Nonclassical 2,4-Diamino-8-deazafolate Analogues as Inhibitors of Dihydrofolate Reductases from Rat Liver, *Pneumocystis carinii,* and *Toxoplasma gondii*[1]

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The synthesis and biological activity of 42 6-substituted-2,4-diaminopyrido[3,2-*d*]pyrimidines (2,4-diamino-8-deazafolate analogues) are reported. The compounds were synthesized in improved yields compared to previous classical analogues using modifications of procedures reported previously by us. Specifically, the *S*-phenyl-; mono-, di-, and trimethoxyphenyl-; and mono-, di-, and trichlorophenyl-substituted analogues with H or CH_3 at the N10 position and methyl and trifluoromethyl phenyl ketone analogues with H, CH_3, and $CH_2C{\equiv}CH$ at the N10 position were synthesized. The S10 and N10 α- and β-naphthyl analogues along with the N10 CH_3 analogues were also synthesized. These compounds were evaluated as inhibitors of dihydrofolate reductases (DHFR) from *Pneumocystis carinii* (pc) and *Toxoplasma gondii* (tg); selectivity ratios were determined against rat liver (rl) DHFR as the mammalian reference enzyme. Against pcDHFR the IC_{50} values ranged from 0.038×10^{-6} M for 2,4-diamino-6-[(*N*-methyl-2′-naphthylamino)methyl]pyrido[3,2-*d*]pyrimidine (**28**) to 5.5×10^{-6} M for 2,4-diamino-6-[(2′,4′-dimethoxyanilino)methyl]pyrido[3,2-*d*]pyrimidine (**15**). N10 methylation in all instances increased potency. None of the analogues were selective for pcDHFR. Against tgDHFR the most potent analogue was 2,4-diamino-6-[(*N*-methylanilino)methyl]pyrido[3,2-*d*]pyrimidine (**5**) (IC_{50} 0.0084×10^{-6} M) and the least potent was 2,4-diamino-6-[(2′-naphthylamino)methyl]-pyrido[3,2-*d*]pyrimidine (**37**) (IC_{50} 0.16×10^{-6} M). N10 methylation afforded an increase in potency up to 10-fold. In contrast to pcDHFR, several of the 8-deaza analogues were significantly selective for tgDHFR, most notably 2,4-diamino-6-[(2′-chloro-*N*-methylanilino)-methyl]pyrido[3,2-*d*]pyrimidine (**13**), 2,4-diamino-6-[(3′,4′,5′-trimethoxyanilino)methyl]pyrido-[3,2-*d*]pyrimidine (**29**), and 2,4-diamino-6-[(2′,4′,6′-trichloroanilino)methyl]pyrido[3,2-*d*]pyrimidine (**32**) which combined high potency at 10^{-8} M along with selectivities of 8.0, 5.0, and 12.4, respectively. The potency of these three analogues are comparable to the clinically used agent trimetrexate while their selectivities for tgDHFR are 17−43-fold better than trimetrexate.

Opportunistic infections in patients with acquired immunodeficiency syndrome (AIDS) remain the principal cause of morbidity and mortality. Of these infections, those by *Pneumocystis carinii* and *Toxoplasma gondii* are the most severe and the most prevalent.[2,3] Though primary and secondary prophylaxis for *P. carinii* with the antifolates trimethoprim−sulfamethoxazole or pentamidine have been successful and the period of survival for AIDS patients is prolonged by this therapy, the persistence of immunodeficiency results in multiple episodes of *P. carinii* infection resulting in pneumonias, some of which are atypical.[4−6] Thus, current regimens are successful in 50−75% of cases with considerable loss of effectiveness in patients with two or more episodes. *T. gondii* infections are currently treated with a combination of the antifolate pyrimethamine and sulfadiazine. In both *P. carinii* and *T. gondii* treatments, adverse reactions are common and the relapse rates are high, and in up to 50% of these cases the effects are severe enough to warrant discontinuation of therapy.[7,8] Thus, the search for alternate therapeutic agents and combinations have resulted in a number of newer agents and combinations each with its attending drawbacks and adverse effects.[9] Resistant strains of these organisms to current therapy are certain to develop as has been observed in other similar infections. In addition, patients who cannot tolerate or do not respond to current agents urgently require alternate therapies.

Allegra *et al.*[10,11] reported trimethoprim (TMP) and pyrimethamine, which are currently used agents, as comparatively weak inhibitors of *P. carinii* (pc) dihydrofolate reductase (DHFR) and *T. gondii* (tg) DHFR, which must be used with sulfonamides to provide synergistic effects. However trimetrexate (TMQ) was 100−10000 times more potent than TMP or pyrimethamine against pcDHFR and tgDHFR. The pyrido[2,3-*d*]pyrimidine piritrexim (PTX) was also found to have similar potency as TMQ.[12,13a] Both TMQ and PTX are more lipid soluble than classical antifolates such as methotrexate (MTX). They do not require the transport mechanism that MTX and other classical antifolates require and are thus able to penetrate these organisms by passive diffusion. This lipid solubility also allows penetration into the CNS where *T. gondii* infections can occur. In clinical trials,[14] TMQ along with host rescue with leucovorin, which provides adequate reduced folate in host cells but not in *P. carinii* or *T. gondii* because it is not taken up efficiently by these organisms, has proved to be a viable alternate therapy, and has been approved by the FDA. However, relapse within 3

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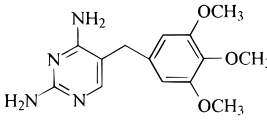
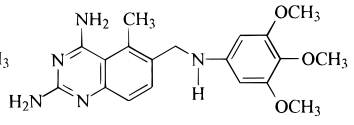
months has been noted in 60% of patients (33% in another study[15]) who received TMQ alone as initial therapy, thus lending further urgency to the development of other DHFR inhibitors which are potent and selective.

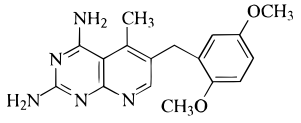
Though TMQ and PTX are significantly inhibitory against pcDHFR and tgDHFR, they lack selectivity against these DHFRs. A pronounced effort has been made to improve the selectivity of TMQ and PTX. Gangjee *et al.*,[16a−d] Rosowsky *et al.*,[17a−c] Piper *et al.*,[18] and Queener *et al.*[13a,b,19] have reported increased selectivity of TMQ- and PTX-based analogues. However, the compounds obtained so far are either not selective enough or lack efficacy due to the lack of transport into *P. carinii* and/or *T. gondii* cells in culture.[20]



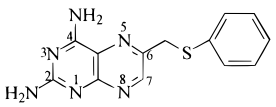
TMP



TMQ



PTX



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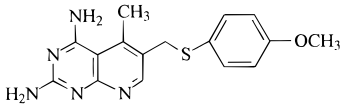

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Compound **1**, a nonclassical pteridine analogue, has been reported by Broughton and Queener,[19] Chio and Queener,[21] and Piper *et al.*[18] to be highly selective against both pcDHFR and tgDHFR. The high selectivity against pcDHFR of 25.9 (vs rat liver DHFR) and against tgDHFR of 319 (vs rat liver DHFR) has made compound **1** one of the most selective analogues against both pcDHFR and tgDHFR. The potency (IC_{50}) of this analogue was poor (9.5×10^{-6} M) against pcDHFR and marginal (0.77×10^{-6} M) against tgDHFR. Compound **1** also lacked potency in *in vitro* cell culture studies[18] which was attributed to its poor DHFR inhibitory effects.[18] Piper *et al.*[18] also reported the 5-CH_3-5-deaza analogue **2** (a pyrido[2,3-*d*]pyrimidine) of compound **1**, which contained a 4-MeOPh moiety rather than a phenyl. This analogue was 17-fold and 12-fold more potent than **1** against pcDHFR and tgDHFR, respectively; however, the analogue lacked the significant selectivity exhibited by compound **1**. Thus a comparison of **1** and **2** indicates that the 5-deaza analogue is highly conducive to potency but is detrimental to selectivity.

The activities and selectivities of compound **1** and its 5-CH_3-5-deaza-4-MeOPh analogue **2** suggested that the N5 of **1** was essential for high selectivity. It was therefore of considerable interest to synthesize the 8-deaza pteridine analogues of **1** as potential potent and selective nonclassical inhibitors of pcDHFR and tgDHFR. In addition, some of the 5-deaza classical antifolates are known to penetrate cells much better than the pteridine analogues, an observation which was confirmed for *T. gondii* cells in culture in the nonclassical 5-CH_3-5-deaza antifolates by Gangjee *et al.*,[22] Piper *et al.*,[18] and Chio and Queener.[21] Thus it was anticipated that in addition to high potency and selectivity, the 8-deaza analogues should retain good cell penetration similar to the 5-deaza analogues. This report consists of the synthesis of a comprehensive series of 42 2,4-diamino-6-substituted-pyrido[3,2-*d*]pyrimidines **3**−**44** and their inhibitory activities against DHFRs from *P. carinii* and *T. gondii* and selectivity ratios against rat liver (rl) DHFR as the reference mammalian enzyme.

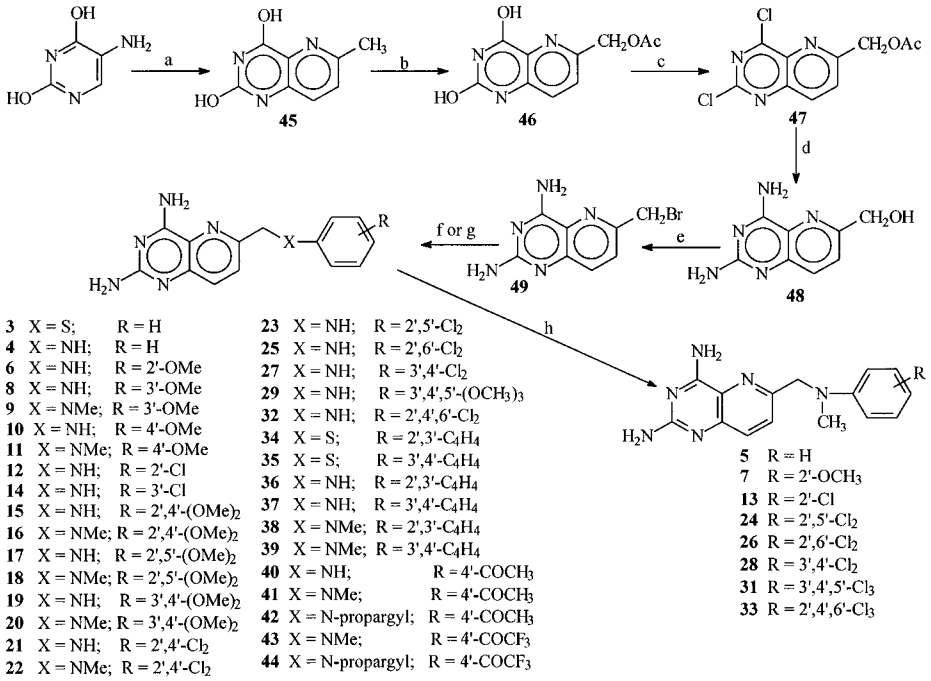
Chemistry

8-Deaza folate analogues have been reported previously by DeGraw *et al.*[23] and Broom *et al.*[24a−c] The target compounds were synthesized adopting Broom's synthetic methodology[24a] as indicated in Scheme 1. The synthesis of the crucial hydroxymethyl intermediate **48**, which has previously been reported by different groups,[24−26] was accomplished from 2,4-dioxo-6-methylpyrido[3,2-*d*]pyrimidine (**45**) which, in turn, was obtained *via* the condensation of 5-aminouracil and crotonaldehyde in HCl under reflux.[27] The yield of **45** as reported by Irwin and Wibberley[27] was only 30%. The low yield was possibly due to its high solubility in water during the workup procedure. Water was removed by evaporation after the reaction was completed (indicated by TLC). A minimum amount of water was then added and the mixture stirred and triturated with ammonium hydroxide. The solid product obtained was filtered and dried. This modified workup procedure significantly increased the yield from 30%[27] to 90%. Treatment of **45** with *m*-chloroperbenzoic acid afforded the intermediate N5-oxide, which underwent the Polonovski rearrangement in Ac_2O−AcOH to afford compound **46**, which in turn was converted to the 2,4-dichloro derivative **47** with $POCl_3$ under reflux. The key intermediate 2,4-diamino-6-hydroxymethyl derivative **48** was obtained by heating **47** at 150−160 °C in liquid ammonia in a closed vessel.

A second method for the synthesis of compound **48** was also carried out without modification of a previously published procedure.[28] The acetophenone precursors 4′-(propargylamino)acetophenone and 4′-(propargylamino)-2,2,2-trifluoroacetophenone required for the synthesis of **42** and **44** were synthesized by previously published methods.[28] Synthesis of 4′-(methylamino)-2,2,2-trifluoroacetophenone required for the synthesis of **43** involved the displacement of aromatic fluorine from the commercially available 2,2,2,4′-tetrafluoroacetophenone and differed from the published method of McNamara *et al.*[28] only in the use of methyl- rather than propargylamine. This approach increased yields of the desired compound 5-fold over the two-step procedure reported by Stewart and Teo.[29,30]

Compound **48** was converted to the bromomethyl analogue **49** by bromination with PBr_3 under anhydrous conditions. Compound **49** was extremely sensitive to moisture due to its arylmethyl bromide moiety. During the workup procedure, the removal of the solid from excess PBr_3 by filtration followed by rapid washing of the product **49** with cold ether was carried out under nitrogen in order to minimize the decomposition of **49** to **48**. Displacement of the bromide of **49** with appropriate nucleophiles (thiophenol, naphthylamines and substituted anilines) in anhydrous DMAc afforded the desired target compounds in yields of 19−57% over the last two steps.

Scheme 1. Synthetic Route of 6-Substituted-2,4-Diaminopyrido[3,2-*d*]pyrimidines[a]



[a] (a) 20% HCl, crotonaldehyde; (b) (i) MCPBA; (ii) acetic anhydride; (c) $POCl_3$, NEt_3; (d) NH_3, 150−160 °C; (e) PBr_3, anhydrous THF; (f) HX−Ar, DMAC, 50−60 °C; (g) HX−Ar, $CaCO_3$, DMAC, 100 °C; (h) $NaCNBH_3$, HCHO, pH 1−3.

The nucleophilic displacement of the bromide **49** was performed in an anhydrous solvent under nitrogen which allowed for an increase in the yield. In order to optimize the yield of the displacement reaction to the final compounds, inorganic bases were generally avoided (except in the synthesis of compounds **40**−**44**) to prevent water formation which resulted from the reaction of the liberated HBr and the inorganic base. The water thus formed reacts with the bromide **49** to afford the alcohol **48** which decreased the yield of the final products.

After the displacement of the bromide **49** with appropriate nucleophiles was complete, the reaction mixture was pretreated to remove polar water soluble impurities and excess nucleophile prior to purification by column chromatography. The pretreatment procedure involved the removal of the solvent DMAc under reduced pressure, followed by addition of water and chloroform. Acidification of the heterogeneous mixture, separation of the organic layer, basification of the water phase with ammonium hydroxide to pH 11, followed by extraction with chloroform and evaporation of the organic solvent afforded the crude products which were then purified by column chromatography. Without this pretreatment, the final compounds which were synthesized directly from the displacement of bromide **49** were extremely difficult to purify due to the retention of side products in the reaction mixture and resulted in yields of less than 10% of the desired products.

Reductive N-methylation of compounds **4**, **6**, **12**, **23**, **25**, **27**, **30**, and **32** to afford the corresponding **5**, **7**, **13**, **24**, **26**, **28**, **31**, and **33** was carried out by a modified procedure previously reported by us.[16a,22] N-Methylation in acetonitrile with sodium cyanoborohydride and formaldehyde was facilitated by dropwise addition of sufficient 1 N HCl to effect solution, as reported previously,[22] and afforded excellent yields of the N-methylated products up to 99% for compound **7**.

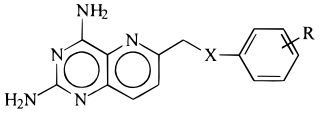
Biological Results and Discussion

The compounds were evaluated as inhibitors of pcDHFR, tgDHFR, and rlDHFR. Selectivity ratios were determined vs rlDHFR as described previously (Table 1).[13b,19] The 8-deaza analogue **3** of compound **1** was a good inhibitor of tgDHFR, and its pcDHFR inhibitory activity was similar to **1**. However, the rlDHFR activity was high; thus compound **3** did not have the high selectivity displayed by **1**. On the basis of the reported selectivity of **1** and **2** along with the results obtained for **3** in this report, it is apparent that both N5 and N8 are required for high selectivity against pcDHFR and tgDHFR, at least for a (methylthio)phenyl substitution.

For compounds **4**−**33**, the side chain S10 of **3** was replaced by N, and a comprehensive structure−activity relationship was developed for the phenyl substitution and N10-methyl and desmethyl analogues. Replacement of the S10 with an N (compounds **4** and **5**) enhances potency against all three DHFRs. Selectivity ratios are, however, decreased compared to **3**. N10 methylation of **4** to **5** increases potency about 10-fold for each enzyme, with a drop in selectivity against both pc- and tgDHFR. This indicates that for the unsubstituted phenyl ring the increase in potency against rlDHFR on N10 methylation is greater than for pc- or tgDHFR.

In the monomethoxy series, the most potent analogue among the N10-H compounds (**6**, **8**, and **10**) is the 4′-OMe compound **10**. N10 methylation of the monomethoxy derivatives significantly increases potency against all three DHFRs; however, the increase is most apparent in the 3′-OMe analogue (compare **8** and **9**) whereas in the 4′-OMe analogue (compare **10** and **11**) the increase is not as significant. The most selective analogue against tgDHFR was the N10-CH_3 2′-OMe compound **7**. The monomethoxy analogues were not selective for pcDHFR.

Table 1. Inhibitory Concentratons (IC_{50}, μM) and Selectivity Ratios against pcDHFR and tgDHFR vs rlDHFR[19,13b]



compd	X	R	pcDHFR	rlDHFR	rl/pc	tgDHFR	rl/tg
1			9.5	246	25.9	0.77	319
2			0.56	0.52	0.93	0.063	8.25
3	S	H	2.0	0.52	0.26	0.13	4.0
4	NH	H	1.7	0.26	0.15	0.085	3.06
5	NMe	H	0.29	0.024	0.08	0.0084	2.86
6	NH	2′-OMe	2.7	0.42	0.16	0.12	3.50
7	NMe	2′-OMe	0.51	0.12	0.24	0.026	4.62
8	NH	3′-OMe	1.7	0.2	0.12	0.1	2.0
9	NMe	3′-OMe	0.097	0.035	0.36	0.015	2.33
10	NH	4′-OMe	0.85	0.073	0.09	0.054	1.35
11	NMe	4′-OMe	0.25	0.018	0.072	0.016	1.13
12	NH	2′-Cl	0.53	0.14	0.26	0.11	1.27
13	NMe	2′-Cl	0.21	0.12	0.57	0.015	8.0
14	NH	3′-Cl	2.0	0.14	0.07	0.13	1.08
14a[a]	NMe	3′-Cl	2.1	0.067	0.032	0.02	3.4
15	NH	2′,4′-$(OMe)_2$	5.5	0.32	0.06	0.14	2.3
16	NMe	2′,4′-$(OMe)_2$	0.16	0.016	0.10	0.014	1.14
17	NH	2′,5′-$(OMe)_2$	4.4	0.28	0.06	0.12	2.33
18	NMe	2′,5′-$(OMe)_2$	0.21	0.05	0.24	0.025	2.0
19	NH	3′,4′-$(OMe)_2$	0.90	0.06	0.07	0.09	0.67
20	NMe	3′,4′-$(OMe)_2$	0.091	0.0027	0.02	0.0098	0.28
21	NH	2′,4′-Cl_2	0.73	0.088	0.12	0.05	1.8
22	NMe	2′,4′-Cl_2	0.5	0.058	0.12	0.05	1.16
23	NH	2′,5′-Cl_2	1.6	0.2	0.13	0.091	2.20
24	NMe	2′,5′-Cl_2	0.15	0.047	0.31	0.025	1.88
25	NH	2′,6′-Cl_2	1.0	0.082	0.082	0.028	2.93
26	NMe	2′,6′-Cl_2	0.17	0.048	0.28	0.03	1.60
27	NH	3′,4′-Cl_2	0.41	0.054	0.13	0.057	0.95
28	NMe	3′,4′-Cl_2	0.038	0.017	0.45	0.027	0.63
29	NH	3′,4′,5′-$(OMe)_3$	2.0	0.2	0.10	0.04	5.00
29a[a]	NMe	3′,4′,5′-$(OMe)_3$	0.13	0.026	0.20	0.0047	5.5
30	NH	3′,4′,5′-Cl_3	0.66	0.044	0.07	0.087	0.51
31	NMe	3′,4′,5′-Cl_3	0.25	0.087	0.35	0.038	2.29
32	NH	2′,4′,6′-Cl_3	2.0	0.57	0.29	0.046	12.39
33	NMe	2′,4′,6′-Cl_3	0.12	0.052	0.43	0.044	1.18
34	S	2′,3′-C_4H_4	0.47	0.16	0.34	0.049	3.3
35	S	3′,4′-C_4H_4	0.38	0.086	0.23	0.048	1.8
36	NH	2′,3′-C_4H_4	0.23	0.04	0.17	0.026	1.54
37	NH	3′,4′-C_4H_4	1.6	0.21	0.13	0.16	1.31
38	NMe	2′,3′-C_4H_4	0.04	0.0073	0.18	0.018	0.41
39	NMe	3′,4′-C_4H_4	0.052	0.0072	0.14	0.016	0.45
40	NH	4′-$COCH_3$	0.41	0.0025	0.01	0.027	0.09
41	NMe	4′-$COCH_3$	0.13	0.0051	0.04	0.015	0.35
42	N-propargyl	4′-$COCH_3$	0.22	0.015	0.07	0.020	0.74
43	NMe	4′-$COCF_3$	0.25	0.032	0.13	0.046	0.69
44	N-propargyl	4′-$COCF_3$	0.12	0.0075	0.06	0.054	0.14
TMP			12	130	11	2.7	48
TMQ			0.042	0.003	0.07	0.01	0.30
PTX			0.031	0.0015	0.048	0.017	0.088

[a] Values from ref 31.

Replacing the 2′-OMe substituent with electron-withdrawing chlorine (compound **12**) increases the inhibitory potency against pc- and rlDHFR but not tgDHFR. This causes a drop in selectivity against tgDHFR compared to compound **6**. N10 methylation of **12** to **13** allows for an increase in tgDHFR inhibitory potency with slight increases against pcDHFR and rlDHFR and parallels the 2′-OMe analogues **6** and **7**. The increase in tgDHFR activity of **13** is accompanied by a significant increase in tgDHFR selectivity which makes **13** the second most selective compound against tgDHFR in this study. The 3′-Cl analogue **14** was equipotent with **12** against rlDHFR and tgDHFR but was less potent than **12** against pcDHFR. Thus the position of the electron-withdrawing monosubstitution was important for both activity and selectivity.

The phenyl ring was next disubstituted. For the isomeric diOMe analogues, the 2′,4′-; 2′,5′-; and 3′,4′-diOMe analogues were evaluated. The 2′,4′- and 2′,5′-diOMe N10-H analogues **15** and **17** were very similar in inhibitory activity and selectivity. N10 methylation of **15** and **17** to afford **16** and **18**, respectively, provided for a 5−34-fold increase in activity against all three DHFRs with selectivity ratios remaining almost unchanged from **15** and **17**. The increase in potency against pcDHFR on N10 methylation was similar to that observed for the 3′-OMe analogue.

The 3′,4′-diOMe analogues **19** and **20** followed the same trend as the previous diOMe analogues regarding increasing potency on N10 methylation with the important difference that this substitution pattern was most conducive to rlDHFR inhibition (rather than pcDHFR);

consequently the molecules lacked selectivity toward both pc- and tgDHFR. On the basis of the monomethoxy and dimethoxy analogues the 3′-OMe substitution is most conducive for potency but not selectivity.

The dichloro-substituted isomers were somewhat different from the diOMe analogues as well as from the monochloro analogues. The 2′,4′-diCl analogue **21** was more potent than the 2′-Cl analogue **12** against rlDHFR and tgDHFR and was also more potent than the N10-methyl 2′-Cl analogue **13** against rlDHFR. N10 methylation of **21** to **22**, in contrast to the electron-donating diOMe analogues, does not increase inhibitory activity against any of the three enzymes. The 2′,5′-diCl analogue **23** was less active than the 2′,4′-diCl compound; however, the decrease for rlDHFR was greater than for tgDHFR, providing for a slight increase in selectivity. N10 methylation of **23** to **24** increases inhibitory activity against pc- and rlDHFR by 5−10-fold but less so for tgDHFR. For the 2′,6′-diCl analogues **25** and **26**, the activity of the N10-H compound **25** was similar to the 2′,5′-diCl analogue **23**. N10 methylation of **25** to **26** increases potency against both rlDHFR and pcDHFR, but interestingly, tgDHFR inhibition remains unchanged. The 2′,6′-diCl substitution demonstrated the same level of selectivity as the 2′,5′-diCl analogues indicating that conformation restriction about the N10−C1′ bond did not increase potency or selectivity. The 3′,4′-diCl analogues **27** and **28** were remarkably similar to the 2′,5′- and 2′,6′-diCl analogues; however, these analogues did not show any selectivity for either enzyme. Therefore in the disubstituted analogues, as was observed for the monosubstituted compounds, the nature and position of the substitution were important for both activity and selectivity.

One 3′,4′,5′-triOMe analogue **29** was synthesized, and it demonstrated low inhibitory activity against pcDHFR similar to the corresponding diOMe N10 unsubstituted analogues described above. However, this analogue did show selectivity for tgDHFR with a ratio of 5. Four isomeric triCl analogues were also evaluated in this series. The N10-methyl 3′,4′,5′-triCl analogue **31** and more particularly the N10-H 2′,4′,6′-triCl analogue **32** were both selective for tgDHFR with **32** being the most selective analogue of the compounds in this report. It should be noted that N10 methylation of the 3′,4′,5′-triCl isomer causes an increase in tgDHFR selectivity by almost 5-fold. Surprisingly, however, N10 methylation of the 2′,4′,6′-triCl isomer decreased tgDHFR selectivity by more than 10-fold and was attributed to a 10-fold increase in rlDHFR inhibitory potency. The reason for this significant increase in potency is not known at this time.

The α- and β-naphthyl-substituted 2,4-diaminopyrido-[3,2-*d*]pyrimidines **34**−**39** were synthesized to determine the influence on inhibitory activities and selectivity of larger than phenyl substitutions on the S10 and N10 moieties. For the S10, both α- and β-naphthyl substitutions (compounds **34** and **35**) increase activity against all three DHFRs compared to the phenyl analogue **3**. However, the selectivity ratios remained almost unaltered. It was interesting to note that while the pcDHFR and tgDHFR activities remained unaltered in going from the α- to the β-naphthyl in analogues **34** and **35**, the rlDHFR activity for the β-naphthyl substitution increased 2-fold compared to the α-naphthyl analogue **34**. For the N10 unsubstituted analogues the α-naphthyl analogue **36** had significantly better inhibitory activity against all three DHFRs than the β-naphthyl analogue **37**. N10 methylation of **36** and **37** to **38** and **39**, respectively, provides for increased potency against all three enzymes (except for **38** against tgDHFR). With the exception of **34**, which had some selectivity against tgDHFR, the other analogues were either not selective or marginally selective.

Compounds **40**−**44** with electron-withdrawing carbonyl groups at the 4′-position were potent inhibitors of tgDHFR and rlDHFR. Compound **41** had IC_{50} values for these two DHFRs which were comparable to those reported previously for TMQ and PTX[31] and were more potent than TMP.[31] Potency of inhibition for pcDHFR was 10-fold lower than that observed for rl- and tgDHFR. Given the potent inhibition of the mammalian and *T. gondii* enzymes observed with **40**−**44**, it would appear that incorporation of a *p*-carbonyl group in the PABA moiety has a greater affect on potency of inhibitors for pcDHFR than does replacement of N8 with CH in the pteridine ring. Compounds **40**−**44** were not selective for pc- or tgDHFR owing to their better inhibition of rlDHFR.

In summary, against pcDHFR, the activities ranged from 0.038×10^{-6} M for **28** to 5.5×10^{-6} M for **15**, a range of 100-fold, and N10 methylation increased activity in all cases, but the increase ranged from a high of 30-fold (for **37** and **39)** to activities that were almost unchanged. The extent of the increase depended upon both the nature and position of the substitution on the phenyl ring. None of the compounds were selective for pcDHFR.

For tgDHFR the activity range was only 20-fold with the most active compound **5** at 0.0084×10^{-6} M and the least active analogue **37** at 0.16×10^{-6} M. All the analogues had IC_{50} values which were submicromolar or better. N-Methylation afforded a 10-fold or less increase in activity with the exception of the diCl, triCl, α-naphthyl, and 4′-OCH_3 analogues where the increase was slight or absent. For pcDHFR activity, the increase was dependent on the nature and position of the phenyl substitution. In contrast to a lack of selectivity for pcDHFR, several of the 8-deaza analogues were significantly selective for tgDHFR, most notably **13**, **29**, and **32** which had selectivities of 8.0, 5.0, and 12.4, respectively. For tgDHFR, 3′,4′-disubstitution was detrimental to selectivity irrespective of the nature or position of the substitution. Thus compounds **19**, **20**, **27**, and **28** were nonselective. In addition, the α- and β-naphthyl N10-methyl analogues **38** and **39** and the 4′-ketone analogues **40**−**44** were nonselective.

Three analogues, compounds **13**, **29**, and **32**, had excellent combinations of both high potency (IC_{50} values from 1.5×10^{-8} to 4.6×10^{-8} M) and good selectivity against tgDHFR. The potencies of these analogues are comparable to TMQ and PTX, and the analogues are from 17- to 43-fold more selective than TMQ and from 56- to 149-fold more selective than PTX. With respect to TMP, the analogues **13**, **29**, and **32** are 180-, 68-, and 59-fold more potent than TMP and compound **32** is only 3.8 times less selective than TMP.

While the manuscript for this paper was in preparation and subsequent to the publication of a preliminary account of this work,[1] a report of a limited series of six

analogues, which included compounds **17**, **28**, and **29**, was submitted and later published by Rosowsky *et al.*[31] In their report, these workers concluded that the design of fused-ring 2,4-diaminopyrimidine systems with the high potency of TMQ and PTX along with the high selectivity of TMP, remained a difficult challenge. Compound **32** of this report, a 2′,4′,6′-triCl analogue, has a potency of 46 nM and a selectivity of 12.4 for tgDHFR. The potency of **32** against tgDHFR is comparable to TMQ and PTX, and the selectivity is 43- and 149-fold better than TMQ and PTX, respectively. Compared to TMP, compound **32** is almost 60 times more potent and only 3.8 times less selective. Thus, compound **32** demonstrates that the 6-substituted-2,4-diaminopyrido[3,2-*d*]pyrimidines are viable lead compounds with significant potency and selectivity. Further studies of the inhibition of the growth of *T. gondii* cells in culture are currently in progress with **32** as a prelude to whole animal studies. The high lipid solubility of **32** bodes well for its activity both in *in vitro* cell culture and in animal studies and will be reported elsewhere.

Experimental Section

All evaporations were carried out *in vacuo* with a rotary evaporator or by short-path distillation. Analytical samples were dried *in vacuo* (0.2 mmHg) in an Abderhalden drying apparatus over P_2O_5 and refluxing ethanol or toluene. Thin-layer chromatography (TLC) was performed on Eastman Kodak silica gel chromatogram plates with fluorescent indicator. Spots were visualized by UV light (254 and 350 nm) or using vanillin as an indicator. All analytical samples were homogeneous on TLC in at least two different solvent systems. Purifications by column and flash chromatography were carried out using Merck silica gel 60 (230−400 mesh). Separations through reversed-phase LC were achieved on a 4.6 mm × 25 cm Microsorb-MV 5 C-18 column with a mobile phase of 20% distilled water in acetonitrile at a flow rate of 1 mL/min. Melting points were determined by capillary method on a Fisher-Johns or Thomas-Hoover melting point apparatus and are uncorrected. UV spectra were obtained on a Hewlett-Packard 8452A diode array spectrophotometer operated in the general scanning mode. ^{1}H NMR spectra were recorded on a Bruker WH-300 (300 MHz) or an IBM AF200 FT-NMR spectrometer. The chemical shifts (δ) values are reported as parts per million (ppm) relative to tetramethylsilane as internal standard: s = singlet, d = doublet, dd = doublet of doublet, t = triplet, q = quartet, m = multiplet, br = broad peak, br s = broad singlet, exch = protons exchangeable by addition of D_2O. Mass spectra were obtained with either a Finnegan MAT94 or a Finnegan MAT95 mass spectrometer. Purity of compounds submitted for high-resolution mass analysis was verified by high-performance liquid chromatography (HPLC) utilizing a Hitachi L6200 intelligent pump equipped with a L3000 photo diode array detector. Elemental analyses were performed by Atlantic Microlab, Inc., Atlanta, GA, or M-H-W Laboratories, Phoenix, AZ. Elemental compositions were within ±0.4% of the calculated values.

General Procedure for the Synthesis of Compounds 3, 4, 6, 8−12, 14−23, 25, 27, 29, 30, 32, 34−39. A suspension of compound **48** (0.48 g, 2.5 mmol) in 10 mL of anhydrous THF was stirred with 0.67 mL of phosphorustribromide overnight. The mixture was rapidly filtered under nitrogen and the precipitate washed with cold ether and dried to afford crude bromide **49** which was used without further purification. To a suspension of freshly prepared bromide **49** in 12 mL of anhydrous dimethylacetamide (DMAc) was added 5 mmol of the appropriate nucleophile (aryl thiol, N-H or N-Me substituted or unsubstituted aniline, or naphthylamine). The suspension was stirred at 50−60 °C under nitrogen for 2−3 days until all the bromide **49** had reacted as evidenced on TLC. DMAc was removed under vacuum, and 15 mL of water and 40 mL of chloroform were then added to the flask. The two-phase mixture was acidified to pH 2 by dropwise addition of 2 N HCl. The water phase was separated, washed with chloroform (2 × 10 mL), and basified to pH 11 with ammonium hydroxide. The resulting precipitate was filtered. The filtrate which contained additional amounts of the desired product was extracted with methylene chloride (3 × 30 mL). The combined methylene chloride extracts were dried over Na_2SO_4, and the solvent was removed under reduced pressure. The solid residue, along with the previously collected precipitate, was combined and dissolved in 10 mL of methanol, and 2 g of silica gel was added and the methanol evaporated to form a plug which was dried and loaded onto a ammonium hydroxide pretreated silica gel column and eluted with 6% methanol in chloroform. The fractions with R_f 0.28−0.35 (in 1: 4 methanol/chloroform) were pooled, and the solvent was evaporated to afford the desired products. The overall yield for the last two steps combined varied from 20 to 57%.

2,4-Diamino-6-[(phenylthio)methyl]pyrido[3,2-*d*]pyrimidine (3). Compound **3** was synthesized from the bromide **49** obtained from **48** (0.48 g, 2.5 mmol) with thiophenol (0.55 g, 5 mmol) to afford 0.24 g (34%) of **3**: TLC MeOH/$CHCl_3$/NH_4OH (3:7:2 drops)/silica gel, R_f 0.67; mp 190−192 °C; ^{1}H NMR (DMSO-d_6) δ 4.37 (s, 2H, 9-CH_2), 6.21 (s, 2H, 2-NH_2, exch), 7.27 (t, 1H, 4′-H), 7.30 (t, 2H, 3′-H and 5′-H), 7.39 (d, 2H, 2′-H and 6′-H), 7.01−7.47 (collapsed br, 2H, 4-NH_2, exch), 7.58 (dd, 2H, 7-H and 8-H); MS (EI) *m*/e 283 (M^+), 174, 132. Anal. ($C_{14}H_{13}N_5S$) C, H, N, S.

2,4-Diamino-6-(aninilomethyl)pyrido[3,2-*d*]pyrimidine (4). Using the same quantities as described for compound **3**, 0.22 g (34%) of compound **4** was obtained: TLC MeOH/$CHCl_3$/NH_4OH (3:7:2 drops)/silica gel, R_f 0.61; mp 220−222 °C; ^{1}H NMR (DMSO-d_6) δ 4.38 (d, 2H, 9-CH_2), 6.14 (s, 2H, 2-NH_2, exch), 6.37 (t, 1H, N_{10}-H, exch), 6.55 (t, 1H, 4′-H), 6.70 (d, 2H, 2′-H and 6′-H), 7.09 (t, 2H, 3′-H and 5′-H), 7.37 (br, 1H, 4-NH_2, exch), 7.60 (br, 1H, 4- NH_2, exch), 7.54 (d, 2H, 7-H and 8-H). Anal. ($C_{14}H_{14}N_6$·0.25NH_4Cl) C, H, N.

2,4-Diamino-6-[(2′-methoxyanilino)methyl]pyrido[3,2-*d*]pyrimidine (6). This compound was obtained using the same quantities described for **3** and afforded 0.18 g (24%) of **6**: TLC MeOH/$CHCl_3$/NH_4OH (3:7:2 drops)/silica gel, R_f 0.67; mp 194−196 °C; ^{1}H NMR (DMSO-d_6) δ 3.81 (s, 3H, 2′-OCH_3), 4.42 (d, 2H, 9-CH_2), 5.78 (t, 1H, N_{10}-H, exch), 6.16 (s, 2H, 2-NH_2, exch), 6.51 (m, 2H, C_6H_4), 6.68 (t, 1H, C_6H_4), 6.81 (d, 1H, C_6H_4), 7.15−7.40 (br, 2H, 4-NH_2, exch), 7.50 (s, 2H, 7-H and 8-H). Anal. ($C_{15}H_{16}N_6O$) C, H, N.

2,4-Diamino-6-[(3′-methoxyanilino)methyl]pyrido[3,2-*d*]pyrimidine (8). Using the same quantities as described for compound **3**, 0.16 g (21%) of compound **8** was obtained: TLC MeOH/$CHCl_3$/NH_4OH (3:7:2 drops)/silica gel, R_f 0.63; mp 180−183 °C dec; ^{1}H NMR (DMSO- d_6) δ 4.37 (d, 2H, 9-CH_2), 6.07 (s, 2H, 2-NH_2, exch), 6.15 (dd, 1H, C_6H_4), 6.25−6.32 (m, 2H, C_6H_4), 6.40 (t, 1H, N_{10}-H, exch), 7.00 (t, 1H, C_6H_4), 7.42 (br, 1H, 4-NH_2, exch), 7.54 (d, 2H, 7-H and 8-H), 7.70 (br, 1H, 4- NH_2, exch). Anal. ($C_{15}H_{16}N_6O$·0.2H_2O) C, H, N.

2,4-Diamino-6-[(3′-methoxy-*N*-methylanilino)methyl]-pyrido[3,2-*d*]pyrimidine (9). *N*-Methyl-3-methoxyaniline (0.45 g, 3.3 mmol) was condensed with intermediate **49** prepared from **48** (2 mmol, 0.38 g) to afford 0.20 g (32%) of compound **9**: TLC MeOH/$CHCl_3$/NH_4OH (3:7:2 drops)/silica gel, R_f 0.69; mp 179−180.5 °C; ^{1}H NMR (DMSO-d_6) δ 3.10 (s, 3H, N_{10}-CH_3), 3.66 (s, 3H, 3′-OCH_3), 4.64 (s, 2H, 9-CH_2), 6.19 (m, 2H, C_6H_4), 6.24 (br s, 2H, 2-NH_2, exch), 6.34 (dd, 1H, C_6H_4), 7.03 (t, 1H, C_6H_4), 7.13 (br, 1H, 4-NH_2, exch), 7.31 (d, 1H, 7-H or 8-H), 7.37 (br, 1H, 4-NH_2, exch), 7.49 (d, 1H, 7-H or 8-H). Anal. ($C_{16}H_{18}N_6O$·0.2H_2O) C, H, N.

2,4-Diamino-6-[(4′-methoxyanilino)methyl]pyrido[3,2-*d*]pyrimidine (10). Anisidine (0.73 g, 5.6 mmol) was condensed with the bromide **49** prepared from **48** (0.48 g, 2.5 mmol) in anhydrous DMAc at room temperature for 3 days. After workup and separation, 0.20 g (27%) of compound **10** was obtained: TLC MeOH/$CHCl_3$/NH_4OH (3:7:2 drops)/silica gel, R_f 0.63; mp 197.5−199.5 °C; ^{1}H NMR (DMSO-d_6) δ 3.63 (s, 3H, 4′-OCH_3), 4.32 (d, 2H, 9-CH_2), 6.00 (t, 1H, N_{10}-H, exch), 6.13 (s, 2H, 2-NH_2, exch), 6.70 (dd, 4H, C_6H_4), 7.35 (br, 1H, 4-NH_2, exch), 7.59 (br, 1H 4-NH_2, exch), 7.60 (s, 2H, 7-H and 8-H). Anal. ($C_{15}H_{16}N_6O$·0.05NH_4Cl) C, H, N.

2,4-Diamino-6-[(4′-methoxy-*N*-methylanilino)methyl]-pyrido[3,2-*d*]pyrimidine (11). The bromide **49** prepared from **48** (0.48 g, 2.5 mmol) was condensed with *N*-methylanisidine (0.68 g, 5 mmol) which was synthesized by methylation of anisidine with 1.2 equiv of methyl iodide in the presence of 1.2 equiv of $^{i}Pr_2NEt$ at 35 °C for 10 h. The yield of compound **11** was 0.42 g (54%): TLC MeOH/$CHCl_3$/NH_4OH (3:7:2 drops)/silica gel, R_f 0.69; mp 240−242 °C dec; ^{1}H NMR (DMSO-d_6) δ 3.00 (s, 3H, N_{10}-CH_3), 3.64 (s, 3H, 4′-OCH_3), 4.56 (s, 2H, 9-CH_2), 6.17 (s, 2H, 2-NH_2, exch), 6.75 (m, 4H, C_6H_4), 7.14 (br, 1H, 4-NH_2, exch), 7.31 (collapsed br, 1H, 4-NH_2, exch), 7.34 (d, 1H, 7-H or 8-H), 7.49 (d, 1H, 7-H or 8-H). Anal. ($C_{16}H_{18}N_6O$·0.4H_2O) C, H, N.

2,4-Diamino-6-[(2′-chloroanilino)methyl]pyrido[3,2-*d*]-pyrimidine (12). Using the same quantities as described for compound **3**, 0.19 g (26%) of compound **12** was obtained: TLC MeOH/$CHCl_3$/NH_4OH (3:7:2 drops)/silica gel, R_f 0.67; mp 232−234 °C dec; ^{1}H NMR (DMSO-d_6) δ 4.51 (d, 2H, 9-CH_2), 6.16 (s, 2H, 2-NH_2, exch), 6.28 (t, 1H, N_{10}-H, exch), 6.58 (t, 1H, C_6H_4), 6.67 (d, 1H, C_6H_4), 7.04 (t, 1H, C_6H_4), 7.27 (d, 1H, C_6H_4), 7.10−7.45 (collapsed br, 2H, 4-NH_2, exch), 7.52 (dd, 2H, 7-H and 8-H). Anal. ($C_{14}H_{13}N_6Cl$) C, H, N, Cl.

2,4-Diamino-6-[(3′-chloroanilino)methyl]pyrido[3,2-*d*]-pyrimidine (14). Using the same quantities as described for compound **3** afforded 0.21 g (28%) of compound **14**: TLC MeOH/$CHCl_3$/NH_4OH (3:7:2 drops)/silica gel, R_f 0.64; mp 178−180 °C; ^{1}H NMR (DMSO-d_6) δ 4.39 (d, 2H, 9-CH_2), 6.15 (br s, 2H, 2-NH_2, exch), 6.54−6.76 (m, 4H, N_{10}-H, exch and C_6H_4), 7.10 (t, 1H, 5′-H), 7.38 (br, 1H, 4-NH_2, exch), 7.63 (br, 1H, 4-NH_2, exch), 7. 52 (q, 2H, 7-H and 8-H). Anal. ($C_{14}H_{13}N_6$-Cl) C, H, N, Cl.

2,4-Diamino-6-[(2′,4′-dimethoxyanilino)methyl]pyrido-[3,2-*d*]pyrimidine (15). Using the same quantities as described for compound **3** afforded 0.31 g (38%) of compound **15**: TLC MeOH/$CHCl_3$/NH_4OH (3:7:2 drops)/silica gel, R_f 0.72; mp >200 °C dec; ^{1}H NMR (DMSO-d_6) δ 3.62 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 4.36 (d, 2H, 9-CH_2), 5.35 (t, 1H, N_{10}-H, exch), 6.14 (s, 2H, 2-NH_2, exch), 6.27 (dd, 1H, 5′-H), 6.39 (d, 1H, 6′-H), 6.48 (d, 1H, 3′-H), 7.26 (br, 2H, 4-NH_2, exch), 7.50 (s, 2H, 7-H and 8-H). Anal. ($C_{16}H_{18}N_6O_2$·0.5H_2O) C, H, N.

2,4-Diamino-6-[(2′,4′-dimethoxy-*N*-methylanilino)methyl]pyrido[3,2-*d*]pyrimidine (16). *N*-Methyl-2,4-dimethoxyaniline was prepared from 2,4-dimethylaniline (2 g, 13 mmol), methyl iodide (1.1 equiv), and triethylamine (1.1 equiv) in acetonitrile at 35 °C for 8 h (60% yield). N-Methylated aniline (0.47 g, 2.8 mmol) reacted with **49** prepared from **48** (0.38 g, 2 mmol) afforded 0.20 g (35%) of the product **16**: TLC MeOH/$CHCl_3$/NH_4OH (3:7:2 drops)/silica gel, R_f 0.73; mp 191−193 °C; ^{1}H NMR (DMSO-d_6) δ 2.59 (s, 3H, N_{10}-CH_3), 3.71 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 4.12 (s, 2H, 9-CH_2), 6.16 (br s, 2H, 2-NH_2, exch), 6.41 (dd, 1H, 5′-H), 6.57 (d, 1H, 3′-H), 6.86 (d, 1H, 6′-H), 7.05−7.4 (br, 2H, 4-NH_2, exch), 7.57 (dd, 2H, 7-H and 8-H). Anal. ($C_{17}H_{20}N_6O_2$) C, H, N.

2,4-Diamino-6-[(2′,5′-dimethoxyanilino)methyl]pyrido-[3,2-*d*]pyrimidine (17). Compound **49** prepared from **48** (0.48 g, 2.5 mmol) was reacted with 2,5-dimethoxyaniline (0.61 g, 4 mmol) at 82 °C for 14 h to afford 0.23 g (28%) of **17**: TLC MeOH/$CHCl_3$/NH_4OH (3:7:2 drops)/silica gel, R_f 0.74; mp 199−201 °C; ^{1}H NMR (DMSO-d_6) δ 3.55 (s, 3H, OCH_3), 3.76 (s, 3H, OCH_3), 4.40 (d, 2H, 9-CH_2), 5.92 (t, 1H, N_{10}-H, exch), 6.06 (br s, 2H, C_6H_3), 6.63 (br s, 2H, 2-NH_2, exch), 6.70 (d, 1H, C_6H_3), 7.58 (q, 2H, 7-H and 8-H), 7.77 (br, 2H, 4-NH_2, exch). Anal. ($C_{16}H_{18}N_6O_2$·0.58H_2O) C, H, N.

2,4-Diamino-6-[(2′,5′-dimethoxy-*N*-methylanilino)methyl]pyrido[3,2-*d*]pyrimidine (18). *N*-Methyl-2,5-dimethoxyaniline (1.74 g, 79%) was prepared from 2,5-dimethoxyaniline (2.5 g, 16 mmol) and methyl iodide (1.2 equiv) at the presence of $^{i}Pr_2NEt$ (1.2 equiv) at 40 °C for 36 h. Then 0.67 g of it was condensed with **49** synthesized from **48** (0.48 g, 2.5 mmol) afforded 0.27 g (32%) of compound **18**: TLC MeOH/$CHCl_3$/NH_4-OH (3:7:2 drops)/silica gel, R_f 0.77; mp 202−204 °C dec; ^{1}H NMR (DMSO-d_6) δ 2.67 (s, 3H, N_{10}-CH_3), 3.66 (s, 3H, OCH_3), 3.75 (s, 3H, OCH_3), 4.32 (s, 2H, 9-CH_2), 6.17 (br s, 2H, 2-NH_2, exch), 6.45 (m, 2H, 4′-H and 6′-H), 6.86 (d, 1H, 3′-H), 6.96−7.42 (br d, 2H, 4-NH_2, exch), 7.57 (q, 2H, 7-H and 8-H). Anal. ($C_{17}H_{20}N_6O_2$) C, H, N.

2,4-Diamino-6-[(3′,4′-dimethoxyanilino)methyl]pyrido-[3,2-*d*]pyrimidine (19). Using the same quantities as described for compound **3** afforded 0.30 g (37%) of the product **19**: TLC MeOH/$CHCl_3$/NH_4OH (3:7:2 drops)/silica gel, R_f 0.67; mp 255−256 °C dec; ^{1}H NMR (DMSO-d_6) δ 3.61 (s, 3H, OCH_3), 3.70 (s, 3H, OCH_3), 4.33 (d, 2H, 9-CH_2), 6.06 (t, 1H, N_{10}-H, exch), 6.15 (m, 3H, 6′-H and 2-NH_2, exch), 6.47 (d, 1H, 2′-H), 6.71 (d, 1H, 5′-H), 7.36 (br, 1H, 4-NH_2, exch), 7.60 (br, 1H, 4-NH_2, exch), 7.52 (d, 2H, 7-H and 8-H). Anal. ($C_{16}H_{18}N_6O_2$·0.8H_2O) C, H, N.

2,4-Diamino-6-[(3′,4′-dimethoxy-*N*-methylanilino)methyl]pyrido[3,2-*d*]pyrimidine (20). *N*-Methyl-3′,4′-dimethoxyaniline (0.66 g, 4 mmol) was prepared from 3,4-dimethoxyaniline (1.5 g, 10 mmol) with stirring with 1.1 equiv of MeI and 1.1 equiv of $^{i}Pr_2NEt$ in acetonitrile and was reacted with **49** [from **48** (0.38 g, 2 mmol)] to afford 0.20 g (29%) of compound **20**: TLC MeOH/$CHCl_3$/NH_4OH (3:7:2 drops)/silica gel, R_f 0.76; mp 207−209 °C dec; ^{1}H NMR (DMSO-d_6) δ 3.03 (s, 3H, N_{10}-CH_3), 3.62 (s, 3H, OCH_3), 3.68 (s, 3H, OCH_3), 4.59 (s, 2H, 9-CH_2), 6.16 (br s, 2H, 2-NH_2, exch), 6.20 (dd, 1H, 6′-H), 6.47 (d, 1H, 2′-H), 6.76 (d, 1H, 5′-H), 7.10−7.35 (br, 2H, 4-NH_2, exch), 7.36 (d, 1H, 7-H or 8-H), 7.51 (d, 1H, 7-H or 8-H). Anal. ($C_{17}H_{20}N_6O_2$·0.5H_2O) C, H, N.

2,4-Diamino-6-[(2′,4′-dichloroanilino)methyl]pyrido-[3,2-*d*]pyrimidine (21). Compound **49** [from **48** (0.19 g, 1 mmol)] was reacted with 2,4-dichloroaniline (0.40 g, 2.0 mmol) to afford 0.12 g (37%) of **21**: TLC MeOH/$CHCl_3$/NH_4OH (3:7:2 drops)/silica gel, R_f 0.71; mp 239−241 °C dec; ^{1}H NMR (DMSO-d_6) δ 4.49 (d, 2H, 9-CH_2), 6.16 (s, 2H, 2-NH_2, exch), 6.43 (t, 1H, N_{10}-H), 6.68 (d, 1H, 6′-H), 7.10 (dd, 1H, 5′-H), 7.36 (d, 1H, 3′-H), 7.15−7.35 (collapsed br, 2H, 4-NH_2, exch), 7.50 (dd, 2H, 7-H and 8-H). Anal. ($C_{14}H_{12}N_6Cl_2$·0.2HCl) C, H, N, Cl.

2,4-Diamino-6-[(2′,4′-chloro-*N*-methylanilino)methyl]-pyrido[3,2-*d*]pyrimidine (22). Bromide **49** prepared from **48** (0.38 g, 2 mmol) was reacted with *N*-methyl-2,4-dichloroaniline (0.70 g, 4 mmol) to afford 0.15 g (22%) of **22**: TLC MeOH/$CHCl_3$/NH_4OH (3:7:2 drops)/silica gel, R_f 0.77; mp 180−182 °C; ^{1}H NMR (DMSO-d_6) δ 2.70 (s, 3H, N_{10}-CH_3), 4.33 (s, 2H, 9-CH_2), 6.20 (s, 2H, 2-NH_2, exch), 7.08 (br, 1H, 4-NH_2, exch), 7.23 (d, 1H, 6′-H), 7.33 (m, 2H, 4-NH_2, exch, and 5′-H), 7.58 (m, 3H, 3′-H, 7-H and 8-H). Anal. ($C_{15}H_{14}N_6Cl_2$) C, H, N, Cl.

2,4-Diamino-6-[(2′,5′-dichloroanilino)methyl]pyrido-[3,2-*d*]pyrimidine (23). Compound **49** [from **48** (0.38 g, 2 mmol)] was reacted with 2,5-dichloroaniline (0.65 g, 4 mmol) to afford 0.15 g (22%) of **23**: TLC MeOH/$CHCl_3$/NH_4OH (3:7:2 drops)/silica gel, R_f 0.73; mp >270 °C dec; ^{1}H NMR (DMSO-d_6) δ 4.50 (d, 2H, 9-CH_2), 6.21 (s, 2H, 2-NH_2, exch), 6.55 (t, 1H, N_{10}-H, exch), 6.60 (dd, 1H, 4′-H), 6.72 (d, 1H, 6′-H), 7.15 (br, 1H, 4-NH_2, exch), 7.29 (d, 1H, 3′-H), 7.35 (br, 1H, 4-NH_2, exch), 7.52 (dd, 2H, 7-H and 8-H). Anal. ($C_{14}H_{12}N_6Cl_2$·0.3H_2O) C, H, N, Cl.

2,4-Diamino-6-[(2′,6′-dichloroanilino)methyl]pyrido-[3,2-*d*]pyrimidine (25). Using the same quantities as described for **23** and the same procedure as described in general procedure except that the temperature of the reaction was raised to 100 °C afforded 0.13 g of **25** (19%): TLC MeOH/$CHCl_3$/NH_4OH (3:7:2 drops)/silica gel, R_f 0.75; mp >190 °C dec; ^{1}H NMR (DMSO-d_6) δ 4.68 (d, 2H, 9-CH_2), 5.85 (t, 1H, N_{10}-H, exch), 6.30 (br s, 2H, 2-NH_2, exch), 6.84 (t, 1H, 4′-H), 7.03 (br, 1H, 4-NH_2, exch), 7.33 (d, 2H, 3′-H and 5′-H), 7.48 (br, 1H, 4-NH_2, exch), 7.55 (s, 2H, 7-H and 8-H). Anal. ($C_{14}H_{12}N_6$-Cl_2·0.35H_2O) C, H, N, Cl.

2,4-Diamino-6-[(3′,4′-dichloroanilino)methyl]pyrido-[3,2-*d*]pyrimidine (27). Using the same quantities as described for compound **3** afforded 0.17g (21%) of **27**: TLC MeOH/$CHCl_3$/NH_4OH (3:7:2 drops)/silica gel, R_f 0.64; mp 187−189 °C dec; ^{1}H NMR (DMSO-d_6) δ 4.40 (d, 2H, 9-CH_2), 6.18 (br s, 2H, 2-NH_2, exch), 6.73 (dd, 1H, 6′-H), 6.81 (t, 1H, N_{10}-H, exch), 6.94 (d, 1H, 2′-H), 7.30 (d, 1H, 5′-H), 7.42 (br, 1H, 4-NH_2, exch), 7.52 (dd, 2H, 7-H and 8-H), 7.65 (br, 1H, 4-NH_2, exch). Anal. ($C_{14}H_{12}N_6Cl_2$·0.4H_2O·0.4HCl) C, H, N, Cl.

2,4-Diamino-6-[(3′,4′,5′-trimethoxyanilino)methyl]pyrido[3,2-*d*]pyrimidine (29). Using the same quantities as described for compound **3** afforded 0.36 g (41%) of **29**: TLC

MeOH/$CHCl_3$/NH_4OH (3:7:2 drops)/silica gel, R_f 0.66; mp 214−216 °C; ^{1}H NMR (DMSO-d_6) δ 3.53 (s, 3H, OCH_3), 3.71 (s, 6H, OCH_3), 4.38 (d, 2H, 9-CH_2), 6.05 (s, 2H, 2′-H and 6′-H), 6.15 (br s, 2H, 2-NH_2, exch), 6.24 (t, 1H, N_{10}-H, exch), 7.39 (br s, 1H, 4-NH_2, exch), 7.53 (d, 2H, 7-H and 8-H), 7.57 (br s, 1H, 4-NH_2, exch). Anal. ($C_{17}H_{20}N_6O_3$) C, H, N.

2,4-Diamino-6-[(3′,4′,5′-trichloroanilino)methyl]pyrido[3,2-*d*]pyrimidine (30). Using the same procedure and the same quantities as described for **3** afforded 0.21 g (22%) of compound **30**: TLC MeOH/$CHCl_3$/NH_4OH (3:7:2 drops)/silica gel, R_f 0.64; mp 248−250 °C dec; ^{1}H NMR (DMSO-d_6) δ 4.42 (d, 2H, 9-CH_2), 6.20 (s, 2H, 2-NH_2, exch), 7.00 (br s, 3H, N_{10}-H, exch, 2′-H and 6′-H), 7.47 (collapsed br, 1H, 4-NH_2, exch), 7.67 (br, 1H, 4-NH_2, exch), 7.53 (dd, 2H, 7-H and 8-H). Anal. ($C_{14}H_{11}N_6Cl_3$) C, H, N, Cl.

2,4-Diamino-6-[(2′,4′,6′-trichloroanilino)methyl]pyrido[3,2-*d*]pyrimidine (32). Compound **32** was synthesized by the procedure as described for **3**. The yield was 0.19 g (23%): TLC MeOH/$CHCl_3$/NH_4OH (3:7:2 drops)/silica gel, R_f 0.70; mp >260 °C dec; ^{1}H NMR (DMSO-d_6) δ 4.67 (d, 2H, 9-CH_2), 5.96 (t, 1H, N_{10}-H, exch), 6.22 (br s, 2H, 2-NH_2, exch), 6.90 (br, 1H, 4-NH_2, exch), 7.36 (br, 1H, 4-NH_2, exch), 7.50 (s, 2H, 3′-H and 5′-H), 7.53 (s, 2H, 7-H and 8-H). Anal. ($C_{14}H_{12}N_6Cl_3 \cdot 0.19C_6H_6$) C, H, N, Cl.

2,4-Diamino-6-[(1′-naphthylthio)methyl]pyrido[3,2-*d*]pyrimidine (34). Compound **49** [from **48** (0.38 g, 2 mmol)] was reacted with 1-naphthylthiol (0.64 g, 4 mmol) to afford 0.23 g (35%) of **34**: TLC MeOH/$CHCl_3$/NH_4OH (3:7:2 drops)/silica gel, R_f 0.68; mp 181−183 °C; ^{1}H NMR (DMSO-d_6) δ 4.44 (s, 2H, 9-CH_2), 6.21 (s, 2H, 2-NH_2, exch), 7.10 (br s, 1H, 4-NH_2, exch), 7.32 (br s, 1H, 4-NH_2, exch), 7.38−7.60 (m, 5H, $C_{10}H_7$, 7-H and 8-H), 7.68 (d, 1H, $C_{10}H_7$), 7.78 (d, 1H, $C_{10}H_7$), 7.92 (d, 1H, $C_{10}H_7$), 8.21 (d, 1H, $C_{10}H_7$). Anal. ($C_{18}H_{15}N_5S \cdot 0.15H_2O$) C, H, N, S.

2,4-Diamino-6-[(2′-naphthylthio)methyl]pyrido[3,2-*d*]pyrimidine (35). Bromide **49** prepared from **48** (0.38 g, 2 mmol) was reacted with 2-naphthylthiol (0.64 g, 4 mmol) to afford 0.27 g (41%) of **35**: TLC MeOH/$CHCl_3$/NH_4OH (3:7:2 drops)/silica gel, R_f 0.70; mp >200 °C dec; ^{1}H NMR (DMSO-d_6) δ 4.48 (s, 2H, 9-CH_2), 6.22 (br s, 2H, 2-NH_2, exch), 7.36 (br, 2H, 4-NH_2, exch), 7.40−8.10 (m, 9H, $C_{10}H_7$, 7-H and 8-H). Anal. ($C_{18}H_{15}N_5S \cdot 0.40H_2O \cdot 0.30AcOH$) C, H, N, S.

2,4-Diamino-6-[(1′-naphthylamino)methyl]pyrido[3,2-*d*]pyrimidine (36). Bromide **49** [from **48** (0.29 g, 1.5 mmol)] was reacted with 1-naphthylamine (0.50 g, 4 mmol) to afford 0.12 g (26%) of **36**: TLC MeOH/$CHCl_3$/NH_4OH (3:7:2 drops)/silica gel, R_f 0.65; mp >200 °C; ^{1}H NMR (DMSO-d_6) δ 4.58 (d, 2H, 9-CH_2), 6.16 (br s, 2H, 2-NH_2, exch), 6.44 (d, 1H, $C_{10}H_7$), 7.02 (t, 1H, N_{10}-H, exch), 7.07−7.20 (m, 2H, $C_{10}H_7$), 7.43 (collapsed br, 2H, 4-NH_2, exch), 7.44 (m, 2H, $C_{10}H_7$), 7.53 (dd, 2H, 7-H and 8-H), 7.73 (m, 1H, $C_{10}H_7$), 8.21 (m, 1H, $C_{10}H_7$). Anal. ($C_{18}H_{16}N_6 \cdot 0.80H_2O$) C, H, N.

2,4-Diamino-6-[(2′-naphthylamino)methyl]pyrido[3,2-*d*]pyrimidine (37). Intermediate **49** prepared from **48** (0.29 g, 1.5 mmol) was reacted with 2-naphthylamine (0.23 g, 1.5 mmol) to afford 0.10 g (22%) of **37**: TLC MeOH/$CHCl_3$/NH_4OH (3:7:2 drops)/silica gel, R_f 0.62; mp >220 °C dec; ^{1}H NMR (DMSO-d_6) δ 4.50 (d, 2H, 9-CH_2), 6.15 (br s, 2H, 2-NH_2, exch), 6.69 (t, 1H, N_{10}-H, exch), 6.83 (s, 1H, $C_{10}H_7$), 7.14 (t, 1H, $C_{10}H_7$), 7.22 (dd, 1H, $C_{10}H_7$), 7.30 (t, 1H, $C_{10}H_7$), 7.41 (collapsed br, 2H, 4-NH_2, exch), 7.55 (m, 3H, $C_{10}H_7$, 7-H and 8-H), 7.65 (d, 1H, $C_{10}H_7$). Anal. ($C_{18}H_{16}N_6$) C, H, N.

2,4-Diamino-6-[(*N*-methyl-1′-naphthylamino)methyl]pyrido[3,2-*d*]pyrimidine (38). *N*-Methyl-1-naphthylamine (0.47 g, 3 mmol) was prepared from 1-naphthylamine (0.93 g, 7.5 mmol) on stirring with 1.1 equiv of MeI and 1.1 equiv of Et_3N in acetonitrile and was reacted with **49** [from **48** (0.38 g, 2 mmol)] to afford 0.13 g (20%) of compound **38**: TLC MeOH/$CHCl_3$/NH_4OH (3:7:2 drops)/silica gel, R_f 0.70; mp 206−208 °C; ^{1}H NMR (DMSO-d_6) δ 2.80 (s, 3H, N_{10}-CH_3), 4.41 (s, 2H, 9-CH_2), 6.22 (br s, 2H, 2-NH_2, exch), 7.15 (br, 1H, 4-NH_2, exch), 7.20 (d, 1H, $C_{10}H_7$), 7.33 (br, 1H, 4-NH_2, exch), 7.44 (t, 1H, $C_{10}H_7$), 7.54 (m, 4H, $C_{10}H_7$, 7-H and 8-H), 7.58 (dd, 1H, $C_{10}H_7$), 7.62 (d, 1H, $C_{10}H_7$), 8.34 (s, 1H, $C_{10}H_7$). Anal. ($C_{19}H_{18}N_6 \cdot 0.20H_2O$) C, H, N.

2,4-Diamino-6-[(*N*-methyl-2′-naphthylamino)methyl]pyrido[3,2-*d*]pyrimidine (39). *N*-Methyl-2-naphthylamine (0.64 g, 4 mmol) was prepared from 1-naphthylamine (0.93 g, 7.5 mmol) on stirring with 1.1 equiv of MeI and 1.1 equiv of Et_3N in acetonitrile and was reacted with bromide **49** made from **48** (0.38 g, 2 mmol) to afford 0.22 g (33%) of compound **39**: TLC MeOH/$CHCl_3$/NH_4OH (3:7:2 drops)/silica gel, R_f 0.71; mp 215−217 °C; ^{1}H NMR (DMSO-d_6) δ 3.18 (s, 3H, N_{10}-CH_3), 4.80 (s, 2H, 9-CH_2), 6.84 (br s, 2H, 2-NH_2, exch), 7.01 (d, 1H, $C_{10}H_7$), 7.14 (br t, 2H, $C_{10}H_7$ and 4-NH_2, exch), 7.26−7.39 (m, 4H, $C_{10}H_7$ and 4-NH_2, exch), 7.49 (d, 2H, 7-H and 8-H), 7.63−7.71 (m, 3H, $C_{10}H_7$). Anal. ($C_{19}H_{18}N_6 \cdot 0.34HCl$) C, H, N.

General Procedure for the Reductive N-Methylation of 4, 6, 12, 23, 25, 27, 30, and 32. To a suspension of **6** (120 mg, 0.40 mmol) in 10 mL of acetonitrile was added 0.15 mL of 37% HCHO (1.8 mmol), followed by the addition of $NaCNBH_3$ (0.08 g, 1.2 mmol). The reaction mixture was stirred for 5 min and followed by the addition of 2 N HCl dropwise over 2−3 min until the suspension dissolved (pH 2). After a while a white solid started to precipitate out of the solution. The reaction was continued at room temperature and monitored by TLC ($CHCl_3$:MeOH, 7:3). After 15 h, the starting material had reacted, at which time the solvent (acetonitrile) was removed using a rotary evaporator. The residue was dissolved in 10 mL of water and 1 mL of ethyl acetate and the resulting solution basified with ammonium hydroxide to pH 10. The white solid which precipitated was filtered, washed with ethyl acetate (2 × 0.5 mL), and dried in vacuum overnight to afford 0.12 g (99%) of **7**.

2,4-Diamino-6-[(*N*-methylanilino)methyl]pyrido[3,2-*d*]pyrimidine (5). Compound **4** (0.10 g, 0.38 mmol) was reacted with 37% HCHO (0.15 mL, 1.8 mmol) and $NaCNBH_3$ (0.080 g, 1.2 mmol) for 4.5 h using the procedure described above. After column chromatographic purification using MeOH/CH_3Cl/NH_4OH (50:450:1) as eluent, 0.063 g (60%) of **5** was obtained: TLC MeOH/$CHCl_3$/NH_4OH (3:7:2 drops)/silica gel, R_f 0.68; mp 182−184 °C; ^{1}H NMR (DMSO-d_6) δ 3.10 (s, 3H, N_{10}-CH_3), 4.66 (s, 2H, 9-CH_2), 6.24 (br s, 2H, 2-NH_2, exch), 6.61 (t, 1H, 4′-H), 6.75 (d, 2H, 2′-H and 6′-H), 7.14 (m, 2H, 3′-H and 5′-H), 7.20 (br, 1H, 4-NH_2, exch), 7.33 (d, 1H, 7-H or 8-H), 7.40 (br, 1H, 4-NH_2, exch), 7.51 (d, 1H, 7-H or 8-H). Anal. ($C_{15}H_{16}N_6 \cdot 0.44HCl$) C, H, N.

2,4-Diamino-6-[(2′-methoxy-*N*-methylanilino)methyl]pyrido[3,2-*d*]pyrimidine (7): TLC MeOH/$CHCl_3$/NH_4OH (3:7:2 drops)/silica gel, R_f 0.70; mp 177.5−179.6 °C; ^{1}H NMR (DMSO-d_6) δ 2.66 (s, 3H, N_{10}-CH_3), 3.82 (s, 3H, 2′-OCH_3), 4.29 (s, 2H, 9-CH_2), 6.17 (s, 2H, 2-NH_2, exch), 6.90 (m, 4H, C_6H_4), 7.09 (br s, 1H, 4-NH_2, exch), 7.28 (br s, 1H, 4-NH_2, exch), 7.53 (d, 1H, 7-H or 8-H), 7.62 (d, 1H, 7-H or 8-H). Anal. ($C_{16}H_{18}N_6O \cdot 0.5H_2O$) C, H, N.

2,4-Diamino-6-[(2′-chloro-*N*-methylanilino)methyl]pyrido[3,2-*d*]pyrimidine (13). Compound **12** (0.112 g, 0.37 mmol) was reacted with 37% HCHO (0.15 mL, 1.8 mmol) and $NaCNBH_3$ (0.080 g, 1.2 mmol) for 3 h using the procedure as described in the general procedure. After purification through a column eluted with 6% MeOH in chloroform (v/v), 0.102 g (88%) of **13** was obtained: TLC MeOH/$CHCl_3$/NH_4OH (3:7:2 drops)/silica gel, R_f 0.63; mp 212−214.5 °C; ^{1}H NMR (DMSO-d_6) δ 2.70 (s, 3H, N_{10}-CH_3), 4.33 (s, 2H, 9-CH_2), 6.17 (s, 2H, 2-NH_2, exch), 7.04−7.28 [m, 5H, 4-NH_2 (exch), 4′-H, 5′-H and 6′-H], 7.44 (dd, 1H, 3′-H), 7.55 (d, 1H, 7-H or 8-H), 7.65 (d, 1H, 7-H or 8-H). Anal. ($C_{15}H_{15}N_6Cl$) C, H, N, Cl.

2,4-Diamino-6-[(2′,5′-dichloro-*N*-methylanilino)methyl]pyrido[3,2-*d*]pyrimidine (24). Compound **23** (0.053 g, 0.16 mmol) was reductively N-methylated for 12 h to form **24** using the procedure as described as in the general procedure to afford 0.043 g (78%) of **24**: TLC MeOH/$CHCl_3$/NH_4OH (3:7:2 drops)/silica gel, R_f 0.72; mp >260 °C dec; ^{1}H NMR (DMSO-d_6) δ 2.79 (s, 3H, N_{10}-CH_3), 4.46 (s, 2H, 9-CH_2), 6.44 (s, 2H, 2-NH_2, exch), 7.15 (dd, 1H, 4′-H), 7.33 (d, 1H, 6′-H), 7.52 (d, 1H, 4′-H), 7.60−8.10 (m, 4H, 4-NH_2, exch, 7-H and 8-H). Anal. ($C_{15}H_{14}N_6Cl_2 \cdot 0.7HCl \cdot 0.8H_2O$) C, H, N, Cl.

2,4-Diamino-6-[(2′,6′-dichloro-*N*-methylanilino)methyl]pyrido[3,2-*d*]pyrimidine (26). Compound **25** (0.093 g, 0.28 mmol) was used to synthesize compound **26** using the procedure described for **7**. After 12 h, the crude product was worked

up and purified *via* a column with 6% MeOH in chloroform (v/v) as eluent to afford 0.091 g (94%) of the **26**: TLC MeOH/$CHCl_3$/NH_4OH (3:7:2 drops)/silica gel, R_f 0.77; mp 208−209 °C; ^{1}H NMR (DMSO-d_6) δ 2.73 (s, 3H, N_{10}-CH_3), 4.43 (s, 2H, 9-CH_2), 6.17 (br s, 2H, 2-NH_2, exch), 7.22 (m, 3H, 4-NH_2, exch and 4′-H), 7.48 (d, 2H, 3′-H and 5′-H), 7.60 (d, 1H, 7-H or 8-H), 7.87 (d, 1H, 7-H or 8-H). Anal. ($C_{15}H_{14}N_6Cl_2$) C, H, N, Cl.

2,4-Diamino-6-[(3′,4′-dichloro-*N*-methylanilino)methyl]-pyrido[3,2-*d*]pyrimidine (28). Using the procedure described for compound **7**, 0.091 g (75%) of compound **28** was prepared by the reductive N-methylation of **27** (0.116 g, 0.35 mmol): TLC MeOH/$CHCl_3$/NH_4OH (3:7:2 drops)/silica gel, R_f 0.73; mp 205−207 °C; ^{1}H NMR (DMSO-d_6) δ 3.12 (s, 3H, N_{10}-CH_3), 4.69 (s, 2H, 9-CH_2), 6.19 (s, 2H, 2-NH_2, exch), 6.74 (dd, 1H, 6′-H), 6.93 (d, 1H, 2′-H), 7.06 (br s, 1H, 4-NH_2 exch), 7.33 (m, 3H, 5′-H, 4-NH_2 and 7-H or 8-H), 7.52 (d, 1H, 7-H or 8-H). Anal. ($C_{15}H_{14}N_6Cl_2 \cdot 1.0H_2O$) C, H, N, Cl.

2,4-Diamino-6-[(3′,4′,5′-trichloro-*N*-methylanilino)methyl]pyrido[3,2-*d*]pyrimidine (31). Compound **30** (0.050 g, 0.14 mmol) was reacted with 37% HCHO (0.075 mL, 0.9 mmol) and $NaCNBH_3$ (0.050 g, 0.74 mmol) for 24 h using the procedure described for **7**. After purification *via* a column with MeOH/$CHCl_3$ (3:47) as eluent, 0.028 g of **31** (55%) was obtained: TLC MeOH/$CHCl_3$/NH_4OH (3:7:2 drops)/silica gel, R_f 0.69; mp >250 °C dec; ^{1}H NMR (DMSO-d_6) δ 3.13 (s, 3H, N_{10}-CH_3), 4.72 (s, 2H, 9-CH_2), 6.24 (br s, 2H, 2-NH_2, exch), 6.98 (s, 2H, 2′-H and 6′-H), 7.05 (br s, 1H, 4-NH_2, exch), 7.38 (d, 1H, 7-H or 8-H), 7.39 (br, 1H, 4-NH_2, exch), 7.54 (d, 1H, 7-H or 8-H). Anal. ($C_{15}H_{23}N_6Cl_3 \cdot 0.42H_2O$) C, H, N, Cl.

2,4-Diamino-6-[(2′,4′,6′-trichloro-*N*-methylanilino)methyl]pyrido[3,2-*d*]pyrimidine (33). This compound was synthesized from **32** (0.080 g, 0.22 mmol) using the procedure described for compound **7**. After column chromatography using the same eluent used for **3**, 0.033 g (40%) of the product **33** was obtained: TLC MeOH/$CHCl_3$/NH_4OH (3:7:2 drops)/silica gel, R_f 0.71; mp 193−195 °C; ^{1}H NMR (DMSO-d_6) δ 2.72 (s, 3H, N_{10}-CH_3), 4.41 (s, 2H, 9-CH_2), 6.20 (s, 2H, 2-NH_2, exch), 7.18 (br, 1H, 4-NH_2, exch), 7.33 (br, 1H, 4-NH_2, exch), 7.59 (d, 1H, 7-H or 8-H), 7.67 (s, 2H, 3′-H and 5′-H), 7.82 (d, 1H, 7-H or 8-H). Anal. ($C_{15}H_{14}N_6Cl_3$) C, H, N, Cl.

General Procedure for Preparation of 40−44. Freshly prepared 2,4-diamino-6-(bromomethyl)pyrido[3,2-*d*]pyrimidine (**49**) (9 mmol), 2.25 equiv of *N*-(alkylamino)-*p*-aminoacetophenone analogue, and 2.7 equiv of dry $CaCO_3$ were suspended in 80 mL of dry DMAc under an atmosphere of dry argon and stirred at 100 °C in an oil bath. The reaction was monitored by TLC ($CHCl_3$/MeOH, 7:3). After disappearance of the reactive bromomethyl intermediate **49** (6−12 h), the reaction mixture was cooled and the solvent removed under reduced pressure on a rotary evaporator with applied heat (60 °C). Residual DMAc was removed by coevaporation with 3−4 volumes of xylene. The dark brown residue was resuspended in water and filtered to remove impure product as a dark orange precipitate. The aqueous filtrate was extracted with $CHCl_3$, dried over Na_2SO_4, and concentrated *in vacuo*. Solid residues were combined and applied to a silica gel column packed with $CHCl_3$. Unreacted starting materials and byproducts were eluted with $CHCl_3$/MeOH (85:15). Desired products were then eluted with $CHCl_3$/MeOH (75:25) as DMAc adducts.

4-[[(2,4-Diaminopyrido[3,2-*d*]pyrimidin-6-yl)methyl]-amino]acetophenone (40). The DMAC adduct was recrystallized from acetone/water as yellow crystals: 57% yield; mp 255−256 °C dec; MS (FAB) *m*/*e* 309 (MH^+); UV_{max} (ϵ_{max}) (pH 1) 324 (24 696); ^{1}H NMR (DMSO-d_6) δ 2.39 (s, 3H, 4′-$COCH_3$), 4.5 (d, 2H, 9-CH_2), 6.17 (br s, 2H, 2-NH_2), 6.7 and 7.7 (q, 4H, C_6H_4), 7.2 (t, 1H, N_{10}-H), 7.5 (m, 2H, 7-H and 8-H), and 7.4 to 7.5 (br s, 2H, 4-NH_2); high-resolution mass calcd for $C_{16}H_{16}N_6O$ 309.146 38, obsd 309.147 05.

4′-[[(2,4-Diaminopyrido[3,2-*d*]pyrimidin-6-yl)methyl]-methylamino]acetophenone (41). The DMAC adduct was purified by dissolution in 0.1 N HCl, treatment with Norit decolorizing charcoal, filtration through Celite, and neutralization with 0.1 N NaOH. The product was collected as an off-white powder: 39% yield; mp 280−282 °C dec; MS (FAB) *m*/*e* 323 (MH^+); UV_{max} (ϵ_{max}) (pH 1), 336 (27 095); ^{1}H NMR (DMSO-d_6) δ 2.4 (s, 3H, 4′-$COCH_3$), 3.2 (s, 3H, N_{10}-CH_3), 4.8 (s, 2H, 9-CH_2), 6.2 (br s, 2H, 2-NH_2), 6.8 and 7.7 (q, 4H, C_6H_4), 7.1 (br s, 2H, 4-NH_2), 7.3 and 7.5 (q, 2H, 7-H and 8-H); high-resolution mass calcd for $C_{19}H_{18}N_6O$ 323.162 03, obsd 323.160 42.

4-[[(2,4-Diaminopyrido[3,2-*d*]pyrimidin-6-yl)methyl]-proparglylamino]acetophenone (42). The DMAC adduct was purified by dissolution in EtOH/0.1 N HCl, treatment with Norit decolorizing charcoal, filtration through Celite, neutralization with 0.1 N NaOH, and concentration *in vacuo*. The product was collected as a light tan powder: 39.5% yield; mp 240−241 °C dec; MS (FAB) *m*/*e* 347 (MH^+); UV_{max} (ϵ_{max}) (pH 1) 326 (25 262); ^{1}H NMR (DMSO-d_6) δ 2.4 (s, 3H, 4′-$COCH_3$), 3.2 (t, 1H, N_{10}-*H*CCCH$_2$), 4.5 (d, 2H, HCCC*H*$_2$-N_{10}), 4.8 (s, 2H, 9-*C*H$_2$), 6.3 (br s, 2H, 2-NH_2), 6.8 and 7.8 (q, 4H, C_6H_4), 7.4 (br s, 2H, 4-NH_2), 7.5 (m, 2H, 7-H and 8-H); high-resolution mass calcd for $C_{19}H_{18}N_6O$ 347.162 03, obsd 347.162 12.

4-[[(2,4-Diaminopyrido[3,2-*d*]pyrimidin-6-yl)methyl]-methylamino]trifluoroacetophenone (43). The DMAC adduct was purified as described for **42** above. The product was collected as a pale yellow powder: 25.6% yield; mp 210−211 °C dec; MS (FAB) *m*/*e* 377 (MH^+); UV_{max} (ϵ_{max}) (pH 1) 366 (28 878); ^{1}H NMR (DMSO-d_6) δ 3.2 (s, 3H, N_{10}-CH_3), 4.8 (s, 2H, 9-*C*H$_2$), 6.2 (br s, 2H, 2-NH_2), 7.0 and 7.8 (q, 4H, C_6H_4), 7.1 (br s, 2H, 4-NH_2), 7.4 and 7.5 (q, 2H, 7-H and 8-H). Anal. ($C_{17}H_{15}N_6F_3O \cdot H_2O$) C, H, N.

4-[[(2,4-Diaminopyrido[3,2-*d*]pyrimidin-6-yl)methyl]-propargylamino]trifluoroacetophenone (44). The DMAC adduct was purified as described for **42** above. The product was collected as a white powder: 26% yield; mp 226−227 °C dec; MS (FAB) *m*/*e* 401 (MH^+); UV_{max} (ϵ_{max}) (pH 1) 354 (30 030); ^{1}H NMR (DMSO-d_6) δ 3.3 (t, 1H, *H*CCCH$_2$-N_{10}), 4.6 (d, 2H, HCCC*H*$_2$-N_{10}), 4.9 (s, 2H, 9-*C*H$_2$), 6.2 (s, 2H, 2-NH_2), 7.0 and 7.8 (q, 4H, C_6H_4), 7.1 and 7.3 (br s, 1H ea, 4-NH_2), 7.5 (collapsed m, 2H, 7-H and 8-H). Anal. ($C_{19}H_{15}N_6F_3O$) C, H, N.

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