(s, 1, 3-H), 6.96 and 7.00 (2s, 2, 5-H and 7-H), 7.56 and 7.65 (2s, 3, NH_2 and pyrimidine 6-H), 7.90 and 8.31 (2s, 2, NH and NH). Anal. ($C_{22}H_{31}Cl_2N_5O_3$) C, H, N.

2,4-Diamino-5-[(1,2-dihydro-2,2,4-trimethyl-8-(methylsulfinyl)-6-quinolyl)methyl]pyrimidine (8l). Compound **8f** (1.0 g, 2.9 mmol) was dissolved in MeOH and added to a solution of $NaIO_4$ (0.66 g, 3.07 mmol) in water at 0 °C. The reaction was then allowed to stir at room temperature overnight. The inorganic precipitate was removed and the product was extracted into CH_2Cl_2 and dried. It was then purified by column chromatography on silica gel, eluting with CH_2Cl_2–MeOH (19:1) to yield 0.47 g (45%) (EtOH) of **8l**, mp 246–248 °C. NMR (Me_2SO-d_6): 1.24 (d, 6, Me_2), 1.90 (d, 3, Me, J = 1.2 Hz), 2.73 (s, 3, SOMe), 3.48 (s, 2, CH_2), 5.42 (br s, 1, quinoline-3-H), 5.65 (br s, 2, NH_2), 6.04 (br s, 2, NH_2), 6.11 (m, 1, NH), 7.02 (s, 2, Ar), 7.50 (s, 1, pyrimidine-6-H). Anal. ($C_{18}H_{23}N_5OS \cdot 0.3H_2O$) C, H, N, S.

2,4-Diamino-5-[(1,2,3,4-tetrahydro-8-methoxy-2,2,4-trimethyl-6-quinolyl)methyl]pyrimidine (9b). Compound **8b** (0.65 g, 2 mmol) was dissolved in 50 mL of EtOH with 2 equiv of hydrochloric acid and reduced on a Parr hydrogenator with 0.2 g of PtO_2. After removal of the catalyst and solvent, a 0.5 M $NaHCO_3$ solution was added, and the product was extracted into CH_2Cl_2, followed by column chromatography as above described; yield 0.41 g (63%), mp 214–216 °C (absolute EtOH). NMR ($CDCl_3$): δ 1.14–1.30 [m, 9, Me, Me_2 (2 different isomers)], 1.41–1.80 (m, 2, CH_2), 2.88 (m, 1, C*H*Me), 3.60 (d, 2, CH_2), 3.75 (s, 3, OMe), 4.01 (br, 1, NH), 4.58 (br, 2, NH_2), 4.67 (br, 2, NH_2), 6.38 (d, 1, Ar), 6.65 (d, 1, Ar), 7.77 (s, 1, pyrimidine-6-H). Anal. ($C_{18}H_{25}N_5O$) C, H, N.

2,4-Diamino-5-[(1,2,3,4-tetrahydro-2,2,4-trimethyl-6-quinolyl)methyl]pyrimidine (9a). This compound was prepared from **8a** as described for **9b** and isolated as the hydrochloride; mp 245–250 °C dec (absolute EtOH–Et_2O). Anal. ($C_{17}H_{23}N_5 \cdot 2HCl \cdot 0.33H_2O$) C, H, N.

B. Enzyme Assays. Assay conditions for *E. coli* and rat liver DHFR were as previously described.[15,23] Homogeneous chicken liver DHFR was obtained from J. Freisheim (Medical College of Ohio) and assayed like the rat liver enzyme. I_{50} is the concentration of inhibitor that decreases the velocity of the standard assay by 50%. The enzyme, NADPH (65 μM), and varying concentrations of inhibitor were preincubated for 2 min at 30 °C, and the reaction was initiated by the addition of dihydrofolate (45 μM final concentration). Steady-state velocities were measured, and plots of the percentage inhibition vs the logarithm of inhibitor concentration were used to estimate I_{50} values. In two cases the inhibition measured at the solubility limit of the compound is given as I_{32} or I_{41}. Since the *E. coli* DHFR assay contained 1 nM enzyme, a 2 nM lower limit was arbitrarily set for I_{50} values. With this cutoff, error due to depletion of free inhibitor by enzyme binding[24] was limited to 25%. Apparent K_i values for some of the more potent *E. coli* DHFR inhibitors were determined by the method of Henderson[25] as described by Baccanari and Joyner.[26]

Acknowledgment. We wish to thank Dr. Lee Kuyper and Dr. Joseph Chan for the molecular mechanics calculations and for providing Figures 1 and 2. We thank Pauline Baker for expert technical assistance. The determination of I_{50} values for *E. coli* and rat liver DHFR inhibition were carried out under the direction of Robert Ferone, and Dr. Lynn Elwell's staff conducted the antibacterial assays. The NMR spectral determinations were carried out by Dr. Stuart Hurlbert and his staff. The log *P* measurements were carried out under the aegis of Dr. David Brent. Microanalyses were carried out by Atlantic Microlabs, Inc., Norcross, GA.

Supplementary Material Available: A table listing NMR data for certain 1,2-dihydroquinolines and 1-aryl-2,4-dimethylpyrroles isolated as oils are available, as well as a table of MIC data for all compounds of this paper against 17 organisms (2 pages). Ordering information is given on any current masthead page.

(23) Kuyper, L. F.; Roth, B.; Baccanari, D. P.; Ferone, R.; Beddell, C. R.; Champness, J. N.; Stammers, D. K.; Dann, J. G.; Norrington, F. E.; Baker, D. J.; Goodford, P. J. *J. Med. Chem.* **1985**, *28*, 303.

(24) Cha, S. *Biochem. Pharmacol.* **1975**, *24*, 2177.

(25) Henderson, P. J. F. *Biochem. J.* **1973**, *135*, 101.

(26) Baccanari, D. P.; Joyner, S. S. *Biochemistry* **1981**, *20*, 1710.

2,4-Diamino-5-benzylpyrimidines as Antibacterial Agents. 13. Some Alkenyl Derivatives with High in Vitro Activity against Anaerobic Organisms

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A series of 2,4-diamino-5-(3,5-dialkenyl-4-methoxy- or -4-hydroxybenzyl)pyrimidines was prepared from [(allyloxy)benzyl]pyrimidines by Claisen rearrangements, and the resulting allyl phenols were further modified by methylation and rearrangement to 1-propenyl analogues. Analogous 3,4-dimethoxy-5-alkenyl derivatives were prepared by similar techniques. High in vitro antibacterial activity was obtained against certain anaerobic organisms, such as *Bacteroides* species and *Fusobacterium*, which was equal to or better than the control, metronidazole, in several cases. The profile was similar against *Neisseria gonorrhoeae* and *Staphylococcus aureus*. The 3,5-bis(1-propenyl)-4-methoxy derivative **8** was 1 order of magnitude more active against *Escherichia coli* dihydrofolate reductase than its saturated counterpart, and it was also more active than trimethoprim, **1**. However, it was considerably less active in vitro against the Gram-negative organisms. The 3,4-dimethoxy-5-alkenyl, -5-alkyl, and -5-alkoxy analogues had very high broad-spectrum antibacterial activity. However, pharmacokinetic studies of four of the compounds in dogs and rats and in vivo studies with an abdominal sepsis model in rats showed no advantages over trimethoprim.

Although trimethoprim (TMP, **1**)[1-4] has excellent broad-spectrum antibacterial activity, it is not useful against all types of organisms. The search has continued for other inhibitors of dihydrofolate reductase (DHFR, E.C. 1.5.1.3) which will show greater activity against

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(1) Roth, B.; Falco, E. A.; Hitchings, G. H.; Bushby, S. R. M. *J. Med. Pharm. Chem.* **1962**, *5*, 1103.

(2) Bushby, S. R. M.; Hitchings, G. H. *Br. J. Pharmacol. Chemother.* **1968**, *33*, 72.

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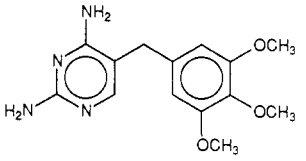


1

Pseudomonas species, certain anaerobic organisms, *Neisseria gonorrhoeae*, and other types of pathogens.

In previous papers, we have described a series of 3,5-dialkyl-4-(substituted-benzyl)pyrimidines, some of which had potent antigonococcal activity.[5-7] This activity was related to the shapes and hydrophobic nature of the substituents. In this paper, we explore unsaturated analogues, particularly 1-propenyl and allyl derivatives and cyclic dihydrobenzofuranyl analogues, where conformational possibilities are limited, in order to see whether their patterns of activity for various DHFRs might differ from those in previous series and thus widen the scope of antibacterial activity for the benzylpyrimidines. The synthesis, DHFR profile, and antibacterial activities are presented and compared with data for alkyl and alkoxy analogues, and finally, the pharmacokinetic profile of a few derivatives is described. For a compound to be more useful clinically than TMP, it must not only have increased potency or a broader antibacterial spectrum but must have a very favorable safety and pharmacokinetic profile as well.

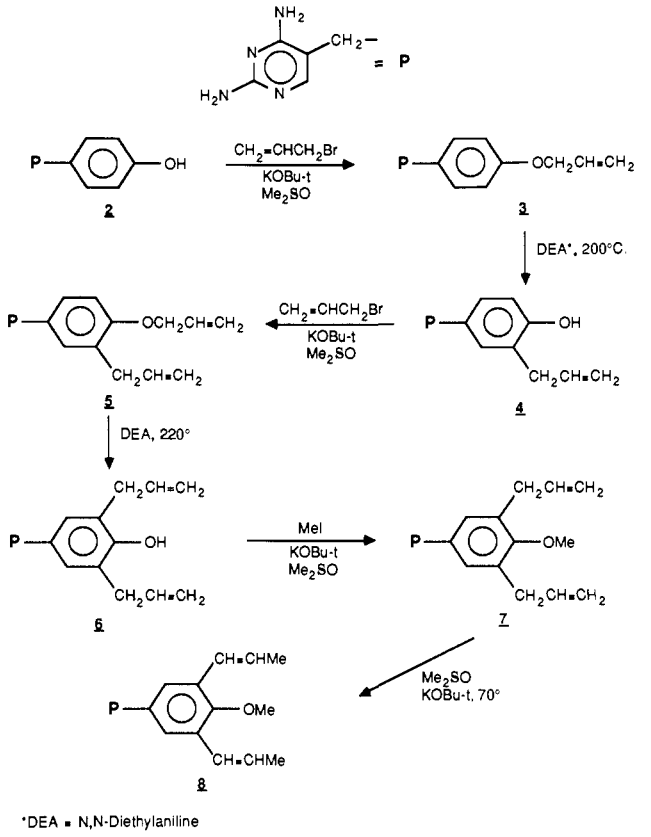
Chemistry

Scheme I illustrates the preferred route to *m*-allyl and 1-propenyl analogues of **1**, which involves the well-known Claisen rearrangement of 4-allyl ethers. This type of reaction works very well in the presence of the 2,4-diaminopyrimidine moiety at temperatures up to 220 °C. Rearrangement of allyl to 1-propenyl analogues was also readily accomplished in alkaline medium. The 1-propenyl derivatives prepared all had the trans configuration, as determined by NMR spectroscopy.

An alternative route, shown in Scheme II, involved condensation of an allylic phenol with **10**, as described in paper 6 of this series.[8] Yields in this condensation were poor, however. A preferable route involved the allylation of **12**, followed by Claisen rearrangement, to produce **11**. Compound **11** was a versatile intermediate, which could be hydrated to **14** or methylated to **15**, followed by rearranging to **16** or reduction to **17**.

Scheme III illustrates the preparation of some 2-alkyl-6-allylic phenols, which, when condensed with **10**, cyclized in part to give **28–31** and usually also produced some phenolic benzylpyrimidines; only **32** was completely characterized in an experiment with anhydrous HCl. The cyclic compounds were readily distinguished from the phenols (which, due to flanking by lipophilic substituents, were not readily soluble in alkali) by their UV spectra. Gradual ionization of the phenol produced a continuous hyperchromic and bathochromic shift in the long-wavelength maximum, with loss of the original isosbestic points. NMR spectra were of course also diagnostic. Compound **32** was methylated to give **33** and also rearranged to the 1-propenyl derivative **34**. Compounds **35–37** were prepared by routine alkylation of phenols. Details of these reactions are provided in the Experimental Section.

Scheme I



*DEA = N,N-Diethylaniline

Biological Data and Discussion

A. Enzyme Kinetic Data and Structural Interpretation. Table I compares the inhibitory activities of the various (*m*-alkenylbenzyl)pyrimidines and some of their saturated analogues and related ethers against DHFR from various sources. I_{50} values were determined for *Escherichia coli*, *N. gonorrhoeae*, and rat liver DHFR. The more active *E. coli* DHFR inhibitors ($I_{50} < 0.5 \times 10^{-8}$ M) sometimes showed slow, tight binding inhibition. Since these I_{50} values were difficult to assess precisely, kinetic inhibition constants (K_i values)[9,10] were determined for these compounds and for all of the compounds tested against *Staphylococcus aureus*.

All of the trisubstituted derivatives were good *E. coli* DHFR inhibitors, with I_{50} values varying about 20-fold from $\sim 0.2 \times 10^{-8}$ to 3.2×10^{-8} M, compared to 0.6×10^{-8} M for **1**. As expected from a large body of data, the 3,4,5-trisubstituted derivatives were all more active against *E. coli* DHFR than their mono- or disubstituted analogues.[11]

The pronounced effect of alkyl substituent shape on the inhibitory activities of (3,5-dialkylbenzyl)pyrimidines, coupled with the enhanced binding to *N. gonorrhoeae* DHFR over that of 1,[5] prompted the exploration of un-

(3) Evaluations on New Drugs: Trimethoprim-Sulfamethoxazole. *Drugs*, 1971, *1*, 7.
(4) Symposium on Trimethoprim-Sulfamethoxazole. *J. Infect. Dis.* 1973, *128* (supplement).
(5) Roth, B.; Rauckman, B. S.; Ferone, R.; Baccanari, D. P.; Champness, J. N.; Hyde, R. M. *J. Med. Chem.* 1987, *30*, 348.
(6) Roth, B.; Aig, E. *J. Med. Chem.* 1987, *30*, 1998.
(7) Roth, B.; Baccanari, D. P.; Sigel, C. W.; Hubbell, J. P.; Eaddy, J.; Kao, J. C.; Grace, M. E.; Rauckman, B. S. *J. Med. Chem.* 1988, *31*, 122.
(8) Stuart, A.; Paterson, T.; Roth, B.; Aig, E. *J. Med. Chem.* 1983, *26*, 667.
(9) Henderson, P. J. F. *Biochem. J.* 1973, *135*, 101.
(10) Baccanari, D. P.; Joyner, S. S. *Biochemistry* 1981, *20*, 1710.
(11) Roth, B.; Cheng, C. C. Recent Progress in the Medicinal Chemistry of 2,4-Diaminopyrimidines. In *Progress in Medicinal Chemistry*; Ellis, G. P., West, G. B., Eds.; Elsevier Biomedical: Amsterdam, 1982; Vol. 19, p 289.

Scheme II

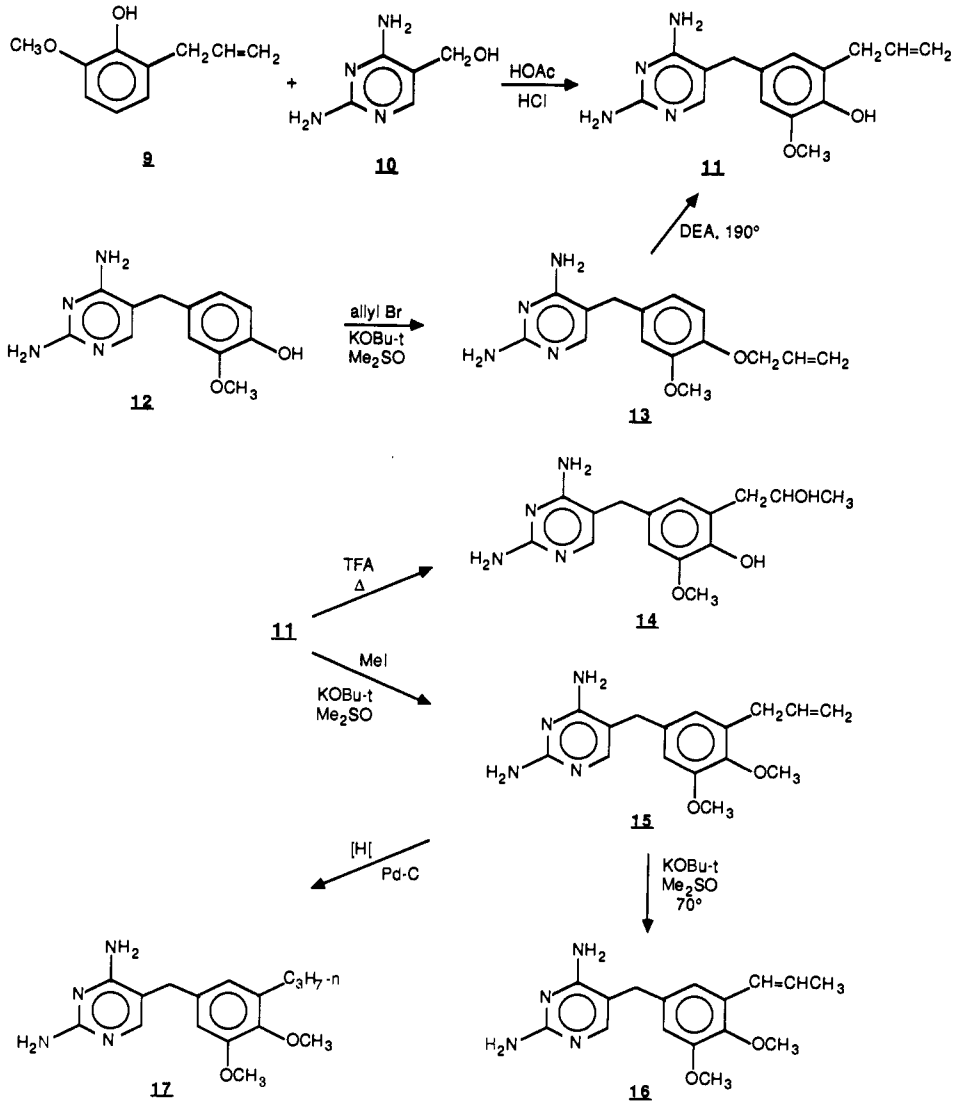
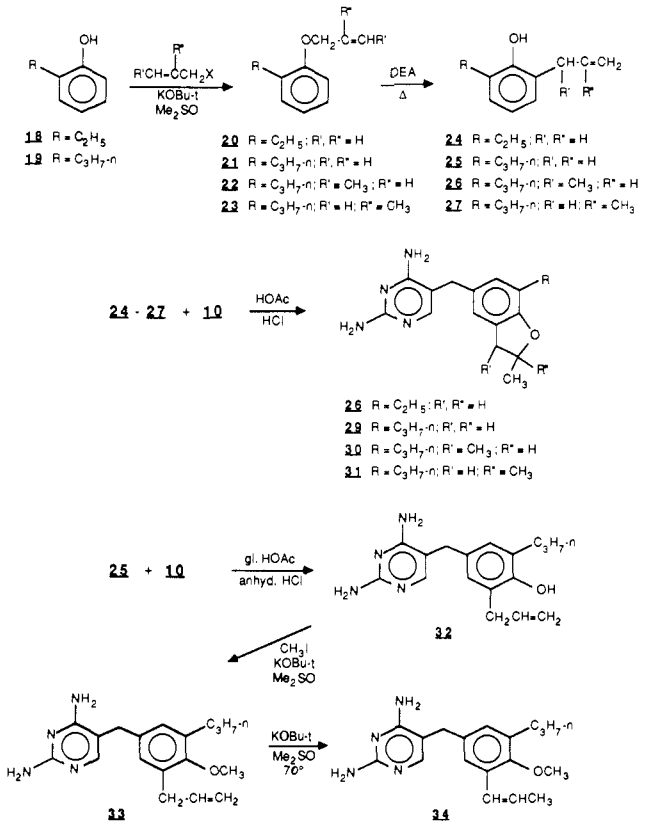


Table I. Comparison of 2,4-Diamino-5-(alkenylbenzyl)pyrimidines as Inhibitors of Dihydrofolate Reductase Enzymes

compd no.	benzene substituents 3	4	5	$10^8 I_{50}$, M, vs DHFR: *E. coli*	rat liver	*N. gonorrhoeae*	K_i, nM: *S. aureus*	*E. coli*
1	OCH_3	OCH_3	OCH_3	0.5–0.7	26000–37000	45	5.1	1.3
3		$OCH_2CH{=}CH_2$		60	32000 (I_{42})			
4	$CH_2CH{=}CH_2$	OH		15	9450			
5	$CH_2CH{=}CH_2$	$OCH_2CH{=}CH_2$		7.3	1100			
6	$CH_2CH{=}CH_2$	OH	$CH_2CH{=}CH_2$	2.4, 3.9	960	2.9		
7	$CH_2CH{=}CH_2$	OCH_3	$CH_2CH{=}CH_2$	0.69	1800	4.8	2.1	
8	$CH{=}CHCH_3$	OCH_3	$CH{=}CHCH_3$	<0.5	2700	3.6		0.8
11	OCH_3	OH	$CH_2CH{=}CH_2$	0.12, 0.22	1470	6.6		0.8
12	OCH_3	OH		6.6	3500			
13	OCH_3	$OCH_2CH{=}CH_2$		5.4	4900	25.5		
14	OCH_3	OH	$CH_2CHOHCH_3$	0.84	3600	14		
15	OCH_3	OCH_3	$CH_2CH{=}CH_2$	<1	5000	11.2	2.8	1.8
16	OCH_3	OCH_3	$CH{=}CHCH_3$	0.32, 0.39	5700	9.3	1.4	0.9
17	OCH_3	OCH_3	$CH_2CH_2CH_3$	0.37	3700	6.7		0.4
32	$CH_2CH{=}CH_2$	OH	$CH_2CH_2CH_3$	2.4	200	2.0		
33	$CH_2CH{=}CH_2$	OCH_3	$CH_2CH_2CH_3$	1.5	1500	4.1		1.6
34	$CH{=}CHCH_3$	OCH_3	$CH_2CH_2CH_3$	3.6	2400	3.3		
35	OCH_3	OCH_3	$OCH_2CH_2CH_3$	<0.45	9450	8.0	1.4	1.0
36	OCH_3	OCH_3	$OCH_2CH{=}CH_2$	0.81	15000	14.8	3.0	
37	OCH_3	OCH_3	OCH_2CH_3	0.40	14000	17	2.5	0.5
38[a]	$CH_2CH_2CH_3$	OH	$CH_2CH_2CH_3$	0.7	200	2.0		1.7
39[a]	$CH_2CH_2CH_3$	OCH_3	$CH_2CH_2CH_3$	4.4	2100	6.0	3.2	
40[a]	CH_2CH_3	OCH_3	$CH_2CH_2CH_3$	1.2	3200	4.4		

[a] Reference 5.

Scheme III



saturated analogues. *m*-1-Propenyl derivatives in particular achieve a rigidity in structure not experienced with an alkyl substituent. Furthermore, such substituents would lie in plane with the benzene ring, in the manner of **1**.[6] It is of interest then that the 3,5-bis(1-propenyl)-4-methoxy derivative **8** was about 1 order of magnitude more inhibitory to *E. coli* DHFR than its saturated counterpart **39**. Furthermore, its K_i value showed it to be bound to the enzyme with almost twice the affinity of **1**. It of course has a one atom longer chain in both meta positions, and at least one of the extra methyl groups may contribute to van der Waals attractive forces. Replacement of one of the propenyl groups by methoxy (compound **16**) did not alter the K_i appreciably, suggesting that only one of the methyl moieties of **8** has a positive contribution to binding. Replacement of the residual propenyl substituent of **16** by ethoxy (**37**) improved the K_i nearly 2-fold. The reason cannot be pinpointed, however, since bond angles and the tendency of atoms to remain in plane would be altered.[12,13] Furthermore, the 4-methoxy group could change its orientation 180°, as could the benzene ring itself. For these reasons, we do not attempt to explain relative *E. coli* DHFR inhibition experienced by the other analogues.

The (3,5-dialkenylbenzyl)pyrimidines, like their saturated counterparts, were found to have relative activities against *E. coli* DHFR that depended on whether the 4-substituent was a hydroxy or a methoxy group. In the 4-methoxy series unsaturation was found to enhance activity, whereas the reverse was found true with the phenols. For example, **8** was at least 10-fold more active than **39**, but **38** was more inhibitory than **6**. With the 3,5-dialkyl analogues, the 4-hydroxy compounds were almost always more active than their 4-methoxy counterparts. This is not necessarily true with the unsaturated derivatives (cf. **6** and **7** for example), suggesting that the various compounds are oriented somewhat differently in the enzyme pocket.

Looking at the K_i values which were determined, the compound most inhibitory to *E. coli* DHFR was the 3,4-dimethoxy-5-*n*-propyl derivative **17**, which was about equivalent to **37**; the latter, a triether, was several times more selective for bacterial DHFR, so this derivative, rather than **17**, was one of the group chosen later for more extended study.

Rat liver and *N. gonorrhoeae* DHFR I_{50} values showed insignificant effects on binding as a function of unsaturation. This was somewhat disappointing, particularly as concerned the bis(1-propenyl) derivative **8** complexed to rat liver DHFR. The closely analogous chicken liver DHFR had been observed to have an inner pocket which fit a *m*-methoxy group of **1** rather snugly;[14] thus, a poor fit for a rigid 1-propenyl substituent might be expected to decrease binding and thus enhance selectivity, but this was not the case.

The various 3,5-dialkenyl and 3,5-dialkyl derivatives are excellent inhibitors of *N. gonorrhoeae* DHFR, reaching 22 times the activity of **1**. The 3,4-dimethoxy or 3-methoxy-4-hydroxy compounds were less inhibitory in all cases, but nevertheless were considerably more active than **1**. Meta substituents of benzylpyrimidines apparently lie completely buried in a hydrophobic pocket in this enzyme, in contrast to *E. coli* DHFR, where all three oxygens of **1** are partially exposed to solvent.[5,14] This enzyme has been sequenced;[15] replacement of Leu-28 (*E. coli* DHFR) by Phe, a larger hydrophobic residue, may be at least partially responsible for this effect.

The lipophilic derivatives all show slightly greater inhibition of *S. aureus* DHFR than the standard, an effect which had been previously ascribed from in vitro MIC data to be solely due to the greater ease of penetration of lipophilic compounds into Gram-positive bacteria. We see here that the compounds are actually more inhibitory to the *S. aureus* enzyme than the control. The lipophilic effect can be seen in Table II, where activities against Gram-positive organisms are significantly higher than is the case with the Gram-negative bacteria as lipophilicity is increased.

B. In Vitro Antibacterial Activities. Table II lists the relative in vitro antibacterial activities of the new compounds of Table I against 11 organisms. By comparing Tables I and II it is obvious that the high *E. coli* DHFR affinity of some of the compounds did not translate into corresponding in vitro inhibition. However, a good correlation was present for *S. aureus*. In particular, compounds **15–17** and **35–37** are highly active against the Gram-positive organisms and have activities within two dilutions of that of **1** against all the other organisms shown. All of these compounds are 3,4-dimethoxy-5-substituted derivatives with a three-atom chain, and all are essentially equivalent in vitro. The only compound for which all MIC values are essentially equivalent to those of **1** (± one dilution) is **11**, an allylic phenol which is not only highly active against bacterial DHFRs but rat liver DHFR as well, with resultant poor selectivity. Compound **37**, which

(12) Radom, L.; Hehre, W. J.; Pople, J. A.; Carlson, G. L.; Fateley, W. G. *J. Chem. Soc., Chem. Commun.* **1972**, 308.
(13) Anderson, G. M., III; Kollman, P. A.; Domelsmith, L. N.; Houk, K. N. *J. Am. Chem. Soc.* **1979**, *101*, 2344.
(14) Matthews, D. A.; Bolin, J. T.; Burridge, J. M.; Filman, D. J.; Volz, K. W.; Kaufman, B. T.; Beddell, C. R.; Champness, J. N.; Stammers, D. K.; Kraut, J. *J. Biol. Chem.* **1985**, *260*, 381.
(15) Baccanari, D. P.; Tansik, R. L.; Paterson, S. J.; Stone, D. *J. Biol. Chem.* **1984**, *259*, 12291.

Table II. Relative in Vitro Antibacterial Activities of 2,4-Diamino-5-(alkenylbenzyl)pyrimidines against Various Microorganisms, Compared to That of Trimethoprim

compd no.	MIC of compd/MIC of trimethoprim[a,d]										
	St.p.	*Hal*	*Pas.m.*	*Myc.s.*	*Sal.t.*	*Sh.f.*	*E.c.*	*Ser.m.*	*Kl.pn.*	*Ent.a.*	*Pr.v.*
1[c]	1	1	1	1	1	1	1	1	1	1	1
3	10	100	100	50	100	100	72	>20	100	10	>100
4	2	10	10	5	100	10	16	20	20	10	50
5	2	10	500	>100	1000	500	72	>10	>100	>100	>50
6	1	1	100	5	10	50	21	>10	20	20	50
7	1	5	20	10	100	100	72	>10	>10	100	100
8	0.1	1	100	2	100	100	72	>5	100	100	>50
11	1	0.3	1	3	3	1	1	3	0.3	1	3
13	3	10	10	3	10	10	7.6	10	10	10	3
14	3	3	3	3	10	3	4	>10	10	3	30
15	0.3	0.1	10	3	3	10	6	3	10	10	10
16	0.1	0.1	10	3	3	10	6	1	10	10	3
17	0.1	0.1	10	3	3	10	6	3	10	10	10
32	1	3	30	10	100	30	5	3	30	30	30
33	2	1	10	0.5	100	100	38	>10	100	100	>50
34	0.1	1	20	2	20	100	26	>5	100	100	>100
35	0.3	0.3	3	3	10	10	6	3	10	3	3
36	0.3	0.3	10	3	10	10	NT	3	10	10	10
37	0.3	0.3	1	1	1	1	2.6	3	1	1	1

[a] Numbers greater than 1 reflect activities less than that of 1. Serial dilutions were normally 0.1, 0.3, 1, 3, 10, etc., but occasionally dilutions of 1, 5, and 10 were used. [b] Average of six strains, also divided by MIC for trimethoprim. [c] Normal MIC values for 1 were 0.3, 0.1, 0.1, 1.0, 0.1, 0.1, 0.1, 1.0, 0.3, 0.1, and 1.0 μg/mL, respectively, for the above organisms, ± about one dilution. The numbers shown were obtained by dividing by the control for that particular experiment. [d] *St.p.* = *Streptococcus pyogenes* CN10, *S.a.* = *Staphylococcus aureus* CN491, *Pas.m.* = *Pasteurella multocides* ATCC6587, *Myc.s.* = *Mycobacterium smegmatis S3254*, *Sal.t.* = *Salmonella typhosa* CN512, *Sh.f.* = *Shigella flexneri* CN6007, *E.c.* = *Escherichia coli*, *Ser.m.* = *Serratia marcescens* CN2398, *Kl.pn.* = *Klebsiella pneumoniae* CN3632, *Ent.a.* = *Enterbacter aerogenes* 2201/86, *Pr.v.* = *Proteus vulgaris* CN329.

Table III. In Vitro Antibacterial Activity of 2,4-Diamino-5-(alkenylbenzyl)pyrimidines vs Anaerobic Organisms Compared to That of Trimethoprim and Metronidazole (MIC, μg/mL)

compd no.	organism					
	B. fragilis[a]	*F. nucleatum*	*Clostridium perfringens*	*Clostridium difficile*	*Bacteroides vulgatus*	*Bacteroides* theta-iotaomicron
1	3.1	0.8	>25	>25	6.2	12.5
4	5.2	0.8	>25	25		
5	3.6	0.8	>25	>25		
6	1.1	0.8	>25	>25	0.8	0.4
7	0.3	0.8	25	>25	0.2	0.2
8	0.1	1.6	25	25	0.8	0.4
11	1.6	0.2	>25	>25	1.6	1.6
13	10.4	0.8	>25	>25		
15	1.1	0.4	>25	>25	1.6	1.6
16	0.7	0.8	>25	>25	0.8	1.6
17	1.1	0.4	>25	>25	1.6	1.6
32	0.6	0.4	>25	>25	0.8	0.8
33	0.2	0.8	12.5	25	0.2	0.1
34	0.3	0.8	12.5	12.5	0.4	0.4
36	2.6	1.6	>25	>25		
metronidazole[b]	0.3 (0.1)	>25 (0)	0.8 (0.3)	0.2 (0)	0.2 (0)	0.2 (0)

[a] Average of three strains. [b] Average of 10 determinations (standard deviation).

differs from 1 only in an extra methylene group and is twice as inhibitory against *E. coli* DHFR, did not show quite as high activity when tested against six strains of *E. coli* in vitro.

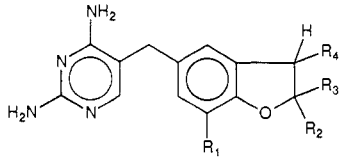
The summation of broad-spectrum antibacterial activities (Table II) shows very clearly that the dialkenyl derivatives do not show sufficient activity against the Gram-negative organisms, including *E. coli*, to be considered as candidate antibacterial agents with a broadened spectrum over that of 1. Similar decreased antibacterial activities have been observed many times with lipophilic derivatives of 1.[16] In fact, the most interesting broad-spectrum results lay with the more hydrophilic 3,4-dimethoxy- or 3-methoxy-4-hydroxy-5-substituted derivatives, which then became the prime candidates for further examination.

Screening against anaerobic organisms showed a profile of activity which was very similar to that found for *N. gonorrhoeae* DHFR. The dialkenyl or alkenyl alkyl derivatives were at least as active as the standard metronidazole in some cases; the dimethoxy or 3-methoxy-4-hydroxy derivatives, although not quite as effective, were nevertheless a considerable improvement over 1.

Table III compares tests of the alkenyl-substituted compounds against a series of anaerobic organisms, with metronidazole and 1 as standards. For three *Bacteroides* species, several of the compounds had activity equal to or greater than metronidazole and considerably greater activity than that shown by 1. Compound **33** was the most active of these, followed closely by **7**, **34**, **8**, and **32**. Thus the most inhibitory compounds of those tested against these organisms were 3-alkenyl-5-alkyl-4-methoxy or 3,5-

(16) Roth, B.; Aig, E.; Rauckman, B. S.; Strelitz, J. Z.; Phillips, A. P.; Ferone, R.; Bushby, S. R. M.; Sigel, C. W. *J. Med. Chem.* 1981, *24*, 933.

Table IV. Inhibition of Dihydrofolate Reductases and in Vitro Antibacterial Activities of 2,4-Diamino-5-(2,3-dihydrobenzofuranyl)pyrimidines, Compared to That of Trimethoprim and a Methylenedioxy Derivative



compd no.	dihydrobenzofuranyl substituents				$10^8 I_{50}$, M, vs DHFR[d]				ratio MIC compound/MIC trimethoprim vs organism[e]							
	R_1	R_2	R_3	R_4	*E.c.*	rat liver	*P.b.*	*N.g.*	*St.p.*	*S.a.*	*Kl.pn.*	*Sal.t.*	*E.c.*	*Sh.d.*	*Ent.a.*	*Pr.v.*
41[a]	CH_3	CH_3	CH_3		5.5	3700		19	3	3	10	10	10	10	30	10
28	C_2H_5	CH_3			1.9	1100	10	11	1	1	30	30	30	30	30	30
29	C_3H_7-*n*	CH_3			2.1	625	9.3	6.6	1	1	100	100	30	30[c]	100	30
30	C_3H_7-*n*	CH_3		CH_3	1.3	204	1.2	1.4	1	1	100	100	30	100	30	100
31	C_3H_7-*n*	CH_3	CH_3		28	990	24	4.6	3	3	100	300	100	300	100	100
	benzene substituents															
	3	4	5													
42[b]	OC_2H_5	OCH_2O			4.1	3700		14	1	10	30	30	30	10	10	10
1	OCH_3	OCH_3	OCH_3		0.6	30000	12	45	1	1	1	1	1	1	1	1

[a] Reference 8. [b] Reference 17. [c] *S. flexneri.* [d] *E.c. = E. coli* CN314, *P.b. = Plasmondium berghei, N.g. = Neisseria gonorrhoeae.* [e] *St.p. = S. pyogenes* CN10, *S.a. = S. aureus* CN491, *Kl.pn. = K. pneumoniae* CN3632, *Sal.t. = S. typhosa* CN512, *Sh.d. = Shigella dysentariae* CN1513, *Ent.a. = E. aerogenes*, 2201/86, *Pr.v. = P. vulgaris* CN329.

dialkenyl-4-methoxy derivatives, all of which were 10–100 times more active than **1** on *Bacteroides*. Compound **11**, the allyl methoxy phenol with the highest general in vitro antibacterial activity, was the most active against *Fusobacterium*. Of the 3,4-dimethoxy-5-substituted compounds, **16** had marginally higher activity than the others and was about 7 times more active than **1** against *Bacteroides*. Although none of the compounds showed activity approaching that of metronidazole against *Clostridium* species, all of the alkenyl compounds were highly active against *Fusobacterium nucleatum*, an organism relatively insensitive to metronidazole.

Table IV presents the DHFR I_{50} values and in vitro antibacterial activities of the dihydrobenzofuranyl derivatives which resulted from cyclization of the allylic phenols. These are compared to **1** and to a methylenedioxy derivative (**42**). The dihydrobenzofuran derivatives **28** and **29** consist of enantiomeric mixtures, and **30** consists of two pairs of diastereoisomers. Compound **30** was found by NMR to be 62% trans.

A comparison of **41**, **28**, and **29** against four DHFR species show that inhibition increases directly with the length of the *m*-alkyl group for rat and *N. gonorrhoeae* DHFRs; with the other two enzymes, ethyl or *n*-propyl were essentially equivalent but better than methyl for *N. gonorrhoeae* DHFR. All of these bicyclic derivatives are less active than **1** against microbial DHFR. The most active compound of this group is **30**, a diastereomeric mixture which has outstanding activity against *Plasmodium berghei* and *N. gonorrhoeae* DHFRs compared to **1**. Unfortunately, it is also almost 200 times more active than **1** against rat liver DHFR, a fact that could make it unacceptably toxic to the host except for short-time use.

The methylenedioxy analogue (**42**)[17] is included in Table IV for comparative purposes. Its overall enzyme inhibitory activity, as well as that against whole organisms, is very similar to that of **41**, despite its longer ethoxy chain, which might be expected to increase *E. coli* enzyme inhibition. All of the dihydrobenzofurans contained a methyl group next to the ring oxygen; **42** was the nearest unsubstituted analogue available to assess the effect of the methyl substitution. The closest comparison is to **29**, which is more inhibitory to all enzymes tested than is **42**, suggesting a possible positive effect for this substituent. The presence of two methyl substituents at this position (**31**) is obviously deleterious to binding, particularly to *E. coli* DHFR.

The compounds of this table all have about the same activity as **1** against the Gram-positive organisms, but are increasingly less active on the remaining bacteria as lipophilicity is increased. One might think that **30** and **31** should show greater differences in their in vitro activity than they do, since **30** is so much more inhibitory to DHFR, and the lipophilicities are about the same. These data indicate that the problem of cell penetrability is very complex.

C. In Vivo Testing. 1. Pharmacokinetic Studies in Dogs. On the basis of broad-spectrum antibacterial screening data, four compounds were chosen for measurement of pharmacokinetic parameters in the dog. The dog was chosen for these studies, since from previous work with analogues of **1**, the kinetic data with this species correlated better with human results than did data with other laboratory animals. In previous work, day-to-day variations in plasma concentration profiles for the reference compound **1** were observed. Therefore in the present experiments the pharmacokinetics of each analogue and **1** were estimated after simultaneous administration of both compounds to each dog. To show that the absorption or elimination of the analogue was not affected by coadministration with **1**, some compounds were also given alone and the pharmacokinetic profiles determined. Representative plasma concentration–time data for compound **32** that illustrate this point are shown in Figure 1.

For the iv studies, 5 mg/kg (free base/body weight) of each of compound **11**, **15**, **16**, and **37** was coadministered with 5 mg/kg of **1**. All of these compounds were eliminated more rapidly than **1**, which had a mean $t_{1/2}$ time of 3.44 h. Compounds **11** and **15** had mean plasma $t_{1/2}$ values that were less than 1 h, while the half-lives for **16** and **37** were 2.25 and 3.13 h, respectively (Table V). The mean bioavailabilities of the four analogues was estimated by comparison of the AUC (area under the plasma concentration–time curve) values after iv and po administration; the

(17) Grunberg, E.; Hoffer, M. Ger. Offen. 2,252,807, 1973; *Chem. Abstr.* **1973**, *79*, 53355*g*.

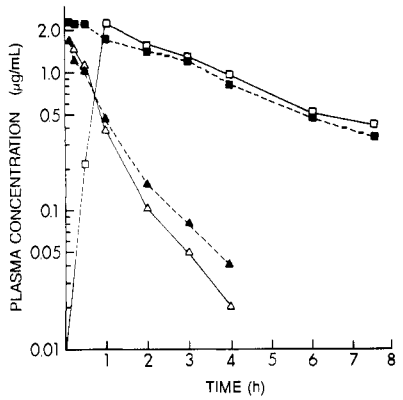


Figure 1. Plasma concentrations (μg/mL) of **32** in a dog after iv administration of **32** alone (△) and of **32** with **1** (▲). Plasma concentrations of **1** when given po alone (□) and iv with **32** (■). The dose of each compound administered in all experiments was 5 mg/kg.

Table V. Plasma Half-Life and Bioavailability of Trimethoprim Analogues in the Dog

compd no.	half-life, h	bioavailability (*F*)
1	3.44	0.99
11	0.70	ND[a]
15	0.84	ND[a]
16	2.25	0.25[b]
37	3.13	0.79

[a] Not determined due to low plasma concentrations. [b] Free base.

only analogue that was well absorbed was **37**; the other compounds had relatively low plasma concentration after po dosing.

To investigate whether the low bioavailability of **16** was due to poor absorption or extensive first-pass metabolism, the bioavailabilities of various salts of **16** were determined in two dogs; lactate and acetate salts were 68% and 94% adsorbed, indicating that solubility was an important parameter in achieving good oral absorption for that compound.

2. Disposition Studies in Rats. To aid in the design and interpretation of in vivo efficacy model studies in the rat, the bioavailability and plasma elimination rates of candidate compounds were estimated. Compounds **1**, **16**, **32**, and **37** were administered subcutaneously at a dose of 25 mg/kg. The mean plasma $t_{1/2}$ values were as follows: **1**, 1.2 h; **16**, 0.84 h; **32**, 0.53 h; and **37**, 0.84 h. As a relative assessment of bioavailability, AUC_{0-3h} values (μg/mL × h) were calculated: **1**, 4.99; **16**, 2.78; **32**, 1.98; and **37**, 3.03.

3. Efficacy. Compounds **16** and **32** were tested for efficacy in a dual-isolate intraabdominal sepsis model in male Wistar rats.[18,19] Animals were challenged with intraperitoneal insertion of gelatin capsules containing a standardized inoculum of *E. coli* and *Bacteroides fragilis*. Antimicrobial therapy was started 4 h after implantation with control drugs or compounds, which were administered by intramuscular injection at 8-h intervals for 10 days. Challenged, untreated animals showed a biphasic disease. Initially, there was an acute peritonitis phase with *E. coli* bacteremia and high (40–50%) mortality. Rats that survived this stage went on to develop multiple intraabdominal abscesses in which *B. fragilis* was the predominant microorganism. Compound **16** and **32** were tested in this infection model basically because both compounds were reasonably active against *E. coli* (in vitro MIC's against six isolates ranged from 0.1 to 1.0 μg/mL; see Table II, where the numbers are shown as ratios to 1) as well as superior activity against *B. fragilis* (Table III). Compared to the untreated controls, compounds **32** and **16** (at doses of 8 mg/injection) reduced abscess formation 62% and 35%, respectively. Compound **1** and metronidazole at this dose level reduced abscess formation 35% and 73%, respectively. Among the test compounds, there appeared to be no clear-cut correlation between in vitro MIC's against *B. fragilis* and the reduction of abscesses. For example, compounds **1** and **16** performed equally well in vivo, and yet compound **16** was about 4-fold more active against *B. fragilis* in vitro (Table III). Compound **32** was the most active in terms of reducing the incidence of abscess formation, and yet was virtually indistinguishable from **16** with respect to in vitro activity against *B. fragilis*. Clearly, factors such as tissue penetration and metabolism contribute signficantly to the performance of drugs or drug candidates in animal infection models. These factors have not been well-characterized for these particular types of compounds.

Conclusions

Results showing the greatest promise for a candidate compound with broadened spectrum of therapeutic activity lay among the data on anaerobic organisms. These bacterial often occur in wound abscesses and are difficult to treat, since, for one reason, it is hard to reach the site.

A pharmacokinetic profile in animals which is similar to that of **1** was considered a reasonable criteria for selection of compounds for more extensive investigation. This included studies that might provide an indication of the duration of activity and the potential of a candidate compound to reach the target site.

Results obtained in the dog showed that all of the four compounds studied (**11**, **15**, **16**, and **37**) had shorter half-lives than **1**, although **37**, a compound very similar to **1**, had a reasonably long half-life, as well as good oral bioavailability. In preliminary studies, it had been found that some dialkenyl derivatives, particularly **8**, were too insoluble to be strong candidates for further consideration.

Efficacy studies in an abdominal sepsis model in rats with two of the compounds proved disappointing, very likely due to pharmacokinetic properties which were inferior to the standard **1**. Although studies against anaerobes were not carried out with **37**, it was concluded that this compound was too similar to trimethoprim to provide any significant advantage as a broad-spectrum antibacterial; furthermore, its activity against *E. coli* in vitro was not compelling.

This series of compounds was then not considered practical for further examination in the search for a DHFR inhibitor with extended antibacterial activity. It is clear that the properties of various bacterial DHFRs differ sufficiently from each other that such a utopia may not be found. This holds as well for the problem of crossing cell boundaries, which differs considerably among bacterial species. However, it does seem possible that compounds such as those described here might be developed successfully for the treatment of specific infections. The disposition and toxicological properties would of course require further examination, but the problem of development of appropriate formulations to achieve drug delivery to appropriate sites should not prove insurmountable.

Experimental Section

A. Chemistry. Melting points were determined with a Thomas-Hoover apparatus and are uncorrected. Where analyses are indicated only by symbols of the elements, results obtained

(18) Bartlett, J. G.; Onderdonk, A. B.; Louie, T. J.; Kasper, D. L.; Gorbach, S. L. *Arch. Surg.* **1978**, *113*, 853.
(19) Bartlett, J. G.; Louie, T. J.; Gorbach, S. L.; Onderdonk, A. B. *Rev. Infect. Dis.* 1981, *3*, 535.

were within ±0.4% of the theoretical values. Nuclear magnetic resonance (NMR) spectra were recorded on Varian XL-100 and T-60 or Bruker WM 250 spectrometers; chemical shifts are reported in parts per million (δ) from internal tetramethylsilane. Mass spectra were determined on a Varian MAT 731 instrument. Ultraviolet spectra were recorded on a Cary 118 spectrophotometer. Thin-layer chromatography was carried out on silica gel, with $CHCl_3$/MeOH, CH_2Cl_2/MeOH, $CHCl_3$/EtOH/NH_3, or EtOAc/MeOH mixtures as solvents. Column chromatographic separations were carried out on silica gel, with $CHCl_3$/MeOH or CH_2Cl_2/MeOH mixtures. Yields quoted refer to products that were chromatographically homogeneous, except in reactions giving rise to isomeric products, in which case figures correspond to the yield of mixed isomers or to amounts separated after chromatography, as stated. Phenols used as starting materials were commercial samples. The biological assays were carried out by methods described in parts 3 and 5 of this series.[19,20]

2,4-Diamino-5-[4-(allyloxy)benzyl]pyrimidine (3). 2,4-Diamino-5-(4-hydroxybenzyl)pyrimidine (**2**)[21] was treated with allyl bromide according to the method of Roth et al.[22] to produce **3** as white crystals, mp 169–170 °C (CH_2Cl_2). Anal. ($C_{14}H_{16}N_4O$) C, H, N.

2,4-Diamino-5-(3-allyl-4-hydroxybenzyl)pyrimidine (4). Compound **3** (6.1 g) was mixed with 10 mL of *N,N*-diethylaniline and heated at 200 °C for 2.5 h. Crystals separated, which were filtered, washed with ether, and recrystallized from EtOH: mp 189–190.5 °C (75%); NMR (Me_2SO-d_6) δ 3.24 (d, 2, CH_2CH, J = 7.0 Hz), 3.47 (s, 2, benzylic CH_2), ca. 5.0 (m, 2, CH_2=), 5.75 (br s, 2, NH_2), ca. 5.9 (m, 1, CH=), 6.06 (br s, 2, NH_2), 6.70 (d, 1, J = 8.0 Hz, arom H), 6.86 (dd, 1, J = 8, 1 Hz, arom H), 6.90 (d, 1, J = 1 Hz, arom H), 7.44 (s, 1, pyrimidine C_6-H), 9.15 (br s, 1, OH). Anal. ($C_{14}H_{16}N_4O$) C, H, N.

2,4-Diamino-5-[3-allyl-4-(allyloxy)benzyl]pyrimidine (5). Compound **4** was alkylated with allyl bromide as for **3**, which produced **5**: mp 126–128 °C (prep LC, Me_2CO); NMR (Me_2SO-d_6) δ 3.29 (d, 2, CH_2CH, J = 6.4 Hz), 3.5 (s, benzylic CH_2), 4.47 (m, 2, OCH_2CH), ca. 4.96 (m, 2, CH_2=), ca. 5.34 (m, 2, OCH_2CH=CH_2), 5.57 (br s, 2, NH_2), ca. 5.9 (m, 2, OCH_2CH= and CH_2CH=), 5.9 (br s, 2, NH_2), 6.92 (m, 3, arom H), 7.44 (s, 1, pyrimidine C_6-H). Anal. ($C_{17}H_{20}N_4O$) C, H, N.

2,4-Diamino-5-(3,5-diallyl-4-hydroxybenzyl)pyrimidine (6). The Claisen rearrangement of **5** was carried out at 220 °C (2.5 h) in *N,N*-diethylaniline. The product (**6**) (75%) melted at 196–197.5 °C (EtOH): NMR (Me_2SO-d_6) δ 3.29 (2d, 4, (CH_2CH=)$_2$, J = 6.25 Hz), 3.47 (s, 2, benzylic CH_2), 5.03 (m, 4, (CH_2=)$_2$), 5.62 (br s, 2, NH_2), ca. 5.95 (m, 2, (CH=)$_2$), 5.96 (br s, 2, NH_2), 6.76 (s, 2, arom), 7.39 (s, 1, pyrimidine C_6-H), 8.05 (s, 1, OH). Anal. ($C_{17}H_{20}N_4O$) C, H, N.

2,4-Diamino-5-(3,5-diallyl-4-methoxybenzyl)pyrimidine (7). A mixture of 1.68 g (0.006 mol) of **6**, 0.24 g (0.006 mol) of NaOH in 30 mL of H_2O, 30 mL of CH_2Cl_2, 1.88 g of benzyltributylammonium chloride, and 0.94 g (0.0066 mol) of MeI was stirred at room temperature for 1 h. The layers were separated, the aqueous layer was washed well with Et_2O, and the combined organic layers were evaporated and triturated with Et_2O, which produced a semisolid. This was extracted with alkali, which did not serve to remove all residual phenol, so the product was purified by flash chromatography (silica gel, 400–230 mesh, Me_2CO). The resultant white solid melted at 121–123 °C: NMR (Me_2SO-d_6) δ 3.34 (2d, 4, (CH_2CH=)$_2$, J = 5.62 Hz), 3.51 (s, 2, benzylic CH_2), 3.62 (s, 3, OCH_3), ca. 5.0 (m, 4, (CH_2=)$_2$), 5.66 (br, s, 2, NH_2), ca. 5.92 (m, 2, (CH=)$_2$), 6.04 (br s, 2, NH_2), 6.90 (s, 2, arom), 7.43 (s, 1, pyrimidine C_6-H).

2,4-Diamino-5-[3,5-bis(1-propenyl)-4-methoxybenzyl]pyrimidine (8). To a solution of 1.06 g (0.0034 mol) of **7** in 10 mL of Me_2SO was added 0.2 g (0.0017 mol) of *t*-BuOK. The mixture was heated with stirring to 70 °C and held there for 15 min, after which it was allowed to stand at room temperature over a weekend, and then diluted with several volumes of water. The resultant precipitate was isolated and recrystallized, first from dilute AcOH and then from MeOH, which produced white crystals of **8**: mp 235–237 °C; NMR (Me_2SO-d_6) δ 1.87 (dd, 6, $(CH_3)_2$, J_{AC} = 1.0 Hz, J_{BC} = 5.2 Hz (CH_A=CH_BCH_{3C})), 3.53 (s, 2, benzylic CH_2), 3.58 (s, 3, OCH_3) 5.67 (br s, 2, NH_2), ca. 6.0 (dq, 2, J_{BC} = 5.2 Hz, J_{AB} = 15.1 Hz), 6.06 (br s, 2, NH_2), ca. 6.59 (dq, 2, J_{AC} = 1.0 Hz, J_{AB} = 15.1 Hz), 7.24 (s, 2, arom), 7.55 (s, 1, pyrimidine C_6-H). Anal. ($C_{18}H_{22}N_4O$) C, H, N.

2,4-Diamino-5-(3-allyl-4-hydroxy-5-methoxybenzyl)pyrimidine (11). Method A: From 9[23] and 10.[8] The method described for **32**, including the column purification, was used to obtain **11**: mp 193–194 °C (3.1%); UV (0.1 N HCl) λ_{max} 275 (ϵ 7250), 215 (sh) nm (34 400); (pH 7, phosphate) λ_{max} 281 nm (ϵ 7800); (pH 8.9, tris) λ_{max} 285 (ϵ 9450), 230 nm (18 700); (0.1 N NaOH) λ_{max} 290 nm (ϵ 11 300). Anal. ($C_{15}H_{18}N_4O_2$) C, H, N.

Method B: From 13. To 2.6 g (0.0099 mol) of 2,4-diamino-5-[3-methoxy-4-(allyloxy)benzyl]pyrimidine (**13**) was added 3 mL of *N,N*-diethylaniline. The mixture was heated under N_2 at 186–190 °C for 4.5 h. The resultant tan solid was slurried in absolute Et_2O, filtered, and recrystallized twice from absolute EtOH to yield 1.23 g (43%) of **11**: mp 194.5–195 °C; NMR (Me_2SO-d_6) δ 3.24 (d, 2, CH_2CH), J = 6.8 Hz), 3.46 (s, 2, benzylic CH_2), 3.74 (s, 3, OCH_3), ca. 5.05 (m, 2, CH_2=), 5.67 (br s, 2, NH_2), ca. 5.9 (m, 1, CH=), 6.02 (br s, 2, NH_2), 6.49 (d, 1, J = 1.9 Hz, arom H), 6.70 (d, 1, J = 1.9 Hz, arom H), 7.43 (s, 1, pyrimidine C_6-H), 8.27 (s, 1, OH). Anal. ($C_{15}H_{18}N_4O_2$) C, H, N.

2,4-Diamino-5-[3-methoxy-4-(allyloxy)benzyl]pyrimidine (13). To a mixture of 19.39 g (0.0787 mol) of **12**[1] in 580 mL of Me_2SO was added 8.85 g (0.0787 mol) of *t*-BuOK in 95 mL of *t*-BuOH and then 9.52 g (0.0787 mol) of allyl bromide. The mixture was stirred at room temperature overnight and then evaporated to a brown oil, which was triturated with water until it had solidified, followed by washing well with Et_2O. Upon recrystallization from MeOH, there was obtained 12.1 g (54%) of **13**, mp 151.5–155 °C. Anal. ($C_{15}H_{18}N_4O_2$) C, H, N.

2,4-Diamino-5-[4-hydroxy-3-methoxy-5-(2-hydroxypropyl)benzyl]pyrimidine (14). A mixture of 5 g of **11** and 10 mL of TFA was heated at 90 °C for 1 h and then allowed to stand at room temperature for 72 h. The clear solution was poured on ice and neutralized with NH_4OH. A precipitate separated (1.69 g), which was shown by TLC to contain at least four components. This was purified by flash column chromatography, followed by recrystallization from EtOH: mp 196–197 °C (**14**); ^{13}C NMR (Me_2SO-d_6) δ 22.97 (q, $CH_2CHOHCH_3$), 32.30 (t, benzylic CH_2), 40.09 (t, $CH_2CHOHCH_3$), 55.64 (q, OCH_3), 66.43 (d, $CH_2CHOHCH_3$), 106.79 (s, pyrimidine C_5), 110.37 (d, benzene C_2), 123.05 (d, benzene C_6), 125.92 (s, benzene C_3), 129.28 (s, benzene C_1), 142.59 (s, benzene C_4), 147.32 (s, benzene C_5), 153.23 (d, pyrimidine C_6), 161.01, 162.50 (pyrimidine C_2 and C_4); MS m/e 304. Anal. ($C_{15}H_{20}N_4O_3$) C, H, N.

2,4-Diamino-5-(3,4-dimethoxy-5-allylbenzyl)pyrimidine (15). To 0.276 g (0.0115 mol) of NaH (50% oil dispersion) in 5 mL of DMF at 0 °C was added dropwise 2.86 g (0.01 mol) of **11** in 10 mL of DMF. The mixture was stirred for 25 min. Methyl iodide (1.63 g, 0.0115 mol) in 5 mL of DMF was then added dropwise. A thick slurry formed, which was stirred at room temperature for 2 h. Water was added, which dissolved the solid. The pH was adjusted to 8.5, and the solution was extracted with 2 × 100 mL of CH_2Cl_2. The organic layers were combined, dried, and concentrated to a yellow oil, which was purified by column chromatography, with silica gel and 3% MeOH/CH_2Cl_2 to give 0.63 g (21%) of **15**, mp 154–155 °C. Anal. ($C_{16}H_{20}N_4O_2$) C, H, N.

2,4-Diamino-5-[3,4-dimethoxy-5-(1-propenyl)benzyl]pyrimidine Hydrochloride (16). Compound **15** (0.63 g, 0.002 mol) was dissolved in 20 mL of Me_2SO and heated in an oil bath to 70 °C. Approximately 0.05 g of *t*-BuOK was added to the clear yellow solution, which was then heated for an additional hour. After cooling, the mixture was worked up as for **15**. The resultant oil was converted to the hydrochloride salt (0.055 g, 8.2%): mp 269–270 °C (absolute EtOH); NMR (Me_2SO-d_6) δ 1.86 (dd, 3, CH_3CH=, J_{BC} = 6.0 Hz; J_{AC} = 1.0 Hz, CH_A=CH_BCH_{3C}), 3.61 (s, 2, benzylic CH_2), 3.66 (s, 3, OCH_3), 3.77 (s, 3, OCH_3), 6.2 (dq, 1, J_{BC} = 6.0 Hz, J_{AB} = 15.0 Hz), 6.6 (dq, 1, J_{AC} = ca. 1 Hz, J_{AB}

(20) Rauckman, B. S.; Roth, B. *J. Med. Chem.* **1980**, *23*, 384.
(21) Cresswell, R. M.; Mentha, J. W.; Seaman, R. Ger. Offen. 2,065,367, 1966; *Chem. Abstr.* **1973**, *79*, 42545*t*.
(22) Roth, B.; Aig, E.; Lane, K.; Rauckman, B. S. *J. Med. Chem.* **1980**, *23*, 535.
(23) Claisen, L. *Chem. Ber.* **1912**, *45*, 3157.

= 15.0 Hz), 6.87 (d, 1, arom, J = 1 Hz), 7.01 (d, 1, aromatic, J = 1 Hz), 7.51 (s, 1, pyrimidine C_6-H), 7.60 (br s, 2, NH_2), 8.0 (br s, 2, NH_2). Anal. ($C_{16}H_{20}N_4O_2$·HCl) C, H, N.

2,4-Diamino-5-(3,4-dimethoxy-5-*n*-propylbenzyl)pyrimidine (17). Compound **15** (0.5 g, 0.0016 mol) in 100 mL of EtOH was reduced with Pd/5% C on a Parr shaker. The product (**17**) melted at 155–156 °C (EtOH): MS m/e 302; NMR (Me_2SO-d_6) δ 0.9 (t, 3, CH_3, J = 7.3 Hz), 1.44 (q, 2, CH_2, J = 7.3 Hz), 2.54 (t, 2, CH_2, J = 7.3 Hz), 3.50 (s, 2, benzylic CH_2), 3.65 (s, 3, OCH_3), 3.73 (s, 3, OCH_3), 5.64 (br s, 2, NH_2), 6.02 (br s, 2, NH_2), 6.58 (d, 1, aromatic, J = 1.9 Hz), 6.75 (d, 1, arom, J = 1.9 Hz), 7.48 (s, 1, pyrimidine C_6-H). Anal. ($C_{16}H_{22}N_4O_2$) C, H, N.

2-Allyl-6-*n*-propylphenol (25). A mixture of 136 g (1 mol) of *o*-(*n*-propyl)phenol (**18**), 121 g (1 mol) of allyl bromide, 140 g of anhydrous K_2CO_3, and 200 mL of acetone was heated under reflux for 8 h, after which it was cooled to room temperature. Water (900 mL) was added until all of the solid had dissolved, followed by petroleum ether to form two clear immiscible layers. The aqueous layer was discarded, and the ether layer was extracted three times with 350-mL portions of 2 N NaOH to remove residual *o*-(*n*-propyl)phenol, followed by drying and removal of the solvent. The crude **allyl *o*-(*n*-propyl)phenyl ether (21)** (114.5 g, 64.5%) was used directly in the next reaction. A 60-g portion was mixed with 26 g of *N*,*N*-diethylaniline and heated under N_2 to 240 °C for 3.5 **h**, after which it was cooled and dissolved in petroleum ether. The solution was extracted with dilute sulfuric acid to remove diethylaniline, followed by extraction with NaOH. The alkaline extract was neutralized and extracted with petroleum ether, followed by removal of the solvent. The crude 2-allyl-6-*n*-propylphenol (**25**) (53 g) was vacuum distilled twice using a short column; bp 58–59 °C (2 mm). This was shown to be essentially pure by VPC. Anal. ($C_{12}H_{16}O$) C, H.

2,4-Diamino-5-[(2-methyl-7-*n*-propyl-2,3-dihydrobenzofuran-5-yl)methyl]pyrimidine Hydrochloride (29). A mixture of 5.6 g (0.04 mol) of **10**, 7.8 g (0.044 mol) of **25**, 400 mL of glacial AcOH, and 12 mL (0.144 mol) of concentrated hydrochloric acid was heated on the steam bath for 6 h, after which the solvent was removed in vacuo. The residue was treated with a mixture of acetone/ether, and 10.5 g of insoluble solid was isolated. This was purified by recrystallization from water and then EtOH, followed by conversion to the free base with excess NaOH to remove possible uncyclized material. Reconversion to the hydrochloride yielded 3.45 g (26%) of **29**, which melted at 226–228 °C (absolute EtOH). This compound had a single pK_a value, between pH 1 and 14, of 7.32, indicating that it was not a phenol: UV (cation, 0.1 N HCl) λ_{max} 276 (ϵ 6700), 222 (sh) (28 700), 203 nm (19 200); (pH 7 phosphate) λ_{max} 283 (ϵ 7700), 233 (sh) (18 400), 201.5 nm (19 100); (pH 9 tris and pH 13 0.1 N NaOH) λ_{max} 288 (ϵ 9800), 237 (sh) nm (15 200); NMR (Me_2SO-d_6) δ 0.88 (t, 3, J = 7.25 Hz, $CH_2CH_2CH_3$), 1.36 (d, 3, J = 6.2 Hz, $CHCH_3$), 1.61 (hex., 2, J = 7.25 Hz, $CH_2CH_2CH_3$), 2.43 (t, 2, J = 7.25 Hz, $CH_2CH_2CH_3$), 2.73, 3.26 (2q, 1 each, J_{gem} = 15.5 Hz, J_{CH_2CH} = 7.8, 9.1 Hz), 3.56 (s, 2, benzylic CH_2), 4.87 (m, 1, methine, J = 9.1, 7.8, 6.2 Hz), 6.84, 6.89 (2d, 1 each, J = 2 Hz, $C_{2,6}$ arom H), 7.39 (s, 1, pyrimidine C_6-H), 7.55 (br s, 2, NH_2), 7.4–8.4 (vbr, 2, NH_2), 11.92 (br s, 1, 1-NH^+). Anal. ($C_{17}H_{22}N_4O$·HCl) C, H, N.

2,4-Diamino-5-[(2-methyl-7-ethyl-2,3-dihydrobenzofuran-5-yl)methyl]pyrimidine Hydrochloride (28). The route described for **29** was used. *o*-Ethylphenol (**18**) was converted to its allyl ether (**20**) as described for **21**. This product was heated in diethylaniline as described for **25** to provide **2-ethyl-6-allylphenol (24)**, which boiled at 44–45 °C (50 μM). The condensation of **24** with **10** was carried out as for **29**. The product (**28**) was obtained as the hydrochloride: mp 279–281 °C (absolute EtOH); UV (cation, 0.1 N HCl) λ_{max} 275 nm (ϵ 6500); (neutral species, 0.1 N NaOH) λ_{max} 287 (ϵ 9250), 236 (sh) nm (16 700). Anal. ($C_{16}H_{20}N_4O$·HCl) C, H, N.

2-(*n*-Propyl)phenyl Crotyl Ether (22). *o*-*n*-Propylphenol (**19**) was treated with crotyl chloride by the method used for **21** to produce **22** as an oil (51%). Anal. ($C_{13}H_{18}O$) C, H.

2-*n*-Propyl-6-(1-methylallyl)phenol (26). Compound **22** was rearranged to **26** as described for **25**. The resultant oil was purified by fractional distillation using a spinning band column; bp 39 °C (1 mm). Anal. ($C_{13}H_{18}O$) C, H.

2,4-Diamino-5-[(2,3-dimethyl-7-*n*-propyl-2,3-dihydrobenzofuran-5-yl)methyl]pyrimidine Hydrochloride (30). Compound **26** was condensed with **10** as described for **29** to produce **30** as the hydrochloride, 10% yield, mp 257–259 °C (MeOH). This product consisted of a 38:62 mixture of cis:trans isomers: NMR (Bruker WM 250 MHz, Me_2SO-d_6) δ 0.872 (t, major $CH_2CH_2CH_3$, J = 7.35 Hz), 0.879 (t, minor $CH_2CH_2CH_3$, J = 7.35 Hz), 1.078 (d, major 3-CH_3, J = 6.99 Hz), 1.231 (d, minor 3-CH_3, J = 6.62 Hz), 1.251 (d, major 2-CH_3, J = 6.62 Hz), 1.396 (d, minor 2-CH_3, J = 6.25 Hz), 1.529 ((6), $CH_2CH_2CH_3$, J = 7.3 Hz), 2.416 (t, $CH_2CH_2CH_3$, J = 7.5 Hz), 2.991 ((5), minor 3-CH, J = 7.7 Hz), 3.39 ((5), major 3-CH), 3.559 (s, benzylic CH_2), 4.264 (dq, minor 2-CH, J = 8.46, 6.25 Hz), 4.816 (dq, major 2-CH), 6.82 and 6.89 (2 and 6 benzene protons), 7.387 (s, pyrimidine C_6-H), 7.558 (br s, 2-NH_2), ca. 7.8, 8.2 (vbr, 4-NH_2), ca. 11.6 (vbr, 1-NH^+).[24] Anal. ($C_{18}H_{24}N_4O$·HCl) C, H, N.

2,4-Diamino-5-[(2,2-dimethyl-7-*n*-propyl-2,3-dihydrobenzofuran-5-yl)methyl]pyrimidine Hydrochloride (31). *o*-*n*-Propylphenol (**19**) was treated with methallyl chloride in a similar manner to that described for **21** to produce the crude methallyl ether (**23**), which was rearranged as for **25** to produce **2-*n*-propyl-6-methallylphenol (27)**, which was partially purified by dissolving in alkali and reprecipitating with acid. Compound **27** was condensed with **10** as described for **29**. This yielded **31** (15%) as the hydrochloride: mp 262–265 °C (MeOH); NMR (Me_2SO-d_6) δ 0.865 (t, 3, $CH_2CH_2CH_3$, J = 7 Hz), 1.382 (s, 6, gem$(CH_3)_2$), 1.48 (m, 2, $CH_2CH_2CH_3$), 2.41 (m, (2), $CH_2CH_2CH_3$), 2.943 (s, 2, $CH_2C(CH_3)_2$), 3.545 (s, 2, benzylic CH_2), 6.826, 6.863 (2 d, 1 each, $C_{2,6}$ benzene protons), 7.391 (s, 1, pyrimidine C_6-H), 7.52 (br s, 2, NH_2), 8.0 (vbr s, NH_2), 11.85 (br s, 1, 1-NH^+). Anal. ($C_{18}H_{24}N_4O$·HCl) C, H, N.

2,4-Diamino-5-(3-allyl-4-hydroxy-5-*n*-propylbenzyl)pyrimidine (32). In an experiment similar to that for **29** except that gaseous HCl was bubbled into glacial AcOH (12 g/400 mL) and water was excluded, a syrupy product was obtained, a portion of which was converted to the free base with $NaHCO_3$. This yielded a yellow solid, which was purified on a silica gel chromatographic column, eluted with $CHCl_3$/MeOH 18:1. A crystalline product (**32**) (0.36 g) was isolated: mp 173–173.5 °C; UV (cation, 0.1 N HCl) λ_{max} 273 (ϵ 6750), 218 (sh) nm (1500); (ca. neutral species, pH 9) λ_{max} 284 (ϵ 8390), 236 (sh) nm (14 000); (anion, 0.1 N NaOH) λ_{max} 291 (ϵ 10 600), 242 (sh) nm (15 200). Anal. ($C_{17}H_{22}N_4O$) C, H, N. Attempts to achieve a separation of products **29** and **32** by extraction with alkali proved unsuccessful, probably as a consequence of the steric hindrance offered by the lipophilic groups flanking the phenolic function. Virtually none of the phenol was extracted into the aqueous layer by this means.

2,4-Diamino-5-(3-allyl-4-methoxy-5-*n*-propylbenzyl)pyrimidine (33). Compound **32** was methylated as previously described[22] to produce **33**; mp 123–126 °C (MeOH). Anal. ($C_{18}H_{24}N_4O$) C, H, N.

2,4-Diamino-5-[3-(1-propenyl)-4-methoxy-5-*n*-propylbenzyl]pyrimidine (34). The rearrangement of **33** was carried out as described for **8**. The product was recrystallized from EtOH; mp 174.5–175 °C. Anal. ($C_{18}H_{24}N_4O$) C, H, N.

2,4-Diamino-5-[3,4-dimethoxy-5-(*n*-propyloxy)benzyl]pyrimidine (35). 2,4-Diamino-5-(3,4-dimethoxy-5-hydroxybenzyl)pyrimidine[25] was alkylated with *n*-propyl iodide by using the method of **3**. After removal of the solvent, the product was extracted with dilute sodium hydroxide to remove residual phenolic material, and recrystallized from EtOH; mp 190–191 °C. Anal. ($C_{16}H_{22}N_4O_3$) C, H, N.

2,4-Diamino-5-[3,4-dimethoxy-5-(allyloxy)benzyl]pyrimidine Hydrochloride (36). 2,4-Diamino-5-(3,4-dimethoxy-5-hydroxybenzyl)pyrimidine[25] was alkylated with allyl bromide by using the method of **3** once more. The product was treated as for **35** and then converted to the hydrochloride salt; mp 234–237 °C (EtOH). Anal. ($C_{16}H_{20}N_4O_3$·HCl) C, H, N.

2,4-Diamino-5-(3,4-dimethoxy-5-ethoxybenzyl)pyrimidine (37). 2,4-Diamino-5-(3,4-dimethoxy-5-hydroxybenzyl)pyrimidine[25] was alkylated with ethyl iodide as for compound **35**. Since good separation from starting material was not effected by NaOH extraction, the product was purified by column chromatography

(24) We are indebted to Prof. Charles G. Moreland, North Carolina State University for this spectrum.

(25) Rey-Bellet, G.; Reiner, R. *Helv. Chim. Acta* **1970**, *53*, 945.

using silica gel and eluting with CH_2Cl_2/MeOH 12:1, followed by crystallization from absolute EtOH; mp 184–186 °C. Anal. ($C_{15}H_{20}N_4O_3$) C, H, N.

B. Enzyme Assays. Dihydrofolate reductase from *S. aureus* (strain S8862) was partially purified by ammonium sulfate fractionation and gel filtration on LKB Ultrogel AcA-54. The preparation was stable and free of interfering NADPH oxidase activity. The standard assay was performed in 0.1 M imidazole chloride buffer pH 6.4, 12 mM mercaptoethanol, 60 μM NADPH, and 45 μM dihydrofolate in a final volume of 1 mL at 30 °C. One enzyme unit is defined as the amount of enzyme needed to reduce 1 μmol of dihydrofolate/min based on an extinction coefficient of 12.3×10^3 M^{-1} cm^{-1} at 340 nm.[26] The concentration of inhibitor necessary to inhibit enzymic activity by 50% (I_{50}) was determined by modification of this assay. The enzyme, NADPH, and varying concentrations of inhibitor were preincubated for 2 min at 30 °C, and the reaction was initiated by the addition of dihydrofolate. Steady-state velocities were measured, and plots of the percentage inhibition vs the logarithm of inhibitor concentration were used to estimate I_{50} values. K_i calculations were performed by using the method of Henderson[9] as described by Baccanari and Joyner.[10] A dihydrofolate K_m value of 5.4 μM (D. Baccanari, unpublished results) was used in the *S. aureus* K_i calculations. Assays for *E. coli*, rat liver, *N. gonorrhoeae*, and *P. berghei* dihydrofolate reductase were performed as previously described.[27,7,28]

C. Disposition Studies. The disposition of several TMP analogues after intravenous (iv) and oral (po) administration to beagle dogs was studied. The dogs were fasted for 18 h before being dosed and were fed a standard ration 5 h after being dosed. For the iv studies, 5 mg/kg (free base/body weight) of each of four analogues (**11**, **15**, **16**, and **37**) was coadministered with 5 mg/kg of **1** dissolved in propylene glycol to two male beagles. Simultaneous administration of **1** with each analogue allowed direct comparison of the pharmacokinetics of each analogue with **1** during the same period of time. Plasma was obtained from blood samples that were obtained by jugular venipuncture prior to dosing and at 0.17, 0.25, 0.5, 1.0, 2.0, 3.0, 4.0, 6.0, 8.0, and 24.0 h after dosing.

For the oral absorption studies, each of five compounds (**11**, **15**, **16**, **32**, and **37**) was administered in a gelatin capsule at a dose of 5 mg/kg (free base/body weight) to two male beagle dogs. In attempts to improve absorption, **16** was also administered in dilute lactic acid solution and as the acetate salt in a gelatin capsule. Plasma was obtained from blood samples that were drawn prior to dosing and at 0.25, 0.5, 1.0, 2.0, 3.0, 4.0, 6.0, 8.0, and 24.0 h after dosing. The plasma was stored at −20 °C until assayed.

Determination of Drug Concentrations in Dog Plasma. The concentrations of the benzylpyrimidines in plasma were measured by making appropriate modifications to the sensitive and specific quantitative TLC methods developed in these laboratories for **1** and related compounds.[29] Compounds **11**, **15**, **16**, and **37** were extracted from the plasma with 5 mL of 2-propanol/methylene chloride (1:19, v/v) after adjustment of the plasma to pH 12 (pH 8 for compound **11**). Compound **32** was extracted from plasma at pH 12 with 5 mL of 2-propanol/methylene chloride (1:4). After the extraction solvents were evaporated, the samples were reconstituted with 100 μL of methanol/chloroform (1:9) and spotted onto silica gel 60 F-254 TLC plates (EM Science, Cherry Hill, NJ) with an electronically driven TLC multispotter (Analytical Specialties, Libertyville, IL). Plates spotted with **15**, **16**, and **37** were developed in toluene/2-propanol/ammonium hydroxide (60:40:1); those with **11** used ethyl acetate/methanol/ammonium hydroxide (80:20:1); those with **32** used chloroform/2-propanol/ammonium hydroxide (80:20:1). The plates were scanned with a Schoeffel SD 3000 spectrodensitometer (Kratos Schoeffel Instruments, Westwood, NJ) set to measure fluorescence. Peak areas from the samples and standards were recorded on a Honeywell Electronik 124 recorder and integrated with an Autolab System IV integrator.

Pharmacokinetic parameters were estimated from plasma concentrations of the benzylpyrimidines after iv administration. For those compounds that had measurable plasma concentrations following po administration, pharmacokinetic data were also determined. The half-life of each analogue was calculated using a least-squares linear-regression analysis of the postabsorption segment of the plasma concentration–time curve. AUC was calculated by applying the trapezoidal rule.[30] The bioavailability (F) after a po dose was estimated as the ratio of the AUC values obtained after iv and po dosing.

Pharmacokinetic Studies in Rats. The pharmacokinetics of **1**, **16**, **32**, and **37** were studied in rats. For each compound, 21 male Wistar rats, weighing 150–200 g, were divided into groups of three and housed in standard cages. After being fasted for 18 h, they were dosed subcutaneously with each analogue at a dose of 25 mg/kg dissolved in water/polyethylene glycol (3:7). The groups were sacrificed at 0.25, 0.5, 1.0, 1.5, 2.0, 3.0, and 4.0 h after the dose. Plasma was obtained from blood that was collected from the hepatic portal vein. The plasma was stored at −20 °C until assayed.

The drug concentrations in the rat plasma were assayed as described above. The half-life of each compound was calculated with a least-squares linear-regression analysis of the postabsorption segment of the plasma concentration–time curve. The AUC of each compound was calculated by applying the trapezoidal rule.

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(26) Hillcoat, B. L.; Nixon, P. F.; Blakely, R. L. *Anal. Biochem.* **1967**, *21*, 178.
(27) Kuyper, L. F.; Roth, B.; Baccanari, D. P.; Ferone, R.; Beddell, C. R.; Champness, J. N.; Stammers, D. K.; Dann, J. G.; Norrington, F. E.; Baker, D. J.; Goodford, P. J. *J. Med. Chem.* **1985**, *28*, 303.
(28) Ferone, R.; Burchall, J. J.; Hitchings, G. H. *Mol. Pharmacol.* **1969**, *5*, 49.
(29) DeAngelis, R. L.; Sigel, C. W. In *Densitometry in Thin Layer Chromatography*; Touchstone, J. C., Sherma, J. A., Eds.; Wiley: New York, 1979; pp 251–273.
(30) Gibaldi, M.; Perrier, D. In *Pharmacokinetics, Drugs, and the Pharmaceutical Sciences. I*; Marcel Dekker: New York, 1975; pp 293–296.