2,4-Diaminopyrido[3,2-*d*]pyrimidine Inhibitors of Dihydrofolate Reductase from *Pneumocystis carinii* and *Toxoplasma gondii*

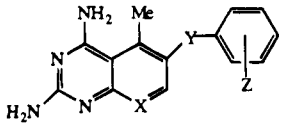
Andre Rosowsky,*,† Ronald A. Forsch,† and Sherry F. Queener‡

Dana-Farber Cancer Institute and Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, Massachusetts 02115, and Department of Pharmacology and Toxicology, Indiana University School of Medicine, Indianapolis, Indiana 46202

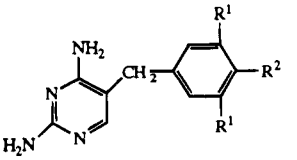
Received March 27, 1995®

Six previously unknown 2,4-diamino-6-(anilinomethyl)- and 2,4-diamino-6-[(*N*-methylanilino)-methyl]pyrido[3,2-*d*]pyrimidines (**5**–**10**) were synthesized from 2,4-diamino-6-(bromomethyl)-pyrido[3,2-*d*]pyrimidine hydrobromide (**11**·HBr) by treatment with the appropriate aniline or *N*-methylaniline in dimethylformamide at room temperature, with or without $NaHCO_3$ present. Compounds **5**–**10** were tested as inhibitors of dihydrofolate reductase from *Pneumocystis carinii*, *Toxoplasma gondii*, and rat liver as a part of a larger effort directed toward the discovery of lipophilic nonclassical antifolates combining high enzyme selectivity and high potency. Of the six analogues tested, the most potent and selective against *T. gondii* DHFR was 2,4-diamino-6-[(3′,4′,5′-trimethoxy-*N*-methylanilino)methyl]pyrido[3,2-*d*]pyrimidine (**7**), which had an IC_{50} of 0.0047 μM against this enzyme as compared with 0.026 μM against the rat liver enzyme. The potency of **7** against *T. gondii* DHFR was similar to that of trimetrexate (TMQ, **1**) and piritrexim (PTX, **2**) but was >500-fold greater than that of trimethoprim (TMP, **3**). However, while **7** was more selective than either TMQ (19×) or PTX (63×) against this enzyme, its selectivity in comparison with TMP was 8-fold lower. 2,4-Diamino-6-[(3′,4′,5′-trimethoxy-anilino)methyl]pyrido[3,2-*d*]pyrimidine (**6**) was 17-fold less active than **7** and was also less selective. 2,4-Diamino-6-[(3′,4′-dichloro-*N*-methylanilino)methyl]pyrido[3,2-*d*]pyrimidine (**10**) had an IC_{50} of 0.022 μM against *P. carinii* DHFR and was comparable in potency to TMQ and PTX. The species selectivity of **10** for *P. carinii* versus rat liver DHFR was greater than that of either TMQ (21-fold) or PTX (31-fold). On the other hand, even though **10** was slightly more active than TMQ against the *P. carinii* enzyme, its selectivity was 7-fold lower than that of TMP. Thus, the goal of combining high enzyme binding activity, which is characteristic of the fused-ring compounds TMQ and PTX, with high selectivity for *T. gondii* and *P. carinii* DHFR versus rat liver DHFR, which is characteristic of the monocyclic compound TMP, remained unmet in this limited series.

A major problem in the clinical management of patients with AIDS and other immunodeficiency syndromes is infection by opportunistic pathogens that are usually benign to healthy individuals but become potentially lethal when the immune system is damaged.[1] Two especially troublesome organisms in this context are *Pneumocystis carinii* and *Toxoplasma gondii*.[2,3] Nonclassical lipophilic inhibitors of dihydrofolate reductase (DHFR) became an attractive class of drug candidates for the treatment of *P. carinii* and *T. gondii* opportunistic infections when thee anticancer drug trimetrexate (TMQ, **1**) was shown to elicit clinical remissions in selected patients when used in combination with leucovorin ((6*R*,6*S*)-5-formyl-5,6,7,8-tetrahydrofolic acid) to ablate hematologic and gastrointestinal toxicity.[4–6] The need for leucovorin in this regimen stems from the fact that TMQ binds less tightly to *P. carinii* or *T. gondii* DHFR than it does to human DHFR;[7–10] in other words, enzyme species selectivity is unfavorable. Fortunately leucovorin can be used to selectively protect host tissues.[5–7,11] Differential protection is possible because, unlike mammalian cells, *P. carinii* and *T. gondii* lack the ability to actively transport reduced folates. Thus these organisms are entirely dependent on *de novo* folate biosynthesis[12] and are sensitive to sulfonamide and sulfone inhibitors of dihydropteroate synthetase, a key step in this pathway.[13,14] Another lipophilic DHFR inhibitor which has been found to closely resemble TMQ in laboratory animal models of *P. carinii* and *T. gondii* infection is the 2,4-diamino-5-methylpyrido[2,3-*d*]pyrimidine derivative piritrexim (PTX, **2**), which likewise possesses unfavorable DHFR species selectivity and yet can be used with leucovorin as a selective host tissue protectant.[8,11]



1 (TMQ): X = CH, Y = CH_2NH, Z = 3′,4′,5′-$(OMe)_3$
2 (PTX): X = N, Y = CH_2, Z = 2′,5′-$(OMe)_2$



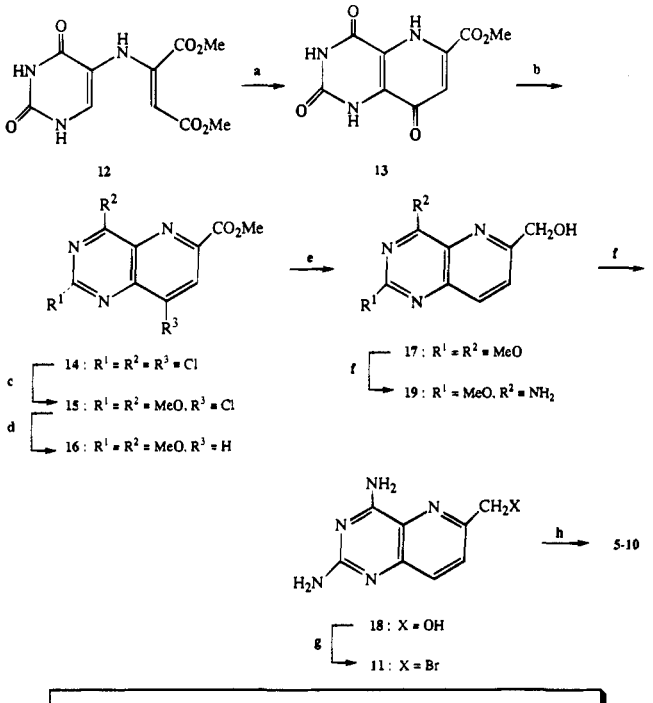
3 (TMP): $R^1 = R^2 = OMe$
4 (epiroprim): R^1 = OEt, R^2 = 1-pyrrolo

While lipophilic DHFR inhibitors with the desired species selectivity exist, such as trimethoprim (TMP, **3**), the DHFR affinity of these compounds tends to be lower

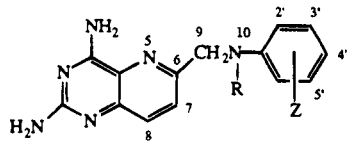
† Dana-Farber Cancer Institute.
‡ Indiana University.
® Abstract published in *Advance ACS Abstracts*, June 1, 1995.

0022-2623/95/1838-2615$09.00/0

Scheme 1



than that of TMQ or PTX.[7-10] Briefly stated, therefore, the problem at the level of the target enzyme (i.e., initially disregarding other important issues like cellular uptake, plasma pharmacokinetics, and metabolism) has been to find compounds that combine the high inhibitory activity of TMQ and PTX with the species selectivity of TMP.[15] A step in this direction has been the development of the second-generation TMP analogue epiroprim (**4**).[16,17] A number of lipophilic fused 2,4-diaminopyrimidine ring systems related to TMQ and PTX have likewise been examined, including pteridines,[10-17] pyrido[2,3-*d*]pyrimidines,[18] quinazolines,[10,17,19] and others.[19-24] However, inhibitors combining high potency and high selectivity have thus far been very few and have in almost all instances shown greater selectivity for the *T. gondii* enzyme than for the *P. carinii* enzyme. In the work reported here, six previously unknown 2,4-diaminopyrido[3,2-*d*]pyrimidines with substituted anilinomethyl or (*N*-methylanilino)methyl groups at the 6-position were synthesized, and their activity as inhibitors of DHFR from *P. carinii*, *T. gondii*, and rat liver was compared.



5: R = H, Z = 2',5'-$(OMe)_2$
6: R = H, Z = 3',4',5'-$(OMe)_3$
7: R = Me, Z = 3',4',5'-$(OMe)_3$
8: R = Me, Z = 4'-Cl
9: R = Me, Z = 3'-Cl
10: R = Me, Z = 3',4'-Cl_2

Chemistry

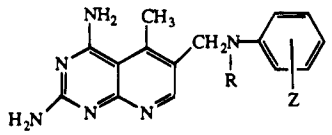
2,4-Diamino-6-(bromomethyl)pyrido[3,2-*d*]pyrimidine (**11**, Scheme 1), the key starting point for the preparation of **5**–**10**, was obtained essentially according to literature methods[25-27] but with several important modifications. Condensation of 5-aminouracil with dimethyl acetylenedicarboxylate afforded the expected fumarate intermediate **12** (67% yield). However when **12** was heated for 45 min in refluxing Dowtherm A (a 3:1 mixture of diphenyl ether and biphenyl) as described,[25] an intractable black product was obtained whose ^{1}H-NMR spectrum showed a complex pattern of signals near δ 7.5, rather than the singlet reported for the cyclized product **13**.[25] After numerous attempts to optimize the reaction conditions, ring closure was found to be accomplished best by preheating the Dowtherm A, adding **12** over a period of 1 min, and then cooling rapidly after another 2 min (i.e., a total contact time of no more than 3 min). Extending the length of heating to as little as 15 min resulted in extensive decomposition. Large-scale purification of **13** by crystallization from DMF[25] was found to be inconvenient, and an alternative method involving fractional extraction in a Soxhlet apparatus was therefore worked out. The crude product was first extracted for 18 h with refluxing acetone to remove residual Dowtherm A, as well as any starting material and noncyclized byproducts, and was then extracted for ca. 2 days with 1:1 $CHCl_3$–MeOH while monitoring aliquots of the residual solid for disappearance of the ^{1}H-NMR singlet at δ 7.5, corresponding to **13**. Cooling of the $CHCl_3$–MeOH extract to room temperature led to deposition of **13** as a very pure solid in 44% yield.

Chlorination of **13** in $POCl_3$ likewise presented a problem, in that the reported method involving addition of the reaction mixture to ice[25] gave low recovery of the chlorinated product **14**. We found that the yield could be improved by pouring the slurry into a stirred mixture of EtOAc and aqueous $NaHCO_3$ to minimize exposure of the chlorination product **14** to HCl; however, even with this modification, the yield was no better than 45%. The remaining steps in the synthesis of **11** via **15**–**18** were as described.[27] The amination step with methanolic ammonia required an *internal* temperature of 170–175 °C. Lower internal temperatures gave considerable amounts of a monomethoxy byproduct which we assume was **19**.[25]

Bromination of **18** was performed with HBr in glacial AcOH, which in our hands proved superior to PBr_3 in THF,[26] probably because the latter gave a heterogeneous reaction mixture. The hygroscopic **11**·HBr was isolated by precipitation with Et_2O and was used without further purification. Stirring **11**·HBr for 4 days in dry DMF containing excess 2,5-dimethoxyaniline and $NaHCO_3$ afforded the monoalkylation product **5**, but only in very low yield (9%). A similar low yield (7%) of **6** was obtained when **11**·HBr was stirred for 3 days with 3,4,5-trimethoxyaniline. This was in sharp contrast to the 61% yield of N-monoalkylation product reported with diethyl *N*-(4-aminobenzoyl)-L-glutamate.[26] We surmise that the low yields of **5** and **6** reflect the fact that when electron-donating MeO groups rather than an electron-withdrawing CONH group are present on the aromatic ring, N,N-dialkylation is favored even when the amine is used in excess (i.e., alkylation of the secondary amine is faster than that of the primary amine). In agreement with this interpretation, when **11**·HBr was stirred with excess *N*-methyl-3,4,5-trimethoxyaniline and $NaHCO_3$ in dry DMF at room temperature for 4 days, a 43% yield of **7** was obtained after purification by silica gel column chromatography. When *N*-methyl-4-chloroaniline, *N*-methyl-3-chloroaniline, and *N*-methyl-3,4-dichloroaniline were used, the

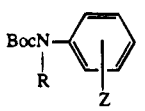
products **8**–**10** were likewise obtained in 40–42% yield. However, when the same reaction was attempted with *N*-methyl-2,5-dimethoxyaniline, only traces of the desired product could be detected, presumably reflecting the sterically unfavorable effect of *ortho* substitution next to the amino group.

^{1}H-NMR spectra of compounds **5**–**10** in d_6-DMSO solution were consistent with their assigned structures. The MeO protons of **6** and **7** gave closely spaced singlets at δ 3.0–3.1. The N^{10}-CH_3 protons of **7** likewise gave an overlapping singlet in this region. The 2- and 4-amino groups produced the expected broad signals in the δ 5.8–6.2 and 7.1–7.3 regions respectively, which disappeared upon addition of D_2O. Other notable features included the 9-CH_2 protons, which appeared as a signal at δ 4.15 in the spectrum of **6**, δ 4.38 in that of **7**, and δ 4.6–4.7 in those of **8**–**10**. Similar differences in the chemical shift of the 9-CH_2 group have been reported for the pyrido[2,3-*d*]pyrimidines **20**–**22**.[18]



20,**21**: R = H,Me; Z = 3',4',5'-$(OMe)_3$
22: R = Me, Z = 3',4'-Cl_2

The low yields obtained in the direct alkylation of 3,4,5-trimethoxyaniline and 2,5-dimethoxyaniline with **11** led us to briefly explore an alternative approach involving temporary use of a *tert*-butyloxycarbonyl (Boc) group to block N,N-dialkylation. Addition of benzyl bromide to the sodium salt of *N*-(*tert*-butyloxycarbonyl)-2,5-dimethoxyaniline (**23**) in DMF solution was found to afford an excellent yield (84%) of **24**. Similarly, a good yield was obtained when the sodium salt of methyl 4-[(*tert*-butyloxycarbonyl)amino]benzoate (**25**) was alkylated with benzyl bromide, though in this case the majority of the isolated product turned out to be **26** (70% yield) because of premature cleavage of the ester group.



23: R = H, Z = 2',5'-$(OMe)_2$
24: R = $PhCH_2$, Z = 2',5'-$(OMe)_2$
25: R = H, Z = 4'-CO_2Me
26: R = $PhCH_2$, Z = 4'-CO_2H
27: R = (2,4-diaminopyrido[3,2-*d*]pyrimidin-6-yl)methyl, Z = 2',5'-$(OMe)_2$

On the other hand, when 4-nitrobenzyl bromide was used instead of benzyl bromide, no alkylation of the carbamate nitrogen in **25** was observed. Moreover, when the reaction was performed with **11** instead of benzyl bromide, the yield of the N-alkylated product **27** was only 4%. On the basis of these disappointing results in model reactions, transient protection with a Boc group was not considered further as a route to **5** and **6**.

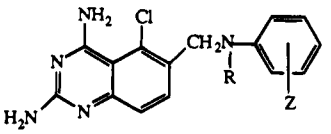
Biological Activity

The potencies and species selectivities of **5**–**10** against *P. carinii*, *T. gondii*, and rat liver DHFR were determined as described earlier[10,15] and are summarized in Table 1. Also included for comparison purposes are published data for **20**–**22**,[18] the recently described 2,4-diamino-5-chloroquinazolines **28**–**30**,[20] and the reference compounds TMQ (**1**), PTX (**2**), and TMP (**3**).

Table 1. Inhibition of Dihydrofolate Reductase by 2,4-Diamino-6-[*N*-arylamino)methyl]pyrido[3,2-*d*]pyrimidines and Other Lipophilic Antifolates

	IC_{50} (μM)1a,b		
compd	rat liver	*P. carinii*	*T. gondii*
5	0.43	1.4 (0.31)	0.10 (4.3)
6	0.30	2.6 (0.12)	0.082 (3.7)
7	0.026	0.13 (0.20)	0.0047 (5.5)
8	0.022	0.062 (0.35)	0.015 (1.5)
9	0.067	2.1 (0.032)	0.020 (3.4)
10	0.032	0.022 (1.5)	0.098 (0.33)
20	0.0021	0.086 (0.024)	0.0074 (0.28)
21	0.0076	0.013 (0.58)	0.00085 (8.9)
22	0.042	0.100 (0.42)	0.027 (1.6)
28	0.044	0.051 (0.86)	0.030 (1.5)
29	0.0059	0.033 (0.18)	0.0052 (1.1)
30	0.012	0.012 (1.0)	0.0064 (1.9)
TMQ (1)	0.003	0.042 (0.071)	0.010 (0.29)
PTX (**2**)	0.0015	0.031 (0.048)	0.017 (0.088)
TMP (3)	130	12 (11)	2.7 (44)

[a] Inhibition was determined spectrophotometrically as described earlier; see refs 10 and 15. Numbers in parentheses are enzyme selectivity ratios, defined as IC_{50}(rat liver)/IC_{50} (*P. carinii*) or IC_{50} (rat liver)/IC_{50} (*T. gondii*). Values of 1.0 or less indicate absence of selectivity for the *P. carinii* or *T. gondii* enzyme versus the rat liver enzyme. [b] Data for **20**–**22** are cited from ref 18, and those for **28**–**30**, TMQ, PTX, and TMP are from ref 20 and other papers cited therein. The IC_{50} of **21** against *T. gondii* DHFR was inadvertently listed in ref 18 as 0.58 nM instead of 0.85 nM (S. F. Queener, personal communication).



28: R = H, Z = 2',5'-$(OMe)_2$
29,**30**: R = H,Me; Z = 3',4',5'-$(OMe)_3$

The IC_{50} values of the 2,4-diaminopyrido[3,2-*d*]pyrimidines **5**–**10** as inhibitors of rat liver DHFR ranged from 0.022 (**8**) to 0.43 μM (**5**). Where its effect could be assessed, N^{10}-methyl substitution appeared to favor binding; for example **7** (IC_{50} = 0.026 μM) was 11-fold more active than **6** (IC_{50} = 0.30 μM). This differed from the 5-methylpyrido[2,3-*d*]pyrimidine **21** (IC_{50} = 0.0076 μM)[18] and 5-chloroquinazoline **30** (IC_{50} = 0.012 μM),[20] which were both several times less active than their N^{10}-unsubstituted counterparts **20** (IC_{50} = 0.0021 μM) and **29** (IC_{50} = 0.0059 μM).[18,20] The 2',5'-dimethoxy and 3',4',5'-trimethoxy analogues **5** (IC_{50} = 0.43 μM) and **6** (IC_{50} = 0.30 μM) were approximately equipotent. With the 5-chloroquinazolines, the 2',5'-dimethoxy analogue **28** (IC_{50} = 0.044 μM) was less active than the 3',4',5'-trimethoxy analogue **29** (IC_{50} 0.0059 μM).[30] Compound **5** was 10-fold less active than **28**, and **6** was 50-fold less active than **29**. When the three ring systems with identical N^{10}-methyl-3',4',5'-trimethoxy sustitution were compared, activity increased in the order **21** > **30** > **7**. None of the 8-deaza compounds was as active as TMQ or PTX, but all of them exceeded the potency of TMP.

Against *P. carinii* DHFR, IC_{50} values for the 8-deaza analogues varied from 0.022 (**10**) to 2.6 μM (**6**). The 2',5'-dimethoxy analogue **5** (IC_{50} = 1.4 μM) and 3',4',5-trimethoxy analogue **6** (IC_{50} = 2.6 μM) were approximately equiactive, as in the case of the rat liver DHFR. N^{10}-Methylation likewise increased activity against the *P. carinii* enzyme, with**7** (IC_{50} = 0.13 μM) being 20-fold more active than **6**. Activity was also dramatically enhanced by 4'-chloro substitution (compare **8** and **10** versus **9**). As in the case of the rat liver enzyme, the

3′,4′,5′-trimethoxy analogues **6** and **7** was less active than the correponding 5-methylpyrido[2,3-*d*]pyrimidines **20** (IC_{50} = 0.086 μM) and **21** (IC_{50} = 0.013 μM),[18] as well as the corresponding 5-chloroquinazolines **29** (IC_{50} = 0.033 μM) and **30** (IC_{50} = 0.012 μM).[20] The most potent pyrido[3,2-*d*]pyrimidine tested was **10**, with an IC_{50} of 0.022 μM. Thus, its activity against *P. carinii* DHFR was comparable to that of TMQ and PTX and was >500-fold higher than that of TMP. Compound **10** showed slight selectivity for *P. carinii* DHFR, but the other pyrido[3,2-*d*]pyrimidines examined were all better inhibitors of the rat liver enzyme than of the *P. carinii* enzyme, and none had the favorable species selectivity of TMP. Thus, the design of fused-ring 2,4-diaminopyrimidine systems exhibiting the desired combination of high enzyme binding potency, characteristic of TMQ and PTX, and high enzyme species selectivity, characteristic of the monocyclic 2,4-diaminopyrimidines TMP and epiroprim,[28] remains a difficult challenge.

Experimental Section

IR spectra were obtained on a Perkin-Elmer Model 781 double-beam recording spectrophotometer; peaks below 1200 cm^{-1} are omitted. ^1H NMR spectra were obtained at 60 MHz on a Varian EM360L instrument, using Me_4Si as the reference. TLC analyses were done on fluorescent Analab silica gel plates, with spots visualized under 254-nm UV light. Column chromatography was performed on Baker 60–200- or 70–230-mesh silica gel or on Baker flash silica gel (40-μm particle size). Solvents for moisture-sensitive reactions were dried over Linde 4A molecular sieves (Fisher, Boston, MA). Melting points (not corrected were measured in a Mel-Temp apparatus (Cambridge Laboratory Devices, Cambridge, MA) using open Pyrex capillary tubes. Microanalyses were done by Quantitative Technologies, Whitehouse, NJ, and were within ±0.4% of theoretical values unless otherwise specified.

2,4-Diamino-6-(bromomethyl)pyrido[3,2-*d*]pyrimidine Hydrobromide (11·HBr). Although the preparation of this compound has been described,[26] we found it useful to modify four of the seven reported steps, specifically those leading to 11·HBr (step 6), 13 (step 1), **14** (step 2), and **18** (step 5). The physical constants of intermediates **12**–**18** were all in agreement with published values and are therefore not restated here. Because of its low stability during purification and storage, 11·HBr was freshly prepared as needed and was used without purification.

Step 1. Dimethyl 2-[(2,4-dioxopyrimidin-5-yl]amino]fumarate (**12**) (6.73 g, 0.025 mol)[25] was added in small portions over a period of 1 min to Dowtherm A (a 3:1 mixture of diphenyl ether and biphenyl) preheated to the boiling point. The reaction mixture was boiled for exactly another 2 min, and the flask was immersed at once into a large amount of crushed ice (care must be taken that only solid ice be present initially to reduce the risk of breaking the flask). After being left to cool, the mixture was diluted with 50 mL of petroleum ether (bp 30–60 °C), and the solid was filtered, combined with the product from another identical run, and extracted with acetone for 18 h in a Soxhlet apparatus. The acetone was then replaced with a 1:1 mixture of $CHCl_3$ and MeOH, and extraction was continued for 2 days, with periodic monitoring of the residue in the Soxhlet cup by ^1H-NMR to follow the disappearance of the δ 7.5 signal corresponding to the uracil C-6 proton. Cooling of the $CHCl_3$–MeOH extract yielded 6-carbomethoxy-2,4,8-trioxopyrido[3,2-*d*]pyrimidine (**13**) as a yellow solid (5.2 g, 44% combined yield of two runs).

Step 2. A mixture of **13** (17.5 g, 0.06 mol), *N*,*N*-diethylaniline (18 mL), and $POCl_3$ (450 mL) was refluxed for 12 h and then concentrated to near-dryness by rotary evaporation. The residual syrup was poured slowly into a well-stirred ice-cooled mixture of EtOAc (200 mL) and a suspension of $NaHCO_3$ (50 g) in H_2O (200 mL). The aqueous layer was acidic after this addition. The organic layer was washed with H_2O until the washes were nearly neutral and was then evaporated to a gummy black solid. Repeated trituration with hot heptane and cooling of the combined triturates deposited 6-carbomethoxy-2,4,8-trichloropyrido[3,2-*d*]pyrimidine (**14**) as a pink powder (9.7 g, 45%): mp 181–183 °C (lit.[23] mp 195–198 °C). The product (9.7 g, 0.033 mol) was dissolved directly in dry MeOH (100 mL), and to this was added a solution of Na (1.54 g, 0.067 mol) in dry MeOH (50 mL). After 2 h at room temperature, the reaction was quenched by sequential addition of glacial AcOH (2 mL) and H_2O (450 mL). The precipitate was filtered, dried, and recrystallized from EtOAc to obtain 6-carbomethoxy-8-chloro-2,4-dimethoxypyrido[3,2-*d*]pyrimidine (**15**) as an off-white solid (8.4 g, 89%).

Step 3. A portion of **15** (4.82 g, 0.017 mol) was dissolved in DMF (145 mL) containing NaOAc (1.93 g, 0.024 mol), and the solution was shaken with 5% Pd–C (0.5 g) under 3 atm of H_2 for 1 h. The catalyst was filtered and washed, first with cold DMF (30 mL) and then hot EtOAc (120 mL). The combined washings were evaporated, and the residue was triturated with cold H_2O and filtered. The solid was washed with H_2O and dried on a lyophilizer to obtain 6-carbomethoxy-2,4-dimethoxypyrido[3,2-*d*]pyrimidine (**16**) as a white solid (2.4 g, 56%): TLC R_f 0.5 (silica gel, $CHCl_3$).

Step 4. A solution of **16** (1.81 g, 0.0073 mol) in dry THF (50 mL) was maintained at 20 °C while $LiBH_4$ (0.21 g, 0.0095 mol) was added in small portions with stirring. When a homogeneous solution was obtained (15 min), the reaction was quenched with MeOH (40 mL), left to stir another 10 min, and evaporated to dryness. The residue was triturated with cold H_2O and the product collected and dried on a lyophilizer to obtain (6-(hydroxymethyl)-2,4-dimethoxypyrido[3,2-*d*]pyrimidine (**17**) as an off-white solid (1.05 g). A second crop (0.13 g) was recovered from the H_2O wash by adding a saturating amount of NaCl, extracting with $CHCl_3$, evaporating the $CHCl_3$ layer, and triturating with Et_2O; total yield 1.18 g (73%).

Step 5. Anhydrous NH_3 was bubbled into dry MeOH (100 mL) at −10 °C to give a final volume of ca. 150 mL, and this was immediately added to **17** (3.75 g, 0.017 mol) in a 250 mL Teflon-lined autoclave (Berghof/America, Concord, CA). The vessel was sealed, and the internal temperature was brought to 140 °C (jacket temperature ca. 170 °C). After 4 days the internal temperature was raised to 170–175 °C and maintained for 20 h (internal pressure of 60 atm). After being allowed to cool completely, the autoclave contents were removed, the solvent was evaporated, and the residue was taken up in hot H_2O (350 mL). A small amount of insoluble material was filtered off, and the solution was concentrated to 100 mL and refrigerated overnight. Filtration and drying on a lyophilizer afforded pure 2,4-diamino-6-(hydroxymethyl)-pyrido[3,2-*d*]pyrimidine (**18**) as a tan-colored solid (1.36 g, 42%). A gummy second crop weighing 0.87 g (17%) was recovered from the mother liquor; total yield 2.23 g (69%).

Step 6. A suspension of **18** (191 mg, 1 mmol) in glacial AcOH (10 mL) was heated quickly to 65 °C, and the resulting solution was cooled promptly back to room temperature. A single portion of 30% HBr in AcOH (20 mL) was then added. A precipitate formed initially and then dissolved. The mixture was stirred at room temperature for 2 days, and was then added over a period of 1 min with vigorous stirring to 150 mL of Et_2O chilled in an ice bath. The precipitate was filtered, washed with Et_2O, and dried immediately *in vacuo* for 2 h at room temperature to obtain 11·HBr as a tan-colored solid. Because of its strongly hygroscopic character, the product was dissolved at once in DMF (5 mL) and used in the next step.

2,4-Diamino-6-[(2′,5′-dimethoxyanilino)methyl]pyrido-[3,2-*d*]pyrimidine (5). 2,5-Dimethoxyaniline (765 mg, 5 mmol) was added in a single portion to a solution of 11·HBr (from 191 mg, 1 mmol of **18**) in dry DMF (5 mL). The solution was left at room temperature for 3 days. The solvent was evaporated to dryness, and the residue was partitioned between $CHCl_3$ and dilute NH_4OH. The organic layer was evaporated and the residue chromatographed on silica gel ("flash" grade, 10 g, 1.5 × 15 cm) with 50:1, 40:1, and 30:1 $CHCl_3$–MeOH (50 mL each) as the eluents. Pooled fractions containing pure **5** (R_f 0.3, silica gel, 10:1 $CHCl_3$–MeOH) were evaporated to a glass, which was taken up in CH_2Cl_2 (5 mL) and left in the hood to permit slow evaporative crystallization. The solid was collected and dried *in vacuo* at 60 °C over P_2O_5: yield 24 mg (7%); mp 195–198 °C; IR (Kr) ν 3460, 3400 br,

3170, 2990 w, 2930, 2830, 1620, 1575, 1525, 1460, 1390, 1360 w, 1340 w, 1325 w, 1290, 1245, 1220 cm^{-1}. Anal. ($C_{16}H_{18}N_6O_2 \cdot 0.2H_2O$) C, H, N.

2,4-Diamino-6-[(3′,4′,5′-trimethoxyanilino)methyl]pyrido[3,2-*d*]pyrimidine (6). 3,4,5-Trimethoxyaniline (915 mg, 5 mmol), $NaHCO_3$ (420 mg, 5 mmol), and **11**·HBr (from 500 mg, 2.62 mmol of **18**) in dry DMF (5 mL) were stirred at room temperature for 5 days. The solvent was evaporated, and the residue was worked up as in the preceding experiment except that the silica gel column (22 g, 2 × 22 cm) was eluted successively with $CHCl_3$, 40:1 $CHCl_3$–MeOH, and 20:1 $CHCl_3$–MeOH (50 mL each). Evaporative crystallization of the glassy product from CH_2Cl_2 containing a few drops of MeOH, followed by drying *in vacuo* over P_2O_5 at 60 °C, afforded a yellow solid (85 mg, 9%): mp 128–131 °C; IR (KBr) ν 3370, 3190, 2990, 2930, 2830, 1610, 1570, 1510, 1450, 1415, 1385, 1365 sh, 1340 w, 1325 w, 1275 sh, 1235, 1200 cm^{-1}; ^{1}H-NMR (d_6-DMSO) δ 3.10 (m, >9H, 3-OMe, 4-OMe, 5-OMe, and H_2O), 4.15 (m, 2H, CH_2), 5.85 (m, 4H, 2′-H, 6′-H, and NH_2), 7.33 (m, 4H, C_7-H, C_8-H, and NH_2). Anal. ($C_{17}H_{20}N_6O_3 \cdot 0.75H_2O$) C, H, N.

2,4-Diamino-6-[(3′,4′,5′-trimethoxy-*N*-methylanilino)methyl]pyrido[3,2-*d*]pyrimidine (7). A solution of 3,4,5-trimethoxyaniline (915 mg, 5 mmol) in 95% formic acid (10 mL) was refluxed for 30 min and evaporated to dryness. The residue was dissolved in EtOAc and the solution washed with 5% $NaHCO_3$ and rinsed with H_2O. The organic layer was dried ($MgSO_4$) and evaporated to obtain 3,4,5-trimethoxyformanilide (584 mg, 55%),[29] which was dissolved directly in dry THF (20 mL) and added dropwise over 10 min under N_2 to a stirred ice-cold suspension of $LiAlH_4$ (114 mg, 3 mmol) in dry THF (10 mL). After being left to stand at room temperature overnight the reaction was quenched with saturated aqueous Na_2SO_4, the inorganic salts were filtered off, and the filter cake was washed thoroughly with Et_2O. Evaporation of the combined THF and Et_2O solutions yielded *N*-methyl-3,4,5-trimethoxyaniline[29] as an oil (464 mg, 85%). A mixture of **11**·HBr (from 191 mg, 1 mmol of **18**), *N*-methyl-3,4,5-trimethoxyaniline (220 mg, 1.1 mmol), and $NaHCO_3$ (252 mg, 3 mmol) in dry DMF was stirred at room temperature for 4 days. The solvent was evaporated and the residue partitioned between $CHCl_3$ and H_2O. TLC (Analab silica gel, 10:1 $CHCl_3$–MeOH) showed some starting amine (R_f 0.7) along with **7** (R_f 0.3) and some minor products (R_f 0.2 and origin). The $CHCl_3$ layer was evaporated and the residue chromatographed on silica gel (20 g, 2 × 20 cm) using $CHCl_3$ to elute the amine followed by 40:1 $CHCl_6$–MeOH to elute **7**. Pooled fractions containing pure **7** were evaporated, and the gummy residue was taken up in CH_2Cl_2 (7 mL) containing a few drops of MeOH. Passive evaporation in the hood led to deposition of a solid, which was dried *in vacuo* at 60 °C over P_2O_5 to obtain **7** as an off-white powder (170 mg, 46%): mp 203–204 °C; IR (KBr) ν 3480, 3440, 3340, 3200, 2980, 2950, 2820, 1640, 1620, 1570, 1515, 1455, 1385, 1345, 1330 w, 1285, 1255, 1245, 1230, 1200 cm^{-1}; ^{1}H-NMR (d_6-DMSO) δ 3.08 (m, >12H, N^{10}-Me, 3-OMe, 4-OMe, 5-OMe, and H_2O), 4.38 (s, 2H, CH_2N), 5.83 (m, 4H, phenyl protons and NH_2), 6.8–7.2 (m, 4H, C_7-H, C_8-H, NH_2). Anal. ($C_{18}H_{22}N_6O_3 \cdot 0.5H_2O$) C, H, N.

2,4-Diamino-6-[(4′-chloro-*N*-methylanilino)methyl]pyrido[3,2-*d*]pyrimidine (8). A solution of 4-chloroaniline (2.55 g, 0.02 mol) in 95% HCO_2H was refluxed for 30 min and evaporated to dryness under reduced pressure. The residue was taken up in Et_2O and washed in succession with 5% citric acid, H_2O, and 5% $NaHCO_3$. The aqueous layer was re-extracted with Et_2O, and the combined Et_2O layers were dried ($MgSO_4$) and evaporated. Recrystallization of the solid residue from H_2O yielded 4-chloroformanilide as a white powder (2.35 g, 76%), mp 101–102 °C (lit.[30] mp 102 °C). A solution of the anilide (1.56 g, 0.01 mol) in dry THF (10 mL) was added dropwise over 20 min to a solution of $LiAlH_4$ (0.38 g, 0.01 mol) in dry THF (10 mL) under N_2 at 0 °C. The mixture was allowed to come to room temperature and stirred overnight and was then cooled in ice and quenched with saturated Na_2SO_4. The solids were filtered and washed thoroughly with Et_2O, and the combined THF and Et_2O were dried ($MgSO_4$) and evaporated to obtain *N*-methyl-4-chloroaniline[31] as a straw-colored liquid (1.1 g, 77%) of suitable purity to be used without distillation. The amine (212 mg, 1.5 mmol) and $NaHCO_3$ (420 mg, 5 mmol) were added to **11**·HBr (from 191 mg, 1 mmol of **18**) in dry DMF (5 mL), and the mixture was left to stir at room temperature for 11 days and evaporated. The residue was suspended in 20:1 $CHCl_3$–MeOH (30 mL), and the suspension was sonicated and filtered. The filtrate was evaporated, the residue was triturated with hexane to remove some unreacted amine, and the remaining solid was chromatographed on silica gel (20 g, 2 × 21 cm) using 40:1 $CHCl_3$–MeOH as the eluent. Pooled pure fractions (R_f 0.3, silica gel, 10:1 $CHCl_3$–MeOH) were evaporated to a gum, which was taken up in CH_2Cl_2 (7 mL) containing a few drops of MeOH. The solution was allowed to passively evaporate in the hood until a solid formed. The solid was dried in vacuo over P_2O_5 at 60 °C to obtain **8** as an off-white powder (136 mg, 43%): mp 185–186 °C; IR (KBr) ν 3440, 3380, 3620, 3170, 2920 sh, 1620, 1595, 1570, 1500, 1445, 1385, 1365, 1335, 1315 w, 1300 w, 1245, 1230 w, 1205 cm^{-1}; ^{1}H-NMR (d_6-DMSO) δ 3.08 (s, 3H, NMe), 4.65 (s, 2H, NCH_2), 6.15 (D_2O exchangeable br s, 2H, NH_2), 6.77 (d, J = 9 Hz, 2H, phenyl protons), 7.18 (d, J = 9 Hz, and D_2O exchangeable br s, 4H, phenyl protons and NH_2), 7.32 (d, J = 9 Hz, 1H, C_7-H), 7.57 (d, J = 9 Hz, 1H, C_8-H). Anal. ($C_{15}H_{15}ClN_6 \cdot 0.05CH_3OH \cdot 0.2H_2O$) C, H, Cl, N.

2,4-Diamino-6-[(3′-chloro-N-methylanilino)methyl]pyrido[3,2-*d*]pyrimidine (9). 3-Chloroformanilide, mp 45–50 °C (lit.[32] mp 57–58 °C), was prepared from 3-chloroaniline (2.55 g, 0.02 mol) as in the preceding experiment, yield 3.04 g (98%). *N*-Methyl-3-chloroaniline, prepared from the anilide (2.29 g, 0.015 mol) by reduction with $LiAlH_4$ (0.57 g, 0.015 mol) as in the preceding experiment was isolated as a straw-colored oil (1.96 g, 94%) and used directly. The amine (217 mg, 1.53 mmol), $NaHCO_3$ (252 mg, 3 mmol), and **11**·HBr (from 100 mg, 0.524 mmol of **18**) were stirred in dry DMF (5 mL) at room temperature for 4 days, and the product was worked up the same way as **8** to obtain **9** as an off-white solid (72 mg, 44%): mp 169–174 °C; IR (KBr) ν 3440, 3380 sh, 3340 sh, 3170, 2910, 1735 w, 1710 sh, 1630, 1615, 1595, 1565, 1495, 1445, 1385, 1365, 1340, 1320 w, 1245, 1225 cm^{-1}; ^{1}H-NMR (d_6-DMSO) δ 3.12 (s, 3H, NMe), 4.67 (s, 2H, NCH_2), 6.15 (D_2O exchangeable br s, 2H, NH_2), 6.78 (m, 3H, phenyl protons), 7.22 (partly D_2O exchangeable m, 3H, phenyl proton and NH_2), 7.33 (d, J = 8 Hz, 1H, C_7-H), 7.55 (d, J = 8 Hz, 1H, C_8-H). Anal. ($C_{15}H_{15}ClN_6 \cdot 0.05CH_3OH \cdot 0.2H_2O$) C, H, Cl, N.

2,4-Diamino-6-[(3′,4′-dichloro-*N*-methylanilino)methyl]pyrido[3,2-*d*]pyrimidine (10). 3,4-Dichloroformanilide, mp 105–106 °C (lit.[33] mp 112–112 °C), was prepared from 3,4-dichlroaniline (3.24 g, 0.02 mol) as in the preceding experiment; yield 3.74 g (98%). *N*-Methyl-3,4-dichloroaniline, prepared from the anilide (1.9 g, 0.01 mol) by reduction with $LiAlH_4$ (0.38 g, 0.01 mol) as in the preceding experiment, was isolated as an oil (1.58 g, 90%) and used directly. The amine (266 mg, 1.51 mmol), $NaHCO_3$ (252 mg, 3 mmol), and **11**·HBr (from 100 mg, 0.524 mmol of **18**) were stirred in dry DMF (5 mL) at room temperature for 6 days, and the product was worked up the same way as **8** to obtain **10** as an off-white solid (77 mg, 42%): mp 199–202 °C; IR (KBr) ν 3450, 3330, 3180, 2920, 1740, 1620, 1595, 1570, 1495, 1450, 1385, 1375 sh, 1365 sh, 1340, 1260 sh, 1245, 1230, 1210 sh cm^{-1}; ^{1}H-NMR (d_6-DMSO) δ 3.10 (s, 3H, NMe), 4.67 (s, 2H, NCH_2), 6.13 (D_2O exchangeable br s, 2H, NH_2), 6.72 (dd, $J_{6',5'}$ = 8 Hz, $J_{6',2'}$ = 3 Hz, 1H, 6′-H), 6.93 (d, $J_{2',6'}$ = 3 Hz, 2′-H), 7.15–7.65 (partly D_2O exchangeable m, 3H, 3′-H, C_7-H, C_8-H, NH_2). Anal. ($C_{15}H_{14}Cl_2N_6 \cdot 0.05CH_3OH$) C, H, Cl, N.

2,4-Diamino-6-[[2′,5′-dimethoxy-*N*-(*tert*-butyloxycarbonyl)anilino]methyl]pyrido[3,2-*d*]pyrimidine (27). A solution of 2,5-dimethoxyaniline (0.77 g, 0.005 mol) and di-*tert*-butyl pyrocarbonate (1.16 g, 0.005 mol) in dry THF (20 mL) was refluxed for 21 h and evaporated to dryness. The residue was partitioned between EtOAc and 5% citric acid, and the organic layer was evaporated to obtain the Boc derivative **23** as an amber-colored oil (1.4 g, ca. 100%), which was used without further purification. A solution of crude **23** (253 mg, 1 mmol) in dry DMF (5 mL) was added during 5 min to a suspension of NaH (120 mg, 5 mmol) (from 200 mg of a 60% mineral oil dispersion) in dry DMF (30 mL) cooled in an ice bath and kept under N_2. The mixture was stirred at 0 °C under N_2 for another 20 min, and a solution of **11**·HBr (from 191 mg, 1 mmol of **18**) in dry DMF (10 mL) was added

dropwise over 15 min. The mixture was allowed to come to room temperature and left to stir overnight before being quenched with glacial AcOH (1 mL) and evaporated to dryness on the rotary evaporator. The residue was partitioned between EtOAc and H_2O, and a small amount of insoluble solid was discarded. The EtOAc layer, whose TLC (silica gel, 10:1 $CHCl_3$–MeOH) showed unchanged **23** at R_f 0.8, two unknown minor impurities (one yellow) at R_f 0.5, and a dark spot at R_f 0.3 (**27**), was chromatographed on silica gel (20 g, 2 × 20 cm). Successive elution with $CHCl_3$, 50:1 $CHCl_3$–MeOH, and 40:1 $CHCl_3$–MeOH removed **23** (>90% recovery) and the twin R_f 0.5 spots. Further elution with 20:1 $CHCl_3$–MeOH yielded the product. Evaporation of pooled fractions with R_f 0.3 afforded **27** as a white solid (19 mg, 4%): mp 224–228 °C; IR (KBr) ν 3420, 3330, 3160, 2970, 2930, 2830, 1695, 1660 sh, 1640, 1620 sh, 1565, 1505, 1450, 1395, 1365, 1340, 1310, 1295, 1275, 1250, 1215 cm^{-1}; ^1H-NMR ($CDCl_3$ + d_6-DMSO) δ 1.37 (s, 9H, t-Bu), 3.68 (s, 3H, MeO), 3.75 (s, 3H, MeO), 4.87 (s, 2H, CH_2N), 6.05 (D_2O exchangeable br m, >4H, 2 × NH_2), 6.80 (m, 3H, phenyl protons, 7.72 (s, 2H, C_7- and C_8-H). Anal. ($C_{21}H_{26}N_6O_4 \cdot 0.05CH_3OH \cdot 0.5H_2O$) C, H, N.

Acknowledgment. This work was supported in part by Grant RO1-AI29904 (A.R.) and Contract NO1-AI35171 (S.F.Q.) from the NIAID, Division of AIDS.

References

(1) Masur, H. Problems in the management of opportunistic infections in patients infected with human immunodeficiency virus. *J. Infect. Dis.* **1990**, *161*, 858–864.
(2) Barlett, M. S.; Smith, J. W. *Pneumocystis carinii*, an opportunist in immunocompromized patients. *Clin. Microbiol. Rev.* **1991**, *4*, 137–149.
(3) Luft, B. J.; Remington, J. S. Toxoplasmic encephalitis in AIDS. *Clin. Infect. Dis.* **1992**, *15*, 211–222.
(4) Allegra, C. J.; Chabner, B. A.; Tuazon, C. U.; Ogata-Arakaki, D.; Baird, B.; Drake, J. C.; Simmons, J. T.; Lack, E. E.; Shelhamer, J. H.; Balis, F.; Walker, R.; Kovacs, J. A., Lane, H. C.; Masur, H. Trimetrexate for the treatment of *Pneumocystis carinii* pneumonia in patients with the acquired immunodeficiency syndrome. *N. Engl. J. Med.* **1989**, *317*, 978–985.
(5) Masur, H.; Polis, M. A.; Tuazon, C. U.; Ogata-Arakaki, D.; Kovacs, J. A.; Katz, D.; Hilt, D.; Simmons, T.; Feuerstein, I.; Lundgren, B.; Lane, H. C.; Chabner, B. A.; Allegra, C. J. Salvage trial of trimetrexate-leucovorin for the treatment of cerebral toxoplasmosis. *J. Infect. Dis.* **1989**, *160*, 312–320.
(6) Sattler, F. R.; Frame, P.; Davis, R.; Nichols, L.; Shelton, B.; Akil, B.; Baughman, R.; Hughlett, C.; Weiss, W.; Boylen, C. T.; van der Horst, C.; Black, J.; Powderly, W.; Steigbigel, R. T.; Leedom, J. M.; Masur, H.; Feinberg, J.; and the AIDS Clinical Trials Group 029/031 Research Team. Trimetrexate with leucovorin versus trimethoprim-sulfamethoxazole for moderate to severe episodes of *Pneumocystis carinii* pneumonia in patients with AIDS: a prospective, controlled multicenter investigation of the AIDS Clinical Trials Group Protocol 029/031. *J. Infect. Dis.* **1994**, *170*, 165–172.
(7) Allegra, C. J.; Kovacs, J. A.; Drake, J. C.; Swan, J. C.; Chabner, B. A.; Masur, H. Potent in vitro and in vivo antitoxoplasma activity of the lipid-soluble antifolate trimetrexate. *J. Clin. Invest.* **1987**, *79*, 478–482.
(8) Kovacs, J. A.; Allegra, C. J.; Swan, J. C.; Drake, J. C.; Parrillo, J. E.; Chabner, B. A.; Masur, H. Potent antipneumocystis and antitoxoplasma activities of piritrexim, a lipid-soluble antifolate. *Antimicrob. Agents Chemother.* **1988**, *32*, 430–433.
(9) Sirawaraporn, W.; Edman, J. C.; Santi, D. V. Purification and properties of recombinant *Pneumocystis carinii* dihydrofolate reductase. *Protein Express. Purif.* **1991**, *2*, 313–316.
(10) Broughton, M. C.; Queener, S. F. *Pneumocystis carinii* dihydrofolate reductase used to screen potential antipneumocystis drugs. *Antimicrob. Agents Chemother.* **1991**, *35*, 1348–1355.
(11) Queener, S. F.; Barlett, M. S.; Jay, M. A.; Durkin, M. M.; Smith, J. W. Activity of lipid-soluble inhibitors of dihydrofolate reductase against *Pneumocystis carinii* in culture and in a rat model of infection. *Antimicrob. Agents Chemother.* **1987**, *31*, 1323–1327.
(12) Kovacs, J. A.; Allegra, C. J.; Beaver, J.; Boarman, D.; Lewis, M.; Parrillo, J. E.; Chabner, B.; Masur, H. Characterization of de novo folate synthesis in *Pneumocystis* carinii and *Toxoplasma gondii*: potential for screening therapeutic agents. *J. Infect. Dis.* **1989**, *160*, 312–320.
(13) Allegra, C. J.; Boarman, D.; Kovacs, J. A.; Morrison, P.; Beaver, J.; Chabner, B. A.; Masur, H. Interaction of sulfonamide and sulfone compounds with *Toxoplasma gondii* dihydropteroate synthase. *J. Clin. Invest.* **1990**, *85*, 371–379.
(14) Merali, S.; Zhang, Y.; Sloan, D.; Meshnick, S. Inhibition of *Pneumocystis carinii* dihydropteroate synthetase by sulfa drugs. *Antimicrob. Agents Chemother.* **1990**, *34*, 1075–1078.
(15) Chio, L.-C.; Queener, S. F. Identification of highly potent and selective inhibitors of *Toxoplasma gondii* dihydrofolate reductase. *Antimicrob. Agents Chemother.* **1993**, *37*, 1914–1923.
(16) Walzer, P. D.; Foy, J.; Steele, P.; White, M. Synergistic combinations of Ro 11-8958 and other dihydrofolate reductase inhibitors with sulfamethoxazole and dapsone for therapy of experimental pneumocystosis. *Antimicrob. Agents Chemother.* **1993**, *37*, 1436–1443.
(17) Chang, H. R.; Arsenijevic, D.; Comte, R.; Polak, A.; Then, R. L.; Pechère, J.-C. Activity of epiroprim (Ro 11-8958), a dihydrofolate reductase inhibitor, alone and in combination with dapsone against *Toxoplasma gondii*. *Antimicrob. Agents Chemother.* **1994**, *38*, 1803–1807.
(18) Gangjee, A.; Shi, J.; Queener, S. F.; Barrows, L. R.; Kisliuk, R. L. Synthesis of 5-methyl-5-deaza nonclassical antifolates as inhibitors of dihydrofolate reductases and as potential antipneumocystis, antitoxoplasma, and antitumor agents. *J. Med. Chem.* **1993**, *36*, 3437–3443.
(19) Rosowsky, A.; Hynes, J. B.; Queener, S. F. Structure-activity and structure-selectivity studies on diaminoquinazolines and other inhibitors of *Pneumocystis carinii* and *Toxoplasma gondii* dihydrofolate reductase. *Antimicrob. Agents Chemother.* **1994**, *39*, 79–86.
(20) Rosowsky, A.; Mota, C. E.; Wright, J. E.; Queener, S. F. 2,4-Diamino-5-chloroquinazoline analogues of trimetrexate and piritrexim: synthesis and antifolate activity. *J. Med. Chem.* **1994**, *37*, 4522–4528.
(21) Rosowsky, A.; Mota, C. E.; Queener, S. F.; Waltham, M.; Ercikan-Abali, E.; Bertino, J. R. 2,4-Diamino-5-substituted-quinazolines as inhibitors of a human dihydrofolate reductase with a site-directed mutation at position 22 and of the dihydrofolate reductases from *Pneumocystis carinii* and *Toxoplasma gondii*. *J. Med. Chem.* **1995**, *38*, 745–752.
(22) Rosowsky, A.; Freisheim, J. H.; Hynes, J. B.; Queener, S. F.; Barlett, M.; Smith, J. W.; Lazarus, H.; Modest, E. J. Tricyclic 2,4-diaminopyrimidines with broad antifolate activity and the ability to inhibit *Pneumocystis carinii* growth in cultured human lung fibroblasts in the presence of leucovorin. *Biochem. Pharmacol.* **1989**, *38*, 2677–2684.
(23) Rosowsky, A.; Mota, C. E.; Wright, J. E.; Freisheim, J. H.; Heusner, J. J.; McCormack, J. J.; Queener, S. F. 2,4-Diaminothieno[2,3-*d*]pyrimidine analogues of trimetrexate and piritrexim as potential inhibitors of *Pneumocystis carinii* and *Toxoplasma gondii* dihydrofolate reductase. *J. Med. Chem.* **1993**, *36*, 3103–3112.
(24) Gangjee, A.; Devraj, R.; McGuire, J. J.; Kisliuk, R. L.; Queener, S. F.; Barrows, L. R. Classical and nonclassical furo[2,3-*d*]-pyrimidines as novel antifolates: synthesis and biological activities. *J. Med. Chem.* **1994**, *37*, 1169–1176.
(25) Srinivasan, A.; Broom, A. Pyridopyrimidines. 10. Nucleophilic substitutions in the pyrido[3,2-*d*]pyrimidine series. *J. Org. Chem.* **1979**, *44*, 435–440.
(26) Srinivasan, A.; Broom, A. Pyridopyrimidines. 12. Synthesis of 8-deaza analogues of aminopterin and folic acid. *J. Org. Chem.* **1981**, *46*, 1777–1781.
(27) Srinivasan, A.; Amarnath, V.; Broom, A. D.; Zou, F. C.; Cheng, Y.-C. A potent multisubstrate analogue inhibitor of human thymidylate synthetase. *J. Med. Chem.* **1984**, *27*, 1710–1717.
(28) Then, R. L.; Hartman, P. G.; Kompis, I.; Santi, D. Selective inhibition of dihydrofolate reductase from problem human pathogens. *Adv. Exp. Med. Biol.* **1993**, *338*, 533–536.
(29) Coppola, G.; Schuster, H. The chemistry of 2*H*-3,1-benzoxazine-2,4(1*H*)-dione (isatoic anhydride). 21. A mild process for the preparation of 10-alkyl-9-acridanones and its application to the synthesis of acridone alkaloids. *J. Heterocycl. Chem.* **1989**, *26*, 957–964.
(30) Chattaway, F. D.; Orton, K. J. P.; Hurtley, W. H. Notizen über einige anilide. (Notes concerning several anilides.) *Ber.* **1899**, *32*, 3635–3638.
(31) Roberts, R. M.; Vogt, P. J. Ortho esters, imidic esters and amidines. VII. N-Alkoxyformanilides from alkyl orthoformates and primary aromatic amines; rearrangement of alkyl N-arylformimidates. *J. Am. Chem. Soc.* **1956**, *78*, 4778–4781.
(32) Davis, O. C. M. The quantitative decomposition of the anilides: a study of steric influence. *J. Chem. Soc.* **1909**, *95*, 1397–1403.
(33) Huffman, C. W. Formylation of amines. *J. Org. Chem.* **1958**, *23*, 727–729.

JM950221T