Inhibition of *Pneumocystis carinii*, *Toxoplasma gondii*, and *Mycobacterium avium* Dihydrofolate Reductases by 2,4-Diamino-5-[2-methoxy-5-(ω-carboxyalkyloxy)benzyl]pyrimidines: Marked Improvement in Potency Relative to Trimethoprim and Species Selectivity Relative to Piritrexim

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A series of previously undescribed 2,4-diamino-5-[2-methoxy-5-alkoxybenzyl]pyrimidines (**3a**−**e**) and 2,4-diamino-5-[2-methoxy-5-(ω-carboxyalkyloxy)benzyl]pyrimidines (**3f**−**k**) with up to eight CH_2 groups in the alkoxy or ω-carboxyalkyloxy side chain were synthesized and tested for the ability to inhibit partially purified dihydrofolate reductase (DHFR) from *Pneumocystis carinii* (Pc), *Toxoplasma gondii* (Tg), *Mycobacterium avium* (Ma), and rat liver in comparison with two standard inhibitors, trimethoprim (**1**) and piritrexim (**2**). The latter drug is known to be extremely potent but shows a marked preference for binding to mammalian DHFR, whereas the former is very selective for the parasite enzymes but is a much weaker inhibitor. The underlying strategy for the synthesis of compounds **3a**−**k** was that a hybrid structure embodying some features of both **1** and **2** might possess a more favorable combination of potency and selectivity than either parent drug. The choice of analogues **3f**−**k** was based on the idea that the acidic ω-carboxyl group might interact preferentially with a basic center in the active site of DHFR from any of the parasite species relative to the active site of mammalian DHFR. In addition, the ω-carboxyl group was expected to improve water solubility relative to **1** or **2**. In standardized spectrophotometric assays with dihydrofolate as the substrate and NADPH as the cofactor, 2,4-diamino-5-[(2-methoxy-4-carboxybutyloxy)benzyl]pyrimidine (**3g**) inhibited Pc DHFR with an IC_{50} of 0.049 μM and rat DHFR with IC_{50} of 3.9 μM. Its potency against Pc DHFR was 140-fold greater than that of **1** and close to that of **2**, and its selectivity index, defined as the ratio IC_{50}(rat liver)/IC_{50}(*P. carinii*), was 8-fold higher than that of **1** and >10^4-fold higher than that of **2**. Although it was less potent and less selective against Tg than Pc DHFR, it was very potent as well as highly selective against Ma DHFR, with an IC_{50} of 0.0058 μM and an IC_{50}(rat liver)/IC_{50}(*M. avium*) ratio of >600. Because of this favorable combination of potency and selectivity relative to **1** and **2**, compound **3g** may be viewed as a promising lead in the search for new antifolates with potential clinical activity against *P. carinii* and other opportunistic pathogens in patients with AIDS.

Introduction

Trimethoprim (**1**, TMP) and piritrexim (**2**, PTX) are
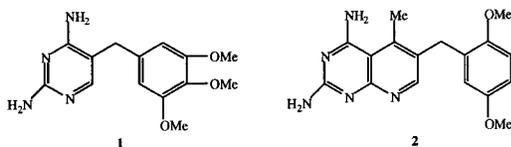
lipid-soluble antifolates that have been used clinically for the prophylaxis and treatment of *Pneumocystis carinii* and *Toxoplasma gondii* infections in patients with AIDS.[1] TMP was developed over 40 years ago as an antibacterial drug and continues to be widely prescribed, generally in combination with a sulfonamide such as sulfamethoxazole (this combination, also called

cotrimoxazole, is popularly known as Bactrim). A number of clinical studies evaluating a variety of TMP-sulfa drug combinations for treatment or prevention of life-threatening *P. carinii* and *T. gondii* infections in AIDS have been reported in the past decade.[2,3] Cotrimoxazole is relatively inexpensive and thus plays an especially important role in controlling AIDS opportunistic infections in economically disadvantaged countries. Initially developed as an anticancer drug,[4] PTX has recently shown some promise against head-and-neck squamous cell carcinoma and bladder carcinoma[5] and has been the subject of one limited clinical study against *P. carinii* pneumonia in patients with AIDS.[6] Both TMP and PTX have as their target the enzyme dihydrofolate reductase (DHFR), which plays a ubiquitous role in one-carbon metabolism by mediating the biosynthesis of DNA, RNA, and the essential amino acid methionine.[7]

Effective use of TMP against *P. carinii* and *T. gondii* requires coadministration of a sulfa drug because these parasites are able to synthesize reduced folate cofactors

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Scheme 1[a]
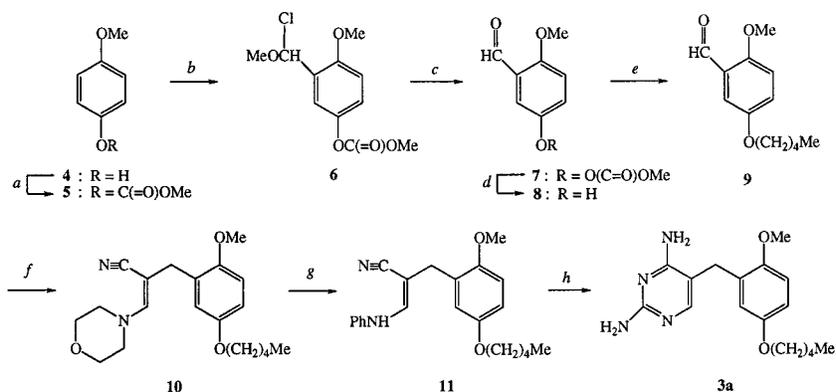
[a] Reagents: (a) MeOC(=O)Cl/C_5H_5N; (b) MeOCHCl$_2$/TiCl$_4$; (c) HCl, 0°C; (d) HCl/MeOH, reflux; (e) Br(CH$_2$)$_5$Me/K$_2$CO$_3$/DMSO; (f) 3-morpholinopropionitrile/NaOMe/DMSO; (g) PhNH$_2$·HCl/EtOH; (h) H$_2$NC(=NH)NH$_2$/EtOH.

de novo. Indeed, these organisms lack a membrane-transport protein for reduced folates and thus cannot rely on their host for their supply of these essential cofactors.[8] However, complete inhibition of one-carbon metabolism in *P. carinii* and *T. gondii* cannot be achieved by blocking the action of DHFR with a weak inhibitor like TMP but requires de novo synthesis of reduced folate cofactors in the parasite to be turned off as well. An unfortunate disadvantage of sulfa drugs is that some patients tend to become severely allergic to them and therefore cannot tolerate chronic treatment with TMP-sulfa drug combinations.[9a] Although allergic side effects can be lessened with corticosteroids, the use of steroids in AIDS can itself pose significant risk.[9b] Thus, a TMP analogue potent enough against *P. carinii* or *T. gondii* to circumvent the need to administer a sulfa drug would be highly desirable.

Tighter-binding DHFR inhibitors than TMP have the potential to be effective without coadministration of a sulfa drug, provided that they bind sufficiently better to the *P. carinii* and/or *T. gondii* enzyme than to the enzyme of the host. A modest level of species selectivity for both *P. carinii* and *T. gondii* DHFR compared with mammalian DHFR is shown by TMP, but its potency as an inhibitor of the enzyme is only in the micromolar range.[10a-d] By contrast, PTX inhibits DHFR at nanomolar concentrations but unfortunately binds much more tightly to the mammalian enzyme than it does to the *P. carinii* or *T. gondii* enzyme.[10a-d] For this reason, in the clinical trial to assess the efficacy of PTX against *P. carinii* pneumonitis in AIDS patients, 5-formyl-5,6,7,8-tetrahydrofolate (leucovorin) was coadministered in order to prevent life-threatening hematologic toxicity.[6]

As part of a larger search for lipophilic DHFR inhibitors that would combine the species selectivity of TMP with the potency of PTX,[11] we were interested in synthesizing analogues of general structure **3**, in which the 2,4-diaminopyrimidine moiety of TMP was retained but the pattern of substitution in the right-hand benzyl moiety was modified from that of TMP (i.e., 3′,4′,5′-trimethoxy) to that of PTX (i.e., 2′,5′-dimethoxy). Moreover, we postulated, on the basis of reported 3D structures of the ternary complexes of *P. carinii* and mammalian DHFR with TMP[12] and PTX,[13] that replacement of the *O*-methyl group at the 5′-position by long-chain *O*-alkyl or *O*-(ω-carboxyalkyl) groups might

increase potency and species selectivity in tandem. The present paper describes the synthesis of the previously unknown compounds **3a–k** and also reports data on

3a-e (R = Me, n = 4-8)
3f-k (R = CO$_2$H, n = 3-8)
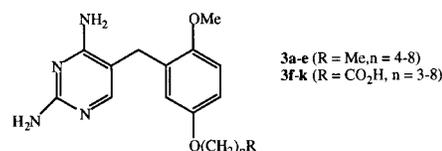
their activity as inhibitors of partially purified preparation of DHFR from rat liver, *P. carinii*, and *T. gondii*. Because of the well-documented prevalence of tuberculosis as a life-threatening opportunistic disease in AIDS patients,[14] we also report the activity of these compounds against the enzyme from *Mycobacterium avium*. A number of DHFR inhibitors have been found to inhibit proliferation of *M. avium* in culture or in an animal model,[15] but the potential benefit of lipophilic antifolates in combination with leucovorin and sulfa drugs for the prophylaxis or therapy of opportunistic tuberculosis infection in AIDS patients appears to be untested at this point.

Chemistry

As shown in Scheme 1, treatment of 4-methoxyphenol (**4**) with methyl chloroformate in the presence of pyridine afforded the carbonate ester **5** (92% yield), which on reaction with MeOCHCl$_2$ and TiCl$_4$ as described earlier[16] was converted to the masked aldehyde **6**. On treatment with HCl at 0 °C for 30 min, compound **6** underwent cleavage to aldehyde **7**. More vigorous acidolysis of **7** cleaved the carbonate ester to form the phenol **8**, and further reaction with 1-bromopentane in the presence of K$_2$CO$_3$ in DMSO solution afforded the previously unknown ether **9**. Condensation of **9** with 3-morpholinopropionitrile in anhydrous DMSO containing a catalytic amount of NaOMe[17a] led to the 3-morpholinoacrylonitrile **10**, which on sequential heating with aniline hydrochloride and guanidine[17b] was converted into 2,4-diamino-5-[(2-methoxy-5-pentyloxy)benzyl]pyrimidine (**3a**) via the 3-anilinoacrylonitrile **11**. Exchange of the morpholino group by an anilino group was an essential step in the sequence because **10** itself failed to undergo cyclization with guanidine. Compounds **10** and **11** were each used without purification.

Scheme 2[a]
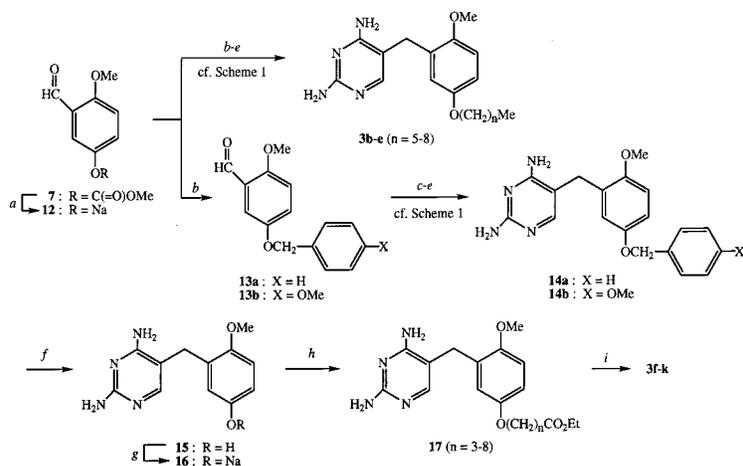
[a] Reagents: (a) NaOMe/MeOH; (b) RBr/DMSO; (c) 3-morpholinopropionitrile/NaOMe/DMSO; (d) PhNH$_2$·HCl/EtOH; (e) H$_2$NC(=NH)NH$_2$; (f) TsOH/MeOH; (g) NaOEt/EtOH; (h) Br(CH$_2$)$_n$CO$_2$Et/DMSO; (i) NaOH/aqueous DMSO.

The overall yield of **3a** for the seven steps starting from **4** was 30%. The final product was purified by flash chromatography on silica gel (10:1 EtOAc/MeOH) followed by recrystallization from aqueous EtOH. In addition to other alkyl and aryl proton signals, the ^{1}H NMR spectrum of **3a** in DMSO-d_6 solution featured the expected singlets at δ 3.47 for the CH$_2$ group between the phenyl and pyrimidine rings, δ 3.73 for the 2-OMe group, and δ 7.39 for the C^6 proton on the pyrimidine ring.

Although the synthesis of **3a** as described above was workable, we felt that an improvement would be to use NaOMe for the deprotection of **7**. We reasoned that cleavage of the carbonate protecting group would afford a DMSO-soluble Na salt of the phenol that could be alkylated directly. Thus, **7** was treated with 1 equiv of NaOMe in MeOH at room temperature, the solution was evaporated to dryness, and the residue was triturated with Et$_2$O to obtain the Na salt (**12**) as a solid. After being dried thoroughly under vacuum, the solid was dissolved in dry DMSO and allowed to react with 1-bromohexane at room temperature to obtain the *O*-hexyl derivative of **8**, which was then subjected to the same sequence of steps as **9** (cf. Scheme 1) to obtain 2,4-diamino-5-[(2-methoxy-5-hexyloxy)benzyl]pyrimidine (**3b**) with a five-step yield of 58% starting from **8**. The same improved sequence using other bromoalkanes afforded the longer-chain analogues **3c**–**e** in overall yields of 63% (**3c**), 58% (**3d**), and 47% (**3e**).

Because of a concern that the strongly basic condensation reactions in Scheme 1 might cause side reactions involving the α-CH$_2$ or carbonyl group, a somewhat different sequence of steps was followed to obtain the carboxy analogues **3f**–**k**. Thus, as shown in Scheme 2, compound **8** was converted to **12** with NaOMe in MeOH, the dried salt was condensed with benzyl bromide in DMSO, and the resulting *O*-benzyl derivative **13a** was converted to the previously unknown diaminopyrimidine **14a** (59% yield), from which we had intended to cleave the *O*-benzyl group by catalytic hydrogenolysis over Pd/C. In the event, this debenzylation proved to be quite difficult, and we therefore abandoned **14a** in favor of the *O*-(4-methoxybenzyl) derivative **14b**. When **14b** was left to stand for 2 days at room temperature in MeOH solution containing a 2-fold excess of TsOH·

H$_2$O, it was converted to 2,4-diamino-5-[(2-methoxy-5-hydroxy)benzyl]pyrimidine (**15**) in 89% yield. The Na salt **16** was then generated with a stoichiometric amount of NaOEt in EtOH and was isolated by evaporation of the solvent. After being rigorously dried in a vacuum, **16** was taken up in dry DMSO and allowed to react with 1 equiv of ethyl 5-bromopentanoate at room temperature while monitoring the progress of the reaction by TLC (silica gel, 5:1 EtOAc/MeOH). The resulting ester **17** (n = 5) was saponified by addition of a 2-fold excess of NaOH directly to the alkylation mixture, and the resulting acid **3g** was purified by column chromatography on Dowex 50W-X2 sulfonic acid resin using 1.5% NH$_4$OH as the eluent. Freeze-drying of appropriately pooled fractions followed by redissolution in a minimal volume of dilute NH$_4$OH and acidification with 10% AcOH afforded the product as an off-white powder, usually solvated with AcOH and/or H$_2$O. Purification by ion-exchange chromatography on a DEAE-cellulose (HCO$_3^-$ form) was also tried but was found to be much less satisfactory because of poor solubility. The same sequence of steps was also used to obtain **3f** and **3h**–**k** from other bromo esters via **17** (n = 3, 4, 6–8) with an overall two-step yield of 65–85% based on **15**. The identity of each product was confirmed by ^{1}H NMR and by microanalysis. As with **3a**–**e**, characteristic singlets were observed at δ 3.46, 3.72, and 7.37, and where solvation with AcOH was indicated by microanalysis, an extra singlet not assignable to protons in the diaminopyrimidine derivative was observed at δ1.88. It may be noted that Scheme 2 should, in principle, be applicable to the synthesis of **3a**–**e** but was not used for this purpose because adequate amounts of the simple *O*-alkyl analogues for in vitro testing had already been made via Scheme 1.

Enzyme Inhibition

Compounds **3a**–**k** were tested for their ability to inhibit DHFR from *P. carinii*, *T. gondii*, *M. avium*, and rat liver as described in earlier papers,[10b,c] and selectivity index (SI) values were determined from the ratio IC$_{50}$(rat liver)/IC$_{50}$(*P. carinii*, *T. gondii*, or *M. avium*). The results are shown in Table 1, along with previously

Table 1. Inhibition of *P. carinii*, *T. gondii*, *M. avium*, and Rat Liver DHFR by 2,4-Diamino-5-[2-methoxy-5-(long-chain alkoxy and ω-carboxyalkoxy)benzyl]pyrimidines

	IC_{50}[a] (μM)				SI[b]		
cmpd	*P. carinii*	*T. gondii*	*M. avium*	rat liver	*P. carinii*	*T. gondii*	*M. avium*
1	12	2.7	0.19	130	11	44	680
2	0.031	0.017	ND	0.0015	0.048	0.088	ND
3a	19	6.5	0.31	44	2.3	6.8	140
3b	14	9.2	0.39	29	2.1	3.1	74
3c	5.6	5.0	0.89	11	1.9	2.1	12
3d	44	15	3.3	30	0.69	2.0	9.1
3e	51	32	32	86	1.7	2.7	2.7
3f	0.25	0.18	0.0048	2.6	11	14	560
3g[c]	0.049	0.11	0.0058	3.9	80	35	660
3h	0.80	0.48	0.15	17	21	35	110
3i	2.6	0.18	0.19	12	4.5	65	63
3j	7.1	0.36	0.13	12	1.7	33	92
3k	4.8	0.43	0.15	12	2.5	28	80

[a] Results for trimethoprim (**1**) and piritrexim (**2**) were obtained under the same standardized assay conditions as **3a**−**k**, and are taken from ref 11. ND = not determined. [b] SI = IC_{50}(rat liver)/IC_{50}(*P. carinii*, *T. gondii*, or *M. avium*). [c] In a separate experiment to confirm the results for **3g** against *P. carinii* and rat liver DHFR, the IC_{50} values were found to be 0.055 and 5.4 μM, respectively, giving a calculated SI of 98.

reported data for trimethoprim (**1**) and piritrexim (**2**) also presented for comparison.

The most potent of the *O*-alkyl derivatives against *P. carinii*, *T. gondii*, and rat liver DHFR was **3c** ($n = 6$), with IC_{50} values of 5.6, 5.0, and 11 μM, respectively. Moreover, there was an increase in potency against all three enzymes as the length of the 5′-*O*-alkyl group increased from $n = 4$ to $n = 6$, followed by a decrease in potency as this length increased from $n = 6$ to $n = 8$. However, this pattern was slightly different in the case of the *M. avium* enzyme, which tended to be more sensitive than the others. The most potent of the 5′-(*O*-alkyl) derivatives against this enzyme were **3a** ($n = 4$) and **3b** ($n = 5$), with IC_{50} values of 0.31 and 0.39 μM. Interestingly, while **3a**−**e** were only marginally selective for *P. carinii* and *T. gondii* DHFR relative to the rat enzyme, **3a** was somewhat selective for *M. avium* DHFR, with SI values of 140. Although the SI of **3a** against *M. avium* DHFR was not as high as that of trimethoprim (**1**), which had an SI of 680, these results suggested that efforts directed toward the discovery of more selective analogues than **3a** might be of interest.

As can be seen from Table 1, the potency of **3c**, the best of the 5′-(*O*-alkyl) derivatives, was slightly greater against *P. carinii* DHFR than that of **1**. However, because **3c** inhibited the rat enzyme 12-fold better than **1**, its SI was lower. Moreover, **3c** showed even lower selectivity for the *T. gondii* enzyme. On the other hand, because of the very high potency of piritrexim (**2**) against rat DHFR, the selectivity of **3c** for both the *P. carinii* and the *T. gondii* enzymes relative to **2** was improved by at least 10-fold.

The most potent of the 5′-*O*-(ω-carboxyalkyl) analogues **3f**−**k** against *P. carinii* DHFR was **3g** ($n = 4$), with an IC_{50} of 0.049 μM compared with 12 μM for **1** and 0.031 μM for **2**. Thus, **3g** approached the potency of **2** against this enzyme and was 245 times more potent than **1**. As in the 5-(*O*-alkyl) series, the potency of the 5′-*O*-(ω-carboxyalkyl) derivatives decreased when *n* was greater than 4. Moreover, the potency of **3f** was lower

than that of **3g**, confirming that the optimal value of *n* against *P. carinii* DHFR was 4. The carboxyalkyl derivatives **3f**−**k** were also more potent than **3a**−**e** against both *T. gondii* and *M. avium* DHFR. With the exception of **3c**, this was also true for inhibition of the rat enzyme. As in the case of the *P. carinii* enzyme, **3g** was the best inhibitor of *T. gondii* and *M. avium* DHFR but was also a potent inhibitor of the rat enzyme.

A gratifying aspect of the data in Table 1 was the high selectivity of **3g** for *P. carinii* versus rat DHFR. Although a somewhat lower SI was obtained with **3h**, both compounds were superior to **1** and **2**. The selectivity index of **3g** for this enzyme was 7-fold greater than that of **1** and >10⁴ times greater than that of **2**. Compound **3g** also displayed the best selectivity index of any of the compounds in both the 5′-*O*-alkyl and 5′-*O*-(ω-carboxyalkyl) series against the *M. avium* enzyme. Interestingly, the potency of **3g** was 33-fold higher than that of **1** against both the *M. avium* and rat DHFR, and thus, there was no difference in SI for the *M. avium* enzyme between these two compounds.

It was of interest to note that the pattern of selectivity of **3f**−**k** for *T. gondii* versus rat DHFR differed markedly from the pattern against the *P. carinii* and *M. avium* enzymes in that the most selective compound was **3i** rather than **3g** and that the SI was relatively insensitive to the length of the side chain, with **3i** (SI = 65) being only twice as selective as the other congeners (SI = 28−35). Also worth noting is the greater similarity in potency between **3g** and **2** against *P. carinii* and *T. gondii* DHFR than against rat DHFR, which suggests that the active site of these two enzyme may have features not present in the rat enzyme. While all three of the parasite enzymes have been cloned and sequenced,[18] only the 3D structure of the *P. carinii* enzyme is published.[12b,c]

There was a striking similarity between our results and those published about 20 years ago by Kuyper and co-workers[21] in one of the earliest examples of rational structure-based design of DHFR inhibitors. In a homologous series of TMP analogues in which the 3′-OMe group was replaced by *O*-(ω-carboxyalkyl) substituents containing up to six CH_2 groups, inhibition assays against *E. coli* DHFR revealed a progressive decrease in the K_i values from 2.6 to 0.035 nM as the number of CH_2 groups was increased from 1 to 3, followed by stabilization of the K_1 in the 0.025−0.066 nM range as this number was increased from 3 to 6. The highest affinity for *E. coli* DHFR was observed with the *O*-(5-carboxypentyl) analogue **18**. On the basis of the results

18
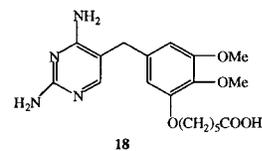
presented here, it would appear (a) that *P. carinii* and *M. avium* DHFR resemble *E. coli* DHFR in showing a binding preference for derivatives with a medium-length *O*-(ω-carboxyalkyl) group at the 3′ position and (b) that this feature is retained even when the OMe groups at the 3′ and 4′ positions are replaced by a single OMe group at the 2′ position.
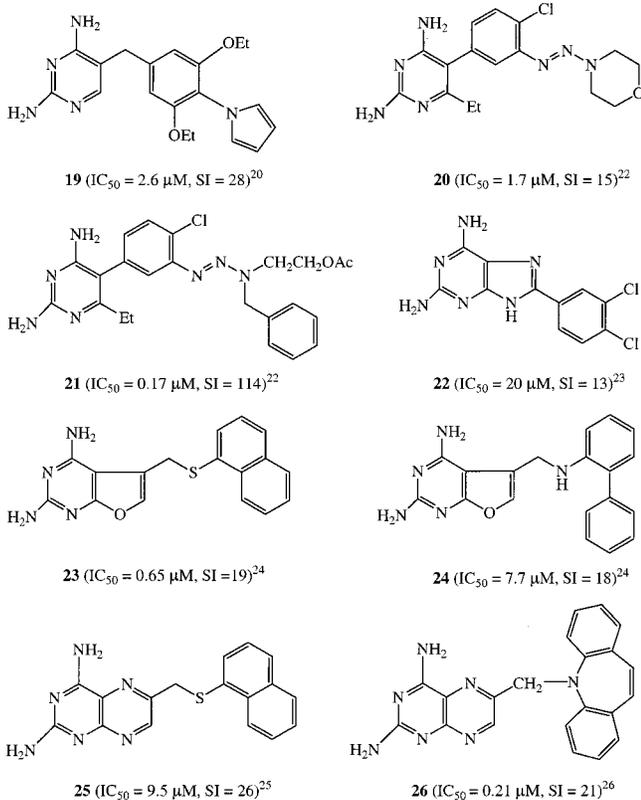
Figure 1. Structures of *P. carinii* DHFR inhibitors reported to be more potent as well as more selective than trimethoprim (**1**).

The published literature contains hundreds of mono-, di-, and tricyclic 2,4-diaminopyrimidines that are more potent than **1** as inhibitors of *P. carinii* versus rat DHFR, but a careful search reveals only a handful whose selectivity and potency are *both* greater than those of **1** (Figure 1). These include the trimethoprim analogue **19** (epiroprim, Ro11-8958),[19,20] the pyrimethamine analogues **20** and **21**,[22] the purine **22**,[23] the furo[2,3-*d*]pyrimidines **23** and **24**,[24] and the pteridines **25** and **26**.[25,26] Thus, compound **3g**, which most nearly approaches **21** in terms of having a highly favorable combination of potency and selectivity, joins this small group and thus may be viewed as a promising lead for structure−activity optimization.

Experimental Section

IR spectra were obtained on a Perkin-Elmer model 781 double-beam recording spectrophotometer. Only peaks with wavenumbers above 1400 cm^{-1} are reported. ^{1}H NMR spectra were recorded at 200 MHz on a Varian VX200 instrument, or in some instances at 60 MHz on a Varian EM360 instrument with Me$_4$Si as the internal standard. Each peak is denoted as a singlet (s), broad singlet (br s), doublet (d), doublet of doublets (dd), triplet (t), or pentet (p). TLC analyses were on Whatman MK6F silica gel plates with UV illumination at 254 nm. Column chromatography was on Baker 7024 flash silica gel (40 μM particle size). Melting points were measured in Pyrex capillary tubes in a Mel-Temp "electrothermal" apparatus (Fisher Scientific, Pittsburgh, PA) and are not corrected. 3-Morpholinopropionitrile was prepared by adding acrylonitrile dropwise to an equimolar amount of morpholine in an ice bath and stirring the mixture at room temperature for 1 h. The resulting light-yellow oil was used directly to prepare **13** and other 2-arylmethyl-3-morpholinoacrylonitriles. Other chemicals were purchased from Sigma-Aldrich (St. Louis, MO) or

Lancaster Synthesis (Windham, NH). Elemental analyses were performed by Quantitative Technologies, Inc. (Whitehouse, NJ) and were within ±0.4% of theoretical values.

2-Methoxy-5-pentyloxybenzaldehyde (9). Step 1. A stirred solution of **4** (24.8 g, 0.2 mol) and pyridine (16.6 g, 17 mL, 0.21 mol) in CH$_2$Cl$_2$ (150 mL) was cooled in an ice bath and treated dropwise with MeOCOCl (19.9 g, 16.2 mL, 0.21 mol) over a period of 15 min. The mixture was then stirred at room temperature for 45 min. After extraction with 1 N HCl, H$_2$O, 0.5 N NaOH, and H$_2$O, the organic solvent was removed by rotary evaporation and the residue distilled under vacuum to obtain 4-(methoxycarbonyloxy)anisole (**5**) as a colorless liquid that crystallized on prolonged standing and was used directly in the next step: yield 33.4 g (92%); bp 112 °C/0.2 Torr; mp 33−34 °C (lit.[27] mp 34.5−36 °C); ^{1}H NMR (200 MHz, CDCl$_3$) δ 3.73 (s, 3H, MeO), 3.83 (s, 3H, MeOCO), 6.85 (d, J = 9 Hz, 2H, aryl 3- and 5-H), 7.11 (d, J = 9 Hz, 2H, aryl 2- and 6-H).

Step 2. To a stirred solution of **5** (20 g, 0.11 mol) in CH$_2$Cl$_2$ (250 mL) at 0 °C was added TiCl$_4$ (49.2 g, 28.5 mL, 0.26 mol) in CH$_2$Cl$_2$ (50 mL). Stirring was continued at 0 °C while a solution of MeOCHCl$_2$ (14.6 g, 11.5 mL, 0.127 mol) was added dropwise over 30 min. When the addition was complete, the solution was warmed to room temperature, left to stand for 30 min, and concentrated to half-volume by rotary evaporation. The resulting solution, containing the α-chloro ether **6**, was poured into a mixture of ice (250 g) and 12 N HCl (10 mL), and then EtOAc (200 mL) was added. The two-phase mixture was stirred vigorously for 30 min, the layers were separated, the aqueous layer was back-extracted with EtOAc, and the combined EtOAc extracts were evaporated to dryness. Recrystallization of the crude solid (24.2 g) from a mixture of isooctane and absolute EtOH afforded 2-methoxy-5-(methoxycarbonyloxy)benzaldehyde (**7**) as white needles (17.7 g, 77%): mp 88−89 °C (lit.[16] mp 88−90 °C); ^{1}H NMR (CDCl$_3$) δ 3.90 (s, 3H, MeO), 3.92 (s, 3H, MeO), 6.99 (d, J = 9 Hz, 1H, aryl 3-H), 7.38 (dd, J = 9 Hz, J = 3 Hz, 1H aryl 4-H), 7.63 (d, J = 3 Hz, 1H, aryl 6-H), 10.45 (s, 1H, CH=O). Evaporation of the supernatant from the first crop afforded an additional 5.3 g (23%), bringing the total to 23 g (100%). The two fractions were combined and used without further purification.

Step 3. A suspension of the carbonate ester **7** (5.28 g, 5 mmol) in a mixture of MeOH (25 mL) and 2 N HCl (50 mL) was refluxed for 6 h, during which most of the solid dissolved. A small amount of insoluble tar was filtered off, the MeOH was evaporated under reduced pressure, and the residue was extracted with EtOAc. Evaporation of the EtOAc extract and recrystallization from H$_2$O containing a small amount of EtOH afforded **8** as a yellow powder (2.49 g, 65%) that was used directly in the next step: mp 109−110 °C (lit.[16] mp 111−113 °C); TLC R_f = 0.2 (silica gel, 2:1 isooctane/EtOAc).

Step 4. A solution of **8** (760 mg, 5 mmol) and K$_2$CO$_3$ (828 mg, 6 mmol) in dry DMSO (5 mL) was treated with 1-bromopentane (744 μL, 906 mg, 6 mmol). After 20 h at room temperature and then 10 min at 60 °C, the mixture was diluted with H$_2$O and extracted with EtOAc. Evaporation under reduced pressure left a solid (1.1 g), which was chromatographed on silica gel (15 g, 2 cm × 12 cm) with 2:1 isooctane/EtOAc as the eluent. Appropriately pooled fractions were evaporated to obtain 2-methoxy-5-pentyloxybenzaldehyde (**9**) as a yellow oil (1.12 g, 100%): TLC bluish spot, R_f = 0.6 (silica gel, 2:1 isooctane/EtOAc); IR (thin film) ν 2960, 2930, 2870, 2750, 1680, 1605, 1580, 1495, 1425 cm^{-1}; ^{1}H NMR (200 MHz, CDCl$_3$) δ 0.85 (m, 3H, pentyl Me), 1.35 (m, 4H, pentyl 3- and 4-CH$_2$), 1.73 (p, J = 7 Hz, 2H, pentyl 2-Me), 3.85 (s, 3H, MeO), 3.90 (t, J = 7 Hz, 2H, OCH$_2$), 6.90 (d, J = 9 Hz, 1H, aryl H-3), 7.09 (dd, J = 9 Hz, J = 3 Hz, 1H, aryl H-4), 7.28 (d, J = 3 Hz, 1H, aryl H-6), 10.40 (s, 1H, CH=O). Anal. (C$_{13}$H$_{18}$O$_3$) C, H.

2,4-Diamino-5-(2-methoxy-5-pentyloxybenzyl)pyrimidine (3a). Step 1. A solution of NaOMe was prepared by dissolving clean metallic Na (23 mg, 1 mmol) in absolute MeOH (3 mL). The solvent was evaporated under reduced pressure, the solid was taken up in DMSO (1.5 mL), and to the solution was added 3-morpholinopropionitrile (728 mg, 5.2

mmol). The solution was placed in an oil bath at 100 °C, and to it was slowly added a solution of **9** (1.05 g, 4.73 mmol) in DMSO (1.5 mL). After 20 min of heating, the reaction mixture was cooled and partitioned between EtOAc and H_2O that had been slightly acidified with dilute aqueous citric acid to prevent the formation of an emulsion. TLC analysis (silica gel, 1:1 isooctane/EtOAc) showed an I_2-absorbing spot with R_f = 0.3 (not UV-absorbing), along with a major UV-absorbing spot (R_f = 0.4) corresponding to the product (**10**) and a faint UV-absorbing impurity (R_f = 0.7). Flash chromatography on silica gel (20 g, 2 cm × 17 cm) afforded 2-(2-methoxy-5-pentyloxy-benzyl)-3-morpholinoacrylonitrile (**10**) as a yellow gum that was used directly the next step: yield 1.16 g (71%); ¹H NMR (200 MHz, CDCl₃) δ 0.88 (m, 3H, pentyl CH₃), 1.41 (m, 4H, pentyl 3- and 4-CH₂), 1.75 (p, J = 7 Hz, 2H, pentyl 2-CH₂), 3.0–3.8 (m, 15H, morpholine CH₂, MeO, pentyl OCH₂, and benzylic CH₂), 6.79 (m, 4H, vinyl and aryl protons).

Step 2. A solution of **10** (0.835 g, 2.43 mmol) and aniline hydrochloride (0.47 g, 3.64 mmol) in absolute EtOH (10 mL) was refluxed for 1 h. In a separate flask, guanidine hydrochloride (1.09 g, 11.4 mmol) was added to a solution of NaOEt prepared by dissolving clean metallic Na (345 mg, 15 mmol) in absolute EtOH (15 mL), and the flask was swirled manually for 5 min. The entire contents of the second flask (including the NaCl) were added to the first, and the combined mixture was refluxed for 18 h and then filtered while hot. TLC analysis of the filtrate (silica gel, 10:1 EtOAc/MeOH) showed the product (**3a**) as a major UV-absorbing spot (R_f = 0.3), along with several fast-moving impurities. Flash chromatography (silica gel, 18 g, 2 cm × 15 cm) with 10:1 EtOAc/MeOH as the eluent afforded an amorphous solid, which on recrystallization from aqueous EtOH afforded **3a** as white flakes (540 mg, 70%): mp 140–141 °C; IR (KBr) ν 3410, 3330, 3230, 3120, 2990, 2950, 2910sh, 2870, 2830, 1665, 1635, 1605, 1565, 1540, 1505, 1480, 1460, 1440, 1420 cm⁻¹; ¹H NMR (200 MHz, DMSO-d_6) δ 0.87 (m, 3H, pentyl Me), 1.33 (m, 4H, pentyl 3- and 4-CH₂), 1.64 (p, J = 7 Hz, 2H, pentyl 2-CH₂), 3.47 (s, 2H, bridge CH₂), 3.73 (s, 3H, OMe), 3.83 (t, J = 7 Hz, 2H, OCH₂), 5.65 (broad s, 2H, NH₂), 6.02 (br s, 2H, NH₂), 6.64 (d, J = 3 Hz, 1H, aryl H-6), 6.70 (dd, J = 8 Hz, J = 3H, 1H, aryl H-4), 6.87 (d, J = 8 Hz, 1H, aryl H-3), 7.39 (s, 1H, pyrimidine H-6). Anal. (C₁₇H₂₄N₄O₂) C, H, N.

General Procedure for a More Direct Synthesis of 2,4-Diamino-5-(2-methoxy-5-alkoxybenzyl)pyrimidines. Compound **8** (1.05 g, 5 mmol) was added to a solution of NaOMe prepared by dissolving clean metallic Na (115 mg, 5 mmol) in absolute MeOH (5 mL). After 5 min, the intensely yellow solution of the Na salt **12** was evaporated to dryness and the residue was triturated with Et₂O until a solid formed. The Et₂O was decanted and the powder dried with aid of a vacuum pump and taken up in DMSO (5 mL). The alkyl halide (5 mmol) was then added and the solution allowed to stand at room temperature overnight. In a separate flask, 3-morpho-linopropionitrile (700 mg, 5 mmol) was added to a solution of freshly prepared NaOMe (1 mmol) in DMSO (2 mL), and the mixture was placed in an oil bath preheated to 100 °C. To this solution were then added the contents of the first flask, and the combined reaction mixture was heated for 20 min. After being cooled to room temperature, the mixture was partitioned between EtOAc and dilute aqueous citric acid. TLC analysis of the organic layer gave predominantly one spot with an R_f of ca. 0.5 (silica gel, 1:1 EtOAc/isooctane) corresponding to the desired 2-arylmethyl-3-morpholinoacrylonitrile. The EtOAc was evaporated and replaced with absolute EtOH (20 mL), and after addition of aniline hydrochloride (0.97 g, 7.5 mmol), the mixture was refluxed for 1 h. Separately, guanidine hydrochloride (1.19 g, 12.5 mmol) was added to a solution of NaOEt obtained by dissolving Na metal (460 mg, 20 mmol) in absolute EtOH (15 mL). After being stirred for 5 min, the contents of the flask (including the precipitated NaCl) were transferred to the flask containing the 2-arylmethyl-3-anilinoacrylonitrile. The mixture was refluxed for 20 h and filtered while hot, and the product was isolated and purified in the same way as **3a**

(see above). The following analogues were prepared by this general method.

2,4-Diamino-5-(2-methoxy-5-hexyloxybenzyl)pyrimidine (3b). Yield 950 mg (58%); glistening white plates, mp 139–140 °C; IR (KBr) ν 3430, 3330, 3250, 3130, 3010, 2950, 2870, 1675, 1645, 1610, 1575, 1545, 1510, 1490, 1475, 1450, 1435, 1400 cm⁻¹; ¹H NMR (200 MHz, DMSO-d_6) δ 0.86 (t, J = 7 Hz, 3H, hexyl Me), 1.30 (m, 6H, hexyl 3- to 5-CH₂), 1.64 (p, J = 7 Hz, 2H, hexyl 2-CH₂), 3.47 (s, 2H, bridge CH₂), 3.73 (s, 3H, OMe), 3.83 (t, J = 7 Hz, 2H, OCH₂), 5.65 (br s, 2H, NH₂), 6.02 (br s, 2H, NH₂), 6.64 (d, J = 3 Hz, 1H, aryl H-6), 6.70 (dd, J = 8 Hz, J = 3 Hz, 1H, aryl H-4), 6.87 (d, J = 8 Hz, 1H, aryl H-3), 7.39 (s, 1H, pyrimidine H-6). Anal. (C₁₈H₂₆N₄O₂) C, H, N.

2,4-Diamino-5-(2-methoxy-5-heptyloxybenzyl)pyrimidine (3c). Yield 1.09 mg (63%); glistening white plates, mp 134–135 °C; IR (KBr) ν 3400, 3310, 3210, 3110, 2990, 2920, 2850, 1665, 1635, 1605, 1575, 1540, 1500, 1485, 1455, 1440, 1415 cm⁻¹; ¹H NMR (200 MHz, DMSO-d_6) δ 0.86 (t, J = 7 Hz, 3H, heptyl Me), 1.28 (m, 8H, heptyl 3- to 6-CH₂), 1.65 (p, J = 7 Hz, 2H, heptyl 2-CH₂), 3.47 (s, 2H, bridge CH₂), 3.73 (s, 3H, OMe), 3.83 (t, J = 7 Hz, 2H, OCH₂), 5.66 (br s, 2H, NH₂), 6.03 (br s, 2H, NH₂), 6.65 (d, J = 3 Hz, 1H, aryl H-6), 6.72 (dd, J = 8 Hz, J = 3 Hz, 1H, aryl H-4), 6.87 (d, J = 8 Hz, 1H, aryl H-3), 7.40 (s, 1H, pyrimidine H-6). Anal. (C₁₉H₂₈N₄O₂) C, H, N.

2,4-Diamino-5-(2-methoxy-5-octyloxybenzyl)pyrimidine (3d). Yield 1.03 mg (58%); mp 135–136 °C; IR (KBr) ν 3400, 3300, 3110, 2980, 2910, 2840, 1660, 1645, 1600, 1565, 1535, 1500, 1480, 1455, 1435, 1415 cm⁻¹; ¹H NMR (200 MHz, DMSO-d_6) δ 0.85 (t, J = 7 Hz, 3H, octyl Me), 1.27 (m, 10H, octyl 3- to 7-CH₂), 1.66 (p, J = 7 Hz, 2H, octyl 2-CH₂), 3.49 (s, 2H, bridge CH₂), 3.75 (s, 3H, OMe), 3.85 (t, J = 7 Hz, 2H, OCH₂), 5.67 (br s, 2H, NH₂), 6.05 (br s, 2H, NH₂), 6.66 (d, J = 3 Hz, 1H, aryl H-6), 6.74 (dd, J = 8 Hz, J = 3 Hz, 1H, aryl H-4), 6.88 (d, J = 8 Hz, 1H, aryl H-3), 7.41 (s, 1H, pyrimidine H-6). Anal. (C₂₀H₃₀N₄O₂) C, H, N.

2,4-Diamino-5-(2-methoxy-5-nonyloxybenzyl)pyrimidine (3e). Yield 877 mg (47%); mp 134–135 °C; IR (KBr) ν 3410, 3320, 3120, 2920, 2850, 1665, 1635, 1605, 1565, 1540, 1500, 1485, 1455, 1440, 1420 cm⁻¹; ¹H NMR (200 MHz, DMSO-d_6) δ 0.86 (t, J = 7 Hz, 3H, nonyl Me), 1.25 (m, 12H, nonyl 3- to 8-CH₂), 1.64 (p, J = 7 Hz, 2H, nonyl 2-CH₂), 3.47 (s, 2H, bridge CH₂), 3.74 (s, 3H, OMe), 3.83 (t, J = 7 Hz, 2H, OCH₂), 5.65 (br s, 2H, NH₂), 6.03 (br s, 2H, NH₂), 6.65 (d, J = 3 Hz, 1H, aryl H-6), 6.72 (dd, J = 8 Hz, J = 3 Hz, 1H, aryl H-4), 6.88 (d, J = 8 Hz, 1H, aryl H-3), 7.40 (s, 1H, pyrimidine H-6). Anal. (C₂₁H₃₂N₄O₂) C, H, N.

2,4-Diamino-5-[2-methoxy-5-(4-benzyloxy)benzyl]pyrimidine (14a). This compound was prepared by the same procedure as for **3b–e** except that the scale was increased 2-fold, the alkylation step with benzyl bromide was performed in MeOH instead of DMSO, and the reaction mixture was refluxed for 18 h. Yield 1.99 g (59%); beige powder, mp 157–158 °C; IR (KBr) ν 3510, 3480, 3420, 3360, 3300, 3160, 3110, 3050, 3030, 1675, 1640, 1605, 1575, 1505, 1460 cm⁻¹; ¹H NMR (200 MHz, DMSO-d_6) δ 3.49 (s, 2H, bridge CH₂), 3.74 (s, 3H, OMe), 4.99 (s, 2H, OCH₂), 5.67 (br s, 2H, NH₂), 6.05 (br s, 2H, NH₂), 6.75–6.95 (m, 3H, aryl protons on the anisole), 7.40 (m, 6H, pyrimidine H-6 and aryl protons on the benzyloxy group). Anal. (C₁₉H₂₀N₄O₂) C, H, N.

2-Methoxy-4-[(4-methoxybenzyl)oxy]benzaldehyde (13b). Compound **8** (2.10 g, 0.01 mol) was added to a solution of NaOMe prepared by dissolving metallic Na (0.23 g, 0.01 mol) in absolute MeOH (40 mL). After 5 min, the solvent was evaporated and the residue triturated with Et₂O until a powder formed, at which point the Et₂O was decanted. The powder was dried in vacuo and taken up in dry DMF (2 mL), and to the solution was added 4-methoxybenzyl chloride (freshly synthesized from 4-methoxybenzyl alcohol and SOCl₂ in toluene). After 20 h at room temperature, the DMF was evaporated under reduced pressure and the residue was partitioned between EtOAc and 1 N NaOH. The organic layer was evaporated, and the residue was recrystallized from EtOH/

H_2O. Drying in a lyophilization apparatus afforded **13b** as a white powder (1.91 g, 70%): mp 75−76 °C; TLC bluish spot, R_f = 0.4 (silica gel, 2:1 isooctane/EtOH); IR (KBr) ν 2970, 2940, 2840, 1675, 1610, 1585, 1515, 1490, 1465, 1450, 1435, 1425 cm^{-1}; ^{1}H NMR (200 MHz, CDCl$_3$) δ 3.82 (s, 3H, OMe), 3.90 (s, 3H, OMe), 4.98 (s, 2H benzylic CH$_2$), 6.91 (m, 4H, aryl protons of 4-methoxybenzyl group), 7.18 (dd, J = 8 Hz, J = 3 Hz, 1H, benzaldehyde H-4), 7.35 (d, J = 8 Hz, 1H, benzaldehyde H-3), 7.42 (d, J = 3 Hz, 1H, benzaldehyde H-6), 10.44 (s, 1H, CH=O). Anal. (C$_{16}$H$_{16}$O$_4$) C, H.

2,4-Diamino-5-[2-methoxy-5-(4-methoxybenzyloxy)benzyl]pyrimidine (14b). This compound was prepared from **13b** in 61% yield via the general procedure described above for **3b**−**e**: white powder, mp 220−221 °C; IR (KBr) ν 3480, 3410, 3340, 3250, 3120, 2950, 3120, 2960, 2920, 2830, 1665, 1620, 1595, 1565, 1510, 1495, 1450, 1415 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 3.48 (br s, >2H, bridge CH$_2$ and partial H$_2$O of solvation), 3.74 (s, 3H, OMe), 3.75 (s, 3H, OMe), 4.89 (s, 2H, OCH$_2$), 5.66 (br s, 2H, NH$_2$), 6.04 (br s, 2H, NH$_2$), 6.72−7.00 (m, 5H, aryl protons), 7.33 (d, J = 8 Hz, 2H, aryl protons), 7.40 (s, 1H, pyrimidine H-6). Anal. (C$_{20}$H$_{22}$N$_4$O$_3$·0.25H$_2$O) C, H, N.

2,4-Diamino-5-(2-methoxy-5-hydroxybenzyl)pyrimidine (15). A stirred suspension of **14b** (183 mg, 0.5 mmol) in MeOH (5 mL) was treated with TsOH·H$_2$O (190 mg, 1 mmol), whereupon the solids dissolved and a new precipitate formed quickly The solution was left at room temperature for 2 days, after which the solvent was evaporated and the residue was suspended in H$_2$O. The mixture was adjusted to pH >10 with NaOH, a trace of insoluble material was filtered, and the filtrate was applied onto a column of Dowex 50W-X2 resin (H$^+$ form, 1.5 cm × 15 cm). The column was washed with H$_2$O until the eluent was neutral and UV-transparent and then with 1.5% NH$_4$OH to remove the product. Appropriately pooled fractions were concentrated and freeze-dried to obtain **15** as a light-brown powder (110 mg, 89%): TLC R_f 0.2 (silica gel, 5:1 EtOAc/MeOH). The analytical sample was prepared by recrystallization from EtOH/H$_2$O: mp 216−218 °C dec; IR (KBr) ν 3430, 3350, 3210, 3050, 2960, 2930, 2830, 2680 w, 2500 br, 1665 sh, 1640, 1615, 1605 sh, 1565, 1540, 1505, 1470, 1445 cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ 3.45 (s, 2H, benzylic CH$_2$), 3.71 (s, 3H, OMe), 5.66 (br s, 2H, NH$_2$), 6.00 (br s, 2H, NH$_2$), 6.44 (d, J = 3 Hz, 1H, aryl H-6), 6.54 (dd, J = 8 Hz, J = 3 Hz, 1H, aryl 4-H), 6.78 (d, J = 8 Hz, 1H, aryl H-3), 7.39 (s, 1H, pyrimidine H-6), 8.80 (s, 1H, phenolic OH). The presence of a small amount of EtOH in the sample was indicated by a small triplet at δ 1.05. Anal. (C$_{12}$H$_{14}$N$_4$O$_2$·0.2EtOH) C, H, N.

General Procedure for the Synthesis of 2,4-Diamino-5-[2-methoxy-5-(ω-carboxyalkoxy)benzyl]pyrimidines 3f−k. Clean metallic Na (23 mg (1.0 mmol) was dissolved in absolute EtOH (2 mL), the solvent was evaporated under reduced pressure, and the residue was redissolved in DMSO (3 mL). To this solution was then added compound **15** (246 mg, 1.0 mmol), and after the reaction mixture was stirred at room temperature for 30 min the bromo ester (1.0 mmol) was added in a single portion. TLC analysis (silica gel, 5:1 EtOAc/MeOH) revealed gradual loss of the spot with R_f = 0.2 (unreacted **15**) and its replacement by a faster-moving spot with R_f = 0.4. The reaction was typically over in 2 h. The product was saponified instantaneously upon addition of 2 N NaOH (1.5 mL) followed by addition of H$_2$O to a final volume of ca. 60 mL. Any insoluble material remaining at this point was filtered off, and the clear solution was applied onto a column of Dowex 50W-X2 (H$^+$ form, 2 cm × 20 cm). The column was washed with H$_2$O until the eluate was neutral and UV-transparent and then with 1.5% NH$_4$OH to remove the product. Appropriately pooled eluates were concentrated to dryness by rotary evaporation followed by lyophilization. The solid was redissolved in H$_2$O with a small volume of dilute NH$_4$OH added as needed and was reprecipitated with 10% AcOH. The collected product was then dried and analyzed. Microchemical analyses typically indicated the presence of fractional amounts of AcOH or H$_2$O of solvation despite careful

drying in vacuo over P$_2$O$_5$. The following compounds were obtained in this manner.

2,4-Diamino-5-[(2-methoxy-5-(3-carboxypropyloxy)benzyl]pyrimidine (3f). White solid (19% yield), mp 244−246 °C; IR (KBr) ν 3340, 3210, 2960 (broad underlying absorbance at 3600−2450), 1665, 1650, 1630 sh, 1565, 1505, 1465, 1410 cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.87 (m, 2H, CH$_2$*CH$_2$*CH$_2$), 2.32 (t, J = 7 Hz, 2H, *CH$_2$*COOH), 3.47 (s, benzylic CH$_2$ partially overlapping a broad H$_2$O peak), 3.72 (s, OMe partially overlapping H$_2$O), 3.84 (t, J = 6 Hz, OCH$_2$ partially overlapping H$_2$O), 5.66 (br s, 2H, NH$_2$), 6.03 (br s, 2H, NH$_2$), 6.68 (m, 2H, aryl H-4 and H-6), 6.87 (d, J = 8 Hz, aryl H-3), 7.37 (s, 1H, pyrimidine H-6). Anal. (C$_{16}$H$_{20}$N$_4$O$_4$·3.2H$_2$O) C, H, N.

2,4-Diamino-5-[(2-methoxy-5-(4-carboxybutyloxy)benzyl]pyrimidine (3g). Beige solid (65% yield), mp 94−100 °C; IR (KBr) ν 3340, 3170, 2940 (broad underlying absorbance at 3400−2700), 1655, 1500, 1460 cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.64 (m, 4H, CH$_2$(*CH$_2$*)$_2$CH$_2$), 2.32 (t, J = 7 Hz, 2H, *CH$_2$*COOH), 3.46 (s, 2H, benzylic CH$_2$), 3.72 (s, 3H, OMe), 3.83 (t, J = 6 Hz, 2H, OCH$_2$), 5.75 (br s, 2H, NH$_2$), 6.12 (br s, 2H, NH$_2$), 6.60 (d, J = 3 Hz, 1H, aryl H-6), 6.73 (dd, J = 8 Hz, J = 3 Hz, 1H, aryl H-4), 6.87 (d, J = 8 Hz, 1H, aryl H-3), 7.37 (s, 1H, pyrimidine H-6). This compound was purified on DEAE-cellulose (HCO$_3^-$ form), but this method was less effective than the use of Dowex 50W-X2 (H$^+$ form) because of precipitation on the column. The presence of AcOH was indicated by a singlet at δ 1.89. Anal. (C$_{17}$H$_{22}$N$_4$O$_4$·0.75AcOH·0.5H$_2$O) C, H, N.

2,4-Diamino-5-[(2-methoxy-5-(5-carboxypentyloxy)benzyl]pyrimidine (3h). Beige powder (85% yield), mp 100−104 °C; IR (KBr) ν 3330, 3190, 2930, 2860 (broad underlying absorbance at 3500−2400), 1655, 1560, 1495, 1455 cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.30−1.70 (m, 6H, CH$_2$(*CH$_2$*)$_3$CH$_2$), 2.19 (t, J = 7 Hz, 2H, *CH$_2$*COOH), 3.46 (s, 2H, benzylic CH$_2$), 3.72 (s, 3H, OMe), 3.82 (t, J = 6 Hz, 2H, OCH$_2$), 5.66 (br s, 2H, NH$_2$), 6.03 (br s, 2H, NH$_2$), 6.64 (d, J = 3 Hz, 1H, aryl H-6), 6.72 (dd, J = 8 Hz, J = 3 H, 1H, aryl H-4), 6.86 (d, J = 8 Hz, 1H, aryl H-3), 7.38 (s, 1H, pyrimidine H-6). The presence of AcOH of was indicated by a singlet at δ 1.89. Anal. (C$_{18}$H$_{24}$N$_4$O$_4$·0.5AcOH) C, H, N.

2,4-Diamino-5-[(2-methoxy-5-(6-carboxyhexyloxy)benzyl]pyrimidine (3i). Light-brown powder (77% yield), mp 200−202 °C; IR (KBr) ν 3330, 3190, 2930, 2850 (broad underlying absorbance at 3500−2700), 1655, 1555, 1535, 1495, 1455 cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.32−1.63 (m, 8H, CH$_2$(*CH$_2$*)$_4$CH$_2$), 2.19 (t, J = 7 Hz, 2H, *CH$_2$*COOH), 3.46 (s, benzylic CH$_2$ partially overlapping a broad H$_2$O peak), 3.72 (s, OMe partially overlapping H$_2$O), 3.82 (t, 2H, J = 6 Hz, OCH$_2$), 5.69 (br s, 2H, NH$_2$), 6.06 (br s, 2H, NH$_2$), 6.64 (d, J = 3 Hz, 1H, aryl H-6), 6.72 (dd, J = 8 Hz, J = 3 H, 1H, aryl H-4), 6.86 (d, J = 8 Hz, 1H, aryl H-3), 7.38 (s, 1H, pyrimidine H-6). Anal. (C$_{19}$H$_{26}$N$_4$O$_4$·0.75H$_2$O) C, H, N.

2,4-Diamino-5-[(2-methoxy-5-(7-carboxyheptyloxy)benzyl]pyrimidine (3j). Light-brown powder (74% yield), mp 108−112 °C; IR (KBr) ν 3330, 3180, 2920, 2850 (broad underlying absorbance at 3500−2700), 1655, 1525 sh, 1495, 1460 cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.29−1.62 (m, 10H, CH$_2$(*CH$_2$*)$_5$CH$_2$), 2.17 (t, J = 7 Hz, 2H, *CH$_2$*COOH), 3.46 (s, 2H, benzylic CH$_2$), 3.72 (s, 3H, OMe), 3.82 (t, 2H, J = 6 Hz, OCH$_2$), 5.71 (br s, 2H, NH$_2$), 6.08 (br s, 2H, NH$_2$), 6.66 (d, J = 3 Hz, 1H, aryl H-6), 6.73 (dd, J = 8 Hz, J = 3 H, 1H, aryl H-4), 6.86 (d, J = 8 Hz, 1H, aryl H-3), 7.38 (s, 1H, pyrimidine H-6). The presence of AcOH was indicated by a singlet at δ 1.89. Anal. (C$_{20}$H$_{28}$N$_4$O$_4$·0.75AcOH) C, H, N.

2,4-Diamino-5-[(2-methoxy-5-(7-carboxyheptyloxy)benzyl]pyrimidine (3k). Light-brown powder (80% yield), mp 142−144 °C; IR (KBr) ν 3330, 3190, 2920, 2850 (broad underlying absorbance at 3500−2700), 1655, 1555, 1495, 1460 cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.25−1.63 (m, 12H, CH$_2$(*CH$_2$*)$_6$CH$_2$), 2.17 (t, J = 7 Hz, 2H, *CH$_2$*COOH), 3.47 (s, benzylic CH$_2$ partially overlapping a broad H$_2$O peak), 3.72 (s, OMe, partially overlapping H$_2$O), 3.82 (t, OCH$_2$ partially overlapping H$_2$O), 5.73 (br s, 2H, NH$_2$), 6.09 (br s, 2H, NH$_2$), 6.68 (d, J = 3 Hz, 1H, aryl H-6), 6.73 (dd, J = 8 Hz, J = 3 H,

1H, aryl H-4), 6.88 (d, $J = 8$ Hz, 1H, aryl H-3), 7.38 (s, 1H, pyrimidine H-6). The presence of AcOH was indicated by a singlet at δ 1.90. Anal. ($C_{21}H_{30}N_4O_4 \cdot 0.5AcOH \cdot 0.25 H_2O$) C, H, N.

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