saturated NaCl, dried, and concentrated in vacuo to give 0.25 g of a foamy gum. Crystallization from EtOAc/heptane afforded 124 mg (50%) of carboxamide **15** as a white powder: mp 113.5–115.5 °C; IR ($CHCl_3$) 3500, 3400, 2950, 1680 cm^{-1}. Anal. ($C_{23}H_{32}ClN_3O$) C, H, N.

(±)-α-[2-[Bis(1-methylethyl)amino]ethyl]-α-(2-chlorophenyl)-2-piperidinebutanamide (16). Pyridyl carboxamide **15** (2.25 g, 5.62 mmol) was dissolved in 30 mL of acetic acid and reduced over 0.225 g of PtO_2 catalyst at a hydrogen pressure of 50 psi. After 45 min the theoretical amount of H_2 had been taken up, and the catalyst was filtered off and the solvent removed in vacuo. The residue was dissolved in water and the solution made alkaline with 6 N KOH. Extraction three times with ether, washing with water and saturated NaCl, drying, and solvent removal afforded the piperidyl carboxamide **16** (1.96 g, 85%) as a crunchy foam: ^1H NMR ($CDCl_3$) δ 7.3 (cm, 4 H), 5.60 (br s, 2 H), 3.2–1.0 (comp alkyl), 1.00 (d, 12 H, Me). Anal. ($C_{23}H_{38}N_3ClO$) C, H, N.

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Registry No. **3**, 116078-61-6; **4**, 116078-62-7; **5**, 116078-63-8; **6**, 116078-64-9; **7a**, 116078-65-0; **7b**, 116078-66-1; **7c**, 116078-67-2; **8a**, 116078-68-3; **8b**, 116078-69-4; **8c**, 116078-70-7; **9a**, 116078-71-8; **9b**, 116078-72-9; **9c**, 116078-73-0; **9d**, 116078-74-1; **10a**, 116078-75-2; **10c**, 91257-05-5; **11a**, 116078-76-3; **11b**, 116078-77-4; **11c**, 116078-78-5; **12a**, 116078-79-6; **12b**, 116078-80-9; **12c**, 116078-81-0; *cis*-**13a**, 116078-82-1; *cis*-**13a**·HCl, 116078-83-2; *trans*-**13a**, 116078-84-3; *trans*-13a·HCl, 116078-85-4; **13b**, 102582-32-1; **13b'**, 102582-33-2; *cis*-**13c**, 116078-86-5; *trans*-**13c**, 116078-87-6; **14**, 116078-88-7; **15**, 116078-89-8; **16** (diastereomer 1), 116078-90-1; **16** (diastereomer 2), 116078-91-2; *N*-benzyl-*N*-(isopropylamino)ethyl chloride, 40737-53-9; 1-(chloroacetyl)piperidine, 1440-60-4; α-chloro-*N*,*N*-diisopropylacetamide, 7403-66-9; 2-picolyl chloride, 4377-33-7; 2-vinylpyridine, 100-69-6; (2-chlorophenyl)acetonitrile, 2856-63-5; 1-(2-chloroethyl)piperidine, 1932-03-2; *N*-benzyl-*N*-(isopropylamino)ethyl chloride hydrochloride, 66903-14-8; *N*,*N*-dimethylphenylacetamide, 18925-69-4; (*N*,*N*-diisopropylamino)ethyl chloride, 96-79-7; 2-picolyl chloride hydrochloride, 6959-47-3; ethylene oxide, 75-21-8; *N*-benzyl-*N*-isopropylamine, 102-97-6.

Synthesis and Antifolate Activity of 5-Methyl-5,10-dideaza Analogues of Aminopterin and Folic Acid and an Alternative Synthesis of 5,10-Dideazatetrahydrofolic Acid, a Potent Inhibitor of Glycinamide Ribonucleotide Formyltransferase

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The title compounds were prepared in extensions of a general synthetic approach used earlier to prepare 5-alkyl-5-deaza analogues of classical antifolates. Wittig condensation of 2,4-diaminopyrido[2,3-*d*]pyrimidine-6-carboxaldehyde (**2a**) and its 5-methyl analogue **2b** with [4-(methoxycarbonyl)benzylidene]triphenylphosphorane gave 9,10-ethenyl precursors **3a** and **3b**. Hydrogenation (DMF, ambient, 5% Pd/C) of the 9,10-ethenyl group of **3b** followed by ester hydrolysis led to 4-[2-(2,4-diamino-5-methylpyrido[2,3-*d*]pyrimidin-6-yl)ethyl]benzoic acid (**5**), which was converted to 5-methyl-5,10-dideazaaminopterin (**6**) via coupling with dimethyl L-glutamate (mixed-anhydride method using *i*-BuOCOCl) followed by ester hydrolysis. Standard hydrolytic deamination of **6** gave 5-methyl-5,10-dideazafolic acid (**7**). Intermediates **3a** and **3b** were converted through concomitant deamination and ester hydrolysis to **8a** and **8b**. Peptide coupling of **8a**,**b** (using $(EtO)_2POCN$) with diesters of L-glutamic acid gave intermediate esters **9a** and **9b**. Hydrogenation of both the 9,10 double bond and the pyrido ring of **9a** and **9b** (MeOH–0.1 N HCl, 3.5 atm, Pt) was followed by ester hydrolysis to give 5,10-dideaza-5,6,7,8-tetrahydrofolic acid (**11a**) and the 5-methyl analogue **11b**. Biological evaluation of **6**, **7**, **11a**, and **11b** for inhibition of dihydrofolate reductase (DHFR) isolated from L1210 cells and for growth inhibition and transport characteristics toward L1210 cells revealed **6** to be less potent than methotrexate in the inhibition of DHFR and cell growth. Compounds **6**, **11a**, and **11b** were transported into cells more efficiently than methotrexate. Growth inhibition IC_{50} values for **11a** and **11b** were 57 and 490 nM, respectively; the value for **11a** is in good agreement with that previously reported (20–50 nM). In tests against other folate-utilizing enzymes, **11a** and **11b** were found to be inhibitors of glycinamide ribonucleotide formyltransferase (GAR formyltransferase) from one bacterial (*Lactobacillus casei*) and two mammalian (Manca and L1210) sources with **11a** being decidely more inhibitory than **11b**. Neither **11a** nor **11b** inhibited aminoimidazolecarboxamide ribonucleotide formyltransferase. These results support reported evidence that **11a** owes its observed antitumor activity to interference with the purine de novo pathway with the site of action being GAR formyltransferase.

In earlier reports we reviewed evidence that modifications of the classical antifolate structure at positions 5 and 10 might lead to new antifolates with enhanced selectivity of antitumor action.[1-3] In this connection, we recently described a versatile synthetic approach to 5-deaza analogues of aminopterin (AMT) and methotrexate (MTX).[3,4] The general route allows the introduction of 5-substituents

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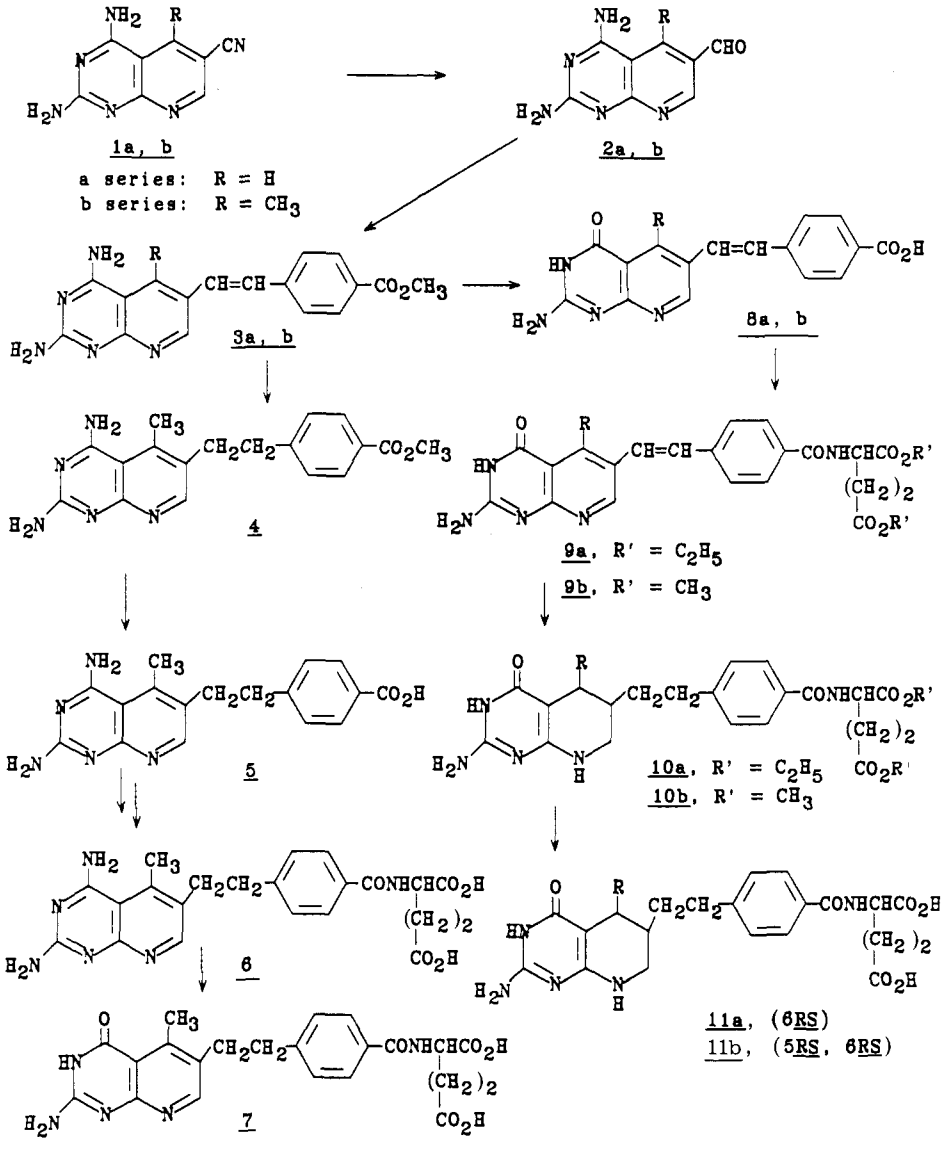
Scheme I



in unequivocal fashion and also permits variations in the 9,10-bridge region, including accessibility to 10-deaza types. In this report we describe extension of the route as shown in Scheme I to include 5-methyl-5,10-dideazaaminopterin (**6**) and 5,10-dideaza-5,6,7,8-tetrahydrofolic acid (**11a**, DDATHF), a reported antitumor agent,[5-11] and its 5-methyl analogue **11b**.

In our earlier account of syntheses of 5-methyl-5-deaza analogues of AMT and MTX we described preparations of the carbonitriles **1a,b** (shown in Scheme I).[3] Conversion of **1b** to the corresponding aldehyde **2b** was also described. In the present work, both aldehydes **2a,b** were prepared and converted through Wittig condensation with [4-(methoxycarbonyl)benzylidene]phosphorane[12] to the 9,10-ethenyl precursors **3a,b**. In the route to 5-methyl-

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Table I. Biochemical Properties of MTX and the Title Compounds 6, 7, and 11a and 11b in L1210 Cells[a]

compd	DHFR inhibn: K_i (n = 3) ± SE, pM	cell growth inhibn: IC_{50} (n = 4) ± SE, nM	mediated transport (n = 3–4) influx: K_m, μM ± SE	mediated transport (n = 3–4) efflux: k, min^{-1} ± SE
MTX	5.45 ± 0.6	2.1 ± 0.1	3.95 ± 0.6	0.22 ± 0.3
5-MeTHF			4.1 ± 0.6[b]	
6	28.3 ± 5.3	23.2 ± 1.2	1.25 ± 0.4	0.50 ± 0.6
7	>1000	36400 ± 2000	18.8 ± 0.7	
11a	>1000	57.2 ± 3	2.48 ± 0.3	
11b	>1000	490 ± 17	1.62 ± 0.3	

[a] Methods used are described in ref 19–21. [b] Previously reported values for (6R,S)-5-methyltetrahydrofolate (see ref 22).

Table II. Evaluation of Title Compounds 6, 7, 11a, and 11b as Enzyme Inhibitors

	IC_{50}, M				
	DHFR[a]	TS[b]	GAR formyltransferase[c]		
compd	*L casei*	*L. casei*	*L. casei*	Manca	L1210
MTX	1.6×10^{-8}	1.2×10^{-4}	$>2 \times 10^{-5}$		$>2 \times 10^{-5}$
6	2.3×10^{-8}	2.9×10^{-6}			
7	1.6×10^{-5}	4.4×10^{-5}	$>2 \times 10^{-5}$	$>2 \times 10^{-5}$	$>2 \times 10^{-5}$
11a	1.9×10^{-5}	$>1 \times 10^{-4}$	1×10^{-8}	5×10^{-7}	5×10^{-7}
11b	$>1 \times 10^{-4}$	$>1 \times 10^{-4}$	7.2×10^{-7}	1.4×10^{-5}	8×10^{-6}

[a] Methods used were reported in ref 23. [b] Methods used were reported in ref 24. [c] See the Experimental Section. Against AICAR formyltransferase from the same three sources, each of the IC_{50} values was $>2 \times 10^{-5}$ M.

5,10-dideazaaminopterin (6), the methyl-substituted compound **3b** was hydrogenated selectively in the ethenyl bridge to give the intermediate ester 4. The hydrogenation was carried out in dimethylformamide under ambient conditions in the presence of 5% Pd on C. Mild ester hydrolysis of 4 then gave 4-amino-4-deoxy-5-methyl-5,10-dideazapteroic acid (**5**). Coupling of **5** with dimethyl L-glutamate through the mixed-anhydride method (using isobutyl chloroformate) was followed by hydrolysis of the diester to give title compound **6**. Hydrolytic deamination of **6** (in refluxing 1 N NaOH) led to 5-methyl-5,10-dideazafolic acid (**7**).

Our interest in the tetrahydrofolate analogues was due at first to the structural relationship between **11b** and the plasma folate 5-methyltetrahydrofolate. We envisioned **11b** as a potential antagonist for the transport and metabolism of 5-methyltetrahydrofolate. Before we had finished the synthesis of **11b**, however, the importance of this structural type was greatly enhanced with the finding by other investigators of broad antitumor effectiveness of **11a**. The antitumor activity of **11a** has been attributed to its inhibition of the purine de novo biosynthetic pathway. The site of action was deduced from results of cytotoxicity reversal studies to be glycinamide ribonucleotide formyltransferase (GAR formyltransferase, EC 2.1.2.2).[10,11] There was no indication from the reversal studies of inhibition of aminoimidazolecarboxamide ribonucleotide formyltransferase (AICAR formyltransferase, EC 2.1.2.3).

The need to make biological activity comparisons between **11a** and **11b** (and related compounds yet to be prepared) then prompted us to apply our method to the synthesis of both **11b** and **11a**. Adaptation of the route to **11a** was readily achieved. Both **11a** and **11b** were prepared by the approach outlined in Scheme I. Hydrolysis of the intermediate esters **3a** and **3b** with concomitant deamination gave **8a** and **8b**. Peptide coupling with diesters of L-glutamic acid followed to give **9a** and **9b**. Diethyl phosphorocyanidate was used as the carboxyl-activating reagent. The yield for the conversion of **8a** to **9a** was significantly improved (46% vs 15%) when dimethyl sulfoxide was used as solvent instead of dimethylformamide (DMF). Hydrogenation of both the olefinic bridge and the pyrido ring of the **9** types to give intermediate esters **10a** and **10b** was effected with a hydrogen pressure of 3.5 atm and Pt catalyst in methanol containing 0.1 N HCl in slight excess over **9a** and **9b**. Finally, mild ester hydrolysis gave the targets **11a** and **11b**.

Both Taylor et al.[5] and DeGraw et al.[13] have reported separate and independent syntheses of 5,10-dideazaaminopterin. Taylor et al have reported syntheses of **11a** by other routes.[5-9] The functional group transformations featured in those reported syntheses were not designed to be adaptable to unambiguous syntheses of 5-alkyl analogues such as **6** and **11b**. The methodology used in the present work features the straightforward approach shown in Scheme I based on the ready accessibility of appropriately substituted precursors of the **1** type.[3]

Compounds **6**, **7**, **11a**, and **11b** were evaluated for inhibition of dihydrofolate reductase (DHFR, EC 1.5.1.3) from L1210 cells, effect on growth of L1210 cells in culture, and transport characteristics. The results are listed in Table I in comparison with MTX. Compound **6** is 5-fold less inhibitory than MTX toward DHFR while **7**, **11a**, and **11b** are not inhibitors toward this enzyme. In the cell growth inhibition tests, **6** is 11-fold less potent than MTX. The IC_{50} for cell growth inhibition by **11a** (57 nM) is in good agreement with that reported (20–50 nM).[10a,11] The 5-methyl compound **11b** is about one-ninth as potent in the cell growth inhibition test as **11a**. Compounds **11b** and **11a** are 230 and 27 times less inhibitory to cell growth than MTX, respectively. In the transport studies, **6**, **11a**, and **11b** displayed greater facility toward inward flux than MTX or the plasma folate 5-methyltetrahydrofolate. It is important to note that compound **11b** is a mixture of four diastereomers and compound **11a** is a mixture of two diastereomers (Scheme I).

Results from evaluation of **6**, **7**, **11a**, and **11b** as inhibitors of DHFR and thymidylate synthase (TS, EC 2.1.1.45) (both from *Lactobacillus casei*) and GAR and AICAR formyltransferase from one bacterial (*L. casei*) and two mammalian (Manca and L1210) sources are reported in Table II. Compound **6** is strongly inhibitory toward bacterial DHFR. Its moderate inhibition of TS is 40 times stronger than MTX and 15 times stronger than the 4-oxo analogue, compound **7**. The 5,10-dideazatetrahydrofolates **11a** and **11b** are inhibitors of GAR formyltransferase from each source, with compound **11a** being the more potent inhibitor. The *L. casei* enzyme is more sensitive to inhibition than the mammalian enzymes. Neither **11a** nor **11b** was an inhibitor of AICAR formyltransferase, having IC_{50} values above 2×10^{-5} M. These results support the previous reports based on cytotoxicity studies that the site of action of **11a** is GAR formyltransferase.[10,11]

Numerous studies on the biochemical and antitumor properties of **11a** have been reported,[10,11] but more complete understanding of the biochemical basis for the inhibition of purine biosynthesis by **11a** (and related compounds) will require studies of their polyglutamate derivatives as inhibitors of the folate-requiring enzymes. Radiolabeled forms would be of value in such studies. The intermediate 9,10-ethenyl pteroic acid type intermediate **8a** offers obvious potential for synthesizing polyglutamate derivatives through peptide-coupling reactions with

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available polyglutamate benzyl esters[14] followed by hydrogenation. Tritium-labeled forms of **11a** or polyglutamate derivatives are also readily accessible through tritiation instead of hydrogenation as in the conversion of **9a** to **11a**.

Experimental Section

Examinations by TLC were performed on Analtech precoated (250-μm) silica gel G (F) plates. Column chromatographic purifications were done with silica gel (Merck, 60 A, 230–400 mesh for flash chromatography). Because of solubility limitations, crude products to be purified were dispersed in silica gel (3 g/g of crude product) for application to the column. Dispersal was achieved by evaporating in vacuo a solution of the crude product in DMF containing suspended silica gel. Except where other conditions are specified, evaporations were performed with a rotary evaporator and a H_2O aspirator. Products were dried in vacuo (1 mm) at 22–25 °C over P_2O_5 and NaOH pellets. Final products were dried and then allowed to equilibrate under ambient conditions. Analytical results indicated by element symbols were within ±0.4% of the calculated values. Spectral determinations and some of the elemental analyses were performed in the Molecular Spectroscopy Section of Southern Research Institute. Some elemental analyses were also performed at Atlantic Microlab, Inc., Atlanta, GA. The ^1H NMR spectra data reported were determined with a Nicolet NMC 300 NB spectrometer using Me_4Si as internal reference. Chemical shifts (δ) listed for multiplets were measured from the approximate centers, and relative integrals of peak areas agreed with those expected for the assigned structures. Mass spectra were recorded on a Varian MAT 311A mass spectrometer in the electron-impact (EI) or fast-atom-bombardment (FAB) mode. UV spectra were determined with a Perkin-Elmer Model Lambda 9 spectrometer. Samples were first dissolved in 0.1 N NaOH, and the solutions were then diluted 10-fold with the medium given in the listings. Maxima are expressed in nanometers with the molar absorbance given in parentheses. Molecular weights used in all calculations conform with the compositions listed with the indicated elemental analyses.

2,4-Diaminopyrido[2,3-*d*]pyridine-6-carboxaldehyde (2a). Treatment of **1a**[3] (2.0 g, 10.7 mmol) with HCO_2H (35 mL of 95–97%) and Raney Ni (about 14 g damp) as reported for the preparation of the 5-methyl analogue **2b**[3] afforded **2a** in nearly theoretical yield (2.0 g); mass spectrum (EI), m/z 189, M^+ for $C_8H_7N_5O$; virtually homogeneous by TLC (MeOH). Later the conversion was carried out with Ni–Al (50/50) alloy (instead of Raney Ni) as follows. A solution of **1a** (18.0 g, 96.7 mmol) in HCO_2H (600 mL of 95–97%) was diluted with H_2O (210 mL), and the Ni–Al alloy (54 g) was then added. The mixture was stirred and refluxed for 2 h and then filtered while hot (Celite mat). The filtrate was evaporated (H_2O aspirator, bath to 50–60 °C), and residual HCO_2H was removed with the aid of added portions of EtOH and subsequent evaporation. The yellow solid was then dissolved in hot H_2O (1.8 L), and the solution was clarified by filtration before it was treated with 1 N NaOH with mechanical stirring to produce pH 8.5. The mixture containing **2a** as a yellow solid was allowed to cool gradually with continued stirring before it was finally chilled in an ice–H_2O bath. The collected and H_2O-washed solid was dried in vacuo at 70 °C. The crude product, obtained in essentially theoretical yield, proved suitable for the conversion to **3a** described below.

Methyl 4-[2-(2,4-Diaminopyrido[2,3-*d*]pyrimidin-6-yl)ethenyl]benzoate (3a). The solution formed by treating [4-(methoxycarbonyl)benzyl]triphenylphosphonium bromide[12] (5.25 g, 10.7 mmol) with NaOMe (0.578 g, 10.7 mmol) in DMF (100 mL) was added to a suspension of **2a** (2.02 g, 10.7 mmol) in DMF (150 mL). The mixture was stirred at 23–25 °C in a stoppered flask under N_2 for 3.5 days. Undissolved solid (mainly **2a**) was then removed, and the filtrate was evaporated (<1 mm, bath to 35 °C) to a thick oil which gave a yellow solid when stirred with MeCN. The solid was collected with the aid of MeCN and then washed several times on the funnel with Et_2O. The air-dried solid was stirred with H_2O (50 mL) before it was collected again and washed successively with MeCN and Et_2O. This solid was prepared for chromatographic purification by mixing it with silica gel in warm DMF (150 mL) and then evaporating the mixture to dryness (<1 mm, bath to 35 °C). The solid dispersion that remained was applied atop a column of silica gel (see explanation above). Elution under N_2 pressure (flash technique) by $CHCl_3$–MeOH (3:1 by volume) followed. Thin-layer chromatograms on early fractions showed only the reaction product. These fractions were combined (totaling 900 mL) and evaporated to give **3a** as a yellow solid in 22% yield (0.74 g); mass spectrum (EI), m/z 321, M^+ for $C_{17}H_{15}N_5O_2$.

In a scaled-up run (5.14 times that described above), the phosphorane solution was added in a thin stream to the stirred suspension of **2a** maintained at 60–65 °C. Rapid stirring at this temperature was continued for 24 h. The isolation procedure was the same as that described above for the smaller run except that elution from the silica gel column was done with 2:1 $CHCl_3$–MeOH. The yield was 29% [5.08 g from 10.4 g (55.0 mmol) of **2a**].

Methyl 4-[2-(2,4-Diamino-5-methylpyrido[2,3-*d*]pyrimidin-6-yl)ethenyl]benzoate (3b). NaOMe (2.1, g, 40.4 mmol) was added in one portion to a stirred suspension of [4-(methoxycarbonyl)benzyl]triphenylphosphonium bromide[12] (19.8 g, 40.4 mmol) in anhydrous DMF (150 mL) under N_2 at 20–25 °C, and the solution that formed was added to a suspension of the pulverized aldehyde **2b** (8.2 g, 40.4 mmol) in DMF (850 mL). This mixture was stirred at 20–25 °C under N_2 in a closed container for 7 days. Insoluble solid (mainly **2b**) was removed by filtration, and the filtrate was evaporated (<1 mm, bath to 40 °C). The viscous orange oil that remained was stirred with MeCN (200 mL) to give a yellow solid which was collected with the aid of MeCN and was then triturated and washed on the funnel with Et_2O. The air-dried solid (6.15 g) was subjected to column chromatographic purification on silica gel as described for the preparation of **3a**. Elution by $CHCl_3$–MeOH (2:1) led to several early fractions homogeneous by TLC which were pooled and evaporated to give **3b** as a yellow solid in 34% yield (4.55 g); mass spectrum (FAB), m/z 336, $(MH)^+$ for $C_{18}H_{17}N_5O_2$.

4-[2-(2,4-Diamino-5-methylpyrido[2,3-*d*]pyrimidin-6-yl)ethyl]benzoic Acid (5) via the Methyl Ester 4. A solution of **3b** (2.50 g, 7.45 mmol) in DMF (600 mL) was treated with 5% Pd on C (2.5 g), and the stirred mixture was kept under H_2 until absorption from a gas buret had ceased. Evaporation of the filtered solution left a residue which produced a mass spectrum and thin-layer chromatograms ($CHCl_3$–MeOH, 2:1) showing it to be a mixture of desired product **4** and unchanged **3b**. On the chromatograms, **3b** moved slightly ahead of **4**. Column chromatography on silica gel (as described for purification of **3a**) with elution by $CHCl_3$–MeOH (2:1) led to early fractions containing both **3b** and **4** according to TLC, but later fractions contained only **4**. The later fractions were combined and evaporated to give **4** (1.09 g), homogeneous according to TLC. The early fractions consisting of **3b** and **4** were combined, and the mixture was again subjected to column chromatographic separation as before to give additional **4** (180 mg). The total yield was 51% (1.27 g); mass spectrum (FAB), m/z 338, $(MH)^+$ for $C_{18}H_{19}N_5O_2$ (**4**).

For saponification, a solution of **4** (1.01 g, 3.00 mmol) in Me_2SO (60 mL) containing 1 N NaOH (3.6 mL) was stirred at 20–25 °C. Solid began separating after several h. After 24 h, MeCN was added to the mixture to promote precipitation of the Na salt and facilitate filtration. The solid was collected with the aid of MeCN, dried, and dissolved in H_2O (30 mL). The Norit-treated and filtered (Celite) solution was treated with AcOH to pH 4.6 to cause precipitation of **5**. Warming the mixture for a few minutes promoted coagulation of the precipitate and made collection easier. The yield of **5** was 64% (655 mg); mass spectrum (FAB), m/z 324, $(MH)^+$. Anal. $C_{17}H_{17}N_5O_2 \cdot H_2O$ (C, H, N).

***N*-[4-[2-(2,4-Diamino-5-methylpyrido[2,3-*d*]pyrimidin-6-yl)ethyl]benzoyl]-L-glutamic Acid (6) (5-Methyl-5,10-dideazaaminopterin).** A mixture of **5**·H_2O (565 mg, 1.65 mmol), Et_3N (183 mg, 1.81 mmol), and DMF (35 mL) was stirred at 20–25 °C for 30 min and then cooled to 0–5 °C and treated with *i*-BuOCOCl (247 mg, 1.81 mmol). Stirring at 0–5 °C was continued for 2 h before more Et_3N (1.81 mmol) was added followed by dimethyl L-glutamate hydrochloride (384 mg, 1.81 mmol). The cooling bath was allowed to warm during 3–4 h to 23–25 °C, and stirring was

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continued for 2 days. The mixture was then filtered, and the filtrate was evaporated (<1 mm, bath to 35 °C). The solid residue was stirred with 5% $NaHCO_3$ solution to give the crude dimethyl ester of **6** (475 mg), which was prepared for column chromatographic purification by mixing it with silica gel (2 g) in MeOH solution followed by evaporation (see under **3a**). The dry residue was applied to a silica gel column, and elution by $CHCl_3$–MeOH (2:1) followed. Early fractions observed by TLC to be homogeneous were combined and evaporated to give **6** dimethyl ester (236 mg). Later fractions were combined and reprocessed to give additional diester (30 mg). The final yield of **6** dimethyl ester, homogeneous according to TLC, was 34% (266 mg); mass spectrum (FAB), m/z 481, $(MH)^+$ for $C_{24}H_{28}N_6O_5$.

A solution of **6** dimethyl ester (236 mg, 0.491 mmol) in Me_2SO (10 mL) containing 1 N NaOH (1.25 mL) was kept under N_2 at 23–25 °C for 18 h. After removal of the solvent (<1 mm, bath to 35 °C) the residue was dissolved in H_2O (10 mL), and the solution was treated with AcOH to pH 3.6 to cause precipitation of **6**; yield 75% (180 mg). Spectral data: mass (FAB) m/z 453, $(MH)^+$; UV λ_{max} 235 nm (ϵ 39 300), 320 (7490), 33 (6790) at pH 1; 236 nm (ϵ 38 800), 270 (sh), 334 (6090) at pH 7; 238 nm (ϵ 38 000), 273 (sh), 343 (6560) at pH 13; ^1H NMR (Me_2SO-d_6) δ 1.98 and 2.03 (2 m, $CHCH_2CH_2$, nonequivalent), 2.32 (t, CH_2CO), 2.66 (s, CH_3), 2.85 (m, CH_2, C^9H_2 or $C^{10}H_2$), 2.95 (m, CH_2, C^9H_2 or $C^{10}H_2$), 4.36 (q, $NHCHCO$), 6.84 (br s, NH_2), 7.30 and 7.80 (AB spin system, C_6H_4), 7.4 (m, NH_2), 8.30 (s, C^7H), 8.42 (d, $CONHCH$). Anal. ($C_{22}H_{24}N_6O_5 \cdot H_2O$) C, H, N.

***N*-[4-[2-(2-Amino-1,4-dihydro-5-methyl-4-oxopyrido[2,3-*d*]pyrimidin-6-yl)ethyl]benzoyl]-L-glutamic Acid (7) (5-Methyl-5,10-dideazafolic Acid).** A solution of **6**·H_2O (78 mg, 0.16 mmol) in 1 N NaOH (4 mL) was refluxed under N_2 for 5 h, cooled, and acidified with 1 N HCl to pH 3 to give **7** in 85% yield (65 mg). Spectral data: mass (FAB), m/z 454, $(MH)^+$; UV λ_{max} 233 nm (ϵ 32 700), 276 (18 300), 342 (8000) at pH 1; 228 nm (ϵ 35 600), 265 (16 300), 320 (5300) at pH 7; 233 nm (ϵ 37 600), 268 (15 400), 333 (6700) at pH 13. Anal. ($C_{22}H_{23}N_5O_6 \cdot 1.5H_2O$) C, H, N.

4-[2-(2-Amino-1,4-dihydro-4-oxopyrido[2,3-*d*]pyrimidin-6-yl)ethenyl]benzoic Acid (8a). A stirred suspension of **3a** (1.92 g, 5.94 mmol) in 1 N NaOH (50 mL) heated at reflux under N_2 became a clear solution within 30 min. The solution was refluxed under N_2 for 6 h, allowed to cool at about 25 °C, and then acidified (to pH 4.5–5.0) by treatment with glacial AcOH (about 8 mL). The resulting mixture was warmed with stirring for a few minutes to promote granulation of the initially gel-like precipitate. After overnight storage at 5 °C, the yellow precipitate was collected and washed successively with H_2O, EtOH, and Et_2O; yield 81% (1.56 g); mass spectrum (EI), m/z 308, M^+. Anal. ($C_{16}H_{12}N_4O_3 \cdot 0.8H_2O$) C, H, N. In another run, the sample of **3a** from the larger preparation described above (5.0 g) was hydrolyzed during a 6-h reflux period in 1 N NaOH (100 mL), and acidification with 2 N H_2SO_4 gave **8a** (3.59 g). The mass spectra of this sample in both the EI and the FAB modes were as expected for **8a**. Its ^1H NMR spectrum was consistent with that to be expected from a mixture of cis and trans isomers of **8a** in approximately equimolar amounts. Spectral data: mass (EI), m/z 308, M^+; mass (FAB) m/z 309, $(MH)^+$; ^1H NMR (Me_2SO-d_6) δ 6.75 (AB spin system, cis CH=CH, J = 12.5 Hz), 6.85 (br s, NH_2), 7.36 and 7.86 (AB spin system, C_6H_4 of cis), 7.46 (AB spin system, trans CH=CH, J = 16.4 Hz), 7.72 and 7.94 (AB spin system, C_6H_4 of trans), 8.05 (d, C^7-H of cis), 8.41 (d, C^5-H of cis), 8.45 (d, C^5-H of trans), 9.00 (d, C^7-H of trans).

4-[2-(2-Amino-1,4-dihydro-5-methyl-4-oxopyrido[2,3-*d*]-pyrimidin-6-yl)ethenyl]benzoic Acid (8b). Hydrolysis and deamination of **3b** (520 mg, 1.55 mmol) was carried out as described for the conversion of **3a** to **8a**. The yield of **8b** was 90% (500 mg); mass spectrum (FAB), m/z 323, $(MH)^+$. Anal. ($C_{17}H_{14}N_4O_3 \cdot H_2O$) C, N; H: calcd, 5.06; found, 4.59.

Diethyl *N*-[4-[2-(2-Amino-1,4-dihydro-4-oxopyrido[2,3-*d*]pyrimidin-6-yl)ethenyl]benzoyl]-L-glutamate (9a). Pulverized **8a**·H_2O (326 mg, 1.00 mmol) was added in portions to a stirred solution of *N*-methylmorpholine (202 mg, 2.00 mmol) and $(EtO)_2POCN$ (326 mg, 2.00 mmol) in Me_2SO (50 mL) at 20–23 °C. After 2 h, nearly complete solution had occurred. More *N*-methylmorpholine (202 mg, 2.00 mmol) was added followed by diethyl L-glutamate hydrochloride (240 mg, 1.00 mmol). The mixture was stirred at 20–25 °C for 48 h with solution occurring during this time. The solvent was removed by evaporation in vacuo (<1 mm, bath to 30 °C), and the residue was stirred with cold H_2O (20 mL). The stirred suspension was treated with sufficient 10% $KHCO_3$ solution (about 10 mL) to bring the pH to 9. The collected and dried sample weighed 411 mg. Its mass spectrum [FAB, m/z 494, $(MH)^+$ for $C_{25}H_{27}N_5O_6$] and thin-layer chromatogram ($CHCl_3$–MeOH, 4:1) indicated this sample to be predominantly the expected **9a** free of unchanged **8a**. The preparative procedure described above was repeated on twice the previous scale. The 789-mg sample produced in the second run was combined with the 411-mg sample from the first run. The combined sample was dissolved in DMF (150 mL) for dispersal with silica gel and chromatographic purifiation as described above for **3a**. The mixture of cis and trans isomers appeared to elute together with use of $CHCl_3$–MeOH (2:1). The pooled and evaporated fractions left a yellow solid residue (720 mg) containing a small amount of residual DMF evident in the ^1H NMR spectrum and indicated by results from elemental analysis. The yield was 46% based on the following formulation. Anal. ($C_{25}H_{27}N_5O_6 \cdot 0.1C_3H_7NO \cdot 1.25H_2O$) C, H; N: calcd, 13.65; found, 14.08. Spectral data: mass, m/z 494, $(M + 1)^+$; ^1H NMR (Me_2SO-d_6) δ 1.1–1.3 (2 t, overlapping, CH_3CH_2), 2.0 and 2.1 (2 m, $CHCH_2CH_2$, nonequivalent), 2.43 (t, $CH_2CH_2CO_2Et$), 4.0–4.2 (2 q, overlapping, CH_3CH_2O), 4.43 (q, $NHCHCH_2$), 6.75 (AB spin system, cis CH=CH, J = 12.3 Hz), 6.95 (br s, NH_2), 7.37 and 7.80 (AB spin system, C_6H_4 of cis), 7.46 (AB spin system, trans CH=CH, J = 17.0 Hz), 7.73 and 7.92 (AB spin system, C_6H_4 of trans), 8.08 (d, C^7-H of cis), 8.42 (d, C^5-H of cis), 8.46 (d, C^5-H of trans), 8.75 (d, CONH), 8.91 (d, C^7-H of trans). Signals due to residual DMF appear at δ 2.74 and 2.90 (CH_3) and 7.95 (HCO), and their integral values were in good agreement with the composition indicated by the elemental analysis results.

Dimethyl *N*-[4-[2-(2-Amino-1,4-dihydro-5-methyl-4-oxopyrido[2,3-*d*]pyrimidin-6-yl)ethenyl]benzoyl]-L-glutamate (9b). A stirred suspension of pulverized **8b**·$2H_2O$ (500 mg, 1.4 mmol) in DMF (50 mL) containing Et_3N (380 mg, 3.8 mmol) was treated with a solution of $(EtO)_2POCN$ (850 mg, 5.2 mmol) in DMF (10 mL). After 30 min at 23–25 °C, dimethyl L-glutamate hydrochloride (500 mg, 2.36 mmol) was added in one portion. The mixture was stirred at 20–25 °C in a stoppered flask under N_2 for 4 days. DMF was then evaporated in vacuo and the semisolid residue was stirred with portions of H_2O (each of which was removed by decantation) until the residue became solid. The solid was then pulverized under 5% $NaHCO_3$ solution, collected by filtration, washed with H_2O, and dried. The crude product (500 mg) was dissolved in the minimum volume of $CHCl_3$–MeOH (10:1), and the solution was applied to a silica gel column (see under preparation of **3a**). Elution by $CHCl_3$–MeOH–AcOH (15:1:0.5) afforded fractions containing only **9b**. The pooled and evaporated solutions gave **9b** in 40% yield (280 mg); mass spectrum (FAB), m/z 480, $(MH)^+$. Anal. $C_{24}H_{25}N_5O_6 \cdot 1.5H_2O$ (C, H, N).

***N*-[4-[2-(2-Amino-1,4,5,6,7,8-hexahydro-4-oxopyrido[2,3-*d*]pyrimidin-6-yl)ethyl]benzoyl]-L-glutamic Acid (11a) (5,10-Dideazatetrahydrofolic Acid) via the Diethyl Ester 10a.** Hydrogenation of the sample of **9a** described above (700 mg, 1.34 mmol) in MeOH (175 mL) containing 0.1 N HCl (14.2 mL) and PtO_2 (270 mg) was carried out in a Parr shaker with H_2 pressure kept near 3.6 kg/cm^2 (51 psi). After 20 h, the catalyst was removed by filtration, and the nearly colorless filtrate was evaporated to dryness. The residue (708 mg) produced a mass spectrum (FAB) with a peak of m/z 500, $(MH)^+$ for the diethyl ester **10a** ($C_{25}H_{33}N_5O_6$). No unchanged **9a** was detected in the mass spectrum or by TLC. The solid was redissolved in MeOH (150 mL), and the solution was treated with 1 N NaOH (7.05 mL). This solution was stirred under N_2 in a closed container at 23–25 °C for 48 h. MeOH was then removed by evaporation, and the concentrated solution was diluted with H_2O (20 mL). After clarification, the solution was treated with AcOH to pH 4.0 to give **11a** as a nearly white solid; yield 69% (422 mg). Spectral data: mass (FAB), m/z 444, $(MH)^+$; UV λ_{max} 242 nm (ϵ 17 600), 279 (19 300) at pH 1; 222 nm (ϵ 29 200), 278 (15 600) at pH 7; 240 nm (ϵ 19 400), 272 (13 200) at pH 13; ^1H NMR (Me_2SO-d_6) δ 1.58 (m, $C^6HC^9H_2$), 1.85 (d of d, C^5H), 1.95 and 2.08 (2 m, $CHCH_2CH_2$, nonequivalent), 2.35 (t, CH_2CO), 2.50 (m, C^5H, obscured by peak due to Me_2SO-d_5), 2.71

(m, $CH_2CH_2C_6H_4$) overlapping 2.80 (m, C^7H), 3.18 (m, C^7H), 4.39 (m, NHC*H*O), 5.95 (s, NH_2), 6.26 (m, N^8H), 7.32 and 7.80 (AB spin system, C_6H_4), 8.50 (d, CON*H*CH). Anal. $C_{21}H_{25}N_5O_6 \cdot 0.75H_2O$.

***N*-[4-[2-(2-Amino-5-methyl-1,4,5,6,7,8-hexahydro-4-oxopyrido[2,3-*d*]pyrimidin-6-yl)ethyl]benzoyl]-L-glutamic Acid (11b) via the Dimethyl Ester 10b.** Hydrogenation of **9b** (230 mg, 0.454 mmol) in MeOH (50 mL) containing 0.1 N HCl (4.54 mL) and PtO_2 (50 mg) was carried out in a Parr shaker with H_2 pressure kept near 3.4 kg/cm^2 (48–49 psi) for 18 h. The mixture was filtered (Celite mat), and the filtrate (now colorless) was treated with saturated $NaHCO_3$ solution to raise the pH to 7–8. Evaporation left a solid residue which was stirred with $CHCl_3$ (50 mL) and H_2O (10 mL) to give two clear layers. The H_2O layer was extracted once more with $CHCl_3$ and then the $CHCl_3$ layers were combined and washed once with H_2O (15 mL). The $CHCl_3$ solution was then dried (Na_2SO_4), filtered, and evaporated to give the dimethyl ester **10b** (170 mg); mass spectrum (FAB), m/z 486, $(MH)^+$ for $C_{24}H_{31}N_5O_6$. Side-by-side TLC comparisons ($CHCl_3$–MeOH–AcOH, 9:1:0.25) showed **10b** as a single UV-quenching spot and absence of the less mobile starting compound **9b**. For the ester hydrolysis, this sample was dissolved in MeOH (25 mL), and the solution was treated with 1 N NaOH (2 mL). After 48 h at 23–25 °C, the solution was evaporated (bath at 20–25 °C) until most of the MeOH had been removed. The concentrated residue was diluted with H_2O (20 mL), and the solution was left overnight in a refrigerator. The colorless solution was then treated with 20% AcOH to pH 4 to cause precipitation of **11b** as a white solid; yield 110 mg (51% overall based on starting amount of **9b**). Spectral data: mass (FAB), m/z 458, $(MH)^+$; UV λ_{max} 242 nm (ϵ 17 600), 278 (20 200) at pH 1; 220 nm (ϵ 30 800), 275 (17 600) at pH 7; 241 nm (ϵ 19 200), 271 (13 500) at pH 13; ^{1}H NMR (Me_2SO-d_6) δ 0.84 (d, CH_3), 1.55 (br, $CH_2CH_2C_6H_4$), 1.95 and 2.08 (2 m, $CHCH_2CO$, nonequivalent), 2.33 (t, CH_2CO), 2.6–3.2 (overlapping m, reduced ring protons plus $CH_2CH_2C_6H_4$), 4.40 (q, NHC*H*O), 5.90 (s, NH_2), 6.26 (m, N^8-H), 7.32 (m, and 7.80 (AB spin system, C_6H_4), 8.50 (d, CON*H*CH), 9.65 (br, s, N^3-H), 12.35 (br s, CO_2H). Anal. $C_{22}H_{27}N_5O_6 \cdot H_2O$ (C, H, N).

Assay of GAR Formyltransferase and AICAR Formyltransferase. GAR formyltransferase was assayed by a modification of a continuous spectrophotometric method.[15] Cell extracts (0.1 mg of protein) were incubated with 0.05 M Tris·HCl, pH 7.5, 0.1 M 2-mercaptoethanol, 60 μM (6*S*)-10-formyltetrahydrofolate[16] and 0.24 mM α,β-GAR[17] in 0.9 mL at 30 °C. The incubation mixtures were flushed with argon before initiating the reaction with enzyme. The formation of tetrahydrofolate was measured by following the increase in absorbance at 298 nm with a molar absorbance of 19 700. AICAR formyltransferase was assayed similarly except that 25 mM KCl was included, GAR was replaced by 55 μM AICAR (Sigma), and incubation was at 37 °C.[18]

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Registry No. **1a**, 101810-72-4; **2a**, 80360-04-9; **2b**, 101810-74-6; **3a**, 115758-35-5; **3b**, 110202-57-8; **4**, 110202-58-9; **5**, 110202-59-0; **6**, 110202-60-3; **6** (dimethyl ester), 115758-38-8; **7**, 115758-41-3; (*Z*)-**8a**, 115758-36-6; (*E*)-**8a**, 115758-37-7; **8b**, 110202-61-4; (*Z*)-**9a**, 115758-39-9; (*E*)-**9a**, 115758-40-2; **9b**, 110202-62-5; (6*R*)-**10a**, 110672-49-6; (6*S*)-**10a**, 110672-50-9; (5*R*,6*R*)-**10b**, 115888-54-5; (5*R*,6*S*)-**10b**, 115887-72-4; (5*S*,6*R*)-**10b**, 115887-73-5; (5*S*,6*S*)-**10b**, 115887-74-6; (6*R*)-**11a**, 106400-81-1; (6*S*)-**11a**, 106400-18-4; (5*R*,6*R*)-**11b**, 115887-68-8; (5*R*,6*S*)-**11b**, 115887-69-9; (5*S*,6*R*)-**11b**, 115887-70-2; (5*S*,6*S*)-**11b**, 115887-71-3; DHFR, 9002-03-3; TS, 9031-61-2; GAR formyltransferase, 9032-02-4; AICAR formyltransferase, 9032-03-5; *p*-$(MeOCO)C_6H_4CH_2PPh_3^+Br^-$, 1253-46-9; H-Glu(OMe)-OMe·HCl, 23150-65-4; H-Glu(OEt)-OEt·HCl, 1118-89-4.

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