Lipophilic Antifolates as Agents against Opportunistic Infections. 1. Agents Superior to Trimetrexate and Piritrexim against *Toxoplasma gondii* and *Pneumocystis carinii* in *in Vitro* Evaluations[1,2]

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2,4-Diaminopteridines (21 compounds) and 2,4-diamino-5-methyl-5-deazapteridines (34 compounds) along with three 2,4-diamino-5-unsubstituted-5-deazapteridines and four 2,4-diaminoquinazolines, each with an aryl group attached to the 6-position of the heterocyclic moiety through a two-atom bridge (either CH_2NH, $CH_2N(CH_3)$, CH_2S, or CH_2CH_2), were synthesized and evaluated as inhibitors of the growth of *Toxoplasma gondii* in culture and as inhibitors of dihydrofolate reductase enzymes from *T. gondii*, *Pneumocystis carinii*, and rat liver. Exceptionally high levels of combined potency and selectivity as growth inhibitors of *T. gondii* and as inhibitors of the microbial enzymes relative to the mammalian enzyme were found among the 5-methyl-5-deazapteridines but not for the other heterocyclic types. Thirty of the 34 5-methyl-5-deaza compounds gave growth inhibition IC_{50} values lower than that of pyrimethamine (0.4 μM) with 14 compounds below 0.1 μM, values that compare favorably with those for piritrexim and trimetrexate (both near 0.02 μM). As inhibitors of *T. gondii* DHFR, all but three of the 34 5-methyl-5-deaza compounds gave IC_{50} values in the order of magnitude with those of piritrexim (0.017 μM) and trimetrexate (0.010 μM), and 17 compounds of this group gave IC_{50} values versus *P. carinii* DHFR similarly comparable with those of piritrexim (0.031 μM) and trimetrexate (0.042 μM). Thirteen of these congeners gave both *T. gondii* growth inhibition and DHFR inhibition IC_{50} values of 0.10 μM or less, thus indicating facile penetration of the cell membrane. Eleven of these inhibitors of both *T. gondii* growth and DHFR have selectivity ratios (IC_{50} rat liver divided by IC_{50} *T. gondii*) of 5 or greater for the parasite DHFR. The highest selectivity ratio of nearly 100 belongs to the 5-methyl-5-deaza compound whose 6-substituent is $CH_2CH_2C_6H_3(OCH_3)_2$-2,5. This compound is over 10^3-fold more selective for *T. gondii* DHFR than bridge homologue piritrexim (selectivity ratio 0.088), a compound now in clinical trials. The candidate with $CH_2NHC_6H_3(CH_3)_2$-2,5 in the 6-position gave the highest *P. carinii* DHFR selectivity ratio of 4.0, which is about 60-fold more selective than trimetrexate (0.071) and 80-fold more selective than piritrexim (0.048) toward this enzyme. The 10 best compounds with respect to potency and selectivity includes six compounds bearing 2,5-disubstituted phenyl groups in the side chain (with little, if any, difference in effects of methyl, methoxy, or ethoxy), two side chains bearing 1-naphthyl groups, and two with 5,6,7,8-tetrahydro-1-naphthyl groups. Bridge groups represented in the 10 choice compounds are CH_2NH, $CH_2N(CH_3)$, CH_2CH_2, and CH_2S. The high levels of both potency and selectivity among these agents suggest that *in vivo* studies now underway may lead to agents that could replace trimetrexate and piritrexim in treatment of toxoplasmosis and *P. carinii* pneumonia.

Patients with AIDS, as well as patients with other immunosuppressive disorders, are susceptible to numerous protozoal, fungal, viral, and bacterial infections.[3,4] Toxoplasmosis[5] and *Pneumocystis carinii* pneumonia (PCP)[6] are examples that are now major causes of morbidity and mortality in immunocompromised patients.[7−13]

Current regimens for treatment of toxoplasmosis include pyrimethamine in combination with one of the following: sulfadiazine, clindamycin, clarithromycin, or azithromycin.[11] Atovaquone is also now in use.[12] Regimens for treatment of *P. carinii* pneumonia include the combinations trimethoprim−dapsone, trimethoprim−sulfamethoxazole, clindamycin−primaquine, and pentamidine−atovaquone.[13] Other agents studied in humans include lipophilic antifolates trimetrexate and piritrexim.[11,13]

Antifolates trimethoprim and pyrimethamine are used in combination with sulfa drugs in regimens that inhibit the ability of the microorganism to synthesize reduced folates. In these treatments, the antifolate inhibits dihydrofolate reductase (DHFR), and the sulfa drug inhibits utilization by the microorganism of 4-aminobenzoic acid in its vital biosynthesis of dihydropteroic acid.[7,8,14]

Adverse reactions that frequently occur with these regimens often necessitate discontinuation of the therapy. Other serious complications are the emergence of drug resistance and the occurrence of relapses after discontinuation of therapy (reviewed in ref 3). New agents or combinations of agents of greater therapeutic efficacy than agents now available are needed.

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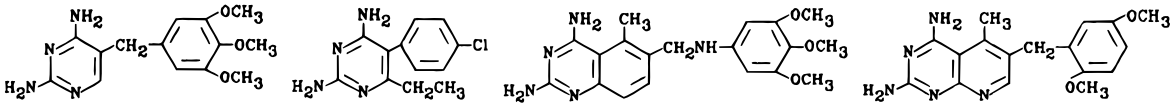
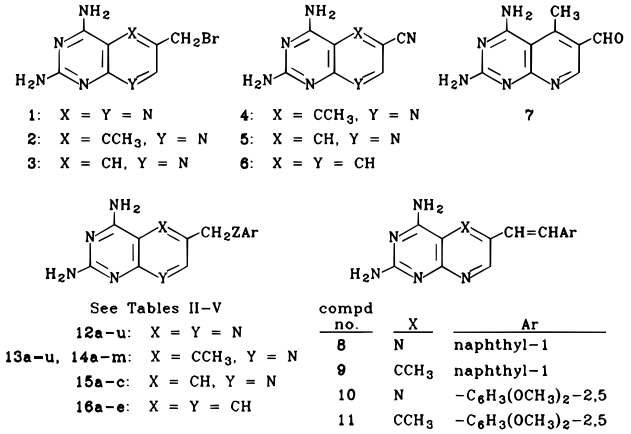
Table 1. Comparative Inhibition Data *vs T. gondii* Cell Growth and DHFR from *P. carinii*, *T. gondii*, and Rat Liver for Known Lipophilic Antifolates



compound	*T. gondii* cell growth inhibn IC_{50} (μM)[a]	IC_{50} (μM) *vs* DHFR[b]				
		P. carinii	rat liver	selectivity rl/pc	*T. gondii*	selectivity rl/tg
trimethoprim	nd	12	133	11.1	2.73	48.7
pyrimethamine	0.4	3.65	2.3	0.63	0.39	5.9
trimetrexate	*ca.* 0.02[c]	0.042	0.003	0.071	0.010	0.29
piritrexim	0.02	0.031	0.0015	0.048	0.017	0.088

[a] Methods described in ref 18. [b] Methods described in ref 14. [c] Reported (in ref 7) to be somewhat more inhibitory than piritrexim.

Scheme 1



Trimetrexate and piritrexim, now undergoing clinical trials,[11,13] are similar in their inhibitory effects against DHFR from *T. gondii* and *P. carinii*, and each is a more potent DHFR inhibitor than pyrimethamine or trimethoprim. Included in Table 1 are inhibition results from these four antifolates against DHFRs from *T. gondii*, *P. carinii*, and rat liver.[14–16] Unlike pyrimethamine or trimethoprim, both piritrexim and trimetrexate are more inhibitory toward mammalian DHFR than the pathogen DHFRs. Thus neither piritrexim nor trimetrexate offers beneficial selectivity; they are selective for the mammalian DHFR. These compounds can, however, be co-administered with the reduced folate leucovorin which, because of its requirement for active or carrier-mediated cell membrane transport, protect host cells but not the microorganism from the effects of DHFR inhibition.[17] Trimetrexate/leucovorin has been approved for the treatment of PCP in AIDS patients who are unable to take standard trimethoprim–sulfamethoxazole therapy.[13]

Two lipophilic antifolates of the 2,4-diaminopteridine class that we prepared initially for antitumor testing,[21,24] the 1-naphthylamino compound **12a** and the phenylthio compound **12b** (structures in Scheme 1 and Table 2), showed favorable differential activity toward DHFR from *P. carinii* and *T. gondii* in comparisons with mammalian DHFR[14,19,22] (see Table 2). Phenylthio compound **12b** is quite weak compared with naphthylamino compound **12a** with respect to *T. gondii* growth inhibition in culture and inhibition of DHFR from the three sources. But relative to trimethoprim, which is a selective inhibitor,[20] **12b** is more inhibitory and has greater selectivity toward the DHFRs from both pathogens. The favorable DHFR inhibition selectivities by **12a** and **12b** prompted a project aimed toward identifying therapeutically useful agents of greater potency and selectivity than agents now in use.

The known antiparasitic activity of piritrexim, a 5-methyl-substituted member of the 5-deazapteridine (pyrido[2,3-*d*]pyrimidine) class, combined with the accessibility of key intermediates from which ring-system analogues of piritrexim may be derived, prompted us to do a comparative study of the properties of 2,4-diaminopteridines and 2,4-diamino-5-methyl-5-deazapteridines bearing the same lipophilic group in the 6-position.

We report our results on candidates whose structural types are shown in Scheme 1 with their lipophilic side chains identified in Tables 2–4. In Table 2, direct comparisons are made of 21 pairs of pteridine types (**12a–u**) with 5-methyl-5-deazapteridines (**13a–u**) bearing the same side chains. Shown in Table 3 are test results on 13 other 5-methyl-5-deaza candidates. Table 4 shows results on three 5-unsubstituted-5-deaza analogues (**15a–c**) and four quinazoline (5,8-dideaza) analogues (**16a–d**) along with side-by-side comparisons with side-chain analogues from the 5-methyl-5-deaza group. Table 5 consists of a collection of the overall 10 best compounds from this study with potency and selectivity for DHFR from the pathogenic organisms considered together.

Key synthetic precursors to the compounds listed in Tables 2–4 are shown in Scheme 1. Piper *et al.* reported bromomethyl compounds **1**,[21,23] **2**,[25–28] and **3**,[27,28] the nitriles **4**[25] and **5**,[25] and the aldehyde **7**.[27,30] 2,4-Diaminoquinazoline-6-carbonitrile (**6**) was reported by Davoll and Johnson.[31] The reports describing the intermediates include procedures for converting them to pure dialkyl esters of classical antifolate types. Those procedures proved readily adaptable to preparations of the compounds of Tables 2–4, which were prepared by one of three general methods: (1) displacement reactions of the bromomethyl compounds with substituted aromatic amines and thiols, (2) reductive alkylation between 6-carbonitriles and anilino types, and (3) by catalytic hydrogenation of olefins **8–11** (Scheme 1) derived from **1**, **2**, or **7** using the Wittig reaction. The method used for each candidate is given in the Experimental Section. The reported methodology[21–30] was also applied by Gangjee *et al.*[33] in syntheses of analogues along with some of the compounds included in this study.

Comparison of the test results in Table 2 from pteridine lead compound **12a** with its 5-methyl-5-deaza counterpart **13a** bearing the same side chain shows **13a**

Table 2. Comparisons of Inhibitory Effects of Pteridines **12a**−**u** and 5-Methyl-5-deazapteridines **13a**−**u** Bearing the Same Side Chains against *T. gondii* Cell Growth and DHFR from *P. carinii*, *T. gondii*, and Rat Liver

			IC_{50}, μM[b]				
compd no.	side chain ZAr (Scheme 1)	*T. gondii* cell growth inhibn[a] IC_{50}, μM	*P. carinii*	rat liver	selectivity rl/pc	*T. gondii*	selectivity rl/tg
12a	$NHC_{10}H_7$-1	0.25	0.13[c]	1.26[c]	9.7[c]	0.076[c]	16.6[c]
13a		0.012	0.22	0.11	0.5	0.24	0.46
12b	SC_6H_5	10	9.5[c]	246[c]	25.9[c]	0.77[c]	319[c]
13b		0.2	0.44	0.43	1	0.034	12.6
12c	$SC_{10}H_7$-1	3	4.2	8.2	2	7	1.2
13c		0.04	0.29	0.55	1.9	0.03	18.3
12d	$SC_6H_4CH_3$-3	4	21.2	8.48	0.4	1.8	4.7
13d		0.2	0.17	0.33	2	0.065	5.1
12e	$SC_6H_4CH_3$-4	5	30	26	0.87	15	1.7
13e		0.3	0.53	0.5	0.93	0.057	8.7
12f	$SC_6H_4OCH_3$-3	5	9.9	7.7	0.78	1.6	4.8
13f		0.2	0.34	0.6	1.8	0.11	5.3
12g	$SC_6H_4OCH_3$-4	3	17.2	10.2	0.59	14	0.73
13g		0.3	0.56	0.52	0.93	0.063	8.3
12h	$SC_6H_3(OCH_3)_2$-3,4	5	58.2	36.3	0.62	23.2	1.6
13h		5	0.15	0.18	1.2	0.03	5.9
12i	SC_6H_4Cl-3	>10	42.2	26.6	0.63	5.7	4.7
13i		0.15	0.068	0.19	2.8	0.09	2.1
12j	SC_6H_4Cl-4	>10	42.1	14.3	0.34	16.8	0.85
13j		0.3	0.36	0.37	1.03	0.09	4.1
12k	NHC_6H_4Cl-4	0.7	1.5	0.3	2.1	0.59	0.52
13k		0.15	0.11	0.072	0.65	0.032	2.2
12l	NHC_6H_3-2-CH_3-5-OCH_3	1	>3	>3		0.44	
13l		0.03	0.038	0.15	3.9	0.023	6.3
12m	NHC_6H_3-2-CH_3-6-OCH_3	5	15.8	5.7	0.36	1.85	3.1
13m		0.3	1	0.32	0.32	0.1	0.3
12n	$NHC_6H_3(OCH_3)_2$-2,5	5	6.2	22.9	3.7	6.9	3.3
13n		0.2	0.011	0.010	0.94	0.014	0.73
12o	$N(CH_3)C_6H_3(OCH_3)_2$-2,5	1.6	3.9	0.47	0.1	0.21	2.2
13o		0.2	0.7	0.46	0.7	0.028	16.4
12p	$NHC_6H_3(OCH_3)_2$-3,5	1	0.96	0.88	0.92	0.11	8
13p		0.06	0.049	0.031	0.6	0.0035	8.9
12q	$NHC_6H_2(OCH_3)_3$-3,4,5	11	7	1.9	0.27	1	1.9
13q		1.0	0.013	0.0054	0.42	0.0027	2
12r	NHC_6H_3-2-OCH_3-5-CF_3	2	21	21	1.0	10.6	2.2
13r		0.02	0.044	0.02	0.5	0.022	1
12s	NHC_6H_3-3-OCH_3-5-CF_3	1	0.68	1.9	2.8	0.89	2.1
13s		0.2	0.02	0.017	0.9	0.018	1
12t	$CH_2C_{10}H_7$-1	1	97	60	0.62	0.82	73.2
13t		0.1	0.064	0.135	2.1	0.026	5.2
12u	$CH_2C_6H_3(OCH_3)_2$-2,5	5	11.1	23.2	2.1	5.4	4.3
13u		0.1	0.34	0.77	2.3	0.0079	97.5

[a] Average of 3−4 runs with excellent agreement; done as described in ref 18. *b* Methods described in ref 14. [c] Results listed are from ref 14.

Table 3. Inhibition Data vs *T. gondii* Cell Growth and DHFR from *P. carinii*, *T. gondii*, and Rat Liver for 5-Methyl-5-deazapteridines **14a**−**m**

			IC_{50},μM				
compd no.	side chain ZAr (Scheme 1)	*T. gondii* cell growth inhibn $IC_{50,\mu M}$	*P. carinii*	rat liver	selectivity rl/pc	*T. gondii*	selectivity rl/tg
14a	$NHC_6H_3(CH_3)_2$-2,5	0.01	0.03	0.12	4.0	0.016	7.5
14b	NHC_6H_3-2-CH_3-4-OCH_3	0.6	0.17	0.029	0.2	0.0072	4
14c	NHC_6H_3-2-OCH_3-5-CH_3	0.05	0.068	0.16	2.4	0.015	10.7
14d	$NHCH_6H_3(OCH_3)_2$-2,4	0.2	0.091	0.061	0.7	0.01	6.1
14e	$N(CH_3)C_6H_3(OCH_3)_2$-2,4	0.3	0.35	0.23	0.7	0.053	4.3
14f	$N(CH_3)C_6H_3(OCH_3)_2$-3,5	0.01	0.05	0.013	0.3	0.004	3.2
14g	$NHC_6H_3(OCH_3)_2$-3,4	0.6	0.051	0.0065	0.13	0.0027	2.4
14h	$N(CH_3)C_6H_3(OCH_3)_2$-3,4	0.15	0.029	0.0052	0.2	0.0029	1.8
14i	$NHC_6H_3(OC_2H_5)_2$-2,5	0.06	0.125	0.37	3	0.022	16.4
14j	$N(CH_3)C_6H_3(OC_2H_5)_2$-2,5	0.06	0.167	0.53	3.2	0.029	18.3
14k	$N(CH_3)C_6H_3$-2-OCH_3-5-CF_3	0.2	0.093	0.23	2.5	0.038	6.2
14l	$NHC_6H_3(CH_2)_4$-2,3	0.06	0.1	0.13	1.3	0.026	5
14m	$N(CH_3)C_6H_3(CH_2)_4$-2,3	0.02	0.15	0.14	0.9	0.016	8.8

to be more potent as a *T. gondii* growth inhibitor but less selective than **12a** toward DHFR from both organisms and also less inhibitory toward *T. gondii* DHFR. This particular comparison favoring the pteridine derivative **12a** over its 5-methyl-5-deaza counterpart **13a** in potency measures is the only example favoring the pteridine in the 21 pairings listed in Table 2. Comparison of results from the phenylthio compounds **12b** and **13b** shows a typical difference in relative potencies of counterparts from the two ring systems, although the weaker inhibitor **12b** does display greater selectivity in the DHFR inhibition comparisons. Of the pteridine

Table 4. Comparative Inhibition Data for Side Chain Analogues 5-Unsubstituted-5-Deazapteridines **15a**−**c** and 5,8-Dideazapteridines **16a**−**d** with 5-Methyl-5-deazapteridines **13a,k,q,n**

			IC_{50}, μM				
compd no.	side chain ZAr (Scheme 1)	*T. gondii* cell growth inhibn IC_{50}, μM	*P. carinii*	rat liver	selectivity rl/pc	*T. gondii*	selectivity rl/tg
15a	$NHC_{10}H_7$-1	0.8	0.26	0.23	0.88	0.15	1.5
16a		0.06	0.21	0.055	0.3	0.027	2
13a		0.012	0.22	0.11	0.5	0.24	0.46
15b	NHC_6H_4Cl-4	4	0.97	0.72	0.74	0.3	2.4
16b		0.01	0.6	0.073	0.12	0.075	1.0
13k		0.15	0.11	0.072	0.65	0.032	2.2
16c	NHC_6H_3-4-Cl-2-CH_3	0.2	0.33	0.023	0.07	0.033	0.7
15c	$NHC_6H_2(OCH_3)$-3,4,5	15	2	0.81	0.41	0.13	6.2
13q		1.0	0.013	0.0054	0.42	0.0027	2.0
16d	$NHC_6H_3(OCH_3)_2$-2,5	0.6	0.75	0.46	0.6	0.14	3.3
13n		0.2	0.011	0.010	0.94	0.014	0.73

Table 5. Comparative Inhibition Data vs *T. gondii* Cell Growth and DHFR from *P. carinii*, *T. gondii*, and Rat Liver for 5-Methyl-5-deazapteridines Selected from Tables 2−4 Showing Highest Overall Potency and Selectivity

			IC_{50}, μM				
compd no.	side chain ZAr	*T. gondii* cell growth inhibn IC_{50}, μM	*P. carinii*	rat liver	selectivity rl/pc	*T. gondii*	selectivity rl/tg
		Ar = 2,5-Disubstituted Phenyl					
13l	NHC_6H_3-2-CH_3-5-OCH_3	0.03	0.038	0.15	3.9	0.023	6.3
13u	$CH_2C_6H_3(OCH_3)_2$-2,5	0.1	0.34	0.77	2.3	0.0079	97.5
14a	$NHC_6H_3(CH_3)_2$-2,5	0.01	0.03	0.12	4.0	0.016	7.5
14c	NHC_6H_3-2-OCH_3-5-CH_3	0.05	0.068	0.16	2.4	0.015	10.7
14i	$NHC_6H_3(OC_2H_5)_2$-2,5	0.06	0.125	0.37	3	0.022	16.4
14j	$N(CH_3)C_6H_3(OC_2H_5)_2$-2,5	0.06	0.167	0.53	3.2	0.029	18.3
		Ar = 1-Naphthyl ($C_{10}H_7$-1)					
13c	$SC_{10}H_7$-1	0.04	0.29	0.55	1.9	0.03	18.3
13t	$CH_2C_{10}H_7$-1	0.1	0.064	0.135	2.1	0.026	5.2
		Ar = Tetrahydronaphthyl ($C_6H_3(CH_2)_4$-2,3)					
14l	$NHC_6H_3(CH_2)_4$-2,3	0.06	0.1	0.13	1.3	0.026	5
14m	$N(CH_3)C_6H_3(CH_2)_4$-2,3	0.02	0.15	0.14	0.9	0.016	8.8

candidates of Table 2, however, only **12a** shows combined overall potency and selectivity that compare favorably with values for pyrimethamine. In contrast, of the 5-methyl-5-deazapteridines, 19 of the candidates in the Table 2 pairings are at least as effective as pyrimethamine in inhibiting *T. gondii* cell growth *in vitro*, and all are more inhibitory than pyrimethamine toward DHFR from both *P. carinii* and *T. gondii*. These results led to our discontinuing work on the pteridine derivatives in order to concentrate our efforts on 5-deazapteridines, mostly 5-methyl-substituted.

Table 3 shows results from 13 additional 5-methyl-5-deazapteridine types (**14a**−**m**), all with CH_2NH or $CH_2N(CH_3)$ bridges between the heteroaromatic ring and the lipophilic side chain. Of the 34 5-methyl-5-deazapteridine types in Tables 2 and 3, 30 candidates are more inhibitory to growth of *T. gondii* than pyrimethamine; 13 of these are of exceptionally high potency with growth IC_{50} values of 0.1 μM or less.

As inhibitors of DHFR from *P. carinii*, 17 of the 5-methyl-5-deaza types have IC_{50} values of less than 0.10 μM, placing them in the order of magnitude with piritrexim (0.031 μM) and trimetrexate (0.042 μM) as inhibitors of the enzyme from this parasite. Against DHFR from *T. gondii*, all but three of the 5-methyl-5-deaza types have IC_{50} values of less than 0.1 μM, values which compare favorably with those for trimetrexate (0.010 μM) and piritrexim (0.017 μM).

A measure of the penetration of the *T. gondii* cell membrane by these candidates is gained by correlating the effects on cell parasite inhibition with inhibitory effect on the isolated enzyme.[19] An example of apparently poor cell membrane penetration is compound **13q**, which, despite potent inhibition of *T. gondii* DHFR, is not a good growth inhibitor.

Several 5-methyl-5-deaza types show excellent overall potency toward *T. gondii*. In addition, 13 candidates display favorable cell penetration as evidenced by both growth inhibition IC_{50} and DHFR inhibition IC_{50} values of 0.10 μM or less. In selectivity, all but two (**13r** and **14f**) of this group with high potency versus cell growth and enzyme have *T. gondii* DHFR selectivity ratios of 5 or above. The highest ratio of nearly 100 belongs to candidate **13u** (Table 3), a 10-deaza compound (CH_2-CH_2 bridge). These high selectivity ratios for the pathogen DHFR greatly exceed those of trimetrexate (0.29) and piritrexim (0.088), which are selective for mammalian DHFR. Thus compound **13u**, a bridge homolog of piritrexim, is over 300-fold more selective than trimetrexate and over 1000-fold more selective than piritrexim toward *T. gondii* DHFR.

Against *P. carinii* DHFR, approximately half of the 5-methyl-5-deaza analogues produced IC_{50} values of 0.10 μM or less, but the highest selectivity ratios toward the *P. carinii* enzyme are near 4 (for **13l** and **14a**). Although this ratio represents a lower level of selectivity toward *P. carinii* DHFR than those shown by several of the analogues toward the *T. gondii* DHFR, it is decidedly better than those for trimetrexate (0.071) and piritrexim (0.048), which are selective for the mammalian enzyme.

Early in this study we synthesized some 5-unsubstituted-5-deaza analogues (**15a**−**c**, Table 4). These compounds lacked the potency and selectivity of 5-methyl-

5-deaza types, and we did not pursue them further. In the 5,8-dideazapteridine (quinazoline) series, the heteroaromatic ring system of trimetrexate, we studied four analogues (**16a**−**d**, Table 4). The quinazoline types are potent inhibitors of *T. gondii* cell growth and DHFR from *T. gondii*. Also their selectivity ratios, at least toward *T. gondii* DHFR, are much more favorable than those of trimetrexate, but they are not particularly impressive when compared with ratios from several of the 5-methyl-5-deazapteridines.

In consideration of the effect of the bridge group on the *in vitro* results, beginning with comparisons of CH_2-NH with $CH_2N(CH_3)$, Tables 2 and 3 contain seven pairs of this type among 5-methyl-5-deaza candidates (**13n**,**o**; **13p**, **14f**; **13r**, **14k**; **14d,e**; **14i**,**j**; **14g**,**h**; **14l**,**m**). We observed that, in most comparisons, little activity benefit resulted from introduction of a methyl group at N^{10}. An exception is in 2,5-dimethoxyphenyl compounds **13n,o**; the N^{10}-CH_3 compound **13o** is less inhibitory toward mammalian DHFR than N^{10}-H compound **13n**, resulting in a higher selectivity ratio for **13o**. Against *P. carinii* DHFR, **13o** is considerably less potent than **13n**. Gangjee *et al.*[33] reported similar findings on this pair of candidates and also reported that introduction of higher N^{10}-alkyl groups leads to a decrease in activity.

In the 10-thia series (CH_2S bridge), of the nine 5-methyl-5-deaza types listed in Table 2 (**13b**−**j**), seven candidates (**13b**−**h**) gave selectivity ratios for *T. gondii* DHFR of greater than 5. The 1-naphthylthio compound **13c** has the highest selectivity ratio,[18] and this compound is also one of the most potent of all candidates in this report toward *T. gondii* growth inhibition (IC_{50} 0.04 μM). The 1-naphthylamino compound **13a** also displays excellent potency against *T. gondii* growth (IC_{50} 0.012), but **13a** is not a selective inhibitor of *T. gondii* DHFR (selectivity ratio 0.5). The high potency against *T. gondii* in culture of these 1-naphthyl compounds is at least partially attributable to facile passive diffusion through the cell membrane by these compounds of high lipophilic character. While most of the 10-thia compounds are nearly as potent toward *T. gondii* as piritrexim, their potency levels versus *P. carinii* DHFR are not particularly remarkable, although they are more potent and selective toward this enzyme than pyrimethamine.

The 10-deaza compounds, **12t,u** and **13t,u**, have produced interesting findings. As previously mentioned, the 2,5-dimethoxyphenyl compound **13u** has a selectivity ratio of nearly 100. It also has sufficient potency toward *T. gondii* cell growth and DHFR inhibition to make it a highly promising candidate as an agent against toxoplasmosis. The pteridine counterpart **12u** remained consistent with the trend mentioned earlier and is not effective. In another pteridine/5-methyl-5-deazapteridine pair, 10-deazapteridine analogue **12t** bearing a 1-naphthyl group showed weak effects in the *in vitro* evaluations, but it produced a selectivity ratio toward *T. gondii* DHFR of 73. We hoped that its 5-methyl-5-deaza counterpart **13t** would maintain high selectivity while affording greater potency. The expected enhanced potency was realized in **13t**, even toward *P. carinii* DHFR, but its *T. gondii* selectivity ratio is only about 5. The overall activity of **13t**, however, certainly including its activity and selectivity toward *P. carinii* DHFR, makes it also a compound of active interest.

In addition to **13t**, five other 5-methyl-5-deazapteridine derivatives (**13i**, **13l**, **14a**, **14c**,and **14k**) exert potent inhibition of *P. carinii* DHFR comparable with that of piritrexim or trimetrexate and also gave selectivity ratios from 2.3 to 4.0. These ratios are about 30−50-fold greater than for trimetrexate and 60−80-fold greater than for piritrexim. Among the six analogues, beginning with **13t**, the groups in the 10-position are CH_2, S, NH, NH, NH, and NCH_3. Of these, the 2,5-dimethylphenyl compound **14a** is the most selective toward *P. carinii* DHFR. Candidate **14a** is also at least as potent as the benchmark antifolates in inhibiting *T. gondii* DHFR as well as growth of the organism in culture; the exciting difference between **14a** and trimetrexate or piritrexim is its selectivity of 4.0 toward *P. carinii* DHFR and 7.5 toward *T. gondii* DHFR.

The best compounds from Tables 2−4, chosen with respect to potency and selectivity toward the DHFR from both microorganisms as well as growth inhibition of *T. gondii* in culture, are compiled in Table 5. Assessment of the results in terms of structural features of the overall best shows that the (2,4-diamino-5-methyl-5-deazapteridin-6-yl)methyl compounds bearing a 2,5-disubstituted phenyl group with either NH, NCH_3, S, or CH_2 in the 10-position bridge are generally superior in potency and selectivity over other candidates bearing variously substituted phenyl side chains. Alkyl and alkoxy groups appear to be equally effective (compare **13l** and **14c**, and **14a** and **14i**). Other compounds of significant overall potency and selectivity are those with added hydrocarbon bulk (naphthyl and tetrahydronaphthyl) apparently conferring a favorable degree of lipophilicity. An important feature of these potent and selective compounds is the excellent correlation between IC_{50} values for inhibition of the growth of *T. gondii* in culture and of DHFR from this organism, thereby indicating efficient cellular entry.

The results to date clearly suggest that among these candidates there could be agents capable of exerting significantly greater therapeutic efficacy against *T. gondii* and *P. carinii* than agents now in use or in clinical trial. Further studies are in progress. Selected candidates have been resynthesized and converted to H_2O-soluble hydrochloride derivatives for *in vivo* evaluations now underway.

Experimental Section

Examinations by TLC were performed on Analtech precoated (250 μm) silica gel G(F) plates. Purifications by preparative TLC were done on Analtech silica gel G(F) plates (2 mm). Column chromatographic purifications were done with silica gel (Merck, 60 A, 230−400 mesh for flash chromatography). When solubility limitations made it necessary, crude products to be purified were dispersed in silica gel for application to the column. Dispersal was achieved by evaporating *in vacuo* a solution of the crude product in DMF containing suspended silica gel (3 g of 60−200 mesh per g of crude product).[25−28] Evaporations were performed with a rotary evaporator; higher boiling solvents (DMF, Me_2NAc, Me_2SO) were removed *in vacuo* (<1 mm, bath to 35 °C) and more volatile solvents with a H_2O aspirator. Products were dried *in vacuo* (<1 mm) at 22−25 °C over P_2O_5 and NaOH pellets. Final products were dried and then allowed to equilibrate with ambient conditions of the laboratory. Elemental analysis results (for C, H, and N) indicated in Table 6 were within ±0.4% of the calculated values. Spectral determinations and

Table 6. ^{1}H NMR Spectral Data (Me_2SO-*d*6) for Target Compounds

compd no., molecular formula[a,b]	chemical shifts (δ, relative to TMS)
12a, $C_{17}H_{15}N_7 \cdot 0.4H_2O$	4.62 (m, C*H*$_2$NH), 6.54 (d, 2′-H), 6.60 (s, NH_2), 6.94 (t, CH_2NH), 7.14 (d, 4′-H), 7.24 (t, 3′-H), 7.46 (m, 6′-H and 7′-H), 7.68 (s, NH_2), 7.78 (t, 5′-H or 8′-H), 8.26 (t, 8′-H or 5′-H), 8.72 (s, 7-H)
12b, $C_{13}H_{12}N_6S$	4.39 (s, CH_2), 6.65 (br s, NH_2), 7.2 (m, 4′-H),7.3 (m, 3′-H and 5′-H), 7.4 (m, 2′-H and 6′-H), 7.64 (br s, NH_2), 8.69 (s, 7-H)
12c, $C_{17}H_{14}N_6S$	4.46 (s, CH_2), 6.62 (br s, NH_2), 7.34 (br s, NH_2), 7.46 (t, 3′-H), 7.50−7.60 (2m, 6′-H and 7′-H overlapping), 7.70 (d, 2′-H), 7.83 (d, 4′-H), 7.95 (m, 5′-H), 8.25 (m, 8′-H), 8.60 (s, 7-H)
12d, $C_{14}H_{14}N_6S \cdot 0.2H_2O$	2.26 (s, 3′-CH_3), 4.38 (s, CH_2), 6.67 (br s, NH_2), 7.0 (t, 5′-H), 7.18 (m, 4′-H and 6′-H), 7.22 (s, 2′-H), 7.46 and 7.67 (2 br s, NH_2, nonequivalent), 8.68 (s, 7-H)
12e, $C_{14}H_{14}N_6S \cdot 0.3H_2O$	2.26 (s, 4′-CH_3), 4.34 (s, CH_2), 6.66 (br s, NH_2), 7.12 (d, 3′-H and 5′-H), 7.29 (d, 2′-H and 6′-H), 7.42 and 7.65 (2 br s, NH_2, nonequivalent), 8.62 (s, 7-H)
12f, $C_{14}H_{14}N_6OS \cdot 0.2H_2O$	3.73 (s, 3′-OCH_3), 4.41 (s, CH_2), 6.66 (br s, NH_2), 6.75 (m, 4′-H), 6.92 (t, 2′-H), 6.96 (m, 6′-H), 7.21 (t, 5′-H), 7.43 and 7.66 (2 br s, NH_2, nonequivalent), 8.70 (s, 7-H)
12g, $C_{14}H_{14}N_6OS \cdot 0.3H_2O$	3.73 (s, 4′-OCH_3), 4.25 (s, CH_2), 6.64 (br s, NH_2), 6.88 (m, 3′-H and 5′-H), 7.31 (m, 2′-H and 6′-H), 7.36 and 7.63 (2 br s, NH_2, nonequivalent), 8.53 (s, 7-H)
12h, $C_{15}H_{16}N_6O_2S \cdot 0.6H_2O$	3.69 and 3.72 (2s, 3′-OCH_3 and 4′-OCH_3), 4.29 (s, CH_2), 6.63 (br s, NH_2), 6.8 to 6.94 (m, 2′-H, 5′-H, and 6′-H, overlapping), 7.36 and 7.62 (2 br s, NH_2, nonequivalent), 8.54 (s, 7-H)
12i, $C_{13}H_{11}ClN_6S \cdot 0.4H_2O$	4.47 (s, CH_2), 6.71 (br s, NH_2), 7.20 to 7.48 (m, 4′-H, 5′-H, 6′-H, and 1 H of a nonequivalent NH_2, overlapping), 7.51 (m, 2′-H), 7.74 (br s, 1 H of a nonequivalent NH_2), 8.74 (s, 7-H)
12j, $C_{13}H_{11}ClN_6S \cdot 0.5H_2O$	4.41 (s, CH_2), 6.74 (br s, NH_2), 7.36 (m, 3′-H and 5′-H overlapping), 7.44 (m, 2′-H and 6′-H, overlapping), 7.52−7.74 (2 br s, NH_2, nonequivalent), 8.7 (s, 7-H)
12k, $C_{13}H_{12}ClN_7 \cdot 0.5H_2O$	4.41 (d, C*H*$_2$NH), 6.49 (t, CH_2N*H*), 6.59 (br s, NH_2), 6.72 (m, 2′-H and 6′-H), 7.12 (m, 3′-H and 5′-H), 7.68 and 7.79 (2 br s, NH_2, nonequivalent), 8.69 (s, 7-H)
2l, $C_{15}H_{17}N_7O \cdot 0.75H_2O$	2.10 (s, 2′-CH_3), 3.58 (s, 5′-OCH_3), 4.48 (d, C*H*$_2$NH), 5.75 (t, CH_2N*H*), 6.08 (m, 4′-H and 6′-H, overlapping), 6.60 (br s, NH_2), 6.87 (d, 3′-H), 7.64 (br s, NH_2), 8.68 (s, 7-H)
12m, $C_{15}H_{17}N_7O \cdot 0.2H_2O$	2.24 (s, 6′-CH_3), 3.7 (s, 2′-OCH_3), 4.42 (d, C*H*$_2$NH), 4.90 (m, CH_2N*H*), 6.55 (br s, NH_2), 6.62−6.76 (br m, 3′-H, 4′-H, and 5′-H, overlapping), 7.59 and 7.39 (2 br s, NH_2, nonequivalent), 8.65 (s, 7-H)
12n, $C_{15}H_{17}N_7O_2 \cdot 0.1H_2O$	3.58 (s, 5′-OCH_3), 3.76 (s, 2′-OCH_3), 4.46 (d, C*H*$_2$NH), 5.86 (d, CH_2N*H*), 6.07 (m, 4′-H), 6.13 (m, 6′-H), 6.60 (br s, NH_2), 6.72 (d, 3′-H), 7.60 (br s, NH_2), 8.66 (s, 7-H)
12o, $C_{16}H_{19}N_7O_2 \cdot 0.4H_2O$	2.7 (s, NC*H*$_3$), 3.67 (s, 5′-OCH_3), 3.77 (s, 2′-OCH_3), 4.35 (s, C*H*$_2$N), 6.47 (m, 4′-H and 6′-H, overlapping), 6.6 (br s, NH_2), 6.88 (t, 3′-H), 7.41 and 7.61 (2 br s, NH_2, nonequivalent), 8.7 (s, 7-H)
12p, $C_{15}H_{17}N_7O2 \cdot 0.1H_2O$	3.66 (s, 3′- and 5′-OCH_3), 4.42 (d, C*H*$_2$NH), 5.76 (m, 4′-H), 5.90 (d, 2′- and 6′-H), 6.36 (CH_2N*H*), 6.58 (br s, NH_2), 7.66 and 7.82 (2 br s,NH_2, nonequivalent), 8.70 (s, 7-H)
12q, $C16_6H_{19}N_7O_3 \cdot H_2O$	3.53 (s, 4′-OCH_3), 3.72 (s, 3′- and 5′-OCH_3), 4.44 (br s, C*H*$_2$NH), 6.04 (br s, 2′- and 6′-H), 6.10 (br s, CH_2N*H*), 6.60 (br s, NH_2), 7.68 and 7.86 (2 br s, NH_2, nonequivalent), 8.72 (s, 7-H)
12r, $C_{15}H_{14}F_3N_7O \cdot 0.3H_2O$	3.89 (s, 2′-OCH_3), 4.52 (d, C*H*$_2$NH), 6.17 (t, CH_2N*H*), 6.61 (br s, NH_2), 6.83 (s, 6′-H), 6.90 (d, 4′-H), 6.98 (d, 3′-H), 7.47 and 7.66 (2 br s, NH_2, nonequivalent), 8.69 (s, 7-H)
12s, $C_{15}H_{14}F_3N_7O \cdot 0.3H_2O$	3.75 (s, 5′-OCH_3), 4.48 (d, C*H*$_2$NH), 6.41, 6.5, and 6.67 (3s, 2′-H, 4′-H, and 6′-H), 6.60 (br s, NH_2), 6.78 (t, CH_2N*H*), 7.70 and 7.83 (2 br s, NH_2, nonequivalent), 8.72 (s, 7-H)
12t, $C_{18}H_{16}N_6 \cdot 0.6H_2O$	3.21 (t, CH_2C*H*$_2$), 3.55 (t, C*H*$_2$$CH_2$), 6.53 (br s, NH_2), 7.41 (m, 2′ and 3′-H, overlapping), 7.56 (m, 6′-H and 7′-H, overlapping), 7.62 (br s, NH_2), 7.78 (q, 4′-H), 7.93 (m, 5′-H), 8.24 (d, 8′-H), 8.58 (s, 7-H)
12u, $C_{16}H_{18}N_6O_2$	3.02 (m, C*H*$_2$C*H*$_2$), 3.63 and 3.69 (2s, 2′-OCH_3 and 5′-OCH_3), 6.5 (br s, NH_2), 6.71 (m, 4′-H and 6′-H, overlapping), 6.85 (d, 3′-H), 7.48 and 7.55 (2 br s, NH_2, nonequivalent), 8.49 (s, 7-H)
13a, $C_{19}H_{18}N_6 \cdot 1.1H_2O$	2.72 (s, 5-CH_3), 4.44 (d, C*H*$_2$$NH_2$), 6.18 (s, NH_2), 6.47−6.55 (two m, CH_2N*H* and 3′-H, overlapping), 6.98 (broad s, NH_2), 7.12 (d, 4′-H), 7.26 (t, 3′-H), 7.35−7.48 (two m, 6′-H and 7P-H, overlapping), 7.76 (m, 5′-H), 8.22 (m, 8′-H), 8.50 (s, 7-H)
13b, $C_{15}H_{15}N_5S \cdot 0.25H_2O$	2.71 (s, 5-CH_3), 4.28 (s, CH_2), 6.26 (br s, NH_2), 7.0 (br s, NH_2), 7.23 (m, 4′-H), 7.28−7.42 (br s, 2′-H, 3′-H, 5′-H, and 6′-H, overlapping), 8.34 (s, 7-H)
13c, $C_{19}H_{17}N_5S \cdot 0.25H_2O$	2.72 (s, 5-CH_3), 4.33 (s, CH_2), 6.26 (br s, NH_2), 7.02 (br s, NH_2), 7.48 (t, 3′-H), 7.52−7.60 (2m, 6′-H and 7′-H overlapping), 7.65 (m, 4′-H), 7.92 (m, 5′-H), 8.25 (m, 8′-H overlapping with s due to 7-H)
13d, $C_{16}H_{17}N_5S \cdot 0.7H_2O$	2.27 (s, 3′-CH_3), 2.72 (s, 5-CH_3), 4.28 (s, CH_2), 6.45 (br s, NH_2), 7.04 (d, 4′-H), 7.1−7.28 (br m, NH_2, 2′-H, 5′-H, and 6′-H, overlapping), 8.37 (s, 7-H)
13e, $C_{16}H_{17}N_5S \cdot 0.7H_2O$	2.27 (s, 4′-CH_3), 2.7 (s, 5-CH_3), 4.22 (s, CH_2), 6.39 (br s, NH_2), 7.12 (br d, NH_2 and either 2′-H and 6′-H or 3′-H and 5′-H, overlapping), 7.27 (d, either 2′-H and 6′-H or 3′-H and 5′-H), 8.28 (s, 7-H)
13f, $C_{16}H_{17}N_5OS \cdot 0.9H_2O$	2.72 (s, 5-CH_3), 3.75 (s, 3′-OCH_3), 4.32 (s, CH_2), 6.43 (br s, NH_2), 6.8 (m, 4′-H or 6′-H), 6.94 (d, 2′-H and either 4′-H or 6′-H, overlapping), 7.07−7.28 (br m, 5′-H and NH_2, overlapping), 8.39 (s, 7-H)
13g, $C_{16}H_{17}N_5OS \cdot 0.7H_2O$	2.68 (s, 5-CH_3), 3.73 (s, 4′-OCH_3), 4.15 (s, CH_2), 6.38 (br s, NH_2), 6.89 (m, 3′-H and 5′-H), 7.11 (br s, NH_2), 7.3 (m, 2′-H and 6′-H), 8.17 (s, 7-H)
13h, $C_{17}H_{19}N_5O_2S \cdot 0.9H_2O$	2.68 (s, 5-CH_3), 3.68 and 3.71 (2 s, 3′-OCH_3 and 4′-OCH_3), 4.17 (s, CH_2), 6.35 (br s, NH_2), 6.88 (m, 2′-H, 5′-H, and 6′-H overlapping), 7.1 (br s, NH_2), 8.18 (s, 7-H)

Table 6 (Continued)

compd no., molecular formula[a,b]	chemical shifts (δ, relative to TMS)
13i, $C_{15}H_{14}ClN_5S\cdot0.9H_2O$	2.71 (s, 5-CH_3), 4.36 (s, CH_2), 6.34 (br s, NH_2), 7.1 (br s, NH2), 7.24−7.36 (br m, 4′-H, 5′-H, and 6′-H, overlapping), 7.48 (s, 2′-H), 8.4 (s, 7-H)
13j, $C_{15}H_{14}ClN_5S\cdot0.6H_2O$	2.72 (s, 5-CH_3), 4.30 (s, CH_2), 6.41 (br s, NH_2), 7.14 (br s, NH_2), 7.38 (m, 2′-H, 3′-H, 5′-H, and 6′-H overlapping), 8.34 (s, 7-H)
13k, $C_{15}H_{15}ClN_6\cdot1.25H_2O$	2.66 (s, 5-CH_3), 4.2 (d, CH_2NH), 6.12 (t, CH_2NH), 6.38 (br s, NH_2), 6.64 (d, 2′-H and 6′-H), 7.10 (d, 3′-H, 5′-H, and NH_2, overlapping), 8.47 (s, 7-H)
13l, $C_{17}H_{20}N_6O\cdot0.1(C_2H_5)_2O\cdot0.5H_2O$	2.04 (s, 2′-CH_3), 2.68 (s, 5-CH_3), 3.62 (s, 5′-OCH_3), 4.29 (d, CH_2NH), 5.22 (t, CH_2NH), 6.05 (d, 6′-H), 6.09 (q, 4′-H), 6.25 (br s, NH_2), 6.86 (d, 3′-H), 7.02 (br s, NH_2), 8.44 (s, 7-H)
13m, $C_{17}H_{20}N_6O\cdot0.6H_2O$	2.25 (s, 6′-CH_3), 2.73 (s, 5-CH_3), 3.71 (s, 2′-OCH_3), 4.01 (br s, CH_2NH), 4.18 (s, CH_2NH), 6.28 (br s, NH_2), 6.68−6.8 (br m, 3′-H, 4′-H, and 5′-H overlapping), 7.05 (br s, NH_2), 8.4 (s, 7-H)
13n, $C_{17}H_{20}N_6O_2\cdot0.2H_2O$	2.67 (s, 5-CH_3), 3.62 (s, 5′-OCH_3), 3.73 (s, 2′-OCH_3), 4.27 (d, CH_2NH), 5.15 (t, CH_2NH), 6.07 (m, 4′-H), 6.12 (m, 6′-H), 6.20 (br s, NH_2), 6.70 (d, 3′-H), 6.95 (br s, NH_2), 8.42 (s, 7-H)
13o, $C_{18}H_{22}N_6O_2\cdot0.6H_2O$	2.55 (s, NCH_3), 2.68 (s, 5-CH_3), 3.68 and 3.75 (2 s, 2′-H and 5′-H), 4.17 (s, CH_2N), 6.29 (br s, NH2), 6.46−6.56 (m, 4′-H and 6′-H, overlapping), 6.87 (d, 3′-H), 7.03 (br s, NH_2), 8.45 (s, 7-H)
13p, $C_{17}H_{20}N_6O_2\cdot0.4H_2O$	2.65 (s, 5-CH_3), 3.64 (s, 3′-H and 5′-H), 4.18 (d, CH_2NH), 5.76 (m, 4′-H), 5.82 (m, 2′-H and 6′-H), 5.94 (t, CH_2NH), 6.23 (br s, NH_2), 7.00 (br s, NH_2), 8.46 (s, 7-H)
13q, $C_{18}H_{22}N_6O_3\cdot H_2O$	2.68 (s, 5-CH_3), 3.54 (s, 4′-OCH_3), 3.70 (s, 3′-OCH_3 and 5′-OCH_3), 4.19 (d, CH_2NH), 5.68 (t, CH_2NH), 5.96 (s, 2′-H and 6′-H), 6.22 (s, NH_2), 6.98 (s, NH_2), 8.50 (s, 7-H)
13r, $C_{17}H_{17}F_3N_6O\cdot0.4H_2O$	2.68 (s, 5-CH_3), 3.86 (s, 2′-OCH_3), 4.34 (d, CH_2NH), 5.52 (t, CH_2NH), 6.3 (br s, NH_2), 6.76 (s, 6′-H), 6.94 (q, 3′-H and 4′-H, overlapping), 7.06 (br s, NH_2), 8.44 (s, 7-H)
13s, $C_{17}H_{17}F_3N_6O\cdot0.6H_2O$	2.67 (s, 5-CH_3), 3.64 (s, 5′-OCH_3), 4.26 (d, CH_2NH), 6.32 (br s, NH_2), 6.40 (m, 2′-H, 6′-H, and CH_2NH, overlapping), 6.55 (s, 4′-H), 7.08 (br s, NH_2), 8.50 (s, 7-H)
13t, $C_{20}H_{19}N_5\cdot0.4H_2O$	2.62 (s, 5-CH3), 3.01 and 3.25 (2 t, CH_2CH_2), 6.2 (br s, NH_2), 6.96 (br s, NH_2), 7.31 (d, 2′-H), 7.41 (t, 3′-H), 7.55 (m, 6′-H and 7′-H), 7.79 (d, 4′-H) 7.94 (d, 5′-H), 8.16 (d, 8′-H), 8.32 (s, 7-H)
13u, $C_{18}H_{21}N_5O_2$	2.68 (s, 5-CH_3), 2.72 and 2.84 (2 m, CH_2CH_2), 3.67 and 3.73 (2 s, 2′-OCH_3 and 5′-OCH_3), 6.14 (br s, NH_2), 6.73 (m, 4′-H and 6′-H), 6.84−6.98 (br m, 3′-H and NH_2, overlapping), 8.28 (s, 7-H)
14a, $C_{17}H_{20}N_6\cdot0.3H_2O$	2.07 (s, 5′-CH_3), 2.17 (s, 2′-CH_3), 2.69 (s, 5-CH_3), 4.28 (d, CH_2NH), 5.05 (t, CH_2NH), 6.22 (br s, NH_2), 6.35 (d, 4′-H and 6′-H, overlapping), 6.86 (d, 3′-H), 6.99 (br s, NH_2), 8.44 (s, 7-H)
14b, $C_{17}H_{20}N_6O$	2.11 (s, 2′-CH_3), 2.68 (s, 5-CH_3), 3.62 (s, 4′-OCH_3), 4.23 (d, CH_2NH), 4.77 (m, CH_2NH), 6.29 (br s, NH_2), 6.46 (d, 6′-H), 6.59 (q, 5′-H), 6.66 (d, 3′-H), 7.05 (br s, NH_2), 8.43 (s, 7-H)
14c, $C_{17}H_{20}N_6O\cdot1.7H_2O$	2.16 (s, 5′-CH_3), 2.67 (s, 5-CH_3), 3.74 (s, 2′-OCH_3), 4.26 (d, CH_2NH), 4.93 (t, CH_2NH), 6.2 (br s, NH_2), 6.39 (t, 4′-H and 6′-H, overlapping), 6.7 (d, 3′-H), 6.97 (br s, NH_2), 8.44 (s, 7-H)
14d, $C_{17}H_{20}N_6O_2\cdot0.5(C_2H_5)_2O\cdot0.3H_2O$	2.67 (5-CH_3), 3.65 and 3.76 (2 s, 2′-OCH_3 and 4′-OCH_3), 4.22 (d, CH_2NH), 4.61 (t, CH_2NH), 6.2 (br s, NH_2), 6.35 (q, 5′-H), 6.49 (q, 3′-H and 6′-H, overlapping), 6.96 (br s, NH_2), 8.42 (s, 7-H)
14e, $C_{18}H_{22}N_6O_2\cdot0.25H_2O$	2.51 (s, NCH_3), 2.71 (s, 5-CH_3), 3.71 and 3.8 (2 s, 2′-OCH_3 and 4′-OCH_3), 4.03 (s, CH_2N), 6.18 (br s, NH_2), 6.4 (q, 5′-H), 6.54 (d, 3′-H), 6.9 (d, 6′-H), 6.94 (br s, NH_2), 8.37 (s, 7-H)
14f, $C_{18}H_{22}N_6O_2\cdot0.25H_2O$	2.6 (s, 5-CH_3), 2.9 (s, NCH_3), 6.67 (s, 2′-OCH_3 and 4′-OCH_3), 4.5 (s, CH_2N), 5.89 (m, 2′-H, 4′-H, and 6′-H, overlapping), 6.2 (br s, NH_2), 6.97 (br s, NH_2), 8.15 (s, 7-H)
14g, $C_{17}H_{20}N_6O_2\cdot0.4H_2O$	2.67 (s, 5-CH_3), 3.62 and 3.68 (2 s, 3′-OCH_3 and 4′-OCH_3), 4.16 (d, CH_2NH), 5.5 (t, CH_2NH), 6.12 (q, 6′-H), 6.19 (br s, NH_2), 6.37 (d, 2′-H), 6.72 (d, 5′-H), 6.97 (br s, NH_2), 8.48 (s, 7-H)
14h, $C_{18}H_{22}N_6O_2\cdot0.3H_2O$	2.62 (s, 5-CH_3), 2.79 (s, NCH_3), 3.64 and 3.71 (2 s, 3′-OCH_3 and 4′-OCH_3), 4.39 (s, CH_2N), 6.2 (br s, NH_2), 6.29 (q, 6′-H), 6.5 (d, 2′-H), 6.8 (d, 5′-H), 6.97 (br s, NH_2), 8.25 (s, 7-H)
14i, $C_{18}H_{22}N_6O_2\cdot0.25H_2O$	1.25 and 1.31 (2 t, 2′-OCH_2CH_3 and 5′-OCH_2CH_3), 2.67 (s, 5-CH_3), 3.86 and 3.96 (2 m, 2′-OCH_2CH_3 and 5′-OCH_2H_3), 4.29 (d, CH_2NH), 5.1 (t, CH_2NH), 6.06 (q, 4′-H), 6.12 (d, 6′-H), 6.2 (br s, NH_2), 6.69 (d, 3′-H), 6.97 (br s, NH_2), 8.42 (s, 7-H)
14j, $C_{20}H_{26}N_6O_2\cdot0.25H_2O$	1.27 (t, 2′-OCH_2CH_3 and 5′-OCH_2CH_3), 2.55 (s, NCH_3), 2.68 (s, 5-CH_3), 3.95 (m, 2′-OCH_2CH_3 and 5′-OCH_2CH_3), 4.18 (s, CH_2N), 6.17 (br s, NH_2), 6.45 (q, 4′-H), 6.5 (d, 6′-H), 6.83 (d, 3′-H), 6.95 (br s, NH_2), 8.48 (s, 7-H)
14k, $C_{18}H_{19}F_3N_6O\cdot1.4H_2O$	2.60 (s, NCH_3), 2.68 (s, 5-CH_3), 3.9 (s, 2′-OCH_3), 4.22 (s, CH_2N), 6.21 (br s, NH_2), 6.96 (br s, NH_2), 7.15 (d, 3′-H), 7.21 (s, 6′-H), 7.33 (d, 4′-H), 8.48 (s, 7-H)
14l, $C_{20}H_{24}N_6\cdot0.25H_2O$	1.68 (s, 6′-H_2 and 7′-H_2), 2.47 (s, NCH_3), 2.69 (t, 5-CH_3, 5′-H_2, and 8′-H_2 overlapping), 4.05 (s, CH_2N), 6.2 (br s, NH_2), 6.79 (m, 3′-H), 6.96 (br s, NH_2), 7.07 (d, 2′-H and 4′-H, overlapping), 8.43 (s, 7-H)
14m, $C_{19}H_{22}N_6\cdot0.25H_2O$	1.66 and 1.76 (2 m, 6′-H_2 and 7′-H_2), 2.41 (t, 5′-H_2 or 8′-H_2), 2.65 (m, 5-CH_3 and either 5′-H_2 or 8′-H_2, overlapping), 4.28 (d, CH_2NH), 5.04 (t, CH_2NH), 6.17 (br s, NH_2), 6.34 (q, 2′-H and 4′-H), 6.86 (t, 3′-H), 6.94 (br s, NH_2), 8.41 (s, 7-H)
15a, $C_{18}H_{16}N_6\cdot H_2O$	4.45 (d, CH_2NH), 6.23 (s, NH_2), 6.49 (d, 2′-H or 4′-H), 6.77 (t, CH_2NH), 7.11 (d, 2′-H or 4′-H), 7.23 (t, 3′-H), 7.30−7.55 (m, 6′-H, 7′-H, and NH_2, overlapping), 7.75 (m, 5′-H or 8′-H), 8.24 (8′-H or 5′-H), 8.42 (s, 5-H), 8.70 (s, 7-H)

Table 6 (Continued)

compd no., molecular formula[a,b]	chemical shifts (δ, relative to TMS)
15b, $C_{14}H_{13}ClN_6 \cdot 0.5H_2O$	4.21 (d, CH_2NH), 6.31 (t, CH$_2$NH), 6.37 (br s, NH$_2$), 6.62 (m, 2′-H and 6′-H), 7.09 (m, 3′-H and 5′-H), 7.55 (br s, NH$_2$), 8.38 (d, 5-H), 8.64 (d, 7-H)
15c, $C_{17}H_{20}N_6O_3 \cdot 0.5CH_3CO_2H \cdot 0.7H_2O$	3.50 (s, 4′-OCH$_3$), 3.67 (s, 3′-OCH$_3$ and 5′-OCH$_3$), 4.20 (d, CH_2NH), 5.92 (s, 2′-H, 6′-H and CH$_2$NH, overlapping), 6.32 (s, NH$_2$), 7.50 (s, NH$_2$), 8.38 (s, 5-H), 8.65 (s, 7-H)
16a, $C_{19}H_{17}N_5 \cdot CH_3CO_2H \cdot 0.7H_2O$	4.46 (d, CH_2NH), 6.0 (br s, NH$_2$), 6.45 (d, 2′-H), 6.82 (t, CH$_2$NH), 7.07 (d, 4′-H), 7.12−7.22 (m, 8-H and 3′-H, overlapping), 7.3 (br s, NH$_2$), 7.42 (m, 6′-H and 7′-H, overlapping), 7.56 (m, 7-H), 7.74 (m, 5′-H or 8′-H), 8.06 (s, 5-H), 8.24 (m, 5′-H or 8′-H)
16b, $C_{15}H_{14}ClN_5 \cdot 1.5CH_3CO_2H$	4.2 (d, CH_2NH), 5.98 (s, NH$_2$), 6.3 (t, CH$_2$NH), 6.61 (d, 2′-H and 6′-H), 7.07 (d, 3′-H and 5′-H), 7.18 (d, 8-H), 7.24 (br s, NH$_2$), 7.48 (q, 7-H), 7.98 (s, 5-H)
16c, $C_{16}H_{16}ClN_5 \cdot 1.7CH_3CO_2H$	2.16 (s, 2′-CH$_3$), 4.31 (d, CH_2NH), 5.69 (t, CH$_2$NH), 6.1 (br s, NH$_2$), 6.41 (d, 6′-H), 6.92 (q, 5′-H), 7.01 (d, 3′-H), 7.16 (d, 8-H), 7.31 (br s, NH$_2$), 7.5 (m, 7-H), 7.96 (s, 5-H)
16d, $C_{17}H_{19}N_5O_2 \cdot 2CH_3CO_2H \cdot 0.5H_2O$	3.57 and 3.72 (2 s, 2′-OCH$_3$ and 5′-OCH$_3$), 4.26 (d, CH_2NH), 5.46 (t, CH$_2$NH), 6.05 (m, 4′-H and 6′-H, overlapping), 6.1 (br s, NH$_2$), 6.68 (d, 3′-H), 7.16 (d, 8-H), 7.3 (br s, NH$_2$), 7.51 (d, 7-H), 8.0 (s, 5-H)

[a] Anal. C, H, N except for **12l** (H: calcd 5.74; found 5.23) and **13t** (C: calcd 71.36; found 71.81; N: calcd 20.81; found 20.32). ^{1}H NMR spectra confirmed solvates with CH_3CO_2H and $(C_2H_5)_2O$ in the molar ratios indicated by elemental analysis results. [b] The mass spectrum (FAB mode) of each sample was as expected for the assigned structure.

elemental analyses were performed in the Molecular Spectroscopy Section of Southern Research Institute. ^{1}H NMR spectra on target compounds are listed in Table 6. The ^{1}H NMR spectra were determined with a Nicolet NMC 300 NB spectrometer using Me_4Si as internal reference. Chemical shifts (δ) listed for multiplets were measured from the approximate centers, and relative integrals of peak areas agreed with those expected for the assigned structures. Mass spectra were recorded on a Varian MAT 311A mass spectrometer in the fast-atom-bombardment (FAB) mode. No attempt was made to observe melting points since the target compounds decompose at high temperatures without actually melting.

IC_{50} values for the various enzymes were determined as reported.[14] Briefly, DHFR activity was assayed spectrophotometrically with continuous recording in the absence of inhibitor and in the presence of several concentrations of inhibitor to yield an inhibition curve ranging from 10 to 90% of the value without inhibitor. Each assay was run in duplicate, and each was calculated relative to a blank that contained no substrate. Values calculated for percent inhibition were converted to probits, and the equation of the resulting straight line (least squares fit) was used to calculate the IC_{50} value. Curve fitting was done with Cricket Graph (Computer Associates). Linear regressions and correlation coefficients were obtained with InStat (GraphPad Software). Correlation coefficients for the linear regressions ranged from 0.7 to 0.99 and were typically above 0.9.

Each sample was tested in the form shown in Table 6, which is the formulation indicated by elemental analysis and spectral (^{1}H NMR and mass) results. All were free base forms except **15c** and **16a**−**d**, which were acetates. In both the *T. gondii* growth inhibition[18] and the DHFR inhibition[14] assays the sample was initially dissolved in Me_2SO to give a stock solution that was diluted with H_2O to make the series of dilutions required for determining the IC_{50} values. The carryover of Me_2SO was less than 0.1% in the enzyme assay solutions and less than 1% in the growth inhibition assay solutions. These levels of Me_2SO have no significant effect on either the DHFR assay under the conditions used (saturating substrate and 37 °C) or on the growth of *T. gondii*.

Preparations of 10-Thia Compounds 12b−j and 13b−j. The general procedure used is similar to that reported earlier for the preparation of **12b**[24] from **1**. The procedure for the preparation of 2,4-diamino-5-methyl-6-[(1-naphthylthio)-methyl]pyrido[2,3-*d*]pyrimidine (**13c**) is representative. A mixture of K_2CO_3 (3.88 g, 28.1 mmol of anhydrous powder) and 1-naphthalenethiol (4.50 g, 28.1 mmol) in Me_2NAc (42 mL) was stirred at 20−23 °C for 2 h before pulverized **2** (3.70 g, 8.50 mmol as **2**·1.7HBr·0.5AcOH[27,28]) was added. The mixture was stirred under N_2 in a stoppered flask for 24 h. The mixture, now containing solid **13c**, was filtered, and the solid was washed on the funnel with a little Me_2NAc. The filtrate was set aside while the solid was washed with H_2O and then dried. The filtrate was evaporated *in vacuo*, and the residue was stirred with H_2O before the solid was collected and dried. The two crops (1.10 and 1.25 g) were each essentially homogeneous by TLC ($CHCl_3$−MeOH, 5:1; containing 0.5% NH_4OH); yield 80% (2.35 g). A sample for testing and elemental analysis (see Table 6) was obtained following column chromatography on silica gel with elution as indicated for the TLC examination.

***N*^{10}-Substituted 6-(Aminomethyl)-2,4-pteridinediamines 12a and 12k−u.** These compounds were prepared by reaction of bromomethyl compound **1**[22,23] with appropriate amines. The reported preparation of 6-[(1-naphthylamino)methyl]-2,4-pteridinediamine (**12a**)[24] is typical of the procedure used.

***N*^{10}-Substituted 6-(Aminomethyl)-2,4-diamino-5-methylpyrido[2,3-*d*]pyrimidines 13a, 13k−u, and 14a−m.** All compounds of this group except **13a** and **13q** were prepared by method A, reaction of bromomethyl compound **2**[25−28] with the appropriate amine. Method B, reductive amination of nitrile **4**[25,29] with the appropriate amine, was used to prepare **13a** and **13q**. *N*-Methylaniline precursors used in method A to prepare N^{10}-methyl target compounds **13o** and **14e,f,h,j,k,m** were derived from the corresponding anilines by the general *N*-monomethylation procedure of Krishnamurthy.[34] Each *N*-methylaniline derivative produced the expected mass spectral result, and each was purified to homogeneity (TLC) by column chromatography on silica gel with elution by cyclohexane−EtOAc combinations, typically 5:1.

Method A. Procedures described earlier for preparations from **2** and appropriate amines of such compounds as 5-methyl-5-deazamethotrexate diethyl ester[25] and N^{10}-propargyl-5-methyl-5-deazaaminopterin diethyl ester[27] as well as related compounds[28] proved readily adaptable. Like the esters mentioned, these products were also purified by silica gel column chromatography.

Method B. The general procedure for the reductive condensation[32] of **4** with primary amines as described for the preparation of 5-methyl-5-deazaaminopterin diethyl ester[25] and other examples[28] was used. Each product was purified by column chromatography on silica gel as described earlier.

***N*^{10}-Substituted 6-(aminomethyl)-2,4-diaminopyrido-[2,3-*d*]pyrimidines 15a and 15c** were prepared from nitrile **5**[27,28] and the appropriate primary amines as indicated for analogues under method B above. The analogous 6-(4-chlorophenyl) compound (**15b**) was prepared from bromomethyl compound **3**[27,28] and 4-chloroaniline as indicated for 5-methyl analogues by method A above.

***N*^{10}-Substituted 6-(aminomethyl)-2,4-diaminoquinazolines 16a−d** were prepared from nitrile **6**[31] and the corresponding amines using the reported reductive condensation procedure.[32] Reaction solutions in AcOH were filtered from catalyst, concentrated to low volumes, and then streaked onto preparative TLC plates. After development with $CHCl_3$−MeOH (2:1), product bands were excised and extracted with

EtOH. Evaporation gave the products as solvates with AcOH as indicated in Table 6. The ^{1}H NMR spectra of each product confirmed the level of solvation indicated by analyses for C, H, and N.

10-Deaza Analogues 12t,u and 13t,u. 2,4-Diamino-6-[2-(1-naphthyl)ethyl]pteridine (12t). The two-step sequence is similar to that used in reported syntheses of 10-deazaaminopterin[26a] and 10-ethyl-10-deazaamino-pterin.[26b] **Step 1. 2,4-Diamino-6-[2-(1-naphthyl)ethenyl]pteridine (8).** A solution of **1** (1.98 g of 85% purity, 5.00 mmol) and $(C_6H_5)_3P$ (1.39 g, 5.30 mmol) in Me_2NAc (100 mL) was stirred at 65−70 °C for 1.5 h, then cooled to 23−25 °C, and treated with 1-naphthaldehyde (0.78 g, 5.00 mmol) followed by NaOMe (0.60 g, 11.1 mmol). The resulting dark red solution was stirred under N_2 at 20−23 °C for 20 h. The mixture, which had deposited a precipitate and developed a yellow-green color, was then chilled in an ice−H_2O bath before the pale yellow precipitate was collected and washed once on the funnel with the minimum of cold (0−5 °C) Me_2NAc. The filtrate was set aside for concentration *in vacuo* while the solid was washed successively with toluene, Et_2O, and H_2O. The dried solid (654 mg) produced spectral data summarized below, showing it to be *trans*-**8**. The filtrate was concentrated to a thick syrup which gave a yellow solid when stirred with toluene. The collected solid was washed successively with toluene, Et_2O, H_2O, and Et_2O before it was dried to give a solid (592 mg) whose ^{1}H NMR spectrum showed it to be mostly *trans*-**8** but with some *cis*-isomer present. The combined yield was 79%. Spectral data: mass *m*/*z* 315 (each crop), MH^+ for $C_{18}H_{14}N_6$; ^{1}H NMR (for first crop) δ 6.70 (br s, NH_2), 7.47 (d, CH=C*H*$C_{10}H_7$, J = 16 Hz, *trans*), 7.53−7.65 (m, overlapping, 3′-H, 6′-H, 7′-H), 7.78 and 8.15 (two br s, NH_2, nonequivalent), 7.80−8.00 (m, overlapping, 4′-H, 5′-H, 8′-H), 8.58 (d, 2′-H), 8.70 (d, C*H*=C$HC_{10}H_7$, J = 16 Hz), 8.95 (s, C^7-H); ^{1}H NMR (second crop) same as first crop except for signals of weaker levels at δ 7.00 (d, CH=C*H*$C_{10}H_7$, J = 13 Hz, *cis*), and 8.10 (s, C^7-H for *cis*); the other vinyl proton signal is apparently under the multiplets from the aromatic hydrogens. **Step 2. Hydrogenation of 8 To Give 12t.** A mixture of *trans*-**8** (192 mg, 0.60 mmol) and 10% Pd on C (190 mg) in glacial AcOH (40 mL) was stirred under H_2 (over H_2O in a gas burette) at atmospheric pressure until 41 mL of H_2 had been absorbed (5 h required). The catalyst was removed by filtration, and the filtrate was treated with 3% H_2O_2 (34 mL). After 2 h at 20−23 °C, the solution was evaporated (to about 10 mL) and then diluted with H_2O. The cloudy suspension was filtered and then made basic (pH 8−9) with NH_4OH to precipitate the product: yield 78% (148 mg); mass spectrum *m*/*z* 317, MH^+. A sample for testing and elemental analysis was purified by column chromatography on silica gel (230−400 mesh) with elution by $CHCl_3$−MeOH−AcOH (5:1:0.3).

2,4-Diamino-6-[2-(2,5-dimethoxyphenyl)ethyl]pteridine (12u) *via* 10. After a solution of **1** (672 mg of 85%, 2.00 mmol) and $(C_6H_5)_3P$ (556, 2.12 mmol) in Me_2NAc (40 mL) had been heated at 65−70 °C for 1.5 h and then cooled to 20−25 °C, 2,5-dimethoxybenzaldehyde (368 mg, 2.20 mmol) was added followed by NaOMe (240, 4.44 mmol). The solution was kept at 20−25 °C under N_2 in a stoppered flask for 4 days before it was concentrated *in vacuo* to about 8 mL. Addition of H_2O caused precipitation of the expected olefinic intermediate **10** as a *cis*/*trans* mixture: TLC, R_f 0.85 and 0.70, $CHCl_3$−MeOH−AcOH (15:5:0.1); yield 87% (562 mg). This sample, combined with a smaller sample (100 mg) that produced an identical thin-layer chromatogram, was hydrogenated in glacial AcOH (200 mL) containing PtO_2 (170 mg) at 50 psi during 4 h. The filtered solution, which gave mass spectral results with peaks corresponding to tetrahydro and dihydro overreduced products, was treated with 3% H_2O_2 (5 mL) and stirred open to the air for 18 h. The solution was evaporated (to about 20 mL) and diluted with H_2O (45 mL). The cloudy solution was clarified (Norit, Celite) and then made basic (pH 9) with NH_4OH to precipitate **12u** (400 mg, 60% yield); mass spectrum, *m*/*e* 327, MH^+. A sample for testing was eluted from a silica gel column with $CHCl_3$−MeOH (9:1 changing to 5:1) after application in AcOH−$CHCl_3$ (1:4).

2,4-Diamino-5-methyl-6-[2-(1-naphthyl)ethyl]pyrido[2,3-*d*]pyrimidine (13t) was prepared *via* olefinic precursor **9**. **Step 1.** (1-Naphthylmethyl)triphenylphosphonium chloride was prepared from 1-(chloromethyl)naphthalene as described below for (2,5-dimethoxybenzyl)triphenylphosphonium chloride (intermediate to **13u**). **Step 2. 2,4-Diamino-5-methyl-6-[2-(1-naphthyl)ethenyl]pyrido[2,3-*d*]pyrimidine (9)** was prepared from **7** (3.90 g, 19.2 mmol) and the phosphonium salt from step 1 (8.53 g, 19.4 mmol) in Me_2SO (100 mL) with NaH (0.78 g of 60% in oil, 19.5 mmol) as described below for **11** (intermediate to **13u**). The yield of *cis*/*trans*-**9** before chromatography was 73% (4.60 g). Chromatography as described below for **11** gave *cis*/*trans*-**9** in 29% yield (1.79 g); mass spectrum, *m*/*z* 328, MH^+ for $C_{20}H_{17}N_5$. **Step 3. Hydrogenation of 9 To Give 13t.** Following treatment of **9** with CF_3CO_2H as described below for the preparation of **13u** (from **11**), the salt was hydrogenated in DMF containing 5% Pd/C as for the preparation of **13u**. The intended free base of **13t** from **8** treatment of the reduced and purified salt with dilute NH_4OH apparently retained sufficient CF_3CO_2H to slightly affect its elemental analysis results (see Table 6). A sample of this material (225 mg) was dissolved in MeOH (100 mL) containing HCl (4.5 mL of 2 N), and the solution was evaporated to dryness. The solid residue was stirred with CH_3CN to give pure **13t**·2HCl·H_2O (200 mg).

2,4-Diamino-5-methyl-6-[2-(2,5-dimethoxyphenyl)ethyl]pyrido[2,3-*d*]pyrimidine (13u) was prepared *via* the olefin **11** as follows. **Step 1. (2,5-Dimethoxybenzyl)triphenylphosphonium Chloride.** A mechanically stirred solution of 2,5-dimethoxybenzyl chloride (10.3 g, 55.2 mmol) and triphenylphosphine (18.8 g, 71.7 mmol) in xylene (130 mL) was refluxed for 2 h while the mixture became thick with solid product. The mixture was allowed to cool to 25 °C, and the solid was collected and washed successively with xylene and Et_2O; yield 72% (17.9 g). **Step 2. 2,4-Diamino-5-methyl-6-[2-(2,5-dimethoxyphenyl)ethenyl]pyrido[2,3-*d*]pyrimidine (11).** A stirred solution of equimolar amounts (15.6 mmol each) of **7** (3.17 g) and the above phosphonium salt (7.00 g) in Me_2SO (80 mL) was treated with NaH (0.63 g of 60% in oil, 15.7 mmol). The resulting solution was kept under N_2 at 20−23 °C for 24 h. Most of the solvent was removed by distillation *in vacuo* (bath to 40 °C). The residue was stirred with portions of Et_2O until the Et_2O-insoluble solid could be collected, dried in the air, and then washed thoroughly with H_2O followed by a little EtOH and finally with Et_2O; crude yield 74% (3.4 g) of *cis*/*trans*-**11**. This sample was fractionated on a silica gel column with elution by $CHCl_3$−MeOH containing 0.2% NH_4OH to give pure *cis*-**11** (1.04 g) and pure *trans*-**11** (140 mg) before fractions consisting of *trans*-**11** contaminated by unchanged **7** began eluting. Spectral data: mass, *m*/*z* 338 (each pure fraction), MH^+ for $C_{18}H_{19}N_5O_2$; ^{1}H NMR (for *cis*-**11**) δ 2.64 (s, 5-CH_3), 3.40 (s, 2′- or 5′-OCH_3), 3.73 (5′- or 2′-OCH_3), 6.26 (br s, NH_2), 6.33 (d, 6′-H), 6.68 (d, CH=C*H*$C_6H_3(OCH_3)_2$, J = 11.9 Hz), 6.76 (q, 4′-H), 6.82 (d, C*H*=C$HC_6H_3(OCH_3)_2$, J = 11.9 Hz), 6.92 (d, 3′-H), 6.99 (br s, NH_2), 8.40 (s, 7-H); ^{1}H NMR (for *trans*-**11**) δ 2.73 (s, 5-CH_3), 3.78 (s, 2′- or 5′-OCH_3), 3.81 (s, 5′- or 2′-OCH_3), 6.34 (br s, NH_2), 6.86 (q, 4′-H), 6.98 (d, 3′-H), 7.07 (br s, NH_2), 7.19 (d, CH=C*H*$_6H_3(OCH_3)_2$, J = 16.4 Hz), 7.28 (d, 6′-H), 7.42 (d, C*H*=C$HC_6H_3(OCH_3)_2$, J = 16.4 Hz), 8.74 (s, 7-H). In a larger run, **7** (7.00 g, 34.5 mmol) and the phosphonium chloride (17.8 g, 39.6 mmol) with NaH (1.58 g of 60%, 39.5 mmol) afforded the *cis*/*trans* mixture in 77% crude yield (8.90 g). Column chromatography with elution as in the smaller run but with intent only to exclude other matter gave the *cis*/*trans* mixture in 20% yield (2.34 g); TLC ($CHCl_3$−MeOH, 9:1, containing 0.2% NH_4OH) showed only the isomers with the *cis* (R_f 0.33) in dominance over the *trans* (R_f 0.27). **Step 3. Hydrogenation of 11 To Give 13u**. The reaction conditions were adapted from those reported by Gangjee *et al.*[34] for the preparation of an analogous compound. In a typical run, **11** (1.79 g, 5.30 mmol of the *cis*/*trans*) was dissolved in CF_3CO_2H, the solution evaporated to dryness, and the residue dissolved in DMF. Catalyst (5% Pd/C, 1.0 g) was added, and the mixture was stirred under H_2 (over H_2O in a gas buret) at ambient conditions. After 15−20 min, treatment with H_2 was stopped; a specimen of the reaction mixture was filtered, the solution evaporated, and the residue examined by mass

spectroscopy. If any unchanged **11** remained, hydrogenation was resumed (for 5−10 min). When mass spectral examination showed conversion of **11** to be complete with only **13u** (m/z 340, MH^+) and low levels of overhydrogenated coproducts (m/z 342 and 344) to be present, the main portion of the hydrogenated solution was filtered and evaporated. Examination of the residue by TLC ($CHCl_3$−MeOH, 9:1, containing 0.2% NH_4OH) showed two separate relatively faint spots due to coproducts above the intense spot (R_f 0.3) due to **13u**. This mixture of trifluoroacetate salts was triturated with the minimum of cold MeOH (dry ice−Me_2CO bath) and quickly collected by filtration with the aid of cold MeOH. Examination by TLC showed that the upper spots due to overhydrogenated coproducts had been removed. The trifluoroacetate salt thus obtained (1.2 g) was then stirred with 0.5 N NH_4OH (55 mL) in an effort to obtain **13u** free base, but the solid obtained (0.93 g) gave elemental analysis results indicating incomplete conversion. This solid was dissolved in warm DMF (50−60 °C), and the solution was treated with 3% Na_2CO_3 to produce pH 8−9 (test paper). Evaporation followed by thorough washing with H_2O gave pure **13u** in 41% yield (0.73 g, dried at 78 °C).

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