Synthesis and Biological Activities of 5-Deaza Analogues of Aminopterin and Folic Acid

Tsann-Long Su,* Jai-Tung Huang, Joseph H. Burchenal, Kyoichi A. Watanabe,* and Jack J. Fox

Sloan-Kettering Institute for Cancer Research, Memorial Sloan-Kettering Cancer Center, Sloan-Kettering Division of Graduate School of Medical Sciences, Cornell University, New York, New York 10021. *Received September 9, 1985*

N-[*p*-[[(2,4-Diaminopyrido[2,3-*d*]pyrimidin-6-yl)methyl]amino]benzoyl]-L-glutamic acid (**1a**, 5-deazaaminopterin) and the 5-methyl analogue (**1b**) were synthesized in 14 steps from 5-cyanouracil (**4a**) and 5-cyano-6-methyluracil (**4b**), respectively, by exploitation of the novel pyrimidine to pyrido[2,3-*d*]pyrimidine ring transformation reaction. The 5-cyanouracils **4** were treated with chloromethyl methyl ether to the 1,3-bis(methoxymethyl)uracils (**5**, which were treated with malononitrile in NaOEt/EtOH to give the pyrido[2,3-*d*]pyrimidines **6**. Diazotization of **6** in concentrated HCl afforded the 7-chloro derivatives **8** in high yield. After reduction of **8**, the 7-unsubstituted products **9** were reduced in the presence of Ac_2O and the products, 6-(acetamidomethyl)pyridopyrimidines **10**, were converted into the 6-acetoxymethyl derivatives **12** via nitrosation. After removal of the *N*-methoxymethyl groups from **12**, the 6-(acetoxymethyl)pyrido[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-diones **14** were converted into 2,4-diamino-6-(hydroxymethyl)pyrido[2,3-*d*]pyrimidine (**15a**) and its 5-methyl analogue **15b** by the silylation–amination procedure. Compounds **15** were brominated to the 6-bromomethyl derivatives **16**, which were treated with diethyl (*p*-aminobenzoyl)-L-glutamate, and the products **17** were saponified to afford 5-deazaaminopterin (**1a**) and its 5-methyl analogue **1b**. Compound **1b** was also prepared by an alternative procedure in 10 steps from cyanothioacetamide and ethyl β-(ethoxymethylene)acetoacetate via 2,4-diamino-6-(hydroxymethyl)-5-methylpyrido[2,3-*d*]pyrimidine (**15b**). 5-Deaza-5-methylfolic acid (**2**) was also prepared in four steps from **15b**. The aminopterine analogues **1** showed significant anticancer activity in vitro and in vivo, whereas the folic acid analogue **2** did not exhibit any significant toxicity.

The 5-deaza analogue **1a** of aminopterine (Figure 1) was recently synthesized and reported to be significantly active, both in vitro and in vivo.[1,2] Stone et al. have reported that 5-deazafolic acid (**1**; R = OH, R′ = H) is a potent inhibitor of dihydrofolate reductase. The 5-deaza-5-methylpteridine derivative[3] has exhibited cytotoxicity against various experimental tumors as potently as methotrexate,[4] one of the most effective antimetabolites currently used in the treatment of various solid tumors.[5,6] These reports and our recent development[7,8] of a method of facile preparation of pyrido[2,3-*d*]pyrimidines from 5-cyano-1,3-dimethyluracil prompted us to synthesize 5-deaza- and 5-deaza-5-methylaminopterin (**1a** and **1b**; Figure 1) as well as 5-deaza-5-methylfolic acid (**2**) by exploitation of our novel ring-transformation reaction.[9]

Our strategy, as shown in Scheme I, contains four critical steps: pyrimidine to pyridopyrimidine ring transformation (**5** to **6**), removal of the 7-amino group (**6** to **9**), deprotection of the 1,3 N substituents (**11** to **12**), and conversion of the 2,4-dioxo derivative into the 2,4-diaminopyridopyrimidine (**12** to **14**). We chose methoxymethyl (MOM) as the removable N-protecting group. 5-Cyano-1,3-bis(methoxymethyl)uracil (**5a**) was obtained in high yield by treatment of 5-cyanouracil[10] (**4a**) with methoxymethyl chloride. We found, however, that **5a** can be more readily prepared from 1,3-bis(methoxymethyl)uracil by bromination followed by NaCN treatment. Almost quantitative conversion of **5a** into the pyrido[2,3-*d*]pyrimidine **6a** was effected by reaction with malononitrile in EtONa/EtOH. Diazotization of **6a** in concentrated HCl in MeOH afforded the 7-chloro derivative **8a** almost exclusively. Reduction of **8a** over Pd/C afforded the 7-unsubsituted pyridopyrimidine **9a**. The overall yield of **9a** from **4a** was about 65%.

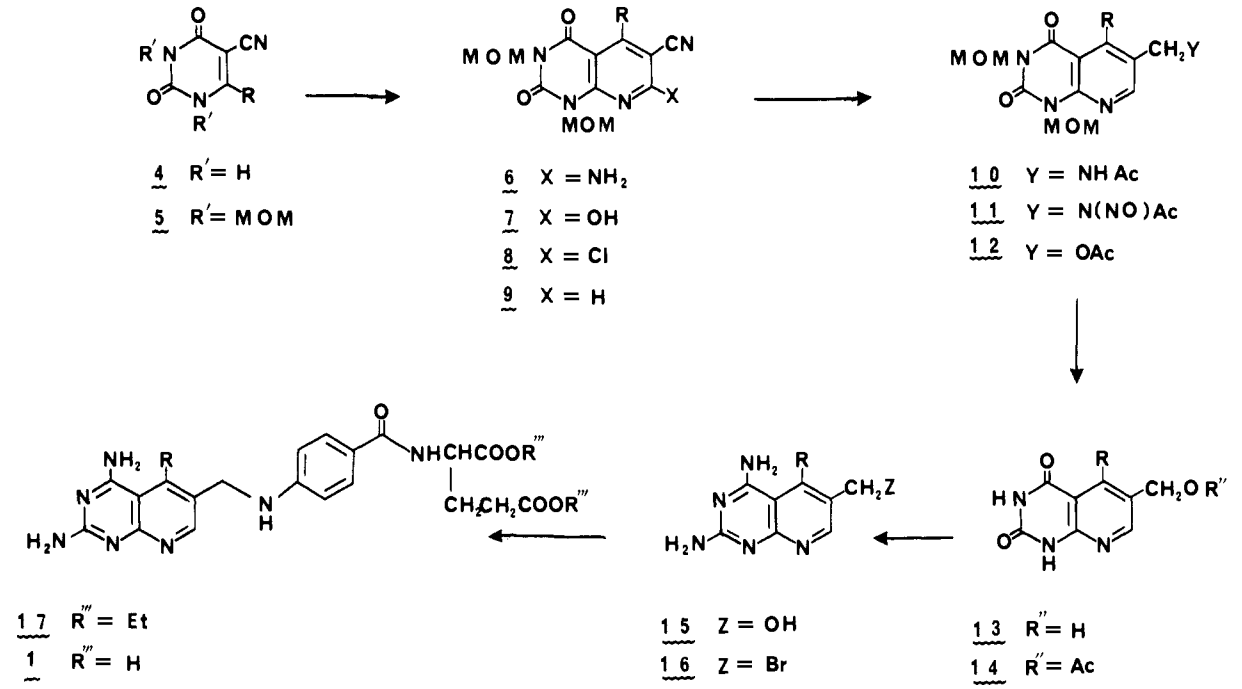
Reduction of the 6-cyano derivative **9a** over Raney Ni in Ac_2O/AcOH afforded the acetamide **10a** in high yield. Nitrosation of **10a** gave the crystalline *N*-nitroso product **11a**, which was converted into the acetate **12a** in high yield by the method of White.[11] Attempts at one-pot conversion of **11a** into the deprotected pyridopyrimidine **13a** by treatment of **11a** in 6 N HCl at reflux resulted in the formation of a mixture of **13a** and the 6-(chloromethyl)-pyridopyrimidine in varying ratios. Compound **13a** was prepared more efficiently by treatment of **12a** with BCl_3/CH_2Cl_2. After acetylation of **13a**, the product **14a** was aminated by the silylation–amination procedure developed by Vorbruggen and Krolikiewicz[12] to afford 2,4-diamino-6-(hydroxymethyl)pyrido[2,3-*d*]pyrimidine (**15a**). Bromination of **15a** with HBr in dioxane[13] gave the bromomethyl derivative **16a**, which was coupled with diethyl (*p*-aminobenzoyl)-L-glutamate. Saponification of the product **17a** afforded the desired 5-deazaaminopterin, *N*-[*p*-[(2,4-diaminopyrido[2,3-*d*]pyrimidin-6-yl)amino]-benzoyl]-L-glutamic acid (**1a**), in high yield.

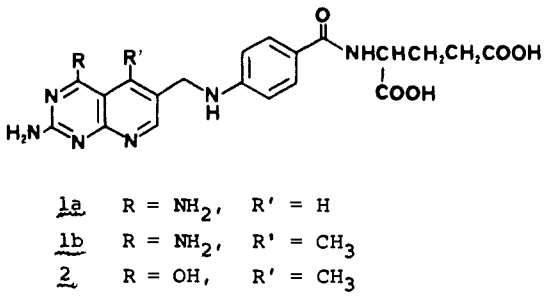
The 5-deaza-5-methylaminopterin analogue (**1b**) was also synthesized in a similar manner with a few modifications. 5-Cyano-6-methyluracil (**4b**) was methoxymethylated almost quantitatively to **5b**, which was treated with cyanoacetamide in EtONa/EtOH. The reaction was very slow as expected from our previous findings that the

(1) Temple, C.; Elliot, R. D.; Montgomery, J. A. *J. Org. Chem.* **1982**, *47*, 761.
(2) Taylor, E. C.; Palmer, D. C.; George, T. J.; Fletcher, S. R.; Tseng, C. P.; Harrington, P. J.; Beardsley, G. P. *J. Org. Chem.* **1983**, *48*, 4852.
(3) Stone, S. R.; Montgomery, J. A.; Morrison, J. F. *Biochem. Pharmacol.* **1984**, *33*, 175.
(4) Grivsky, E. M.; Lee, S.; Sigel, S. W.; Duch, D. S.; Nichol, C. A. *J. Med. Chem.* **1980**, *23*, 327; Duch, D. S.; Edelstein, M. P.; Bowers, S. W.; Nichol, C. A. *Cancer Res.* **1982**, *42*, 3987.
(5) Bertino, J. R. *Antineoplastic Agents*, Part II; Sartorelli, A. C., Johns, D. G., Eds.; Springer-Verlag: Berlin, 1975; pp 468–483.
(6) Ensminger, W. D.; Grindey, G. B.; Hoglind, J. A. *Advances In Cancer Chemotherapy*; Rosowsky, A., Ed.; Marcel Dekker: New York, 1979; Vol. 1, pp 61–109.
(7) Hirota, K.; Kitade, Y.; Senda, S.; Halat, M. J.; Watanabe, K. A.; Fox, J. J. *J. Org. Chem.* **1981**, *46*, 846.
(8) Su, T.-L.; Watanabe, K. A. *J. Heterocycl. Chem.* **1982**, *19*, 1261; **1984**, *21*, 1543.
(9) During the course of this investigation, the synthesis of **1b** and its 10-*N*-methyl analogue, as well as the 6-(hydroxymethyl) and 6-(bromomethyl) intermediates corresponding to **15b** and **16b**, were reported: Piper, J. R.; McCaleb, G. S.; Montgomery, J. A. Thirty-Sixth Southeastern Regional Meeting of the American Chemical Society, Raleigh, NC, Oct 26, 1984.
(10) Johnson, T. B. *J. Am. Chem. Soc.* **1920**, *42*, 513. Shaw, G. *J. Chem. Soc.* **1955**, 1834.
(11) White, E. H. *J. Am. Chem. Soc.* **1955**, *77*, 6011.
(12) Vorbruggen, H.; Krolikiewicz, K. *Chem. Ber.* **1984**, *117*, 1523.
(13) Srinivasan, A.; Broom, A. D. *J. Org. Chem.* **1980**, *45*, 3746.

0022-2623/86/1829-0709$01.50/0

Scheme I





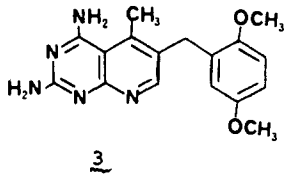


Figure 1.

presence of a methyl group in the 1,3-dimethyluracil ring at C-6 dramatically suppressed the reactivity in the pyrimidine to pyrimidine[14] and pyrimidine to pyridine[7,15] ring-transformation reactions. 7-Amino-6-cyano-1,3-bis(methoxymethyl)-5-methylpyrido[2,3-*d*]pyrimidine-2,4-(1*H*,3*H*)-dione (**6b**) was obtained in about 60% yield.

Conversion of **6b** to **12b** was achieved in a similar manner for the preparation of **12a** from **6a**. Compound **12b**, however, was recovered unchanged after treatment with 6 N HCl, although **12a** was deprotected smoothly to **13a** under similar conditions. Deprotection of **12b** was effected by treatment with BCl_3 in CH_2Cl_2, and unprotected 5-deaza-5-methylpteridine (**13b**) was obtained in good yield. 5-Deaza-5-methylaminopterin (**1b**) was prepared from **13b** in a manner similar to that described for the preparation of **1a** from **13a**.

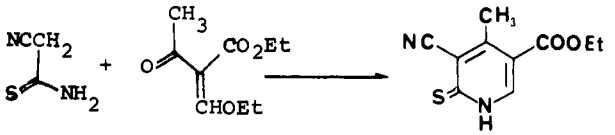
Compound **15b**, one of the key intermediates for the synthesis of **1b**, was also prepared by an alternative route somewhat similar to the methods reported by Taylor et al.[2] and Elslager and Davoll[16] (Scheme II). Condensation of cyanothioacetamide and ethyl β-(ethoxymethylene)-acetoacetate gave 3-cyano-5-(ethoxycarbonyl)-4-methylpyridine-2(1*H*)-thione (**18**) in 40% yield. The pyridine **18** was S-methylated with MeI/K_2CO_3 in DMF to afford **19**, which was reduced with LAH to the 5-(hydroxymethyl)-pyridine derivative **20**. After protection of the hydroxymethyl function by methoxymethylation, the product **21** was oxidized to the sulfone **22** with *m*-chloroperbenzoic acid. Direct treatment with guanidine afforded the 5-deaza-5-methylpteridine **23** which was deprotected by treatment with concentrated HCl to give 2,4-diamino-6-(hydroxymethyl)-5-methylpyrido[2,3-*d*]pyrimidine (**15b**) in modest overall yield. Compound **15b** thus obtained was identical in all respects with **15b** prepared from **4** via the ring-transformation reaction (Scheme I).

The 5-deaza-5-methylfolate analogue **2** was also prepared as follows (Scheme II): Selective hydrolytic deamination of **15b** afforded **24**, which was converted into the bromide **25**. Condensation of **25** with diethyl (*p*-aminobenzoyl)-L-glutamate afforded the ester **26**, which was saponified to afford the folate analogue **2** in good yield.

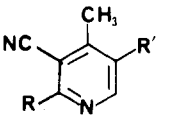
For cell culture studies, a modification[17] of the technique of Fischer[18] was employed. The 5-deaza-5-methylaminopterin (**1b**) showed a moderate degree of activity (ID_{50} 1.8 × 10^{-8}–2.0 × 10^{-7} M) against P-388 and L-1210 leukemic cells in vitro, which is 30–200 times the concentration of aminopterin or methotrexate required for similar activity in these cell lines. In vivo, **1b** was tested in BDF mice with transplanted L-1210/0 leukemia by using a procedure previously described.[19] The mice were inoculated ip with

(14) Hirota, K.; Watanabe, K. A.; Fox, J. J. *J. Heterocycl. Chem.* **1977**, *14*, 537; *J. Org. Chem.* **1978**, *43*, 1193.
(15) Hirota, K.; Kitade, Y.; Senda, S.; Halat, M. J.; Watanabe, K. A.; Fox, J. J. *J. Am. Chem. Soc.* **1979**, *101*, 4423.
(16) Elslager, E. F.; Davoll, J. *Lectures in Heterocyclic Chemistry*; Castle, R. N., Townsend, L. B., Eds.; Hetero. Corp.: Orem, UT, 1974; Vol. 2, p 120.
(17) Burchenal, J. H.; Chou, T.-C.; Lokys, L.; Smith, R. S.; Watanabe, K. A.; Su, T.-L.; Fox, J. J. *Cancer Res.* **1982**, *42*, 2598.
(18) Fischer, G. A. *Ann. N.Y. Acad. Sci.* **1958**, *76*, 673.

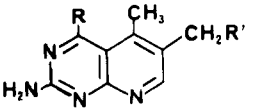
Scheme II



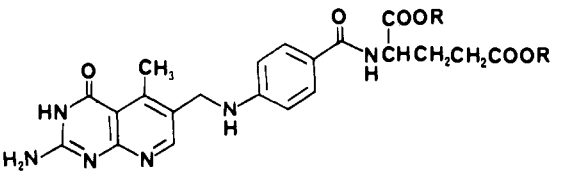
18



19 R = SCH_3, R' = COOEt
20 R = SCH_3, R' = CH_2OH
21 R = SCH_3, R' = $CH_2OCH_2OCH_3$
22 R = SO_2CH_3, R' = $CH_2OCH_2OCH_3$



23 R = NH_2, R' = OCH_2OCH_3
15b R = NH_2, R' = OH
24 R = OH, R' = OH
25 R = OH, R' = Br



26 R = Et
2 R = H

10^6 cells on day zero and treated ip with drug. Compound **1b** was toxic at 400 mg/kg per day × 5 and moderately active therapeutically at 200 and 300 mg/kg per day when treated on days 1, 2, 5, 7, and 9, with an increase in life span of 47–56%. Thus, in vivo, the dose of **1b** needed for comparable activity was 600 times that of aminopterin and 240 times that of methotrexate. The folate analogue **2**, however, was without significant activity in vitro or in vivo. In contrast to **1b**, 5-deazaaminopterin **1a** shows activity roughly comparable to aminopterin and methotrexate.

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). Elemental analyses were performed by M.H.W. Laboratories, Phoenix, AZ. ^1H NMR spectra were recorded on a JEOL PFT-100 or JEOL FX90Q spectrometer with Me_4Si as the internal standard. Chemical shifts are reported in parts per million (δ) and signals are quoted as s (singlet), d (doublet), t (triplet), q (quartet), or m (multiplet). IR spectra were recorded on a Perkin-Elmer Infracord Model 137B spectrometer and UV spectra on a Gilford RESPONSE UV–vis spectrophotometer.

5-Cyano-1,3-bis(methoxymethyl)uracil (5a). To a mixture of uracil (44.8 g, 0.4 mol) and anhydrous K_2CO_3 (276 g, 2.0 mol) in dry DMF (600 mL) was added dropwise $ClCH_2OCH_3$ (96.7 g, 1.2 mol) below −15 °C. The mixture was allowed to warm to room temperature and was stirred overnight. Inorganic salts were removed by filtration, and the filtrate was concentrated to ~100 mL, which was partitioned between water (600 mL) and $CHCl_3$ (300 mL). The aqueous layer was washed with $CHCl_3$ (300 mL × 4). The combined organic layers were washed with water (600 mL × 2), dried (Na_2SO_4), and concentrated in vacuo. The residue was chromatographed on a silica gel column (40 × 8 cm) with $CHCl_3$ as the eluent. The major UV-absorbing fraction was concentrated to afford 48.8 g (51%) of 1,3-bis(methoxymethyl)uracil as a syrup: ^1H NMR (Me_2SO-d_6) δ 3.41 (3 H, s, Me), 3.45 (3 H, s, Me), 5.14 (2 H, s, CH_2), 5.39 (2 H, s, CH_2), 5.82 (1 H, d, H-5), 7.29 (1 H, d, H-6).

Twenty grams of the above syrup (0.1 mol) was dissolved in HOAc (100 mL), and Br_2 (17.4 g, 0.11 mol) in HOAc (10 mL) was added to the solution. The mixture was stirred for 1.5 h at room temperature and then concentrated in vacuo. The residue was dissolved in $CHCl_3$ (600 mL), washed with water (200 mL × 5), dried (Na_2SO_4), and evaporated in vacuo and the residue crystallized from Et_2O to give 19.8 g (71%) of 5-bromo-1,3-bis(methoxymethyl)uracil: mp 67–68 °C; ^1H NMR (Me_2SO-d_6) δ 3.43 and 3.46 (3 H, s, Me), 5.15 and 5.44 (2 H, s, CH_2), 7.67 (1 H, s, H-6).

A mixture of 5-bromo-1,3-bis(methoxymethyl)uracil (20.0 g, 71.6 mmol) and NaCN (5.31 g, 108 mmol) in dry DMF (300 mL) was heated at 80–90 °C for 5 h and then concentrated in vacuo. The residue was dissolved in a mixture of water (500 mL) and EtOAc (200 mL). The aqueous layer was separated and extracted with EtOAc (200 mL × 4). The combined organic layers were dried (Na_2SO_4) and concentrated in vacuo and the residue chromatographed on a silica gel column with $CHCl_3$ as the eluent. Compound **5a** (16.0 g, 98% from the 5-bromo derivative) was obtained as a syrup: ^1H NMR (Me_2SO-d_6) δ 3.46 (6 H, s, 2Me), 5.20, 5.34 (each 2 H, s, CH_2), 7.97 (1 H, s, H-6); IR (KBr) 2210 cm^{-1} for CN. Anal. C, H. N.

5-Cyano-1,3-bis(methoxymethyl)-6-methyluracil (**5b**) was prepared in a similar manner from 5-cyano-6-methyluracil[20] (60.50 g, 0.4 mol) as colorless crystals: 42 g (44%); mp 91–92 °C; UV λ_{max} (H_2O) 217, 276 nm (ϵ 10600, 12400), λ_{min} 240 (2240); ^1H NMR (Me_2SO-d_6) δ 2.59, 3.28, 3.34 (each 3 H, s, Me), 5.20, 5.34 (each 2 H, s, CH_2). Anal. C, H, N.

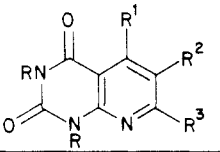
7-Amino-6-cyano-1,3-bis(methoxymethyl)pyrido[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (6a). A mixture of **5a** (13.5 g, 0.06 mol) and $CH_2(CN)_2$ (5.95 g, 0.09 mol) in NaOEt/EtOH [freshly prepared by dissolving Na (2.76 g, 0.12 mol) in EtOH (450 mL)] was stirred for 1 h at room temperature. The crystalline precipitates were collected by filtration and recrystallized from EtOH to give **6a**: 14.9 g (85%); mp 274–275 °C; IR (KBr) 2210 cm^{-1} (CN). See Table I for ^1H NMR data. Anal. C, H, N.

7-Amino-6-cyano-1,3-bis(methoxymethyl)-5-methylpyrido[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (6b). A mixture of **5b** (23.9 g, 0.1 mol) and $CH_2(CN)_2$ (19.8 g, 0.3 mol) in NaOEt/EtOH [freshly prepared from 5.75 g (0.25 mol) of Na and EtOH (500 mL)] was heated at 60 °C with stirring for 2 h. After the mixture was cooled in an ice bath, crystalline precipitates were collected and recrystallized from MeOH/$CHCl_3$ to afford **6b**: 13.8 g (45.3%); mp 228–229 °C; IR (KBr) 2215 cm^{-1} (CN). See Table

(19) Burchenal, J. H.; Cioracco, K.; O'Toole, T.; Kiefner, R.; Dowling, M. D.; Chus, C.; Watanabe, K. A.; Wempen, I.; Fox, J. J. *Cancer Res.* **1976**, *36*, 1520.

(20) Kanatomo, S.; Hase, T.; Nagai, S. *Chem. Pharm. Bull.* **1981**, *29*, 229.

Table I. ^{1}H NMR Parameters (δ) for Pyrido[2,3-*d*]pyrimidines[a]



compd	R	R^1	R^2	R^3	H-5	H-7	N-CH_2-O	OCH_3	5-CH_3	other
6a	CH_2OCH_3	H	CN	NH_2	8.41 s		5.51 s, 5.26 s	3.15 s, 3.30 s		8.01 **br** s (NH_2)
6b	CH_2OCH_3	CH_3	CN	NH_2			5.53 s, 5.25 s	3.34 s, 3.30 s	2.78 s	7.85 **br** s (NH_2)
7a	CH_2OCH_3	H	CN	OH	8.04 s		5.44 s, 5.22 s	3.31 s, 3.27 d		
7b	CH_2OCH_3	CH_3	CN	OH			5.49 s, 5.22 s	3.30 s, 3.27 s	2.64 s	
8a	CH_2OCH_3	H	CN	Cl	9.00 s		5.55 s, 5.26 s	3.37 s, 3.33 s		
8b	CH_2OCH_3	CH_3	CN	Cl			5.57 s, 5.31 s	3.37 s, 3.33 s	2.95 s	
9a	CH_2OCH_3	H	CN	H	9.17 d	8.99 d	5.65 s, 5.33 s	3.36 s, 3.33 s		
9b	CH_2OCH_3	CH_3	CN	H		9.05 s	5.65 s, 5.32 s	3.35 s, 3.34 s	2.93 s	
10a	CH_2OCH_3	H	CH_2NHAc	H	8.64 d	8.29 d	5.63 s, 5.33 s	3.34 s, 3.22 s		8.50 t (NH), 4.43 d (CH_2NH), 1.87 (Ac)
10b	CH_2OCH_3	CH_3	CH_2NHAc	H		8.50 s	5.64 s, 5.32 s	3.33 s (6 H)	2.74 s	8.25 t (NH), 4.32 d (CH_2NH), 1.85 s (Ac)
11a	CH_2OCH_3	H	$CH_2N(NO)Ac$	H	8.55 d	8.14 d	5.59 s, 5.31 s	3.32 s, 3.31 s		5.00 s (CH_2N), 2.79 s (Ac)
11b	CH_2OCH_3	CH_3	$CH_2N(NO)Ac$	H		8.17 s	5.60 s, 5.31 s	3.31 s (6 H)	2.73 s	4.97 s (CH_2N), 2.84 s (Ac)
12a	CH_2OCH_3	H	CH_2OAc	H	8.55 d	8.15 d	5.59 s, 5.31 s	3.33 s, 3.32 s		5.11 s (CH_2O), 2.79 (Ac)
12b	CH_2OCH_3	CH_3	CH_2OAc	H		8.62 s	5.65 s, 5.33 s	3.34 s (6 H)	2.77 s	5.20 s (CH_2O), 2.05 (Ac)
13a	H	H	CH_2OH	H	8.54 d	8.19 d				11.52 **br** s (NH), 4.55 d (CH_2OH), 5.39 t (CH_2OH)
13b	H	CH_3	CH_2OH	H		8.40 s			2.71 s	11.23, 11.44 br (NH), 4.53 d (CH_2OH), 5.21 t (CH_2OH)
14a	H	H	CH_2OAc	H	8.63 d	8.27 d				5.14 s (CH_2OAc), 2.06 (Ac), 11.75, 11.31 br (NH)
14b	H	CH_3	CH_2OAc	H		8.47 s			2.72 s	5.14 s (CH_2OH), 2.04 s (Ac), 11.57, 11.31 **br** (NH)

Table II. UV Spectral Data for Some Pyrido[2,3-*d*]pyrimidines[a]

compd	λ_{max} (H_2O)	λ_{min} (H_2O)	compd	λ_{max} (H_2O)	λ_{min} (H_2O)
6a	229 (39000)	257 (4430)	**10a**	214 (40100)	265 (1850)
	284 (15200)	299 (6050)		244 (10700) sh	
	329 (17700)			310 (7120)	
6b	232 (24700)	257 (1890)	**10b**	221 (41200)	265 (550)
	284 (7200)	298 (3900)		244 (9160) sh	
	325 (8990)			306 (5260)	
7a	227 (60800)	254 (5370)	**11a**	215 (40100)	233 (15700)
	282 (23700)	296 (1250)	**11a**	243 (16600)	278 (2670)
	323 (32400)	296 (1250)		309 (6200)	
7b	228 (60100)	254 (4150)	**11b**	221 (39800)	274 (2080)
	282 (23400)	295 (9410)		249 (10500) sh	
	322 (31400)			305 (5430)	
8a	221 (50100)	246 (5850)	**12a**	215 (40400)	234 (8900)
	272 (16600)	288 (4830)		246 (9920)	266 (1610)
	311 (9850)			307 (6670)	
				318 (5210) sh	
8b	230 (41300)	249 (5630)			
	273 (13000)	289 (4680)	**12b**	220 (40000)	266 (2680)
	310 (6840)			250 (8880) sh	
	310 (6840)			250 (8880) sh	
				303 (6410)	
9a	217 (37800)	239 (7810)			
	263 (18700)	282 (3690)	**14a**	212 (39200)	231 (7860)
	307 (6910)			246 (11000)	264 (1120)
	320 (4800) sh			309 (6980)	
9b	223 (36200)	242 (7940)	**14b**	218 (40200)	236 (9220)
	265 (16100)	283 (3190)		246 (10100)	266 (1360)
	304 (5180)			306 (6300)	
	315 (4240) sh				

[a] Units: η, nm (ϵ).

I for ^{1}H NMR data and Table II for UV data. Anal. C, H, N.

7-Chloro-6-cyano-1,3-bis(methoxymethyl)pyrido[2,3-*d*]-pyrimidine-2,4(1*H*,3*H*)-dione (8a). A solution of $NaNO_2$ (40 g, 0.58 mol) in water (100 mL) was added slowly to a suspension of **6a** (13.5 g, 46.3 mmol) in concentrated HCl (300 mL) with stirring over a period of 2.5 h. The mixture, after being stirred for an additional 1 h, was poured onto ice (500 g). Colorless precipitates (containing two products as detected by TLC, $CHCl_3$/MeOH (10:1)) were collected by filtration, washed with water and EtOH, and then chromatographed over a column of silica gel (40 × 4 cm) with $CHCl_3$ as the eluent. Compound **8a** was eluted first from the column and recrystallized from $CHCl_3$/EtOH: 10.2 g (71%); mp 178–179 °C; IR (KBr) 2220 cm^{-1} (CN). See Table I for ^{1}H NMR data and Table II for UV data. Anal. C, H, N.

6-Cyano-1,3-bis(methoxymethyl)pyrido[2,3-*d*]pyrimidine-2,4,7(1*H*,3*H*,8*H*)-trione (7a) was then eluted from the column: 1.1 g (8% after recrystallization from EtOH); mp >350 °C; IR (KBr) 2225 cm^{-1} (CN). See Table I for ^{1}H NMR data and Table II for UV data.

The 5-methyl analogues **8b** (mp 128–129 °C) and **7b** (mp >345 °C) were also prepared in a similar manner in 71% and 5% yields, respectively, after recrystallization from EtOH. See Table I for ^{1}H NMR data and Table II for UV data. Anal. C, H, N.

Conversion of 7a into 8a. To an ice-cold mixture of dry DMF (1.08 g, 14.7 mmol) and dry $CHCl_3$ (20 mL) was added dropwise a solution of $SOCl_2$ (1.75 g, 14.7 mmol) in dry $CHCl_3$ (5 mL) followed by a solution of **7a** (430 mg, 1.47 mmol) in $CHCl_3$ (20 mL). The mixture was heated at reflux overnight, cooled in an ice bath, and then carefully neutralized with 20% NH_4OH. The organic layer separated was washed with 20% NH_4OH (10 mL × 2) and water (10 mL × 2), dried (Na_2SO_4), and concentrated to dryness and the residue crystallized from EtOH to give 276 mg of **8a**, mp 178–179 °C, unaltered on admixture with an authentic sample of **8a**.

In a similar manner, **7b** was converted almost quantitatively into **8b**.

6-Cyano-1,3-bis(methoxymethyl)pyrido[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (9a). A mixture of **8a** (8.0 g, 25.7 mmol), MgO (16.0 g), and 10% Pd/C (4 g) in dioxane (300 mL) was hydrogenated in a Parr apparatus with initial pressure of 50 psi. The mixture was filtered through a Celite pad, the filtrate concentrated, and the residue crystallized from $CHCl_3$/EtOH to afford **9a**: 6.29 g (88%); mp 185–186 °C; IR (KBr) 2220 cm^{-1}. See Table I for ^{1}H NMR data and Table II for UV data. Anal. C, H, N.

In a similar manner, **9b** [6.4 g (81%); mp 144–145 °C], was prepared from 8.67 g (27.3 mmol) of **8b**: IR (KBr) 2220 cm^{-1}. See Table I for ^{1}H NMR data and Table II for UV data. Anal. C, H, N.

6-(Acetamidomethyl)-1,3-bis(methoxymethyl)pyrido[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (10a). A mixture of **9a** (4.8 g, 17.3 mmol), Raney Ni (3 g), and Ac_2O (80 mL) in AcOH (160 mL) was hydrogenated in a Parr apparatus with initial pressure of 50 psi. The catalyst was filtered and washed with $CHCl_3$. The combined filtrate and washings were concentrated in vacuo. The residue was dissolved in $CHCl_3$ (300 mL), washed with water, dried (Na_2SO_4), and concentrated to dryness and the residue crystallized from $CHCl_3$/EtOH to afford 4.69 g (84%) of **10a** as colorless crystals, mp 207–208 °C. See Table I for ^{1}H NMR data and Table II for UV data. Anal. C, H, N.

In a similar manner, **10b** (mp 209–210 °C), was obtained in 89% yield from **9b**. See Table I for ^{1}H NMR and Table II for UV data. Anal. C, H, N.

[(*N*-Nitrosoacetamido)methyl]-1,3-bis(methoxymethyl)-pyrido[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (11a). To a suspension of **10a** (12.52 g, 38.8 mmol) in a 1:5 mixture of AcOH and Ac_2O (30 mL) was added portionwise $NaNO_2$ (10 g) below 5 °C. The mixture was stirred at room temperature for 2 h and then poured into ice (500 g). The precipitates were collected by filtration, and the filtrate was extracted with EtOAc (300 mL × 3). The extracts were washed with water, dried (Na_2SO_4), and concentrated to dryness. The solid residue was combined with the collected precipitates and recrystallized from $CHCl_3$/EtOH to afford 12.1 g of **11a** (89%), mp 143–144 °C. See Table I for ^{1}H NMR data and Table II for UV data. Anal. C, H, N.

Compound **11b** (mp 149–150 °C) was obtained in a similar manner from **10b** in 95% yield. See Table I for ^{1}H NMR data and Table II for UV data. Anal. C, H, N.

6-(Acetoxymethyl)-1,3-bis(methoxymethyl)pyrido[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (12a). Method A. Compound **11a** (7.02 g, 0.02 mmol) in Ac_2O (350 mL) was heated at reflux until evolution of N_2 ceased (~45 min). The clear solution was concentrated and the residue, after being coevaporated several times with EtOH, was recrystallized from EtOH to give 5.8 g of **12a** (90%) as colorless needles, mp 159–160 °C. See Table I for ^{1}H NMR data and Table II for UV data. Anal. C, H, N.

Method B. Compound **10a** (6.4 g, 20 mmol) was treated with $NaNO_2$ (20 g) as described for the preparation of **11a**. The reaction mixture, instead of being poured into ice, was diluted with Ac_2O (300 mL) and heated at reflux for 1 h. The mixture was concentrated in vacuo and the residue triturated with 400 mL of ice-cold water. The solid was collected and recrystallized from EtOH to give 5.88 g (91%) of **12a** (mp 159–160 °C) unaltered upon admixture with an authentic sample of **12a**.

Compound **12b** was prepared from **11b** by method A in 91% yield and from **10b** by method B in 92% yield; mp 128–129 °C. See Table I for ^{1}H NMR data and Table II for UV data. Anal. C, H, N.

6-(Hydroxymethyl)pyrido[2,3-*d*]pyrimidine-2,4-(1*H*,3*H*)-dione (13a). To a solution of **12a** (9.69 g, 30 mmol) in dry CH_2Cl_2 (100 mL) was added dropwise 1M BCl_3/CH_2Cl_2 (160 mL) at −78 °C. The mixture, after being stirred at −78 °C for 3 h, was allowed to warm to room temperature and the stirring continued overnight. The mixture was cooled in an ice bath, and MeOH (5 mL) was slowly added. After stirring for 20 min, the mixture was concentrated in vacuo and the residue coevaporated several times with MeOH and then recrystallized from water (80 mL). Recrystallization of the solid from MeOH/water afforded analytically pure **13a**: 5.6 g (83%); mp >300 °C. See Table I for ^{1}H NMR data and Table III for UV data. Anal. C, H, N.

In a similar manner, **12b** was converted in 87% yield into **13b**, mp >300 °C. See Table I for ^{1}H NMR data and Table III for UV data. Anal. C, H, N.

6-(Acetoxymethyl)pyrido[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (14a). A suspension of **13a** (5.0 g, 24 mmol) in a 2:3 mixture of Ac_2O and pyridine (80 mL) was stirred at room temperature for 48 h. The solvent was removed in vacuo and the residue recrystallized from $CHCl_3$/MeOH (3:1) to give 4.8 g of **14a** (80%), mp 263–264 °C. See Table I for ^{1}H NMR data and Table II for UV data. Anal. C, H, N.

In a similar manner, the 5-methyl analogue **14b** (mp 294–295 °C) was obtained in 93% yield from **13b**. See Table I for ^{1}H NMR data and Table II for UV data. Anal. C, H, N.

2,4-Diamino-6-(hydroxymethyl)pyrido[2,3-*d*]pyrimidine (15a). To a suspension of **14a** (1.2 g, 5.1 mmol) and *p*-TsOH (0.5 g) in hexamethyldisilazane (50 mL) in a stainless-steel container was added liquid NH_3 (15 mL). The mixture was heated at 160 °C in an oil bath for 4 h with stirring and then placed in an oven for 5 days at 155–160 °C. The mixture was concentrated, and the residue was refluxed in 50% aqueous MeOH (200 mL) for 4 h. The brown solution was filtered from the solid, and the filtrate was concentrated to ~20 mL. After cooling overnight at 4 °C, **15a**, precipitated as light brown crystals, was collected by filtration: 0.9 g (74.1%); mp >340 °C. See Table III for UV data. ^{1}H NMR (Me_2SO-d_6) δ 4.52 (2 H, br, CH_2), 5.23 (1 H, br, OH, exchangeable), 6.27 (2 H, br, NH_2, exchangeable), 7.48 (2 H, br, NH_2, exchangeable), 8.31 (1 H, d, H-7), 8.58 (1 H, d, H-5).

Compound **15b** (mp >340 °C) was prepared in a similar manner from **14b** in 55% yield. See Table III for UV data. ^{1}H NMR (Me_2SO-d_6) δ 2.53 (3 H, S, CH_3), 4.58 (2 H, s, CH_2), 5.50 (1 H, br, OH, exchangeable), 8.09 (2 H, br, NH_2, exchangeable), 8.64 (1 H, s, H-7), 8.91 (1 H, br, NH, exchangeable), 9.21 (1 H, br, NH, exchangeable). Anal. C, H, N.

2,4-Diamino-6-(bromomethyl)-5-methylpyrido[2,3-*d*]pyrimidine (16b). Dry HBr was bubbled into a suspension of **15b** (1.0 g, 4.9 mmol) in dry dioxane (80 mL) to saturation, and the mixture was stirred for 20 h at room temperature. After concentration of the mixture in vacuo, traces of HBr were removed by several coevaporations with PhMe. The residue was triturated with Et_2O (100 mL) and the solid collected by filtration and dried over P_2O_5 in vacuo to give 1.38 g (quantitative) of **16b**: ^{1}H NMR (Me_2SO-d_6) δ 2.66 (3 H, s, Me), 4.79 (2 H, s, CH_2), 5.83 (2 H, br, NH_2, exchangeable), 8.77 (1 H, s, H-7), 9.11 (2 H, br, NH_2, exchangeable). This compound was unstable and was used directly in the next step without further characterization.

Diethyl *N*-[*p*-[[(2,4-Diamino-5-methylpyrido[2,3-*d*]pyrimidin-6-yl)methyl]amino]benzoyl]-L-glutamate (17b). To a solution of **16b** (1.37 g, 4.9 mmol) in dry *N*,*N*-dimethylacetamide (40 mL, distilled over CaH_2) was added diethyl *N*-(*p*-aminobenzoyl)glutamate (2.3 g, 7.1 mmol), and the solution was stirred for 3 days at room temperature. The mixture was concentrated in vacuo, and the residue was suspended in water (100 mL). After neutralization with dilute NH_4OH to pH 7, the mixture was extracted with $CHCl_3$ (3 × 200 mL). The combined extracts were dried (Na_2SO_4) and concentrated to ~20 mL, and the solution was poured onto a silica gel column that was then washed with $CHCl_3$/MeOH (19:1, v/v). A small amount of unreacted diethyl (*p*-aminobenzoyl)-L-glutamate was eluted with the solvent, and product **17b** precipitated out in the column. The contents of the column were transferred into a beaker and extracted with boiling $CHCl_3$/MeOH (7:3). Upon concentration of the extracts, compound **17b** was obtained: 1.71 g (93%); mp 239–240 °C dec; ^{1}H

Table III. UV Spectral Behavior of Some Pyrido[2,3-*d*]pyrimidines[a]

compd	λ_{max} (0.1 N HCl)	λ_{min} (0.1 N HCl)	λ_{max} (H_2O)	λ_{min} (H_2O)	λ_{max} (0.1 N NaOH)	λ_{min} (0.1 N NaOH)
13a	220 (28900)	266 (1550)	218 (32100)	265 (700)	246 (13600)	258 (9830)
	249 (6320) sh		247 (6690) sh		267 (11000)	290 (2750)
	309 (8890)		307 (5620)		336 (5620)	
13b	220 (29800)	266 (790)	248 (8620)	265 (1820)	246 (13700)	258 (9840)
	249 (5750) sh		306 (6910)		267 (11100)	290 (2430)
	309 (8790)				336 (5490)	
15a	219 (26300)	263 (5700)	222 (25700)	234 (16400)	247 (19000)	238 (18100)
	279 (6060)	295 (2960)	245 (17000)	291 (2270)	269 (9700) sh	
	319 (6210)		272 (8190) sh		345 (6710)	294 (1330)
	331 (5690) sh		332 (6380)			
15b	223 (28400)	293 (3490)	223 (28600)	285 (2750)	226 (23600)	262 (7170)
	246 (12700) sh		246 (12300) sh		247 (16900) sh	
	317 (8750)		272 (4710) sh		271 (7570)	292 (980)
	327 (8120) sh		316 (9490) sh		341 (6900)	
			328 (8660) sh			
1a	220 (38500)	295 (810)	218 (34500)	258 (13000)	249 (26600)	241 (22600)
	245 (21500) sh		279 (18900) sh		279 (28400)	260 (22000)
	278 (9160) sh		297 (20400)		298 (26100) sh	
	315 (10000)				342 (1000) sh	
	329 (7910) sh					
1b	222 (39300)	257 (12100)	222 (30800)	253 (9210)	248 (21700) sh	
	245 (17300) sh		276 (15000) sh		286 (24500)	259 (15900)
	279 (17500) sh		298 (17900)		295 (24200) sh	
	301 (9210)		332 (7500) sh		340 (9300) sh	

[a] Units: η, nm (ϵ).

NMR (Me_2SO-d_6) δ 1.16 (3 H, t, CH_2Me), 1.18 (3 H, t, CH_2Me), 1.92–2.20 (2 H, br m, $CH_2CH_2CO_2Et$), 2.34–2.50 (2 H, br m, $CH_2CH_2CO_2Et$), 2.54 (3 H, s, Me), 4.12 (2 H, q, CH_2Me), 4.16 (2 H, q, CH_2Me), 4.30–4.54 (3 H, br m, CH_2NH and CONHCH), 6.60 (2 H, br, NH_2, exchangeable), 6.67 (2 H, d, Ph), 7.60 (2 H, br, NH_2, exchangeable), 7.68 (2 H, d, Ph), 8.11 (1 H, br, CH_2NH, exchangeable), 8.22 (1 H, br, CONHCH), 8.38 (1 H, s, H-7). (C, H, N).

Diethyl *N*-[*p*-[[(2,4-Diaminopyrido[2,3-*d*]pyrimidin-6-yl)methyl]amino]benzoyl]-L-glutamate (17a). Compound **15a** (0.5 g, 2.6 mmol) was treated with HBr in dioxane as described for the preparation of **16b**. After concentration of the mixture and several coevaporations with PhMe, the residue was treated with diethyl *N*-(*p*-aminobenzoyl)-L-glutamate (1.15 g, 3.6 mmol) in dry *N*,*N*-dimethylacetamide for 3 days as described for the synthesis of **17b**. Compound **17a** was obtained in 36% yield (0.50 g) as a dihydrate: mp 233 °C dec [lit.[1] mp 262 °C dec, an anhydrous sample; ^{1}H NMR (Me_2SO-d_6) δ 1.16 (6 H, br t, CH_2CH_3), 2.05 (2 H, deformed dt, $CHCH_2CH_2CO$), 2.41 (2 H, t, CH_2CH_2CO), 4.07 (4 H, m, CH_2CH_3), 4.32 (3 H, m, CH_2NH, CHNH), 6.63 (2 H, d, Ph), 6.76 (2 H, br s, NH_2), 7.66 (2 H, d, Ph), 7.93 (2 H, br s, NH_2), 8.24 (1 H, d, CONH), 8.47 (1 H, d, 5-H, $J_{5,7} \sim 2.0$ Hz), 8.66 (1 H, d, 7-H, $J_{5,7} \sim 2.0$ Hz). These data are very similar to those reported, except for the chemical shifts for dissociable protons. Anal. C, H, N.

***N*-[*p*-[[(2,4-Diamino-5-methylpyrido[2,3-*d*]pyrimidin-6-yl)methyl]amino]benzoyl]-L-glutamic Acid (1b) (L-5-Deaza-5-methylaminopterin).** Compound **13** (1.61 g, 3.16 mmol) was dissolved in a mixture of MeOH (400 mL) and 1 N NaOH (7 mL), and the solution was stirred for 3 days at room temperature. After concentration in vacuo to ~7 mL, the concentrated solution was neutralized with 1 N HCl (7 mL). Compound **2**, 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: 1.21 g (85%); mp 235–237 °C dec; ^{1}H NMR (Me_2SO-d_6) δ 2.09–2.30 (2 H, br m, $CH_2CH_2CO_2H$), 2.40–2.50 (2 H, br m, $CH_2CH_2CO_2H$), 2.56 (3 H, s, Me), 4.30 (3 H, br m, CH_2NH and CONHCH), 6.61 (1 H, br, CH_2NH, exchangeable), 6.65 (2 H, d, Ph), 7.27 (2 H, br, NH_2, exchangeable), 7.68 (2 H, d, Ph), 8.04 (3 H, br, NH_2 and CONH, exchangeable), 8.40 (1 H, s, H-7). See Table III for UV data. Anal. C, H, N.

In a similar manner, **1a** was obtained as a yellow powder (0.2 g, 45%) from **17a** (0.5 g, 1.0 mmol); mp >220 °C dec. See Table III for UV data. ^{1}H NMR (Me_2SO-d_6) δ 2.05 (m, CH_2CH_2CO), 2.38 (t, CH_2CO), 4.33 (m, CHN, CH_2N), 6.52 (d, Ph), 7.05 (d, Ph), 6.69 (NH_2), 7.61 (NH_2), 8.26 (m, NH, CO_2H), 8.40 (br s, 5-H), 8.70 (br s, 7-H). Anal. C, H, N.

2-Amino-6-(hydroxymethyl)-5-methyl-4(3*H*)-oxopyrido[2,3-*d*]pyrimidine (24). Compound **15b** (2.0 g, 7.2 mmol) in 1 N NaOH (100 mL) was heated under reflux for 4 h. The solution was cooled, a small amount of insoluble impurities removed by filtration, and the filtrate neutralized to pH 7 with 2 N HCl. Compound **24** precipitated as microcrystals was collected by filtration, washed thoroughly with H_2O, Me_2CO, and Et_2O, and dried in vacuo over P_2O_5: 1.21 g (82%); mp >340 °C; UV λ_{max} (H_2O) 218 nm (ϵ 7.82×10^3), 271 (3.16×10^3), 318 (1.98×10^3), λ_{min} (H_2O) 249 (1.85×10^3), 290.5 (1.12×10^3), λ_{max} (0.1 N HCl) 215 (2.23×10^4), 243 (1.47×10^4, sh), 279 (1.35×10^4), 350 (9.69×10^3), λ_{min} (0.1 N HCl) 255.5 (5.16×10^3), 295.5 (1.90×10^3), λ_{max} (0.1 N NaOH) 216 (4.74×10^4), 241 (1.99×10^4), 271 (8.72×10^3), 332 (7.64×10^3), λ_{min} (0.1 N NaOH), 230.5 (1.87×10^4), 259.5 (7.25×10^3), 290.5 (1.86×10^3). Anal. C, H, N.

2-Amino-6-(bromomethyl)-5-methylpyrido[2,3-*d*]pyrimidin-4(3*H*)-one (25). A suspension of **24** (1.03 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 toluene. The residue was triturated with Et_2O and the solid collected and dried over P_2O_5 in vacuo. This bromide was not stable and, thus, was used directly in the next step.

Diethyl *N*-[*p*-[[(2-Amino-5-methyl-4(3*H*)-oxopyrido[2,3-*d*]pyrimidin-6-yl)methyl]amino]benzoyl]-L-glutamate (26). To a solution of **25** (prepared from 1.03 g of **24**) in dry *N*,*N*-dimethylacetamide (30 mL, distilled over CaH_2) was added diethyl (*p*-aminobenzoyl)-L-glutamate (2.42 g, 7.5 mmol), and the mixture was stirred at room temperature for 3 days. After concentration of the mixture in vacuo, the residue was triturated several times with warm $CHCl_3$ to remove unreacted diethyl (*p*-aminobenzoyl)-L-glutamate. The residue was then precipitated with Et_2O, and microcrystals were collected and dried in vacuo to give 1.90 g (75%) of **26**: mp >320 °C; ^{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, $CH_2CH_2CO_2Et$), 2.52 (3 H, s, 5-Me), 4.03 (2 H, q, CH_2Me), 4.08 (2 H, q, CH_2Me), 4.33–4.54 (3 H, br m, CH_2NH, CONHCH), 6.65 (2 H, d, Ph), 6.65–6.68 (3 H, br, NH_2, CH_2NH, exchangeable), 7.64 (2 H, d, Ph), 7.98 (1 H, s, H-7), 8.11 (1 H, br, NH). Anal. C, H, N.

***N*-[*p*-[[(2-Amino-5-methyl-4(3*H*)-oxopyrido[2,3-*d*]pyrimidin-6-yl)methyl]amino]benzoyl]-L-glutamic Acid (2).** A solution of **26** (1.80 g, 3.5 mmol) in 0.1 N NaOH (200 mL) was heated to 80 °C under N_2 and cooled to room temperature; insoluble impurities were filtered off, and the filtrate was acidified with 3 N HCl to pH 3. The crystalline precipitates were collected

by filtration, washed with water, EtOH, and Et_2O, and dried in vacuo over P_2O_5 to afford 1.12 g (70%) of **2**: mp >300 °C; ^1H 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, $CH_2CH_2CO_2H$), 2.54 (3 H, s, 5-Me), 4.36 (3 H, m, CH_2NH_2 and CONHCH), 6.61 (2 H, d, Ph), 6.97 (3 H, br, NH_2 and CH_2NH, exchangeable), 7.65 (2 H, d, Ph), 8.00 (1 H, s, H-7), 8.08 (1 H, br, NH, exchangeable), 8.17 (1 H, br, NH, exchangeable); UV λ_{max} (H_2O) 216.5 nm (ε 2.86 × 10^4), 280.5 (1.86 × 10^4), 302 (sh, 1.56 × 10^4), 342 (sh, 5.49 × 10^3). Anal. C, H, N.

3-Cyano-5-(ethoxycarbonyl)-4-methylpyridine-2(1*H*)-thione (18). A mixture of cyanothioacetamide (30.05 g, 0.3 mol), ethyl β-(ethoxymethylene)acetoacetate[21] (52.8 g, 0.3 mol) and 2-(dimethylamino)ethanol (2 mL) in anhydrous EtOH (400 mL) was heated under reflux for 90 min, and then the mixture was cooled in a ice bath. The solid precipitates, collected by filtration, were extracted with boiling $CHCl_3$ (6 × 500 mL). The $CHCl_3$ extracts were concentrated in vacuo, and the residue was crystallized from $CHCl_3$/EtOH (10:1) to afford 29.3 g (41%) of **18** as yellow needles: mp 232–233 °C; IR (KBr) 2230 (CN), 1680 cm^{-1} (ester); ^1H NMR (Me_2SO-d_6) δ 1.30 (3 H, t, CH_2Me), 2.69 (3 H, s, 4-Me), 4.25 (2 H, q, CH_2Me), 8.33 (1 H, s, H-6). Anal. C, H, N, S.

Ethyl 3-Cyano-4-methyl-2-(methylthio)nicotinate (19). A mixture of **18** (22.3 g, 0.1 mol), K_2CO_3 (30 g, 0.22 mol), and MeI (28.4 g, 0.2 mol) in dry DMF (150 mL) was stirred at room temperature for 3 h and then diluted with cold water (500 mL). The yellow precipitates were collected by filtration and crystallized from $CHCl_3$/EtOH to give 23.1 g (98%) of **19** as pale yellow needles: mp 134–135 °C; IR (KBr) 2220 (CN), 1720 cm^{-1} (ester); ^1H NMR (Me_2SO-d_6) δ 1.40 (3 H, t, CH_2Me), 2.67 (3 H, s, 4-Me), 2.88 (3 H, s, SMe), 4.94 (2 H, q, CH_2Me), 8.31 (1 H, s, H-6). Anal. C, H, N, S.

3-Cyano-5-(hydroxymethyl)-4-methyl-2-(methylthio)-pyridine (20). To a stirred suspension of **19** (47.2 g, 0.2 mol) in dry ether (1 L) was added portionwise $LiAlH_4$ (11.4 g, 0.3 mol) at −15 to −10 °C. The mixture was stirred at −10 °C for 3 h, and then excess $LiAlH_4$ was destroyed with 1 N HCl. Cold water (500 mL) was added to the mixture and the ethereal layer was separated. The aqueous layer was extracted with AcOEt (3 × 300 mL). The combined organic extracts were washed with water, dried (Na_2SO_4), and concentrated, and the residue was chromatographed over a silica gel column (50 × 8 cm) with $CHCl_3$ as the eluent that eluted the unreacted **19** (5.2 g). The column was then washed with $CHCl_3$/MeOH (50:1, v/v) to elute **20** that was obtained as colorless crystals after concentration of the solvent and recrystallization of the residue from EtOH: 16.3 g (50%); mp 117–118 °C; IR (KBr) 2220 cm^{-1} (CN); ^1H NMR (Me_2SO-d_6) δ 2.50 (3 H, s, Me), 2.60 (3 H, s, Me), 4.49 (2 H, d, CH_2OH, collapsed to a singlet upon addition of D_2O), 5.41 (1 H, t, CH_2OH, exchangeable), 7.98 (1 H, s, H-6). Anal. C, H, N, S.

3-Cyano-5-[(methoxymethoxy)methyl]-4-methyl-2-(methylthio)pyridine (21). A solution of **20** (44.01 g, 0.27 mol) and *N*,*N*-dimethylaniline (80.6 g, 0.54 mol) in dry $CHCl_3$ (500 mL) was treated with CH_3OCH_2Cl (43.2 g, 0.54 mol) for 5 h at room temperature. The mixture was successively washed with 2% HCl (4 × 200 mL), saturated $NaHCO_3$, and water, then dried (Na_2SO_4), and concentrated. The residue was crystallized from *n*-C_6H_{14}/ether to afford **21**: 54.1 g (84%); mp 50–51 °C; IR (KBr) 2220 cm^{-1} (CN); ^1H NMR (Me_2SO-d_6) δ 2.55 (3 H, s, Me), 2.62 (3 H, s, Me), 3.40 (3 H, s, OMe), 4.53 (2 H, s, CH_2), 4.71 (2 H, s, CH_2), 7.76 (1 H, s, H-6). Anal. C, H, N, S.

3-Cyano-5-[(methoxymethoxy)methyl]-4-methyl-2-(methylsulfonyl)pyridine (22). A mixture of **21** (54.0 g, 0.23 mol) and *m*-chloroperbenzoic acid (118 g, 0.68 mol) in EtOH (600 mL) was stirred for 1 h at room temperature and then concentrated in vacuo. The residue was dissolved in AcOEt (800 mL), and the solution was washed (2% NaOH and water), dried (Na_2SO_4), and concentrated. The residue was crystallized from ether to give 45.9 g (75%) of **22**: mp 64–65 °C; IR (KBr) 2250 cm^{-1} (CN); ^1H NMR ($CDCl_3$) δ 2.63 (3 H, s, Me), 3.35 (3 H, s, Me), 3.42 (3 H, s, Me), 4.67 (2 H, s, CH_2), 4.80 (2 H, s, CH_2), 8.27 (1 H, s, H-6). Anal. C, H, N, S.

2,4-Diamino-6-[(methoxymethoxy)methyl]-5-methylpyrido[2,3-*d*]pyrimidine (23). A mixture of **22** (5.40 g, 20 mmol) and guanidine carbonate (3.60 g, 20 mmol) in Ph_2O (20 mL) was located 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, dissolved in EtOH/H_2O (5:1, 300 mL), and decolorized (Norit A), and the solution was concentrated to ~150 mL. Colorless crystals deposited were collected by filtration, washed with EtOH and Et_2O, and dried to afford 3.34 g (67%) of **23**: mp 273–274 °C; ^1H NMR (Me_2SO-d_6) δ 2.49 (3 H, s, Me), 3.32 (3 H, s, Me), 4.52 (2 H, s, CH_2), 4.66 (2 H, s, CH_2), 6.21 (2 H, br, NH_2, exchangeable), 7.40 (2 H, br, NH_2, exchangeable), 8.23 (1 H, s, H-7); UV λ_{max} (H_2O) 223 nm (ε 3.35 × 10^4), 245 (1.94 × 10^4, sh), 2.72 (8.41 × 10, sh), 317.5 (9.63 × 10^3, sh), 327.5 (9.97 × 10^3), λ_{min} (H_2O) 290 (4.10 × 10^3), λ_{max} (0.1 N NaOH) 221 (3.30 × 10^4), 247 (2.33 × 10^4), 272 (1.06 × 10^4), 340 (9.17 × 10^4), λ_{min} (0.1 N NaOH) 235.5 (2.28 × 10^4), 264 (9.89 × 10^3) 294 (7.88 × 10^3), λ_{max} (0.1 N HCl) 223 (3.47 × 10^4), 245 (1.63 × 10^4, sh), 271 (5.93 × 10^3, sh), 3.16 (1.05 × 10^4), 326 (9.76 × 10^3), λ_{min} (0.1 N HCl) 287.5 (4.46 × 10^3). Anal. C, H, N.

2,4-Diamino-6-(hydroxymethyl)-5-methylpyrido[2,3-*d*]-pyrimidine (15b). A mixture of **23** (17.21 g, 69 mmol) and concentrated HCl (20 mL) in MeOH (800 mL) was heated at 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 over P_2O_5 in vacuo to afford **15b**: 16.32 g (85%); mp >340 °C. The ^1H NMR spectrum of this product was identical with that of **15b** prepared earlier by the ring-transformation reaction.

Acknowledgment. This investigation was supported in part by funds from the National Cancer Institute and U.S. Department of Health and Human Services, Grants CA-08748 and CA-18856.

(21) Yasuda, H. *Yakugaku Zasshi* **1959**, *79*, 836.