3.54 (d, 1 H, J = 5 Hz), 1.64 (d, 3 H, J = 7 Hz); ^{13}C NMR ($CDCl_3$) δ 144.8, 138.2, 137.0, 133.6, 129.0, 125.9, 124.7, 123.7, 120.9, 114.5, 108.7, 62.5, 21.7; UV (95% EtOH) λ_{max} 222 nm, 251.

Anal. Calcd for $C_{16}H_{15}NO_3S$: C, 63.77; H, 5.02; N, 4.64; S, 10.64. Found: C, 63.69; H, 5.06; N, 4.59; S, 10.61.

1-(Phenylsulfonyl)-2,3-diiodoindole (13). To a solution of lithium diisopropylamide prepared from diisopropylamine (0.68 g, 6.69 mmol) and *n*-butyllithium (1.58 M in hexane; 3.83 mL, 6.05 mmol) in dry THF (30 mL) under argon at −78 °C was added via syringe over 3 min a solution of **6** (2.25 g, 5.87 mmol) in dry THF (25 mL). After being stirred for 1.5 h at −78 °C, the golden yellow solution was treated dropwise over 4 min with a solution of iodine (1.91 g, 7.53 mmol) in dry THF (20 mL) and the mixture was allowed to warm slowly to room temperature overnight. The reaction mixture was cooled to 0–5 °C and treated with 5% aqueous sodium thiosulfate (200 mL) and CH_2Cl_2 (200 mL). The layers were separated, and the organic phase was washed again with sodium thiosulfate (150 mL). The combined aqueous portions were extracted with CH_2Cl_2 (2 × 100 mL) and the combined organic extracts were washed with water (2 × 150 mL) and brine (2 × 150 mL), dried (Na_2SO_4), and evaporated in vacuo to give 3.35 g of crude **13** as a light-tan crystalline solid. Column chromatography over Florisil with 1:1 ether–hexane afforded 2.93 g (98%) of analytically pure **13** as a colorless crystalline solid: mp 166–167 °C; IR ($CHCl_3$) 1585 (m), 1450 (s), 1435 (s), 1380 (s), 1265 (s), 1180 (m), 1140 (s), 1085 (s), 1020 (s), 935 (m), 675 (m), 585 cm^{-1} (m); ^{1}H NMR ($CDCl_3$) δ 8.28–7.73 (m, 3 H), 7.58–7.05 (m, 6 H); ^{13}C NMR ($CDCl_3$) δ 138.0, 137.8, 134.1, 133.4, 129.1, 127.1, 125.8, 124.6, 122.5, 115.5, 90.4, 88.5; UV (95% EtOH) λ_{max} 210 nm, 266.

Anal. Calcd for $C_{14}H_9NO_2SI_2$: C, 33.03; H, 1.78; N, 2.75; S, 6.30; I, 49.85. Found: C, 33.04; H, 1.81; N, 2.73; S, 6.25; I, 49.85.

Acknowledgment. This investigation was supported by Grant CH-200 from the American Cancer Society, and in part by PHS Grant CA-24422, awarded by the National Cancer Institute, DHHS, by Merck Sharp and Dohme Research Laboratories, and by Biomedical Research Support Grants RR-05392 and RR-07056 from the Biomedical Research Support Branch, Division of Research Facilities and Resources, National Institutes of Health.

Registry No. **1**, 80360-13-0; **2**, 40900-03-6; **4**, 120-72-9; **5**, 26340-47-6; **6**, 80360-14-1; **7**, 18344-49-5; **8**, 40899-71-6; **9**, 70957-04-9; **10a**, 58550-84-8; **10b**, 80360-15-2; **10c**, 80360-16-3; **10d**, 80360-17-4; **10e**, 80360-18-5; **10f**, 80360-19-6; **10g**, 80360-20-9; **11a**, 80360-21-0; **11b**, 80360-22-1; **11c**, 40899-92-1; **11d**, 80360-23-2; **11e**, 80360-24-3; **12**, 80360-25-4; **13**, 80360-26-5; **14**, 1796-25-4; **15**, 80360-27-6; CH_3I, 74-88-4; $ClCO_2Et$, 541-41-3; PhCHO, 100-52-7; $(CH_3)_3SiCl$, 75-77-4; $(PhS)_2$, 882-33-7; PhCOCl, 98-88-4; $HCON(CH_3)_2$, 68-12-2; CH_3CHO, 75-07-0; $PhSO_2Cl$, 98-09-9; Me_3CCOCl, 3282-30-2; I_2, 7553-56-2.

Supplementary Material Available: Detailed experimental procedures for the compounds listed in Table I (12 pages). Ordering information is given on any current masthead page.

Pyrido[2,3-*d*]pyrimidines. Synthesis of the 5-Deaza Analogues of Aminopterin, Methotrexate, Folic Acid, and N^{10}-Methylfolic Acid

Carroll Temple, Jr.,* Robert D. Elliott, and John A. Montgomery

Kettering-Meyer Laboratory, Southern Research Institute, Birmingham, Alabama 35255

Received September 18, 1981

Reaction of bromoacetic acid with *N,N*-dimethylformamide and phosphorus oxychloride gave a triformylmethane derivative, which was condensed with 2,4-diaminopyrimidin-6(1*H*)-one (**2**) in water at reflux to give 2-amino-4(3*H*)-oxopyrido[2,3-*d*]pyrimidine-6-carboxaldehyde (**4**). The structure of **4** was confirmed by conversion to the 2,4-dinitrophenylhydrazone and oxidation to the known 6-carboxylic acid (**6**). Similarly, condensation of **1** with 2,4,6-triaminopyrimidine gave 2,4-diaminopyrido[2,3-*d*]pyrimidine-6-carboxaldehyde (**5**). Reductive alkylation of diethyl (*p*-aminobenzoyl)-L-glutamate (**9**) with **5** in 70% acetic acid over Raney nickel gave diethyl *N*-[4-[[(2,4-diaminopyrido[2,3-*d*]pyrimidin-6-yl)methyl]amino]benzoyl]-L-glutamate (**10**), which was saponified with base to give the corresponding glutamic acid **11** (5-deazaaminopterin). The latter was methylated with formaldehyde and sodium cyanoborohydride to give 5-deazamethotrexate (**12**). Reductive alkylation of **9** with **4** gave diethyl *N*-[4-[[(2-amino-4(3*H*)-oxopyrido[2,3-*d*]pyrimidin-6-yl)methyl]amino]benzoyl]-L-glutamate (**13**), which was converted to the corresponding glutamic acid **14** (5-deazafolic acid). The preferred route for the preparation of **14** involved the hydrolysis of **10** with base at reflux, which resulted in replacement of the 4-amino group and saponification of the ester groups. Methylation of **14** with formaldehyde and sodium cyanoborohydride gave 5-deaza-10-methylfolic acid (**15**), which was also prepared by alkaline hydrolysis of the 4-amino group of **12**.

Aminopterin and methotrexate are folic acid antagonists that inhibit the enzyme dihydrofolate reductase.[1] In addition, quinazoline (5,8-dideazapteridine) analogues of folic acid, aminopterin, and methotrexate have been identified as potent inhibitors of both dihydrofolate reductase and thymidylate synthetase.[2] In another series, 2,4-diaminopyrido[2,3-*d*]pyrimidines (5-deazapteridines) are known to be dihydrofolate reductase inhibitors.[3] The synthesis of 5-deazafolic acid via a condensation reaction involving triformylmethane has been reported,[4] and the preparation of 5-deazaaminopterin via a long sequence of reactions involving the elaboration of either a pyridine[5]

(1) Blakley, R. L. *Front. Biol.* **1969**, *13*, 157, 172.
(2) Calvert, A. H.; Jones, T. R.; Dady, P. J.; Grzelakowska, B.; Paine, R. M.; Taylor, G. A.; Harrap, K. R. *Eur. J. Cancer* **1980**, *16*, 713. Scanlon, K. J.; Moroson, B. A.; Bertino, J. R.; Hynes, J. B., *Mol. Pharmacol.* **1979**, *16*, 261. Bird, O. D.; Vaitkus, J. W.; Clarke, J. *Ibid.* **1970**, *6*, 573. Jones, T. R.; Calvert, A. H.; Jackman, A. L.; Brown, S. J.; Jones, M.; Harrap, K. R. *Eur. J. Cancer* **1981**, *17*, 11.
(3) Irwin, W. J.; Wibberley, D. G. *Adv. Heterocycl. Chem.* **1969**, *10*, 149. Grivsky, E. M.; Lee, S.; Sigel, C. W.; Duch, D. S.; Nichol, C. A. *J. Med. Chem.* **1980**, *23*, 327.
(4) Hurst, D. T. "An Introduction to the Chemistry and Biochemistry of Pyrimidines, Purines, and Pteridines"; Wiley: New York, 1980; p 231. Tseng, C.-P. *Diss. Abstr. Int. B* **1980**, *40*, 3752.
(5) Elslager, E. F.; Davoll, J. *Lect. Heterocycl. Chem.* **1974**, *2*, S-97.

0022-3263/82/1947-0761$01.25/0

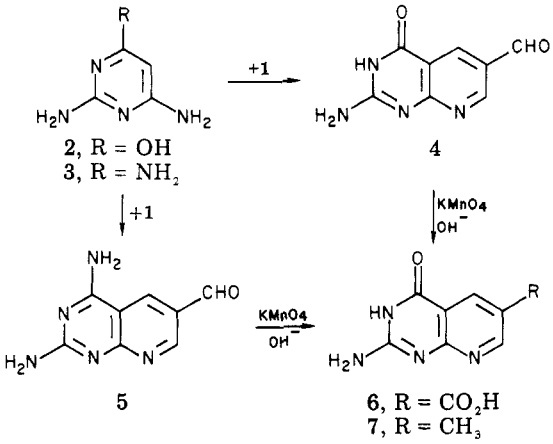
or pyrimidine[6] intermediate has been described. Recently the synthesis of 5-deaza-5(8*H*)-oxoaminopterin[7] and 5-deaza-10-formylfolic acid was reported.[8] The latter was shown to be an inhibitor of AICAR transformylase (EC 2.1.2.3). In this paper we describe the preparation of the 5-deaza analogues of folic acid, aminopterin, and their N^{10}-methyl derivatives.

In one of the methods reported by Arnold for the preparation of triformylmethane, bromoacetic acid was treated with the *N,N*-dimethylformamide–phosphorous oxychloride complex to give an intermediate quaternary salt (1), which was hydrolyzed with aqueous potassium carbonate.[9] The condensation of triformylmethane with several 4-aminopyrimidines was reported to give 5-deaza-6-formylpteridines.[10] However, the isolation of triformylmethane was laborious, and methods were sought for the conversion of the methyl group of 2-amino-6-methyl-4(3*H*)-oxopyrido[2,3-*d*]pyrimidine (**7**)[11] to a reactive group (e.g., bromomethyl), which would allow coupling with (*p*-aminobenzyl)-L-glutamic acid. This work and that of others[12a] was unsuccessful. As a result we have investigated the use of crude **1** or its partially hydrolyzed derivatives in condensation reactions with 4-aminopyrimidines.

$$\mathrm{CH[CH{=}N^{+}(CH_3)_2]_2{\cdot}3Cl^{-}} \xrightarrow{OH^-} \mathrm{CH(CHO)_3}$$
1

By analogy with the condensations of 4-aminopyrimidines with malondialdehyde derivatives to give pyrido[2,3-*d*]pyrimidines,[12] the reaction of **1** with 2,4-diaminopyrimidin-6(1*H*)-one (**2**) in water at reflux gave 2-amino-4(3*H*)-oxopyrido[2,3-*d*]pyrimidine-6-carboxaldehyde (**4**). The presence of a formyl group in this product was confirmed by condensation with 2,4-dinitrophenylhydrazine to give the corresponding 2,4-dinitrophenylhydrazone **8**. In addition, oxidation of **4** with alkaline potassium permanganate gave the known 2-amino-4-(3*H*)-oxopyrido [2,3–*d*]pyrimidine-6-carboxylic acid (**6**).[12,13] An authetic sample of **6** was prepared by alkaline potassium permanganate oxidation of 2-amino-6-methyl-4-(3*H*)-oxopyrido[2,3-*d*]pyrimidine (**7**), which was synthesized by the method of Stark and Breitmaier.[11]

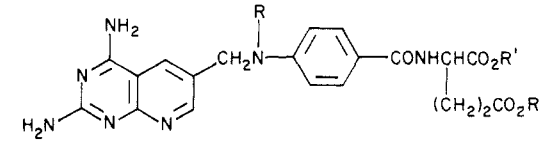
Although the mechanism of the condensation reaction is unknown, two of the formyl groups or potential formyl groups of **1** must react with the enamine moiety of the 4-aminopyrimidine with the elimination of either water or dimethylamine. The initial reaction involves the electrophilic attack of one formyl group or derivative either with the 5-position of the pyrimidine ring or with the 4-amino group to give a Schiff base followed by cyclization of the resulting monocyclic intermediate to give the desired bicyclic ring system. Price and co-workers have observed that pyrido[2,3-*d*]pyrimidines were readily formed from 4-aminopyrimidines and malondialdehydes containing electron-withdrawing groups.[12a] Compound **1** can be considered a malondialdehyde derivative substituted with



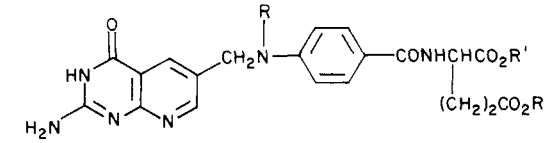
an electron-withdrawing group, which is substantiated by the condensation of **1** with **2** to give **4** under mild conditions.

Under conditions that were used for the preparation of **4**, the condensation of **1** with 2,4,6-triaminopyrimidine (**3**) gave 2,4-diaminopyrido[2,3-*d*]pyrimidine-6-carboxaldehyde (**5**). The structure of **5** was confirmed by the alkaline potassium permanganate oxidation of the formyl group and hydrolysis of the 4-amino group to give **6**. It has been established that in the 2,4-diaminopyrido[2,3-*d*]pyrimidine ring system, the 4-amino function undergoes alkaline hydrolysis readily.[14]

Reductive alkylation of diethyl (*p*-aminobenzoyl)-L-glutamate (**9**) with **5** and hydrogen in 70% acetic acid containing Raney nickel gave a 32% yield of 5-deazaaminopterin diethyl ester (**10**). Saponification of the ester groups of **10** in a mixture of dimethyl sulfoxide–water at ambient temperature gave an 87% yield of 5-deazaaminopterin (**11**). Methylation of the latter was accomplished by treatment of **11** with formaldehyde and sodium cyanoborohydride in aqueous solution at pH 6.4 to give an 85% yield of 5-deazamethotrexate (**12**). The structure of **12** was established by oxidation with alkaline potassium permanganate to give the previously prepared 2-amino-4(3*H*)-oxopyrido[2,3-*d*]pyrimidine-6-carboxylic acid (**6**), which indicated that methylation had occurred either on the 4- or 10-amino group. Methylation of the 4-amino group was eliminated from consideration by alkaline hydrolysis of the 4-amino group to give 5-deaza-10-methylfolic acid (**15**).



10, R = H; R′ = Et
11, R = R′ = H
12, R = Me; R′ = H



13, R = H; R′ = Et
14, R = R′ = H
15, R = Me; R′ = H

The reductive alkylation of **9** with **4** and hydrogen in acetic acid containing Raney nickel gave a 44% yield of

(6) Taylor, E. C.; Palmer, D. C.; Fletcher, S. R.; Harrington, P. J., 8th International Congress of Heterocyclic Chemistry, Karl-Franzens Universitat, Graz, Austria, 1981.
(7) Srinivasan, A.; Broom, A. J. *J. Org. Chem.* **1980**, *45*, 3746.
(8) Smith, G. K.; Mueller, W. T.; Benkovic, P. A.; Benkovic, S. J. *Biochemistry* **1981**, *20*, 1241.
(9) Arnold, A. *Collect. Czech. Chem. Commun.* **1961**, *26*, 3051.
(10) Temple, C., Jr.; Montgomery, J. A. "Synthesis of Potential Anticancer Agents", Cancer Chemotherapy National Service Center, Southern Research Institute Report 85, 1966; Report 86, 1967.
(11) Stark, E.; Breitmaier, E. *Tetrahedron* **1973**, *29*, 2209.
(12) (a) Bernetti, R.; Mancini, F.; Price, C. C. *J. Org. Chem.* **1962**, *27*, 2863. (b) Herlbert, B. S.; Valenti, B. F. *J. Med. Chem.* **1968**, *11*, 708.
(13) Mulvery, D. M.; Cottis, S. G.; Tieckelmann, H. *J. Org. Chem.* **1964**, *29*, 2903.
(14) Tratner, R.; Elion, G.; Hitchings, G.; Sharefkin, D. *J. Org. Chem.* **1964**, *29*, 2674.

diethyl 5-deazafolate (13) of 78% purity as determined by high-pressure liquid chromatography. The structure proof for 13 is based on mass spectral data and base hydrolysis of the ester groups to give 5-deazafolic acid 14. The preferred route for the preparation of 14 involved the hydrolysis of 10 in aqueous sodium hydroxide at reflux temperature, which resulted in replacement of the 4-amino group as well as hydrolysis of the ester functions to give a 79% yield of 5-deazafolic acid (14). In addition, methylation of 14 with formaldehyde and sodium cyanoborohydride gave an 84% yield of 15, which was identical with that prepared by the alkaline hydrolysis of 12. For estimation of the extent of racemization, if any, in the base hydrolysis of 10 to 14 the rotation of a solution of *N*-(*p*-aminobenzoyl)-L-glutamic acid in 1 N NaOH was determined before and after heating at reflux temperature for 4 h. There was no decrease in optical activity within the limits of experimental error.

Preliminary results indicated that the 5-deazapteridines 10–12, 14, and 15 have significant biological activity in cell culture experiments. Work is also in progress on the preparation of derivatives of these compounds, and the results of these investigations will be reported at a later date.

Experimental Section

Melting points were determined on a Kofler Keizbank unless otherwise indicated. Absence of melting-point data indicates an indefinite melting point. The ultraviolet absorption spectra were determined with a Carey Model 17 spectrophotometer. The ^{1}H NMR spectra were determined with a Varian XL-100-15 spectrometer operating at 100 MHz (internal Me_4Si or DSS). Chemical shifts quoted in the case of multiplets are measured from the approximate center. Mass spectral data were taken with a Varian MAT 311A instrument equipped with a combination EI/FI/FD ion source. High-pressure liquid chromatography (HPLC) was carried out on an ALC-242 liquid chromatograph equipped with a UV detector (254 nm), an M-6000 pump, and a 30 cm × 4 mm (i.d.) column of Bondapak C_{18} (Waters Associates, Inc.). The ultraviolet spectra of eluted peaks were determined with an interfaced Beckman Model 25 spectrophotometer. The solvent systems used were the following: A, pH 3.6, 0.1 M $^-$OAc–MeCN (9:1); B, a linear gradient of the above solvent going from 9:1 to 1:1 over a period of 20 min.

2-Amino-4(3*H*)-oxopyrido[2,3-*d*]pyrimidine-6-carboxaldehyde (4). Phosphorus oxychloride (27.5 mL, 46.0 g, 300 mmol) was added with stirring over 25 min to *N*,*N*-dimethylformamide (110 g, 1.50 mol), which was cooled with an ice bath. After being stirred at room temperature for 1 h, the reaction mixture was treated with bromoacetic acid (13.9 g, 100 mmol). The resulting solution, protection with a calcium chloride tube, was heated at 90 °C for 10 h and evaporated to dryness in vacuo. The dark brown oil (~60 g) was dissolved in H_2O (300 mL) and neutralized with 50% sodium hydroxide to pH 7. After the addition of 2,4-diaminopyrimidin-6(1*H*)-one hydrate (2; 6.00 g, 41.7 mmol), the mixture was refluxed for 3 h and filtered hot to give the product: yield, 6.50 g (82%); mass spectrum, *m/e* 190 (M^+). HPLC [0.1 M NH_4OAc (pH 3.6)–CH_3CN (9:1)] chromatograms indicated that this sample was 76% VI. A sample (1.00 g) was dissolved in 4 N HCl (15 mL), concentrated to one-half volume, and cooled, and the solid that precipitated was removed by filtration. The filtrate was evaporated to dryness in vacuo, and the residue was recrystallized from a mixture of water and ethanol: yield, 115 mg; mp 300 °C; UV λ_{max} (0.1 N HCl) 276 nm ($10^{-3}\epsilon$ 10.9), 301 (8.75), 348 (6.20); UV (pH 7) 265 nm ($10^{-3}\epsilon$ 8.91), 308 (12.5); UV (0.1 N NaOH) nm 252 (sh, $10^{-3}\epsilon$ 13.7), 256 (13.8), 339 (12.6).

Anal. Calcd for $C_8H_6N_4O_2 \cdot 0.6C_2H_6O \cdot 0.3H_2O$: C, 49.50; H, 4.61; N, 25.10. Found: C, 49.34; H, 4.34; N, 25.02.

2,4-Diaminopyrido[2,3-*d*]pyrimidine-6-carboxaldehyde (5). Phosphorus oxychloride (27.5 mL, 46.0 g, 300 mmol) was added over 15 min with stirring to *N*,*N*-dimethylformamide (11.0 g, 150 mmol), which was cooled with an ice bath. After being stirred at room temperature for 1 h, the reaction mixture was treated with bromoacetic acid (13.9 g, 100 mmol). The resulting solution, protection by a calcium chloride tube was heated at 92 °C for 10 h and evaporated to dryness in vacuo. The colored oil (~30 g) was dissolved in H_2O (1000 mL), and the solution was neutralized with 50% sodium hydroxide to pH 7. After the addition of 2,4,6-triaminopyrimidine (3; 5.00 g, 40.0 mmol), the solution was refluxed for 3 h and filtered hot through a fluted filter. The filtrate was cooled and the solid that precipitated was collected by filtration and dried in vacuo over P_2O_5: yield, 2.53 g (33%); mass spectrum, *m/e* 189 (M^+). HPLC [0.1 M NH_4OAc (pH 3.6)–CH_3OH (9:1)] indicated that this product was 86% pure. A sample (200 mg) was dissolved in 0.1 N HCl (15 mL) and diluted with acetone (225 mL) to precipitate impure 5: yield, 91 mg. The filtrate was evaporated to dryness under reduced pressure and the residue was dried in vacuo over P_2O_5 to give 5: yield, 128 mg; mp, gradual darkening and decomposition with white sublimate when taken to 360 °C; UV λ_{max} (0.1 N HCl) 258 nm ($10^{-3}\epsilon$ 16.4), 317 (9.12), 326 (sh, 8.42); UV (pH 7) 263 nm ($10^{-3}\epsilon$ 15.0), 316 (10.1), 345 (10.8); UV (0.1 N NaOH) 254 nm ($10^{-3}\epsilon$ 13.2), 267 (13.5), 316 (8.56), 347 (10.0); ^{1}H NMR (CF_3CO_2D, 6% w/v) δ 9.48 (s), 9.75 (s, 5-CH, 7-CH), 10.21 (s, 6-CHO).

Anal. Calcd for $C_8H_7N_5O \cdot HCl \cdot 1.3H_2O$: C, 38.57; H, 4.30; N, 28.12. Found: C, 38.44; H, 4.15; N, 28.14.

2-Amino-4(3*H*)-oxopyrido[2,3-*d*]pyrimidine-6-carboxylic Acid (6). Method A. To a solution of 7 (177 mg, 1.00 mmol) in 1 N NaOH (60 mL) at reflux temperature was added with stirring an aqueous solution of 0.2 M potassium permanganate over a period of about 1 h. After the excess permanganate was destroyed with sodium bisulfite, the resulting hot mixture was filtered (Celite). The filtrate was adjusted to pH 3 with HCl and allowed to stand at room temperature for 18 h. The solid that precipitated (170 mg) was collected by filtration and dissolved in 2 N NaOH, and the solution was cooled to deposit the sodium salt of the product. The salt was collected by filtration and dissolved in H_2O, and the solution was adjusted to pH 2–3 with HCl. The solid that deposited was collected by filtration and dried in vacuo over P_2O_5: yield, 67 mg (29%); mp 264 °C; HPLC [0.1 M Na_2HPO_4 (pH 7)–CH_3CN (92:8)] showed that this sample was homogeneous; UV λ_{max} (0.1 N HCl) 216 nm ($10^{-3}\epsilon$ 35.9), 266 (14.5), 306 (6.70), 315 (sh, 5.35); UV (pH 7) 216 nm ($10^{-3}\epsilon$ 26.8), 232 (sh, 17.8), 283 (11.4), 310 (sh, 5.93), 321 (sh, 5.37); UV (0.1 N NaOH) 246 nm ($10^{-3}\epsilon$ 22.8), 292 (10.1), 332 (7.20); ^{1}H NMR (NaOD, 5% w/v) δ 8.76 (d, 7-CH, J = 1.5 Hz), 9.06 (d, 5-CH).

Anal. Calcd for $C_8H_6N_4O_3 \cdot 0.6HCl$: C, 42.13; H, 2.92; N, 24.57. Found: C, 42.04; H, 2.80; N, 24.41.

Method B. Oxidation of 4 (180 mg, 0.950 mmol) as described in method A and acidification of the resulting solution to pH 3 gave 6: yield, 140 mg; mass spectrum, *m/e* 206 (M^+). The ^{1}H NMR spectrum and HPLC (coinjection) chromatogram of this product were identical with that of 6 prepared in method A.

Method C. Treatment of 5 (186 mg, 0.980 mmol) by the procedure described in method A resulted in hydrolysis of the 4-amino group and oxidation of the formyl group to give 6: yield, 158 mg; field-desorption mass spectrum, *m/e* 206 (M^+). The HPLC chromatogram (coinjection) of this product was identical with that of 6 prepared in method A.

Method D. A solution of 12 (5.00 mg, 0.010 mmol) in 2 mL of 1 N NaOH was treated with $KMnO_4$ (1.62 mg, 0.010 mmol), heated at 95 °C for 3 h, filtered, and adjusted to pH 8 with 1 N HCl. An HPLC chromotogram indicated the presence of 6 (~22% yield) and unreacted 12 (~50% recovery). The ultraviolet spectrum (240–360 nm) of the eluted 6 was identical with the ultraviolet spectrum of an authentic sample.

2-Amino-4(3*H*)-oxopyrido[2,3-*d*]pyrimidine-6-carboxaldehyde 2,4-Dinitrophenylhydrazone (8). A solution of crude 4 (200 mg, 1 mmol) in concentrated sulfuric acid (3 mL) was added with stirring to a solution of 2,4-dinitrophenylhydrazine (200 mg) in concentrated sulfuric acid (3 mL). After 5 min the solution was diluted with 30% aqueous ethanol to precipitate the hydrazone: yield, 380 mg. This material was suspended in water (25 mL) and neutralized with 1 N sodium hydroxide. The product that precipitated was collected by filtration, washed with water, and dried in vacuo over P_2O_5: yield, 276 mg (~70%); mp >350 °C; UV λ_{max} (pH 7) 268 nm (sh, $10^{-3}\epsilon$ 9.93), 309 (9.54), 383 (15.4).

Anal. Calcd for $C_{14}H_{10}N_8O_5 \cdot 1.2H_2O$: C, 42.90; H, 3.20; N, 28.60. Found: C, 42.80; H, 3.21; N, 28.52.

Diethyl *N*-[4-[[(2,4-Diaminopyrido[2,3-*d*]pyrimidin-6-yl)methyl]amino]benzoyl]-L-glutamate (10). A solution of **5** (1.47 g, 5.90 mmol) in warm 70% acetic acid (59 mL) was cooled to 25 °C, treated with diethyl (*p*-aminobenzoyl)-L-glutamate (**9**; 2.28 g, 7.08 mmol), and hydrogenated in the pressure of Raney nickel (6.3 g, weighed wet) at 25 °C and atmospheric pressure for 17 h. The mixture was filtered and the catalyst was washed with 70% acetic acid (25 mL). The combined filtrate and wash was evaporated to dryness under high vacuum, and a solution of the residue in ethanol was filtered into 2 N Na_2CO_3 (60 mL). The mixture was stirred to give a homogeneous powder, which was collected, washed with H_2O, and dried. A solution of the powder in boiling ethanol (415 mL) was filtered hot and evaporated to dryness in vacuo. The residue was triturated with $CHCl_3$ (85 mL) and collected by filtration, and the solid was washed with additional $CHCl_3$ (40 mL). A suspension of the solid in boiling EtOH (140 mL) was stirred for 20 min and refrigerated. The product was collected by filtration and dried in vacuo (P_2O_5): yield, 945 mg (32%); mp 262 °C (Kofler Heizbank); HPLC (solvent B) indicated 97% purity; mass spectrum, *m/e* 496 $(M + 1)^+$; UV λ_{max} (0.1 N HCl) 218 nm ($10^{-3}\epsilon$ 42.4), 280 (sh, 19.3), 300 (22.0); UV (pH 7) 218 nm ($10^{-3}\epsilon$ 36.4), 249 (20.2), 280 (sh, 22.3), 297 (23.6), 355 (sh, 6.10); UV (0.1 N NaOH) 249 nm ($10^{-3}\epsilon$ 22.0), 280 (23.8), 297 (sh, 22.5), 345 (7.23); ^1H NMR (Me_2SO-d_6, 6% w/v) δ 1.18 (m, CH_3), 2.05 (m, CH_2CH_2CO), 2.43 (t, CH_2CO), 4.08 (m, CH_2O), 4.32 (m, CH_2N, CHN), 6.31 (s), 7.51 (s, NH_2), 6.67 (d), 7.69 (d, C_6H_4), 6.71 (s, CH_2NH), 8.25 (d, CONH), 8.41 (d, 5-CH, J = 2.0 Hz), 8.66 (d, 7-CH, J = 2.0 Hz).

Anal. Calcd for $C_{24}H_{29}N_7O_5$: C, 58.17; H, 5.90; N, 19.79. Found: C, 57.91; H, 6.24; N, 19.55.

Evaporation of the filtrate and trituration of the residue with EtOH gave an additional 123 mg of less pure product: mp 246 °C.

***N*-[4-[[(2,4-Diaminopyrido[2,3-*d*]pyrimidin-6-yl)methyl]amino]benzoyl]-L-glutamic Acid (11).** A solution of **10** (359 mg, 0.724 mmol) in Me_2SO (10 mL) under N_2 was treated with 1 N NaOH (1.81 mL, 1.81 mmol), stirred in a stoppered flask under N_2 for 6 h, and evaporated to dryness in vacuo at ~30 °C. A solution of the residue in H_2O (18 mL) was filtered and acidified to pH 3.6 with 1 N HCl. The precipitate was collected by filtration, washed with H_2O at pH 3.6, and dried in vacuo (P_2O_5); yield, 297 mg (87%); mp, indefinite (softens above 200 °C); HPLC (solvent A) indicated 99% purity; mass spectrum, *m/e* 440 $(M + 1)^+$; UV λ_{max} (0.1 N HCl) 218 nm ($10^{-3}\epsilon$ 40.5), 280 (sh, 16.9), 300 (18.8); UV (pH 7) 218 nm ($10^{-3}\epsilon$ 38.5), 245 (19.2), 280 (23.9), 296 (sh 22.7); UV (0.1 N NaOH) 248 nm ($10^{-3}\epsilon$ 22.0), 280 (24.4), 296 (sh, 22.7), 345 (7.75); ^1H NMR (Me_2SO-d_6, 6% w/v) δ 2.00 (m, CH_2CH_2CO), 2.29 (t, CH_2CO), 4.36 (m, CHN, CH_2N), 6.66 (d), 7.68 (d, C_6H_4), 7.41 (NH_2), 8.04 (m, NH_2, NH, CO_2H), 8.52 (d), 8.70 (d, 5-CH, 7-CH).

Anal. Calcd for $C_{20}H_{21}N_7O_5 \cdot 1.9H_2O$: C, 50.72; H, 5.28; N, 20.70. Found: C, 50.86; H, 5.43; N, 20.50.

***N*-[4-[[(2,4-Diaminopyrido[2,3-*d*]pyrimidin-6-yl)methyl]methylamino]benzoyl]-L-glutamic Acid (12).** A suspension of **11** (100 mg, 0.211 mmol) in O_2-free H_2O (5 mL) under N_2 was adjusted to pH 6.4 with 1 N NaOH to give a solution, which was treated with 38% HCHO (83.1 μL, 1.14 mmol) followed by $NaBH_3CN$ (19.9 mg, 0.317 mmol). The solution was maintained at pH 6.4 by gradual addition of 1 N HCl over a period of 45 min. The solution was stirred under N_2 for 23 h, filtered, and acidified to pH 3.6 with 1 N HCl. The product was collected by filtration, washed with H_2O at pH 3.6, and dried in vacuo (P_2O_5): yield, 97 mg (94%); mp, indefinite (softens and darkens above 217 °C); HPLC (solvent A) indicated 99% purity; mass spectrum, *m/e* 454 $(M + 1)^+$; UV λ_{max} (0.1 N HCl) 221 nm ($10^{-3}\epsilon$ 37.1), 311 (19.0); UV (pH 7) 219 nm ($10^{-3}\epsilon$ 35.1), 247 (18.1), 305 (25.2); UV (0.1 N NaOH) 249 nm ($10^{-3}\epsilon$ 19.9), 305 (25.0), 355 (sh, 6.15); ^1H NMR (Me_2SO-d_6, ~5% w/v) δ 2.00 (m, CH_2CH_2CO), 2.28 (t, CH_2CO), 3.12 (s, CH_3), 4.32 (m, CHN), 4.66 (s, CH_2N), 6.78 (d), 7.72 (d, C_6H_4), 8.31 (d, 5-CH), 8.59 (d, 7-CH).

Anal. Calcd for $C_{21}H_{23}N_7O_5 \cdot 2H_2O$: C, 51.53; H, 5.56; N, 20.03. Found: C, 51.54; H, 5.47; N, 20.35.

***N*-[4-[[(2-Amino-4(3*H*)-oxopyrido[2,3-*d*]pyrimidin-6-yl)methyl]amino]benzoyl]-L-glutamic Acid (14). Method A.** A suspension of **10** (100 mg, 0.202 mmol) in O_2-free 1 N NaOH (4 mL) was stirred at reflux temperature under N_2 for 4.25 h and acidified to pH 3.1 with 6 N HCl. The precipitate was collected by filtration and dried in vacuo. A solution of the solid in 1 N HCl (0.5 mL) was diluted with H_2O (0.5 mL), filtered, diluted with H_2O (9 mL), and adjusted to pH 3.1 with 1 N NaOH. The precipitate was collected by filtration, washed with H_2O at pH 3.1, and dried in vacuo (P_2O_5): yield, 74 mg (79%); mp, indefinite; HPLC (solvent A) indicated 100% purity; mass spectrum, *m/e* 441 $(M + 1)^+$; UV λ_{max} (0.1 N HCl) 213 nm ($10^{-3}\epsilon$ 37.0), 280 (23.9), 297 (sh, 20.6), 350 (7.35); UV (pH 7) 216 nm ($10^{-3}\epsilon$ 40.8), 278 (24.9), 295 (sh, 23.8); UV (0.1 N NaOH) 243 nm ($10^{-3}\epsilon$ 22.9), 278 (24.0), 295 (sh, 22.7), 345 (sh, 7.58); ^1H NMR (CF_3CO_2D, ~6% w/v) δ 2.56 (CH_2CH_2CO), 2.82 (t, CH_2CO), 5.11 (m, CHN, CH_2N), 7.87 (d), 8.15 (d, C_6H_5), 8.98 (s), 9.10 (s, 5-CH, 7-CH).

Anal. Calcd for $C_{20}H_{20}N_6O_6 \cdot 1.1H_2O$: C, 52.20; H, 4.86; N, 18.26. Found: C, 52.00; H, 4.92; N, 18.54.

Method B. A mixture of **4** (206 mg, 1.00 mmol), diethyl (*p*-aminobenzoyl)-L-glutamate (**9**) (400 mg, 1.24 mmol) and Raney nickel (1 g, weighed wet) in HOAc (10 mL) was hydrogenated at 25 °C and atmospheric pressure for 22 h and filtered through Celite and the catalyst was washed with additional HOAc (25 mL). The combined filtrate and wash was evaporated to dryness under high vacuum and the residue extracted first with Et_2O (50 mL) and then with boiling EtOH (25 mL). The solid was collected by filtration, washed with hot EtOH (25 mL), and dried in vacuo (P_2O_5) to give 217 mg of crude diethyl 5-deazafolate (**13**): field-desorption mass spectrum, *m/e* 497 $(M + 1)^+$. A solution of crude **13** (82 mg) in O_2-free 0.1 N NaOH (10 mL) was stirred under N_2 for 3 days, filtered, acidified to pH 3.3 with 1 N HCl, heated to boiling, and then refrigerated. The product was collected in a centrifuge, washed with H_2O, and dried in vacuo (P_2O_5) to give **14**: yield, 27 mg. The sample was estimated to be 44% pure by HPLC.

***N*-[4-[[(2-Amino-4(3*H*)-oxopyrido[2,3-*d*]pyrimidin-6-yl)methyl]methylamino]benzoyl]-L-glutamic Acid (15). Method A.** A suspension of **14** (60 mg, 0.13 mmol) was methylated by the procedure used for the preparation of **12**. The reaction solution after filtration was diluted with O_2-free H_2O (3 mL) and acidified to pH 3.1 with 1 N HCl. The product was collected, washed with H_2O at pH 3.1, and dried in vacuo (P_2O_5): yield, 53 mg (84%), mp, indefinite; HPLC (solvent A) indicated 97% purity; mass spectrum, *m/e* 455 $(M + 1)^+$; UV λ_{max} (0.1 N HCl) 215 nm ($10^{-3}\epsilon$ 35.1), 280 (19.0), 306 (20.8), 355 (sh, 6.85); UV (pH 7) 216 nm ($10^{-3}\epsilon$ 38.0), 274 (19.0), 306 (27.3); UV (0.1 N NaOH) 242 nm ($10^{-3}\epsilon$ 22.9), 275 (sh, 17.4), 307 (25.4); ^1H NMR (Me_2SO-d_6, 5% w/v) δ 2.02 (m, $CH_2CH_2CO_2H$), 2.35 (t, CH_2CO), 3.09 (s, CH_3), 4.37 (m, CHN), 4.73 (s, CH_2N), 6.82 (d), 7.75 (d, C_6H_4), 8.03 (d, 5-CH), 8.19 (d, NH), 8.55 (d, 7-CH).

Anal. Calcd for $C_{21}H_{22}N_6O_5 \cdot H_2O \cdot 0.75HCl$: C, 52.14; H, 5.16; N, 17.37. Found: C, 52.12; H, 5.12; N, 17.47.

Method B. A solution of compound **12** (50 mg) was hydrolyzed by procedure A for the preparation of compound **14** to give a 64% yield of compound **15**. HPLC and UV data indicated that this product was identical with that prepared in method A above.

Acknowledgment. This investigation was supported by Southern Research Institute and by Grant CA-23141 awarded by the National Cancer Institute, National Institutes of Health. We are indebted to Dr. W. C. Coburn, Jr., and other members of the Molecular Spectroscopy Section of Southern Research Institute, who performed most of the microanalytical and spectral determinations.

Registry No. **1**, 80360-02-7; **2**, 56-06-4; **3**, 1004-38-2; **4**, 80360-03-8; **5**, 80360-04-9; **5**·HCl, 80360-05-0; **6**, 80360-06-1; **7**, 49739-04-0; **8**, 80360-07-2; **9**, 13726-52-8; **10**, 80360-08-3; **11**, 80360-09-4; **12**, 80360-10-7; **13**, 80360-11-8; **14**, 51989-25-4; **15**, 80360-12-9.