2,4-Diamino-5-benzylpyrimidines and Analogues as Antibacterial Agents. 6. A One-Step Synthesis of New Trimethoprim Derivatives and Activity Analysis by Molecular Modeling[1]

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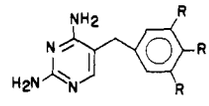
Received August 2, 1982

A new route to 2,4-diamino-5-(4-hydroxybenzyl)pyrimidines has been developed that involves the condensation of 2,4-diamino-5-(hydroxymethyl)pyrimidine with phenols in acidic medium. The use of phenol and its 2,6-dialkyl derivatives produces 5-(4-hydroxybenzyl)pyrimidines exclusively. However, 2,6-dimethoxyphenol produces a mixture of 5-(3-hydroxy-2,4-dimethoxybenzyl)- and 5-(4-hydroxy-3,5-dimethoxybenzyl)pyrimidines. The phenolic condensation has been used to prepare a series of alkyl-substituted 5-(4-hydroxybenzyl)- and 5-(4-alkoxybenzyl)pyrimidines. The use of 1,2,3-trimethoxybenzene in place of a phenol produces 2,4-diamino-5-(2,3,4-trimethoxybenzyl)pyrimidine, a trimethoprim isomer with low antibacterial activity. The use of molecular models of several of the new ortho-substituted derivatives in the active site of dihydrofolate reductase has provided a rational explanation for their activities relative to trimethoprim.

A new synthetic route to trimethoprim (TMP, 1), a



1 R = OCH_3 (TRIMETHOPRIM)

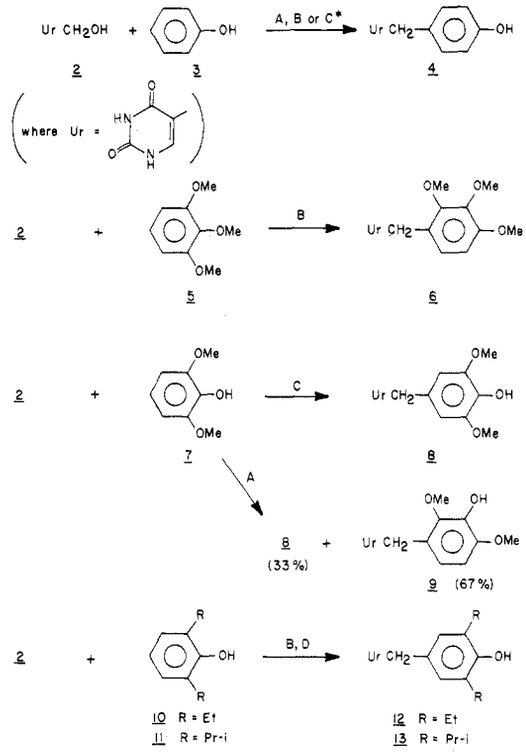
broad-spectrum antibacterial agent,[2] which involved the condensation of a phenolic Mannich base with 2,4-diaminopyrimidine, was described in part 2 of this series.[3] A subsequent paper (part 4) described a modification that permitted condensations with the less reactive 2,6-dialkylphenols but which required an additional step, the dethiation of 6-(methylthio)pyrimidine condensation products.[4]

This paper describes a new route to such analogues, which involves the one-step condensation of 2,4-diamino-5-(hydroxymethyl)pyrimidine with phenols in acid medium. One stimulus for this study originated in a brief report by Brossmer,[5] which describes a high-yield synthesis of certain 5-benzyluracils by the condensation of 5-(hydroxymethyl)uracil (2) with molten phenols in the presence of mineral acid. A related condensation has also been described, which involves the reaction of *N*-acetyl-5-(bromomethyl)uracil with alkoxybenzenes in the presence of a Lewis acid.[6]

5-(Hydroxymethyl)uracil is readily available from uracil plus formaldehyde.[7] However, 5-(hydroxymethyl)-2,4-diaminopyrimidine, a much more attractive intermediate for our needs, has also been reported to be easily prepared by the two-step reduction of 5-cyano-2,4-diaminopyrimidine.[8,9] This in turn can be obtained by condensing ethoxymethylenemalononitrile with guanidine.[10] With this background, the procedures described here were developed.

Chemistry. A number of exploratory condensations with four solvent systems were conducted with 5-(hydroxymethyl)uracil (2), as shown in Scheme I. Although the Brossmer reaction with phenol proceeded best under that author's conditions (solvent A) and did indeed produce a single product, a satisfactory result was also obtained by heating equimolar amounts of 2 and 3 in 0.1 N HCl. Alkaline catalysis was much less satisfactory (~20% yield of 4). 1,2,3-Trimethoxybenzene condensed smoothly

Scheme I



with 2 in acid but produced none of the desired 3,4,5-trimethoxybenzyl isomer. This result is to be expected,

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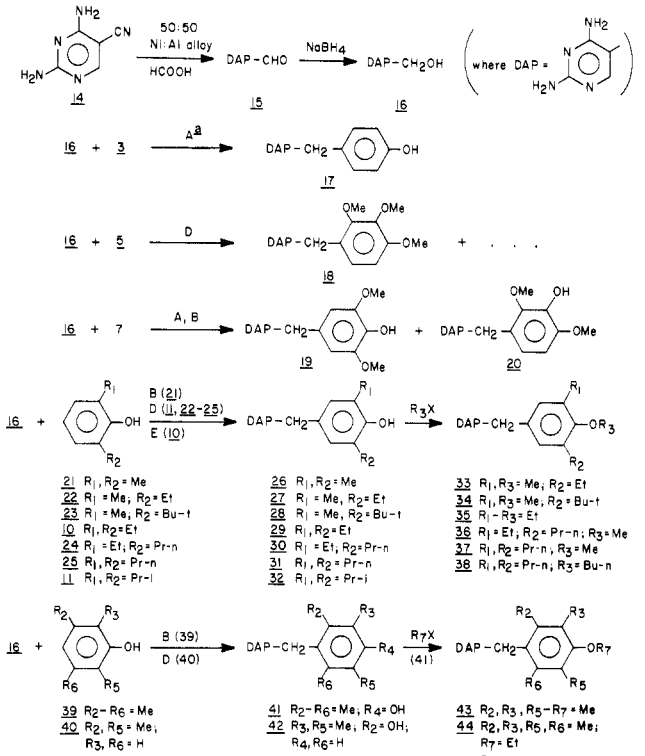
Scheme II



[a] For solvents A–D, see Scheme I; E = *p*-toluenesulfonic acid.

due to the predominant ortho, para directing influence of the methoxy substituents; a similar finding was reported earlier.[6] 2,6-Dimethoxyphenol gave a mixture of the 3,4,5- and 2,3,4-substituted benzyl isomers, **8** and **9**, with the latter in predominance under acidic conditions. However, in alkali the phenoxide had the dominating directive influence, and **8** only was produced, but in low yield.

In contrast to **7**, the 2,6-dialkylphenols did not condense with **2** under the Brossmer conditions (solvent A). However, use of solvent B with 2,6-diethylphenol (**10**) gave an 82% yield of **12** as the sole product. The corresponding 2,6-diisopropylphenol (**11**) afforded the analogous product (**13**) in only 22% yield, however, and 2,6-di-*tert*-butylphenol produced a barely discernible reaction, as observed by TLC. This decrease in yield with increasing ortho bulk can be attributed to inhibition of resonance between the phenolic group and the ring. Solvent D, tried in two cases, gave better yields of **13** from **11**. This proved to be the solvent of choice for reactions with **16** (Scheme II), because of improved solubility of the reactants.

Conversion of **14** to **15** (Scheme II) gave erratic results in our hands using published procedures.[10,8] However, use of nickel–aluminum alloy in 75% formic acid according to van Es and Staskum[11] gave consistent 80% reduction to **15**. Borohydride reduction of **15** to **16** by the method of Tieckelman et al.[8] was very satisfactory.

Scheme II outlines the condensations of **16** with the phenols and alkoxy derivatives that were investigated. Yields are shown in Table I, which lists the products obtained and their physical properties. Yields were generally lower than those obtained in the uracil series, presumably due to the decreased stability of the derived carbonium ion.

Table I. 2,4-Diamino-5-(substituted-benzyl)pyrimidines from Condensations with 16

no.	benzene substituents 2	3	4	5	6	mp, °C	reaction solvent[a]	recrystn solvent[b]	yield,[c] %	emp formula	anal.
17[d]			OH			298–300	A	A	55	*d*	
18[e,f]	OCH_3	OCH_3	OCH_3			224.5–226	D	B	19	$C_{14}H_{18}N_4O_3$	*f*
19		OCH_3	OH	OCH_3			A	A	25[g]	$C_{13}H_{16}N_4O_3 \cdot HCl$	*h*
20	OCH_3	OH	OCH_3			246–248	B	A	33[i]	$C_{13}H_{16}N_4O_3 \cdot HCl \cdot H_2O$	C, H, N, Cl
26		CH_3	OH	CH_3		280	B	C	50	$C_{13}H_{16}N_4O \cdot HCl$[j]	C, H, N, Cl
27		CH_3	OH	C_2H_5		278–281 dec	D	A	27	$C_{14}H_{18}N_4O \cdot HCl$	C, H, N
28		CH_3	OH	*t*-C_4H_9		290–294 dec	D	A	29	$C_{16}H_{22}N_4O \cdot HCl$	C, H, N
29		C_2H_5	OH	C_2H_5		277–279	D	A	52	$C_{15}H_{20}N_4O \cdot HCl$[k]	C, H, N, Cl
							E		66		
30		C_2H_5	OH	*n*-C_3H_7		266–269	D	A	43	$C_{16}H_{22}N_4O \cdot HCl$	C, H, N
31		*n*-C_3H_7	OH	*n*-C_3H_7		268–270 dec	D	A	30	$C_{17}H_{24}N_4O \cdot HCl$	C, H, N
32		*i*-C_3H_7	OH	*i*-C_3H_7		244–246	D	B	20	$C_{17}H_{24}N_4O$[l]	C, H, N
41	CH_3	CH_3	OH	CH_3	CH_3	330	B	A	45	$C_{15}H_{20}N_4O \cdot HCl$[m]	C, H, N
42	OH	CH_3		CH_3		279–281	D		21	$C_{13}H_{16}N_4O \cdot HCl$[n]	H, N, Cl; C[o]
49	OH	$CH_2CH{=}CH_2$		CH_3		203–204	D	D	9	$C_{15}H_{18}N_4O$	H, N; C[p]

[a] A = the phenol; B = 0.1 N HCl; C = 0.1 N NaOH; D = AcOH–HCl; E = *p*-toluenesulfonic acid. [b] A = H_2O; B = EtOH; C = precipitated with $NaHCO_3$; D = dilute EtOH. [c] Yield of purified product. [d] See ref 4. [e] See ref 6. [f] Analysis given under method B for **18**. [g] Total yield of **19** and **20**; see Experimental Section, reaction of **16** with **5**, method A. [h] See ref 7. [i] Total yield of **19** and **20**; see Experimental Section, reaction of **16** with **5**, method B. [j] NMR (TFA) δ 2.3 (s, 6, CH_3), 3.83 (s, 2, benzylic CH_2), 6.95 (s, 2, aromatic), 7.8 (s, 1, pyrimidine C_6 H). [k] NMR (TFA) δ 1.25 [t, 6, *J* = 8.0 Hz, $(CH_2CH_3)_2$], 2.7 [q, 4, *J* = 8.0 Hz, $(CH_2CH_3)_2$], 3.84 (s, 2, benzylic CH_2), 6.98 (s, 2, aromatic), 7.65 (s, 1, pyrimidine C_6 H). [l] NMR (TFA) δ 1.25 [d, 12, *J* = 7.0 Hz, $[CH(CH_3)_2]_2$], 3.28 [m, 2, $[CH(CH_3)_2]_2$], 3.86 (s, 2, benzylic CH_2), 7.05 (s, 2, aromatic), 7.65 (s, 1, pyrimidine C_6 H). [m] NMR (TFA) δ 2.18, 2.3 (s, 12, Me groups), 3.89 (s, 2, benzylic CH_2), 7.1 (s, 1, pyrimidine C_6 H). [n] NMR (TFA) δ 2.25, 2.28 (s, 6, CH_3 groups), 3.9 (s, 2, benzylic CH_2), 6.9, 7.05 (s, 2, aromatic H), 8.03 (s, 1, pyrimidine C_6 H). [o] C: calcd, 55.62; found, 54.24. [p] C: calcd, 66.65; found, 66.0.

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Table II. 2,4-Diamino-5-(alkoxybenzyl)pyrimidines

no.	benzene substituents 2	3	4	5	6	mp, °C	recrystn solvent[a]	emp formula	anal.
33		CH_3	OCH_3	C_2H_5		279-282	A	$C_{15}H_{20}N_4O\cdot HCl$	C, H, N
34		CH_3	OCH_3	t-C_4H_9		257-258	B	$C_{17}H_{24}N_4O\cdot HCl$	C, H, N
35		C_2H_5	OC_2H_5	C_2H_5		166-167	C	$C_{17}H_{24}N_4O$	C, H, N
36		C_2H_5	OCH_3	n-C_3H_7		268-272	B	$C_{17}H_{24}N_4O\cdot HCl$	C, H, N
37		n-C_3H_7	OCH_3	n-C_3H_7		263-267	B	$C_{18}H_{26}N_4O\cdot HCl$	C, H, N
38		n-C_3H_7	n-OC_4H_9	n-C_3H_7		274-276	D	$C_{21}H_{32}N_4O\cdot HCl$	C, H, N
43	CH_3	CH_3	OCH_3	CH_3	CH_3	311-313	E	$C_{16}H_{22}N_4O$	C, H, N
44	CH_3	CH_3	OC_2H_5	CH_3	CH_3	276-278	F	$C_{17}H_{24}N_4O$	C, H, N
50	OCH_3	CH_3		CH_3		176-177	G	$C_{14}H_{18}N_4O$	C, H, N

[a] A = 95% EtOH; B = absolute EtOH/Et_2O; C = H_2O/EtOH (20:15); D = absolute EtOH; E = DMF; F = H_2O/EtOH (15:80); G = H_2O/EtOH (2:1).

Table III. Inhibitory Activity of 2,4-Diamino-5-benzylpyrimidine Derivatives against Dihydrofolate Reductase[a]

no.	benzene substituents 2	3	4	5	DHFR 50% inhibn × 10^8, M *E. coli*	rat liver	*N. gonorrhoea*
1		OCH_3	OCH_3	OCH_3	0.7	34 000	45
51[b]		OCH_3	OCH_3		6.0	8 600	53
18	OCH_3	OCH_3	OCH_3		92	5 800	161
20	OCH_3	OH	OCH_3		130	>4 000	
49	OH	$CH_2CH{=}CH_2$		CH_3	100	31 000	
48	c-$OCH(CH_3)CH_2$			CH_3	20	45 000	127
46	c-$CH_2CH(CH_3)O$			CH_3	5.5	3 700	19
42	OH	CH_3		CH_3	195	14 000	
50	OCH_3	CH_3		CH_3	70	7 000	

[a] For assay method, see ref 36. [b] Reference 14.

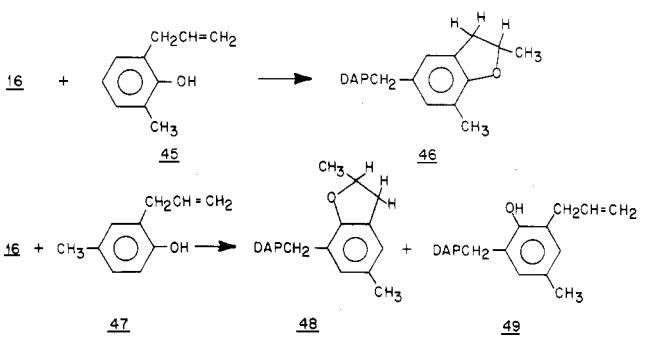
1,2,3-Trimethoxybenzene (**5**) and **16** produced the 2,3,4-trimethoxybenzyl isomer (**18**)[6] of trimethoprim exclusively when the reagents were heated for no longer than 3 h, in analogy to the 5-hydroxyuracil reaction. Upon longer heating, hydrolysis of the middle methoxy group of **5** occurred, and **19** and **20** were produced as well. Compound **18** was also prepared from 2,3,4-trimethoxybenzaldehyde by condensation with β-morpholinopropionitrile. Again, two isomeric products, **19** and **20**, were obtained by condensations of **16** with **7**.

The most useful result of this study was the finding that alkylphenols did indeed condense with **16** to produce the desired 2,4-diamino-5-(4-hydroxy-3,5-dialkylbenzyl)pyrimidines. Thus, by using a common intermediate, a number of TMP derivatives could be obtained in a one-step synthesis. Even though yields were often low, this was sometimes preferable to the Mannich condensation of alkylphenols with 2,4-diamino-6-(methylthio)pyrimidine, which then required dethiation. Again, yields were decreased in the carbonium ion condensation with increased size of substituents ortho to the phenol. In a single case tried, use of molten *p*-toluenesulfonic acid as solvent gave an improved yield. Use of a phenol blocked in the para position (**40**) as a reactant produced an *o*-hydroxybenzylpyrimidine (**42**).

The application of glacial acetic acid and *p*-toluenesulfonic acid as solvents suggested that 5-(acetoxymethyl)- and 5-[(tosyloxy)methyl]pyrimidine ester derivatives might be formed as intermediates. Evidence obtained by chromatographic analysis of reactions carried out in these solvents showed initial rapid formation of a new species, which was subsequently converted to the appropriate products. Glacial acetic acid (D) proved to be the most versatile solvent, since it generally dissolved the reactants.

Scheme III shows the reaction of **16** and two allylic phenols. This resulted in the concomitant reaction of the allylic function with the phenol to produce dihydrobenzofurans **46** and **48**, along with some uncyclized material (**49**) in the latter case. This type of cyclization was not unexpected. Such reactions have been well documented.[12] Compound **46** has been reported in the patent literature, although made by a different route, using a preformed bicyclic aldehyde.[13]

Scheme III



The phenolic benzylpyrimidines were successfully alkylated to the corresponding ethers by methods previously discussed.[3,4] These products are described in Table II.

Biological Activity and Discussion

Table III compares the inhibitory activities of several of the benzylpyrimidine derivatives described here against dihydrofolate reductase (DHFR) enzymes from various sources. The 2,3,4-trimethoxy analogue of trimethoprim (**18**) is compared with TMP (**1**), along with the 3,4-dimethoxy analogue (**51**),[14] to aid in the evaluation of the various methoxy substitution effects. Several other ortho-substituted derivatives are compared, as well as the two isomeric dihydrobenzofuran derivatives, **46** and **48**.

Compound **18** is considerably less active than either **1** or **51** against *Escherichia coli* DHFR, although it is sixfold

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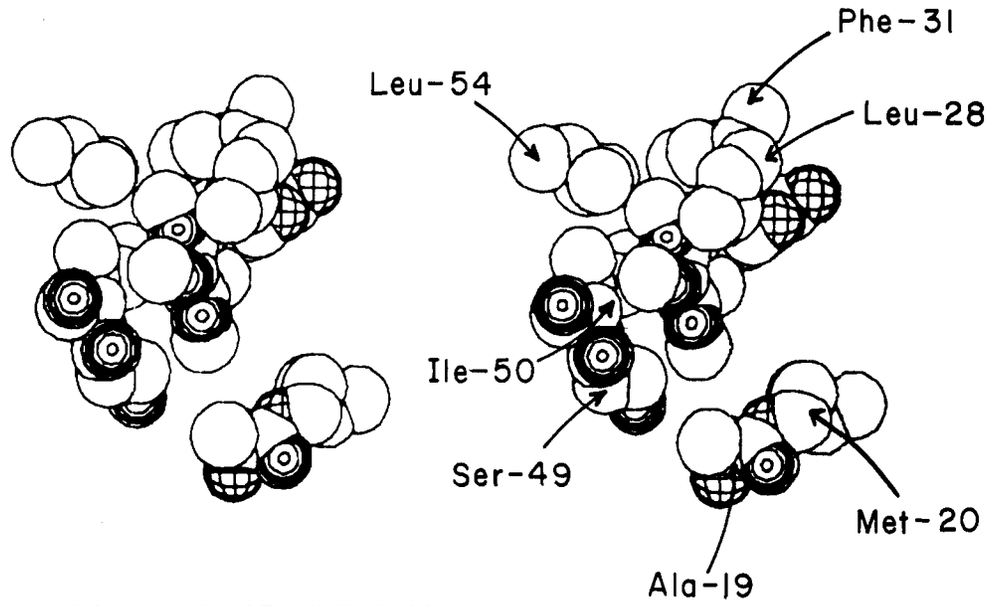

Figure 1. Stereoview of the active site of *E. coli* dihydrofolate reductase in binary complex with trimethoprim, showing interactions of the aromatic moiety of the inhibitor. Hydrogen atoms are not shown. Carbon atoms are open circles, oxygen atoms are concentric circles, and nitrogen atoms are cross-hatched circles. The α-carbon atoms of neighboring side chains are shown with the arrows.

more active than **1** against the rat liver enzyme. These results now become understandable in the light of recent information on the three-dimensional structure of DHFR complexes with TMP and other inhibitors, as determined by X-ray crystallography.[15-19] Let us consider the shapes of these various substituted benzenes and their interactions with the side chains of DHFR. The 3,5-dimethoxy groups of TMP lie approximately in the plane of the benzene ring, bent away from the 4-substituent, which of necessity lies out of plane.[20-22,3] However, the methoxy groups of **18** will of necessity provide quite a different conformation for that aromatic moiety, since the 2- and 3-methoxy groups will be forced out of plane, probably in opposing directions, because of the vicinal substituents on either side. The 4-methoxy group in this case can lie in plane, bent away from the 3-substituent.

Figure 1 shows a stereoview of TMP in the active site of *E. coli* DHFR, without the hydrogen atoms.[16,23,24] The benzene ring is seen to be aligned almost perpendicularly in the cleft, with the oxygen atoms of the methoxy groups apparently in contact with external solvent. The top methoxy group (which we will call 3-methoxy) is surrounded by side chains of Leu-28 on the right, Phe-31 at the roof of the cleft, and Leu-54 and Ile-50 on the left. The 4-methoxy group of TMP is in contact with side chains of Ile-50 and Ser-49, near the terminus of α-helix C.[24] The 5-methoxy group, near Ser-49, is observed to have fewer contacts with the enzyme. Met-20 is found on the lower right, with its side chain facing away from the aromatic moiety of TMP and with solvent between it and the inhibitor.

The structure of the ternary complex of *E. coli* DHFR with the coenzyme NADPH and an inhibitor is not as yet known.[25] However, the structure of the ternary complex of *Lactobacillus casei* DHFR with NADPH and methotrexate (MTX) was described in 1978[17] and recently refined to 1.7 Å.[24,26] By modeling experiments, one can place trimethoprim in the cleft of *L. casei* DHFR with the pyrimidine ring in the same position as that occupied by the corresponding ring of MTX, and with torsional angles similar to those observed in the *E. coli* enzyme, without problems of fit. The 5-methoxy group is in good contact with the Leu-19 side chain, which replaces Met-20 of *E. coli* DHFR; the "teen" loop appears to have moved up about 3 Å relative to the *E. coli* DHFR,[17] and this side chain is in an "up" orientation to give good contacts with the inhibitor, with no intervening solvent. Furthermore, the nicotinamide ring of the coenzyme is also in good contact with the 5-methoxy group, lying just behind it, from the view of Figure 1. Matthews et al.[17] have discussed these elements of the *L. casei* DHFR ternary complex with MTX, compared to the *E. coli* DHFR binary structure. They have suggested that the large cooperative effect in

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the binding of MTX and analogues to the ternary, as opposed to the binary, complex of DHFR enzymes, which has been observed in several laboratories,[27-34] is caused by the juxtaposition of the nicotinamide ring and Leu-19 side chain to the pteridine ring of MTX. Birdsall et al.[33] observed a greater than 100-fold cooperativity in TMP binding in the presence of the cofactor and suggested similarly that a conformational change involving Leu-19 (*L. casei* DHFR), as well as proximity of the nicotinamide ring, might be involved. Concerning *E. coli* DHFR, it should also be mentioned that there are two molecules in the unit cell and that the conformations of the "teen" loop, which includes Met-20, differ slightly in the two molecules, possibly as a result of crystal packing forces.[15,16,24] The proposed conformational change in *L. casei* DHFR on binding of the coenzyme could actually be an artifact.[17,24]

Baccanari et al.[35] have determined binary dissociation constants and ternary K_i values for the various possible *m*- and *p*-methoxy substituted TMP analogues, as well as for the parent unsubstituted benzylpyrimidine, using *E. coli* and L1210 (mouse) DHFR. Although the conditions were not the same for the two types of assay, the binary K_D values were found to be approximately the same for all of the six compounds, whereas in the case of the *E. coli* DHFR ternary complex, there was a stepwise tenfold increase in binding in going from unsubstituted to mono- to dimethoxylation and about a sixfold increase in going from compound **51** to **1**. In other words, there was an increasing cooperativity in binding in the ternary complex, as well as an increased specificity in binding to the bacterial enzyme, on increasing the meta- or para-methoxylation. These data suggest that the explanation of Matthews et al. and of Birdsall et al. for MTX and TMP cooperativity in the presence of NADPH is correct.[17,33]

The low activity of **51** compared to **1** for *E. coli* DHFR is then caused in part by the lack of a 5- or 3-substituent to bind either in the pocket produced by adding the coenzyme and movement of Met-20 or in the "3" pocket shown in Figure 1. If the cofactor is assumed to be present, modeling of **18** to the *E. coli* enzyme suggests that a 2-substituent would not fit into the cavity without movement of Phe-31. A fit in the 6-position also appears difficult with this out-of-plane substituent; it would probably require side-chain or cofactor adjustments. It should be noted that compound **20**, which lacks an out-of-plane 3-substituent, has equally low (within experimental error) activity for the bacterial enzyme, suggesting again that the *o*-methoxy substituent may be largely responsible for this result. Further speculation is unwarranted in the absence of the *E. coli* ternary structure.

Further clues to possible ortho effects are offered by the pairs **48** and **49** and **42** and **50** (Table III). Note that in each case the *o*-hydroxy derivative is less active than its alkylated counterpart; the out-of-plane nature of the *o*-methoxy group cannot provide the sole explanation then for poor binding. A polar substituent is then further contraindicated, as might be expected in the hydrophobic milieu provided by the side-chain contacts. In addition, Filman[36] has discovered that the nicotinamide ring of the cofactor is surrounded by three oxygen atoms of the protein, in the plane of the ring, with each approximately 3.2 Å from the 2-, 4-, and 6-positions. He suggests that this phenomenon may be related to the catalytic activity. A hydroxy group nearby might then adversely affect the positioning of the cofactor.

The fact that compound **48** has its ortho oxygen in a five-membered ring, with the attached carbon pulled away from possible interfering atoms, may explain its relatively higher activity, which is obviously increased further on moving the ring to the meta, para locus with **46**. The latter compound also has relatively good activity against the gonococcal enzyme, compared to TMP, in contrast to the other derivatives of Table III.

One other point of interest concerning compound **18** and some of the other ortho-substituted derivatives is that they have higher activity against a mammalian enzyme than does TMP. Matthews[19] has crystallized TMP with chicken liver DHFR in the presence of the cofactor and has found that the inhibitor conformation is different from that found in *E. coli* DHFR, with no contacts of methoxy groups to the cofactor. Furthermore, no cooperativity has been observed on binding of TMP to a vertebrate enzyme.[35] On the other hand, the presence of a 5-methoxy group is definitely deleterious to binding to a vertebrate enzyme (cf. **51** and **1**, Table III). It would seem then that the lack of a 5-methoxy group, rather than the presence of a 2-methoxy substituent, gives **18** its relatively high activity, which is, within experimental error (about ±50%), the same as the value listed for **51**.

Table IV compares the in vitro antibacterial activity of **18** with **1**, against a battery of organisms. The consistent low activity of **18** suggests strongly that the DHFR en-

Table IV. Comparison of in Vitro Antibacterial Activities of 18 and 1

organisms		MIC,[a] μg/mL 18	1
Streptococcus pyogenes	CN10	1.0	0.3
Streptococcus faecalis	CN478	1.0	0.1
Streptococcus agalactiae	CN1143	3.0	0.3
Staphylococcus aureus	CN491	10	0.3
Bordiella bronchiseptica	CN385	30	0.3
Vibrio cholerae	ATCC14035	10	0.3
Pasteurella multocida	ATCC6587	100	0.1
Candida albicans	CN1863	>100	>100
Mycobacterium smegmatis	S3254	3.0	1.0
Salmonella typhimurium	S8587	30	0.1
Salmonella typhosa (LT-2)	CN512	10	0.03
Shigella flexneri	CN6007	30	0.3
Escherichia coli	CN314	10	0.1
Serratia marcescens	CN2398	>100	3.0
Serratia marcescens	UNC18	>100	0.3
Klebsiella pneumoniae	CN3632	100	0.3
Enterococcus aerogenes	2200/86	30	0.3
Citrovorum freundii	2200/77	30	0.3
Proteus vulgaris	CN329	100	1.0
Proteus mirabilis	S2409	>100	3.0
Pseudomonas aeruginosa	CN200	>100	100
Pseudomonas aeruginosa	S5641	>100	>100

[a] The assay was conducted using Wellcotest sensitivity test agar plus 7% lysed horse blood.

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zymes of the various bacteria present the same problems of poor fit for such a compound.

The biological activities of the various 5-(3,5-dialkylbenzyl)pyrimidines that are described in this paper will be compared in a separate publication, which gathers the various compounds that have been prepared by different methods of synthesis, so that more meaningful comparisons are possible.

Experimental Section

Melting points were determined with a Hoover or Thiele tube melting point apparatus and are uncalibrated. Where analyses are indicated only by symbols of the elements, analytical results obtained for these elements were within ±0.4% of the theoretical values. Nuclear magnetic resonance (NMR) spectra were recorded on Varian HA-100, XL-100, and T-60 spectrophotometers; chemical shifts are reported in parts per million (δ) from internal tetramethylsilane. Ultraviolet spectra were recorded on Unicam SP700 and SP800 or Cary 118 spectrophotometers. Thin-layer chromatography was carried out on silica gel, with $CHCl_3$/EtOH/0.88NH_3 (70:28:2), dioxane, and EtOAc as solvents. Paper chromatography, with *n*-BuOH/5 N AcOH (7:3) as solvent, was conducted by the ascending technique. Column chromatographic separations were carried out on silica gel, with $CHCl_3$/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 column chromatography, as stated. Phenols employed were commercial samples or were synthesized by published procedures. The biological assays were carried out by methods described in parts 3 and 5 of this series.[37,38]

5-(2,3,4-Trimethoxybenzyl)uracil (6). A mixture of 1.42 g (0.01 mol) of **2** and 1.7 g (0.01 mol) of **5** in 30 mL of H_2O and 0.3 mL of concentrated hydrochloric acid was heated on the steam bath for 5 h. After the mixture was heated for 10 min, a colorless solid began to separate. The hot mixture was filtered after 5 h and recrystallized from 50% EtOH to give **6**: yield 1.9 g (65%); mp 242–243 °C; NMR (TFA) δ 3.8 (s, 2, benzylic CH_2), 3.98 (s, 3, OMe), 4.08 [s, 6, $(OMe)_2$], 6.92 (d, 1, aromatic, J = 9.0 Hz), 7.1 (d, 1, aromatic, J = 9.0 Hz), 7.35 (s, 1, pyrimidine C_6 H); UV (0.1 N HCl) λ_{max} 266 nm (ϵ 8650); UV (0.1 N NaOH) λ_{max} 280 nm (ϵ 6700). Anal. ($C_{14}H_{16}N_2O_5$) C, H, N.

Reactions of 2 with Phenols. The following four solvent systems were studied: (A) the phenol with about 2% of concentrated hydrochloric acid, according to Brossmer;[4] (B) 0.1 N HCl; (C) 0.1 N NaOH; (D) glacial AcOH containing 1% of concentrated hydrochloric acid. Various times and ratios of reactants were employed. Reactions with four phenols are described below.

5-(4-Hydroxybenzyl)uracil (4).[4] Method A. The method of Brossmer was repeated to give the title compound in 92% yield (70% EtOH): paper chromatography showed a single component; NMR (TFA) δ 3.72 (s, 2, benzylic CH_2), 6.98 (d, 2, aromatic protons, J = 9.0 Hz), 7.2 (d, 2, aromatic protons, J = 9.0 Hz), 7.3 (s, 1, pyrimidine C_6 H).

Method B. A mixture of 1.42 g (0.01 mol) of **2** and 0.94 g (0.01 mol) of **3** was suspended in 30.3 mL of solvent B. The mixture was heated at 90 °C for 2 h, whereupon an additional 0.5 g of **3** was added, followed by heating for another hour. Upon refrigeration and filtration, 1.85 g of product was isolated, which upon recrystallization from water gave **4** as the sole product (1.59 g, 73%), mp >320 °C.

Method C. Method B was repeated with solvent C, which gave **4** as the sole product in 20% yield.

Reaction of 2 with 7. Method A. Compound **2** (1.0 g, 0.007 mol) and concentrated hydrochloric acid (0.3 mL) were added to molten 2,6-dimethoxyphenol (**7**, solvent A, 15 g), and the mixture was heated at 85–95 °C for 1 h, during which time the pyrimidine dissolved. Addition of ether and filtration gave a mixture of crude products **8** and **9** (1.85 g, 85%). Recrystallization from water/EtOH (7:1) gave a similar mixture, although the level of the minor component had been reduced: NMR (TFA) δ 3.72, 3.78 (s, overall 2, benzylic CH_2), 3.98, 4.05 (s, overall 6, OMe protons), 6.65, 6.85 (s, overall 2, aromatic), 7.3 (s, 1, pyrmidine C_6 H). Integration of the aromatic and methoxy protons established that the product was a mixture of **5-(4-hydroxy-3,5-dimethoxybenzyl)uracil (8)** and **5-(3-hydroxy-2,4-dimethoxybenzyl)uracil (9)** in a ratio of 67:33 in the crude product and 88:12 after crystallization.

Method B. The above reaction was repeated in solvent B, which produced 65% of a mixture having the same composition as was obtained by method A.

Method C. A mixture of 1.42 g (0.01 mol) of **2** and 2.0 g (0.013 mol) of **7** was dissolved in 30 mL of solvent C and heated on the steam bath for 2.5 h. The product was chilled and neutralized, which precipitated 0.54 g of a product; upon recrystallization from water, this gave a homogeneous crystalline solid (0.48 g, 20%), mp 218–222 °C, which the NMR spectrum showed to be **5-(4-hydroxy-3,5-dimethoxybenzyl)uracil (8)**: NMR (TFA) δ 3.7 (s, 2, CH_2), 3.93 [s, 6, 3,5-$(OMe)_2$], 6.65 (s, 2, aromatic), 7.3 (s, 1, pyrimidine C_6 H). Anal. ($C_{13}H_{14}N_2O_5$) C, H, N.

5-(3,5-Diethyl-4-hydroxybenzyl)uracil (12). A mixture of 0.7 g (0.005 mol) of **2** and 1.5 g (0.01 mol) of **10** was refluxed for 5 h in solvent B, after which the mixture was chilled and the product isolated (1.5 g). Recrystallization from water produced **12** as a single component (1.14 g, 82%): mp 208–210 °C; NMR (TFA) δ 1.26 (t, 6, CH_2CH_3, J = 8.0 Hz), 2.7 (q, 4, CH_2CH_3, J = 8.0 Hz), 3.7 (s, 2, benzylic CH_2), 6.96 (s, 2, aromatic), 7.27 (s, 1, pyrimidine C_6 H). Anal. ($C_{15}H_{18}N_2O_3$) C, H, N.

5-(4-Hydroxy-3,5-diisopropylbenzyl)uracil (13).[3] Method A. Equimolar portions of **2** and **11** were refluxed in solvent B for 10 h. This produced a tacky solid, which, when triturated with acetone and recrystallized from dilute EtOH, produced a single product (**13**) in 20% yield: mp 274–275 °C; NMR (TFA) δ 1.3 [d, 12, J = 7.0 Hz, $(CH_3)_2CH$], 3.3 [m, 2,$(CH_3)_2CH$], 3.75 (s, 2, benzylic CH_2), 7.05 (s, 2, aromatic), 7.2 (s, 1, pyrimidine C_6 H).

Method B. Compounds **2** (1.4 g, 0.01 mol) and **11** (1.8 g, 0.01 mol) were added to glacial acetic acid (100 mL) containing 1.0 mL of concentrated hydrochloric acid (solvent D) and heated on the steam bath for 8 h. The mixture was poured into water, and the precipitate was isolated (2.0 g). Recrystallization from dilute EtOH produced **13** as a single product (1.6 g, 53%), mp 270–275 °C.

2,4-Diaminopyrimidine-5-carboxaldehyde (15).[8] 2,4-Diamino-5-cyanopyrimidine[10] (**14**; 13.5 g), dissolved in 150 mL of HCOOH, was treated with 50:50 Ni–Al alloy (13 g) by heating at 120 °C for 1.5 h, followed by cooling to 80 °C and filtering. The filtrate was evaporated to dryness in vacuo and made alkaline with 2 N NaOH, which produced a colorless precipitate. This was isolated, washed with H_2O, EtOH, and Et_2O, and dried, yielding 10.8 g (80%) of crude **15**, which upon recrystallization from H_2O melted at 273–275 °C dec. Anal. ($C_5H_6N_4O$) C, H, N.

2,4-Diamino-5-(2,3,4-trimethoxybenzyl)pyrimidine (18).[6] Method A. From 16 plus 5. A mixture of 2.8 g (0.02 mol) of **16**,[8] 3.4 g (0.02 mol) of **5**, 60 mL of HOAc, and 6 mL of concentrated hydrochloric acid was heated in an oil bath at 100 °C for 3 h. The solvent was removed in vacuo, and the residue was separated on a silica gel column with 3% MeOH/CH_2Cl_2. A pure fraction was isolated (**18**; 1.97 g, 34%): mp 224.5–226 °C (EtOH); NMR (Me_2SO-d_6) δ 3.47 (s, 2, CH_2), 3.75 (s, 3, OMe), 3.76 [s, 6, $(OCH_3)_2$], 5.64 (br s, 2, NH_2), 6.03 (br s, 2, NH_2), 6.73 and 6.79 (2 d, 1 each, arom 5′ and 6′ H, J = 8.6 Hz), 7.38 (s, 1, pyrimidine C_6 H). Anal. ($C_{14}H_{18}N_4O_3$) C, H, N. Following fractions that were collected consisted of a mixture of three or more products, including phenols **19** and **20**, as judged by TLC and NMR comparisons. No trimethoprim (**1**) was found among the products. When the reaction mixture was heated for 16 h on the steam bath, very little **18** was obtained; instead, a complex mixture containing **19** and **20** was isolated.

Method B. From 2,3,4-Trimethoxybenzaldehyde and Morpholinopropionitrile. 3-Morpholinopropionitrile (3.66 g, 0.026 mol) in 10 mL of Me_2SO was treated with 0.14 g (0.0026 mol) of NaOMe, followed by the addition of a warm solution of 2,3,4-trimethoxybenzaldehyde (3.92 g, 0.02 mol) in 10 mL of Me_2SO over a 10-min period. The internal temperature was maintained at 65–69 °C during the addition and for a 45-min period afterward. The mixture was then cooled in an ice bath,

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and 20 mL of water was added. The product was extracted into CH_2Cl_2, followed by drying and removal of the solvent. The resultant red oil weighed 7.53 g [90% calculated as crude 3-morpholino-2-(2,3,4-trimethoxybenzyl)acrylonitrile]. This material was dissolved in 100 mL of EtOH and refluxed for 1 h with 3.3 g (0.025 mol) of aniline hydrochloride, followed by the addition of 4.59 g (0.048 mol) of guanidine hydrochloride and 4.34 g (0.0803 mol) of NaOMe. The resultant mixture was refluxed for 1 h; the condenser was then set downward and 50 mL of EtOH was distilled off. Refluxing was then continued for an additional hour, followed by chilling. The solid material was separated and washed well with water, followed by recrystallization twice from 50% EtOH: yield 2.21 g (51%) of **18**; mp 223.5–224.5 °C. The NMR spectrum was identical with that obtained by method A.

Condensations of 16 with Phenols. The methodology and solvent systems used were, in general, the same as those studied for the uracil condensations. A fifth solvent system (E), molten *p*-toluenesulfonic acid, was used in one instance. The most commonly used method was to heat equimolar amounts of **16** and phenol in solvent D for about 6 h, followed by quenching in water or removal of the solvent, and then purification. Compounds made, solvent systems, and yields of purified products are shown in Table I. The use of alkoxyphenols produced mixtures in acidic medium, as described below.

Reactions of 16 with 7. Method A. A mixture of 1.0 g (0.007 mol) of **16**, 1.5 g (0.01 mol) of **7**, and 0.1 mL of concentrated hydrochloric acid was heated at 90 °C for 2 h. The melt was cooled, 200 mL of Et_2O was added, and the mixture was allowed to stand overnight. An oil separated, which was triturated with water, neutralized with $NaHCO_3$, and triturated again with acetone; 0.3 g of a crystalline solid was obtained, which was shown by TLC to be a two-component mixture: NMR (TFA) δ 3.85 (s, 2 overall, benzylic CH_2), 3.95, 4.08 (s, 6 overall, OMe), 6.62, 6.85 (s, 2 overall, aromatic), 7.58, 7.86 (s, 1 overall, pyrimidine C_6 H). A second crop of product (0.25 g) was deposited from the acetone trituration upon standing. This was shown by TLC and paper chromatography to be a single component, identical in R_f with the minor component in the above mixture: NMR (TFA) δ 3.86 (s, 2, benzylic CH_2), 3.96, 4.08 (s, 3 each, OMe), 6.88 (s, 2, aromatic), 7.88 (s, 1, pyrimidine C_6 H). The major component in the first fraction had an R_f value identical with that of an authentic specimen of **2,4-diamino-5-(4-hydroxy-3,5-dimethoxybenzyl)pyrimidine**.[3] Integration of spectra established that the first crop was a 34:66 mixture of **2,4-diamino-5-(3-hydroxy-2,4-dimethoxybenzyl)pyrimidine (20)** and **2,4-diamino-5-(4-hydroxy-3,5-dimethoxybenzyl)pyrimidine (19)**, whereas the second crop was pure **20**, mp 246–248 °C. The overall ratio of isomers isolated was 63.5:36.5 of 20:19, with a total yield of 25.5%. (See procedure B below for analysis of **20**).

Method B. The reaction was repeated with solvent B; heating was continued for 5 h, followed by neutralization of the mixture, which precipitated an oil that solidified on standing (0.80 g, 29%). This crop was homogeneous and identical in NMR and R_f with **20**. The substance was converted to its hydrochloride salt by dissolving in the minimum of 2 N HCl, filtering, and evaporating to dryness, followed by crystallization from water, mp >250 °C. Anal. (**20**) ($C_{13}H_{16}N_4O_3 \cdot HCl \cdot H_2O$) C, H, N, Cl. The filtrate from the reaction deposited a further crop (0.1 g), which was shown by NMR and TLC to be a mixture of **19** and **20**; the total yield of mixed isomers was 33%.

2,4-Diamino-5-[(2,7-dimethyl-2,3-dihydrobenzofuran-5-yl)methyl]pyrimidine (46). A mixture of 4.2 g (0.03 mol) of **16** and 4.5 g (0.03 mol) of 2-allyl-6-methylphenol (**45**) in 300 mL of glacial AcOH containing 9 mL of concentrated hydrochloric acid was heated at 90 °C for 24 h. The mixture was cooled, evaporated to dryness in vacuo, and triturated with acetone, followed by recrystallization of the residue twice from water: yield 1.7 g (18.5%); mp 286–287 °C dec. Neutralization yielded the free base (**46**): mp 214–216 °C; NMR (TFA) δ 1.6 (d, 3, J = 7.0 Hz, CH_3), 2.25 (s, 3, aromatic CH_3 protons), 2.9, 3.45 (q, 1 each, J_{gem} = 16 Hz, J_{CH_2CH} = 7, 8 Hz, nonequivalent CH_2 protons), 3.85 (s, 2, benzylic CH_2), 5.25 (m, 1, methine), 6.9, 6.95 (s, 2 overall, aromatic), 7.7 (s, 1, pyrmidine C_6 H). Anal. ($C_{15}H_{18}N_4O$) C, H, N.

Reaction of 16 with 47. Compound **16** (2.8 g, 0.02 mol) and 2-allyl-4-methylphenol (**47**; 15 g, 0.1 mol) in glacial acetic acid (200 mL) containing 6 mL of concentrated hydrochloric acid were heated at 90 °C for 24 h. The reaction mixture was cooled and then evaporated to dryness in vacuo, and the residue was treated with 100 mL of acetone. The mixture was stirred for 1 h, and the crude solid was filtered and dried (3.6 g). Recrystallization from water afforded the mixed isomers **48** and **49** as the hydrochloride salts (2.4 g, 39%). Thin-layer chromatography revealed two components. Treatment with hot 0.4 N NaOH, followed by filtering and drying, gave a solid (0.53 g), which on crystallization from dilute ethanol gave pure **2,4-diamino-5-[(2,5-dimethyl-2,3-dihydrobenzofuran-2-yl)methyl]pyrimidine (48)** as colorless needles (0.44 g, 9%), mp 210–211 °C. The alkaline filtrate from this experiment was adjusted to pH 8 with glacial acetic acid to precipitate the product (**49**) as a colorless solid (0.61 g). Recrystallization from dilute ethanol afforded pure **2,4-diamino-5-(2-hydroxy-3-allyl-5-methylbenzyl)pyrimidine (49)** as colorless needles (0.43 g, 9%), mp 203–204 °C. Anal. (**48**) ($C_{15}H_{18}N_4O$) H, N; C: calcd, 66.65; found, 66.0. Anal. (**49**), Table I.

2,4-Diamino-5-(alkoxybenzyl)pyrimidines. These compounds were prepared by simple alkylation of the corresponding phenols[3] and are described in Table II.

Acknowledgment. We thank Dr. Lee Kuyper for helpful discussions and criticism of this manuscript, Robert Ferone and associates for the enzyme assays, Dr. Lynn Elwell and his associates for the antibacterial assays, and Drs. B. S. Hurlbert and A. J. Everett and their staffs for the NMR spectra and the elemental analyses.

Registry No. **2**, 4433-40-3; **3**, 108-95-2; **4**, 17187-50-7; **5**, 634-36-6; **6**, 6981-02-8; **7**, 91-10-1; **8**, 21253-60-1; **9**, 84876-23-3; **10**, 1006-59-3; **11**, 2078-54-8; **12**, 84876-24-4; **13**, 84876-25-5; **15**, 20781-06-0; **16**, 42310-45-2; **17**, 30077-67-9; **18**, 6981-04-0; **19**, 83322-41-2; **20**, 84876-27-7; **26**, 84876-30-2; **27**, 84876-31-3; **28**, 84876-32-4; **29**, 57506-37-3; **30**, 84876-33-5; **31**, 84876-34-6; **32**, 42310-33-8; **33**, 84876-37-9; **34**, 84894-75-7; **35**, 36821-98-4; **36**, 84876-38-0; **37**, 84876-39-1; **38**, 84876-40-4; **41**, 84876-35-7; **42**, 84876-36-8; **43**, 84876-41-5; **44**, 84876-42-6; **45**, 3354-58-3; **46**, 66893-29-6; **47**, 6628-06-4; **48**, 84876-28-8; **49**, 84876-29-9; **50**, 84876-43-7; 2,4-diamino-5-cyanopyrimidine, 16462-27-4; 3-morpholinopropionitrile, 4542-47-6; 2,3,4-trimethoxybenzaldehyde, 2103-57-3; guanidine hydrochloride, 50-01-1; 3-morpholino-2-(2,3,4-trimethoxybenzyl)acrylonitrile, 84876-26-6.