to a suspension of 0.025 mol (1.2 g) of 55% sodium hydride with the temperature maintained around 0 °C during the addition. The mixture was kept at room temperature for 30 min before a solution of 0.04 mol (7.8 g) of ethyl 2-bromoisobutyrate in 20 mL of anhydrous THF was added and the reaction mixture refluxed for 7 h. After cooling, 80 mL of water was added and the mixture was acidified to pH 1 with 6 N HCl and extracted with 200 mL of ether. The extract was washed with saturated NaCl solution and dried over anhydrous $MgSO_4$. Excess ethyl 2-bromoisobutyrate and ether were evaporated off under reduced pressure. The residue was subjected to column chromatography (ether–hexane, 1:9). A colorless oily liquid (6.5 g) was obtained in a yield of 88.4%.

10: A solution of 0.019 mol (6.5 g) of **10a** in 20 mL of ethanol was added with stirring to 0.08 mol (6.4 mL) of a solution of 50% NaOH, and the mixture was refluxed for 1 h. Ethanol was removed under reduced pressure, and 50 mL of water was added. The mixture was acidified to pH 1 with 6 N HCl and extracted with 100 mL of ether. The extract was washed with water, dried over anhydrous $MgSO_4$, and concentrated. The residual solid was recrystallized from hexane to give 5.5 g (yield, 93.2%) of colorless crystals, mp 91–92 °C.

Acknowledgment. We thank S. Koyama, K. Iwase, and S. Ayabe for their technical assistance.

Registry No. **1**, 113795-02-1; **1a**, 92-69-3; **2**, 113795-03-2; **2a**, 60859-24-7; **3**, 113795-04-3; **3a**, 6335-83-7; **4**, 113795-05-4; **4a**, 34591-21-4; **5**, 113795-06-5; **5a**, 1821-12-1; **5b**, 36940-99-5; **6**, 113795-07-6; **6a**, 57344-26-0; **7**, 113795-08-7; **7a**, 5581-75-9; **8**, 113795-09-8; **8a**, 25827-79-6; **8b**, 25827-80-9; **8c**, 113795-10-1; **9**, 113795-11-2; **9a**, 41859-54-5; **10**, 113795-12-3; **10a**, 113795-13-4; **11**, 113795-14-5; **12**, 113795-15-6; **13**, 113795-16-7; **14**, 113795-17-8; **15**, 113795-18-9; **16**, 113795-19-0; **17**, 113795-20-3; **18**, 113795-21-4; **18a**, 1453-06-1; **19**, 113795-22-5; **19a**, 13621-26-6; **20**, 113795-23-6; **20a**, 100-55-0; **21**, 113795-24-7; **21a**, 61892-95-3; **22**, 113795-25-8; **22a**, 59-67-6; **23**, 113795-26-9; **23a**, 5521-55-1; **24**, 113795-27-0; **24a**, 51037-30-0; **I** (R_1 = H), 108-95-2; **II** (X = Br, n = 3), 109-64-8; **II** (X = Br, n = 4), 110-52-1; **II** (X = Br, n = 5), 111-24-0; **II** (X = Br, n = 6), 629-03-8; **II** (X = Br, n = 7), 4549-31-9; **II** (X = Br, n = 8), 4549-32-0; **II** (X = Br, n = 9), 4549-33-1; **II** (X = Br, n = 10), 4101-68-2; **III** (R_1 = Ph, n = 3, X = Br), 113795-28-1; **III** (R_1 = $Ph(CH_2)_2$, n = 3, X = Br), 108357-59-1; **III** (R_1 = $Ph(CH_2)_3$, n = 3, X = Br), 113795-29-2; **III** (R_1 = $Ph(CH_2)_4$, n = 3, X = Br), 113795-30-5; **III** (R_1 = $Ph(CH_2)_5$, n = 3, X = Br), 113795-31-6; **III** (R_1 = $Ph(CH_2)_6$, n = 3, X = Br), 113795-32-7; **III** (R_1 = $PhCONH(CH_2)_2$, n = 3, X = Br), 113795-33-8; **III** (R_1 = $Ph(CH_2)_4$, n = 4, X = Br), 113795-34-9; **III** (R_1 = $Ph(CH_2)_4$, n = 5, X = Br), 113795-35-0; **III** (R_1 = $Ph(CH_2)_4$, n = 6, X = Br), 113795-36-1; **III** (R_1 = $Ph(CH_2)_4$, n = 7, X = Br), 113795-37-2; **III** (R_1 = $Ph(CH_2)_4$, n = 8, X = Br), 113795-38-3; **III** (R_1 = $Ph(CH_2)_4$, n = 9, X = Br), 113810-77-8; **III** (R_1 = $Ph(CH_2)_4$, n = 10, X = Br), 113795-39-4; **III** (R_1 = H, n =3, X = Br), 588-63-6; **VI** (n = 5), 113795-40-7; **VI** (n = 3), 101594-58-5; **VII** (n = 6), 113795-41-8; **VII** (n = 4), 38841-95-1; p-$Br(CH_2)_4OC_6H_4(CH_2)_4$-2,4-$(Me)_2C_6H_3$, 113795-42-9; p-$Br(CH_2)_4OC_6H_4(CH_2)_4$-2,5-$(Me)_2C_6H_3$, 113795-43-0; p-$HOC_6H_4(CH_2)_4$-2,4-$(Me)_2C_6H_3$, 113795-44-1; p-$HOC_6H_4(CH_2)_4$-2,5-$(Me)_2C_6H_3$, 113795-45-2; p-$HOC_6H_4(CH_2)_6Ph$, 113795-46-3; $Ph(CH_2)_5COCl$, 21389-46-8; $ClCO(CH_2)_3$-2,4-$(Me)_2C_6H_3$, 113795-47-4; $ClCO(CH_2)_3$-2,5-$(Me)_2C_6H_3$, 113795-48-5; $Ph(CH_2)_3COCl$, 18496-54-3; p-$MeOC_6H_4CO(CH_2)_3$-2,4-$(Me)_2C_6H_3$, 113795-49-6; p-$MeOC_6H_4CO(CH_2)_3$-2,5-$(Me)_2C_6H_3$, 113795-50-9; p-$MeOC_6H_4(CH_2)_4$-2,4-$(Me)_2C_6H_3$, 113795-51-0; p-$MeOC_6H_4(CH_2)_4$-2,5-$(Me)_2C_6H_3$, 113795-52-1; sodium isobutyrate, 996-30-5; 2,2-dimethyl-6-[4-(4-phenybutyl)phenoxy]hexanol, 113795-53-2; ethyl 2-bromoisobutyrate, 600-00-0.

Chemical Synthesis and Biological Activities of 5-Deazaaminopterin Analogues Bearing Substituent(s) at the 5- and/or 7-Position(s)[1]

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Condensation of cyanothioacetamide (**4**) with ethyl α-(ethoxymethylene)acetoacetate (**5b**), ethyl 4-ethoxy-2-(ethoxymethylene)-3-oxobutanoate (**5c**), ethyl 2-(ethoxymethylene)-3-oxo-4-phenylpropanoate (**5d**) afforded exclusively the corresponding 6-substituted pyridines (**6b–d**). Cyclization of **4** with 3-carbethoxybutane-2,4-dione (**5e**) gave 3-cyano-5-(ethoxycarbonyl)-4,6-dimethylpyridine-2(1*H*)-thione (**6e**), whereas reaction of **4** with 3-carbethoxy-1-phenylpropane-1,3-dione (**5f**) yielded two products, 3-cyano-5-(ethoxycarbonyl)-4-methyl-6-phenylpyridine-2(1*H*)-thione (**6f**) and the 6-methyl-4-phenyl isomer **6g**. The structural assignments for **6f** and **6g** are made on the basis of ^1H and ^{13}C NMR spectral analyses of the 2-(methylthio)nicotinates (**7f,g**) prepared from **6f** and **6g** by treatment with MeI/K_2CO_3. Nicotinates **7b,d–g** were converted into their corresponding 2,4-diaminopyrido[2,3-*d*]pyrimidines **12b,d–g** in five steps, via reduction, protection, oxidation, condensation with guanidine, and deprotection. The 7-mono- and 5,7-disubstituted-5-deazaaminopterins (**1b,d–g**) were prepared from the respective pyrido[2,3-*d*]pyrimidines **12b,d–g**. Preliminary biological studies showed that 7-methyl and 5,7-dimethyl analogues (**1b** and **1e**) were less active than methotrexate against human leukemic HL-60 and murine L-1210 cells in tissue culture. Compound **1e** produced an ILS of 71% at 100 mg/kg per day × 5 (ip) in BDF mice inoculated ip with 10^6 L-1210 cells.

Certain deaza analogues of methotrexate (MTX) and aminopterin (AP) have been reported to exhibit potent antitumor activity. Quinazoline[2–6] and pyrido[2,3-*d*]pyrimidine (5-deazapteridine),[7–10] for example, are found to be effective inhibitors of both dihydrofolate reductase

(1) This investigation was supported in part by funds from the National Cancer Institute, National Institutes of Health, U.S. Department of Health and Human Services (Grant No. CA-08748 and CA-18856).

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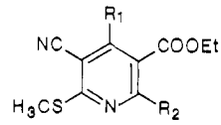
0022-2623/88/1831-1209$01.50/0

Table I. ^{1}H NMR Parameters for Pyridines (in $CDCl_3$)

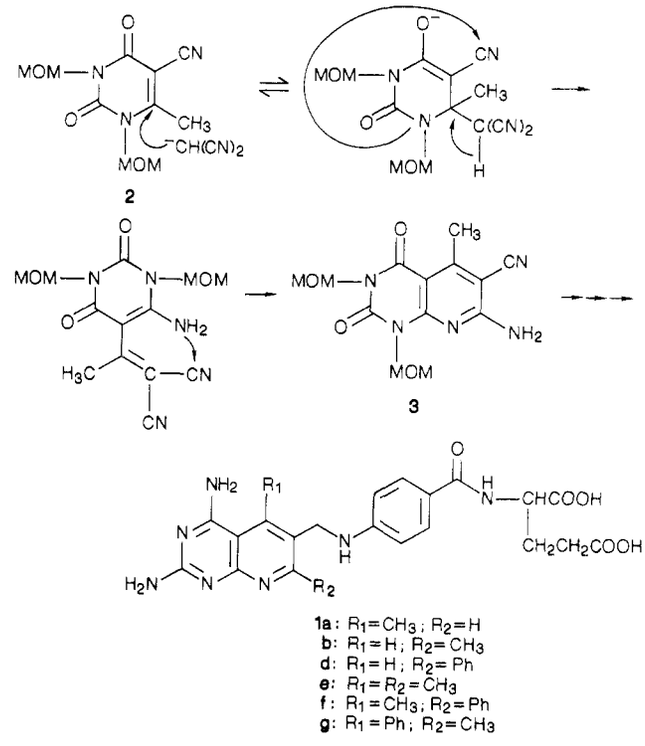


compd	R_1	R_2	H-4	Ar H	CH_3	SCH_3	OCH_2	CH_2CH_3	other
7b	H	CH_3	8.31 s		2.67 (s)	2.88 (s)	4.44 (q)	1.40 (t)	
7c	H	$CH_2OC_2H_5$	8.26 s			2.70 (s)	4.37 (q)	1.40 (t)	4.96 (s, CH_2O), 3.63 (q, OCH_2), 1.24 (t, CH_2CH_3)
7d	H	C_6H_5	8.22 s	7.36–7.64 (m)		2.68 (s)	4.18 (q)	1.10 (t)	
7e	CH_3	CH_3			2.62 (s, C-6), 2.56 (s, C-4)	2.47 (s)	4.42 (q)	1.40 (t)	
7f	CH_3	C_6H_5		7.40–7.70 (m)	2.57 (s)	2.66 (s)	4.16 (q)	1.02 (t)	
7g	C_6H_5	CH_3		7.29–7.50 (m)	2.64 (s)	2.66 (s)	3.99 (q)	0.89 (t)	

(DHFR) and thymidylate synthase (TS), thereby exerting strong inhibitory activity against various tumors both in vitro and in vivo. We have recently reported[11] the synthesis of 5-methyl-5-deazaaminopterin (**1a**) from 1,3-bis(methoxymethyl)-5-cyano-6-methyluracil (**2**) by exploitation of our pyrimidine to pyridopyrimidine ring transformation reaction.[12] In the same report, we also described an alternative synthesis of this compound in 10 steps from cyanothioacetamide (**4**) and ethyl β-(ethoxymethylene)-acetoacetate (**5**) via 2,4-diamino-6-(hydroxymethyl)-5-methylpyrido[2,3-*d*]pyrimidine (**6**). The products obtained by the two routes appeared at first to be identical with respect to UV, MS, and elemental analyses. These samples, however, did not give consistent results in our antitumor assays. The product of ring transformation was approximately 100 times more potent than the product of cyanothioacetamide route in the L-1210 cell growth test. The ^{1}H NMR spectrum of a mixture of these two samples showed pairs of peaks in both the δ 8.40 (the hydrogen in the pyridine ring) and 2.56 (the methyl group on the pyridine) region.

The ring transformation reaction of 1,3-dialkyl-5-cyano-6-methyluracil into the pyrido[2,3-*d*]pyrimidine system proceeds by Michael addition of the active methylene of malononitrile to C_6 of the pyrimidine, followed by a double cyclization involving an $S_NANRORC$ mechanism[13] (Scheme I) leading to the formation of the 5-methyl derivative **3** as the only possible product. The 5-deazaaminopterin analogue derived from **3** must, therefore, bear the methyl substituent at the C_5 position, i.e., **1a**. On the other hand, condensation of **4** and **5b** may result in the formation of two possible products, 4-methyl-3-cyano-5-(ethoxycarbonyl)pyridine-2(1*H*)-thione (**6a**) and the isomeric 6-methyl congener **6b** (Scheme II). Apparently, the expected 4-methylpyridine **6a** was not obtained, but the exclusive formation of the isomeric **6b** did occur. Compound **6b** was then converted into 7-methyl-5-deazaaminopterin (**1b**) (not the 5-methyl isomer **1a** as reported previously[11]).

Scheme I



1a: $R_1=CH_3$; $R_2=H$
b: $R_1=H$; $R_2=CH_3$
d: $R_1=H$; $R_2=Ph$
e: $R_1=R_2=CH_3$
f: $R_1=CH_3$; $R_2=Ph$
g: $R_1=Ph$; $R_2=CH_3$

The chemistry that led to the unexpected formation of **6b** by condensation of **4** and **5b**, and the interesting biological activity exhibited by the 7-methyl analogue **1b** of aminopterin, prompted us to investigate the effect of substituents in **5** on the direction of cyclization and also the structure of the cyclization product. This report also describes the synthesis of several 5- and 7-substituted 5-deazaaminopterins **1** (from the cyclization product **6**) and their biological activities.

Condensation of cyanothioacetamide (**4**) with α-(ethoxymethylene)-β-acylacetate (**5b–d**) gave exclusively the 6-substituted pyridines **6b–d**. No 4-substituted isomer such as **6a** was detected in the reaction mixture. Cyclization of **4** with ethyl diacetoacetate (**5e**) afforded 3-cyano-5-(ethoxycarbonyl)-4,6-dimethylpyridine-2(1*H*)-thione (**6e**), whereas reaction of **4** with 3-carbethoxy-1-phenylpropane-1,3-dione (**5f**) yielded two products: 3-cyano-5-(ethoxycarbonyl)-4-methyl-6-phenylpyridine-2-(1*H*)-thione (**6f**) and 3-cyano-5-(ethoxycarbonyl)-6-methyl-4-phenylpyridine-2(1*H*)-thione (**6g**). The assignments of these isomers, **6f** and **6g**, are based on NMR analyses of the S-methylated pyridines **7** (Tables I and II), which were prepared by treatment of **6** with methyl iodide and potassium carbonate in DMF. The proton resonances for the two CMe in **7e** appear at δ 2.56 and 2.62 (Table I), while the chemical shift of the C_6-Me signal in **7b** is δ 2.67.

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Scheme II

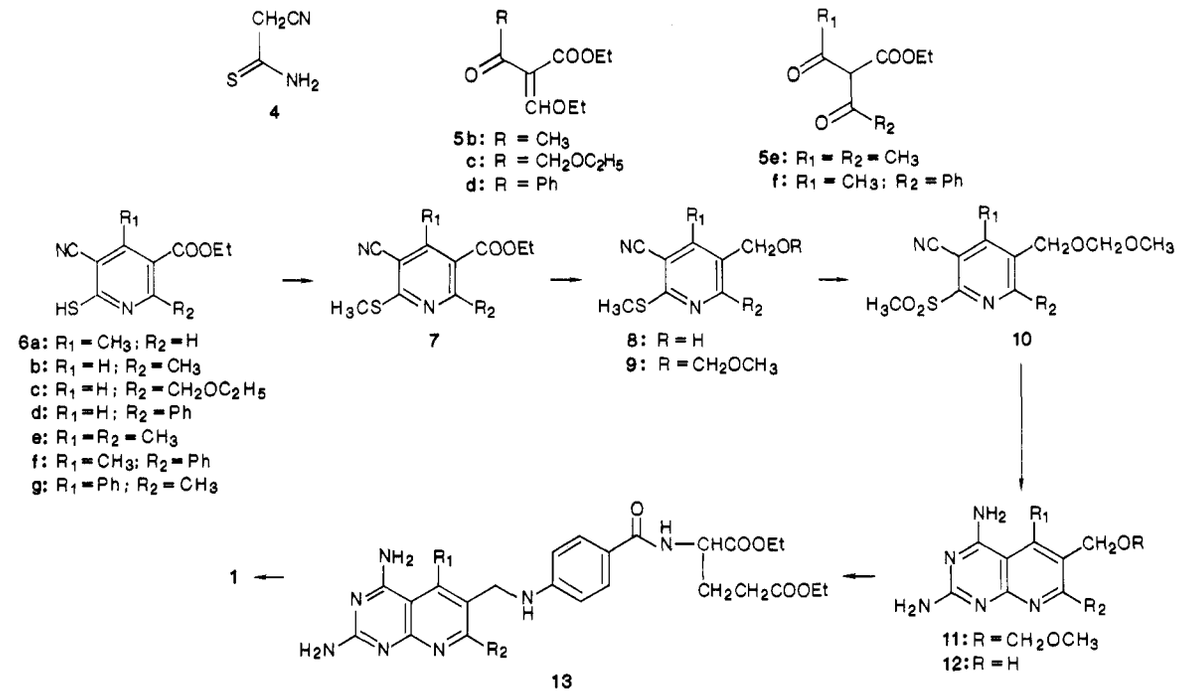


Table II. ^{13}C NMR Data for 2-(Methylthio)pyridines (in $CDCl_3$)

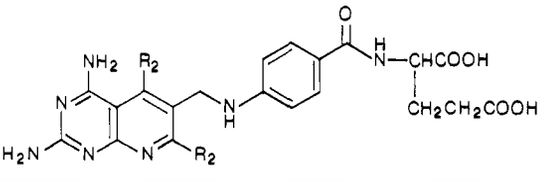
compd	R_1	R_2	C_2	C_3	C_4	C_5	C_6	C=O	CN	CH_3	CH_2CH_3	CH_2CH_3	SCH_3	other
7b	H	CH_3	164.64	104.40	142.59	120.38	163.72	165.62	115.02	25.52	61.60	14.26	13.25	
7c	H	$CH_2OC_2H_5$	164.26	105.37	142.37	120.65	162.20	165.78	114.64		61.71	14.04	13.17	72.06 (CH_2), 66.86 (CH_2), 15.07 (CH_3)
7d	H	C_6H_5	164.97	104.72	142.48	121.78	160.96	166.10	114.85		61.76	13.61	13.28	
7e	CH_3	CH_3	163.45	103.32	149.09	124.82	158.25	166.86	114.67	18.26, 23.52	61.71	13.98	13.01	
7f	CH_3	C_6H_5	163.94	124.55	150.28	127.64	158.09	167.40	114.64	18.32	61.87	13.51	13.23	
7g	C_6H_5	CH_3	164.04	124.61	151.85	127.42	158.63	166.64	114.53	23.35	61.44	13.28	13.28	

The protons of C_6-Me apparently have a higher ppm than those of C_4-Me. The ^{13}C NMR of **7e** (Table II) exhibits two CMe carbon signals at 18.26 and 23.52 ppm, whereas the chemical shift for C_6-Me in **7b** is 25.52 ppm. The proton and ^{13}C resonance signals for the CMe group in **7f** appear at δ 2.57 and 18.32, respectively, whereas the corresponding signals of **7g** are seen at δ 2.64 and 23.35. Compound **7f** is therefore assigned the 4-methyl-6-phenylpyridine structure.

Conversion of **7** into the corresponding 5-deazapteridine analogues **1** were achieved by following the procedure we reported previously.[11] Reduction of **7** with lithium aluminum hydride (LAH) afforded the 5-(hydroxymethyl)-2-(methylthio)pyridines **8**, which, after protection of the hydroxy function by methoxymethylation to **9**, were oxidized to the sulfones **10** with *m*-chloroperbenzoic acid (*m*-CPBA). Condensation of **10** with guanidine afforded the corresponding 2,4-diaminopyrido[2,3-*d*]pyrimidines **11**. Deprotection of **11** to the free hydroxymethyl derivatives **12** followed by bromination of **12** with HBr in dioxane[14] gave the crude 6-(bromomethyl)-5-deazapteridines, which were directly coupled with diethyl (*p*-aminobenzoyl)-L-glutamate. Subsequent saponification of the product **13** afforded **1** in high yield.

The ID_{50} values for these analogues for inhibition of cell growth in vitro are listed in Table III. The 5-methyl-5-deaza analogue **1a** is most potent while any derivative containing a phenyl substituent on the 5-deazapteridine ring showed little activity. As expected,[15] moving the methyl group from position 5 to 7 is extremely detrimental

Table III. Inhibition of Growth and DHFR of L-1210 by the 5- and/or 7-Substituted 5-Deazaaminopterin Analogues



compd	R_1	R_2	IC_{50},[a] μM	K_i,[b] μM
	H	H	0.020	
1a	Me	H	0.00011	$(5.23 \pm 0.7) \times 10^{-12}$
1b	H	Me	0.080	$>4 \times 10^{-6}$
1d	H	Ph	16.94	$>4 \times 10^{-6}$
1e	Me	Me	0.125	2.01×10^{-6}
1f	Me	Ph	18.88	$>4 \times 10^{-6}$
1g	Ph	Me	18.88	$>4 \times 10^{-6}$
MTX			0.0045	$(5.62 \pm 0.8) \times 10^{-12}$

[a] Methods used are described in ref 20. [b] Methods used are described in ref 21.

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Table IV. Inhibition of HL-60 Cell Growth and [6-^{3}H]dUrd Incorporation into DNA

	1a	1b	1e	MTX
Median-Effect Concentration for Cell-Growth Inhibition[a] (ED_{50}, μM)				
24 h	2290	144	42	609
48 h	0.15	2.0	8.2	<0.05
72 h	<0.001	0.011	0.59	≪0.05
Cell-Growth Inhibition at 1 μM[a] (% inhibition)				
24 h	25.2	4.7	10.3	34.2
48 h	61.1	32.5	19.8	64.0
72 h	92.2	77.6	50.8	87.7
Inhibition of [6-^{3}H]dUrd Incorporation into DNA[a] (ED_{50}, μM)				
	≪4	123	568	0.10

[a] See the Experimental Section.

to activity against L-1210 cells. It is interesting to note that the 7-methyl and 5,7-dimethyl analogues (**1b** and **1e**) exhibited cell-growth inhibition, though they are extremely weak inhibitors of DHFR from L-1210 cells (about 1 000 000 times less active than MTX or **1a**, Table III), and are 1200-fold less potent than MTX in inhibiting [6-^{3}H]-dUrd incorporation into DNA (Table IV). Table IV shows time-dependent cytotoxicity in inhibiting HL-60 leukemic cell growth by **1a**, **1b**, **1e**, and MTX. However, increase in exposure time to compounds from 24 to 72 h markedly increased cytotoxicity. Exposure to the compound (1 μM) for 24–72 h yielded a similar degree of growth inhibition of HL-60 cells for **1a** (25–92% inhibition) and MTX (34–88% inhibition) whereas **1b** and **1e** exhibited a little weaker activity (Table IV). These compounds showed great differences in potency in inhibiting [6-^{3}H]dUrd incorporation into DNA in HL-60 cells (Table IV). The ED_{50} for MTX, **1a**, **1b**, and **1e** are 0.1 ≪4, 123, and 568 μM, respectively. Compounds **1c**, **1f**, and **1g** have much less biological activity in all studies (data not shown). Preliminary study showed that compound **1e** produced an increase in lifespan at maximum tolerated dose of 100 mg/kg per day × 5 (ip) of 71% in BDF mice inoculated ip with 10^6 L-1210 cells.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary apparatus and are uncorrected. Column chromatography was performed on silica gel G60 (70–230 mesh, ASTM, Merck). Thin-layer chromatography was performed on Analtech Uniplates with short-wavelength UV light for visualization. Elementary analyses were performed by M. H. W. Laboratories, Phoenix, AZ, or Spang Microanalytical Laboratory, Eagle Harbor, MI. ^{1}H NMR and ^{13}C NMR data were recorded on a JEOL-FX90Q spectrometer with Me_4Si as the internal standard. Chemical shifts were reported in ppm (δ), and the signals are described as s (singlet), d (doublet), t (triplet), q (quartet), m (miltiplet), dd (double doublet), dt (double triplet), br s (broad singlet). Values reported for coupling constants are first order. UV spectra were recorded on a Gilford Response UV–vis spectrophotometer.

Ethyl 4-Ethoxy-2-(ethoxymethylene)-3-oxobutanoate (5c). A mixture of ethyl 4-ethoxyacetoacetate[15] (17.4 g, 0.1 mol), triethyl orthoformate (14.8 g, 0.1 mol), and Ac_2O (20.4 g, 0.2 mol) was heated under reflux in an oil bath for 40 min. The bath temperature was raised to 190 °C, and the reaction mixture was concentrated at ambient pressure. The residue was distilled in vacuo. The fraction with bp_{13} 170–173 °C was collected and crystallized from n-C_6H_{14}/Et_2O to afford 11.3 g (49%) of **5c**: mp 42–45 °C; ^{1}H NMR ($CDCl_3$) δ 1.36 (3 H, t, CH_2*Me*), 1.39 (3 H, t, CH_2*Me*), 3.80 (2 H, q, C*H*$_2$Me), 4.34 (2 H, q, C*H*$_2$Me), 4.95 (2 H, s, CH_2), 8.22 (1 H, s, H-4). Anal. ($C_{11}H_{18}O_5$) C, H.

3-Cyano-5-(ethoxycarbonyl)-6-methylpyridine-2(1*H*)-thione (6b). A mixture of **4** (10.02 g, 0.1 mol), **5b** (22.3 g, 0.12 mol), and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (4 mL) in anhydrous EtOH (200 mL) was heated at 60 °C for 1 h, then cooled in an ice bath. The solid precipitates, collected by filtration, were extracted with boiling $CHCl_3$ (6 × 200 mL). The $CHCl_3$ extracts were concentrated in vacuo, and the residue was crystallized from $CHCl_3$/EtOH to give 9.10 g of **6b** (41%): mp 232–233 °C; IR (KBr) 2230 cm^{-1} (CN); ^{1}H NMR ($CDCl_3$) δ 1.41 (3 H, t, CH_2*Me*), 2.68 (3 H, s, 6-Me), 4.38 (2 H, q, C*H*$_2$Me), 8.30 (1 H, s, H-4). Anal. ($C_{10}H_{10}N_2O_2S$) C, H, N, S.

By the same procedure but with **5c** or **5d**, the corresponding products, **3-cyano-5-(ethoxycarbonyl)-6-(ethoxymethyl)-pyridine-2(1*H*)-thione (6c)** and **3-cyano-5-(ethoxycarbonyl)-6-phenylpyridine-2(1*H*)-thione (6d)**, were obtained. **6c**: 12.0 g (45%); mp 128–129 °C: IR (KBr) 2230 cm^{-1} (CN); ^{1}H NMR ($CDCl_3$) δ 1.37 (3 H, t, CH_2*Me*), 1.39 (3 H, t, CH_2*Me*), 3.80 (2 H, q, C*H*$_2$Me), 4.34 (2 H, q, C*H*$_2$Me), 4.95 (2 H, s, CH_2O), 8.22 (1 H, s, H-4). Anal. ($C_{12}H_{14}N_2O_3S$) C, H, N, S. **6d**: 15.7 g (58%); mp 218–219 °C; IR (KBr) 2225 cm^{-1} (CN); ^{1}H NMR ($CDCl_3$) δ 1.08 (3 H, t, CH_2*Me*), 4.14 (2 H, q, C*H*$_2$Me), 7.34–7.72 (5 H, m, Ph), 8.31 (1 H, s, H-4). Anal. ($C_{15}H_{12}N_2O_2S$) C, H, N, S.

3-Cyano-4,6-dimethyl-5-(ethoxycarbonyl)pyridine-2-(1*H*)-thione (6e). A mixture of **4** (20 g, 0.2 mol), **5e** (37.9 g, 0.22 mol), and DBU (5 mL) in anhydrous EtOH (300 mL) was stirred at room temperature for 2 days. The precipitate was collected by filtration and recrystallized from EtOH to afford **6e**: 17.5 g (78%); mp 214–215 °C; IR (KBr) 2220 cm^{-1} (CN); ^{1}H NMR ($CDCl_3$) δ 1.39 (3 H, t, CH_2*Me*), 2.56 (3 H, s, 6-Me), 2.54 (3 H, s, 4-Me), 4.41 (2 H, q, C*H*$_2$Me). Anal. ($C_{10}H_{12}N_2O_2S$) C, H, N, S.

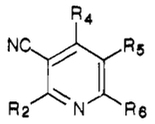
3-Cyano-5-(ethoxycarbonyl)-4-methyl-6-phenylpyridine-2(1*H*)-thione (6f) and 3-Cyano-5-(ethoxymethyl)-6-methyl-4-phenylpyridine-2(1*H*)-thione (6g). A mixture of **4** (54 g, 0.54 mol), **5f** (189 g, 0.81 mol), and piperidine (37 mL) in anhydrous EtOH (600 mL) was stirred at room temperature for 1 day and then heated under reflux for another day. The mixture was concentrated in vacuo, and the residue was dissolved in $CHCl_3$, washed with water, dried (Na_2SO_4), concentrated, and chromatographed on a silica gel column (10 × 50 cm) with $CHCl_3$/n-C_6H_{14} (4:1 v/v) as the eluent. Compound **6f** was eluted first from the column followed by **6g**. After crystallization from EtOH, **6f** (26.5 g, 16.4%) had mp 147–148 °C: IR (KBr) 2230 cm^{-1} (CN); ^{1}H NMR ($CDCl_3$) δ 0.91 (3 H, t, CH_2*Me*), 2.54 (3 H, s, 4-Me), 4.03 (2 H, q, C*H*$_2$Me), 7.40–7.62 (5 H, m, Ph). Anal. ($C_{16}H_{14}N_2O_2S$) C, H, N, S. **6g** (6.8 g, 4.2%): mp 252–253 °C; IR (KBr) 2230 cm^{-1} (CN); ^{1}H NMR ($CDCl_3$) δ 0.81 (3 H, t, CH_2*Me*), 2.61 (3 H, s, 6-Me), 3.92 (2 H, q, C*H*$_2$Me), 7.36–7.53 (5 H, m, Ph). Anal. ($C_{16}H_{14}N_2O_2S$) C, H, N, S.

Synthesis of 2-(Methylthio)pyridines (7b–g). A mixture of **6** (1 equiv), MeI (2 equiv), and K_2CO_3 (2 equiv) in DMF was stirred at room temperature for 3–4 h, and the mixture was diluted with cold water. The precipitated solid was collected, washed (water), air-dried, and crystallized from $CHCl_3$/EtOH to afford pure ethyl nicotinates **7**. The ^{1}H NMR and ^{13}C NMR spectral data for **7b–g** are listed in Tables I and II, respectively. The yield and melting points of these 2-(methylthio)nicotinates are reported in Table V.

Reduction of Ethyl Nicotinates 7 to 3-Cyano-5-(hydroxymethyl)-2-(methylthio)pyridines (8). To a stirred suspension of **7** (0.2 mmol) in dry Et_2O (1 L) was added portionwise LAH (9.1 g, 0.24 mmol) at −15 to −10 °C. The mixture was stirred at −10 °C for 3 h, and then excess LAH was destroyed with 1 N HCl to ca. pH 3. Cold water (500 mL) was added to the mixture, and the ethereal layer was separated. The aqueous layer was extracted with EtOAc (3 × 300 mL). The combined organic extracts were washed (H_2O), dried (Na_2SO_4), and concentrated, and the residue was chromatographed on a silica gel column (8 × 50 cm) with $CHCl_3$ as the eluent, which eluted unreacted **7**. The column was then washed with $CHCl_3$/MeOH (50:1, v/v) to elute **8**, which was obtained as colorless crystals after concentration of the solvent and recrystallization of the residue from EtOH. Yields and melting points of **8b–g** are listed in Table V. Anal. C, H, N, S.

3-Cyano-5-[(methoxymethoxy)methyl]-7-methyl-2-(methylthio)pyridine (9b). A solution of **8b** (44.0 g, 0.27 mol) and *N*,*N*-dimethylaniline (80.5 g, 0.54 mol) in dry $CHCl_3$ (500 mL) was treated with $MeOCH_2Cl$ (43.2 g, 0.54 mol) for 5 h at room temperature. The mixture was washed successively with 2% HCl (4 × 200 mL), water, and saturated $NaHCO_3$, dried (Na_2SO_4), and concentrated. The residue was crystallized from n-C_6H_{14}/Et_2O to afford **9b**.

Table V. Yields and Melting Points of New Pyridine Intermediates



compd	R_2	R_4	R_5	R_6	mp, °C	yield, %	formula
7b	SCH_3	H	COOEt	CH_3	134–135	98	$C_{11}H_{12}N_2O_2S$
7c	SCH_3	H	COOEt	CH_2OEt	73–75	98	$C_{13}H_{16}N_2O_3S$
7d	SCH_3	H	COOEt	C_6H_5	85–86	84	$C_{16}H_{14}N_2O_2S$
7e	SCH_3	CH_3	COOEt	CH_3	61–62	89	$C_{11}H_{14}N_2O_2S$
7f	SCH_3	CH_3	COOEt	C_6H_5	72–73	90	$C_{17}H_{16}N_2O_2S$
7g	SCH_3	C_6H_5	COOEt	CH_3	85–86	92	$C_{17}H_{16}N_2O_2S$
8b	SCH_3	H	CH_2OH	CH_3	117–118	50	$C_9H_{10}N_2OS$
8c	SCH_3	H	CH_2OH	CH_2OEt	67–69	45	$C_{11}H_{14}N_2O_2S$
8d	SCH_3	H	CH_2OH	C_6H_5	147–148	40	$C_{14}H_{12}N_2OS$
8e	SCH_3	CH_3	CH_2OH	CH_3	121–128	56	$C_{10}H_{12}N_2OS$
8f	SCH_3	CH_3	CH_2OH	C_6H_5	145–146	47	$C_{15}H_{14}N_2OS$
8g	SCH_3	C_6H_5	CH_2OH	CH_3	123–124	58	$C_{15}H_{14}N_2OS$
9b	SCH_3	H	$CH_2OCH_2OCH_3$	CH_3	50–51	84	$C_{11}H_{14}N_2O_2S \cdot {}^1/_4H_2O$
9c	SCH_3	H	$CH_2OCH_2OCH_3$	CH_2OEt	syrup	94	$C_{13}H_{18}N_2O_3S$
9d	SCH_3	H	$CH_2OCH_2OCH_3$	C_6H_5	107–108	83	$C_{16}H_{16}N_2O_2S$
9e	SCH_3	CH_3	$CH_2OCH_2OCH_3$	CH_3	63–64	88	$C_{12}H_{16}N_2O_2S$
9f	SCH_3	CH_3	$CH_2OCH_2OCH_3$	C_6H_5	119–120	82	$C_{17}H_{18}N_2O_2S$
9g	SCH_3	C_6H_5	$CH_2OCH_2OCH_3$	CH_3	96–97	81	$C_{17}H_{18}N_2O_2S$
10b	SO_2CH_3	H	$CH_2OCH_2OCH_3$	CH_3	64–65	75	$C_{11}H_{14}N_2O_4S$
10c	SO_2CH_3	H	$CH_2OCH_2OCH_3$	CH_2OEt	syrup	84	$C_{13}H_{18}N_2O_5S$
10d	SO_2CH_3	H	$CH_2OCH_2OCH_3$	C_6H_5	101–102	92	$C_{16}H_{16}N_2O_4S$
10e	SO_2CH_3	CH_3	$CH_2OCH_2OCH_3$	CH_3	58–59	89	$C_{12}H_{16}N_2O_4S$
10f	SO_2CH_3	CH_3	$CH_2OCH_2OCH_3$	C_6H_5	155–156	94	$C_{17}H_{18}N_2O_4S$
10g	SO_2CH_3	C_6H_5	$CH_2OCH_2OCH_3$	CH_3	syrup	96	$C_{17}H_{18}N_2O_4S$

Similarly, **8c–g** were also methoxymethyalted to **9c–g**. Compounds **9d–g** were directly crystallized, whereas **9c** was purified by chromatography on a silica gel column with *n*-C_6H_{14}/EtOAc (9:1) as the eluent. Yields and melting points for **9b–g** are given in Table V. Anal. C, H, N, S.

Oxidation of (Methylthio)pyridines 9 to the Corresponding Methyl Sulfones 10. A mixture of **9** (0.23 mol) and *m*-chloroperbenzoic acid (*m*-CPBA) (0.69 mol) in EtOH (600 mL) was stirred for 1 h at room temperature, and then the solvent was removed in vacuo. The residue was redissolved in EtOAc (800 mL), and the solution was washed (2% NaOH and water), dried (Na_2SO_4), and concentrated. Compounds **10b** and **10d–f** were purified directly from the residue by crystallization from ether. Sulfones **10c** and **10g** were purified by chromatography on a silica gel column with $CHCl_3$ as the eluent. Yields and melting points of **10b–g** are listed in Table V. Anal. C, H, N, S.

2,4-Diamino-6-[(methoxymethoxy)methyl]-7-mono- and -5,7-disubstituted-pyrido[2,3-*d*]pyrimidines (11b–g). A mixture of **10** (20 mmol) and guanidine carbonate (3.60 g, 20 mmol) in Ph_2O (20 mL) was heated at 180–185 °C with vigorous stirring for 2 h. After cooling, the mixture was diluted with EtOH/Et_2O (1:1, 200 mL). The precipitates were collected, redissolved in EtOH/H_2O (5:1, 300 mL), and decolorized (Norit A), and the solution was concentrated to ca. 150 mL. Colorless crystals deposited were collected, washed with EtOH and Et_2O, and dried to give **11**. Yields and melting points for **11b–g** are reported in Table VI. Anal. C, H. N.

2,4-Diamino-6-(hydroxymethyl)-7-mono- and -5,7-disubstituted-pyrido[2,3-*d*]pyrimidines (12b–g). A mixture of **11** (70 mmol) and concentrated HCl (20 mL) in MeOH (800 mL) was heated under reflux for 4 h and then concentrated in vacuo. The residue was suspended in water (300 mL) and neutralized to pH 7 with 1 N NaOH. The solid was filtered, washed successively with water, EtOH, and Et_2O, and dried in vacuo over P_2O_5 to give **12**, which was sufficiently pure to be used in the next step. Analytical samples were obtained by recrystallization from EtOH. Yields and melting points for **12b–g** are listed in Table VI. Anal. C, H, N.

Diethyl *N*-[*p*-[[(2,4-Diamino-7-methylpyrido[2,3-*d*]pyrimidin-6-yl)methyl]amino]benzoyl]-L-glutamate (13b). A suspension of **12b** (1.05 g, 5 mmol) in dry dioxane (150 mL) was saturated with dry HBr. The mixture was stirred overnight at room temperature, and the solvent was removed in vacuo (<35 °C). Traces of HBr were removed azeotropically by several coevaporations with PhMe, and the residue was dissolved in dry *N*,*N*-dimethylacetamide (30 mL, distilled over CaH_2). To the solution was added diethyl (*p*-aminobenzoyl)-L-glutamate (3.22 g, 10 mmol), and the mixture was stirred for 3 days at room temperature. After concentration of the mixture in vacuo, the residue was triturated thoroughly with warm $CHCl_3$ to remove unreacted diethyl (*p*-aminobenzoyl)-L-glutamate. The gummy residue was then solidified by trituration with ether, and microcrystals were collected and dried in vacuo to give 2.13 g (84%) of **13b**, mp 239–240 °C.

In a similar manner, the following compounds were prepared.

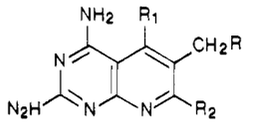
Diethyl *N*-[*p*-[[(2,4-diamino-7-phenylpyrido[2,3-*d*]pyrimidin-6-yl)methyl]amino]benzoyl]-L-glutamate (13d): ^{1}H NMR (Me_2SO-d_6) δ 1.15 (3 H, t, CH_2Me), 1.17 (3 H, t, CH_2Me), 1.82–2.10 (2 H, br m, $CH_2CH_2CO_2Et$), 2.30–2.40 (2 H, br m, $CH_2CH_2CO_2Et$), 4.03 (2 H, q, CH_2Me), 4.08 (2 H, q, CH_2Me), 4.13–4.34 (3 H, br m, CH_2NH and NHC*H*), 6.45–6.58 (3 H, m, 2 H of Ph and CH_2NH), 7.56 (7 H, m, Ph), 7.98–8.17 (3 H, m, NH_2 and CONH), 8.67 (1 H, s, H-5).

Diethyl *N*-[*p*-[[(2,4-diamino-5,7-dimethylpyrido[2,3-*d*]-pyrimidin-6-yl)methyl]amino]benzoyl]-L-glutamate (13e): ^{1}H NMR (Me_2SO-d_6) δ 1.17 (3 H, t, CH_2Me), 1.19 (3 H, t, CH_2Me), 1.78–2.10 (2 H, br m, $CH_2CH_2CO_2Et$), 2.30–2.40 (2 H, br m, $CH_2CH_2CO_2Et$), 2.61 (3 H, s, 7-Me), 2.70 (3 H, s, 5-Me), 4.03 (2 H, q, CH_2Me), 4.08 (2 H, q, CH_2Me), 4.09–4.53 (3 H, br m, CH_2NH and CONHC*H*), 6.16 (1 H, s, CH_2NH), 6.71 (2 H, d, Ph), 7.68 (2 H, d, Ph), exchangeable proton signals at 7.50, 8.07, 8.23, and 8.35.

Diethyl *N*-[*p*-[[(2,4-diamino-5-methyl-7-phenylpyrido-[2,3-*d*]pyrimidin-6-yl)methyl]amino]benzoyl]-L-glutamate (13f): ^{1}H NMR (Me_2SO-d_6) δ 1.16 (3 H, t, CH_2Me), 1.17 (3 H, t, CH_2Me), 1.80–2.10 (2 H, br m, $CH_2CH_2CO_2Et$), 2.30–2.40 (2 H, br m, $CH_2CH_2CO_2Et$), 2.72 (3 H, s, 5-Me), 3.58 (2 H, s, CH_2NH), 4.03 (2 H, q, CH_2Me), 4.08 (2 H, q, CH_2Me), 4.26–4.47 (1 H, m, NHC*H*CO_2Et), 6.3–6.54 (3 H, m, 2 H of Ph and NH), 6.9–7.6 (9 H, m, Ph and NH_2), 8.16 and 8.26 (NH).

Diethyl *N*-[*p*-[[(2,4-diamino-7-methyl-5-phenylpyrido-[2,3-*d*]pyrimidin-6-yl)methyl]amino]benzoyl]-L-glutamate (13g): ^{1}H NMR (Me_2SO-d_6) δ 1.15 (3 H, t, CH_2Me), 1.17 (3 H, t, CH_2Me), 1.85–2.23 (2 H, br m, $CH_2CH_2CO_2Et$), 2.3–2.4 (2 H, br m, $CH_2CH_2CO_2Et$), 2.55 (3 H, s, 7-Me), 3.71 (2 H, br m, CH_2NH), 4.00 (2 H, q, CH_2Me), 4.08 (2 H, q, CH_2Me), 4.17–4.52 (1 H, br m, NHC*H*CO_2Et), 6.46 (2 H, d, Ph), 7.47 (6 H, br m, Ph

Table VI. Yields and Melting Points of 2,4-Diaminopyrido[2,3-*d*]pyrimidine **Derivatives**



compd	R_1	R_2	R	mp, °C	yield, %	formula
11b	H	CH_3	OCH_2OCH_3	273–274	67	$C_{11}H_{15}N_5O_2 \cdot {}^1/_2H_2O$
11c	H	CH_2OEt	OCH_2OCH_3	300	59	$C_{13}H_{19}N_5O_3 \cdot {}^3/_2H_2O$
11d	H	C_6H_5	OCH_2OCH_3	142–143	58	$C_{16}H_{17}N_5O_2 \cdot {}^5/_4H_2O$
11e	CH_3	CH_3	OCH_2OCH_3	214–215	60	$C_{12}H_{17}N_5O_2 \cdot H_2O$
11f	CH_3	C_6H_5	OCH_2OCH_3	290–291	75	$C_{17}H_{19}N_5O_2$
11g	C_6H_5	CH_3	OCH_2OCH_3	224–225	53	$C_{17}H_{19}N_5O_2 \cdot H_2O$
12b	H	CH_3	OH	300	85	$C_9H_{11}N_5O \cdot HCl \cdot {}^1/_2H_2O$
12c	H	CH_2OH	OH	300	74	$C_9H_{11}N_5O_2 \cdot {}^9/_4H_2O$
12d	H	C_6H_5	OH	300	65	$C_{14}H_{13}N_5O \cdot {}^5/_4H_2O$
12e	CH_3	CH_3	OH	300	52	$C_{10}H_{13}N_5O \cdot HCl \cdot {}^5/_4H_2O$
12f	CH_3	C_6H_5	OH	300	62	$C_{15}H_{15}N_5O \cdot HCl \cdot {}^5/_4H_2O$
12g	C_6H_5	CH_3	OH	300	40	$C_{15}H_{15}N_5O \cdot HCl$
13b	H	CH_3	Et_2pABG	239–240	84	$C_{25}H_{31}N_7O_5 \cdot 4H_2O$
13d	H	C_6H_5	Et_2pABG	214–219	70	$C_{30}H_{33}N_7O_5 \cdot {}^{15}/_2H_2O$
13e	CH_3	CH_3	Et_2pABG		31	$C_{26}H_{33}N_7O_5$
13f	CH_3	C_6H_5	Et_2pABG	175–178	53	$C_{31}H_{35}N_7O_5 \cdot 5H_2O$
13g	C_6H_5	CH_3	Et_2pABG		71	$C_{31}H_{35}N_7O_5 \cdot 6H_2O$
1b	H	CH_3	pABGA	235–237	65	$C_{21}H_{23}N_7O_5 \cdot 2H_2O$
1d	H	C_6H_5	pABGA	234–235	48	$C_{26}H_{25}N_7O_5 \cdot {}^3/_2H_2O$
1e	CH_3	CH_3	pABGA	226–227	42	$C_{22}H_{25}N_7O_5$
1f	CH_3	C_6H_5	pABGA	239–240	35	$C_{27}H_{27}N_7O_5 \cdot {}^3/_2H_2O$
1g	C_6H_5	CH_3	pABGA	227–228	30	$C_{27}H_{27}N_7O_5 \cdot {}^5/_4H_2O$

and NH), 7.56 (2 H, d, Ph), exchangeable NH signals at 6.14–6.34 (3 H), 8.15, and 8.25. Anal. C, H, N for diethyl esters **13**.

***N*-[*p*-[[(2,4-Diamino-7-methylpyrido[2,3-*d*]pyrimidin-6-yl)methyl]amino]benzoyl]-L-glutamic Acid (1b).** A solution of **13b** (1.74 g, 3 mmol) in MeOH (400 mL) containing 7 mL of 1 N NaOH was stirred for 3 days at room temperature. After concentration in vacuo to ca. 7 mL, the solution was neutralized with 1 N HCl (7 mL). Compound **1b**, precipitated as pale yellow microcrystals, was collected by filtration, washed with cold water, Me_2CO, and Et_2O, and dried in vacuo over P_2O_5. The melting point and yield are reported in Table VI.

By the same procedure, but with the corresponding diethyl esters **13d–g**, the following 7-substituted 5-deazaaminopterin analogues were prepared.

***N*-[*p*-[[(2,4-Diamino-7-phenylpyrido[2,3-*d*]pyrimidin-6-yl)methyl]amino]benzoyl]-L-glutamic acid (1d)**: ^{1}H NMR (Me_2SO-d_6) δ 1.9–2.20 (2 H, br m, $CH_2CH_2CO_2H$), 2.24–2.32 (2 H, br m, $CH_2CH_2CO_2H$), 4.10–4.59 (3 H, br m, CH_2NH and CONHCH), 6.51 (2 H, d, Ph), 7.43 (5 H, m, Ph), 7.62 (3 H, m, 2 H of Ph and CONH), 8.57 (1 H, s, H-5), and exchangeable NH signals at 6.62 (2 H), 7.92 and 8.01.

***N*-[*p*-[[(2,4-Diamino-5,7-dimethylpyrido[2,3-*d*]pyrimidin-6-yl)methyl]amino]benzoyl]-L-glutamic acid (1e)**: ^{1}H NMR (Me_2SO-d_6) δ 1.78–2.10 (2 H, br m, $CH_2CH_2CO_2H$), 2.3–2.4 (2 H, br m, $CH_2CH_2CO_2H$), 2.54 (3 H, s, 7-Me), 2.66 (3 H, s, 5-Me), 3.72–4.77 (3 H, br m, CH_2NH and CONHCH), 6.70 (2 H, d, Ph), 7.71 (4 H, d, Ph and NH_2), exchangeable NH at 6.20, 7.16, 8.10, and 8.20.

***N*-[*p*-[[(2,4-Diamino-5-methyl-7-phenylpyrido[2,3-*d*]pyrimidin-6-yl)methyl]amino]benzoyl]-L-glutamic acid (1f)**: ^{1}H NMR (Me_2SO-d_6) δ 1.78–2.10 (2 H, br m, $CH_2CH_2CO_2H$), 2.26–2.42 (2 H, br m, $CH_2CH_2CO_2H$), 2.72 (3 H, s, 5-Me), 4.06 (2 H, s, CH_2NH), 4.26 (1 H, m, $NHCHCO_2H$), 6.56 (3 H, m, 2 H of Ph and NH), 7.19–7.67 (9 H, m, Ph and NH_2), 7.99 and 8.10 (NH).

***N*-[*p*-[[(2,4-Diamino-7-methyl-5-phenylpyrido[2,3-*d*]pyrimidin-6-yl)methyl]amino]benzoyl]-L-glutamic acid (1g)**: ^{1}H NMR (Me_2SO-d_6) δ 1.78–2.10 (2 H, br m, $CH_2CH_2CO_2H$), 2.26–2.35 (2 H, br m, $CH_2CH_2CO_2H$), 2.57 (3 H, s, 7-Me), 3.76 (2 H, m, CH_2NH), 4.12–4.47 (1 H, m, NHCH), 6.47 (2 H, d, Ph), 7.49 (5 H, m, Ph), 7.65 (2 H, d, Ph), 6.03, 6.79 (2 H), 8.02 and 8.12 (exchangeable). The melting points for **1b,d–g** are listed in Table VI. Anal. C, H, N for all analogues **1**.

Biological Studies on 5- and/or 7-Substituted 5-Deazaaminopterins (1). HL-60 cells (1.5×10^5/mL) were grown in RPMI 1640 medium containing 10% fetal calf serum, 100 μg/mL streptomycin, and 100 units/mL penicillin, in humidified 5% CO_2 at 37 °C. Five concentrations of each compound were added for up to 72 h exposure. Viable cells were counted with the trypan blue exclusion method.

The incorporation of [6-^{3}H]dUrd (0.05 μM, 1 μCi/mL) into DNA in HL-60 cells at 37 °C for 30 min was measured by the method described previously.[16] Cells were preincubated with the compound for 40 min prior to the addition of [6-^{3}H]dUrd. ED_{50} values were calculated by the median-effect equation and plot[17] with use of microcomputer software.[18] Five concentrations of each compound were used for each ED_{50} determination. For shallow dose–effect curves, ED_{50} values could not be accurately determined and were assigned with < or ≪ marks.

Registry No. **1b**, 113859-37-3; **1d**, 113859-38-4; **1e**, 113859-39-5; **1f**, 113859-40-8; **1g**, 113859-41-9; **4**, 7357-70-2; **5b**, 3788-94-1; **5c**, 113858-89-2; **5d**, 39973-76-7; **5e**, 603-69-0; (±)-**5f**, 113858-94-9; **6b**, 113858-90-5; **6c**, 113858-91-6; **6d**, 113858-92-7; **6e**, 113858-93-8; **6f**, 113858-95-0; **6g**, 97651-26-8; **7b**, 113858-96-1; **7c**, 113858-97-2; **7d**, 113858-98-3; **7e**, 113858-99-4; **7f**, 113859-00-0; **7g**, 113859-01-1; **8b**, 113859-02-2; **8c**, 113859-03-3; **8d**, 113859-04-4; **8e**, 113859-05-5; **8f**, 113859-06-6; **8g**, 113859-07-7; **9b**, 113859-08-8; **9c**, 113859-09-9; **9d**, 113859-10-2; **9e**, 113859-11-3; **9f**, 113859-12-4; **9g**, 113859-13-5; **10b**, 113859-14-6; **10c**, 113859-15-7; **10d**, 113859-16-8; **10e**, 113859-17-9; **10f**, 113859-18-0; **10g**, 113859-19-1; **11b**, 113859-20-4; **11c**, 113859-21-5; **11d**, 113859-22-6; **11e**, 113859-23-7; **11f**, 113859-24-8; **11g**, 113859-25-9; **12b**, 113859-26-0; **12c**, 113859-27-1; **12d**, 113859-28-2; **12e**, 113859-29-3; **12f**, 113859-30-6; **12g**,

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113859-31-7; **13b**, 113859-32-8; **13d**, 113859-33-9; **13e**, 113859-34-0; **13f**, 113859-35-1; **13g**, 113859-36-2; $EtOCH_2COCH_2COOEt$, 41051-14-3; $H_2NC(=NH)NH_2 \cdot Y_2H_2CO_3$, 593-85-1; diethyl (*p*-aminobenzoyl-L-glutamate, 13702-52-8.

Supplementary Material Available: Tables listing UV spectral data for pyrido[2,3-*d*]pyrimidines (**11b–g** and **1b,d–g**) and also ^1H NMR parameters for **11b–g** (2 pages). Ordering information is given on any current masthead page.

Substituted 2-[(2-Benzimidazolylsulfinyl)methyl]anilines as Potential Inhibitors of H^+/K^+ ATPase

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A series of substituted 2-[(2-benzimidazolylsulfinyl)methyl]anilines were synthesized as potential inhibitors of the acid secretory enzyme H^+/K^+ ATPase. Substitutions on the aniline nitrogen atom resulted in potent enzyme inhibition in vitro but weak activity in gastric fistula dogs. Electron-donating substituents on the aniline ring enhanced in vitro and in vivo potency relative to the unsubstituted analogue. The potency showed a correlation to the calculated pK_a of the aniline nitrogen atom. Substitutions on the aniline and benzimidazole rings did not further enhance potency. Di- and trisubstituted aniline derivatives were potent inhibitors of the enzyme system. The preferred combination of substituents were a methoxy group on the benzimidazole ring and a single alkyl group on the aniline ring. One such compound, **76**, was an effective inhibitor of acid secretion in the dog and was selected for further pharmacological study.

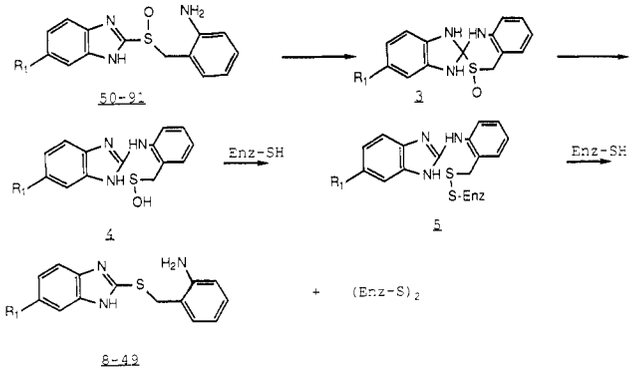
Investigations into the mechanism of gastric acid secretion and the design of new therapeutic agents were greatly stimulated following the discovery of histamine-2 antagonists as therapeutic agents for peptic ulcer disease. The identification of H^+/K^+ ATPase as the proton pump in the parietal cell soon led to the first series of inhibitors of the enzyme, omeprazole (**1**) and timoprazole (**2**).[1-3] Our interest in inhibitors of gastric acid secretion led us to explore structural modifications of substituted benzimidazole derivatives.

The mechanism of omeprazole's inhibitory action on the ATPase was reported recently.[4] In the presence of acid, **1** is transformed into a sulfenic acid, which ultimately oxidizes the enzyme to an inactive disulfide. During the process, **1** becomes reduced to its sulfide precursor. Although reduced **1** retains no in vitro activity, it has been shown in vivo that oxidation of sulfide to **1** occurs.[5]

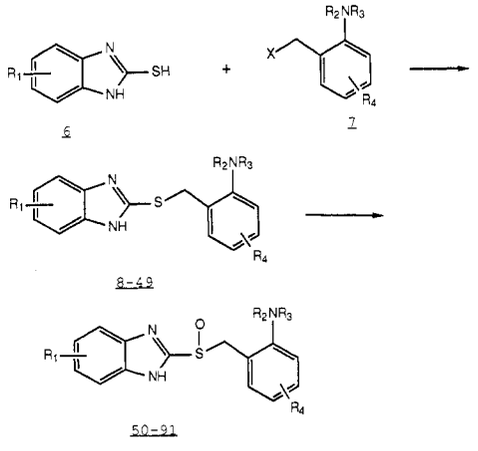
The in vitro inhibitory activity of substituted benzimidazoles was shown to be profoundly influenced by substituents on the benzimidazole and pyridine rings.[6] Thus the rate of decomposition of the sulfoxide should correlate with the basicity of the pyridine nitrogen, and the subsequent stability of the cyclic intermediate should be influenced by the benzimidazole ring substituent.

In view of the dependence on a weakly basic center situated proximal to the sulfoxide group, we replaced the pyridine ring of omeprazole and some analogues with substituted aniline groups (Table I). The observation that many of these aniline-derived compounds were potent inhibitors of H^+/K^+ ATPase was expected on the basis of a mechanistic pathway analogous to that of omeprazole.

Scheme I



Scheme II



A sulfenic acid **4** should be formed by acid-induced decomposition of sulfoxides **50–91** to form the sulfides **8–49** and oxidized enzyme via the covalently bound intermediate **5** (Scheme I). The synthesis of a similar series of compounds was recently disclosed in a patent,[7] and the bio-

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