Synthesis of D,L-7,10-Ethano-5-deazaaminopterin and L-7,10-Ethano-5-deazafolic Acid[1]

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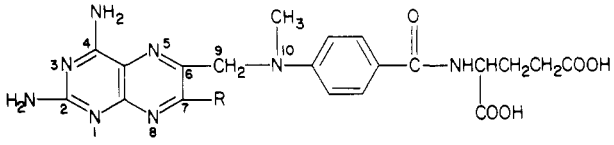
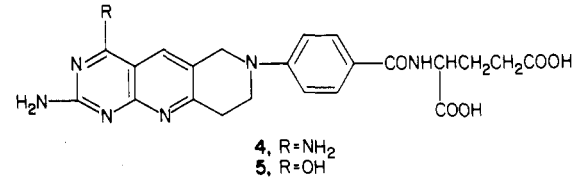
The title compounds have been prepared by several different ring annulation procedures starting from 1-[4-(*tert*-butoxycarbonyl)phenyl]-4-piperidone (**6b**).

The extreme toxicity of methotrexate (1), coupled with its inactivity toward most forms of human cancers, continues to stimulate an intensive search for less toxic and more selective agents for cancer chemotherapy based upon inhibition of dihydrofolate reductase and thymidylate synthetase. A major pathway for methotrexate metabolism is oxidation at C-7 to give the extremely insoluble 7-oxo derivative **2**.[2,3] In an attempt to block this metabolic inactivation pathway for methotrexate, the 7-methyl derivative **3** was prepared (albeit by an ambiguous synthetic route) and found to be inactive against L1210 cells.[3] On the other hand, an increase in lipophilicity in the central portion of molecules designed as inhibitors of thymidylate synthetase and dihydrofolate reductase increases enzyme binding,[4] and in line with this observation, an increase in polar character at N-10 of quinazoline analogues of folic acid normally decreases binding.[5] We therefore considered it of interest to construct analogues of methotrexate and folic acid in which (a) metabolic oxidation at C-7 is blocked by an appropriate substituent which, however, should have a smaller steric requirement than a freely rotating methyl group, and (b) lipophilicity is increased around the central portion of the molecule encompassing the region N-5, C-6, C-9, and N-10. These criteria appear to be met by folic acid and methotrexate analogues in which N-5 is replaced by carbon, and N-10 is joined to C-7 by an ethano bridge, giving rise to the "tied-back" analogues **4** and **5**. These compounds have additional appeal as potential inhibitors of dihydrofolate reductase and thymidylate synthetase because of our recent observation[6] that 5,10-dideaza-5,6,7,8-tetrahydroaminopterin is an extremely potent inhibitor of L1210 leukemia; **4** and **5** also contain a peripheral tetrahydropyridine ring separated from the fused pyrimidine ring by an aromatic pyridine ring spacer.



1, R=H
2, R=OH
3, R=CH_3

Synthesis of D,L-7,10-Ethano-5-deazaaminopterin (4). Our initial approach to the synthesis of **4** was patterned after the reported conversion of 1-pyrrolidino-1-cyclohexene to 2-amino-3-cyano-5,6,7,8-tetrahydroquinoline, which proceeds by the sequence of reactions described in Scheme I.[7] We[8] and others[9] have used the same general method involving alkylation of an enamine with (alkoxymethylene)malononitriles to generate 5-substituted 2-amino-3-cyanopyridines. In the present work, the requisite enamine **7a**, which was readily prepared from 1-[4-(ethoxycarbonyl)phenyl]-4-piperidone (**6a**), was subsequently alkylated with (methoxymethylene)malononitrile in THF at −20 °C to give **8a** (see Scheme II). Reaction with methanolic ammonia at room temperature for 12 h then gave the pyridine amino nitrile **9a**. Although cyclization of **9a** with guanidine free base in refluxing ethanol gave the annulated 2,4-diaminopyrimidine **10a** in very poor yield, this transformation was found to be much more successful using *N,N*-dimethylguanidine free base in DMF at 100 °C. Unfortunately, attempts to effect selective hydrolysis of the ester grouping in **10a** to give the corresponding carboxylic acid (**10c**) were unsuccessful; either no reaction at all took place, or hydrolysis of the 4-amino group, along with probable ring cleavage, accompanied ester saponification under more vigorous conditions. We therefore turned to the preparation of the corresponding *tert*-butyl ester **10b**, which would be expected to undergo selective ester hydrolysis in the presence of the labile 4-amino grouping.



4, R=NH_2
5, R=OH

1-[4-(*tert*-Butoxycarbonyl)phenyl]-4-piperidone (**6b**) was prepared by the sequence of reactions outlined in Scheme III. Conversion of **6b** to the enamine **7b** was best effected with anhydrous magnesium sulfate in THF.[10] Conversion of **7b** to the pyridine amino nitrile **9b** was then most effectively carried out by alkylation of **7b** with (chloromethylene)malonitrile[11] (which proved to be superior to (methoxymethylene)malononitrile) in the presence of triethylamine in THF at −10 °C, followed (without isolation of the intermediate **8b**) by treatment with methanolic ammonia (80% overall yield starting from **6b**). Annulation of the 2,4-diaminopyrimidine ring was then carried out with *N,N*-dimethylguanidine free base in DMF at 100 °C, and the requisite carboxylic acid **10c** prepared in 96% yield from **10b** by reaction with dry HCl in nitromethane[12] at 0 °C (see Scheme II).

(1) This work was supported by a grant (RO1 CA 28351) to Princeton University from the National Cancer Institute, National Institutes of Health.

(2) Breithau, H.; Kuenzlen, E. *Cancer Treat. Rep.* **1982**, *66*, 1733.
(3) Farquhar, D.; Loo, T. L.; Vadlamudi, S. *J. Med. Chem.* **1972**, *15*, 567.
(4) Yan, S. J.; Weinstock, L. T.; Cheng, C. C. *J. Heterocycl. Chem.* **1979**, *16*, 541.
(5) Jones, T. R. Personal communication.
(6) Taylor, E. C.; Harrington, P. J.; Fletcher, S. R.; Beardsley, G. P. *J. Med. Chem.*, in press.
(7) Kurihara, H.; Miahim, H. *J. Heterocycl. Chem.* **1977**, *14*, 1077.
(8) Taylor, E. C.; Dumas, D. J. *J. Org. Chem.* **1980**, *45*, 2485.
(9) DeGraw, J. I.; Tagawa, H. *J. Heterocycl. Chem.* **1982**, *19*, 1461.
(10) Zoretic, P. A.; Barcelos, F.; Branchaud, B. *Org. Prep. Proced. Int.* **1976**, *8*, 211.
(11) Josey, A. D.; Dickinson, C. L.; Dewhirst, K. C.; McKusick, B. C. *J. Org. Chem.* **1967**, *32*, 1940.
(12) Meslin, J. C.; Reliquet, A.; Quiniou, H. *Synthesis* **1980**, 453.

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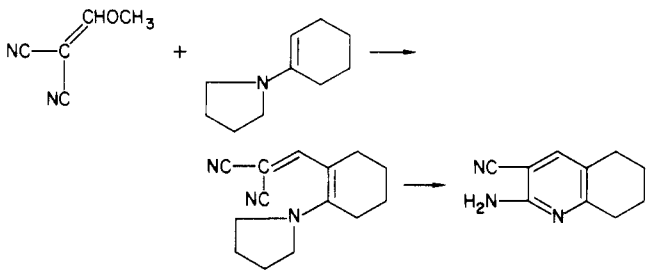
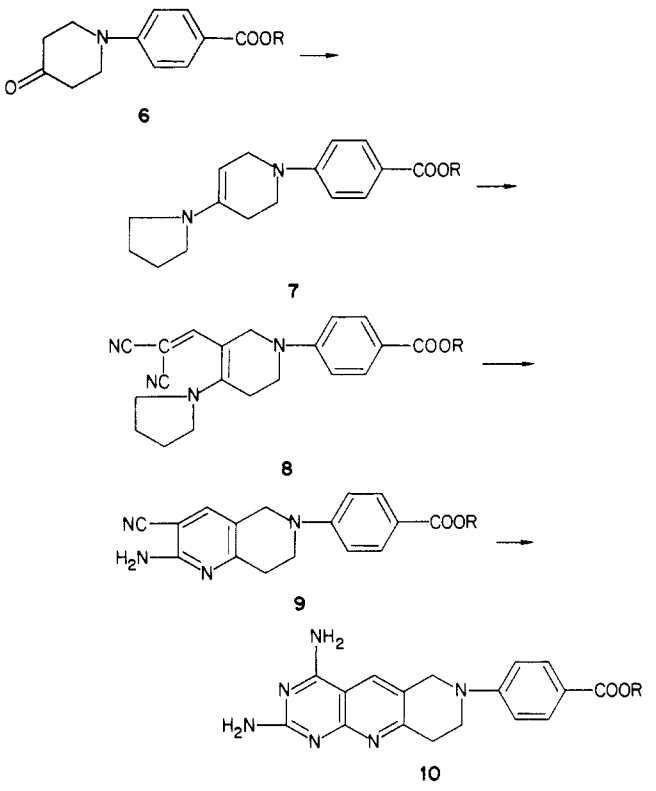
Scheme I

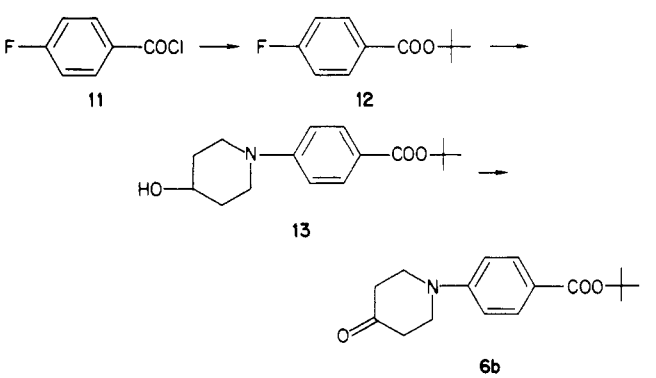


Scheme II

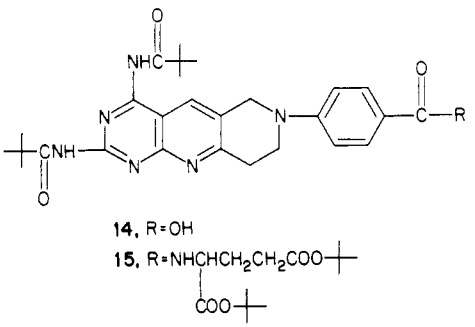


a, R=C_2H_5; b, R=*t*-Bu; c, R=H

Scheme III



The only remaining steps in our projected synthesis of 4 appeared to be trivial; namely, coupling of **10c** with diethyl L-glutamate and selective hydrolysis of the resulting diethyl ester groupings. However, all attempts to effect the peptide coupling reaction by using either diethyl phosphorocyanidate or diphenyl chlorophosphonate as coupling agents were frustrated by the extraordinary insolubility of **10c**. The tetrabutylammonium salt of **10c** was prepared by the use of Triton-B, but attempts to achieve a homogeneous solution of this salt by heating in DMF resulted in extensive decomposition. A number of attempts were therefore made to solubilize **10b,c** by acetylating the 2- and 4-amino groups. It has been reported, for example, that 2,4-diamino-6-methylquinazoline can be converted into a soluble 2,4-dibenzamido derivative with benzoyl chloride/triethylamine–dioxane, which then serves as a substrate for free radical bromination of the methyl group; final deacylation is smoothly effected with sodium methoxide and methanol.[13] However, all attempts to convert **10b** into the corresponding 2,4-dibenzamido derivative were unsuccessful. Thus, reaction of **10b** with benzoyl chloride in dioxane under reflux gave only a dark reaction mixture which appeared to be a mixture of decomposition products. Attempted acetylation with acetyl chloride/pyridine or with acetic anhydride/4-(dimethylamino)pyridine gave only starting material upon workup, while more vigorous reaction conditions led to cleavage of the *tert*-butyl ester grouping. We were successful, however, in obtaining a dipivaloyl derivative of **10b** by heating with pivaloyl chloride and triethylamine in dioxane. Treatment of this compound with HCl/nitromethane, followed by coupling of the resulting carboxylic acid **14** with di-*tert*-butyl L-glutamate/triethylamine/diethyl phosphorocyanidate, finally gave the coupled product **15** in 41% yield. However, attempted deprotection gave complex mixtures of hydrolysis products from which pure **4** could not effectively be recovered.



14, R=OH

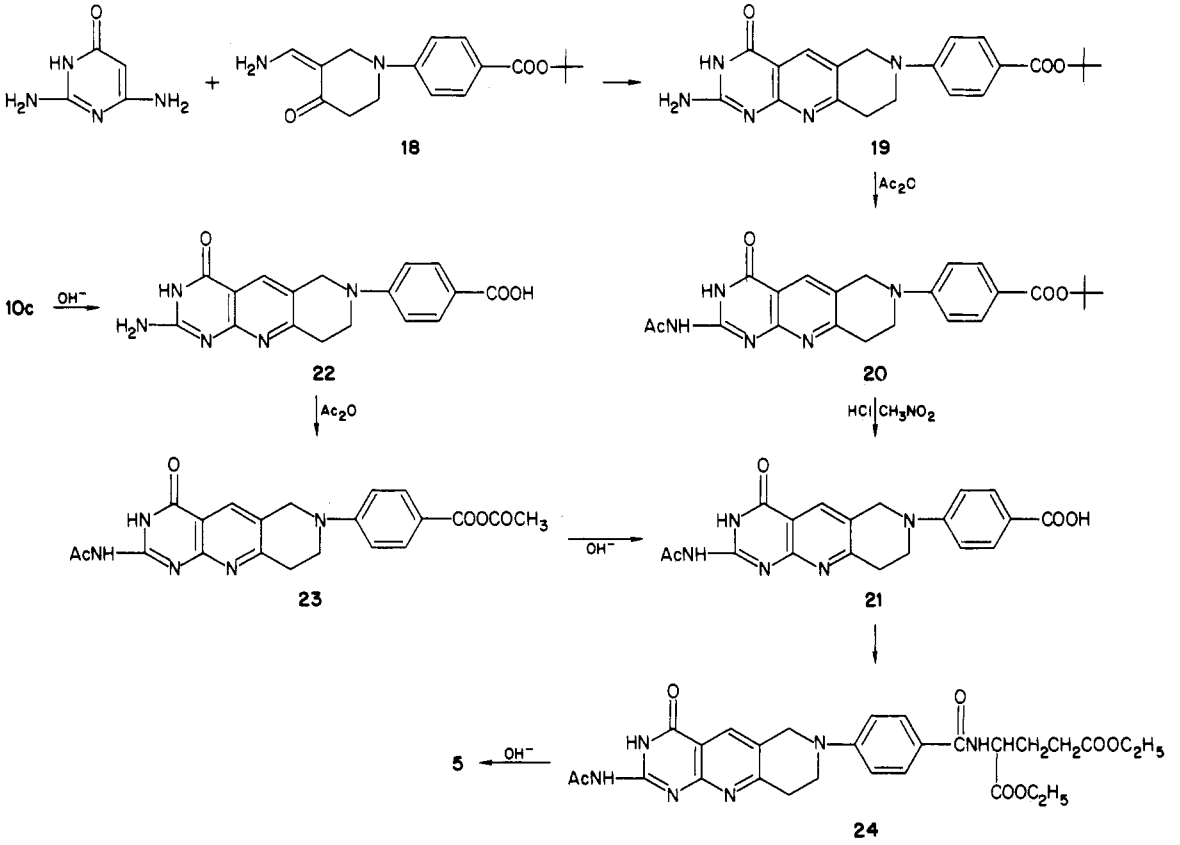
15, R=NHCHCH$_2$CH$_2$COO–, COO–

A synthesis of racemic 7,10-ethano-5-deazaaminopterin (**4**) was finally achieved as follows. Compound **9b** was converted to the free carboxylic acid **9c** with dry HCl in nitromethane, and this compound (which, in contrast to **9b**, was readily soluble in *N*-methylpyrrolidone) was successfully coupled with di-*tert*-butyl glutamate by using diphenyl phosphorochloridate. The resulting peptide was then smoothly cyclized with *N,N*-dimethylguanidine free base in hot *tert*-butyl alcohol (these conditions are known to effect racemization of glutamic acid derivatives[14]). D,L-7,10-Ethano-5-deazaaminopterin (**4**) was then obtained in 88% yield by hydrolysis of the *tert*-butyl ester groupings with dry HCl in nitromethane.

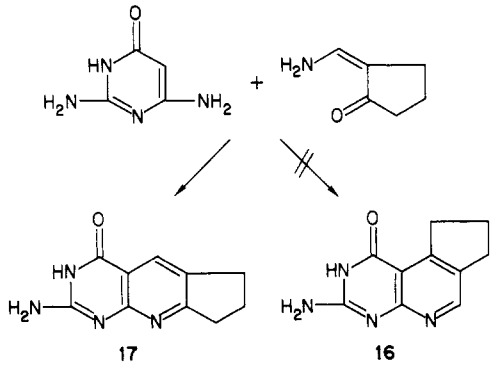
Synthesis of L-7,10-Ethano-5-deazafolic Acid (5). In 1973, Stark and Breitmaier claimed a simple route to 5,6-annulated pyrido[2,3-*d*]pyrimidine derivatives (i.e., **16**) by condensation of 2,4-diamino-6(1*H*)-pyrimidinone with α-aminomethylene ketones.[15] During an investigation of the possible applicability of this appealing synthesis to the preparation of deaza analogues of the thymidylate synthetase cofactor, we recently showed by an independent and unequivocal synthesis that the condensation product is correctly formulated as the 6,7-annulated isomer **17**.[16] On the assumption that a 6,7-annulated pyrido[2,3-*d*]py-

(13) Elslager, E. F.; Davoll, J. *Lect. Heterocycl. Chem.* **1974**, *2*, S-97.
(14) Mautner, H. G.; Kim, Y.-H.; *J. Org. Chem.* **1975**, *40*, 3447.
(15) Stark, E.; Breitmaier, E. *Tetrahedron* **1973**, *29*, 2209.
(16) Taylor, E. C.; Fletcher, S. R. *J. Org. Chem.* **1984**, *49*, 3226.

Scheme IV



rimidine would also be obtained by condensation of 2,4-diamino-6(1*H*)-pyrimidinone with a six-membered cyclic α-aminomethylene ketone, a simple synthesis of **5** could be envisioned utilizing **18** as a reaction partner. In the event, this assumption proved to be correct, and our results are described below.



Treatment of 1-[4-(*tert*-butoxycarbonyl)phenyl]-4-piperidone (**6b**) with potassium hydride in ethyl formate/THF, alkylation of the resulting hydroxymethylene derivative with dimethyl sulfate, and aminolysis with methanolic ammonia gave 1-[4-(*tert*-butoxycarbonyl)-phenyl]-3-(aminomethylene)-4-piperidone (**18**) as a low melting solid in 32% overall yield. This compound was condensed with 2,4-diamino-6-(1*H*)-pyrimidinone in 60% aqueous acetic acid containing a catalytic amount of piperidine. Surprisingly, the *tert*-butyl ester groupings survived these cyclization conditions, and **19** was obtained in 51% yield (see Scheme IV). Treatment of **19** with acetic anhydride then gave the 2-acetamido derivative **20**, which was converted to the free carboxylic acid **21** with dry HCl in nitromethane. The structure of this compound was then firmly established by an unequivocal independent synthesis commencing with **10c**, prepared as described above. Thus, hydrolysis of **10c** with aqueous 1 N sodium hydroxide gave the 5-deazapterin derivative **22**. Acetylation with acetic anhydride under reflux in the presence of a catalytic amount of 4-(dimethylamino)pyridine gave the 2-acetylated mixed anhydride **23**, which was selectively hydrolyzed with sodium hydroxide to give **21**. This compound was identical in every respect with the compound prepared from 2,4-diamino-6(1*H*)-pyrimidinone as described above. Coupling with diethyl L-glutamate in *N*-methylpyrrolidone as solvent was successfully accomplished by using phenyl *N*-phenylphosphoramidochloridate as condensing agent. The coupled product (**24**), which was readily purified by chromatography on silica, was hydrolyzed with methanolic sodium hydroxide at room temperature to give L-7,10-ethano-5-deazafolic acid (**5**).

Biochemical evaluation of these "tied-back" deaza analogues of methotrexate and folic acid will be described independently.

Experimental Section

1-[4-(Ethoxycarbonyl)phenyl]-4-(*N*-pyrrolidino)-1,2,5,6-tetrahydropyridine (7a). A solution of 2.5 g (0.0101 mol) of 1-[4-(ethoxycarbonyl)phenyl]-4-piperidone, 10 mL of benzene, 1.3 mL (0.140 mol) of pyrrolidine, and 30 mg of *p*-toluenesulfonic acid was stirred under reflux for 17 h with constant azeotropic removal of water. The reaction mixture was then allowed to cool gradually to room temperature, and the solid which had separated was collected by filtration and washed with cold benzene to give 2.15 g (71%) of **7a** as a pale yellow solid: mp 141–143 °C; NMR ($CDCl_3$) δ 1.35 (t, 3 H, J = 7 Hz), 2.00–1.73 (m, 4 H), 2.60–2.30 (m, 2 H), 3.20–2.92 (m, 4 H), 3.57 (t, 2 H, J = 6 Hz), 4.03–3.82 (m, 2 H), 4.35–4.17 (m, 1 H), 4.35 (q, 2 H, J = 7 Hz), 7.97, 6.87 (ABq, 4 H, J = 9 Hz); IR (KBr) 1700, 1655, 1600 cm^{-1}.

Anal. Calcd for $C_{18}H_{24}N_2O_2$: C, 71.97; H, 8.05; N, 9.33. Found: C, 71.97; H, 7.82; N, 9.53.

1-[4-(Ethoxycarbonyl)phenyl]-3-(2,2-dicyanovinyl)-4-(*N*-pyrrolidino)-1,2,5,6-tetrahydropyridine (8a). To a stirred solution of 1.0 g (0.0093 mol) of (methoxymethylene)malononitrile

in 20 mL of anhydrous tetrahydrofuran, cooled to −25 °C under nitrogen, was added dropwise a solution of 2.80 g (0.0093 mol) of 1-[4-(ethoxycarbonyl)phenyl]-4-(*N*-pyrrolidino)-1,2,5,6-tetrahydropyridine in 40 mL of tetrahydrofuran. After 2 h, the reaction mixture was warmed to 0 °C and diluted with water and the mixture was extracted with 5 × 200-mL portions of diethyl ether. The combined organic extracts were washed with saturated sodium chloride solution and then dried over anhydrous sodium sulfate. The precipitate which separated on standing was collected by filtration to give 900 mg (26%) of **8a** as a reddish-orange powder, mp 178–181 °C. Concentration of the filtrate gave an oil which was dissolved in diethyl ether and passed through a short column of silica gel. Evaporation of the eluate gave an additional 110 mg of **8a**; total yield 1.10 g (29%). The analytical sample was prepared as bright orange crystals by recrystallization from ethanol: mp 184–185 °C; NMR ($CDCl_3$) δ 1.37 (t, 3 H, J = 7 Hz), 2.18–1.82 (m, 4 H), 2.93–2.62 (m, 2 H), 3.83–3.40 (m, 6 H), 4.33 (q, 2 H, J = 7 Hz), 4.40 (br s, 2 H), 7.20 (br s, 1 H), 7.78, 6.87 (AB q, 4 H, J = 9 Hz); IR (KBr) 2210, 2200, 1698, 1685, 1603 cm^{-1}.

Anal. Calcd for $C_{22}H_{24}N_4O_2$: C, 70.18; H, 6.43; N, 14.88. Found: C, 70.04; H, 6.42; N, 14.46.

2-Amino-3-cyano-6-[4-(ethoxycarbonyl)phenyl]-5,6,7,8-tetrahydropyrido[4,3-*b*]pyridine (9a). A mixture of 0.5 g of 1-[4-(ethoxycarbonyl)phenyl]-3-(2,2-dicyanovinyl)-4-(*N*-pyrrolidino)-1,2,5,6-tetrahydropyridine and 15 mL of saturated methanolic ammonia was stirred at room temperature overnight and then filtered to give 0.41 g (96%) of **9a** as a pale orange powder, mp 220–222 °C. The analytical sample was prepared by recrystallization from toluene/cyclohexane without change in the melting point: NMR (Me_2SO-d_6) δ 1.28 (t, 3 H, J = 7 Hz), 2.84 (t, 2 H, J = 6 Hz), 3.70 (t, 2 H, J = 6 Hz), 4.26 (q, 2 H, J = 7 Hz), 4.38 (s, 2 H), 7.62 (br s, 2 H), 7.74 (s, 1 H), 7.82, 7.00 (ABq, 4 H, J = 9 Hz); IR (KBr) 3420, 3320, 3190, 2220, 1695, 1688, 1648, 1640, 1605 cm^{-1}.

Anal. Calcd for $C_{18}H_{18}N_4O_2$: C, 67.06; H, 5.63; N, 17.38. Found: C, 66.82; H, 5.37; N, 17.17.

2,4-Diamino-7-[4-(ethoxycarbonyl)phenyl]-5-deaza-6,7,8,9-tetrahydropyrido[3,4-*g*]pteridine (10a). A mixture of 0.96 g (0.0078 mol) of freshly dried dimethylguanidine hydrochloride, 0.6 g (0.0088 mol) of sodium ethoxide, and 10 mL of anhydrous dimethylformamide was stirred under nitrogen for 1 h, and then 0.5 g (0.0016 mol) of 2-amino-3-cyano-6-[4-(ethoxycarbonyl)phenyl]-5,6,7,8-tetrahydropyrido[4,3-*b*]pyridine in 25 mL of anhydrous dimethylformamide added. The reaction mixture was heated for 5 h at 100 °C and filtered while hot, and the collected yellow solid was washed successively with water, ether, dimethylformamide, ether, and then acetone to give 0.34 g (58%) of **10a** as a microcrystalline yellow powder: NMR (Me_2SO-d_6) δ 1.27 (t, 3 H, J = 7 Hz), 3.12–2.92 (m, 2 H), 3.86–3.52 (m, 2 H), 4.22 (q, 2 H, J = 7 Hz), 4.54 (s, 2 H), 6.16 (br s, 2 H), 7.36 (br s, 2 H), 7.0, 7.82 (ABq, 4 H, J = 9 Hz), 8.22 (s, 1 H); IR (KBr) 3410, 3340, 3130, 1720, 1640, 1610, 1590 cm^{-1}.

Anal. Calcd for $C_{19}H_{20}N_6O_2$: C, 62.62; H, 5.53; N, 23.06. Found: C, 62.88; H, 5.65; N, 23.37.

1-[4-(*tert*-Butoxycarbonyl)phenyl]-4-hydroxypiperidine (13). A mixture of 13.3 g (0.068 mol) of *tert*-butyl 4-fluorobenzoate, 7.6 g (0.074 mol) of 4-hydroxypiperidine, and 10 g (0.072 mol) of finely powdered anhydrous potassium carbonate in 25 mL of Me_2SO was heated at 120 °C (external temperature) with stirring under nitrogen for 7 h. The mixture was cooled to room temperature and poured into 150 mL of water, and the resulting white precipitate collected by filtration, washed with water, and dissolved in 200 mL of methylene chloride. The solution was washed once with water and the organic phase dried over anhydrous magnesium sulfate and evaporated to give a colorless solid which was recrystallized from carbon tetrachloride/hexane: yield 13.87 g (73%) of **13** as a colorless crystalline solid; mp 122–124 °C; NMR ($CDCl_3$) δ 1.58 (s, 9 H), 1.8–2.2 (m, 4 H), 2.9–4.1 (m, 5 H), 6.85, 7.88 (ABq, 4 H, J = 9 Hz); IR (nujol) 3500, 3600–3000, 1670, 1610 cm^{-1}.

Anal. Calcd for $C_{16}H_{23}NO_3$: C, 69.28; H, 8.36; N, 5.05. Found: C, 69.11; H, 8.40; N, 4.78.

1-[4-(*tert*-Butoxycarbonyl)phenyl]-4-piperidone (6b). Pyridine (2.4 mL) and trifluoroacetic acid (1.1 mL) were added dropwise and sequentially to a solution of 8.1 g (0.029 mol) of 1-[4-(*tert*-butoxycarbonyl)phenyl]-4-hydroxypiperidine and 18.1 g (0.088 mol) of dicyclohexylcarbodiimide in 42 mL of Me_2SO and 78 mL of benzene. The addition was carried out at such a rate that the temperature did not exceed 5 °C. The reaction mixture was stirred in an ice bath for several hours and then allowed slowly to reach room temperature. Ethyl acetate (200 mL) was added, and the precipitated dicyclohexylurea was removed by filtration. The filtrate was washed with 3 × 50-mL portions of water, dried over anhydrous magnesium sulfate, and evaporated. The resulting gummy solid was triturated with cyclohexane and the resulting solid collected by filtration and recrystallized from carbon tetrachloride to give 6.65 g (83%) of **6b** as a colorless solid: mp 140–142 °C; NMR ($CDCl_3$) δ 1.6 (s, 9 H), 2.58 (t, 4 H, J = 6 Hz), 3.75 (t, 4 H, J = 6 Hz), 6.93, 7.95 (ABq, 4 H, J = 9 Hz); IR (nujol) 1725, 1680, 1610 cm^{-1}.

Anal. Calcd for $C_{16}H_{21}NO_3$: C, 69.79; H, 7.69; N, 5.09. Found: C, 69.67; H, 7.75; N, 5.24.

1-[4-(*tert*-Butoxycarbonyl)phenyl]-4-(*N*-pyrrolidino)-1,2,5,6-tetrahydropyridine (7b). A mixture of 0.6 mL (0.0072 mol) of pyrrolidine, 1.0 g (0.0036 mol) of 1-[4-(*tert*-butoxycarbonyl)phenyl]-4-piperidone, 4 g of anhydrous magnesium sulfate, and 30 mL of anhydrous tetrahydrofuran was stirred at room temperature for 2 h. Additional anhydrous magnesium sulfate (1 g) was added and stirring was continued for an additional 2 h. The reaction mixture was then filtered, and the filtrate was evaporated to dryness. Recrystallization of the residual solid from toluene/cyclohexane gave 1.15 g (96%) of **7b**: mp 131–133 °C; NMR ($CDCl_3$) δ 1.6 (s, 9 H), 1.7–2.1 (m, 4 H), 2.4–2.7 (m, 2 H), 2.8–3.3 (m, 4 H), 3.5–3.8 (m, 4 H), 3.8–4.0 (m, 1 H), 6.75–6.95, 7.8–8.0 (ABq, 4 H, J = 9 Hz); IR (nujol) 1690, 1650, 1610 cm^{-1}.

Anal. Calcd for $C_{20}H_{28}N_2O_2$: C, 73.13; H, 8.59; N, 8.53. Found: C, 73.08; H, 8.41; N, 8.66.

2-Amino-3-cyano-6-[4-(*tert*-butoxycarbonyl)phenyl]-5,6,7,8-tetrahydropyrido[4,3-*b*]pyridine (9b). To a solution of 8.3 g (0.025 mol) of 1-[4-(*tert*-butoxycarbonyl)phenyl]-4-(*N*-pyrrolidino)-1,2,5,6-tetrahydropyridine and 3.5 mL of triethylamine in 100 mL of tetrahydrofuran, cooled to −30 °C, was added dropwise a solution of 2.82 g (0.025 mol) of (chloromethylene)-malononitrile in 5 mL of tetrahydrofuran. The reaction mixture rapidly turned deep red. It was stirred at −30 °C for 20 min and then at room temperature for 1 h and was then filtered through Celite, with copious washing of the filter pad with tetrahydrofuran. Evaporation of the filtrate gave a deep red solid which was suspended in 100 mL of saturated methanolic ammonia, and the mixture was stirred at room temperature overnight. The pale yellow solid which had separated was collected by filtration and recrystallized from 400 mL of toluene to give 6.68 g (76%) of **9b**: mp 221–222 °C; NMR (Me_2SO-d_6) δ 1.55 (s, 9 H), 2.9 (t, 2 H, J = 4 Hz), 4.4 (s, 2 H), 6.7 (s, 2 H), 7.0, 7.85 (ABq, 4 H, J = 9 Hz), 8.12 (s, 1 H); IR (nujol) 3420, 3300, 3180, 2200, 1680, 1630, 1600, 1560 cm^{-1}.

Anal. Calcd for $C_{20}H_{22}N_4O_2$: C, 68.55; H, 6.33; N, 15.99. Found: C, 68.74; H, 6.37; N, 15.74.

2,4-Diamino-7-[4-(*tert*-butoxycarbonyl)phenyl]-5-deaza-6,7,8,9-tetrahydropyrido[3,4-*g*]pteridine (10b). Dimethylguanidine hydrochloride (10 g, 0.08 mol) was added in 1 portion to a slurry of 10 g (0.088 mol) of potassium *tert*-butoxide in 50 mL of dry dimethylformamide under nitrogen, and the mixture was stirred at room temperature for 1 h. To this solution was added 6.5 g (0.018 mol) of 2-amino-3-cyano-6-[4-(*tert*-butoxycarbonyl)phenyl]-5,6,7,8-tetrahydropyrido[4,3-*b*]pyridine in one portion, and the mixture was heated at 100 °C under nitrogen with stirring for 5 h. The reaction mixture was cooled and filtered, and the collected yellow precipitate washed copiously with hot water followed by methanol and acetone to give 5.8 g (80%) of **10b**: mp >300 °C; NMR (TFA) δ 1.7 (s, 9 H), 3.8–4.0 (m, 2 H), 4.4–4.6 (m, 2 H), 5.25 (s, 2 H), 7.95, 8.55 (ABq, 4 H, J = 9 Hz), 8.85 (s, 1 H); IR (nujol) 3400, 3340, 3140, 1700, 1640, 1605 cm^{-1}.

Anal. Calcd for $C_{21}H_{24}N_6O_2$: C, 64.27; H, 6.16; N, 21.42. Found: C, 64.03; H, 6.19; N, 21.57.

2,4-Diamino-7-(4-carboxyphenyl)-5-deaza-6,7,8,9-tetrahydropyrido[3,4-*g*]pteridine (10c). A solution of 0.5 g of 2,4-diamino-7-[4-(*tert*-butoxycarbonyl)phenyl]-5-deaza-6,7,8,9-tetrahydropyrido[3,4-*g*]pteridine in 5 mL of 88% formic acid was stirred at room temperature for 72 h. The precipitated yellow solid was collected by filtration and washed well with water followed by methanol and acetone: yield 0.375 g (88%); mp >300

°C; NMR (TFA) δ 3.8–4.05 (m, 2 H), 4.4–4.65 (m, 2 H), 5.25 (s, 2 H), 7.3–7.0 (br, 2 H), 7.95 and 8.55 (ABq, 4 H, J = 9 Hz), 8.9 (s, 1 H).

2,4-Bis(pivaloylamino)-7-[4-(*tert*-butoxycarbonyl)phenyl]-5-deaza-6,7,8,9-tetrahydropyrido[3,4-*g*]pteridine. A suspension of 3.5 g (0.0089 mol) of 2,4-diamino-7-[4-(*tert*-butoxycarbonyl)phenyl]-5-deaza-6,7,8,9-tetrahydropyrido[3,4-*g*]pteridine in a mixture of 2.4 mL (0.0196 mol) of pivaloyl chloride, 5.5 mL (0.04 mol) of triethylamine, and 50 mL of dioxane was heated for 2 h under reflux. The reaction mixture was filtered, 5 mL of methanol was added to the filtrate, and the mixture was filtered once again. The filtrate was evaporated and the residual yellow solid chromatographed on silica. Elution of the column with ether followed by ethyl acetate gave 2.05 g (41%) of the 2,4-dipivaloyl derivative of **10b**: mp 205–210 °C dec; NMR ($CDCl_3$) δ 1.4 (s, 18 H), 1.6 (s, 9 H), 3.15–3.35 (m, 2 H), 3.7–3.9 (m, 2 H), 4.6 (s, 2 H), 6.95, 7.95 (ABq, 4 H, J = 9 Hz), 8.57 (s, 1 H); IR (nujol) 3480, 3160 (br), 1700, 1660, 1600 cm^{-1}.

Anal. Calcd for $C_{31}H_{40}N_6O_4 \cdot H_2O$: C, 64.34; H, 7.32; N, 14.52. Found: C, 64.05; H, 6.83; N, 14.22.

2,4-Bis(pivaloylamino)-7-(4-carboxyphenyl)-5-deaza-6,7,8,9-tetrahydropyrido[3,4-*g*]pteridine (14). Dry hydrogen chloride gas was bubbled slowly through a suspension of 2.0 g of 2,4-bis(pivaloylamino)-7-[4-(*tert*-butoxycarbonyl)phenyl]-5-deaza-6,7,8,9-tetrahydropyrido[3,4-*g*]pteridine in 20 mL of nitromethane cooled in an ice bath. The reaction mixture darkened and a brown solid slowly precipitated from solution. After 30 min, 100 mL of ether was added and the precipitated solid was collected by filtration. This material was suspended in 10 mL of water and the pH adjusted to 4 with aqueous sodium carbonate. The suspended yellow solid was collected by filtration and dried under reduced pressure: yield 0.4 g (22%) of **14**; mp >300 °C; NMR (Me_2SO-d_6) δ 1.3 (s, 18 H), 3.0–3.25 (m, 2 H), 2.7–2.95 (m, 2 H), 4.68 (s, 2 H), 7.05 and 7.85 (ABq, 4 H, J = 9 Hz), 8.3 (s, 1 H).

Di-*tert*-butyl *N*-[4-[7-[2,4-Bis(pivaloylamino)-5-deaza-6,7,8,9-tetrahydropyrido[3,4-*g*]pteridino]]benzoyl]glutamate (15). Hydrogen chloride gas was bubbled for 2 min through a solution of 0.38 g of 2,4-bis(pivaloylamino)-7-[4-(*tert*-butoxycarbonyl)phenyl]-5-deaza-6,7,8,9-tetrahydropyrido[3,4-*g*]pteridine in 15 mL of nitromethane. The mixture was stirred for 30 min at room temperature, 50 mL of diethyl ether was added, and the precipitated **14** was collected by filtration and suspended in 50 mL of acetonitrile containing 1 mL of triethylamine and 0.33 g of diethyl phosphorocyanidate. The reaction mixture was stirred at room temperature for 1 h, 0.59 g of L-di-*tert*-butyl glutamate hydrochloride added, and the resulting mixture stirred at room temperature overnight. The mixture was then evaporated to dryness, the residual solid dissolved in ether and washed with water, and the ether evaporated under reduced pressure. The residual solid was dissolved in methylene chloride/methanol (9:1) and chromatographed on silica gel. Rechromatography of the product with methylene chloride/methanol (97:3) yielded 0.21 g (41%) of the coupled product: mp 142–144 °C; NMR ($CDCl_3$) δ 1.35 (s, 18 H), 1.42 (s, 9 H), 1.50 (s, 9 H), 2.0–2.25 (m, 4 H), 3.28 (t, 2 H, J = 8 Hz), 3.79 (t, 2 H, J = 8 Hz), 4.65 (s, 2 H), 6.95 and 7.85 (ABq, 4 H, J = 9 Hz), 8.58 (s, 1 H); MS calcd for $C_{40}H_{55}N_7O_7$ 745, found 745, 688, 576, 559, 487, 476.

2-Amino-3-cyano-6-(4-carboxyphenyl)-5,6,7,8-tetrahydropyrido[4,3-*b*]pyridine (9c). Hydrogen chloride gas was passed through a suspension of 2.0 g of 2-amino-3-cyano-6-[4-(*tert*-butoxycarbonyl)phenyl]-5,6,7,8-tetrahydropyrido[4,3-*b*]pyridine (**9b**) in 15 mL of nitromethane, cooled in an ice bath, for a period of 5 min. The reaction mixture was allowed to stand at room temperature for 30 min, 20 mL of anhydrous diethyl ether added, and the precipitated colorless solid collected by filtration and dissolved in 10 mL of 1 N sodium hydroxide solution. The mixture was adjusted to pH 5 with acetic acid, and the resulting yellow-green solid was collected by filtration and recrystallized from dimethylformamide to give 1.3 g (77%) of **9c**: mp >300 °C; NMR (Me_2SO-d_6) δ 2.96–3.15 (m, 2 H), 3.70–3.9 (m, 2 H), 4.45 (s, 2 H), 7.05 and 7.85 (ABq, 4 H, J = 9 Hz), 8.4 (s, 1 H), 9.25 (br s, 3 H).

Di-*tert*-butyl *N*-[4-[6-(2-Amino-3-cyano-5,6,7,8-tetrahydropyrido[4,3-*b*]pyridino)]benzoyl]glutamate. 2-Amino-3-cyano-6-(4-carboxyphenyl)-5,6,7,8-tetrahydropyrido[4,3-*b*]-pyridine (**9c**) (1.5 g, 0.0051 mol) was dissolved in 100 mL of hot *N*-methylpyrrolidone, 2.1 mL (0.015 mol) of triethylamine added, and the mixture cooled to 5 °C. Diphenyl phosphorochloridate (1.4 mL, 0.0066 mol) was then added dropwise, and the resulting mixture was stirred for 25 min at 5 °C. L-Di-*tert*-butyl glutamate hydrochloride (1.5 g, 0.0051 mol) was added in one portion, and the reaction mixture was stirred overnight at room temperature. The solvent was evaporated under reduced pressure and the residual solid partitioned between 50 mL of saturated aqueous sodium bicarbonate solution and 50 mL of ethyl acetate. The organic phase was separated, washed with water, dried over anhydrous magnesium sulfate, and evaporated to give a brown oil which slowly solidified to give 1.95 g (71%) of the desired coupled product; NMR ($CDCl_3$) δ 1.45–1.5 (2 s, 18 H), 2.0–2.5 (m, 4 H), 3.0 (t, 2 H, J = 6 Hz), 3.6–3.8 (t, 2 H, J = 6 Hz), 4.35 (s, 2 H), 6.8–7.0 (br s, 1 H), 7.9, 7.8 (ABq, 4 H, J = 9 Hz), 7.5 (s, 1 H); IR (nujol) 3500–3000, 2200, 1715, 1600 cm^{-1}.

Anal. Calcd for $C_{29}H_{37}N_5O_5$: C, 65.02; H, 6.96; N, 13.08. Found: C, 64.24; H, 7.02; N, 13.21.

Di-*tert*-butyl *N*-[4-[7-(2,4-Diamino-5-deaza-6,7,8,9-tetrahydropyrido[3,4-*g*]pteridino)]benzoyl]glutamate. To a solution of 0.33 g (0.0029 mol) of potassium *tert*-butoxide in 20 mL of *tert*-butyl alcohol was added 0.39 g (0.0032 mol) of dimethylguanidine hydrochloride, and the mixture was stirred at room temperature for 20 min. To the mixture was then added 1.90 g of di-*tert*-butyl *N*-[4-[6-(2-amino-3-cyano-5,6,7,8-tetrahydropyrido[4,3-*b*]pyridino)]benzoyl]glutamate, and the saturated mixture was heated under reflux under nitrogen for 24 h. The solvent was evaporated under reduced pressure and the residual solid triturated with water and filtered, and the collected solid suspended in ether and recollected by centrifugation: yield 0.4 g (69%); mp >250 °C; NMR (TFA-d_1) δ 1.7 (s, 18 H), 2.0–3.0 (br s, 4 H), 3.75–4.0 (m, 2 H), 4.3–4.6 (m, 2 H), 4.95–5.3 (m, 3 H), 7.95, 8.25 (ABq, 4 H, J = 9 Hz), 8.8 (s, 1 H); IR (nujol) 3500–3000, 1710, 1600 (br) cm^{-1}.

Anal. Calcd for $C_{30}H_{39}N_7O_5 \cdot 2.5\ H_2O$: C, 57.88; H, 7.07; N, 15.75. Found: C, 57.83; H, 6.92; N, 15.66.

D,L-7,10-Ethano-5-deazaaminopterin (4). Hydrogen chloride gas was passed for 5 min through a suspension of 0.7 g of di-*tert*-butyl *N*-[4-[7-(2,4-diamino-5-deaza-6,7,8,9-tetrahydropyrido[3,4-*g*]pteridino)]benzoyl]glutamate in 20 mL of nitromethane at 0 °C. The resulting pink-colored emulsion was stirred at room temperature for 1 h, 20 mL of diethyl ether added, and the precipitated solid collected by filtration. This solid was resuspended in water and 1 N sodium hydroxide solution was added until a clear solution resulted. This solution was filtered, the filtrate acidified with acetic acid, and the resulting orange precipitate collected by centrifugation and washed thoroughly with water followed by methanol and ether: yield 0.49 g (88%); mp >250 °C. This compound was so insoluble, even in TFA, that its NMR spectrum could not be determined.

Anal. Calcd for $C_{22}H_{23}N_7O_5$: C, 56.77; H, 4.95; N, 21.06. Found: C, 56.51; H, 4.60; N, 20.82.

1-[4-(*tert*-Butoxycarbonyl)phenyl]-3-(aminomethylene)-4-piperidone (18). A solution of 18.4 g (0.067 mol) of 1-[4-(*tert*-butoxycarbonyl)phenyl]-4-piperidone (**6b**) in 6.5 mL (0.08 mol) of ethyl formate was added dropwise to a slurry of 8.4 g (0.073 mol) of 35% potassium hydride in 100 mL of dry diethyl ether cooled by an external ice bath. The reaction mixture was stirred at 0 °C until hydrogen evolution ceased (approximately 30 min), and then 6.2 mL (0.066 mol) of dimethyl sulfate was added. Stirring was continued for 12 h at room temperature, the reaction mixture was poured into half saturated ammonium chloride solution, and the mixture was extracted with ether. The ether extracts were dried over anhydrous magnesium sulfate, 10 g of silica gel was added, and the solvent was evaporated. The impregnated silica gel was applied to the top of a silica gel column which was eluted with hexane followed by a diethyl ether/hexane (1:1) mixture. The eluate was evaporated to dryness and the residual solid (12 g) dissolved in 100 mL of saturated methanolic ammonia. The mixture was stirred at room temperature for 16 h, the solvent evaporated under reduced pressure, and the residual solid triturated with diethyl ether/hexane (1:1): yield 6.5 g (32% from **6b**); mp 132–134 °C.

Anal. Calcd for $C_{17}H_{22}N_2O_3$: C, 67.52; H, 7.33; N, 9.27. Found: C, 67.77; H, 7.48; N, 9.09.

2-Amino-7-[4-(*tert*-butoxycarbonyl)phenyl]-5-deaza-6,7,8,9-tetrahydro-4(3*H*)-pyrido[3,4-*g*]pteridinone (19). A

mixture of 0.6 g (0.002 mol) of 1-[4-(*tert*-butoxycarbonyl)-phenyl]-3-(aminomethylene)-4-piperidone (**18**) and 0.29 g (0.002 mol) of 2,4-diamino-6(1*H*)-pyrimidinone was dissolved in 10 mL of glacial acetic acid containing 5 mL of water. One drop of piperidine was added, and the mixture was heated under reflux in a nitrogen atmosphere for 2 h, cooled to room temperature, diluted with 10 mL of water, and filtered. The collected solid was washed with water followed by methanol and acetone: yield 0.4 g (51%); mp >250 °C; NMR (TFA) δ 1.7 (s, 9 H), 3.8–4.05 (m, 2 H), 4.4–4.55 (m, 2 H), 5.3 (s, 2 H), 7.95 and 8.55 (ABq, 4 H, J = 9 Hz), 8.8 (s, 1 H).

2-Acetamido-7-[4-(*tert*-butoxycarbonyl)phenyl]-5-deaza-6,7,8,9-tetrahydro-4(3*H*)-pyrido[3,4-*g*]pteridinone (20). A mixture of 1.0 g of 2-amino-7-[4-(*tert*-butoxycarbonyl)phenyl]-5-deaza-6,7,8,9-tetrahydro-4(3*H*)-pyrido[3,4-*g*]pteridinone (**19**) and 10 mL of acetic anhydride containing 5 drops of 4-(dimethylamino)pyridine was heated under reflux for 1 h and cooled to room temperature, and 10 mL of ether added. Filtration then gave 0.84 g (76%) of **20**: mp >250 °C; NMR (TFA) δ 1.75 (s, 9 H), 2.65 (s, 3 H), 4.1–4.3 (m, 2 H), 4.5–4.75 (m, 2 H), 5.4 (s, 2 H), 7.95 and 8.58 (ABq, 4 H, J = 9 Hz), 9.25 (s, 1 H).

2-Acetamido-7-(4-carboxyphenyl)-5-deaza-6,7,8,9-tetrahydro-4(3*H*)-pyrido[3,4-*g*]pteridinone (21). Method A. Hydrogen chloride was bubbled for 2 min through a suspension of 50 mg of 2-acetamido-7-[4-(*tert*-butoxycarbonyl)phenyl]-5-deaza-6,7,8,9-tetrahydro-4(3*H*)-pyrido[3,4-*g*]pteridinone (**20**) in 5 mL of nitromethane at room temperature. The reaction mixture was stirred for 1 h, the solvent evaporated under reduced pressure, and the residual solid triturated with ether and filtered: yield 44 mg (96%); mp >250 °C.

Anal. Calcd for $C_{19}H_{17}N_5O_4 \cdot H_2O$: C, 57.43; H, 4.79; N, 17.63. Found: C, 57.76; H, 4.43; N, 16.98.

Method B. Hydrogen chloride gas was bubbled for 2 min through a suspension of 2.0 g of 2,4-diamino-7-[4-(*tert*-butoxycarbonyl)phenyl]-5-deaza-6,7,8,9-tetrahydropyrido[3,4-*g*]pteridine (**10b**) in 50 mL of nitromethane, cooled by an external ice bath. The resulting solution was stirred for 1 h at room temperature, 50 mL of ether added, and the resulting solid precipitate collected by filtration and washed well with ether. This solid (**10c**) was suspended in 70 mL of 1 N sodium hydroxide solution, and the mixture was heated under reflux under nitrogen for 4 h. The resulting yellow homogeneous solution was filtered, and the filtrate was acidified with acetic acid. The resulting gellatinous precipitate was collected by centrifugation, washed with water and methanol, and dried at 100 °C (100 mm) to give 1.6 g (86%) of **22**. This compound was suspended in 15 mL of acetic anhydride, 0.1 g of 4-(dimethylamino)pyridine added, and the reaction mixture heated under nitrogen at 130 °C for 4 h. The mixture was then cooled to room temperature, 20 mL of diethyl ether added, and the mixture filtered to give 2.0 g (93%) of **23**. This compound was dissolved in 20 mL of 1 N sodium hydroxide solution and quickly filtered and the filtrate acidified with acetic acid. The solid which precipitated was collected by filtration and dried at 80 °C (0.1 mm) to give 1.68 g (92%) of **21**, identical in every respect with the compound obtained by Method A as described above.

Diethyl *N*-[4-[7-(2-Acetamido-5-deaza-4(3*H*)-oxo-6,7,8,9-tetrahydropyrido[3,4-*g*]pteridino)]benzoyl]-L-glutamate (24). To a solution of 0.37 g (0.001 mol) of 2-acetamido-7-(4-carboxyphenyl)-5-deaza-6,7,8,9-tetrahydro-4(3*H*)-pyrido[3,4-*g*]pteridinone (**21**) in 5 mL of hot *N*-methylpyrrolidone was added 0.14 mL (0.0012 mol) of triethylamine, the mixture was cooled to room temperature, and 0.27 g (0.001 mol) of phenyl *N*-phenylphosphoramidochloridate added. After 30 min of stirring at room temperature, 0.24 g (0.001 mol) of L-diethyl glutamate hydrochloride and 0.14 mL (0.0012 mol) of triethylamine in 5 mL of *N*-methylpyrrolidone were added, and the reaction mixture was stirred at room temperature overnight. Evaporation of the solvent gave a tan solid which was triturated with 50 mL of water, air dried, dissolved in 100 mL of chloroform/methanol (4:1), and filtered through Celite. Silica gel (5 g) was added, the solvent evaporated, and the impregnated silica gel applied to the top of a silica gel column prepared from 15 g of Merck Kieselgel-60, 230–240 mesh. The product was eluted with chloroform/methanol (95:5); evaporation of the solvent gave 0.31 g (55%) of **24** as a brown powder: mp 185–190 °C; NMR (Me_2SO-d_6) δ 1.25 (2 t, 6 H, J = 7 Hz), 2.3 (s, 3 H), 1.7–2.6 (m, 4 H), 3.0–2.25 (m, 2 H), 2.7–2.95 (m, 2 H), 4.1 (2 q, 4 H, J = 7 Hz), 4.3–4.5 (m, 1 H), 4.65 (s, 2 H), 7.05 and 7.8 (ABq, 4 H, J = 9 Hz), 8.3 (s, 1 H).

Anal. Calcd for $C_{28}H_{32}N_6O_7 \cdot 0.5H_2O$: C, 58.64; H, 5.76; N, 14.66. Found: C, 58.65; H, 5.52; N, 14.73.

L-7,10-Ethano-5-deazafolic Acid (5). Hydrogen chloride gas was passed for a few minutes through a suspension of 0.7 g of diethyl *N*-[4-[7-(2-acetamido-5-deaza-4(3*H*)-oxo-6,7,8,9-tetrahydropyrido[3,4-*g*]pteridino)]benzoyl]-L-glutamate (**24**) in 20 mL of nitromethane at 0 °C. The resulting pink emulsion was stirred at room temperature for 1 h, 20 mL of diethyl ether added, the suspended solid collected by filtration and suspended in water, and 1 N sodium hydroxide solution added until a homogeneous solution resulted. This solution was filtered and the filtrate acidified with acetic acid to give an orange precipitate which was collected by centrifugation and washed well with water, methanol, and ether: yield 0.49 g (88%) of **4**; mp >250 °C. The insolubility of this compound, even in TFA, precluded determination of its NMR spectrum.

Anal. Calcd for $C_{22}H_{22}N_6O_6 \cdot H_2O$: C, 54.55; H, 4.96; N, 17.35. Found: C, 54.19; H, 4.70; N, 17.00.

Synthesis of 4-Amino-4-deoxy-7,10-methano-5-deazapteroic Acid and 7,10-Methano-5-deazapteroic Acid[1]

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The 5-deazapteroic acid analogues **1** and **2** have been prepared by several different strategies starting from 1-[4-(*tert*-butoxycarbonyl)phenyl]-3-pyrrolidinone (**3b**).

A major objective in our laboratories over the past few years has been the synthesis of structural analogues of folic acid, methotrexate, and aminopterin which we hope will exhibit enhanced binding to dihydrofolate reductase and/or thymidylate synthetase and thus greater selectivity for a broader range of human tumors. Arguments supporting the synthesis of "tied-back" analogues of the above pteridines in which C-7 (the site of metabolic inactivation of methotrexate itself) is blocked by a bridge to N-10 have been presented elsewhere.[2] We describe in the present

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