One-Step Synthesis of Novel 2,4-Diaminopyrimidine Antifolates from Bridged Alicyclic Ketones and Cyanoguanidine

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A convenient one-step reaction with cyanoguanidine was used to convert alicyclic ketones to previously undescribed 2,4-diamino-5,6,7,8-tetrahydroquinazolines with a one-, two-, or three-carbon bridge in the carbocyclic ring. Although the yields of the desired products were modest, the principal advantage of this one-step process was that it provided easy access to a variety of novel bridged heterocyclic ring systems whose synthesis from sterically hindered ketones by other methods would have required multiple steps with an even lower overall yield. The products were tested as inhibitors of dihydrofolate reductases from *Pneumocystis carinii, Toxoplasma gondii,* and rat liver with a view to examining the effect of a space-filling bridge on binding. The most potent and selective compound in the group was 4,6-diamino-3,5-diazatricyclo[7.2.1.$0^{2,7}$]dodeca-2,4,6-triene (**13**), whose potency and selectivity approached those of trimethoprim, a drug commonly used to treat *P. carinii* and *T. gondii* infection. 3,5-Diamino-4,6-diazatricyclo[6.2.1.$0^{2,7}$]-undeca-2,4,6-triene (**14**), the analog of **13** with a one-carbon rather than a two-carbon bridge showed similar potency and selectivity against the *T. gondii* enzyme, but was a weak and nonselective inhibitor of *P. carinii* dihydrofolate reductase. The other compounds tested were likewise weak and nonselective.

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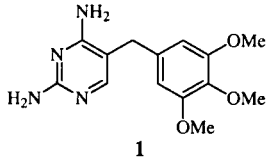
Lipophilic antifolates that bind tightly and selectively to dihydrofolate reductase from the opportunistic pathogens *Pneumocystis carinii* and *Toxoplasna gondii* have been actively sought for a number of years [reviewed in 1-3]. However, especially in the case of inhibitors of the *Pneumocystis carinii* enzyme, the combination of high potency and high selectivity has remained elusive. Classical antifolates such as methotrexate bind very tightly to the *P. carinii* and *T. gondii* dihydrofolate reductase, but cannot be used clinically against these organisms because they lack the membrane-bound active transport protein which is responsible for concentrative methotrexate uptake by mammalian cells [4]. Thus, standard antifolate prophylaxis and therapy of *P. carinii* and *T. gondii* infection relies on the permeability of these organisms to lipophilic molecules like trimethoprim. (**1**) [5,6] or pyrimethamine (**2**) [7,8], generally in combination with a sulfa drug such as sulfamethoxazole. Trimethoprim exhibits a high degree of species selectivity for *P. carinii* and *T. gondii versus* mammalian dihydrofolate reductase, but is not very potent. Pyrimethamine is somewhat more potent but less selective. Two other lipophilic antifolates, trimetrexate (**3**) and piritrexim (**4**), are extremely potent but non-selective, and therefore have to be given together with leucovorin [(6*R*,6*S*)-5-formyl-5,6,7,8-tetrahydrofolate) in order to prevent the hematopoeitic toxicity which would otherwise ensue [9,10]. The success of leucovorin as a protective agent is due in this case to its ability to be actively transported by the cells of the host as opposed to those of the pathogen. Controlled clinical comparison of trimetrexate-leucovorin with standard trimethoprim-sulfamethoxazole showed no dramatic difference, though it was suggested that the trimetrexate-leucovorin combination would be appropriate in patients who experience unacceptable side effects from sulfa drugs. Taken together, the frequent inability of patients to tolerate sulfa drugs and the relatively high cost of leucovorin have prompted attempts in several laboratories to try to design a new generation of lipophific dihydrofolate reductase inhibitors potent enough to be used without a sulfa drug and yet selective enough to not require co-administration of leucovorin [11-14]. However this has proved to be a daunting task, as most such attempts have been thwarted by what seems to be, at least empirically, an inverse correlation between potency and selectivity [15].



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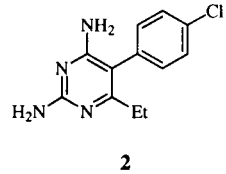

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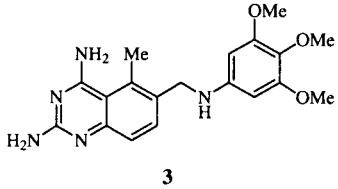

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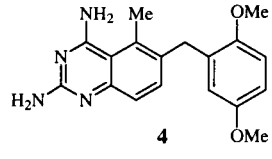

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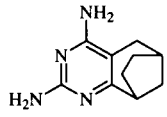
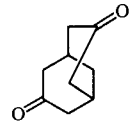
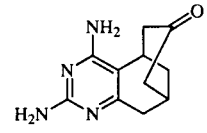
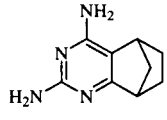
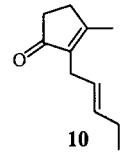
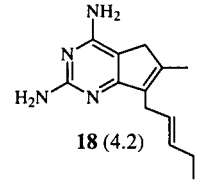
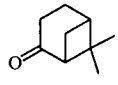
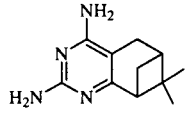
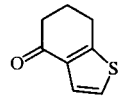
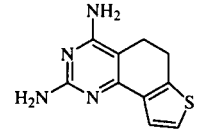
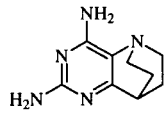
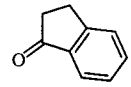
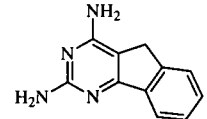
A possible approach to enhancing the selective binding of inhibitors to the active site of dihydrofolate reductase

from different species might be to take advantage of differences in steric bulk in the part of the molecule directly adjacent to the diaminopyrimidine moiety of trimethoprim and pyrimethamine. According to this view, inspired by elegant recent work on *Candida albicans* dihydrofolate reductase [16], the ability of a dihydrofolate reductase inbibitor with space-filling alkyl substituents in this region to make effective van der Waals contacts with hydrophobic residues in the active site would vary according to the topology of the cavity formed by these residues. In other words, if the pocket into which this part of the inhibitor fits is either too narrow or too wide allow effective hydrophobic interaction, binding will be weaker than if the pocket has precisely the right volume and contour to allow a tight fit. To test this hypothesis we thought it might be useful to examine the binding to *P. carinii, T. gondii,* and mammalian (rat liver) dihydrofolate reductase of dicyclic diaminopyrimidine derivatives with a fused carbocyclic moiety that contains a bulky hydrophobic bridge while lacking the substituted aryl group of conventional trimethoprim and pyrimethamine analogues. The synthesis and activity of a small group of such compounds as dihydrofolate reductase inhibitors are the subject of this paper.

Thermal condensation of cyclic ketones with cyanoguanidine under fusion conditions or in a high-boiling inert solvent affords a convenient one-step route to condensed diaminopyrimidine derivatives [17], and has been used to prepare a variety of nonbridged ring systems [18-22]. The largest number of these have been tetrahydroquinazolines, and dihydrobenzo[*f*]quinazolines, though others have also been made by this route. In contrast, there have been few attempts to extend this reaction to *bridged* cyclic ketones. In the present study, cyanoguanidine was condensed with bicyclo[3.2.1]octan-2-one (**5**), norcamphor (**6**), (*R*)-(+)-nopinone (**7**), 3-quinuclidinone (**8**), and bicyclo[3.3.1]-nonane-3-dione (**9**). Three non-bridged ketones, *cis*-jasmone (**10**), 4-keto-1,2,3,4-thianaphthene (**11**), and 1-indanone (**12**) were also used for comparison. The structures of **5-12**, along with those of their respective cyanoguanidine fusion products, **13-20,** which represent some very unusual heterocyclic ring systems, are shown in Table 1. Compound **18** is, to our knowledge, the first reported example of the reaction of cyanoguanidine with a substituted cyclopentenone. Compound **16** can be viewed as a bridged 2,4-diamino-5,6,7,8-tetrahydropyrido[3,2-*d*]pyrimidine, and is notable because of its bioisosteric relationship to the bridged

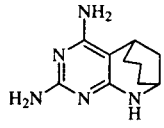
Table 1

Synthesis of Condensed 2,4-Diaminopyrimidines from Cyclic Ketones and Cyanoguanidine

Ketone	Fusion Conditions	Product (% Yield) [a]	Ketone	Fusion Conditions	Product (% Yield) [a]
 **5**	200°C, 1 hour	 **13** (6.3)	 **9**	200°C, 1 hour	 **17** (7.6)
 **6**	190-200°C, 2.5 hours (sealed tube)	 **14** (1.3)	 **10**	230°C, 1.5 hours	 **18** (4.2)
 **7**	200-210°C, 1 hour	 **15** (1.4)	 **11**	200°C, 1 hour	 **19** (28)
 **8**	210°C, 1.5 hours	 **16** (23)	 **12**	200-205°C, 1 hour	 **20** (14)

[a] Compounds **14** and **16** were purified by preparative thin-layer chromtography on silica gel using 92:8 and 85:15 chloroform-methanol, respectively. The other products were purified by column chromatography on silica gel using 85:15 chloroform-methanol. Yields are not corrected for recovered starting material.

2,4-diamino-5,6,7,8- tetrahydropyrido[2,3-*d*]pyrimidine **22**, which was recently discussed as being of potential interest as a dihydrofolate reductase ligand [24].



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A general procedure for the cyanoguanidine fusion reaction is given in the experimental section. Reaction conditions and non-optimized yields obtained for individual reactions summarized in Table 1; systematic chemical and names and physical data for the products are given in Table 2. Briefly, the ketone and an equimolar amount of recrystallized cyanoguanidine were heated under a stream of nitrogen at 190-230° for 1.0-2.5 hours, the mixture was allowed to cool, and the solidified melt was pulverized and thoroughly extracted with an 85:15 mixture of chloroform and methanol. The insoluble residue, assumed to consist of melamine-type polymers resulting from the thermal breakdown and polymerization of cyanoguanidine [23], was discarded, and the organic extract was chromatographed on silica gel using 85:15 chloroform-methanol as the eluent. The yield of purified product varied considerably, from a low of 1.3% (**15**) to a high of 28% (**19**). Among the compounds with a bridged carbocyclic ring, the one that formed in highest yield was **16** (23%). However yields were higher when they were corrected for recovered starting material. For example, the adjusted yield in the synthesis of **13** was 18%, rather than 6.3%. Substantial amounts of unchanged starting ketone were generally recovered from all the reactions, along with tarry byproducts assumed to arise *via* self-condensation reactions of the ketones.

Both the degradative polymerization of cyanoguanidine, and the self-condensation of ketones appear to be unavoidable

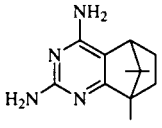
Table 2

Chemical Names and Physical Data for Compounds **13-20**

Compound	Molecular Formula	Chemical Name	^{1}H-NMR Data [a]	Microanalysis and/or Mass Spectral Data
13	$C_{10}H_{14}N_4$	4,6-Diamino-3,5-diazatricyclo-[7.2.1.0^{2,7}]dodeca-2,4,6-triene	δ 1.0-3.0 (complex m, 10H, 4 CH_2, 2H), 4.8 (br s, 4H, 2- and 4-NH_2)	m/z [M+1]$^+$ 191.25, expected 191.26
14	$C_9H_{12}N_4$	3,5-Diamino-4,6-diazatricyclo-[6.2.1.0^{2,7}]undeca-2,4,6-triene	δ 1.3-3.2 (complex m, 8H, 3 CH_2, 2 CH), 4.6 (br s, 2H, 4-NH_2), 5.0 (br s, 2H, 2-NH_2)	m/z [M]$^+$ 176.11, expected 176.22
15	$C_{11}H_{16}N_4$	4,6-Diamino-3,5-diaza-10,10-dimethyltricyclo[7.1.1.0^{2,7}]-undeca-2,4,6-triene	δ 0.9-2.6 (complex m, 10H, 2 CH_3, 2 CH_2, 2 CH), 4.7 (br s, 4H, 2- and 4-NH_2)	Calcd. for $C_{11}H_{16}N_4O$• 0.15H_2O: C, 63.92; H, 7.68; N, 27.11. m/z [M+1]$^+$ 205.16, expected 205.28
16	$C_9H_{13}N_5$	3,5-Diamino-1,4,6-triazatricyclo-[6.2.2.0^{2,7}] dodeca-2,4,6-triene	δ 1.6-3.2 (complex m, 9H, 4 CH_2, 1 CH), 4.8 (br s, 2H, 4-NH_2), 5.2 (br s, 2H, 2-NH_2)	Calcd: C, 56.51; H, 6.86; N, 36.62. Found: C, 56.54; H, 6.68; N, 36.65
17	$C_9H_{14}N_4O$	4,6-Diamino-3,5-diazatricyclo-[7.3.1.0^{2,7}]tetradeca-2,4,6-trien-10-one	δ 1.6-3.6 (complex m, 10H, 4 CH_2, 2 CH), 5.2 (br s, 4-NH_2), 6.1 (s, 2H, 2-NH_2)	m/z [M+1]$^+$ 219.10, expected 219.13
18	$C_{13}H_{18}N_4$	2,4-Diamino-6-methyl-7-(*trans*-pent-2-en-1-yl)-6-methyl-5*H*-cyclopenta[2,1-*d*]pyrimidine	δ 1.0-3.3 (complex m, 10H, 2 CH_3, 2 CH_2), 5.0 (br s, 4H, 2- and 4-NH_2), 5.4 (m, 2H, CH=CH)	m/z [M+1]$^+$ 231.31, expected 231.32
19	$C_{10}H_{10}N_4S$	6,8-Diamino-4,5-dihydrothieno-[2,3-*d*]quinazoline	δ 2.9 (m, 4H, CH_2CH_2), 4.8 (br s, 4H, 2- and 4-NH_2), 7.1 (d, 1H, 2-thienyl), 7.6 (d, 1H, 3-thienyl)	Calcd. for $C_{10}H_{10}N_4S$• 0.4 H_2O: C, 53.13; H, 4.64; N, 24.79. Found: C, 53.13; H, 4.46; N, 24.80
20	$C_{11}H_{10}N_4$	2,4-Diamino-5*H*-indeno[3,2-*d*]-pyrimidine	δ 3.6 (m, 2H, CH_2), 5.9 (br, s, 2H, 4-NH_2), 6.4 (br s, 2H, 2-NH_2), 7.5 (m, 4H, aryl)	Calcd. for $C_{11}H_{10}N_4$• 0.25H_2O: C, 65.48; H, 5.23; N, 27.64. Found: C, 65.27; H 5.04; N, 27.80. m/z [M+1]$^+$ 199.25, expected 199.24

[a] The spectra of **13-16, 18,** and **19** were obtained in deuteriochloroform solution, whereas those of **17** and **20** were obtained in d_6-dimethylsulfoxide.

features of this high-temperature process. However, despite its low yield, the reaction is not without merit when one considers that other routes to sterically hindered 2,4-diamino-5,6-cycloalkanopyrimidines require several steps whose overall yield may not be any higher. For example, in a multistep synthesis of the bridged analog **23** from camphor *via* 3-cyano-2-methoxycamph-2-ene [25] the yield in just the final step, involving reaction of the cyano enol ether with guanidine in an autoclave at 160 170°, is reported to be only 4%.



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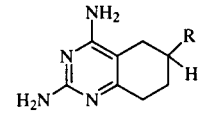
It may be noted that since the cyanoguanidine reaction only works with ketones that contain a free CH_2 group, and the ketones in Table 1 either contain only one such group or, contain a plane a symmetry (as in **9**), all the reactions can only give a single diaminopyrimidine product. However, because **13-17** are chiral, the products from these ketones are assumed to be racemic. As a result, bioassay data obtained with the racemates, will reflect the combined effect of two enantiomers that may or may not have the same potency. Obviously, if the inhibitory effect of the racemic mixture against dihydrofolate reductase were due entirely to one enantiomer, the potency of this species would not vary from that of the racemic mixturc by more than twofold.

The ability of the bridged analogs **13-17** and the nonbridged analogs **18-20** to inhibit dihydrofolate reductase from *P. carinii, T. gondii,* and rat liver was determined spectrophotometrically as described earlier [26,27]. This method depends on the change in ultraviolet absorbance which occurs at 254 nm when dihydrofolate is reduced to tetrahydrofolate and the 1,4-dihydropyridine ring of the nicotinamide adenine nucleotide cofactor is oxidized. The concentration of each compound required to inhibit the enzyme-catalyzed reaction by 50%, defined as the IC_{50}, is shown in Table 3, along with the previously determined IC_{50} values of reference compounds **1-4**. As noted above, **1** and **2** are examples of selective but relatively weak inhibitors whereas **3** and **4** are very potent but completely nonselective. Also shown for comparison are recently published IC_{50} values for two additional examples of nonbridged analogs, namely 2,4-diamino-6-ethyl-5,6,7,8-tetrahydroquinazoline (**24**) and 2,4-diamino-6-*tert*-butyl-5,6,7,8-tetrahydroquinazoline (**25**). Like the other compounds in the series, **24** and **25** were synthesized from the thermal reaction of appropriate ketones with cyanoguanidine [22].

Comparison of tle IC_{50} values for compounds **13-17** as dihydrofolate reductase inhibitors suggests that a space-filling hydrophobic group in the B-ring region may indeed influence



24: R = Et
25: R = *t*-Bu

binding in a species-specific manner. However, this type of substitution unfortunately also seems to lead to a sharp decrease in potency. The most active of the bridged compounds was **13**, with an IC_{50} of 6.1 μM against *P. carinii* dihydrofolate reductase and 5.1 μM against *T. gondii* dihydrofolate reductase. Its selectivity index for the *P. carinii* enzyme was 9.7, and thus was similar to that of trimethoprim but substantially greater than that of pyrimethamine, trimetrexate, or piritrexim. The selectivity index of **13** for the *T. gondii* enzyme was 12, a value somewhat lower than that of trimethoprim, but still significant in comparison with the other reference drugs. While the selectivity of **13** was somewhat greater than that of the nonbridged analogs **23** and **24**, its potency, especially in comparison with **24**, was reduced. Thus it appears that bulky substitution may actually be tolerated better when it lies in the region just beyond the B-ring, rather than being part of a bridge spanning the ring.

Decreasing the number of CH_2 groups in the bridge, as in **14**, resulted in little change in binding to *T. gondii* dihydrofolate reductase but a 20-fold loss of binding to *P. carinii* dihydrofolate reductase, suggesting that the active site of the *P. carinii* enzyme is more sensitive to a small change in size and geometry of the hydrophobic bridge. The selectivity index of **14** for *T. gondii* dihydrofolate reductase, while a little lower than that of **13**, was still significant in comparison with trimetrexate and piritrexim. Interestingly, when the bridge spanned C6 and C8, as in **15**, and additional bulk was introduced in the form of geminal dimethyl groups, there was a loss of binding to both the *T. gondii* enzyme and the rat liver enzyme. Moreover, selectivity for the *T. gondii* enzyme was nearly abolished. When the bridge spanned C5 and C7 and was extended to three carbons, as in **17**, there was a sharp loss of potency against both *P. carinii* and *T. gondii* dihydrofolate reductase. The presence of a polar carbonyl group on the bridge may have been detrimental for binding to the enzyme. The solubility of this compound was also a problem, preventing it from being tested at concentrations greater than 46 μM. Compounds **18-20** were very weak inhibitors, and were also nonselective.

In summary, we have shown that the condensation reaction between cyanoguanidine and bicycloalkanones provides a reasonable route to sterically hindered 2,4-diamino heterocyclic systems whose synthesis would otherwise be uneconomical and extremely laborious. Although the yields in this reaction are low, they provided enough material to allow these novel and otherwise difficultly accessible bridged compounds to be tested *in vitro*.

Table 3

Dihydrofolate Reductase Inhibition by Compounds **13-20** and Other Lipophilic 2,4-Diaminopyrimidines

Compound	IC_{50} (μM) [a]			Selectivity Index [b]	
	P. *carinii*	*T. gondii*	Rat Liver	P. *carinii*	*T. gondii*
1 (trimethoprim)	12	2.7	130	11	48
2 (pyrimethamine)	2.4	0.39	1.5	0.62	5.9
3 (trimetrexate)	0.042	0.01	0.003	0.07	0.30
4 (piritrexim)	0.031	0.017	0.0015	0.048	0.088
13	6.1	5.1	59	9.7	12
14	117	5.6	43	0.37	7.7
15	77	39	112	1.5	2.9
16	26% @ 46 μ*M*	46	27% @ 46 μ*M*	ND	ND
17	260	34	237	0.91	7.0
18	176	30	101	0.57	3.2
19	41% @ 352 μ*M*	101	30	ND	0.3
20	18% @ 205 μ*M*	20	29	ND	1.5
24 [c]	6.9	1.1	3.0	0.43	2.7
25 [c]	0.18	0.018	0.065	0.36	3.6

[a] Enzyme activity was determined at 340 nm according to a standardized and highly reliable method [26,27] which has been in continuous use in this program for a number of years. Each concentration of drug was tested in triplicate. As an example of the reproducibility of the assay, the IC_{50} value (mean ± standard error) obtained in Dr. Queener's laboratory over a five-year period using the reference compound pyrimethamine (**2**) against *P. carinii, T gondii,* and rat liver dihydrofolate reductase has been 2.39 ± 0.42, 0.50 ± 0.23, and 1.52 0.32 μ*M*, respectively. [b] Selectivity Index = IC_{50} (rat liver)/IC_{50} (*P. carinii*) or IC_{50} (rat liver)/IC_{50} (*T. gondii*). [c] Data from reference 22.

EXPERIMENTAL

The ^{1}H-nmr spectra were recorded on a Varian Model EM360L spectrometer with tetramethylsilane as the reference. Analytical thin-layer chromatography was on fluorescent Baker Si250F silica gel plates, with spots being visualized under ultraviolet light at 254 nm. Column chromatography was on Baker 70-230 mesh silica gel or Baker flash-grade silica gel (40 μm particle size). Melting points (uncorrected) were obtained on a Fisher-Johns hot-stage apparatus. Microanalyses were by Robertson Laboratory, Madison, NJ, or QTI Laboratories, Whitehouse, NJ, Mass spectral data were supplied by the Mass Spectrometry Core Facility, University of California/Riverside, and the Molecular Biology Core Facility, Dana-Farber Cancer Institute. Chemicals and solvents were purchased from Aldrich (Milwaukee, WI) or Fisher (Boston, MA). Cyanoguanidine was recrystallized from water before being used.

General Procedure.

An equimolar mixture of the ketone and cyanoguanidine was finely pulverized and placed in a round-bonom flask which was heated at 230-230° in an oil bath for 1.0 or 1.5 hour under a gentle stream of nitrogen. In the case of the volatile ketone norcamphor (**2**) the reaction was carried out in a sealed tube at 190-200° for 2.5 hours. At the end of the reaction, the dark molten mixture was allowed to come to room temperature, and the contents of the flask were scraped out with a strong spatula and triturated with a large volume of 85:15 chloroform-methanol. The insoluble portion, assumed to contain polymeric by-products derived from the breakdown of cyanoguanidine, was discarded. Because the fused melt typically solidified as a glass, care had be taken not to break the flask during this operation. The extract was washed with water, then dried over anhydrous sodium sulfate and evaporated, or was dried and evaporated directly. The product was purified by column chromatography on silica gel with 85:15% chloroform-methanol as the eluent, or on a preparative thin-layer chromatography plate with the same eluent or one containing a little less methanol (*e.g.*, 8%). Significant amounts of unreacted ketone always eluted from the column ahead of the product. Fractions were monitored by thin-layer chromatography, and only those containing a single spot were pooled and evaporated. Despite being homogeneous by thin-layer chromatography and giving the expected spectral and/or microanalytical results, the product typically had a very poorly defined decomposition point starting above 200°.

3,5-Diamino-1,4,6-triazatricyclo[6.2.2.0^{2,7}]dodeca-2,4,6-triene (**16**).

As the best example of the general procedure, 3-quinuclidinone (1 g, 8 mmoles) was thoroughly mixed with powdered cyanoguanidine (0.679 g, 8 mmoles) and the mixture was heated for 1.5 hour at 210° under a gentle flow of nitrogen. The reaction mixture was cooled to room temperature and the fused melt was thoroughly extracted with 85:15 chloroform-methanol (200 ml). The insoluble portion (0.22 g) showed no absorption at 254 nm and was discarded. The solution was washed with water, the water layer was extracted with 85:15 chloroform-methanol, and the combined organic extracts were dried over anhydrous sodium sulfate and evaporated. Column chromatography on silica gel with 85:15 chloroform-methanol as the eluent yielded **16** as a pale yellow solid (0.35 g, 23%). Chemical formulas and yields of the other diaminopyrimidines prepared by this method, **13-15** and **17-20,** are shown in Table 1, and their spectral and microanalytical data are given in Table 2.

Dihydrofolate Reductase Inhibition Assays.

The ability of **13-20** to inhibit *P. carinii, T. gondii,* and rat liver dihydrofolate reductase was determined spectrophotometrically as previously reported [26,27]. The results of these assays are presented in Table 3, along with previously reported data for reference compounds **1-4** and the nonbridged analogs **24** and **25** [22].

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