Synthesis and Antifolate Activity of 2,4-Diamino-5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidine Analogues of Trimetrexate and Piritrexim

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2,4-Diamino-5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidines with di- and trimethoxyaralkyl substitution at the 6-position were synthesized from the N^6-unsubstituted compound and appropriate aralkyl bromides in *N,N*-dimethylformamide solution containing a catalytic amount of sodium iodide. An improved method of preparation of 2,4-diamino-5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidine from 2-amino-6-benzyl-5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidin-4(3*H*)-one was also developed, in which N^2 was protected by reaction with pivalic anhydride and the resulting product was subjected consecutively to reaction with 4-chlorophenylphosphorodichloridate and 1,2,4-triazole, ammonolysis to replace the 4-imidazolido group and remove the N^2-pivaloyl group, and catalytic hydrogenolysis to remove the 6-benzyl group. In assays of the ability of the products to inhibit dihydrofolate reductase from *Pneumocystis carinii*, and *Toxoplasma gondii*, and rat liver the most active of the compounds tested was 2,4-diamino-6-(2'-bromo-3',4',5'-trimethoxybenzyl)-5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidine. The concentration of this compound needed to inhibit enzyme activity by 50% was 0.51 μ*M* against the *P. carinii* enzyme, 0.09 μ*M* against the *T. gondii enzyme*, and 0.35 μ*M* against the rat enzyme. Thus, there was selectivity of binding to *T. gondii* enzyme, but not *P. carinii* enzyme, relative to rat enzyme. 2',5'-Dimethoxybenzyl analogues were less active than the corresponding 3',4',5'-trimethoxybenzyl analogues, and compounds with a CH_2CH_2 or $CH_2CH_2CH_2$ bridge were less active than those with a CH_2 bridge. 2,4-Diamino-6-(2'-bromo-3',4',5'-trimethoxybenzyl)-5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidine showed greater selectivity than trimetrexate or piritrexim for the *P. carinii* and *T. gondii* enzyme, but was less selective than trimethoprim or pyrimethamine. However its molar potency against both enzymes was greater than that of trimethoprim, the antifolate most commonly used, in combination with sulfamethoxazole, for initial treatment of opportunistic *P. carinii* and *T. gondii* infections in patients with AIDS and other disorders of the immune system.

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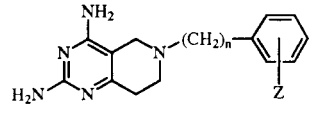
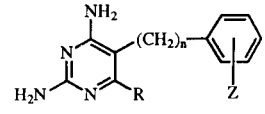
2,4-Diamino-6-aryl(heteroaryl)-5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidines have been found to possess significant antimicrobial, antiparasitic, and antimetabolite activity [1]. For example, oral and parenteral antimalarial effects were observed against *Plasmodium berghei* in mice and *Plasmodium gallinaceum* in chicks. Activity was also seen against several pathogenic strains of *Streptococcus* bacteria. Since their antibacterial effect was reversed by folic acid or 5-formyl-5,6,7,8-tetrahydrofolic acid (leucovorin), the proximal biochemical target of these dicyclic 2,4-diaminopyrimidine derivatives was assumed to be dihydrofolate reductase (DHFR) [1].

The first reported examples of this family (*e.g.*, compound **1**) contained either a 3- or 4-pyridylmethyl group or a benzyl, 3-chlorobenzyl, 3,4-dichlorobenzyl, or 3,5-dichlorobenzyl group at the 6-position [1]. More recently we reported a synthesis of the non-lipophilic *N*-(4-aminobenzoyl)-L-glutamic acid derivative **2**, which contained an extra carbon in the bridge. This compound likewise displayed strong antifolate activity, and inhibited the growth of tumor cells (L1210 murine leukemia) in culture with a potency similar to that of methotrexate (MTX) [2].



1: *e.g.*, n = 1, Z = 4'-Cl
2: n = 2, Z = 4-CONHCH(COOH)CH_2CH_2COOH



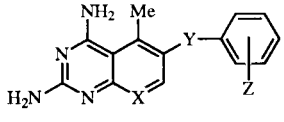
3 (TMP): R = H, n = 1, Z = 3',4',5'-$(OMe)_3$
4: R = Et, n = 0, Z = 4'-Cl_2

Lipophilic DHFR inhibitors are widely used for the treatment and prophylaxis of opportunistic infections in patients with AIDS [3], and are also used when such infections occur following immunosuppressive cancer chemotherapy [4]. Two of the principal opportunistic pathogens associated with immune deficiency are *Pneumocystis carinii* and *Toxoplasma gondii* [5]. While these organisms ordinarily pose little risk to the immunocompetent general population, they cause significant morbidity and mortality in AIDS patients. *Pneumocystis* pneumonia (PCP) is often the first overt sign of AIDS following the initial latent phase [6]; toxoplasmosis of the central nervous system, on the other hand, tends to account for the severe neurological problems, including loss of eyesight, that are commonly encountered in the final months of the disease [7]. Standard first-line treatment for *P. carinii* and

T. gondii infections generally relies on combinations of trimethoprim (**3**) or pyrimethamine (**4**) in combination with a sulfonamide inhibitor of *de novo* folate synthesis [8-10]. However patients often suffer adverse reactions to these agents, requiring cessation of treatment. Moreover, as with other DHFR inhibitors, drug resistance is likely to develop during extended therapy [11].

Two newer antifolates recently investigated as drugs for the treatment of PCP [12] and toxoplasmosis [13] in AIDS patients are the very potent DHFR inhibitors trimetrexate (TMQ, **5**) and piritrexim (PTX, **6**), which were originally synthesized for use in cancer chemotherapy [reviewed in refs 14 and 15, respectively]. These compounds are better inhibitors of human DHFR than of the *P. carinii* or *T. gondii* enzyme [16-20], and thus would not be expected to be therapeutically selective were it not for the fact that *P. carinii* and *T. gondii* organisms, unlike mammalian cells, lack an active transport system for reduced folates. Thus their growth remains inhibited when leucovorin is administered to "rescue" the cells of the host [21]. This is in contrast to trimethoprim, which exhibits a high preference for the parasite enzyme [16,17] and therefore can be used safely without leucovorin "rescue".

An alternative approach to achieving selective inhibition of one-carbon metabolism in *P. carinii* and *T. gondii* without having to co-administer leucovorin to protect host tissues would be to use TMQ and PTX analogues that bind more tightly to the enzyme from these organisms than they do to the human enzyme. As part of a larger program directed toward this goal [22-25] we were interested in determining whether 2,4-diamino-5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidines bearing a di- or trimethoxyphenylalkyl group at the 6-position, as opposed to the chlorinated compounds previously studied [1], might show favorable enzyme-binding selectivity relative to TMQ and PTX. In this paper we report an improved method of preparation of 2,4-diamino-5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidine hydrochloride (**7**•HCl), and its conversion to the heretofore undescribed di- and trimethoxyaralkyl analogues **8-12** (Scheme 1).

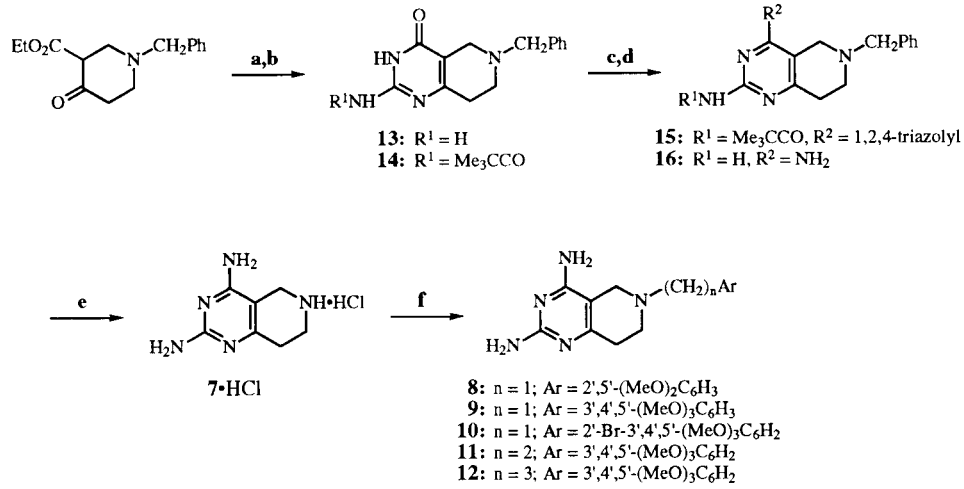


5 (TMQ): X = CH, Y = CH_2NH, Z = 3',4',5'-$(OMe)_3$
6 (PTX): X = N, Y = CH_2, Z = 2',5'-$(OMe)_2$

Chemistry.

The key intermediate 2,4-diamino-5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidine (**7**) was originally synthesized by reaction of guanidine carbonate with 4-amino-3-cyano-1,2,5,6-tetrahydropyridine, which in turn was made from 3',3'-iminodipropionitrile by a Thorpe reaction [1]. An alternative route to **7**, used in this laboratory in connection with the synthesis of **2**, began with 2-amino-6-benzyl-5,6,7,8-tetrahydroquinazolin-4(3*H*)-one (**13**) [2]. Reaction of **13** with phosphorous oxychloride (73%) was followed by displacement of the 4-chloro substituent with hydrazine (71%), hydrogenolysis of the 6-benzyl group over 10% palladium-on carbon (81%), and cleavage of the 4-hydrazino group to an amino group with Davison sponge nickel (92%). In the present work this approach was further refined as shown in Scheme 1. Compound **13** was protected with pivalic anhydride, and the resultant product (**14**, 85%) was treated with 4-chlorophenylphosphorodichloridate and 1,2,4-triazole to obtain the triazolide **15**. Direct reaction of crude **15** with ammonia in

Scheme 1



13: R^1 = H
14: R^1 = Me_3CCO
15: R^1 = Me_3CCO, R^2 = 1,2,4-triazolyl
16: R^1 = H, R^2 = NH_2

7•HCl

8: n = 1; Ar = 2',5'-$(MeO)_2C_6H_3$
9: n = 1; Ar = 3',4',5'-$(MeO)_3C_6H_3$
10: n = 1; Ar = 2'-Br-3',4',5'-$(MeO)_3C_6H_2$
11: n = 2; Ar = 3',4',5'-$(MeO)_3C_6H_2$
12: n = 3; Ar = 3',4',5'-$(MeO)_3C_6H_2$

a: $H_2NC(=NH)NH_2$; **b**: $(Me_3CCO)_2O$; **c**: 4-$ClC_6H_4OPOCl_2$/1,2,4-triazole/pyridine; **d**: NH_4OH/dioxan; **e**: H_2/Pd-C/HCl; **f**: $Ar(CH_2)_nBr$/NaI/DMF [Ar = 2,5-$(MeO)_2C_6H_3$, 3,4,5-$(MeO)_3C_6H_2$, or 2-Br-3,4,5-$(MeO)_3$; n = 1-3]

dioxane simultaneously introduced the 4-amino group and deprotected the 2-amino group, giving **16** (40%). Removal of the 6-benzyl group by catalytic hydrogenolysis in the presence of hydrochloric acid [1,2] then yielded **7**•HCl. As expected, the N^2-pivaloyl group led to markedly improved solubility in the chlorination step. Moreover, 4-chlorophenylphosphorodichloridate was more convenient to use and afforded more reproducible yields than phosphorus oxychloride. Therefore, this is now our method of choice for the preparation of **7**. Reaction of **7**•HCl with 2,5-dimethoxybenzyl, 3,4,5-trimethoxybenzyl, 2-bromo-3,4,5-trimethoxybenzyl, and 3-(3,4,5-trimethoxybenzyl)propyl bromide in *N,N*-dimethylformamide containing a catalytic amount of sodium iodide at 80° for 2 hours yielded compounds **8**, **9**, **10**, and **12**, respectively. Compound **11** could not be obtained satisfactorily from 2-(2,5-dimethoxyphenyl)ethyl bromide by this method, but was formed in modest yield from the corresponding tosylate. As expected from the higher reactivity of benzylic as compared with longer-chained aralkyl halides, the best yields were obtained with 2,5-dimethoxybenzyl bromide (47%) and 2-bromo-3,4,5-trimethoxybenzyl bromide (55%).

The ^{1}H nmr spectra of **8-12** were consistent with their structures, revealing in particular the strong deshielding effect of N^6. For example, the 7- and 8-CH_2 protons in **8** gave a multiplet at δ 2.70-2.85 whereas the singlets for the 5-CH_2 protons and the bridge CH_2 protons were shifted downfield to δ 3.31 and δ 3.75, respectively. Interestingly, the chemical shift for the 2'- and 6'-protons in the 3',4',5'-trimethoxy derivatives **9**, **10**, and **11** varied as a function of the number of CH_2 groups in the bridge. Whereas the 2'- and 6'-protons in **9** (CH_2 bridge) produced a singlet at δ 6.60, this signal was shifted upfield to δ 6.46 in **11** (CH_2CH_2 bridge) and downfield to δ 6.43 in **12** ($CH_2CH_2CH_2$ bridge). This suggests that the unshared pair of electrons on N^6 can affect the ring current in the phenyl ring, and that the spatial orientation of the two halves of the molecule is influenced by the length of the bridge. It may also be noted that, in the spectrum of the 2'-bromo derivative **10** (CH_2 bridge plus bulky *ortho* substitution), the chemical shift of the 6'-proton was shifted *upfield* to δ 6.96. Since the molecular shape of these compounds will undoubtedly influence their ability to bind to the active site, one might well expect to see these differences reflected in their IC_{50} values for DHFR inhibition.

Enzyme Inhibition.

The ability of the pyrido[4,3-*d*]pyrimidine analogues to inhibit DHFR from rat liver, *P. carinii*, and *T. gondii* was evaluated as previously described [19,20]. The rat enzyme was used as a surrogate for the human enzyme in these assays because immunosuppressed rats are a useful laboratory model for *in vivo* studies of *P. carinii* infections [26]. The binding affinities of rat and human DHFR for 2,4-monocyclic and condensed 2,4-diaminopyrimidine derivatives are very similar [26a]. As shown in Table 1, the most active compound against all three enzymes was **10**, with IC_{50} values of 0.35 μ*M* against rat liver DHFR, 0.51 μ*M* against *P. carinii* DHFR, and 0.09 μ*M* against *T. gondii* DHFR. The selectivity of this compound, expressed as the ratio IC_{50}(rat)/IC_{50}(*P. carinii* or *T. gondii*) was 0.69 for the *P. carinii* enzyme and 3.9 for the *T. gondii* enzyme. Compounds **8** and **9**, likewise containing a CH_2 bridge, were less potent than **10** against each of the three enzymes. However, because their IC_{50} values were increased more against rat liver enzyme than against *T. gondii* enzyme, their selectivity ratios were actually somewhat higher. Elongation of the bridge to two or three CH_2 groups diminished activity against all three enzymes, but not to the same degree. This presumably reflected species differences in the topology of the active site. It should be noted, however, that the potency of these compounds as DHFR inhibitors was 5- to 10-fold lower than that of TMQ or PTX, and that they were also much weaker inhibitors than the 'classical' glutamate analogue **2**, for which an IC_{50} value of 0.035 μ*M* was obtained in earlier assays using human DHFR [2].

Comparison of the bioassay data for compounds **8-12** with those reported previously for the reference compounds trimethoprim, pyrimethamine, TMQ, and PTX under the same assay conditions [19,20,27] revealed **10** to be 24-fold more potent, but 31-fold less selective, than trimethoprim against *P. carinii* DHFR, and 16-fold more potent, but 11-fold less selective, against the *T. gondii*

Table 1

Inhibition of Dihydrofolate Reductase by 2,4-Diamino-5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidine Analogues of Trimetrexate (**5**) and Piritrexim (**6**)

Compound	IC_{50}(μ*M*) [a] rat liver	*P. carinii*	*T. gondii*
8	1.4	3.3 (0.42)	0.3 (4.7)
9	2.2	6.9 (0.32)	0.20 (11)
10	0.35	0.51 (0.69)	0.09 (3.9)
11	9.5	30 (0.2)	3.2 (3.0)
12	0.94	9.3 (0.1) [b]	1.7 (0.6)
Trimethoprim (**3**) [c]	130	12 (11)	2.7 (44)
Pyrimethamine (**4**) [c]	2.3	3.7 (0.62)	0.39 (5.9)
Trimetrexate (**5**) [c]	0.003	0.042 (0.071)	0.010 (0.29)
Piritrexim (**6**) [c]	0.0015	0.031 (0.048)	0.017 (0.088)

[a] Assays were performed as specified in references 19 and 20 Numbers in parentheses are selectivity ratios IC_{50}(rat)/IC_{50}(*P. carinii*) and IC_{50}(*T. gondii*). Ratios of less than 1.0 signify lack of selectivity for the microbial enzyme relative to the mammalian enzyme. [b] Recombinant *P. carinii* enzyme was used in this experiment instead of the enzyme from intact organisms. The kinetics of wild-type and recombinant *P. carinii* enzyme, as well as the K_i values of standard inhibitors, are essentially identical (S. F. Queener, unpublished results). [c] Data for these reference compounds are from reference 27.

enzyme. Relative to pyrimethamine, **10** was only 7-fold more potent against the *P. carinii* enzyme and 4-fold more potent against the *T. gondii* enzyme; however, there was a <2-fold difference in selectivity. In comparison to TMQ and PTX, **10** was 12-fold and 16-fold less potent against *P. carinii* DHFR and 9-fold and 5-fold less potent against *T. gondii* DHFR. However, the enzyme specificity of **10** was less unfavorable than that of TMQ or PTX where *P. carinii* versus rat enzyme was concerned, and was superior to that of TMQ and PTX with respect to the *T. gondii* enzyme. Compound **9** was a better inhibitor of *T. gondii* DHFR than trimethoprim, pyrimethamine, TMQ, or PTX, and was more selective than these reference agents with the exception of trimethoprim. Compound **9**, on the other hand, was less potent than **10** but showed the best selectivity against *T. gondii* DHFR, being second only to trimethoprim among the reference drugs.

It may be concluded from these results that the species specificity of DHFR inhibition by most of the 2,4-diamino-5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidine analogues in this study would be more favorable than that of TMQ or PTX, but less favorable than that of trimethoprim. The fact that the best specificity in this series was achieved with compounds containing a one-carbon bridge suggests that a short distance between the two halves of the molecule (n = 0 in pyrimethamine; n = 1 in **1**, trimethoprim, and PTX) favors binding to non-mammalian over mammalian enzyme. This is further supported by the excellent selectivity for *T. gondii* DHFR recently reported for a number of 4,6-diamino-1-aryl-1,1-dimethyl-*s*-triazines and 2,4-diamino-6-arylpteridines [20], which likewise lack a bridge. Given that (i) antimalarials with diverse mechanisms of action were recently found to inhibit the growth of *P. carinii* and *T. gondii* [28-37], (ii) these organisms share certain biochemical and developmental features with *Plasmodia* and other protozoan species, and (iii) the previously described 2,4-diamino-6-aryl(heteroaryl)methyl-5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidines had promising antimalarial activity in laboratory models [1], a broader assessment of the therapeutic potential of this family of compounds against PCP and toxoplasmal infections may be worth exploring.

EXPERIMENTAL

The ir spectra were obtained on a Perkin-Elmer Model 781 double-beam recording spectrophotometer; only peaks with wavenumbers greater than 1400 cm^{-1} are reported. Quantitative uv absorbance spectra were measured on a Varian Model 210 instrument. The ^1H nmr spectra were recorded on a Varian EM360 or in some instances a Varian Model VXR500 instrument, using tetramethylsilane as the reference. Tlc analyses were done on Baker Si250F silica gel plates, with spots being visualized under 254-nm illumination. Column chromatography was on Baker 7024 flash silica gel (40 μm particle size). Solvents for moisture-sensitive reactions were purchased in 'Sure-Seal' bottles from Aldrich (Milwaukee, WI). Melting points were determined in Pyrex capillary tubes using a Mel-Temp Apparatus (Laboratory Devices, Inc., Cambridge, MA) and are not corrected. Microanalyses were done by QTI Laboratories, Whitehouse, NJ. Analytical samples were either obtained by simple evaporation of appropriately pooled chromatography fractions or by recrystallization of these pooled fractions from warm dichloromethane. Tenacious retention of fractional molar amounts of organic solvents in the analytical samples, which is common among lipophilic antifolates [2,38-40], could not be avoided despite careful drying *in vacuo* and was confirmed wherever possible by examination of ^1H nmr spectra.

2,4-Diamino-5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidine Hydrochloride (**7**•HCl).

A mixture of **13** (2.0 g, 7.8 mmoles) [2], 4-*N*,*N*-dimethylaminopyridine (10 mg, 2.1 mmoles), and pivalic anhydride (10 ml) was refluxed for 3 hours, then cooled to room temperature and poured into ether (100 ml). The resulting yellow precipitate was filtered and washed with hexanes. Recrystallization from hot ethanol gave **14** as pale-yellow needles (2.3 g, 85%), mp 228-229°; ir (potassium bromide): ν 3440 br, 3230, 2960, 2920, 1650, 1615, 1580, 1490, 1410 cm^{-1}; ^1H nmr (deuteriochloroform): δ 1.3 (s, 9H, Me_3C), 2.6 (m, 4H, 7- and 8-CH_2), 3.45 (m, 2H, 5-CH_2), 3.70 (s, 2H, benzylic CH_2), 7.35 (s, 5H, phenyl protons).

To a solution of crude **14** (1.7 g, 6.0 mmoles) and 1,2,4-triazole (1.2 g, 18 mmoles) in dry pyridine (30 ml) was slowly added 4-chlorophenylphosphorodichloridate (1.5 ml, 9.0 mmoles) and the solution was stirred at room temperature under argon. After 24 hours the mixture was chilled to 2° and saturated aqueous sodium bicarbonate (15 ml) was added slowly with stirring. The solvent was removed by rotary evaporation and the red residue was taken up in dioxane (5 ml) and 28% ammonium hydroxide (5 ml). The thick solution was stirred at room temperature for 30 minutes, the solvent was removed by rotary evaporation, the residue was redissolved in a small volume or 100:10:1 dichloromethane-methanol-28% ammonium hydroxide, and the solution was filtered through a pad of silica gel. The pad was washed with the same solvent mixture until all the product eluted, and the filtrate was concentrated to dryness. The orange residue was taken up in a mixture of dioxane (5 ml) and 28% ammonium hydroxide (5 ml), and the mixture was refluxed for 6 days and evaporated to dryness under reduced pressure. The residue was taken up in water, and the solution was acidified to *p*H 5 with acetic acid and washed with dichloromethane (2 x 30 ml). The combined dichloromethane washes were extracted with 10% acetic acid (2 x 30 ml), and the aqueous layer was basified to *p*H 8 with aqueous ammonia and re-extracted with chloroform (3 x 30 ml). The combined organic layers were concentrated by rotary evaporation. Flash chromatography of the residue on silica gel with 100:10:1 dichloromethane-methanol-28% ammonium hydroxide as the eluent afforded **16** (0.36 g, 40% yield), which was used in the next step without further purification; ^1H nmr (deuteriochloroform): δ 2.70 (m, 4H, 7- and 8-CH_2), 3.25 (m, 2H, 5-CH_2), 3.70 (m, 2H, benzylic CH_2), 4.8-5.2 (br m, 4H, 2- and 4-NH_2).

A solution of crude **16** (0.36 g, 1.37 mmoles) in a mixture of 95% ethanol (20 ml) and 1 *N* hydrochloric acid (2 ml) was hydrogenated in a Parr apparatus (55 lb/in^2 initial pressure) in the

presence of 10% palladium-on-carbon for 12 hours. Filtration through celite, followed by washing with ethanol, and evaporation of the combined filtrate and wash solutions afforded **7**•HCl as a beige solid (0.19 g, 57%) which was identical in all respects with an authentic specimen obtained as described earlier [2].

2,4-Diamino-6-(2',5'-dimethoxybenzyl)-5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidine (**8**).

A solution of **7**•HCl (100 mg, 0.43 mmole), 2,5-dimethoxybenzyl bromide (200 mg, 0.81 mmole) [41], sodium acetate (180 mg, 2.25 mmoles), and anhydrous sodium iodide (10 mg) in dry *N,N*-dimethylformamide (5 ml) was heated to 80° for 2 hours under argon. The solvent was removed under reduced pressure, the residue was taken up in 15% acetic acid (20 ml), and the solution was washed with ether (3 x 30 ml). The aqueous layer was cooled in an ice bath, basified to *p*H 9 with concentrated ammonia, and extracted with chloroform (2 x 30 ml). The combined organic layers were washed with brine, dried over magnesium sulfate, and concentrated to a yellow oil by rotary evaporation. The oil was chromatographed on silica gel with 10:1 chloroform-methanol as the eluent, and fractions with R_f 0.18 (silica gel, 100:10:1 chloroform-methanol-28% ammonium hydroxide) were pooled and evaporated to a semi-solid (62 mg, 47% yield), mp 151-154° (crystallized from warm dichloromethane): ir (potassium bromide): ν 3430 br, 3320, 3200, 2910, 1630, 1590, 1575 cm^{-1}; ^1H nmr (500 MHz, deuteriochloroform): δ 2.70-2.85 (m, 4H, 7- and 8-CH_2), 3.31 (s, 2H, 5-CH_2), 3.75 (s, 2H, bridge CH_2), 3.76 (s, 3H, 5'-OMe), 3.79 (s, 3H, 2'-OMe), 4.49 (br s, 2H, NH_2), 4.68 (br s, 2H, NH_2), 6.78-7.00 (m, 3H, 3'-, 4'-H, and 6'-H).

Anal. Calcd. for $C_{16}H_{21}N_5O_2$•0.8CH_3OH•0.2$CHCl_3$: C, 55.95; H, 6.75; N, 19.19. Found: C, 55.66; H, 6.73; N, 19.12.

2,4-Diamino-6-(3',4',5-trimethoxybenzyl)-5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidine (**9**).

A solution of **7**•HCl (100 mg, 0.49 mmole), 3,4,5-trimethoxybenzyl bromide (100 mg, 0.38 mmole) [42], sodium acetate (120 mg, 1.47 mmoles), and anhydrous sodium iodide (5 mg) in dry *N,N*-dimethylformamide (3 ml) was heated to 80° for 1 hour under argon. The solvent was evaporated under reduced pressure, the residue was taken up in 15% acetic acid (50 ml), and the solution was washed with dichloromethane (2 x 15 ml). The aqueous layer was cooled in an ice bath, adjusted to *p*H 9 with concentrated ammonia, and re-extracted with dichloromethane (2 x 30 ml). The combined organic layers were washed with brine, dried over magnesium sulfate, and concentrated to a yellow oil, which was chromatographed on silica gel with 100:20:1 dichloromethane-methanol-28% ammonium hydroxide as the eluent. Fractions with R_f 0.20 (silica gel, 100:10:1 chloroform-methanol-28% ammonium hydroxide) were pooled and evaporated to a semi-solid, which upon recrystallization from isopropyl alcohol afforded white needles (101 mg, 76%); mp 180-182°; ir (potassium bromide): ν 3420 br, 3200, 2915, 1645, 1595, 1575 cm^{-1}; ^1H nmr (500 MHz, deuteriochloroform): δ 2.72-2.76 (m, 4H, 7- and 8-CH_2), 3.25 (s, 2H, 5-CH_2), 3.64 (s, 2H, bridge CH_2), 3.85 (s, 3H, 4'-OMe), 3.86 (s, 6H, 3'- and 5'-OMe), 4.57 (br s, 2H, NH_2), 4.82 (br s, 2H, NH_2), 6.60 (s, 2H, 2'- and 6'-H).

Anal. Calcd. for $C_{17}H_{23}N_5O_3$•0.8H_2O: C, 56.75; H, 6.89; N, 19.46. Found: C, 56.95; H, 6.91; N, 19.28.

2,4-Diamino-6-(2'-bromo-3',4',5'-trimethoxybenzyl)-5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidine (**10**).

A solution of **7**•HCl (180 mg, 0.78 mmole), 2-bromo-3,4,5-trimethoxybenzyl bromide (391 mg, 1.15 mmoles) [43], sodium acetate (184 mg), and anhydrous sodium iodide (10 mg) in dry *N,N*-dimethylformamide (6 ml) was heated to 80° for 2 hours under argon. The solvent was removed *in vacuo* and the residue was taken up in 15% acetic acid (20 ml). The solution was washed with ether (3 x 30 ml), the aqueous layer was cooled and basified to *p*H 9, and re-extracted with chloroform (2 x 30 ml). The combined organic layers were washed with brine, dried (magnesium sulfate), and evaporated to an oil, which was chromatographed on silica with 10:1 chloroform-methanol as the eluent. Fractions containing a single tlc spot with R_f 0.22 (silica gel 100:10:1 chloroform-methanol-28% ammonium hydroxide) were pooled and evaporated to a white powder. Recrystallization from methanol afforded **9** as white needles (144 mg, 45%), mp 169-171°; ir (potassium bromide): ν 3455, 3320, 3160, 2935, 1630, 1590, 1575 cm^{-1}; ^1H nmr (500 MHz, deuteriochloroform): δ 2.74-2.85 (m, 4H, 7- and 8-CH_2), 3.36 (s, 2H, 5-CH_2), 3.80 (s, 2H, bridge CH_2), 3.85 (s, 3H, 4'-OMe), 3.90 (s, 3H, 5'-OMe), 3.91 (s, 3H, 3'-OMe), 4.56 (br s, 2H, NH_2), 4.83 (br s, 2H, NH_2), 6.96 (s, 1H, 6'-H).

Anal. Calcd. for $C_{17}H_{22}BrN_5O_3$: C, 48.12; H, 5.23; N, 16.50. Found: C, 48.22; H, 5.16; N, 16.13.

2,4-Diamino-6-[2-(3',4',5'-trimethoxyphenyl)ethyl]-5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidine (**11**).

A stirred solution of **7**•HCl (120 mg, 0.52 mmole) and sodium acetate (100 mg) in dry dimethyl sulfoxide (3 ml) was heated to 80° under argon, and 2-(3,4,5-trimethoxyphenyl)ethyl tosylate [44] (256 mg, 0.7 mmole) was added in small portions. After 20 hours at 80°, the solvent was removed by distillation under reduced pressure, and the residue was taken up in 15% acetic acid (20 ml). The solution was extracted with ether (3 x 30 ml), and the aqueous layer was cooled in an ice bath, basified to *p*H 9 with concentrated aqueous ammonia, and extracted with chloroform (2 x 30 ml). The combined organic layers were washed with brine, dried over anhydrous magnesium sulfate, and concentrated to dryness by rotary evaporation. Chromatography of the solid residue on silica gel, with 100:10:1 chloroform-methanol-28% ammonium hydroxide as the eluent, afforded **10** as a white powder (47 mg, 26%), mp 160-162°; R_f 0.41 (silica gel, 100:10:1 chloroform-methanol-28% ammonium hydroxide); ir (potassium bromide): ν 3420, 3300, 2930, 1630, 1590 cm^{-1}; ^1H nmr (500 MHz, deuteriochloroform): δ 2.73-2.84 (complex m, 8H, 7-CH_2, 8-CH_2, and bridge CH_2CH_2), 3.34 (s, 2H, 5-CH_2), 3.83 (s, 3H, 4'-OMe), 3.85 (s, 6H, 3'- and 5'-OMe), 4.54 (br s, 2H, NH_2), 4.66 (br s, NH_2), 6.46 (s, 2H, 2'- and 6'-H).

Anal. Calcd. for $C_{18}H_{25}N_5O_3$•0.4CH_3OH: C, 59.40; H, 7.22; N, 18.82. Found: C, 59.37; H, 6.88; N, 18.72.

2,4-Diamino-6-[3-(3',4',5'-trimethoxyphenyl)propyl]-5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidine (**12**).

A solution of **7**•HCl (120 mg, 0.52 mmole), 3-(3,4,5-trimethoxyphenyl)propane (164 mg, 0.57 mmole) [45], sodium acetate (85 mg), and anhydrous sodium iodide (10 mg) in anhydrous *N,N*-dimethylformamide (5 ml) was heated to 80° under argon for 2 hours. The solvent was removed by distillation *in vacuo*, and the residue was taken up in 15% acetic acid (20 ml). The solution was extracted with chloroform (2 x 20 ml), and the aqueous layer was cooled in an ice bath, basified to *p*H 8 with

28% ammonium hydroxide, and re-extracted with chloroform (2 x 30 ml). The combined organic layers were washed with brine, dried over magnesium sulfate, and evaporated. Chromatography of the yellow solid on silica gel with 10:1 chloroform-methanol as the eluent afforded a yellow semi-solid, which was recrystallized from dichloromethane to obtain pale-yellow needles (61 mg, 31%), mp 174-176°; R_f 0.15 (silica gel, 100:10:1 chloroform-methanol-28% ammonium hydroxide); ir (potassium bromide): ν 3400 br, 3310, 2940, 1650, 1590 cm^{-1}; ^1H nmr (500 MHz, deuteriochloroform): δ 1.92 (m, 2H, $CH_2CH_2CH_2$), 2.56-2.75 (m, 8H, 7-CH_2, 8-CH_2, and $CH_2CH_2CH_2$), 3.26 (s, 2H, 5-CH_2), 3.83 (s, 3H, 4'-OMe), 3.85 (s, 6H, 3'- and 5'-OMe), 4.52 (br s, 2H, NH_2), 4.66 (br s, 2H, NH_2), 6.43 (s, 2H, 2'- and 6'-H).

Anal. Calcd. for $C_{19}H_{27}N_5O_3 \cdot 0.3CH_2Cl_2$: C, 58.09; H, 6.99; N, 17.56. Found: C, 57.85; H, 7.25; N, 17.36.

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