noethylidene)-7-methoxy-9-oxo-1-azabicyclo[5.2.0]nonane in acidic ethanol solution (11% water and 1% acetic acid).[10]

Registry No. 4, 32363-51-2; **4a**, 32363-54-5; **4b**, 69912-33-0; **5**, 76599-91-2; **6**, 72611-06-4; **7**, 73645-42-8; **7a**, 76599-92-3; **7b**, 76599-93-4; **7**(D_1), 76599-94-5; **7**(D_2), 76599-95-6; **7**(D_3), 76599-96-7; **7**(D_4), 76599-97-8; **8**, 73645-43-9; **8a**, 76599-98-9; **8b**, 76599-99-0; **9**, 76600-00-5; **18**, 58156-40-4; **19**, 76600-01-6; **20**, 66849-14-7; **22**, 72611-26-8; **23**, 73645-44-0; **24**, 76227-55-9; **25**, 69912-22-7; **26**, 76600-02-7; **27**, 72611-27-9; 3-amino-*N*-methyl-2-butenamide, 24392276; CH_3OH, 67-56-1; acetic anhydride, 108-24-7; propionic anhydride, 123-62-6; CH_3OD, 4206-31-9; CH_3NH_2, 74-89-5.

Pyridopyrimidines. 12. Synthesis of 8-Deaza Analogues of Aminopterin and Folic Acid

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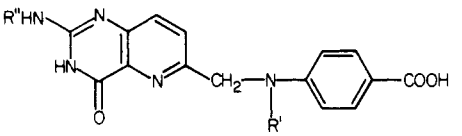
Received December 30, 1980

A new, general approach to the synthesis of numerous 8-deazafolate and 8-deazaaminopterin analogues is described. The key intermediate, 6-(acetoxymethyl)-2,4-dichloropyrido[3,2-*d*]pyrimidine, was prepared by chlorination of the 2,4-dioxo derivative which, in turn, resulted from the acetic anhydride induced rearrangement of 2,4-dioxopyrido[3,2-*d*]pyrimidine 5-oxide. Suitable nucleophilic displacements on the ring followed by activation of the side chain to the bromomethyl derivative gave 2,4-diamino- or 2-amino-4-oxo-6-(bromomethyl)pyrido-[3,2-*d*]pyrimidines which were reacted with a variety of *p*-substituted benzoylglutamates to give, after saponification, the target folate analogues.

Since the discovery that folate antagonists can be effectively used in the treatment of human neoplastic disease,[1] investigations have focused on the synthesis and biological evaluation of analogues of folic acid.[2] The successful use of methotrexate (MTX) in the treatment of certain forms of cancer[3] prompted the preparation of aminopterin analogues[4] which included modifications in the side chain. It has been reported that 8-deazafolic acid is as potent as methotrexate in the inhibition of certain bacterial cell lines and was active against some methotrexate-resistant strains.[5] The present report describes the synthesis of a series of 8-deaza analogues of aminopterin and folic acid having various isosteric substitutions at the 10 position.

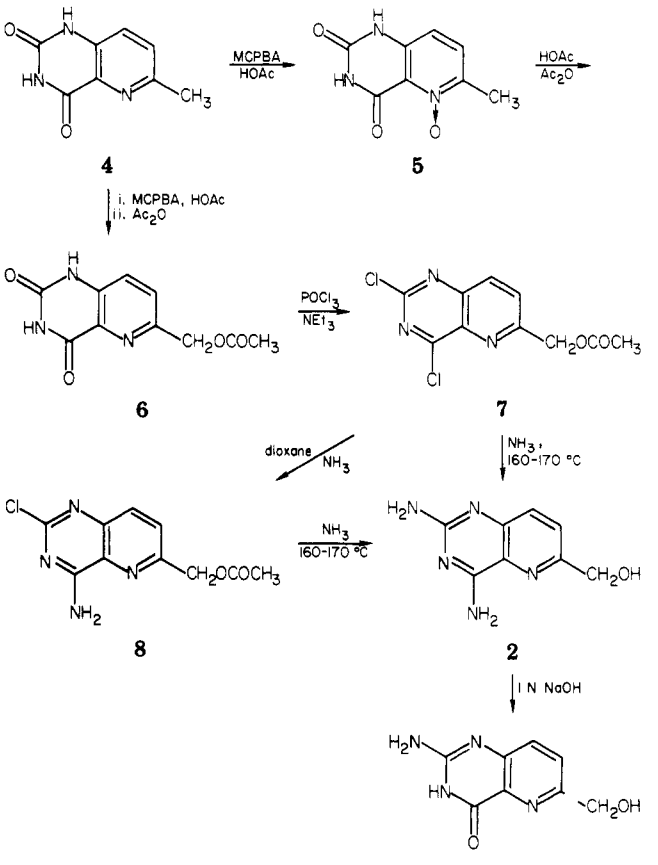
The synthetic procedure of DeGraw et al.[5] involves the preparation of protected 8-deazapteroic acid 1 followed by



1, R′ = $COCF_3$; R″ = $COCH_3$

introduction of glutamic acid by a mixed anhydride or solid-phase method to give 8-deazafolic acid. The intermediates used in the above scheme were not suitable for the preparation of a wide variety of aminopterin and folic acid analogues necessary to investigate structure–activity relationships. Since we were also interested in the effect of replacement of side-chain nitrogen in 8-deazaaminopterin and folic acid, a new general approach was developed.

Scheme I



The syntheses of 2,4-diamino-6-(hydroxymethyl)-pyrido[3,2-*d*]pyrimidine (**2**) and 2-amino-6-(hydroxymethyl)-4-oxopyrido[3,2-*d*]pyrimidine (**3**), the precursors of 8-deaza aminopterin and folic acid, respectively, are

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(3) Livingston, R. B.; Carter, S. K. "Single Agents in Cancer Chemotherapy"; IFI/Plenum: New York, 1970; p 130.

(4) (a) Nair, M. G.; Campbell, P. T. *J. Med. Chem.* **1976**, *19*, 825. (b) DeGraw, J. I.; Kisliuk, R. L.; Gaumont, Y.; Baugh, C. M.; Nair, M. G. *Ibid.* **1974**, *17*, 552. (c) Kim, Y. H.; Gaumont, Y.; Kisliuk, R. L.; Mautner, H. G. *Ibid.* **1975**, *18*, 776.

(5) DeGraw, J. I.; Kisliuk, R. L.; Gaumont, Y.; Baugh, C. M. *J. Med. Chem.* **1974**, *17*, 470.

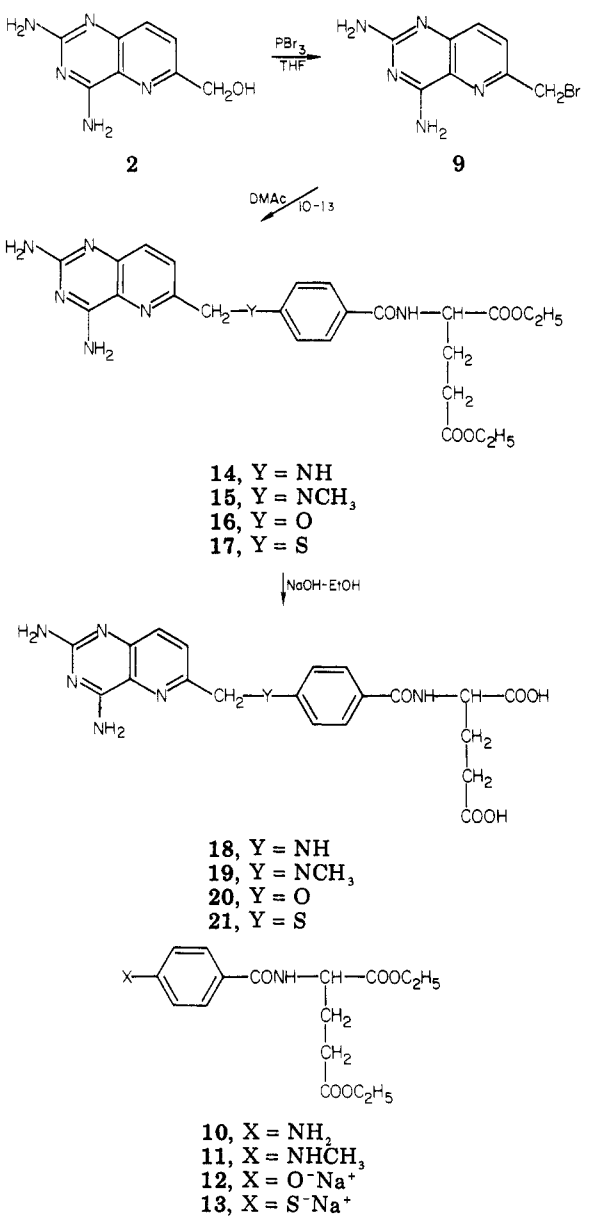
0022-3263/81/1946-1777$01.25/0

shown in Scheme I. The syntheses of these two key intermediates also lend themselves to the incorporation of a modified side chain in two steps. The present procedure is devoid of a protection and deprotection sequence either in the functionalization or in the introduction of the side chain, required in the procedure[5] reported earlier.

The functionalization of the 6-methyl group of 2,4-dioxo-6-methylpyrido[3,2-*d*]pyrimidine[6] (**4**) was carried out as follows: oxidation of **4** with *m*-chloroperoxybenzoic acid in acetic acid gave **5**. Because of the lactam structure of **4**, N-5 is the only site available for oxidation. α-Picoline *N*-oxides are known to rearrange in refluxing acetic anhydride to give α-(acetoxymethyl)pyridine.[7] Refluxing a solution of **5** in acetic acid–acetic anhydride mixture gave **6**, 6-(acetoxymethyl)-2,4-dioxopyrido[3,2-*d*]pyrimidine. The structure was established by mass spectra (M^+, 235) and by proton NMR spectra. The aromatic methyl resonance at δ 2.33 was replaced by the acetyl methyl resonance at δ 2.26, and a new, two-proton signal for the methylene group appeared at δ 5.38. Compound **6** can also be prepared from **4** without the isolation of the *N*-oxide **5** (see Experimental Section). The dioxo derivative **6** was converted to 6-(acetoxymethyl)-2,4-dichloropyrido[3,2-*d*]pyrimidine (**7**) by treatment with phosphoryl chloride/triethylamine at reflux. The presence of two chlorine atoms was confirmed by mass spectra (molecular ion peaks *m/e* 271 and 273 in the ratio of 3:2). The reaction of **7** with ammonia in dry dioxane gave 6-(acetoxymethyl)-4-amino-2-chloropyrido[3,2-*d*]pyrimidine. The assignment of structure **8** is based on the known lability of 4-chloro groups in the pyridopyrimidine series.[8] Treatment of **8** with liquid ammonia in a sealed vessel at 160–170 °C gave **2**, which can also be prepared directly from **7** under similar conditions. Alkaline hydrolysis of **2** gave 2-amino-6-(hydroxymethyl)-4-oxopyrido[3,2-*d*]pyrimidine (**3**). It was established by Hitchings et al.[9] that in 2,4-diaminopyridopyrimidine and 2,4-diaminoquinazoline the 4-amino function is prone to alkaline hydrolysis. The structure of **8** was confirmed by comparing its UV spectrum with that of 2-amino-6-*n*-amyl-4-oxopyrido[3,2-*d*]pyrimidine, prepared by DeGraw and Brown[10] by an unambiguous procedure.

Treatment of **2** with phosphorus tribromide in dry THF gave **9**, which could not be purified because of lability of the bromo group. Condensation of **9** in dimethylacetamide with diethyl *p*-aminobenzoyl-L-glutamate[11] (**10**), diethyl [*p*-(methylamino)benzoyl]-L-glutamate[12] (**11**), the sodium salt of diethyl (*p*-hydroxybenzoyl)-L-glutamate (**12**), prepared from diethyl (*p*-hydroxybenzoyl)-L-glutamate[13]), and the sodium salt of diethyl (*p*-mercaptobenzoyl)-L-glutamate[4] (**13**) followed by chromatographic purification gave the desired compounds **14–17**, respectively. Saponification in dilute base afforded the aminopterin analogues **18–21**. In a similar manner, compound **3** was converted to the bromomethyl derivative **22**. Condensation of **22** with **10**, **11**, and **13** gave the esters **23**, **24**, and **25**, respectively. Hydrolysis of **23**, **24**, and **25** gave the folic acid analogues **26–28**. Compound **26** had similar UV properties (λ_{max} 252 and 305 nm at pH 1 and 285 nm at pH 13) to those reported by DeGraw.[5] The structure of compounds **14–17**, **18–21**, **23–25**, and **26–28** were established by ^1H NMR (by the presence of an aromatic quartet and pyridopyrimidine protons), mass spectra of permethylated derivatives, and analytical data. In compounds **2**, **3**, **4**, **6**, **14**, **16**, **18**, **20**, **23**, and **26**, C^7-H and C^8-H appear as a 2-proton singlet because of fortuitous equivalence of the chemical shift of these protons. The above reactions are summarized in Schemes II and III.

Scheme II



2 **9**

14, Y = NH
15, Y = NCH_3
16, Y = O
17, Y = S

18, Y = NH
19, Y = NCH_3
20, Y = O
21, Y = S

10, X = NH_2
11, X = $NHCH_3$
12, X = O^-Na^+
13, X = S^-Na^+

Experimental Section

The ^1H NMR spectra were recorded on a Varian EM-390 spectrometer, unless otherwise stated, in dimethyl-d_6 sulfoxide with DSS as the internal standard. Exchangeable protons were detected by the addition of D_2O. Melting points were determined on a Thomas-Hoover melting-poing apparatus and are not corrected. Mass spectra were taken on a Varian 112S or LKB-GCMS Model 9000S spectrometer. Permethylations were carried out in dimethyl sulfoxide, using NaH and methyl iodide. UV spectra were taken on a Cary Model 15 or Beckman Acta CIII spectrometer. Elemental analyses were performed by Het-Chem-Co., Harrisonville, MO, and Galbraith Laboratories, Knoxville, TN.

(6) Irwin, W. J.; Wibberley, D. G. *J. Chem. Soc. C* **1967**, 1945.
(7) Boekelheide, V.; Linn, W. J. *J. Am. Chem. Soc.* **1954**, *76*, 1286.
(8) (a) Srinivasan, A.; Broom, A. D. *J. Org. Chem.* **1979**, *44*, 435. (b) Anderson, G. L.; Shim, J. L.; Broom, A. D. *Ibid.* **1977**, *42*, 993. (c) Oakes, V.; Rydon, H. N. *J. Chem. Soc.* **1956**, 443.
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(11) Obtained from Chemical Dynamics Corporation, South Plainfield, NJ.
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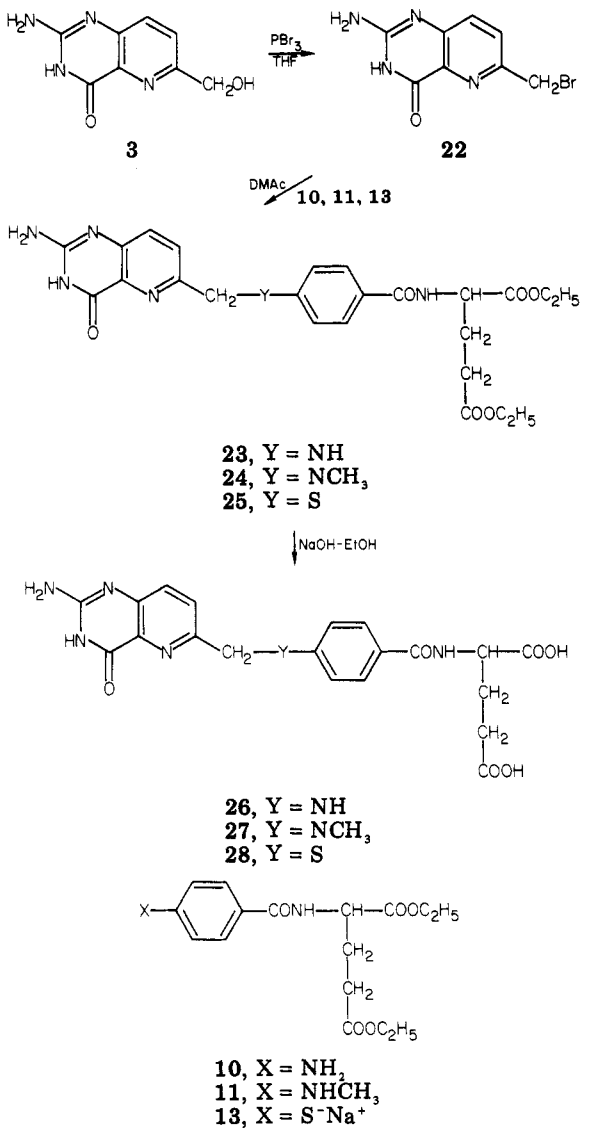
(13) Fairburn, E. I.; Magerlein, B. J.; Stubgerfield, L.; Stapert, E.; Weisblat, D. I. *J. Am. Chem. Soc.* **1954**, *76*, 676.

Scheme III



3 22

23, Y = NH
24, Y = NCH_3
25, Y = S

26, Y = NH
27, Y = NCH_3
28, Y = S

10, X = NH_2
11, X = $NHCH_3$
13, X = S^-Na^+

2,4-Dioxo-6-methylpyrido[3,2-*d*]pyrimidine 5-Oxide (5). A suspension of 1.77 g (10 mmol) of 2,4-dioxo-6-methylpyrido-[3,2-*d*]pyrimidine (4)[6] in 50 mL of glacial acetic acid containing 6 g of *m*-chloroperoxybenzoic acid (85–90%) was stirred and refluxed for 3 h. Acetic acid was removed in vacuo and the residue was stirred with 100 mL of ether and filtered. The solid was crystallized from glacial acetic acid to give 1.6 g (83%) of **5**: mp >300 °C; mass spectrum, *m/e* 193 (M^+), 177 ($M^+ - 16$); UV λ_{max} (pH 1) 239 (ϵ_{max} 26000), 290 (3350), 355 (4300); (pH 7) 235 (23500), 255 (6750), 285 (3600), 360 (4000); (pH 13) 255 (19000), 26 (18000), 372 (3500); ^1H NMR δ 2.33 (s, 3, CH_3), 7.18 and 7.88 (q, 2, C^7H and C^8H).

Anal. Calcd for $C_8H_7N_3O_3$: C, 49.74; H, 3.65; N, 21.75. Found: C, 49.79; H, 3.72; N, 21.66.

6-(Acetoxymethyl)-2,4-dioxopyrido[3,2-*d*]pyrimidine (6). A. A suspension of **5** (0.96 g, 5 mmol) in a mixture of 10 mL of acetic anhydride and 10 mL of glacial acetic acid was refluxed for 30 min. The clear brown solution was evaporated to dryness and the residue was crystallized from ethanol to give 1.0 g (85%) of **6**: mp 288–289 °C; mass spectrum, *m/e* 235 (M^+), 192 ($M^+ - HCNO$); UV λ_{max} (pH 1) 246 (ϵ_{max} 10500), 315 (5500); (pH 7) 247 (10550), 315 (5500); (pH 11) 232 (16500), 268 (9200), 322 (5200); ^1H NMR δ 2.26 (s, 3, $COCH_3$), 5.33 (s, 2, CH_2), 7.90 (s, 2, C^7H and C^8H).

Anal. Calcd for $C_{10}H_9N_3O_4$: C, 51.06; H, 3.85; N, 17.86. Found: C, 51.40; H, 3.86; N, 17.60.

B. For large-scale preparations the following procedure was used. A suspension of 17.7 g (0.1 mol) of **4** in 500 mL of glacial acetic acid containing 60 g of *m*-chloroperoxybenzoic acid was refluxed for 3 h. Acetic anhydride (400 mL) was added to the hot reaction mixture and the refluxing was continued for another 30 min. The clear brown solution was evaporated to dryness and the solid mass stirred with ether (1000 mL) and filtered. The solid was crystallized from ethanol to give 16.9 g (72%) of **6**, identical in all respects with the one obtained in procedure A.

6-(Acetoxymethyl)-2,4-dichloropyrido[3,2-*d*]pyrimidine (7). Compound **6** (6 g, 25.5 mmol) was refluxed with 150 mL of phosphoryl chloride containing 10 mL of triethylamine for 8 h. The volume was reduced to ~20 mL by distillation under reduced pressure. The dark syrup was poured into crushed ice. The cold suspension was extracted with methylene chloride (3 × 200 mL) and washed with cold water until the washings were neutral. The organic layer was dried over anhydrous sodium sulfate and evaporated to dryness. The dark solid was stirred and refluxed with petroleum ether (bp 60–90 °C), treated with decolorizing charcoal, filtered through Celite, concentrated to ~200 mL, and allowed to cool to room temperature. The crystallized solid was filtered and dried to give 4.2 g (62%) of **7**: mp 104–105 °C; mass spectrum, *m/e* 271 (M^+), 228 ($M^+ - COCH_3$), 212 ($M^+ - OCOCH_3$); UV λ_{max} (CH_3OH) 255 (ϵ_{max} 7050), 303 (6500), 318 (6300); ^1H NMR ($CDCl_3$) δ 2.26 (s, 3, $COCH_3$), 5.43 (s, 2, CH_2), 7.87 and 8.27 (q, 2, C^7H and C^8H).

Anal. Calcd for $C_{10}H_7N_3O_2Cl_2$: C, 44.14; H, 2.59; N, 15.44. Found: C, 44.18; H, 2.72; N, 15.38.

6-(Acetoxymethyl)-4-amino-2-chloropyrido[3,2-*d*]pyrimidine (8). A solution of 1.35 g (5 mmol) of **7** in 20 mL of anhydrous dioxane was saturated with gaseous ammonia at room temperature. After the mixture was stirred for 2 h at room temperature the solvent was removed under reduced pressure and the residue was stirred with water and filtered. Crystallization from ethyl acetate gave 0.9 g (72%) of **8**: mp 199–200 °C; mass spectrum, *m/e* 252 (M^+), 209 ($M^+ - COCH_3$); UV λ_{max} (pH 1) 253 (ϵ_{max} 22700), 270 (4700), 280 (9600), 308 (8850), 326 (7700); (pH 7) 273 (5600), 280 (6500), 328 (5500); (pH 11) 238 (27000), 280 (6400), 328 (5500); ^1H NMR δ 2.33 (s, 3, CH_3), 5.41 (s, 2, CH_2), 7.91 and 8.2 (q, 2, C^7H and C^8H), 8.33 and 8.6 (br, 1 each, 4-NH_2).

Anal. Calcd for $C_{10}H_9N_3O_2Cl$: C, 47.53; H, 3.59; N, 22.17. Found: C, 47.56; H, 3.59; N, 21.96.

2,4-Diamino-6-(hydroxymethyl)pyrido[3,2-*d*]pyrimidine (2). A. Compound **8** (0.5 g, 2 mmol) was heated with 10 mL of anhydrous liquid ammonia in a sealed vessel at 160–170 °C for 18 h. Liquid ammonia was allowed to evaporate and the solid was washed with water and ethanol. Crystallization from large volume of water gave 0.3 g (79%) of **2**: mp 290–291 °C; mass spectrum, *m/e* 191 (M^+); UV λ_{max} (pH 1) 243 (ϵ_{max} 13350), 305 (4850), 320 (6500), 331 (5200); (pH 7) 244 (15000), 306 (3600), 334 (4500); (pH 11) 271 (6900), 341 (4200); ^1H NMR δ 4.63 (s, 2, CH_2), 6.23 (br, 2, 2-NH_2), 7.43 (br, 2, 4-NH_2) 7.61 (s, 2, C^7H and C^8H).

Anal. Calcd for $C_8H_9N_5O$: C, 50.25; H, 4.74; N, 36.72. Found: C, 50.04; H, 4.78; N, 36.48.

B. Compound **7** (9.03 g, 33 mmol) was heated with 200 mL of liquid ammonia as described in procedure A to give 5.3 g (84%) of **2**, after crystallization from glacial acetic acid. This compound is identical in all respects with the one obtained in procedure A.

2-Amino-6-(hydroxymethyl)-4-oxopyrido[3,2-*d*]pyrimidine (3). A suspension of 4.8 g (25 mmol) of **2** in 125 mL of 1 N NaOH was refluxed for 10 h. The solution was cooled, filtered, and neutralized with 2 N acetic acid (pH 6). The precipitated solid was filtered, washed with cold water, and crystallized from water to give 3.9 g (81%) of **3**: mp >300 °C; mass spectrum, *m/e* 192 (M^+); UV λ_{max} (pH 1) 248 (ϵ_{max} 13700), 304 (5500), 320 (4800); (pH 7) 264 (11500), 315 (5050), 325 (5000); (pH 13) 236 (24500), 271 (9600), 335 (5700); ^1H NMR δ 4.56 (s, 2, CH_2), 6.6 (br, 2, 2-NH_2), 7.6 (s, 2, C^7H and C^8H). In TFA the C^7H and C^8H appear as doublets each at δ 8.37 and 8.74 with $J_{7,8}$ = 9.0 Hz.

Anal. Calcd for $C_8H_8N_4O_2$: C, 49.93; H, 4.19; N, 29.11. Found: C, 49.76; H, 4.19; H, 28.93.

Diethyl *N*-[4-[[(2,4-Diaminopyrido[3,2-*d*]pyrimidin-6-yl)methyl]amino]benzoyl]-L-glutamate or Diethyl 8-Deazaaminopterin (14). A suspension of 1.91 g (10 mmol) of **2** in 30 mL of anhydrous THF was stirred for 8 h with 2 mL of phosphorus tribromide. The precipitated solid was filtered, washed with cold THF and ether, and dried to give **9**. TLC in $CHCl_3$–CH_3OH (80:20) showed one major compound with minor

impuirites. To a solution of this compound in 50 mL of dimethylacetamide (distilled over CaH_2) was added 6.4 g (20 mmol) of **10**.[11] The resulting solution was stirred for 72 h at room temperature. TLC in $CHCl_3$–CH_3OH (80:20) indicated complete disappearance of compound **2**. The solvent was removed in vacuo, and the residue was suspended in water, stirred with 20 mL of 5% bicarbonate solution, and extracted with three 100-mL portions of chloroform. The chloroform extract was dried over anhydrous sodium sulfate, concentrated to a small volume (~20 mL), and poured onto a column (silica gel, 60–200 mesh). Elution with $CHCl_3$–CH_3OH (96%4) gave unreacted **10**. Elution with $CHCl_3$–CH_3OH (85:15) gave 3.0 g (61%) of pure **14**: mp 94–96 °C; mass spectrum, *m/e* 579 (M^+, hexamethyl derivative); UV λ_{max} (pH 1) 318 (ϵ_{max} 10500); (pH 7) 278 (23000), 355 (7500); (pH 13) 278 (25000), 350 (7300); ^1H NMR δ 4.6 (d, 2, CH_2), 6.36 (br, 2, 2-NH_2), 7.13 (t, 1, $N^{10}H$), 7.8 (s, 2, C^7H and C^8H), 6.98 and 7.98 (q, 4, C_6H_4), 7.81 (br, 2, 4-NH_2), 8.5 (d, 1, CONH).

Anal. Calcd for $C_{24}H_{29}N_7O_5 \cdot 1.0H_2O$: C, 56.13; H, 6.08; N, 19.09. Found: C, 55.96; H, 5.78; N, 18.76.

Diethyl *N*-[4-[[(2,4-Diaminopyrido[3,2-*d*]pyrimidin-6-yl)methyl]methylamino]benzoyl]-L-glutamate or Diethyl 8-Deazamethotrexate (15). To a solution of **9** (prepared from 1.91 g (10 mmol) of **2** and PBr_3 as described earlier) in 50 mL of dimethylacetamide was added 6.86 g (20 mmol) of **11**,[12] and the solution was kept over a steam bath for 5–6 h. TLC in $CHCl_3$–CH_3OH (85:15) indicated disappearance of **9**. The product was isolated in an identical procedure from the column (except $CHCl_3$–CH_3OH (90:10) was used for final elution) to give 2.7 g (53%) of pure **15**: mp 230–232 °C; mass spectrum, *m/e* 579 (M^+, pentamethyl derivative); UV λ_{max} (pH 1) 318 (ϵ_{max} 11800), 333 (7700); (pH 7) 278 (14000), 307 (24000); (pH 13) 278 (20000), 307 (25000); ^1H NMR δ 3.32 (s, 3, $N^{10}CH_3$), 4.86 (s, 2, CH_2), 6.33 (s, 2, 2-NH_2), 7.51 and 7.71 (q, 2, C^7H and C^8H), 6.95 and 7.91 (q, 4, C_6H_4), 7.43 (br, 2, 4-NH_2), 8.48 (d, 1, CONH).

Anal. Calcd for $C_{25}H_{31}N_7O_5$: C, 58.92; H, 6.13; N, 19.24. Found: C, 58.76; H, 6.27; N, 19.06.

Diethyl *N*-[4-[[(2,4-Diaminopyrido[3,2-*d*]pyrimidin-6-yl)methyl]oxy]benzoyl]-L-glutamate or Diethyl 8-Deaza-10-oxaaminopterin (16). To a solution of sodium ethoxide in ethanol (prepared by dissolving 0.51 g (22 mmol) of sodium metal in 100 mL of absolute ethanol) was added 6.46 g of diethyl (*p*-hydroxybenzoyl)-L-glutamate.[13] The resulting solution was stirred at room temperature for 1 h and evaporated to dryness. The residue, the sodium salt of diethyl (*p*-hydroxybenzoyl)-L-glutamate (**12**), was dissolved in 50 mL of dimethylacetamide to which compound **9** (prepared from 1.91 g (10 mmol) of **2** and PBr_3 as described earlier) was added. The resulting solution was stirred at room temperature for 8–10 h. The solvent was removed under diminished pressure and the residue was suspended in water and extracted with chloroform. The organic layer was dried over anhydrous sodium sulfate, concentrated to a small volume (~20 mL), and poured on to a column (silica gel 60–200 mesh). Elution with $CHCl_3$–CH_3OH (96:4) gave unreacted diethyl (*p*-hydroxybenzoyl)-L-glutamate. Elution with $CHCl_3$–CH_3OH (90:10) gave 2.6 g (52%) of **16**: mp 165–167 °C; mass spectrum, *m/e* 566 (M^+, pentamethyl derivative); UV λ_{max} (pH 1) 246 (ϵ_{max} 32000), 318 (8300); (pH 7) 248 (36000), 335 (6700); (pH 13) 250 (38500), 343 (6500); ^1H NMR δ 5.44 (s, 2, CH_2–O), 6.49 (, 2, 2-NH_2), 7.36 and 8.16 (q, 4, C_6H_4), 7.61 (br, 2, 2-NH_2), 7.91 (s, 2, C^7H and C^8H, 8.84 (d, 1, CONH).

Anal. Calcd for $C_{24}H_{28}N_6O_6 \cdot 0.5H_2O$: C, 57.02; H, 5.78; N, 16.62. Found: C, 56.75; H, 5.65; N, 16.52.

Diethyl *N*-[4-[[(2,4-Diaminopyrido[3,2-*d*]pyrimidin-6-yl)methyl]thio]benzoyl]-L-glutamate or Diethyl 8-Deaza-10-thiaaminopterin (17). A solution of the sodium salt of diethyl (*p*-mercaptobenzoyl)-L-glutamate (**13**) was prepared according to the procedure of Mautner et al.[4c] (from 4.0 g (6 mmol) of tetraethyl 4,4′-dithiobis(*N*-benzoyl-L-glutamate) in 50 mL of ethanol and 0.68 g of sodium borohydride). The solution was concentrated to about 10 mL under diminished pressure, to which a solution of **9** (prepared from 1.91 g (10 mmol) of **2** and PBr_3 as described earlier) in 50 mL of dimethylacetamide was added and the resulting solution was stirred for 6 h at room temperature. The product **17** was isolated by a procedure identical with that described from the isolation of diethyl 10-oxaaminopterin: 2.8 (55%); mp 152–154 °C; mass spectrum, *m/e* 582 (M^+, pentamethyl derivative); UV λ_{max} (pH 1) 243 (ϵ_{max} 22000), 275 (15000), 320 (6900); (pH 7) 277 (18000), 335 (5300); (pH 13) 277 (19000), 343 (5300); ^1H NMR δ 4.61 (s, 2, CH_2S), 6.43 (s, 2, 2-NH_2), 7.53 (bnr, 2, 3-NH_2), 7.66 and 7.83 (q, 2, C^7H and C^8H), 7.72 and 8.05 (q, 4, C_6H_4), 9.96 (d, 1, CONH).

Anal. Calcd for $C_{24}H_{28}N_6O_5S$: C, 56.23; H, 5.50; N, 16.39. Found: C, 56.35; H, 5.65; N, 16.46.

Diethyl *N*-[4-[[(2-Amino-3,4-dihydro-4-oxopyrido[3,2-*d*]-pyrimidin-6-yl)methyl]amino]benzoyl]-L-glutamate or Diethyl 8-Deazafolic Acid (23). A suspension of 1.92 g (10 mmol) of **3** in 30 mL of anhydrous THF was stirred with 2 mL of phosphorus tribromide for 12 h. The precipitated solid was filtered and washed with cold THF and ether to give **22**. TLC in $CHCl_3$–CH_3OH (85:15) indicated the presence of one major compound with minor impurities. To a solution of this compound in 50 mL of dimethylacetamide (distilled from CaH_2) was added 6.4 g (20 mmol) of **10**[11] and the resulting solution was stirred at room temperature for 3 days.

The product **23** was isolated by a procedure identical with that described earlier for the isolation of diethyl 8-deazaaminopterin **14**: 3.4 g (68%); mp 180–182 °C; mass spectrum, *m/e* 556 (M^+, pentamethyl derivative); UV λ_{max} (pH 1) 251 (ϵ_{max} 14800), 305 (11000); (pH 7) 275 (17000); (pH 13) 283 (20500); ^1H NMR δ 4.56 (d, 2, CH_2), 6.79 (br, 2, 2-NH_2), 6.81 and 7.91 (q, 4, C_6H_4), 7.78 (s, 2, C^7H and C^8H), 7.13 (t, 1, $N^{10}H$), 8.46 (d, 1, CONH), 11.60 (br, 1, N^3H).

Anal. Calcd for $C_{24}H_{28}N_6O_6$: C, 58.05; H, 5.68; N, 16.92. Found: C, 57.96; H, 5.70; N, 16.99.

Diethyl *N*-[4-[[(2-Amino-3,4-dihydro-4-oxopyrido[3,2-*d*]-pyrimidin-6-yl)methyl]methylamino]benzoyl]-L-glutamate or Diethyl 8-Deaza-N^{10}-methylfolic Acid (24). To a solution of **22** (prepared from 1.92 g (10 mmol) of **3** and PBr_3 as described above) in 50 mL of dimethylacetamide was added 6.86 g (20 mmol) of **11**.[12] The solution was kept over a steam bath for 5–6 h with occasional stirring. TLC in $CHCl_3$–CH_3OH (85:15) indicated complete disappearance of **22**. The product **24** was isolated by a procedure identical with that described earlier for the isolation of diethyl 8-deazamethotrexate **15**: 2.8 g (58%); mp 140–142 °C; mass spectrum, *m/e* 566 (M^+, tetramethyl derivative; UV λ_{max} (pH 1) 250 (ϵ_{max} 15000), 307 (12500); (pH 7) (16000), 305 (28500); (pH 13) 305 (27000); ^1H NMR δ 3.23 (s, 3, $N^{10}CH_3$), 4.91 (s, 2, CH_2), 6.75 (br, 2, 2-NH_2), 6.98 and 7.98 (q, 4, C_6H_4), 7.51 and 7.80 (q, 2, C^7H and C^8H), 8.56 (d, 1, CONH), 11.6 (br, 1, N^3H).

Anal. Calcd for $C_{25}H_{30}N_6O_6$: C, 58.81; H, 5.92; N, 16.46. Found: C, 58.72; H, 6.04; N, 16.32.

Diethyl *N*-[4-[[(2-Amino-3,4-dihydro-4-oxopyrido[3,2-*d*]-pyrimidin-6-yl)methyl]thio]benzoyl]-L-glutamate or Diethyl 8-Deaza-10-thiafolic Acid (25). A solution of the sodium salt of diethyl (*p*-mercaptobenzoyl)-L-glutamate (**13**) was prepared according to the procedure of Mautner et al.[4c] (from 4.0 g (6 mmol) of tetraethyl 4,4′-dithiobis(*N*-benzoyl-L-glutamate) in 50 mL of ethanol and 0.68 g of sodium borohydride). The solution was concentrated to about 10 mL under diminished pressure, to which a solution of **22** (prepared from 1.92 g of **3** and PBr_3 as described earlier) in 50 mL of dimethylacetamide was added, and the resulting solution was stirred for 6 h at room temperature. The product **25** was isolated by a procedure similar to that described earlier for the isolation of diethyl 8-deaza-10-thiaaminopterin (**17**): 2.1 g (41%); mp 202–204 °C; mass spectrum, *m/e* 569 (M^+, tetramethyl derivative); UV λ_{max} (pH 1) 251 (ϵ_{max} 25000), 284 (15800); (pH 7) 274 (22000), 325 (5400); (pH 13) 282 (20000), 335 (6000); ^1H NMR δ 4.6 (s, 2, CH_2-S), 6.73 (br, 2, 2-NH_2), 7.68 and 8.05 (q, 4, C_6H_4), 7.71 and 7.85 (q, 2, C^7H and C^8H), 8.86 (d, 2, CONH), 11.5 (br, 1, N^3H).

Anal. Calcd for $C_{24}H_{27}N_5O_6S$: C, 56.13; H, 5.29; N, 13.63. Found: C, 55.95; H, 5.33; N, 13.49.

General Method for Hydrolysis of Diethyl 8-Deazaaminopterin (14–17) and 8-Deazafolic Acid (23–25) Derivatives to the Corresponding Acids. To a suspension of diethyl 8-deazaaminopterin and folic acid derivatives in ethanol was added 1 N NaOH (2–3 mL of ethanol and 5 mL of 1 N NaOH was added per millimole of the esters). A clear solution resulted after a few hours. If precipitation occurred (or cloudiness persisted) a few drops of 1 N NaOH was added to effect dissolution. The solution was stirred for 48 h at room temperature and filtered if necessary. The filtrate was acidified with 1 N HCl to pH 3–4. The pre-

cipitated solid was filtered, washed with water and ethanol and dried.

***N*-[4-[[(2,4-Diaminopyrido[3,2-*d*]pyrimidin-6-yl)methyl]amino]benzoyl]-L-glutamic Acid or 8-Deazaaminopterin (18).** 18 was prepared in 72% yield from 14: mp 100–102 °C; mass spectrum, *m/e* 551 (M^+, octamethyl derivative); UV λ_{max} (pH 1) 318 (ϵ_{max} 10 000); (pH 7) 278 (22 300), 355 (8000); (pH 13) 278 (25 000), 355 (8000); ^1H NMR (Me_2SO-d_6 + D_2O) δ 4.6 (s, 2, CH_2), 7.8 (s, 2, C^7H and C^8H), 6.9 and 7.91 (q, 4, C_6H_4).

Anal. Calcd for $C_{20}H_{21}N_7O_5 \cdot 1.5H_2O$: C, 51.49; H, 5.18; N, 21.02. Found: C, 51.76; H, 4.96; N, 20.67.

***N*-[4-[[(2,4-Diaminopyrido[3,2-*d*]pyrimidin-6-yl)methyl]methylamino]benzoyl]-L-glutamic Acid or 8-Deazamethotrexate (19).** 19 was prepared in 68% yield from 15: mp 260 °C dec; mass spectrum, *m/e* 551 (M^+, heptamethyl derivative); UV λ_{max} (pH 1) 318 (ϵ_{max} 11 000), 333 (7700); (pH 7) 275 (16 000), 305 (20 400); (pH 13) 278 (19 500), 307 (25 500); ^1H NMR (Me_2SO-d_6 + D_2O) δ 3.27 (s, 3, $N^{10}CH_3$), 6.91 and 7.9 (q, 4, C_6H_4), 7.58 and 7.85 (q, 2, C^7H and C^8H).

Anal. Calcd for $C_{21}H_{23}N_7O_5 \cdot 2.0H_2O$: C, 51.52; H, 5.55; N, 20.03. Found: C, 51.92; H, 5.20; N, 20.49.

***N*-[4-[[(2,4-Diaminopyrido[3,2-*d*]pyrimidin-6-yl)methyl]oxy]benzoyl]-L-glutamic Acid or 8-Deaza-10-oxaaminopterin (20).** 20 was prepared in 62% yield from 16: mp 260–262 °C; mass spectrum, *m/e* 538 (M^+, heptamethyl derivative); UV λ_{max} (pH 1) 246 (ϵ_{max} 32 000), 318 (8300); (pH 7) 248 (35 500), 333 (6600); (pH 13) 250 (36 000), 343 (6300); ^1H NMR (Me_2SO-d_6 + D_2O) δ 5.43 (s, 2, CH_2–O), 7.31 and 8.05 (q, 4, C_6H_4), 7.93 (s, 2, C^7H and C^8H).

Anal. Calcd for $C_{20}H_{20}N_6O_6 \cdot 1.0H_2O$: C, 52.39; H, 4.83; N, 18.33. Found: C, 52.46; H, 4.67; N, 18.60.

***N*-[4-[[(2,4-Diaminopyrido[3,2-*d*]pyrimidin-6-yl)methyl]thio]benzoyl]-L-glutamic Acid or 8-Deaza-10-thiaaminopterin (21).** 21 was prepared in 68% yield from 17: mp 208–210 °C; mass spectrum, *m/e* 554 (M^+, heptamethyl derivative); UV λ_{max} (pH 1) 243 (ϵ_{max} 21 900), 275 (16 000), 320 (6600); (pH 7) 277 (16 000), 335 (5500); (pH 13) 277 (20 000), 343 (5500); ^1H NMR (Me_2SO-d_6 + D_2O) δ 4.63 (s, 2, CH_2-S), 7.66 and 8.03 (q, 4, C_6H_4), 7.98 and 8.01 (q, 2, C^7H and C^8H).

Anal. Calcd for $C_{20}H_{20}N_6O_5S \cdot 0.5H_2O$: C, 51.60; H, 4.54: N, 18.05. Found: C, 51.45; H, 4.75; N, 17.95.

***N*-[4-[[(2-Amino-3,4-dihydro-4-oxopyrido[3,2-*d*]pyrimidin-6-yl)methyl]amino]benzoyl]-L-glutamic Acid or 8-Deazafolic Acid (26).** 26 was prepared in 65% yield from 23: mp 238–240 °C; mass spectrum, *m/e* 552 (M^+, octamethyl derivative); UV λ_{max} (pH 1) 251 (ϵ_{max} 16 000), 305 (11 000); (pH 13) 283 (23 000) (the UV spectrum is similar to that reported by DeGraw et al.[5]); ^1H NMR (Me_2SO-d_6 + D_2O) δ 4.63 (s, 2, CH_2), 6.95 and 7.91 (q, 4, C_6H_4), 7.86 (s, 2, C^7H and C^8H).

***N*-[4-[[(2-Amino-3,4-dihydro-4-oxopyrido[3,2-*d*]pyrimidin-6-yl)methyl]methylamino]benzoyl]-L-glutamic Acid or 8-Deaza-N^{10}-methylfolic Acid (27).** 27 was prepared in 58% yield from 24: mp 267–270 °C; mass spectrum, *m/e* 538 (M^+, hexamethyl derivative); UV λ_{max} (pH 1) 250 (ϵ_{max} 17 000), 307 (12 000); (pH 7) 273 (15 200), 305 (27 000); (pH 13) 305 (26 000); ^1H NMR (Me_2SO-d_6 + D_2O) δ 3.26 (s, 3, $N^{10}CH_3$), 4.86 (s, 2, CH_2), 6.91 and 7.91 (q, 4, C_6H_4), 7.48 and 7.76 (q, 2, C^7H and C^8H).

Anal. Calcd for $C_{21}H_{22}N_6O_6 \cdot 1.5H_2O$: C, 52.34; H, 5.20; N, 17.45. Found: C, 52.60; H, 4.70; N, 17.39.

***N*-[4-[[(2-Amino-3,4-dihydro-4-oxopyrido[3,2-*d*]pyrimidin-6-yl)methyl]thio]benzoyl]-L-glutamic Acid or 8-Deaza-10-thiafolic Acid (28).** 28 was prepared in 72% yield from 25: mp 228–231 °C; mass spectrum, *m/e* 514 (M^+, tetramethyl (incomplete permethylation) derivative); UV λ_{max} (pH 1) 252 (ϵ_{max} 20 000), 284 (15 000); (pH 7) 274 (21 500), 325 (6400); (pH 13) 282 (21 500), 335 (7200); ^1H NMR (Me_2SO-d_6 + D_2O) δ 4.63 (s, 2, CH_2S), 7.71 and 8.05 (q, 4, C_6H_4), 7.81 and 7.95 (q, 2, C^7H and C^8H).

Anal. Calcd for $C_{20}H_{19}N_5O_6S$: C, 52.50; H, 4.18; N, 15.30. Found: C, 52.40; H, 4.31; N, 15.13.

Acknowledgment. This work was supported by a NHSRA traineeship (A.S.) under the auspices of Training Grant CA09038 and by Research Grants CH-125 from the American Cancer Society and CA11935 from the National Cancer Institute, NIH.

Registry No. 2, 76822-61-2; 3, 76807-52-8; 4, 2499-96-9; 5, 76807-53-9; 6, 76807-54-0; 7, 76807-55-1; 8, 76832-40-1; 9, 76807-56-2; 10, 13726-52-8; 11, 2378-95-2; 12 free alcohol, 57963-63-0; 12, 76807-57-3; 13, 76807-58-4; 14, 76807-59-5; 15, 76807-60-8; 16, 76807-61-9; 17, 76807-62-0; 18, 76807-63-1; 19, 76822-62-3; 20, 76807-64-2; 21, 76822-63-4; 22, 76832-41-2; 23, 76807-65-3; 24, 76807-66-4; 25, 76807-67-5; 26, 51989-25-4; 27, 76807-68-6; 28, 76807-69-7.

Reassignment of the Structures of Iodonitroimidazole, Its *N*-Methyl Derivatives, and Related Compounds[1]

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Received June 20, 1980

Condensation of ethyl 4-(2-thioethoxy)benzoate (4) with the compound hitherto described[3,4] as 2-iodo-1-methyl-5-nitroimidazole (2) does not lead to the expected product, ethyl 4-[2-[(1-methyl-5-nitro-2-imidazolyl)thio]ethoxy]benzoate (1), whereas condensation of 4 and the bromoimidazole 3 does give 1. An X-ray crystallographic analysis of the sodium salt of the corresponding acid confirms the structure of 1. Reinvestigation of the synthesis of 2 shows that its precursor, diiodoimidazole, is formed with retention of deuterium when 2-deuterioimidazole is used as the starting material and therefore has structure 16 rather than 14. The origin of this error in the literature was the assignment to a derivative of 16 of the structure 18 rather than 23. The nitration product of 16 has structure 9 rather than 11, and the derivatives assigned structures 2 and 12 are in fact 8 and 10, respectively. Authentic 12 can be prepared by nitration of 2-iodo-1-methylimidazole. Reaction of 9 with hydrobromic acid gives 29 and not, as reported previously, 27.

In the course of studies of methods for the large-scale preparation of 2-alkylthio-substituted nitroimidazoles, for example, 1,[2] we investigated the reactions of thiols with the compound reported as 2-iodo-1-methyl-5-nitro-

0022-3263/81/1946-1781$01.25/0