Effect of N^9-Methylation and Bridge Atom Variation on the Activity of 5-Substituted 2,4-Diaminopyrrolo[2,3-*d*]pyrimidines against Dihydrofolate Reductases from *Pneumocystis carinii* and *Toxoplasma gondii*[1a,b]

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Received October 11, 1996[⊗]

The effect of N^9-methylation and bridge atom variation on inhibitory potency and selectivity of 2,4-diaminopyrrolo[2,3-*d*]pyrimidines against dihydrofolate reductases (DHFR) was studied. Specifically three nonclassical 2,4-diamino-5-((*N*-methylanilino)methyl)pyrrolo[2,3-*d*]pyrimidines with 2′,5′-dimethoxyphenyl (**2**), 3′,4′-dichlorophenyl (**3**), 1′-naphthyl (**4**), one classical analogue with a 4′-L-glutamate substituent (**10**), and four nonclassical 2,4-diamino-5-((phenylthio)methyl)pyrrolo[2,3-*d*]pyrimidines with 3′,4′-dimethoxyphenyl (**5**), 3′,4′-dichlorophenyl (**6**), 1′-naphthyl (**7**), and 2′-naphthyl (**8**) substituents were synthesized. The classical and nonclassical analogues were obtained by displacement of the intermediate 2,4-diamino-5-bromomethylpyrrolo[2,3-*d*]pyrimidine, **14**, with appropriately substituted *N*-methylaniline, thiophenols, or 4-(*N*-methylamino)benzoyl-L-glutamate. Compounds **2**−**8** and **10** were evaluated against *Pneumocystis carinii* (pc), *Toxoplasma gondii* (tg), and rat liver (rl) DHFRs. The *N*-methyl and thiomethyl analogues were more inhibitory than their corresponding anilinomethyl analogues (previously reported) against all three DHFRs. The inhibitory potency of these analogues was greater against rlDHFR than against tgDHFR which resulted in a loss of selectivity for tgDHFR compared to the N^9-H analogues. The classical N^9-methyl analogue **10** was more potent and about 2-fold more selective against tgDHFR than its corresponding desmethyl analogue. All of the analogues, **2**−**8** and **10**, were more selective than trimetrexate (TMQ) against pcDHFR (except **4**) and significantly more selective than TMQ against tgDHFR.

Introduction

Defective cell-mediated immunity renders patients with Acquired Immunodeficiency Syndrome (AIDS) susceptible to opportunistic infections, which remains the principal cause of morbidity and mortality in patients in the United States.[2] The prevention of cerebral toxoplasmosis, caused by the intracellular coccidian protozoan *Toxoplasma gondii*, and of *P. carinii* pneumonia (PCP), caused by *Pneumocystis carinii*, is an essential objective in the management of patients with AIDS. The treatment of these infections by antifolates takes advantage of the fact that these organisms, unlike mammalian cells, lack a carrier-mediated active transport mechanism for the uptake of classical antifolates with polar glutamate side chains.[3] These organisms, however, are permeable to lipophilic nonclassical antifolates, which can also penetrate into the central nervous system where *T. gondii* infections occur.

The standard treatment for *P. carinii* (pc) and *T. gondii* (tg) infections, involving combinations of sulfonamides and trimethoprim (TMP) or pyrimethamine,[4] is often limited by the toxicity of the sulfonamides, used for their synergistic effect.[5] Trimetrexate (TMQ) and piritrexim (PTX), which are 100−10000 times more potent than TMP or pyrimethamine against pc- and tgDHFR are, however, nonselective and strongly inhibit DHFR from mammalian sources.[6] Thus TMQ is administered in conjunction with the reduced folate, leucovorin ((6*R*,6*S*)-5-formyl-5,6,7,8-tetrahydrofolic acid), to selectively rescue host cells.[3,7] Development of inhibitors, possessing both the high potency of TMQ or PTX and the high selectivity of TMP for pcDHFR and/or tgDHFR, is a desirable goal.

Table 1. Inhibitory Concentrations (IC_{50}, μM) against DHFRs and Selectivity Ratios[18,19]

compd	*P. carinii*	rl	selectivity ratio rl/pc	*T. gondii*	selectivity ratio rl/tg
1a[8]	45.7	156	3.4	1.7	92
1b[8]	35.3	14.4	0.4	1.4	10.3
1c[8]	307	59.3	0.2	1.1	53.9
2	>12.0	>12.0	ND	3.40	>4.0
3	28.30	3.00	0.11	1.00	3.0
4	209.0	8.20	0.04	0.87	9.43
5	11.10	16.7	1.50	2.60	6.42
6	58.50	5.30	0.09	11.6	0.46
7	10.60	3.00	0.28	0.81	3.70
8	929.0	82.9	0.09	9.20	9.01
9[8]	0.038	0.044	1.2	0.21	0.21
10	0.044	0.06	1.36	0.15	0.40
TMQ	0.042	0.003	0.07	0.01	0.30
PTX	0.038	0.001	0.04	0.01	0.10
TMP	12.00	133.0	11.1	2.7	49.0
MTX	0.001	0.003	3.0	0.014	0.21

In our efforts directed toward the design and synthesis of lipophilic nonclassical antifolates with selectivity and high potency against pcDHFR and tgDHFR, we recently reported[8] some nonclassical 6−5 fused, 5-substituted 2,4-diaminopyrrolo[2,3-*d*]pyrimidines of general structure **1**, with various substituted lipophilic side chains. These analogues possessed excellent selectivity against tgDHFR (**1a**−**c**, Table 1). However, they were only moderately potent. The present work attempts to

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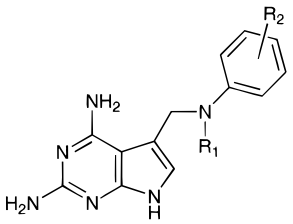
⊗ Abstract published in *Advance ACS Abstracts,* March 15, 1997.

S0022-2623(96)00717-0 CCC: $14.00

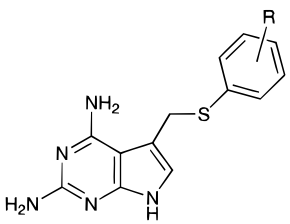
study the effect of N^9-methylation and bridge length variation on both potency and selectivity of these analogues.

Side chain N-methylation of most 6−6 fused nonclassical pyrido[2,3-*d*]pyrimidines and, more significantly, 6−5 fused classical furo[2,3-*d*]pyrimidines substantially increase the DHFR inhibitory potencies[9,10] compared to their N-H analogues. Thus N^9-methylation of the 6−5 fused pyrrolo[2,3-*d*]pyrimidine analogues **1a**−**c**, the most potent and/or selective analogues in the previous series,[8] were similarly anticipated to increase the DHFR inhibitory potency and prompted the synthesis of compounds **2**−**4**.

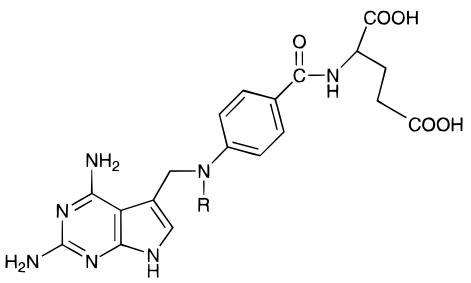


R_1 = H; R_2 =
1a : 2', 5' - $(OCH_3)_2$
1b : 3', 4' - $(Cl)_2$
1c : 2', 3'-$(CH)_4$

R_1 = CH_3; R_2 =
2 : 2', 5' - $(OCH_3)_2$
3: 3', 4' - $(Cl)_2$
4: 2', 3'-$(CH)_4$



R =
5 : 3', 4' - $(OCH_3)_2$
6: 3', 4' - $(Cl)_2$
7: 2', 3'-$(CH)_4$
8: 3', 4'-$(CH)_4$



R =
9 : H
10 : CH_3

In the classical 6−5 fused pyrrolo[2,3-*d*]pyrimidines, reported by Miwa *et al.*,[11,12] the analogue possessing a three-atom bridge, between the heterocycle and the benzoyl L-glutamate, was significantly more cytotoxic than the analogue with a two-atom bridge. Thus, compounds **5**−**8** were designed wherein the bridge nitrogen was replaced by a sulfur which, because of its larger size, was anticipated to increase the bridge length, between the heterocyclic ring and the aromatic side chain, of the nonclassical pyrrolo[2,3-*d*]pyrimidines to about a three-atom distance and consequently increase the potency.

Compound **10** was also synthesized and evaluated in order to study the effect of N^9-methylation on the potency of the classical pyrrolo[2,3-*d*]pyrimidine analogue **9**, which we had reported[8] as being 1000-, 5-, and 325-fold more potent than the corresponding nonclassical analogues against pcDHFR, tgDHFR, and rlDHFR, respectively.

Chemistry

The synthesis of the pyrrolo[2,3-*d*]pyrimidine analogues **2**−**4** was initially attempted *via* the reductive methylation of the precursor NH analogues **1a**−**c** using formaldehyde and sodium cyanoborohydride, employing known procedures.[9,13] No N^9-methylated product was obtained under these reaction conditions, which yielded a complex mixture, probably the result of multiple methylations and/or degradation of the starting material. This led to the exploration of an alternate method.

A versatile intermediate, from which both the N^9-methyl (**2**−**4** and **10**) as well as the thio-substituted analogues (**5**−**8**) could be synthesized, was sought. Transformation of the aldehyde **12** (Scheme 1), which we had previously reported, [8] *via* the alcohol to the bromide would provide such an intermediate from which both series of target compounds could be readily obtained by nucleophilic displacement using the appropriate nitrogen or sulfur nucleophiles.

Thus the aldehyde **12**, obtained from the nitrile **11**, was reduced with $NaBH_4$, to afford the alcohol **13**. Compound **13** was converted to the bromide **14**, with 30% HBr−AcOH, which had R_f 0.62 on TLC which was, as expected, higher than that of the alcohol **13** (R_f 0.43). The hygroscopic HBr salt of **14** was isolated by precipitation with Et_2O and, owing to its instability, was used in subsequent steps without further purification. Nucleophilic displacement of the bromide **14**, at room temperature, with excess *N*-methyl-2,5-dimethoxyaniline,[14] *N*-methyl-3,4-dichloroaniline,[15] and *N*-methylnaphthylamine[16] (**16a**−**c**, respectively), in anhydrous DMF or DMAC, containing K_2CO_3, afforded the desired analogues **2**−**4** in 31−36% yield. The classical analogue **10** was obtained by nucleophilic displacement of the bromide **14** with diethyl (*N*-methylamino)benzoyl-L-glutamate[17] (**16d**) followed by saponification of the resulting diester with 1 N NaOH, at room temperature, and acidification to pH 5 which afforded **10** in 50% yield.

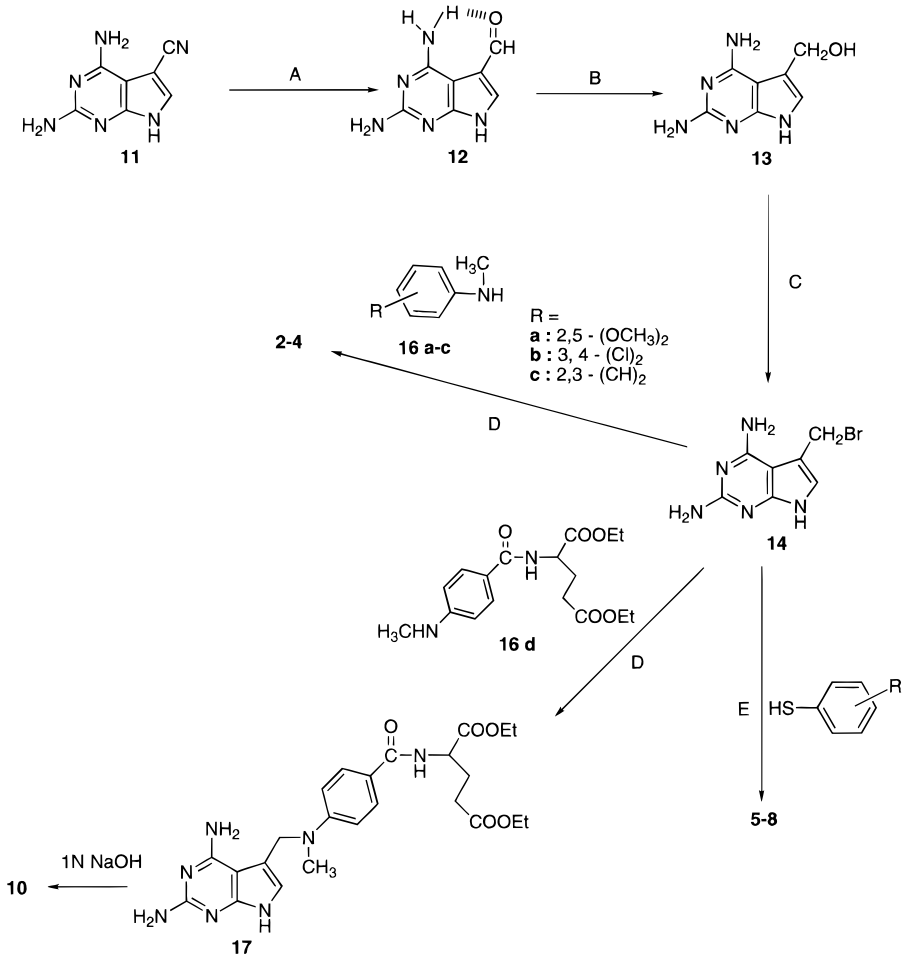
Analogues **5**−**8** were, similarly, obtained *via* nucleophilic displacement of the bromide **14** with 3,4-dimethoxythiophenol, 3,4-dichlorothiophenol, 1-naphthalenethiol, and 2-naphthalenethiol, respectively. The thiols, in each case, were first activated by stirring with sodium hydride in DMF/DMAC, at room temperature, followed by addition of the bromide **14**. The displacement of the bromide, at room temperature, provided products **5**−**8** in 26−38% yield.

Thus a versatile intermediate bromide **14** was obtained from which the target analogues **2**−**8** and **10** were easily accessible. This intermediate **14** could also serve as the precursor for nonclassical carbon-bridged analogues (*via* Wittig reactions) which will be the topic of future reports.

Biological Evaluation and Discussion

Compounds **2**−**8** and **10** were evaluated as inhibitors of *P. carinii*, *T. gondii*, and rat liver DHFR as described previously.[18,19] The inhibitory concentrations (IC_{50}) and the selectivity ratios *vs* rlDHFR are listed in Table 1. Compound **2** was insoluble at concentrations greater than 12 μM, and hence IC_{50} values could not be determined against pcDHFR and rlDHFR. Against tgDHFR N^9-methylation decreased potency compared to the N^9-H analogue **1a**. N^9-Methylation of **1b** and **1c** (compounds **3** and **4**) increased potency against all three

Scheme 1[a]



[a] (A) Raney Ni/HCOOH; (B) $NaBH_4$; (C) 30% HBr−AcOH; (D) DMF or DMAC/K_2CO_3; (E) NaH/DMF or DMAc.

DHFRs but drastically decreased selectivity against pcDHFR and tgDHFR. The decrease in selectivity of **3** and **4** compared with **1b** and **1c** is clearly due to the greater increase in potency against rlDHFR than against pcDHFR and tgDHFR. Thus N^9-methylation did in general increase inhibitory effects against pcDHFR and tgDHFR but was detrimental to selectivity compared to the N^9-H analogues.

Replacement of the nitrogen in the side chain with a sulfur, as in analogues **5**−**8**, affected inhibitor potency against all three DHFRs. Compared with **1a** and **1c**, analogues **5** and **7**, with the 3′,4′-dimethoxyphenyl and a 1-naphthyl substituent, respectively, were 4- and 29-fold more inhibitory against pcDHFR and 9- and 20-fold more inhibitory against rlDHFR, respectively. The 3′,4′-dichlorophenyl analogue, **6**, was about 3-fold more inhibitory against rlDHFR and less active against pcDHFR and tgDHFR than its corresponding nitrogen-containing analogue, **1b**. Replacing the 1-naphthyl of **7** with a 2-naphthyl moiety, as in analogue **8**, resulted in a decrease in inhibitory potency against all three DHFRs. Compound **7** was 28-, 88-, and 11-fold more potent, than **8**, against rlDHFR, pcDHFR, and tgDHFR, respectively. The differential decrease in inhibitory potency against the three DHFRs for **8** further indicates the differences in the three DHFRs. Compound **8** was, however, 3 times more selective for tgDHFR than the corresponding 1-naphthyl analogue **7**. In general, increasing the bridge length by replacement of the N^9 with S increased potency against rlDHFR more so than for pc- or tgDHFR and was detrimental to selectivity compared to the N^9-H analogues.

The classical analogue **10** was a more potent inhibitor of all three DHFRs tested compared to the nonclassical analogues **2**−**8**. It was similar to the N-desmethyl analogue, **9**, against all three DHFRs and was about 2-fold more selective for tgDHFR than **9**.

Both the CH_2NCH_3 and the CH_2S bridge analogues, **2**−**8** and **10**, were more selective than TMQ and PTX against tgDHFR. The 1′-(*N*-(methylnaphthyl)amino)-methyl analogue **4** and the 1′-(naphthylthio)methyl analogue **7** were about 3-fold more potent than TMP against tgDHFR and about 67- and 26-fold more selective than TMQ and PTX, respectively, against tgDHFR. The ((3′,4′-dimethoxyphenyl)thio)methyl analogue **5** possessed the highest selectivity for pcDHFR (rl/pc: 1.5) which was about 21- and 38-fold greater than that of TMQ and PTX, respectively.

Experimental Section

All evaporations were carried out *in vacuo* with a rotary evaporator. Analytical samples were dried *in vacuo* (0.2 mmHg) in an Abderhalden drying apparatus over P_2O_5 and refluxing ethanol or toluene. Melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. Nuclear magnetic resonance spectra for proton (^{1}H NMR) were recorded on a Bruker WH-300 (300 MHz) spectrometer. Data was accumulated by 16K size with a 0.5 s delay time and 70° tip angle. The chemical shift values are expressed in ppm (parts per million) relative to tetramethylsilane as internal standard; s = singlet, d = doublet, dd =

doublet of doublet, t = triplet, q = quartet, m = multiplet, bs = broad singlet. The relative integrals of peak areas agreed with those expected for the assigned structures. Thin layer chromatography (TLC) was performed on POLYGRAM Sil G/UV_{254} silica gel plates with fluorescent indicator, and the spots were visualized under 254 and 366 nm illumination. Proportions of solvents used for TLC are by volume. Eluents used in column chromatography contained 0.1% NH_4OH by volume. Elemental analyses were performed by Atlantic Microlabs Inc., Norcoss, GA. Analytical results indicated by element symbols are within ±0.4% of the calculated values. Fractional moles of water or organic solvents frequently found in some analytical samples of antifolates were not removed in spite of 24−48 h of drying *in vacuo* and were confirmed where possible by their presence in the ^{1}H NMR spectrum. All solvents and chemicals were purchased from Aldrich Chemical Co. and Fisher Scientific and were used as received.

2,4-Diaminopyrrolo[2,3-*d*]pyrimidine-5-carboxaldehyde (12). Compound **12** was synthesized from the corresponding nitrile using HCOOH and Raney nickel:[8] mp >300 °C; TLC R_f 0.49 ($CHCl_3$/MeOH/NH_4OH, 9:3:0.1, silica gel); IR (Nujol) 1600−1650 cm^{-1}; ^{1}H NMR (Me_2SO-d_6) δ 5.84 (s, 2 H, 2-NH_2), 6.90 (bs, 1 H, NH_2), 7.35 (bs, 1 H, NH_2), 7.84 (s, 1 H, 6-CH), 9.51 (s, 1 H, CHO), 11.87 (bs, 1 H, 7-NH).

2,4-Diaminopyrrolo[2,3-*d*]pyrimidine-5-methanol (13). To a solution of **12** (0.80 g, 4.5 mmol) in MeOH (25 mL) was carefully added $NaBH_4$ (1.00 g). The mixture was stirred at room temperature for 2 h. The MeOH was evaporated under reduced pressure, and cold water (5 mL) was carefully added to the residue which was then neutralized with glacial AcOH. The suspension was refrigerated overnight and filtered to yield 0.46 g (56%) of the alcohol **13**: mp >175 °C dec; TLC R_f 0.43 ($CHCl_3$/MeOH/NH_4OH, 10:3:0.2, silica gel); ^{1}H NMR (Me_2SO-d_6) δ 4.48 (s, 2 H, CH_2), 5.43 (m, 3 H, C-OH, NH_2), 6.38 (s, 2 H, NH_2), 6.56 (s, 1 H, 6-CH), 10.47 (s, 1 H, 7-NH).

2,4-Diamino-5-(bromomethyl)pyrrolo[2,3-*d*]pyrimidine (14). The alcohol **13** (1.00 g, 5.60 mmol) was stirred in 30% HBr−AcOH (10 mL) for 12 h at room temperature. The reaction mixture was evaporated under reduced pressure. Et_2O (15 mL) was added to the residue, which was filtered and washed with additional Et_2O. The bromide **14** was used immediately in subsequent reactions: TLC R_f 0.62 ($CHCl_3$/MeOH/NH_4OH, 10:3:0.2, silica gel).

General Procedure for the Preparation of 2,4-Diamino-5-[(substituted *N*-methylanilino)methyl]pyrrolo-[2,3-*d*]pyrimidines (2−4, 17). To a solution of the bromide **14** (0.10 g, 0.40 mmol) in anhydrous DMF or DMAC (5 mL) containing K_2CO_3 (0.10 g) was added *N*-methyl-substituted aniline **16** (1.20 mmol). The mixture was stirred at room temperature until **14** disappeared on TLC (5 h). The solvent was then evaporated under reduced pressure. The residue was dissolved in MeOH, and silica gel (2.00 g) was added to the solution which was then evaporated to dryness to form a plug which was loaded onto a dry silica gel column (2.4 × 20 cm). The column was eluted with a gradient of 1% MeOH in $CHCl_3$ to 10% MeOH in $CHCl_3$. Fractions corresponding to the product (TLC) were pooled and evaporated to dryness under reduced pressure. The residue was triturated in cold Et_2O, and hexane(s) was added dropwise until a suspension formed which was refrigerated for 2 h and filtered to yield the products.

2,4-Diamino-5-[(2′,5′-dimethoxy-*N*-methylanilino)-methyl]pyrrolo[2,3-*d*]pyrimidine (2): yield 0.04 g (30.7%); mp 144−145 °C; TLC R_f 0.65 ($CHCl_3$/MeOH/NH_4OH, 10:3:0.1, silica gel); ^{1}H NMR (Me_2SO-d_6) δ 2.73 (s, 3 H, 9-CH_3), 3.69 (s, 3 H, OCH_3), 3.77 (s, 3 H, OCH_3), 3.97 (s, 2 H, 8-CH_2), 5.55 (bs, 2 H, NH_2), 6.00 (bs, 2 H, NH_2), 6.18 (s, 1 H, 6-CH), 6.61 (m, 1 H, 4′-CH), 6.70 (d, 1 H, 6′-CH), 6.91 (d, 1 H, 3′-CH), 10.59 (s, 1 H, 7-NH). Anal. Calcd for ($C_{16}H_{20}N_6$·0.5H_2O) C, H, N.

2,4-Diamino-5-[(3′,4′-dichloro-*N*-methylanilino)methyl]-pyrrolo[2,3-*d*]pyrimidine (3). Fractions obtained from the column were pooled and acidified (pH 4−5) with 1 N HCl before evaporation and trituration with cold Et_2O: yield 0.05 g (36%); mp >240 °C dec; TLC R_f 0.53 ($CHCl_3$/MeOH/NH_4OH, 10:3:0.1, silica gel); ^{1}H NMR (Me_2SO-d_6) δ 2.87 (s, 3 H, 9-CH_3), 4.51 (s, 2 H, 8-CH_2), 5.49 (bs, 2 H, NH_2), 6.04 (bs, 2 H, NH_2), 6.42 (s, 1 H, 6-CH), 6.87 (m, 1 H, 6′-CH), 7.03 (d, 1 H, 2′-CH), 7.37 (d, 1 H, 5′-CH), 10.56 (s, 1 H, 7-NH). Anal. Calcd for ($C_{14}H_{14}N_6Cl_2$·0.75H_2O·0.41HCl) C, H, N, Cl.

2,4-Diamino-5-[(*N*-methylnaphthylamino)methyl]pyrrolo[2,3-*d*]pyrimidine (4): yield 0.04 g (31%); mp 172−174 °C dec; TLC R_f 0.65 ($CHCl_3$/MeOH/NH_4OH, 10:3:0.1, silica gel); ^{1}H NMR (Me_2SO-d_6) δ 2.75 (s, 3 H, 9-CH_3), 4.25 (s, 2 H, 8-CH_2), 5.49 (bs, 2 H, NH_2), 6.66 (bs, 2 H, NH_2), 6.78 (s, 1 H, 6-CH), 7.33 (m, 1 H, $C_{10}H_7$), 7.43 (m, 1 H, $C_{10}H_7$), 7.47 (m, 2 H, $C_{10}H_7$), 7.62 (m, 1 H, $C_{10}H_7$), 7.90 (d, 1 H, $C_{10}H_7$), 8.16 (d, 1 H, $C_{10}H_7$), 10.58 (s, 1 H, 7-NH). Anal. Calcd for ($C_{18}H_{18}N_6$·0.4H_2O) C, H, N.

***N*-[4-[*N*-[(2,4-Diaminopyrrolo[2,3-*d*]pyrimidin-5-yl)-methyl]methylamino]benzoyl]-L-glutamic acid diethyl ester (17):** yield 0.08 g (38%); mp 143 °C; ^{1}H NMR (Me_2SO-d_6) δ 1.17 (t, 6 H, CH_2CH_3), 2.07 (m, 2 H, Glu β-CH_2), 2.42 (t, 2 H, Glu γ-CH_2), 2.94 (s, 3 H, 9-CH_3), 4.06 (q, 4 H, CH_2CH_3), 4.41 (m, 1 H, Glu α-CH), 4.61 (s, 2 H, 8-CH_2), 6.20 (bs, 4 H, NH_2), 6.47 (s, 1 H, 6-CH), 6.89 (d, 2 H, 3′-, 5′-CH), 7.76 (d, 2 H, 2′-,6′-CH), 8.37 (d, 1 H, CONH), 10.95 (bs, 1 H, 7-NH).

General Procedure for Preparation of 2,4-Diamino-5-[(substituted phenylthio)methyl]pyrrolo[2,3-*d*]-pyrimidine (5−8). To a solution of substituted thiophenol (1.70 mmol) in DMF or DMAC (15 mL) was added NaH (0.04 g, 1.70 mmol). The mixture was stirred at room temperature for 1 h. To this was added the bromide **14** (0.10 g, 0.40 mmol), and the reaction mixture was stirred for 4 h until no more bromide was detected on TLC. The solvent was evaporated under reduced pressure. The residue was dissolved in MeOH (5 mL), and silica gel (1.00 g) was added to the solution, which was evaporated to dryness to form a plug which was loaded onto a dry silica gel column (2.4 cm × 20 cm). The column was eluted with a gradient of 1% MeOH in $CHCl_3$ to 10% MeOH in $CHCl_3$. Fractions corresponding to the product (TLC) were pooled and evaporated to dryness under reduced pressure. The residue was triturated in cold Et_2O, and hexane(s) was added dropwise until a suspension formed which was refrigerated for 2 h and filtered to yield products.

2,4-Diamino-5-[[(3′,4′-dimethoxyphenyl)thio]methyl]-pyrrolo[2,3-*d*]pyrimidine (5). Fractions obtained from the column were pooled and acidified (pH 4−5) with glacial AcOH before evaporation and trituration with cold Et_2O: yield 0.05 g (38%); mp 230−231 °C; TLC R_f 0.76 ($CHCl_3$/MeOH/NH_4OH, 10:3:0.1, silica gel); ^{1}H NMR (Me_2SO-d_6) δ 3.67 (s, 3 H, OCH_3), 3.70 (s, 3 H, OCH_3), 4.21 (s, 2 H, 8-CH_2), 5.43 (s, 2 H, NH_2), 6.12 (s, 2 H, NH_2), 6.45 (s, 1 H, 6-CH), 6.85 (m, 3 H, 2′,5′,6′-CH), 10.48 (s, 1 H, 7-NH). Anal. Calcd for ($C_{15}H_{17}N_5O_2S$·0.2CH_3COOH) C, H, N, S.

2,4-Diamino-5-[[(3′,4′-dichlorophenyl)thio]methyl]pyrrolo[2,3-*d*]pyrimidine (6): yield 0.045 g (32%); mp 228−229 °C dec; TLC R_f 0.66 ($CHCl_3$/MeOH/NH_4OH, 10:3:0.1, silica gel); ^{1}H NMR (Me_2SO-d_6) δ 4.41 (s, 2 H, 8-CH_2), 5.54 (bs, 2 H, NH_2), 6.25 (bs, 2 H, NH_2), 6.62 (s, 1 H, 6-CH), 7.28 (m, 1 H, 6′-CH), 7.52 (d, 1 H, 2′-CH), 7.58 (d, 1 H, 5′-CH), 10.62 (s, 1 H, 7-NH). Anal. Calcd for ($C_{13}H_{11}N_5SCl_2$) C, H, N, S, Cl.

2,4-Diamino-5-[(1′-naphthylthio)methyl]pyrrolo[2,3-*d*]-pyrimidine (7): yield 0.046 g (35%); mp >250 °C dec; TLC R_f 0.66 ($CHCl_3$/MeOH/NH_4OH, 10:3:0.1, silica gel); ^{1}H NMR (Me_2SO-d_6) δ 4.42 (s, 2 H, 8-CH_2), 5.47 (bs, 2 H, NH_2), 6.17 (s, 2 H, NH_2), 6.53 (s, 1 H, 6-CH), 7.46 (m, 1 H, $C_{10}H_7$), 7.56 (m, 1 H, $C_{10}H_7$), 7.59 (m, 2 H, $C_{10}H_7$), 7.77 (m, 1 H, $C_{10}H_7$), 7.91 (d, 1 H, $C_{10}H_7$), 8.18 (d, 1 H, $C_{10}H_7$), 10.53 (s, 1 H, 7-NH). Anal. Calcd for ($C_{17}H_{15}N_5S$·0.5H_2O) C, H, N, S.

2,4-Diamino-5-[(2′-naphthylthio)methyl]pyrrolo[2,3-*d*]-pyrimidine (8): yield 0.035 g (26%); mp >250 °C dec; TLC R_f 0.65 ($CHCl_3$/MeOH/NH_4OH, 10:3:0.1, silica gel); ^{1}H NMR (Me_2SO-d_6) δ 4.50 (s, 2 H, 8-CH_2), 5.51 (bs, 2 H, NH_2), 6.21 (bs, 2 H, NH_2), 6.69 (s, 1 H, 6-CH), 7.48 (m, 3 H, $C_{10}H_7$), 7.88 (m, 4 H, $C_{10}H_7$), 10.65 (s, 1 H, 7-NH). Anal. Calcd for ($C_{17}H_{15}N_5S$·0.5H_2O) C, H, N, S.

***N*-[4-[*N*-[(2,4-Diaminopyrrolo[2,3-*d*]pyrimidin-5-yl)-methyl]methylamino]benzoyl]-L-glutamic Acid (10).** The pure ester **17** was stirred in 1 N NaOH (5 mL) for 24 h at room temperature. Acidification with glacial AcOH to pH 5 (the pH briefly dropped below 5 and was readjusted with NH_4OH solution) afforded a light brown suspension which on

filtration and washing with water followed by Et_2O afforded 0.035 g (50%) of an analytically pure light brown solid, **10**: mp 223−224 °C dec; TLC R_f 0.78 (3% NH_4HCO_3, cellulose); ^{1}H NMR (Me_2SO-d_6) δ 1.93 (m, 1 H, Glu β-CH_2), 2.08 (m, 1 H, Glu β-CH_2), 2.32 (m, 2 H, Glu γ-CH_2), 2.91 (s, 3 H, 9-CH_3), 4.33 (m, 1 H, Glu α-CH), 4.57 (s, 2 H, 8-CH_2), 5.45 (bs, 2 H, NH_2), 5.98 (bs, 2 H, NH_2), 6.41 (s, 1 H, 6-CH), 6.90 (d, 2 H, 3′-, 5′-CH), 7.74 (d, 2 H, 2′-,6′-CH), 8.07 (d, 1 H, CONH), 10.53 (s, 1 H, 7-NH). Anal. Calcd for ($C_{20}H_{23}N_7O_5 \cdot 0.6H_2O \cdot 0.6NH_3$) C, H, N.

Acknowledgment. This work was supported, in part, by NIH Grants GM40998 (A.G.) and AI30900 (A.G.) and NIH Contract N01-AI-87240 (S.F.Q.) Division of AIDS.

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JM960717Q