K. K Salt Hydrate 25.[7] A total of 8.65 mL of 2.5 N (0.0186 mol) of KOH was added slowly to a mixture of 5 g (0.0186 mol) of 1 and 5 mg of disodium edetate in 40 mL of THF. After the mixture warmed to 45 °C to give a complete solution, 200 mL of THF was added to give the solid. The mixture was first cooled with a dry ice–EtOH bath to promote crystallization and then allowed to stand at 25 °C for 16 h. The solid was collected, washed with THF, and dried in vacuo over $CaCl_2$ to give 5.6 g (93%) of **25**.

L. **Ammonium Salt 26.**[7] To a mixture of 0.8 g (0.003 mol) of 1 and 8 mL of CH_3OH was added dropwise 1.1 mL (0.0033 mol) of 3 N NH_4OH under N_2. The N_2 atmosphere was maintained and solvent was removed by warming at 40–45 °C. The residual solid was stirred with 2-PrOH for 16 h, collected on a filter, washed with acetone, and dried in a vacuum desiccator over KOH to give 0.75 g (88%) of **26**.

M. Ammonium Salt Sesquihydrate 27.[7] A total of 7.2 mL (0.0216 mol) of 3 N NH_4OH was slowly added to a mixture of 5 g (0.0186 mol) of 1, 5 mg of disodium edetate, and 40 mL of THF. The resulting solution was diluted with 300 mL of THF. The reaction mixture was first cooled with a dry ice–EtOH bath to promote crystallization and then stirred for 16 h. The solid was collected, washed with THF, and air-dried to give 4.6 g (79%) of **27**.

N. (2-Hydroxyethyl)ammonium Salt 28.[7] A solution of 1.13 mL (1.15 g, 0.0188 mol) of ethanolamine and 10 mL of THF was added to a mixture of 5 g (0.0186 mol) of 1, 5 mg of disodium ededate, and 46 mL of 87% aqueous THF. The reaction mixture was first warmed to 40–45 °C to give a solution and then 200 mL of THF was added to precipitate the product. The product was isolated as described in preparative method M. However, the compound was dried in vacuo over $CaCl_2$ to afford 5.7 g (93%) of **28**.

Preparation of Pooled Human Sputum Substrate. Frozen sputa from patients with chronic bronchitis or asthma were thawed at room temperature and kept chilled on ice. The sputa were mixed by homogenization in a Potter-Elvehjem homogenizer (three strokes) and combined in a large Erlenmeyer flask. Pooled sputa were again homogenized, subdivided into tubes (ca. 18 mL), and stored at −20 °C.

Mucolytic Testing.[12] Frozen pooled sputum was thawed, mixed in a Potter-Elvehjem homogenizer (10 strokes), and kept on ice. Sputum was adjusted to the desired pH, and data were obtained with use of a Wells-Brookfield microviscometer set to a clearance of 5×10^{-4} in. and fitted with a pneumatic transducer. A 2-mL aliquot of the sputum is placed in the center of a viscometer plate and allowed to equilibrate to 37 °C for 2 min. The cone is then rotated at gradually increasing speed up to 100 rpm during 2 min. The rotation is reduced to give a convenient reading for 1 min. The test compounds were added as dry powders or as a 0.2-mL aliquot of solution and readings were recorded for 15 min.

Calculation of Percent Mucolysis. The effects of the various sulfhydryl compounds on sputum viscosity were calculated from the following equation:

$$\% \text{ mucolysis} = \left(\frac{V_1 - V_2}{V_1}\right) \times 100$$

where V_1 represents the initial sputum viscosity reading and V_2 represents the sputum viscosity reading at specified times after compound addition to the sputum.

Stability Studies in D_2O Solution. A total of 50 mg of the compounds (**2**, **27**, NAC·NH_3) was placed in the NMR tube and 1 mL of D_2O was added. The extent of disulfide formation, determined by a Varian T-60 NMR spectrometer, is given in Table VIII from 0 to 20 days. L-*N*,*N*-Diacetylcystine ammonium (1:2) salt sesquihydrate **31**[8a] was employed as reference material. The values on the indicated days were determined by the difference in the areas of the methylene loss in the CH_2SH region, NAC δ 2.84 (Me_2SO-d_6) and **26** δ 3.25 (D_2O), and the methylene gain in the $(SCH_2)_2$ region, **31** δ 3.11 (Me_2SO-d_6) and **16** δ 3.81 (Me_2SO-d_6).

Acknowledgment. We are grateful to Dr. D. C. Cashman and L. W. Jacobs for the mucolytic data, to J. P. Catlett for technical assistance, to C. M. Combs, C. I. Kennedy, J. R. Saucerman, and J. G. Schmidt for analytical and spectral data, to Dr. Dana Brooke and coworkers for the stability and hygroscopicity studies, to J. P. Catlett and Conrad Lyles for the stability studies in D_2O solution, to Harold D. Kinney for supplying intermediates **5** and **17**, and to Dr. D. G. Gallo and Dr. L. A. Riblet and their co-workers for the toxicity studies.

Registry No. 1, 67852-72-6; **2**, 67881-46-3; **3**, 99-05-8; 4, 79-04-9; **5**, 28547-08-2; **7**, 67852-69-1; **8**, 5680-79-5; **9**, 67852-70-4; **10**, 95765-84-7; **12**, 67852-71-5; **13**, 95765-85-8; **14**, 95765-86-9; **15**, 95841-11-5; **16**, 95765-87-0; **17**, 4596-39-8; **18**, 74233-61-7; **19**, 95765-88-1; **20**, 95765-89-2; **21**, 95784-28-4; **23**, 67881-46-3; **25**, 67852-74-8; **26**, 67852-73-7; **28**, 67852-75-9; **29**, 67852-76-0; **30**, 67852-77-1; CH_3COSH, 507-09-5; glycine ethyl ester, 459-73-4; *p*-aminobenzoic acid, 150-13-0.

Synthesis of the Antileukemic Agents 5,10-Dideazaaminopterin and 5,10-Dideaza-5,6,7,8-tetrahydroaminopterin[1]

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Total syntheses from pyridine precursors of 5,10-dideazaaminopterin (1) and 5,10-dideaza-5,6,7,8-tetrahydroaminopterin (2) are described. These compounds exhibit significant in vivo activity against L1210 leukemia that is comparable to that observed with methotrexate.

Despite the established position of methotrexate as a clinical agent for the treatment of lymphocytic leukemia and choriocarcinoma, its extreme toxicity, coupled with its ineffectiveness against most other types of human cancers,[2,3] have provided continuing incentives for the development of less toxic drugs with more selective transport properties that might be effective against a broader range of human cancers. Various deaza analogues of folic acid, methotrexate, and aminopterin (the 10-desmethyl derivative of methotrexate) have shown particular promise; 10-deazaaminopterin, for example, is a

† Princeton University.
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(1) We are indebted to the National Cancer Institute, National Institutes of Health (Grant RO1 CA 28351) for support of this work.
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(3) Condit, P. T. *Ann. N.Y. Acad. Sci.* **1917**, *186*, 475.

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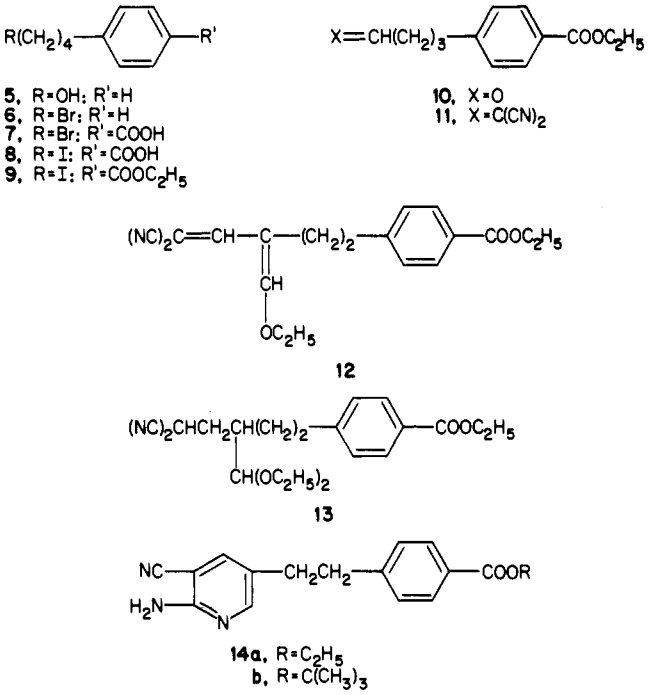
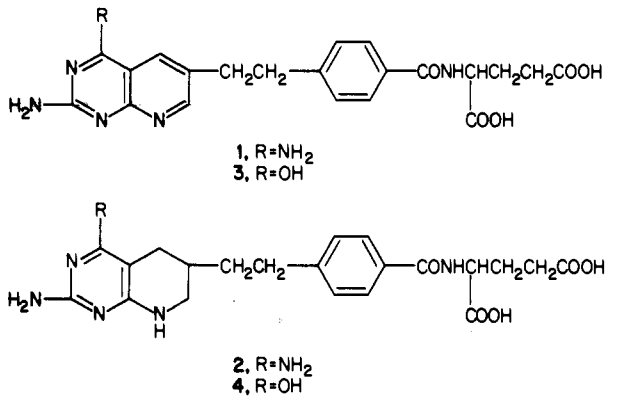
Scheme I



more effective antitumor agent against L1210 leukemia and sarcoma 180 tumors than either methotrexate or aminopterin, apparently because of its more favorable transport properties.[4] 8,10-Dideazaaminopterin appears to be comparable to methotrexate as an inhibitor of L1210 leukemia in mice,[5] and the N-10 propargyl derivative of 5,8-dideazafolic acid is a potent inhibitor of thymidylate synthetase, which is now in clinical trial.[6] We report in this paper the synthesis and preliminary biological evaluation of the active antileukemic agents 5,10-dideazaaminopterin (1) and its tetrahydro derivative (2) and the corresponding folic acid analogues 3 and 4.



Chemistry. Our initial approach to 1–4 centered upon the preparation of 2-amino-3-cyano-5-[2-[4-(ethoxycarbonyl)phenyl]ethyl]pyridine (**14a**) and its corresponding *tert*-butyl ester (**14b**), since ample precedent exists for the cyclization of analogous *o*-amino nitriles to fused 2,4-diaminopyrimidine systems.[7] We have developed two independent routes to these intermediates. The first commenced with 4-phenyl-1-butanol (**5**), which was converted into the bromide **6** and then to the acid **7** by known precedures. The remaining steps involved the intermediates shown in Scheme I and a pyrimidine ring construction procedure analogous to that developed by Ponticello et al.[8] for the synthesis of 2-amino-3-cyano-5-methylpyridine. The conversion of the primary iodo compound **9** to the aldehyde **10** was most conveniently carried out with use of trimethylamine oxide. Condensation of **10** with malononitrile gave the butylidene derivative **11**, which was formylated with trimethyl orthoformate to give a mixture of the enol ether **12** and the corresponding diethyl acetal **13**. Direct treatment of this mixture with ethanolic ammonia yielded the amino nitrile **14a**.

An alternative approach to the projected intermediates **14a,b** utilized methodology previously developed for the synthesis of 5-deazaaminopterin.[10] Thus (see Scheme II), condensation of α-cyanothioacetamide with 2-methyl-3-ethoxyacrolein gave 3-cyano-5-methyl-2(1*H*)-pyridinethione. This was arylated with 4-nitrofluorobenzene, and the resulting 2-[4-(nitrophenyl)thio]-3-cyano-5-methylpyridine (**15**) brominated under free-radical conditions to give a mixture of mono-, di-, and tribromo derivatives. In our previously described synthesis of 5-deazaminopterin,[9] the desired monobromo constituent **16** of this bromination mixture was efficiently retrieved by addition of pyridine, which resulted in direct crystallization from the reaction mixture of its corresponding pyridinium salt. We have now found that, in analogous fashion, addition of triphenylphosphine to the above bromination mixture led to direct crystallization of the triphenylphosphonium salt **17**. Reaction of **17** with 4-carbethoxybenzaldehyde at room temperature for 4 days in the presence of triethylamine then gave the *trans*-styryl derivative **18a** in 88% yield. This compound was readily converted into the *o*-aminonitrile **19a** in 64% yield by reaction with liquid ammonia and cupric bromide at room temperature for 14 days. Attempts to shorten the reaction period by increasing the temperature resulted in concomitant amide formation. The styryl derivative **19a** was smoothly reduced with hydrogen and Pd–C in ethyl acetate at room temperature to give **14a**, identical in every respect with the compound prepared by the method outlined in Scheme I.

To our surprise, however, all attempts to cyclize this *o*-amino nitrile to a fused 2,4-diaminopyrimidine failed. The use of either guanidine free base in hot ethanol or guanidine acetate in DMF led to aroylation of the guanidine rather than cyclization. Similar results were obtained with *N,N*-dimethylguanidine, which is an effective replacement for guanidine in this type of ring annelation reaction.[9] We therefore repeated the reaction sequence described in Scheme II utilizing 4-(*tert*-butoxycarbonyl)-benzaldehyde and attempted guanidine and dimethylguanidine cyclizations on the resulting *tert*-butyl ester **14b**. Negative results were again obtained, however; only ester involvement was observed under mild conditions, and complex, inseparable mixtures were obtained under forcing conditions. It seems that the failure of these guanidine

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Scheme II

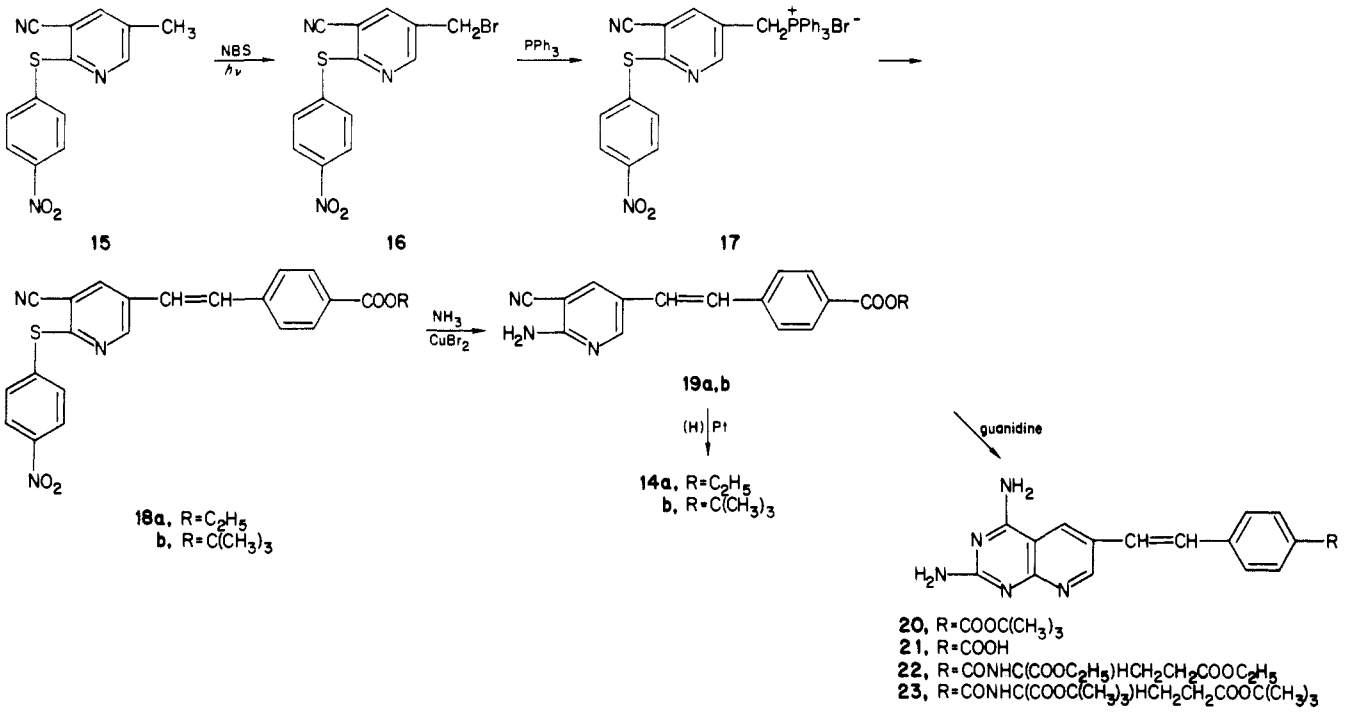


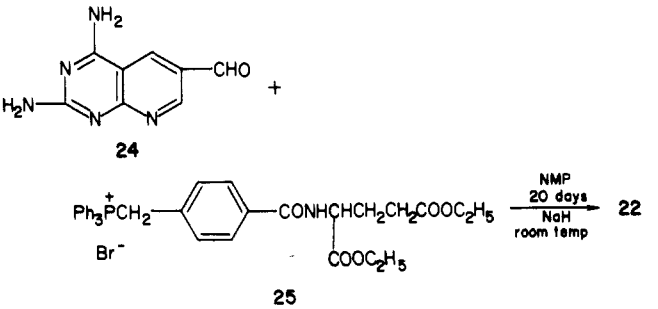
Table I. Effects of Deazapteridines on DHFR and TS

compd	DHFR inhibn: IC50, M	TS inhibn: IC50, M
1	4.3×10^{-8}	9.2×10^{-5}
2	7.1×10^{-8}	9.2×10^{-4}
3	4.9×10^{-5}	7.7×10^{-5}
4	5.6×10^{-4}	$>1 \times 10^{-3}$
MTX	1.7×10^{-9}	1.9×10^{-4}

cyclization reactions with **14a** and **14b** is probably due not only to the electrophilicity of the benzoate carbonyl group but also to a decrease in the electrophilicity of the nitrile substituent (compared with the analogous pyrazine) as a consequence of removal of one of the electronegative ring nitrogen atoms.

Both of the above factors could, in principle, be neutralized by employing the styryl amino nitriles **19a** and **19b** as substrates for the pyrimidine ring annelation reaction, since retention of unsaturation between the pyridine and benzene rings would serve both to decrease the electrophilicity of the benzoate carbonyl group (which would now be a vinylogous urethane) and to increase the electrophilicity of the 3-cyano grouping. In the event, treatment of **19b** with guanidine free base in refluxing *tert*-butyl alcohol indeed resulted in smooth cyclization to **20**, which was then carried on to our target compounds **1–4** as outlined below.

Treatment of the *tert*-butyl ester **20** either with 88% formic acid at room temperature for 72 h or with dry HCl in nitromethane at 0 °C for 1 h gave the free carboxylic acid **21**. Coupling with diethyl L-glutamate and with di-*tert*-butyl L-glutamate was best carried out with diphenyl chlorophosphonate as the coupling reagent in anhydrous *N*-methylpyrrolidone as solvent. The products **22** and **23** were purified by chromatography; although neither compound could be crystallized, NMR and mass spectral data indicated that both compounds had been obtained pure. Since reproducible microanalytical data could not be obtained for either compound, however, the structure of **22** was confirmed by an independent synthesis from 2,4-diamino-6-formyl-5-deazapteridine (**24**)[9] and the Wittig reagent derived from the phosphonium salt **25**, formed from diethyl *N*-[4-(bromomethyl)benzoyl]glutamate.[10] The products obtained by both methods were identical in every respect.



Since **23** was readily soluble in methanol (in contrast to **22**), hydrogenation was attempted with use of hydrogen and 5% Pd–C, but no reduction was observed even after 72 h. A similar failure to effect catalytic reduction under nonacidic conditions of the isomeric 8,10-deaza analogue of **23** has recently been reported.[5] We therefore resorted to catalytic reduction of the diethyl ester **22** in trifluoroacetic acid as solvent utilizing an excess of Pd–C as catalyst (see Scheme III). The resulting mixture of **26** and the tetrahydro derivative **27** was easily separable by column chromatography. Mild hydrolysis of **26** with methanolic sodium hydroxide at room temperature for 72 h gave 5,10-dideazaaminopterin (**1**) in 86% yield. Under similar conditions, the tetrahydro ester **27** was hydrolyzed to 5,10-dideaza-5,6,7,8-tetrahydroaminopterin (**2**) in 42% yield. 5,10-Dideaza-9,10-didehydroaminopterin (**28**) was prepared by hydrolysis of the di-*tert*-butyl ester **23** with dry HCl in nitromethane.

The corresponding folic acid analogues **3** and **4** were prepared as follows (see Scheme IV). Treatment of **21** with aqueous base resulted in hydrolysis of the 4-amino grouping to give **29**. Acetylation then gave the 2-acetamido mixed anhydride **30**, which could be selectively hydrolyzed to **31**. Coupling with diethyl L-glutamate to give **32** was then achieved with phenyl *N*-phenylphosphoramidochloridate in *N*-methylpyrrolidone as solvent. Catalytic reduction of **32** gave a mixture of **33** and **34**, which were separated by chromatography and hydrolyzed with

Scheme III

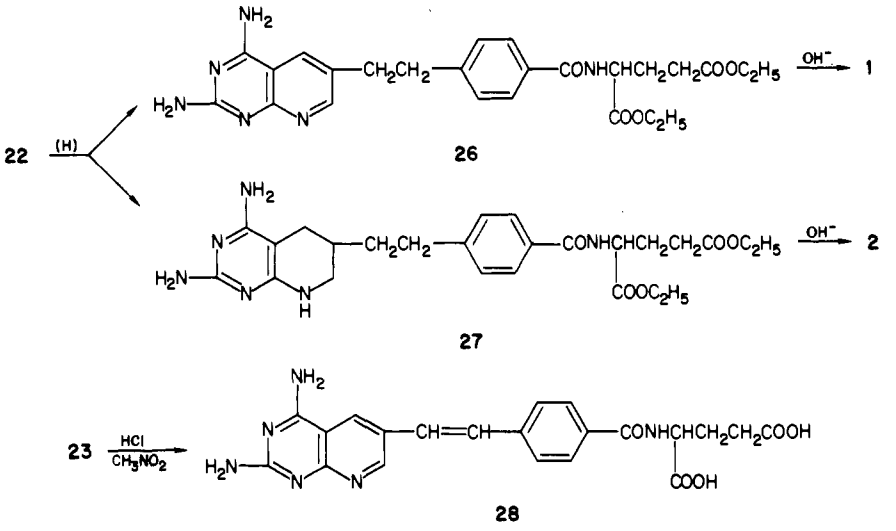


Table II. Activity of Deazapteridines as Substrates for Mouse Liver FPGS in Vitro[a]

compd	N^b	apparent K_m		rel V_{max}	rel V_{max}/K_m
		M	rel		
PteGlu	6	234 ± 43[e]	1.0	1.0	1.0
1	2	200 ± 23	0.86 ± 0.11	0.35 ± 0.02	0.40 ± 0.03
2	2	47 ± 11[f]	0.23 ± 0.01	1.61 ± 0.05	7.10 ± 0.10
3	2	157 ± 5	0.68 ± 0.14	0.90 ± 0.10	1.33 ± 0.14
4	2	9.7 ± 2.1[f]	0.05 ± 0.02	1.24 ± 0.10	27.1 ± 8.90
aminopterin[c]	4	17.6 ± 5.8	0.21 ± 0.04	1.50 ± 0.15	12.4 ± 5.00
1-H_4PteGlu[d]	2	7.1 ± 1.4	0.051 ± 0.01	1.31 ± 0.07	30.0 ± 7.00

[a] Each of the compounds listed was incubated with partially purified mouse liver FPGS (specific activity = 1.2 nmol h^{-1} (mg of protein) for 1 h at 37 °C. Full-saturation curves were obtained with duplicate assays at each of six concentrations usually chosen on the range of 0.2–3 apparent K_m and products were isolated by using our previously described conditions (ref 14). The values listed were derived from analysis of the data by the method of Cleland (ref 15). [b] Lists the number of experiments used for each estimate. [c] Data taken from ref 13. [d] Data taken from ref 16. [e] The relative K_m, V_{max}, and V_{max}/K_m values for each compound are expressed as a proportion of the corresponding value for PteGlu with use of data drawn from saturation curves run in each experiment. The apparent K_m value for PteGlu drawn from a much larger series (ref 13) was 140 ± 47 μM (N = 49). [f] Significant substrate inhibition was observed with these compounds. The data presented are extrapolated from the linear portions of double-reciprocal plots.

methanolic sodium hydroxide to give 5,10-dideazafolic acid (3) and 5,10-dideaza-5,6,7,8-tetrahydrofolic acid (4). Compound 35 was obtained by hydrolysis of 32 with methanolic sodium hydroxide.

Biological Evaluation. Compounds 1–4 were evaluated in vitro as inhibitors of beef liver dihydrofolate reductase (DHFR)[11] and *Lactobacillus casei* thymidylate synthetase (TS).[12] In addition, these compounds were tested as substrates for mouse liver folate polyglutamate synthetase (FPGS).[13] Results are shown in Tables I and II.

Thus, 1 and 2 were found to be potent inhibitors of DHFR but only weak inhibitors of TS. Compounds 3 and 4 were poor inhibitors of both enzymes. All of these 5,10-dideaza compounds were substrates for FPGS. The tetrahydro derivatives 2 and 4 were better substrates than the unreduced parent compounds 1 and 3. The 2-amino-4-oxo compounds 3 and 4 were better substrates than the corresponding 2,4-diamino compounds 1 and 2. In fact, the kinetic values for 4 closely approach those for the natural substrate for FPGS, H4-PteGlu.

Compounds 1–4 were evaluated as inhibitors of the growth of L1210 murine leukemia cells in tissue culture.[17]

Table III. Effects of Deazapteridines on the Growth of L1210 in Tissue Culture

compd	IC50, M	compd	IC50, M
1	1.7×10^{-8}	4	5.9×10^{-8}
2	3.3×10^{-9}	aminopterin	2.1×10^{-9}
3	$>10^{-4}$	MTX	1.0×10^{-8}

Table IV. Effects of Deazapteridines on L1210 Leukemia in BDF_1 Mice

compd	optimal dose, mg/kg	% ILS	survivors
1	4	130	4/21
2	1	111	5/22
MTX	4	85	0/10

Results are shown in Table III.

Thus, 1 and 2 show strong growth inhibitory activity against this tumor in tissue culture, as does 4. This latter observation is of particular interest, since 4 is a tetrahydropterin analogue rather than an aminopterin analogue.

Compounds 1 and 2 were also tested against L1210 in BDF_1 mice.[18,19] Each animal received 10^5 cells intraper-

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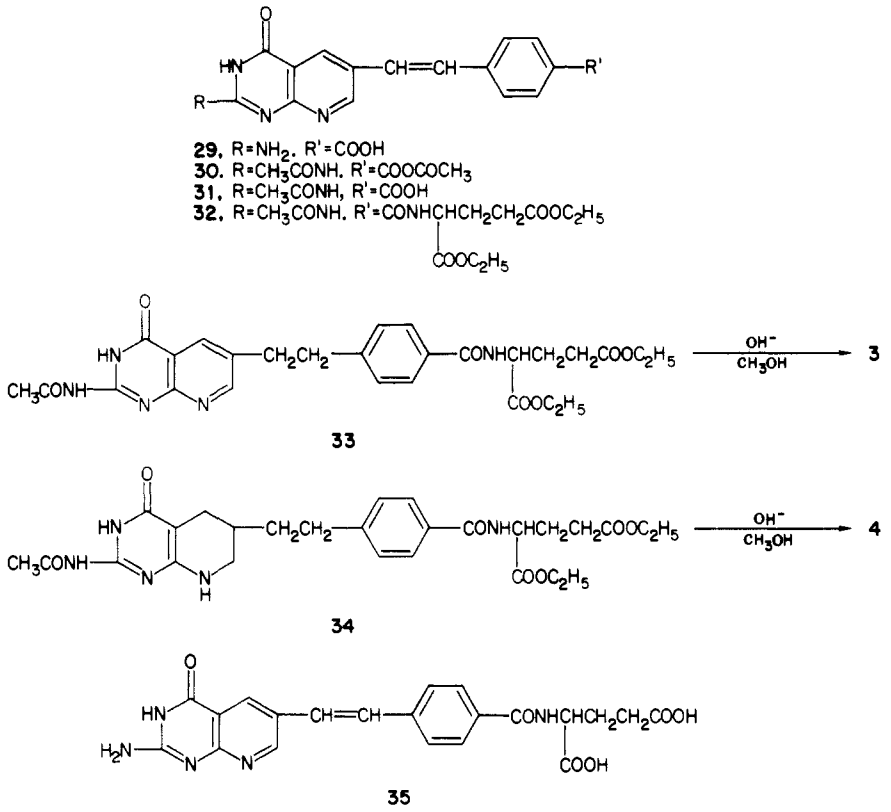
Scheme IV



itoneally on day 0. Drugs were administered intraperitoneally daily for 9 days beginning on day 1. Results are shown in Table IV.

These results show that compounds 1 and 2 have significant in vivo activity against L1210 leukemia that is comparable to that observed with methotrexate.

Experimental Section

4-(4-Iodobutyl)benzoic Acid (8). A mixture of 63.04 g (0.245 mol) of 4-(4-bromobutyl)benzoic acid (7) and 127.10 g (0.736 mol) of finely ground potassium iodide in 1200 mL of acetone was stirred at room temperature for 24 h and suction filtered and the isolated salt washed with fresh acetone. The filtrate was evaporated under reduced pressure and the residual solid triturated with a mixture of 18 mL of diethyl ether and 1 L of water. The aqueous layer was extracted twice with ether, and the combined organic layers were dried over anhydrous $MgSO_4$. Evaporation of the filtrate under reduced pressure and recrystallization of the residual solid from benzene gave 52.58 g (71%) of 8 as colorless needles: mp 155–162 °C; NMR (Me_2SO-d_6) δ 1.72–1.79 (m, 4 H), 2.69 (t, 2 H), 3.29 (t, 2 H), 7.32 (s, 2 H, J = 8.1 Hz), 7.94 (d, 2 H, J = 8.1 Hz); IR (KBr) 3300–2300, 2945, 2865, 1688, 1613, 1430, 1320, 1299 cm^{-1}. Anal. Calcd for $C_{11}H_{13}IO_2$: C, 43.44; H, 4.31; I, 41.73. Found: C, 43.57; H, 4.34; I, 41.61.

Ethyl 4-(4-Iodobutyl)benzoate (9). Concentrated sulfuric acid (10 mL) was slowly added to 20.00 g of 4-(4-iodobutyl)benzoic acid in 45 g of anhydrous ethanol. The reaction mixture was heated under reflux for 4 h, cooled to room temperature, and poured into a mixture of 500 mL of water and 500 mL of CH_2Cl_2. The organic layer was separated, washed with 500 mL of 1 N aqueous potassium hydroxide followed by water, dried over anhydrous magnesium sulfate, and filtered. The filtrate was evaporated under reduced pressure and the residual oil filtered through a short plug of silica gel, using petroleum ether as eluent. Evaporation of the filtrate gave 19.09 g (87%) of analytically pure 9 as a colorless oil: NMR ($CDCl_3$) δ 1.38 (t, 3 H, J = 7.2 Hz), 1.70–1.90 (m, 4 H), 2.68 (t, 2 H), 3.17 (t, 2 H), 4.36 (q, 2 H, J = 7.2 Hz), 7.23 (d, 2 H, J = 9 Hz), 7.97 (d, 2 H, J = 9 Hz); IR (neat) 2975, 2935, 2860, 1720–1710, 1286–1272, 1106 cm^{-1}. Anal. Calcd for $C_{13}H_{17}IO_2$: C, 47.01; H, 5.16; I, 38.20. Found: C, 47.31; H, 5.12; I, 38.52.

4-[4-(Ethoxycarbonyl)phenyl]butanal (10). To a solution of 0.10 mol of anhydrous trimethylamine N-oxide (from 11.11 g of the dihydrate) in 25 mL of chloroform at 55–60 °C was slowly added 16.6 g (0.05 mol) of ethyl 4-(4-iodobutyl)benzoate over a period of 15 min. The reaction mixture was heated under reflux for 20 min, cooled to room temperature, and washed with 28 mL of 2 N aqueous sulfuric acid, 30 mL of 2 N aqueous sodium carbonate, and 30 mL of water. The solution was dried over anhydrous sodium sulfate and filtered, and the filtrate evaporated under reduced pressure. The residual oil was chromatographed on silica gel, with benzene as the eluent. Evaporation of the eluent gave 3.69 g (33%) of 10 as a yellow oil.

[4-[4-(Ethoxycarbonyl)phenyl]butylidene]malononitrile (11). A mixture of 4.31 g (0.0196 mol) of 4-[4-(ethoxycarbonyl)phenyl]butanal, 1.215 g (0.0184 mol) of malononitrile, 0.05 g of *d,l*-alanine, 1 mL of glacial acetic acid, and 14 mL of benzene was heated under reflux with azeotropic removal of water with use of a Dean–Stark trap. The reaction mixture was cooled to room temperature and washed twice with benzene, the combined organic layers were dried over anhydrous sodium sulfate and filtered, and the solvent was removed under reduced pressure. The residual solid was then passed through a short column of silica gel with benzene as the eluent; evaporation of the eluate gave 3.68 g (75%) of 11 as a light yellow oil; NMR ($CDCl_3$) δ 1.38 (t, 3 H, J = 7.2 Hz), 1.73–2.07 (m, 2 H), 2.47–2.83 (m, 4 H), 4.36 (q, 2 H, J = 7.2 Hz), 7.25 (d, 2 H, J = 8.1 Hz), 7.99 (d, 2 H, J = 8.1 Hz); IR (neat) 2242, 1718, 1612, 1280, 1110–1105 cm^{-1}.

2-Amino-3-cyano-5-[2-[4-(ethoxycarbonyl)phenyl]ethyl]pyridine (14a). Method A. A mixture of 3.68 g (0.0137 mol) of [4-[4-(ethoxycarbonyl)phenyl]butylidene]malononitrile, 0.05 g of $ZnCl_2$, 7.76 g (0.0524 mol) of triethyl orthoformate, and 10 mL of acetic anhydride was heated for 18 h at 145 °C. The dark reaction mixture was cooled and evaporated to about half its volume, and 5 mL of acetic anhydride and 2 mL of triethyl orthoformate were added. The resulting mixture was then heated for 10 h at 150 °C and the dark suspension cooled, poured into 25 mL of water, and extracted with three 25-mL portions of benzene. Although the mixture could be separated by silica gel chromatography into 12 (more polar) and 13 (less polar), it was

(19) USA–USSR Monograph, "Methods of Development of New Anti-Cancer Drugs", NCI Monograph 45, 1977, pp 5–177.

found most convenient to convert the mixture of **12** and **13** directly to **14a** by stirring at room temperature for 6 h with ethanolic ammonia (from 6.8 g of ammonia in 50 mL of anhydrous ethanol). The reaction mixture was cooled to 0 °C and filtered, and the isolated solid was washed with anhydrous ethanol and recrystallized from ethanol to give 1.39 g (34%) of **14a** as light yellow crystals: mp 136–137.5 °C; NMR ($CDCl_3$) δ 1.39 (t, 3 H, J = 6.3 Hz), 2.88 (m, 4 H), 4.38 (q, 2 H, J = 6.3 Hz), 5.31 (br, 2 H), 7.18 (d, 2 H, J = 8.1 Hz), 7.44 (d, 1 H, J = 2.7 Hz), 7.92, 8.01 (dd, 2 H); IR (KBr) 3420–3415, 3310, 3285, 2218, 1704, 1639, 1280 1097 cm^{-1}. Anal. Calcd for $C_{17}H_{17}N_3O_2$: C, 69.14; H, 5.80; N, 14.23. Found: C, 69.15; H, 5.89; N, 14.23.

[[3-Cyano-2-[(4-nitrophenyl)thio]-5-pyridinyl]methyl]triphenylphosphonium Bromide (17). A mixture of 60.00 g (0.221 mol) of 2-[(4-nitrophenyl)thio]-3-cyano-5-methylpyridine (**15**), 39.37 g (0.221 mol) of *N*-bromosuccinimide, 3.0 g of benzoyl peroxide, and 60 mL of benzene was refluxed for 16 h while being irradiated with a 275-W sunlamp. Solvent was then removed under reduced pressure. The residue was shaken with a mixture of 1 L of water and 1 L of CH_2Cl_2, andd the organic layer was separated, washed with 1 L of water, dried over anhydrous magnesium sulfate, filtered, and evaporated under reduced pressure. The residual solid was stirred at room temperature with a solution of 58.01 g (0.221 mol) of triphenylphosphine in 500 mL of benzene. Filtration of the reaction mixture gave 77.63 g of **17**; stirring of the mother liquor at room temperature for 6 h afforded an additional 5.67 g of **17** (total yield 83.30 g, 62%). Recrystallization from acetonitrile gave **17** as light yellow crystals, mp <200 °C, with resolidification, and then mp 253–256 °C dec.

2-[(4-Nitrophenyl)thio]-3-cyano-5-[4-(ethoxycarbonyl)styryl]pyridine (18a). A mixture of 4.544 g (7.42 mmol) of **17**, 0.751 g (7.42 mmol) of triethylamine, and 50 mL of chloroform was stirred at room temperature for 15 min, and 1.322 g (7.42 mmol) of 4-(ethoxycarbonyl)benzaldehyde was added. After the mixture was stirred for 4 days at room temperature, 100 mL of water was added, the mixture was filtered, and the organic layer was washed twice with 100-mL portions of water, dried, and filtered. Evaporation of the filtrate under reduced pressure gave a residue, which was chromatographed on silica gel; unreacted aldehyde was eluted with 2:1 petroleum ether/benzene, while the desired styryl derivative **18a** was eluted with benzene. Evaporation of the benzene eluate gave 2.82 g (88%) of **18a** as a light yellow solid: melting point indefinite (turns from a solid to a gum below 100 °C andd then to a clear liquid between 180 and 220 °C); NMR (Me_2SO-d_6) δ 1.34 (t, 3 H, J = 6.3 Hz), 4.32 (q, 2 H, J = 6.3 Hz), 6.73 (d, 1 H, J = 13 Hz), 6.99 (d, 1 H, J = 13 Hz), 7.27 (d, 2 H, J = 9 Hz), 7.74, 7.85, 7.94 (dd, 2 H, 2 H), 8.26, 8.31 (dd, 2 H, 1 H), 8.38 (d, 1 H, J = 1.8 Hz); IR (KBr) 2220, 1707, 1605, 1597, 1575, 1512, 1344, 1295–1277, 1174 cm^{-1}. Anal. Calcd for $C_{23}H_{17}N_3O_4S$: C, 64.08; H, 3.97; N, 9.74; S, 7.43. Found: C, 63.82; H, 4.01; N, 9.51; S, 7.38.

2-Amino-3-cyano-5-[4-(ethoxycarbonyl)styryl]pyridine (19a). A suspension of 2.00 g (4.64 mmol) of **18a**, 1.553 g (6.95 mmol) of cupric bromide, and 50 mL of liquid ammonia was stirred in a pressure tube at room temperature for 13 days. Evaporation of the ammonia afforded a dark residue, which was chromatographed on Florisil with CH_2Cl_2 as eluent. The eluate was evaporated under reduced pressure and chromatographed on silica gel; benzene eluted unreacted starting material while ethyl acetate eluted the product **19a**, which was obtained by evaporation of the ethyl acetate eluate; yield 0.87 g (64%) of a light yellow solid, mp 135–141.5 °C; NMR (Me_2SO-d_6) δ 1.39 (t, 3 H, J = 6.3 Hz), 4.38 (q, 2 H, J = 6.3 Hz), 6.67 (m, 2 H), 7.10 (br, 2 H), 7.45 (d, 2 H, J = 9 Hz), 7.71 (d, 1 H, J = 3.6 Hz), 7.97 (d, 2 H, J = 9 Hz), 8.11 (d, 1 H, J = 3.6 Hz); IR (KBr) 3155, 2218, 1715, 1650–1645, 1593, 1491, 1277, 1100 cm^{-1}. Anal. Calcd for $C_{17}H_{15}N_3O_2$: C, 69.61; H, 5.16; N, 14.33. Found: C, 69.37; H, 5.25; N, 14.22.

2-Amino-3-cyano-5-[2-[4-(ethoxycarbonyl)phenyl]ethyl]pyridine (14a). Method B. Hydrogenation of **19a** (0.87 g, 3.0 mmol) was carried out in 150 mL of ethyl acetate at room temperature with 50–55 psi of hydrogen, using 2.61 g of 5% Pd–C. After 1 h, the catalyst was removed by filtration through Celite and the filtrate evaporated under reduced pressure to give 0.76 g (86%) of **14a** as a colorless solid, mp 138–139 °C, identical in every respect with the material prepared by method A as described above.

2-[(4-Nitrophenyl)thio]-3-cyano-5-[4-(*tert*-butoxycarbonyl)styryl]pyridine (18b). This compound was prepared in 81% yield by the method described above for the preparation of **18a** except that 4-(*tert*-butoxycarbonyl)benzaldehyde was employed rather than the corresponding ethoxycarbonyl compound; melting point indefinite (indicative of a cis–trans mixture); NMR ($CDCl_3$) δ 1.62 (s, 9 H), 6.43 (d, 1 H, J = 13 Hz), 6.90 (d, 1 H, J = 13 Hz), 7.24 (d, 2 H, J = 9 Hz), 7.69 (d, 2 H, J = 8.1 Hz), 7.76 (d, 1 H, J = 2.7 Hz), 7.92 (d, 2 H, J = 8.1 Hz), 8.22 (d, 2 H, J = 9 Hz), 8.34 (d, 1 H, J = 2.7 Hz); IR (KBr) 2220, 1707, 1600, 1577, 1518, 1341, 1290, 1163 cm^{-1}. Anal. Calcd for $C_{25}H_{21}N_3O_4S$: C, 65.35; H, 4.61; N, 9.14; S, 6.98. Found: C, 65.28; H, 4.68; N, 9.20; S, 6.93.

2-Amino-3-cyano-5-[4-(*tert*-butoxycarbonyl))styryl]pyridine (19b). A suspension of 1.95 g (4.24 mmol) of **18b**, 1.42 g (6.37 mmol) of cupric bromide, and 50 mL of liquid ammonia was stirred at room temperature in a pressure tube for 14 days. Evaporation of the ammonia gave a dark residue, which was worked up as described above for the preparation of **19a**: yield 1.14 g (84%) of light yellow crystals, mp 190–195 °C; NMR (Me_2SO-d_6) δ 1.57 (s, 9 H), 6.57–6.60 (m, 2 H), 7.00 (br, 2 H), 7.35 (d, 2 H, J = 8.1 Hz), 7.65 (d, 1 H, J = 2.7 Hz), 7.84 (d, 2 H, J = 8.1 Hz), 8.00 (d, 1 H, J = 2.7 Hz); IR (KBr) 3460, 3360, 2215, 1707, 1623, 1480, 1300, 1287, 1158 cm^{-1}. Anal. Calcd for $C_{19}H_{19}N_3O_2$: C, 71.00; H, 5.96; N, 13.07. Found: C, 70.83; H, 6.03; N, 12.83.

2-Amino-3-cyano-5-[2-[4-(*tert*-butoxycarbonyl)phenyl]ethyl]pyridine (14b). Hydrogenation of **19b** was carried out at described above for the hydrogenation of **19a**; **14b** was obtained in 92% yield as pale yellow needles: mp 155–158 °C (from hexane); NMR (Me_2SO-d_6) δ 1.58 (s, 9 H), 2.86 (m, 4 H), 6.65 (br, 2 H), 7.73, 7.79, 7.88 (dd, 2 H, 1 H), 8.03 (d, 1 H); IR (KBr) 3415, 3310, 3200, 2210, 1710, 1640–1635, 1490, 1288, 1165–1160 cm^{-1}. Anal. Calcd for $C_{19}H_{21}N_3O_2$: C, 70.57; H, 6.55; N, 12.99. Found: C, 70.24; H, 6.85; N, 13.16.

2,4-Diamino-6-[4-(*tert*-butoxycarbonyl)styryl]-5-deazapteridine (20). To a solution of 4.54 mmol of guanidine free base (from 0.433 g (4.54 mmol) of guanidine hydrochloride and 0.114 g (4.94 mmol) of sodium in 25 mL of dry *tert*-butyl alcohol) was added 1.325 g (4.12 mmol) of **19b**, and the deep red suspension was heated under reflux under dry nitrogen for 8 h. The reaction mixture was cooled to room temperature and filtered, and the precipitate was washed with water, acetone, and ether and then dried under reduced pressure; yield 0.911 g (61%) of **20** as a light yellow solid, mp >350 °C; NMR (Me_2SO-d_6) δ 1.55 (s, 9 H), 6.42 (br, 2 H), 6.73 (m, 2 H), 7.30–8.00 (br, 2 H), 7.35 (d, 2 H, J = 9 Hz), 7.81 (d, 2 H, J = 9 Hz), 8.34 (m, 2 H); IR (KBr) 3320–3300, 3200–3140, 2970, 1718, 1626, 1610–1600, 1550, 1450–1445, 1288, 1167, 812 cm^{-1}. Anal. Calcd for $C_{20}H_{21}N_5O_2$: C, 66.10; H, 5.82; N, 19.27. Found: C, 65.88; H, 5.86; N, 18.98.

2,4-Diamino-6-(4-carboxystyryl)-5-deazapteridine (21). Method A. A solution of 1.27 g of the *tert*-butyl ester **20** and 10 mL of 88% formic acid was stirred at room temperature. A yellow solid started to precipitate after about 12 h; after 4 days of stirring, the reaction mixture was filtered and the collected solid washed well with water, methanol, and acetone and dried under reduced pressure to give 0.85 g (79%) of **21**, mp >300 °C.

Method B. The *tert*-butyl ester **20** (0.4 g) was added to a saturated solution of HCl in 20 mL of nitromethane at 0 °C. The reaction mixture quickly became viscous and turned a deep yellow in color. After a few minutes of stirring a granular precipitate formed; after 1 h of stirring, 50 mL of ether was added and the precipitate was collected by filtration. The collected solid was dissolved in 50 mL of 10% sodium carbonate solution; acidification with acetic acid then resulted in the separation of a yellow solid, which was collected by filtration and dried under reduced pressure; yield 0.31 g (92%) of **21**; NMR (Me_2SO-d_6) δ 6.75 (s, 2 H), 7.35, 7.85 (AB q, 4 H, J = 9 Hz), 8.38 (s, 2 H); IR (Nujol) 3400–2300, 3380, 3150, 1700, 1650, 1630, 1590 cm^{-1}.

Diethyl *N*-[4-[2-(2,4-Diamino-5-deaza-6-pteridyl)ethenyl]benzoyl]-L-glutamate (22). Method A. Diphenyl chlorophosphonate (1.4 g, 0.0048 mol) was added dropwise to a solution of 1.0 g (0.0033 mol) of **21** in 120 mL of *N*-methylpyrrolidone containing 1 g of *N*-methylmorpholine cooled to 5 °C. The reaction mixture was stirred for 1 h, an additional 0.5 mL of *N*-methylmorpholine was added, followed by 1.1 g (0.0048 mol) of diethyl L-glutamate hydrochloride, and the reaction

mixture was stirred overnight at room temperature. The solvent was removed under reduced pressure, the residual solid was washed with 50 mL of dry ether and triturated with 100 mL of 1 N aqueous sodium hydroxide, and the resulting suspension was centrifuged. The collected solid was dissolved in 200 mL of 3:1 chloroform/methanol and filtered through Florisil. The filtrate was evaporated to a small volume, 10 g of Florisil added, and the resulting impregnated Florisil added to the top of a Florisil column, which was then eluted sequentially with ethyl acetate followed by ethyl acetate/methanol (9:1, 3:1, and 1:1). The product was collected in the latter two fractions; evaporation of the eluates gave a glassy material, which was triturated with ether and then collected by filtration; yield 0.41 g (26%), mp 183–185 °C; NMR (Me_2SO-d_6/TFA) δ 1.25–1.45 (overlapping t, 6 H, J = 7 Hz)8 2.25–2.50 (m, 2 H), 2.5–2.8 (m, 2 H), 4.05–4.45 (overlapping q, 4 H, J = 7 Hz), 4.8–5.0 (m, 1 H), 6.8, 7.2 (AB q, 2 H, J = 16 Hz), 7.4, 7.85 (AB q, 4 H, J = 9 Hz), 8.6 (s, 1 H), 9.05 (s, 1 H); IR (Nujol) 3500–3000, 1730, 1635, 1605 cm^{-1}; MS, calcd for $C_{25}H_{28}N_6O_5$ 492, found m/e 492, 290, 94, 84.

Method B. The triphenylphosphonium salt prepared from triphenylphosphine and diethyl *N*-[4-(bromomethyl)benzoyl]-glutamate[11] (7.86 g, 0.012 mol) was added portionwise to a slurry of 0.4 g (0.01 mol) of sodium hydride (60% suspension in oil) in 70 mL of dry *N*-methylpyrrolidone over a period of 10 min, and the resulting red reaction mixture was stirred at room temperature under nitrogen for 1 h. To this in situ Wittig reagent was added 2.27 g (0.012 mol) of 2,4-diamino-6-formyl-5-deazapteridine (**24**),[9] and the resulting slurry was stirred at room temperature under nitrogen for 3 weeks. The solvent was then evaporated under reduced pressure, the residual solid triturated with benzene to remove triphenylphosphine oxide, and the residual solid collected by centrifugation. This material was resuspended in water and filtered, and the collected solid was dissolved in 200 mL of chloroform/methanol (1:2). Florisil (10 g) was added, the mixture was evaporated to dryness, and the impregnated Florisil residue was applied to the top of a Florisil column, which was then eluted with ethyl acetate/methanol (9:1 → 1:1). Combination of the fractions gave a mixture of two products, which were rechromatographed on silica gel with chloroform/methanol as eluents. The initial fraction proved to be the phosphorane derived from **25**; compound **22** was obtained by elution with chloroform/methanol (1:9); yield 1.7 g (34.5%). This material was identical in all respects with that prepared as described above by method A.

Di-*tert*-butyl *N*-[4-[2-(2,4-Diamino-5-deaza-6-pteridyl)-ethenyl]benzoyl]-L-glutamate (23). This compound was prepared from 1.5 g (0.0049 mol) of **21** and 2.2 g (0.0074 mol) of di-*tert*-butyl L-glutamate hydrochloride as described above for the preparation of **22**; yield 1.3 g (48%), mp >300 °C. The product was purified by chromatography on silica gel with chloroform/methanol (95:5–4:1): NMR ($CDCl_3$/CD_3OD) δ 1.47, 1.52 (2 s, 18 H), 2.0–2.6 (m, 4 H), 4.5–7.0 (m, 1 H), 6.8 (br s, 2 H), 7.35, 7.78 (AB q, 4 H, J = 9 Hz), 8.38 (s, 1 H), 8.5 (s, 1 H); IR (Nujol) 3350, 3180, 1725, 1640, 1605 cm^{-1}; MS, calcd for $C_{29}H_{36}N_6O_5$ 548, found m/e 548, 446, 290.

Diethyl *N*-[4-[2-(2,4-Diamino-5-deaza-6-pteridyl)ethyl]-benzoyl]-L-glutamate (26). A solution of 0.9 g of **22** in 40 mL of trifluoroacetic acid was hydrogenated under 55 psi of hydrogen for 24 h with 2.5 g of Pd/C as catalyst. The catalyst was removed by filtration through Celite, the TFA filtrate evaporated, and the residual solid triturated with 30 mL of 2 N sodium carbonate solution, followed by washing with water. The resulting solid was purified by column chromatography on silica gel; elution with chloroform/methanol (95:5) afforded a small amount (0.1 g) of the tetrahydro derivative **27**; subsequent elution with chloroform/methanol (1:4) gave 0.52 g (58%) of **26**: mp >200 °C; NMR (Me_2SO-d_6) δ 1.1–1.3 (2 t, 6 H, J = 7 Hz), 1.8–2.6 (m, 4 H), 3.05 (s, 4 H), 3.1–3.8 (br, 5 H), 3.9–4.2 (2 q, 4 H, J = 7 Hz), 4.3–4.5 (m, 1 H), 7.35, 7.85 (AB q, 4 H, J = 9 Hz), 8.6 (br s, 2 H): IR (Nujol) 3320, 3150, 1650 cm^{-1}.

5,10-Dideazaaminopterin (1). A solution of 0.38 g of **26** in 50 mL of methanol containing 4.6 mL of 0.5 N hydroxide solution was stirred at room temperature for 72 h. Acetic acid (5 mL) was added, and the resulting white precipitate was collected by filtration, washed well with water, methanol, and ether and dried under reduced pressure; yield 0.15 g (44%), mp >250 °C; NMR (TFA-d_1) δ 2.2–2.7 (m, 2 H), 2.28 (s, 4 H), 2.7–2.95 (m, 2 H), 5.0–5.2 (m, 1 H), 7.35 and 7.85 (AB q, 4 H, J = 9 Hz), 8.7 (s, 1 H), 9.1 (s, 1 H).

Diethyl *N*-[4-[2-(2,4-Diamino-5-deaza-5,6,7,8-tetrahydro-6-pteridyl)ethyl]benzoyl]-L-glutamate (27). The yield of **27** was considerably improved by more extensive hydrogenation of the styryl derivative **22**. Thus, hydrogenation for 72 h, followed by workup as described above for the preparation of **26**, provided a crude product, which was chromatographed on silica gel with chloroform/methanol (95:5) to give 0.42 g (31%) of **27** as a colorless microcrystalline solid: mp >250 °C; NMR (Me_2SO-d_6) δ 1.6, 1.8 (2 t, 6 H, J = 6 Hz), 1.4–3.8 (m, 13 H), 4.1 (2 q, 4 H, J = 6 Hz), 4.3–4.6 (m, 1 H), 6.8 (s, 2 H), 7.35, 7.85 (AB q, 4 H, J = 9 Hz), 8.7 (d, 1 H, J = 9 Hz); IR (Nujol) 3350, 3150, 1730, 1630 cm^{-1}; MS, calcd for $C_{25}H_{34}N_6O_5$ 498, found m/e 498, 425, 178, 165 (base), 150.

5,10-Dideaza-5,6,7,8-tetrahydroaminopterin (2). This compound was prepared by hydrolysis of 0.35 g of the diethyl ester **27** as described above for the preparation of 1 from **26**; yield 0.13 g (42%), mp >250 °C.

6-(4-Carboxystyryl)-5-deazapterin (29). A suspension of 1.0 g of **21** in 30 mL of 1 N sodium hydroxide solution was heated under reflux under nitrogen for 3 h. The resulting homogeneous orange solution was cooled to room temperature and acidified with 6 mL of glacial acetic acid, and the resulting yellow precipitate was collected by filtration, washed with water, methanol, acetone, and ether and dried under reduced pressure to give 0.88 g (88%) of **29** as a microcrystalline yellow powder: mp >250 °C; NMR (TFA-d_1) δ 6.8, 7.25 (AB q, 2 H, J = 12 Hz), 7.45, 8.2 (AB q, 4 H, J = 9 Hz), 8.55 (s, 1 H), 8.85 (s, 1 H); IR (Nujol) 3500–2500 (br), 1670, 1625, 1600 cm^{-1}.

2-Acetamido-6-[4-(acetoxycarbonyl)styryl]-5-deaza-4-(3*H*)-pteridinone (30). A suspension of 0.88 g of **29** in 20 mL of acetic anhydride containing 0.05 g of 4-(dimethylamino)pyridine was heated under nitrogen at 120 °C for 3 h. The reaction mixture was cooled to room temperature, 50 mL of ether added, and the resulting yellow solid collected by filtration: yield 0.95 g (84%), mp >300 °C; IR (Nujol) 3350, 3150, 1800, 1670, 1600 cm^{-1}.

2-Acetamido-6-(4-carboxystyryl)-5-deaza-4(3*H*)-pteridinone (31). Sodium hydroxide solution (1 N) was slowly added to a suspension of 0.95 g of the mixed anhydride **30** in 50 mL of water until a homogeneous solution resulted. Acidification with acetic acid then resulted in the formation of a yellow precipitate, which was collected by filtration and washed sequentially with water, methanol, acetone, and ether. The residual solid was recrystallized from DMF to give 0.65 g (77%) of **31** as a microcrystalline yellow solid: mp >300 °C; NMR (TFA-d_1) δ 2.5 (s, 3 H), 6.85, 7.32 (AB q, 2 H, J = 12 Hz), 7.45, 8.18 (AB q, 4 H, J = 9 Hz), 8.65 (s, 1 H), 9.02 (s, 1 H); IR (Nujol) 3300–2200 (br), 1685, 1655, 1630, 1600, 1565 cm^{-1}; MS, calcd for $C_{18}H_{14}N_4O_4$ 350, found m/e 350 (base), 308.

Diethyl 2-Acetyl-5,10-dideaza-9,10-didehydrofolate (32). To an ice-cold solution of 1.5 g (0.0043 mol) of **31** in 40 mL of *N*-methylpyrrolidone containing 1.4 mL of *N*-methylmorpholine was added 1.72 g (0.0064 mol) of phenyl *N*-phenylphosphoramidochloridate in one portion, and the mixture was stirred at 0 °C for 30 min. Diethyl L-glutamate hydrochloride (1.53 g, 0.0064 mol) was then added, and the reaction mixture was stirred at room temperature overnight. Solvent was evaporated under reduced pressure and the residual solid triturated with 50 mL of 1 N sodium carbonate solution. The mixture was filtered and the collected solid dissolved in 20 mL of chloroform, which was dried over anhydrous magnesium sulfate and filtered, and the filtrate was evaporated to dryness. Chromatography of the residual solid on silica gel followed by elution with chloroform/methanol (95:5) gave 1.52 g (66%) of **32**: mp >250 °C; NMR ($CDCl_3$/Me_2SO-d_6) δ 1.15–1.45 (2 t, 6 H, J = 6 Hz), 2.0–2.65 (m, 4 H), 2.3 (s, 3 H), 4.0–4.35 (2 q, 4 H, J = 6 Hz), 4.5–4.75 (m, 1 H), 6.7, 6.9 (AB q, 2 H, J = 15 Hz), 7.33, 7.84 (AB q, 4 H, J = 9 Hz), 8.25–8.38 (m, 2 H), 8.62 (d, 1 H, J = 2 Hz), 11.5–12.5 (br, 2 H); IR (Nujol) 3320, 3150, 1730, 1680, 1630, 1600 cm^{-1}. Anal. Calcd for $C_{27}H_{29}N_5O_7$: C, 60.56; H, 5.42; N, 13.08. Found: C, 60.26; H, 5.45; N, 12.84.

Hydrogenation of 32. Preparation of Diethyl 2-Acetyl-5,10-dideazafolate (33) and Diethyl 2-Acetyl-5,10-dideaza-5,6,7,8-tetrahydrofolate (34). A solution of 0.45 g of **32** in 30 mL of trifluoroacetic acid was hydrogenated at 55 psi of hydrogen in the presence of 1.0 g of 5% Pd/C at room temperature for 14

h. The catalyst was removed by filtration, the filtrate evaporated under reduced pressure, and the residual solid partitioned between 100 mL of chloroform and 50 mL of 2 N sodium carbonate solution. The organic phase was separated, dried over anhydrous magnesium sulfate, and evaporated to give a gum, which was chromatographed on silica gel. Compound **33** [0.25 g, 56%, mp 215–217 °C; NMR ($CDCl_3$) δ 1.25, 1.35 (2 t, 6 H, J = 6 Hz), 2.1–2.5 (m, 4 H), 2.55 (s, 3 H), 3.1 (s, 4 H), 4.15, 4.25 (2 q, 4 H, J = 6 Hz), 4.6–4.96 (m, 1 H), 7.05 (s, 1 H), 7.25, 7.75 (AB q, 4 H, J = 9 Hz), 8.35 (d, 1 H, J = 3 Hz), 8.77 (d, 1 H, J = 3 Hz); IR (Nujol) 3200, 3150, 1725, 1675, 1630, 1605 cm^{-1}. Anal. Calcd for $C_{27}H_{31}N_5O_7$: C, 60.32; H, 5.81; N, 13.03. Found: C, 59.98; H, 6.03; N, 12.92] was obtained upon elution with chloroform/methanol (97:3), while compound **34** [0.08 g, 18%, mp >200 °C; NMR ($CDCl_3$/Me_2SO-d_6) δ 1.24, 1.28 (2 t, 6 H, J = 6 Hz), 1.5–3.3 (m, 13 H), 2.18 (s, 3 H), 4.1, 4.18 (2 q, 4 H, J = 6 Hz), 4.4–4.7 (m, 1 H), 6.2 (s, 1 H), 7.28, 7.85 (AB q, 4 H, J = 9 Hz), 8.4 (d, 1 H, J = 8 Hz); IR (Nujol) 3320, 3250, 1730, 1630, 1575 cm^{-1}. Anal. Calcd for $C_{27}H_{35}N_5O_7$: C, 59.87; H, 6.51; N, 12.93. Found: C, 59.66; H, 6.71; N, 12.77] was obtained by elution with chloroform/methanol (95:5).

5,10-Dideazafolic Acid (3). A homogeneous solution of 0.175 g of **33** in 50 mL of methanol containing 3 mL of 1 N sodium hydroxide was stirred at room temperature for 72 h. Addition of 2 mL of acetic acid followed by centrifugation gave 0.125 g (86%) of **3** as a microcrystalline colorless solid: mp >200 °C; NMR (TFA-d_1) δ 2.3–2.7 (m, 2 H), 2.7–3.0 (m, 2 H), 3.25 (s, 5 H), 4.9–5.25 (m, 1 H), 7.35, 7.85 (AB q, 4 H, J = 9 Hz), 8.50 (s, 1 H), 8.90 (s, 1 H).

5,10-Dideaza-5,6,7,8-tetrahydrofolic acid (4) was obtained by alkaline hydrolysis of **34** as described above for the preparation of **3** from **33**: yielded 87%, mp >250 °C; NMR (TFA) δ 1.7–3.9 (m, 13 H), 5.0–5.25 (m, 1 H), 7.45, 7.85 (AB q, 4 H, J = 9 Hz).

5,10-Dideaza-9,10-didehydrofolic acid (35) was obtained in 29% yield by methanolic sodium hydroxide hydrolysis of **32** as described above for the preparation of **3** from **33**: mp >200 °C. The insolubility of **35** in all solvents, including TFA, precluded determination of its NMR spectrum.

Registry No. **1**, 95674-53-6; **2**, 95674-54-7; **3**, 85597-18-8; **4**, 95693-76-8; **7**, 7377-04-0; **8**, 95674-55-8; **9**, 95674-56-9; **10**, 72313-37-2; 11, 95674-57-0; **12**, 95674-58-1; **13**, 95674-59-2; **14a**, 95674-60-5; **14b**, 95674-61-6; **15**, 87373-60-2; **16**, 88553-19-9; **17**, 95693-77-9; **18a**, 95674-62-7; **18b**, 95693-78-0; **19a**, 95674-63-8; **19b**, 95674-64-9; **20**, 95674-65-0; **21**, 95674-66-1; **22**, 95674-67-2; **23**, 95674-68-3; **24**, 80360-04-9; **25**·Br^-, 70583-34-5; **26**, 95674-69-4; **27**, 95693-79-1; **28**, 95674-70-7; **29**, 95674-71-8; **30**, 95674-72-9; **31**, 95674-73-0; **32**, 95674-74-1; **33**, 95674-75-2; **34**, 95674-76-3; **35**, 95693-80-4; malononitrile, 4341-85-9; 4-(ethoxycarbonyl)benzaldehyde, 6287-86-1; 4-(*tert*-butoxycarbonyl)benzaldehyde, 65874-27-3; guanidine, 113-00-8; diethyl L-glutamate hydrochloride, 1118-89-4; diethyl *N*-[4-(bromomethyl)benzoyl]glutamate, 70583-33-4; di-*tert*-butyl L-glutamate hydrochloride, 32677-01-3; dihydrofolate reductase, 9002-03-3; thymidylate synthetase, 9031-61-2; folate polyglutamate synthetase, 63363-84-8.

Synthesis and Biological Activity of 6-Substituted Mitosene Analogues of the Mitomycins[1]

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A series of 1-acetoxymitosene analogues, in which the substituent at C-6 was varied, was prepared by total synthesis and screened for activity against P388 leukemia in mice and induction of λ phage in *Escherichia coli*. Among the 6-substituents prepared, none was as effective as the methyl group in conferring biological activity. However, certain *N*-methylcarbamates were more active than the unsubstituted carbamates.

Previous studies on the relationship between structural modifications in mitomycin analogues and their antitumor activity have included variations in the 7-substituent (quinone ring), carbamate, and the aziridine ring.[2-8] One structural feature not investigated thus far is the 6-substituent in the quinone ring, which always has been a methyl group in analogues with the complexity of pyrrolo[1,2-*a*]indoles. This feature had been explored in simpler indole analogues to see its influence on antibacterial activity.[9,10] However, it has been shown that antibacterial activity does not correlate with antitumor activity in mitomycins.[11] Furthermore, none of the indole analogues showed antitumor activity,[12] although some relatively simple pyrrolo[1,2-*a*]indole analogues (e.g., **15**) are active.[8] For these reasons, it seemed desirable to conduct a study on the synthesis and antitumor activity of pyrrolo[1,2-*a*]indole analogues (mitosenes), based on the structure of **15**, but with different substituents in place of the 6-methyl group.

Chemistry. The synthesis of analogues of **15** presents a problem in that modification of the 6-methyl group has not been accomplished. Therefore, we decided to prepare first the 6-unsubstituted analogue **11** and then introduce novel substituents at C-6. Two routes for the synthesis of **11** were explored. One was based on well-established chemistry,[8,13,14] starting from ethyl 5-methoxyindole-2-

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