $\mu$ M 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<sup>16</sup> 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<sup>-</sup>, and 25-fold less inhibitory to CEM/dCK<sup>-</sup> 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<sup>-</sup>/dCK<sup>-</sup> 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 <sub>50</sub> <sup>a</sup> (µ1	(Iv				
			upon addition <sup>b</sup> of						
compd	cell line	drug only	dCyd (1000 μM)	Ado (1000 μM)	dCyd + Ado (1000 μM)	Guo (50 µM)			
38	L1210 CEM	10 17	16 133	48 21	41 95	44			
39	L1210 CEM	10 15	17 136	55 25	75 136	51			

<sup>a</sup>Concentration of compound required to inhibit L1210 and CEM cell proliferation by 50%. <sup>b</sup>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	$15.6 \pm 2.9$
	50	8	$12.0 \pm 1.9$
38	100	8	2.0
	50	8	$3.0 \pm 0.5$
	25	15	8.7 单 1.4
	12.5	7	9.1 🛳 0.7
39	100	8	2.0
	50	8	$5.3 \pm 3.0$
	25	8	$7.5 \pm 0.5$
	12.5	8	$7.5 \pm 0.5$
none (control)	0	15	<b>8.2 1</b> .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  $\sim 10\%$ , 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  $\mu$ g/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  $\mu$ g/mL.

MdCyd (20) had an IC<sub>50</sub> of 2–7  $\mu$ g/mL against HSV-1 strains (KOS, F, and McIntyre) and thymidine kinase deficient (TK<sup>-</sup>) HSV-1 strains (B2006, ACV<sup>\*</sup>, and BVDU<sup>\*</sup>), an IC<sub>50</sub> of 7  $\mu$ g/mL against HSV-2 strains (G, 196, and Lyons), and an IC<sub>50</sub> of 2  $\mu$ g/mL against vaccinia virus. The observation that HSV-1 wild-type and TK<sup>-</sup> (deficient)

**Table IX.** Inhibition of S-Adenosylhomocysteine Hydrolase by

 Nucleoside Analogues



**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 ( $\blacksquare$ , 2  $\mu$ M;  $\bigcirc$ , 4  $\mu$ M;  $\square$ , 6  $\mu$ M;  $\bigcirc$ , 8  $\mu$ M;  $\triangle$ , 12  $\mu$ M).

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<sup>16</sup> for the antiviral activity of MdCyd (20) would be enlightening. Compounds 38 and MdGuo (39) were inhibitory against HSV-1 at 20 and 70  $\mu$ g/mL, against HSV-2 at 150 and >200  $\mu$ g/mL, and against VV at 7 and 20  $\mu$ g/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  $K_{\rm I} = 13.1$  $\mu$ M and  $k_2 = 0.195$  min<sup>-1</sup>. 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<sup>6a</sup> which inactivates the enzyme by reduction of the enzyme-bound NAD<sup>+</sup> 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<sup>+</sup> to NADH followed by covalent attachment of the resulting 3'-ketonucleoside (e.g. Michael alkylation of enone 6) to the enzyme.<sup>6a</sup> 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  $\mu$ M), 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. <sup>1</sup>H and <sup>13</sup>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<sub>254</sub> 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<sub>2</sub>. Et<sub>2</sub>O was distilled from sodium benzophenone ketyl. DMF was distilled from  $P_4O_{10}$ . tert-Butyldimethylsilyl chloride (TBDMSCI) 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-( $\beta$ -D-ribofuranosyl)purine.<sup>19</sup> TBDMSCl (14.9 g, 99.5 mmol) was added to a stirred solution of 21<sup>18</sup> (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<sub>2</sub>O (150 mL). The solution was washed with H<sub>2</sub>O (10 mL) and the aqueous phase was extracted with Et<sub>2</sub>O (2 × 10 mL). The combined organic phase was washed with ice-cold 0.01 N HCl/H<sub>2</sub>O, saturated NaHCO<sub>3</sub>/H<sub>2</sub>O, and brine,

### Nucleic Acid Related Compounds

dried (Na<sub>2</sub>SO<sub>4</sub>), 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%;  $R_f \sim 0.74$ ), 2',5'-bis-O-TBDMS (22; 7.60 g, 36%;  $R_f \sim 0.32$ ), 3',5'-bis-O-TBDMS (23; 8.84 g, 42%;  $R_f \sim 0.21$ ), and 5'-O-TBDMS (0.82 g, 5%;  $R_f \sim 0.01$ ) derivatives of 2-amino-6-chloro-9-( $\beta$ -D-ribofuranosyl)purine (21). Data for 22: colorless solid (CH<sub>2</sub>Cl<sub>2</sub>/hexane); mp 159–160 °C; UV max 310, 248 nm ( $\epsilon$  8500, 7600); MS m/z 472 (100, M - CMe<sub>3</sub>), 326 (98), 261 (29). Anal. (C<sub>22</sub>H<sub>40</sub>ClN<sub>5</sub>O<sub>4</sub>Si<sub>2</sub>) C, H, N. Data for 23: colorless solid (CH<sub>2</sub>Cl<sub>2</sub>/hexane); mp 165–166 °C; UV max 309, 248 nm ( $\epsilon$  8600, 7500); MS m/z 472 (100, M - CMe<sub>3</sub>), 326 (80), 261 (41). Anal. (C<sub>22</sub>H<sub>40</sub>ClN<sub>5</sub>O<sub>4</sub>Si<sub>2</sub>) C, H, N.

2-Amino-6-chloro-9-(2,5-bis-O-TBDMS- $\beta$ -D-erythro-pentofuran-3-ulosyl)purine (24). Procedure A.<sup>20</sup> Ac<sub>2</sub>O (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<sub>3</sub> (3.46 g, 34.56 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (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 glassmicrofiber 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<sub>3</sub>), 301 (100, M - BH - CMe<sub>3</sub>), 169 (29, B).

2-Amino-6-chloro-9-(3,5-bis-O-TBDMS- $\beta$ -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<sub>2</sub>O (0.05 M, five times), NaHCO<sub>3</sub>/H<sub>2</sub>O, and brine, dried (Na<sub>2</sub>SO<sub>4</sub>) for 1 h, and evaporated to give 26 (7.50 g) as a yellow solid foam: MS m/z 470 (60, M – CMe<sub>3</sub>), 301 (100, M – BH – CMe<sub>3</sub>), 169 (38, B).

2-Amino-6-chloro-9-(2,5-bis-O-TBDMS-3-deoxy-3methylene- $\beta$ -D-*erythro*-pentofuranosyl)purine (25). Procedure B.<sup>12,14</sup> 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<sub>2</sub>O (430 mL). The bright yellow mixture was stirred under N<sub>2</sub> 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<sub>4</sub>Cl/H<sub>2</sub>O. The aqueous phase was extracted with Et<sub>2</sub>O (2 × 20 mL) and the combined organic phase was washed with brine, dried (Na<sub>2</sub>SO<sub>4</sub>), 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<sub>3</sub>), 357 (12, M - B).

2-Amino-6-chloro-9-(3,5-bis-O-TBDMS-2-deoxy-2methylene- $\beta$ -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<sub>3</sub>), 328 (35, M - BCHO).

2-Amino-6-chloro-9-(3-deoxy-3-methylene- $\beta$ -D-erythropentofuranosyl)purine (28). Procedure C. Bu<sub>4</sub>NF/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<sub>2</sub>O/H<sub>2</sub>O) The aqueous phase was washed with Et<sub>2</sub>O and concentrated (<35 °C), and the residue purified by flash chromatography (EtOAc/Me<sub>2</sub>CO, 5:3). Crystallization (MeOH/H<sub>2</sub>O) afforded 28 (1.1 g, 49% overall from 22) as a colorless solid: mp 154 °C dec; UV max 310, 248 nm ( $\epsilon$  8300, 7600); MS m/z 297 (23, M<sup>+</sup>), 169 (100, B), 134 (38, B - Cl). Anal. (C<sub>11</sub>H<sub>12</sub>ClN<sub>5</sub>O<sub>3</sub>) C, H, N.

**2-Amino-6-chloro-9-(2-deoxy-2-methylene-\beta-D-***erythro***-<b>pentofuranosyl)purine (37).** Procedure C was applied to 27 (2.67 g) to give crystalline (MeOH/H<sub>2</sub>O) **37** (0.71 g, 32% from **23)**: mp 163 °C dec; UV max 310, 248 nm ( $\epsilon$  8100, 7500); MS m/z297 (14, M<sup>+</sup>), 169 (100, B). Anal. (C<sub>11</sub>H<sub>12</sub>ClN<sub>5</sub>O<sub>3</sub>) C, H, N.

2,6-Diamino-9-(3-deoxy-3-methylene- $\beta$ -D-erythro-pentofuranosyl)purine (29). A mixture of 28 (0.25 g, 0.84 mmol) and NH<sub>3</sub>/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<sub>2</sub>Cl<sub>2</sub>/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 ( $\epsilon$  10 000, 11 000); MS m/z 278 (70, M<sup>+</sup>), 248 (20, M - CH<sub>2</sub>O), 179 (50, BHCHO), 150 (100, BH). Anal. (C<sub>11</sub>H<sub>14</sub>N<sub>6</sub>O<sub>3</sub>) C, H, N.

**2,6-Diamino-9-(2-deoxy-2-methylene-\beta-D-erythro-pento-furanosyl)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<sub>2</sub>CO): mp 112-114 °C; UV max 257, 281 nm ( $\epsilon$  9000, 10000); MS m/z 278 (60, M<sup>+</sup>), 261 (10, M - OH), 150 (100, BH). Anal. (C<sub>11</sub>H<sub>14</sub>N<sub>6</sub>O<sub>3</sub>·0.75 MeOH) C, H, N.

9-(3-Deoxy-3-methylene- $\beta$ -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<sub>2</sub>O. Recrystallization (MeOH/H<sub>2</sub>O) gave 30 (190 mg, 86%) as a colorless solid: mp 245 °C dec; UV max 255, 274 nm ( $\epsilon$  14 500, 9500); MS m/z 279 (20, M<sup>+</sup>), 151 (100, BH). Anal. (C<sub>11</sub>H<sub>13</sub>N<sub>5</sub>O<sub>4</sub>-0.25H<sub>2</sub>O) C, H, N.

9-(2-Deoxy-2-methylene- $\beta$ -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 ( $\epsilon$  15000, 10000); MS m/z 261 (60, M – H<sub>2</sub>O), 151 (100, BH). Anal. (C<sub>11</sub>H<sub>13</sub>N<sub>5</sub>O<sub>4</sub>•0.5H<sub>2</sub>O) C, H, N.

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

2-Amino-9-(2-deoxy-2-methylene- $\beta$ -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 ( $\epsilon$  14 400, 10 500, 20 500); MS m/z 306 (82, M<sup>+</sup>), 178 (100, BH), 149 (40). Anal. (C<sub>13</sub>H<sub>18</sub>N<sub>6</sub>O<sub>3</sub>) C, H, N.

2-Amino-9-(3-deoxy-3-methylene- $\beta$ -D-erythro-pentofuranosyl)-6-methoxypurine (32). Na metal (80 mg, 3.48 amol) was dissolved in absolute MeOH (50 mL), and 28 (300 ag, 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<sub>3</sub> (1:3). Evaporation of appropriate fractions and crystallization of the residue (MeOH/H<sub>2</sub>O, 1:9) gave colorless 32 (0.242 g, 82%): mp 125-128 °C; UV max 281, 250 nm ( $\epsilon$  8500, 9600); MS m/z 293 (100, M<sup>+</sup>), 263 (40, M - CH<sub>2</sub>O), 165 (80, BH). Anal. (C<sub>12</sub>H<sub>15</sub>-N<sub>5</sub>O<sub>4</sub>·0.5H<sub>2</sub>O) C, H, N.

2-Amino-9-(2-deoxy-2-methylene- $\beta$ -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<sub>2</sub>O) of 41 (236 mg, 80%): mp 178-180 °C; UV max 282, 248 nm ( $\epsilon$ 9200, 9500); MS m/z 293 (30, M<sup>+</sup>), 263 (17, M - CH<sub>2</sub>O), 165 (100, BH). Anal. (C<sub>12</sub>H<sub>15</sub>N<sub>5</sub>O<sub>4</sub>) C, H, N.

2-Amino-9-(3-deoxy-3-methylene- $\beta$ -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<sub>2</sub>S (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<sub>2</sub>S was maintained while NaSH/MeOH (1 M NaOMe/MeOH; 3.03 mL, 3.03 mmol; presaturated with H<sub>2</sub>S for 0.5 h) was added to the solution.<sup>23</sup> Heating was continued for 2 h, H<sub>2</sub>S 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<sub>3</sub>, 1:4). Appropriate fractions were concentrated (~50 mL), H<sub>2</sub>O (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<sub>2</sub>O, 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 ( $\epsilon$  26 700, 6900, 7100); MS m/z 295 (20, M<sup>+</sup>), 167 (100, BH). Anal. (C<sub>11</sub>H<sub>13</sub>N<sub>5</sub>O<sub>3</sub>S), C, H, N.

**2-Amino-9-(2-deoxy-2-methylene**- $\beta$ -D-*erythro*-pento-furanosyl)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 ( $\epsilon$  25 600, 6650, 7000); MS m/z 277 (66, M – H<sub>2</sub>O), 244 (74, M – H<sub>2</sub>O – HS), 167 (100, BH). Anal. (C<sub>11</sub>H<sub>13</sub>N<sub>5</sub>O<sub>3</sub>S-0.50H<sub>2</sub>O) C, H, N.

2-Amino-9-(3-deoxy-3-methylene- $\beta$ -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<sub>3</sub>, 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<sub>2</sub>Cl<sub>2</sub>/hexane) to give 34: mp 104-105 °C; UV max 310, 246 nm ( $\epsilon$  11400, 15200); MS m/z 309 (100, M<sup>+</sup>), 210 (28, BHCHO), 181 (80, BH). Anal. (C<sub>12</sub>H<sub>16</sub>N<sub>6</sub>O<sub>3</sub>S) C, H, N.

2-Amino-9-(2-deoxy-2-methylene- $\beta$ -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<sub>2</sub>O (10 mL) was added to the filtrate. This mixture was concentrated and applied to a column of Dowex 1 × 2 (OH<sup>-</sup>). Elution with H<sub>2</sub>O (80 mL) and MeOH/H<sub>2</sub>O (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 ( $\epsilon$  12 200, 16 200); MS m/z 309 (82, M<sup>+</sup>), 181 (100, BH), 127 (30, M – BH<sub>2</sub>). Anal. (C<sub>12</sub>H<sub>15</sub>N<sub>5</sub>O<sub>3</sub>S), C, H, N.

4-Amino-7-(3,5-O-TPDS- $\beta$ -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<sup>24</sup> (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<sup>+</sup>), 463 (45, M - CHMe<sub>2</sub>), 345 (30, M - BCHO), 134 (100, BH<sub>2</sub>).

4-Amino-7-(2-deoxy-3,5-O-TPDS-2-methylene- $\beta$ -Derythro-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<sup>+</sup>), 461 (60, M - CHMe<sub>2</sub>), 371 (30, M - BH), 343 (40, M - BCHO).

4-Amino-7-(2-deoxy-2-methylene- $\beta$ -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<sub>2</sub>O): mp 215-216 °C; UV (H<sub>2</sub>O, pH 7 buffer) max 270 nm ( $\epsilon$  11 600); MS m/z 262 (30, M<sup>+</sup>), 245 (14, M - OH), 231 (36, M - CH<sub>2</sub>OH), 134 (100, BH). Anal. (C<sub>12</sub>H<sub>14</sub>N<sub>4</sub>O<sub>3</sub>·0.75H<sub>2</sub>O) 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<sub>3</sub> (Flow Laboratories). The thymidine kinaseficient Raji/TK<sup>-</sup> cells, the dCyd kinase-deficient Raji/dCK<sup>-</sup> and CEM/dCK<sup>-</sup> cells, the Ado kinase-deficient CEM/AK<sup>-</sup>, and the double mutant CEM cell line deficient in both dCK and AK (CEM/dCK<sup>-</sup>/AK<sup>-</sup>) were grown in essentially the same culture medium. The cytostatic assays were performed as previously described.<sup>25,28</sup> Briefly, 100-µL aliquots of the cell suspensions  $(5 \times 10^5 \text{ L1210} \text{ or FM3A cells/mL}, \text{ or } 7.5 \times 10^5 \text{ Raji}, \text{ Molt/4F}, or MT-4 cells/mL})$  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<sub>2</sub>-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<sub>50</sub>). 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  $\mu$ L.

**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<sub>B</sub>) and type 2 (strain ROD).

Antiviral Activity. Inhibition of virus-induced cytopathogenicity was measured following established procedures.<sup>23,29</sup> The virus inoculum was 100 CCID<sub>50</sub> (1 CCID<sub>50</sub> 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,<sup>30</sup> 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.<sup>31</sup> which involves measuring the hydrolysis of [2,8-3H]AdoHcy to [2,8-3H]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  $\mu$ M [2,8-<sup>3</sup>H]AdoHcy. The reaction was stopped by addition of 100  $\mu$ L of 5 N formic acid and the reaction mixture was applied to a column  $(1 \times 4 \text{ cm})$  of SP Sephadex C-25 equilibrated in 0.1 N formic acid. The [2,8-3H]inosine product of deamination of [2,8-3H]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,  $K_1$  and  $k_2$  were determined by the method previously described.<sup>32</sup> 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  $\mu$ L) of the supernatant obtained by centrifugation of the reaction mixture was injected into an HPLC column (C-18 reverse phase column, Econosphere, Alltech,  $25 \text{ cm} \times 4.6 \text{ mm}$ ) 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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$$\frac{1}{k_{\rm obs}} = \frac{K_{\rm I}}{k_2[{\rm I}]} + \frac{1}{k_2}$$

The data for 2'-deoxy-2'-methyleneadenosine (44) are shown in Figures 3 and 4. For 44,  $K_1$  and  $k_2$  values of 13.1  $\mu$ M and 0.195 min<sup>-1</sup>, 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,<sup>1-6</sup> podophyllo-

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