silica gel (chloroform–methanol, 9:1) and crystallization from chloroform–petroleum ether. This procedure gave 26 mg (51%) of **12** as the monohydrate, a yellow solid with mp 151–154 °C: $[\alpha]^{26}_{546}$ −23.5 (*c* 0.08, MeOH); IR (KBr) showed no carbonyl bands at 1700 or 1660 cm^{-1}; NMR ($CDCl_3$) δ 3.3 (CH_3N=). Anal. ($C_{59}H_{89}NO_{24}\cdot H_2O$) C, H, N.

1-Deoxy-2′-dihydroolivomycin A (5). An ice-cooled solution of **11** (55 mg, 0.46 mmol) in 10 mL of dry methanol was treated gradually with sodium borohydride (15 mg, excess). The mixture was stirred for 2 h, treated with dilute HCl, and concentrated to dryness. An acetone extract of the residue was passed through a small column of silica gel and reconcentrated. The resulting solid was purified by preparative TLC on silica gel (chloroform–methanol, 8:2) and recrystallization from acetone–ether. This procedure gave 28 mg (50%) of **5** as yellow solid with mp 210 °C dec: $[\alpha]^{26}_{546}$ −17.5 (*c* 0.64, $CHCl_3$); IR (KBr) showed no carbonyl bands at 1700 or 1660 cm^{-1}, but the ester carbonyls were present at 1735 and 1720 cm^{-1}. Anal. ($C_{58}H_{88}O_{25}$) C, H.

3′,4′-*O*-Isopropylideneolivomycin A (13). A mixture of **1** (60 mg, 0.05 mmol), 4 mL of 2,2-dimethoxypropane, and a small amount of *p*-toluenesulfonic acid was stirred at 25 °C for 35 min, treated with a few drops of water, and concentrated. The residue was extracted with chloroform. This extract was washed with dilute sodium bicarbonate solution and with water, dried over sodium sulfate, and concentrated. Purification of the crude product by preparative TLC on silica gel (chloroform–methanol, 9:1) and crystallization from chloroform–petroleum ether gave 41 mg (67%) of **13** as the trihydrate, a yellow solid with mp 178–180 °C; $[\alpha]^{26}_{546}$ −42.00 (*c* 0.05, $CHCl_3$); NMR ($CDCl_3$) showed two new CH_3 groups at δ 1.25 and the phenolic OH groups were still present at δ 9.6 and 15.5. Anal. ($C_{61}H_{86}O_{26}\cdot 3H_2O$) C, H.

3′,4′-*O*-Cyclohexylideneolivomycin A (14). A solution of **1** (60 mg, 0.05 mmol) in 0.5 mL of dry tetrahydrofuran was treated with 0.5 mL (excess) of 1,1-dimethoxycyclohexane and a small crystal of *p*-toluenesulfonic acid. After 16 h at 25 °C, the mixture was poured into dilute sodium bicarbonate solution and extracted with dichloromethane. This extract was washed with water, dried over sodium sulfate, and concentrated. The residual solid was purified by preparative TLC on silica gel (chloroform–methanol, 9:1), which afforded 48 mg (75%) of **14** as the monohydrate yellow solid with mp 155–158 °C: $[\alpha]^{26}_{546}$ −47.00 (*c* 0.1, $CHCl_3$); NMR ($CDCl_3$) showed broadening and increase in area for absorption near δ 1.35. Anal. ($C_{64}H_{92}O_{26}\cdot H_2O$) C, H.

5-Bromo- or 7-Bromoolivomycin A (17). A solution of **1** (30 mg, 0.025 mmol) in 2.5 mL of dry dichloromethane was treated with pyridine perbromide (7 mg, 0.029 mmol). After 20 h at 25 °C, the mixture was diluted with water and dilute sodium bicarbonate solution and dried over sodium sulfate. Concentration and addition of petroleum ether gave 22 mg (68%) of **17** as yellow solid with mp 149–153 °C: $[\alpha]^{26}_{546}$ −17.08 (*c* 0.07, $CHCl_3$); UV (CH_3OH) λ_{max} 415 nm (ε 4.05), 340 (sh), 279 (5.60), 230 (4.36); NMR ($CDCl_3$) showed in the aromatic region 1 proton at δ 6.5 (decrease from 2) and 1 proton at δ 6.75. Anal. ($C_{58}H_{83}BrO_{26}$) C, H, Br. A small amount of a second product was obtained, but it was too unstable for purification and analysis.

5-Nitro- or 7-Nitroolivomycin A (18). A solution of **1** (60 mg, 0.05 mmol) in 5 mL of dry methanol was treated with 2 mL of phosphate buffer (pH 8.2), cooled in an ice bath, and treated with tetranitromethane (15 mg, 0.076 mmol). After 16 h at 5 °C, the mixture was concentrated and the residue was extracted with dichloromethane. This extract was washed with water, dried over sodium sulfate, and concentrated with the addition of petroleum ether. The dark product was purified by preparative TLC on silica gel (chloroform–methanol, 8:2) and crystallization from dichloromethane–ether. This procedure gave 30 mg (48%) of **18** as tan solid with mp 200–203 °C: $[\alpha]^{26}_{546}$ −33.71 (*c* 0.18, MeOH); IR (KBr) 1530, 1350 cm^{-1} (NO_2); UV (CH_3OH) λ_{max} 402 nm (ε 3.80), 273 (4.32), 223 (sh); NMR ($CDCl_3$) showed 1 proton at δ 6.5 (decrease from 2) and 1 proton at δ 6.8 in the aromatic region. Anal. ($C_{58}H_{83}NO_{28}$) C, H, N.

8-*O*-Methylolivomycin A (16). A mixture of **1** (60 mg, 0.05 mmol), *N*,*N*-dimethylformamide dimethyl acetal (2 mL), and a small amount of *p*-toluenesulfonic acid was stirred at 25 °C for 36 h, poured into dilute sodium bicarbonate solution, and extracted two times with dichloromethane. The extract was washed with brine, dried over sodium sulfate, and concentrated. Purification of the solid residue by preparative layer chromatography on silica gel (chloroform–methanol, 9:1) gave 38 mg (62%) of **16** as yellow powder with mp 183–186 °C: $[\alpha]^{26}_{546}$ −21.1 (*c* 0.25, MeOH); IR (KBr) 1740, 1720, 1710, 1620 cm^{-1}; UV (CH_3OH) λ_{max} 410 nm (ε 3.71), 3.15 (sh), 278 (4.31), 224 (3.94); NMR ($CDCl_3$) showed one OCH_3 at δ 3.5 and two OCH_3 at δ 3.6. The phenolic 9-OH was present at δ 15.5, whereas the 8-OH at δ 9.6 was absent. Anal. ($C_{59}H_{86}O_{26}\cdot 1.5H_2O$) C, H.

Acknowledgment. We thank Drs. Terrence Doyle and Robert Grulich of Bristol Laboratories for their interest and help in obtaining NMR spectra of certain compounds.

2,4-Diamino-5-benzylpyrimidines and Analogues as Antibacterial Agents. 2. C-Alkylation of Pyrimidines with Mannich Bases and Application to the Synthesis of Trimethoprim and Analogues[1]

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A new route to 5-(*p*-hydroxybenzyl)pyrimidines has been developed which utilizes phenolic Mannich bases plus pyrimidines containing at least two activating groups. The products can be alkylated on the phenolic oxygen or on the pyrimidine N-1 atom, depending on conditions. This method has been used to prepare trimethoprim, a broad-spectrum antibacterial agent, starting from 2,4-diaminopyrimidine and 2,6-dimethoxyphenol.

The importance of trimethoprim [**13**, 2,4-diamino-5-(3,4,5-trimethoxybenzyl)pyrimidine][2] as a broad-spectrum antibacterial agent[3-6] has dictated the need for new and improved synthetic routes. The original synthesis[2] was lengthy. Stenbuck and co-workers[7] reported a two-step

(1) (a) B. Roth and J. Z. Strelitz, British Patent 1 128 234 (1968); U.S. equivalent, 3 819 629 (1974). (b) This paper was presented in part at the 154th meeting of the American Chemical Society, Chicago, 1967.
(2) For Paper 1 of this series, see: B. Roth, E. A. Falco, G. H. Hitchings, and S. R. M. Bushby, *J. Med. Pharm. Chem.*, **5**, 1103 (1962).
(3) S. R. M. Bushby and G. H. Hitchings, *Br. J. Pharmacol. Chemother.*, **33**, 72 (1968).
(4) J. J. Burchall and G. H. Hitchings, *Mol. Pharmacol.*, 1, 126 (1965).
(5) "Evaluations on New Drugs: Trimethoprim-Sulfamethoxazole", *Drugs*, 1, 7 (1971), and references therein.
(6) "Symposium on Trimethoprim-Sulfamethoxazole", *J. Infect. Dis.*, **128** (supplement), (1973).

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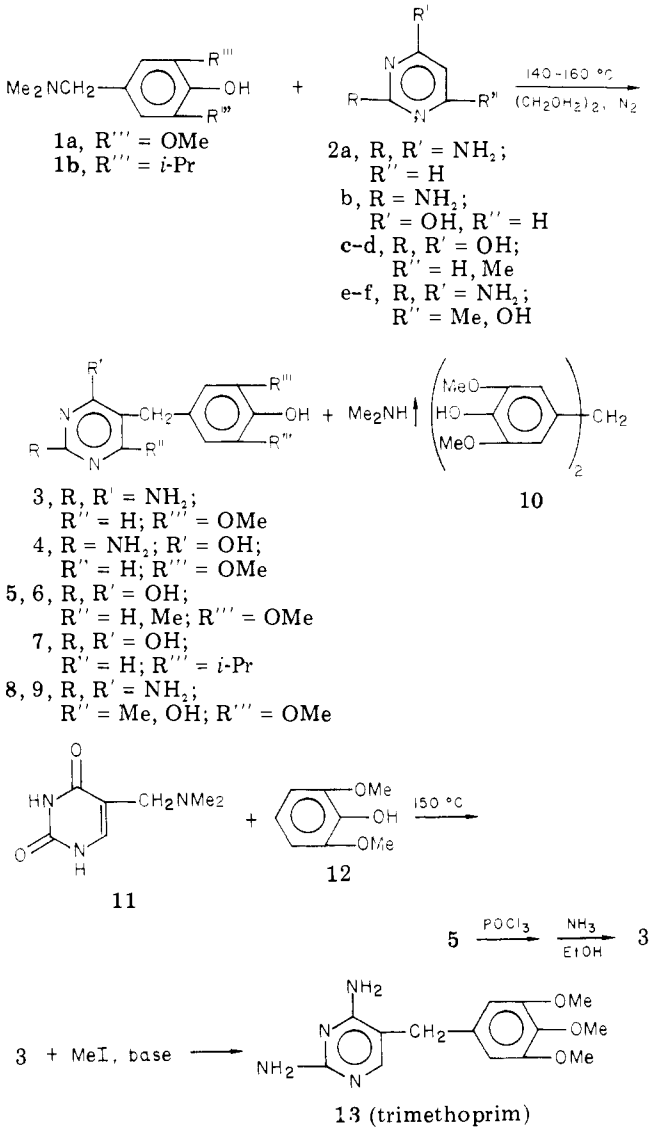
Scheme I



synthesis from β-ethoxypropionitrile and trimethoxybenzaldehyde, followed by guanidine, which became the basis for a commercial route.[8]

This paper reports an entirely different two-step synthesis which can be applied to the preparation of trimethoprim and a variety of analogues. This is outlined in Scheme I.

Phenolic Mannich bases have been known since 1906.[9] The use of Mannich bases for carbon alkylation was reported by Robinson and co-workers in 1937[10] and by Snyder[11] in 1944. A 1943 patent to Salzer and Andersag[12] describes similar reactions. Intermediate vinyl ketone formation was suggested by Snyder,[11] and with some reactions of phenolic Mannich bases,[13] intermediate methene quinone formation was postulated.

Certain pyrimidines with electron-donating groups at the 2 or 4 positions are known to react with formaldehyde and secondary amines to form Mannich bases, either in the 5 position or on an active 2- or 4-methyl group.[14] Extension of this reaction to the synthesis of a 5-benzylpyrimidine by Scheme I seemed plausible.

When 2,6-dimethoxy-4-[(*N*,*N*-dimethylamino)methyl]-phenol (**1a**) was heated to 150 °C with uracil (**2c**) in ethylene glycol, dimethylamine was rapidly evolved, and a 5-benzylpyrimidine was isolated in nearly 70% yield. Isocytosine (**2b**) and 2,4-diaminopyrimidine (**2a**) reacted similarly, as ascertained by NMR and UV spectral studies, but yields rarely exceeded 35%. 6-Aminoisocytosine (**2f**), on the other hand, produced a 5-substituted pyrimidine in greater than 70% yield. No products of reaction on a pyrimidine nitrogen were isolated in any of these reactions. Varying the ratios of reactants, the temperature or the solvent did not improve the yield with 2,4-diaminopyrimidine. No reaction occurred with 2-aminopyrimidine.

The reaction of **1a** with 2,4-diamino-6-methylpyrimidine (**2f**) rather surprisingly gave better yields than did the 6-unsubstituted compound. No product of reaction on the methyl group was detected. The result suggests assistance of the methyl group in electron donation to the 5 position without concomitant steric interference.

When **1a** was heated alone in ethylene glycol to 150 °C, dimethylamine was also evolved, and the diarylmethane **10** was isolated from the reaction mixture.[9] This could arise by electrophilic attack of an intermediate methene quinone on 2,6-dimethoxyphenol created by a reverse Mannich reaction. 3,4,5-Trimethoxybenzyl-*N*,*N*-dimethylamine does not react in this manner nor with the above pyrimidines—an expected result if a methene quinone intermediate is formed.

The condensations described here are best carried out in neutral to alkaline medium. No reaction occurred under the conditions described here when the hydrochloride of the phenolic Mannich base was treated with a pyrimidine. This was also the case with two quaternary salts, 3,4,5-trimethoxybenzyltrimethylammonium iodide (**16**) and 3,4,5-triethoxybenzyltrimethylammonium tosylate (**17**). Normally, we have found it advantageous to add a small amount of sodium methylate to the reaction mixture; however, the Mannich base produces a sufficiently alkaline milieu at high temperatures for reaction to occur.

Uracil plus formaldehyde and dimethylamine produced Mannich base **11**. When this was heated with 2,6-dimethoxyphenol (**12**) at 150 °C, a benzylpyrimidine (**5**) was produced which was identical with that obtained from uracil plus **1a**. This product could be chlorinated and aminated to produce **3**.

Attempts were made to extend the scope of the reaction with phenolic Mannich bases by using the commercially available 2,6-dialkylphenols, which readily yield Mannich bases.[15] These derivatives were much less reactive than their dimethoxy congeners, however, and did not produce significant amounts of 5-benzyl derivatives with 2,4-diaminopyrimidine under the conditions described here. However, benzylation did occur with the more reactive pyrimidine uracil, to produce, for example, **7**.

The alkylation of the various *p*-hydroxybenzylpyrimidines to produce ethers, rather than N-alkylated pyrimidines, was by no means a certainty. The methoxy

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(8) P. Stenbuck and H. M. Hood, U.S. Patent 3 049 544 (1962).
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(14) D. J. Brown, "The Pyrimidines", Wiley-Interscience, New York, 1962, pp 132–134; supplement 1 (1970), pp 91–92.
(15) T. H. Coffield, A. H. Filbey, G. G. Ecke, and A. J. Kolka, *J. Am. Chem. Soc.*, **79**, 5019 (1957).

groups flanking the phenol offer considerable steric hindrance. Pyrimidinones would compete as anions; aminopyrimidines could alkylate at the basic N-1 atom. Compound **3** did indeed produce trimethoprim (**13**), but there were competing reactions, and yields never exceeded 70%. No N-1 alkylated pyrimidine was isolated when 1 equiv of alkali was used in the reaction, but such a compound would probably have readily undergone degradation in alkali.

The methylation of the isocytosine derivative **4** produced a mixture of products, as described under Experimental Section. This is to be expected, since isocytosine has a dissociation constant for proton loss which is very close to that of the phenol; there would thus be competition for alkylation from two anionic groups. Its 6-amino analogue **9**, which is a weaker acid by more than a pH unit, did not give this problem and could be alkylated satisfactorily to produce a 4′ ether.

When **3** was alkylated in the absence of base, methylation took place at N-1 to produce a methiodide. Trimethoprim reacted similarly. Evidence for these structures was obtained from the UV spectra, which were practically identical with the spectra of the protonated starting materials, and from the dissociation constants. These products, as expected, were very strong bases, with pK_a values above 13.

Biological Activity. The antibacterial activity and dihydrofolate reductase (DHFR) enzyme inhibition of some of the compounds described here are presented in Table I. Compound **3**, the 4′-hydroxy analogue of trimethoprim (**13**), is virtually as active as the latter as an antibacterial agent in vitro, and its inhibitory activity vs. *Escherichia coli* DHFR approaches that of **13**. The methyl moiety of the 4′-methoxy substituent appears to contribute slightly in increasing the binding to *E. coli* DHFR; the effect against a mammalian enzyme is the reverse; i.e., **3** is about three to four times more potent an inhibitor against rat liver enzyme than is **13**. This suggests that this unsymmetrical substituent plays a role in the specificity exhibited by **13** against bacterial DHFR enzymes. X-ray crystallographic studies show that the 4-methoxy group of TMP is out of plane with the benzene ring, in contrast with the 3- and 5-substituents.[16,17] Many X-ray and photoelectron spectroscopy experiments have demonstrated this to be true with other vicinally substituted trimethoxybenzene derivatives.[18] Solution properties strongly suggest that this is not restricted to the crystalline state or to the gas phase. For example, Leo et al.[19] have noted that the partition coefficient of 1,2,3-trimethoxybenzene is unexpectedly low in comparison to the predicted additive log P value and have shown that the result can be explained by use of an aliphatic π constant for the middle out-of-plane methoxy group. We have obtained a similar result with **13**, which has a log P value of 0.89 in octanol–water, as compared to 1.60 for the unsubstituted 5-benzyl-2,4-diaminopyrimidine.[20] It will be noted that NMR measurements also show different environments for the 3′,5′- vs. 4′-methoxy substituents in **13** (see Experimental Section).

Table I. Dihydrofolate Reductase Inhibition and Antibacterial Activity of Some 5-(3,5-Dimethoxy-4-substituted-benzyl)pyrimidines

no.	pyrimidine substituents 1	2	4	6	benzene 4-subst	I_{50} vs. dihydrofolate reductases $\times 10^8$, M: *E. coli*	rat liver	*P. berghei*	MIC, μg/mL, vs. microorganisms[a]: *S.p.* CN 10	*S.a.* CN 491	*S.f.* CN 512	*E.c.* CN 314	*K.p.* CN 3632	*S.d.* CN 1523	*S.m.* CN 398	*P.v.* CN 329
13		NH_2	NH_2		OCH_3	0.5–0.7[b]	26 000–43 000[b]	12	0.3	1.0	0.3	0.1–0.3	0.3	0.1	3.0	1.0
3		NH_2	NH_2		OH	1.1	9 200	30	0.5	0.5	0.03	0.125				2.0
8		NH_2	NH_2	CH_3	OH	30		48								
18		NH_2	NH_2	CH_3	OCH_3	10	>40 000	34	30	30	30	30	>100	30	>100	>100
9		NH_2	NH_2	OH	OH				>100	>100	>100	>100	>100	>100	>100	>100
19[c]	CH_3	NH_2	NH_2		OCH_3	320	~41% 40 000		50	50	25	50	>100	25		>100

[a] Abbreviations used are: *S.p.*, *Streptococcus pyogenes*; *S.a.*, *Staphylococcus aureus*; *S.f.*, *Salmonella typhosa*; *E.c.*, *Escherichia coli*; *K.p.*, *Klebsiella pneumoniae*; *S.d.*, *Shig. dysentariae*; *S.m.*, *Serr. marcescens*; *P.v.*, *Proteus vulgaris*. [b] Range of six determinations over a period of time. [c] Methiodide salt.

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(17) T. F. Koetzle and G. J. B. Williams, *J. Am. Chem. Soc.*, **98**, 2074 (1976).
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(19) A. Leo, C. Hansch, and D. Elkins, *Chem. Rev.*, 71, 525 (1971).
(20) E. Oppenheimer and B. Roth, unpublished data.

Table II. 5-(4-Hydroxybenzyl)pyrimidines via Mannich Condensations

no.	pyrimidine substituent 2	pyrimidine substituent 4	pyrimidine substituent 6	benzene substituent 3	benzene substituent 5	% yield[a]	rx solvent[b]	mp, °C	emp formula	anal.
3	NH_2	NH_2		OCH_3	OCH_3	35	A	265-267 dec	$C_{13}H_{16}N_4O_3$	C, H, N
4	NH_2	OH		OCH_3	OCH_3	33	B, C	149-153	$C_{13}H_{15}N_3O_4 \cdot H_2O$	N; C, H[c]
5[d]	OH	OH		OCH_3	OCH_3	68	D	241-242	$C_{13}H_{14}N_2O_5$	C, H, N
6	OH	OH	CH_3	OCH_3	OCH_3	78	B	259-262	$C_{14}H_{16}N_2O_5$	C, H, N
7[e]	OH	OH		i-C_3H_7	i-C_3H_7	33	E	272-279 dec	$C_{17}H_{22}N_2O_3$	C, H, N
8	NH_2	NH_2	CH_3	OCH_3	OCH_3	53	A	240 dec	$C_{14}H_{18}N_4O_3$	H, N; C[f]
9[g]	NH_2	NH_2	OH	OCH_3	OCH_3	72	D	225 dec	$C_{13}H_{16}N_4O_4 \cdot HCl$	C, H, N

[a] Yield of semipurified product, after extraction of nonhetero byproducts. [b] A, DMF; B, EtOH; C, dilute Me_2CO; D, β-methoxyethanol; E, dilute EtOH. [c] C: calcd, 52.87; found, 53.59. H: calcd, 5.80; found, 6.32. [d] This product was also prepared by a reverse Mannich reaction, from 11 plus 2,6-dimethoxyphenol under similar conditions; yield, 16% after three recrystallizations (β-methoxyethanol). [e] NMR (Me_2SO-d_6) δ 1.12 [d, 12, $(CHMe_2)_2$], 3.26 (septet, 2, $CHMe_2$), 3.38 (s, 2, Ar CH_2), 6.79 (s, 2, Ar), 7.03 (s, 1, Pyr 6 H), 7.25 (br s, 1, Ar OH), 10.5 (br, 1, NH), 11.0 (br, 1, NH). [f] C: calcd, 57.92; found, 57.32. [g] This compound was also prepared as the free base: yield 63%; mp 296–298 °C (ethylene glycol). Anal. ($C_{13}H_{16}N_4O_4$) C, H, N.

Compound **3** is rapidly metabolized in vivo and excreted as its glucuronide,[21,22] which militates against its use as a systemic antibacterial agent; furthermore, it is rapidly oxidized in neutral to alkaline media in the absence of an enzyme.

The 6-methyl analogue of **13** (**18**) is considerably less active as an antibacterial than its parent; in addition, it has only one-tenth the inhibitory activity against *E. coli* DHFR. This low activity is not unexpected. G. H. Hitchings[23] first observed in the early 1950's that 2,4-diamino-5-benzylpyrimidines prepared in his laboratory[24,25] were very active as antimalarials when 6-alkyl groups were present; however, removal of the 6-alkyl substituent had the rather remarkable effect of considerably depressing antimalarial activity but markedly increasing antibacterial activity. In the present case, a 6-methyl substituent did not increase antimalarial activity, as evidenced by the *P. berghei* enzyme data.

A 6-hydroxy derivative (**9**), more properly considered as a 6-aminoisocytosine, not surprisingly has no appreciable antibacterial activity. This compound is not ionized at physiological pH (pK_a values approximately 3.5 and 10.7), in contrast with **13**, which has a dissociation constant of 7.1.[26] The 3′,4′-dimethoxy analogue of **9** was shown earlier to have very low antibacterial activity.[2]

Trimethoprim methiodide (**19**) has only $^1/_{100}$ to $^1/_{1000}$ the antibacterial activity of its parent against various organisms in vitro, as shown in Table I. This cannot be explained on the basis of its cationic nature (pK_a = 13) and consequent supposed lack of penetration into bacterial cells, since its activity against *E. coli* DHFR is of a comparable low order of magnitude. If the assumption is made that **13** binds to *E. coli* DHFR in the manner of methotrexate,[27] the approach of the positively charged N-1 atom of **19** to Asp-27 in the postulated active-site region of the enzyme[27] would necessarily be altered, which might well result in crowding and lessening of the opportunity for benzylic hydrophobic interactions. The high pK_a in itself is probably not the causative factor. Many dihydrotriazines, for example, which have high dissociation constants (around 11) are very effective inhibitors of some dihydrofolate reductases.[28]

Experimental Section

All melting points were determined with calibrated thermometers, using either a Hoover or a Thiele tube melting point apparatus or a hot stage microscope. 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 A-60, XL-100, and T-60 spectrometers; chemical shifts are reported in parts per million (δ) from internal tetramethylsilane. Infrared spectra (IR) were obtained on a Beckman IR4 or Perkin-Elmer 267 grating spectrophotometer as KBr disks for solids.

2,6-Dimethoxy-4-[(*N*,*N*-dimethylamino)methyl]phenol (1a). a. The method of Coffield[15] was used to obtain **1a**: yield 93%; mp 83–85 °C (50% EtOH). Anal. ($C_{11}H_{17}NO_3$) C, H, N.

b. Compound **1a** was synthesized as the *hydrochloride* (**1a**·HCl) as follows: 2,6-Dimethoxyphenol (92 g, 0.6 mol) was slowly added to a mixture of 315 mL of 2 N HCl (0.63 mol), 135 g of 25% aqueous Me_2NH (0.75 mol), and 81 g (1 mol) of 37% HCHO. An exothermic reaction occurred and the mixture turned purple. An additional 45 g of Me_2NH solution (0.25 mol) was added, and the mixture was permitted to stand overnight at room temperature, followed by evaporation of the solvent. The resultant tan solid was washed well with Et_2O, followed by recrystallization from ca. 1.5 L of EtOH (Darco G60): yield 122 g (82%); mp 226–227 °C dec. Anal. ($C_{11}H_{17}NO_3$·HCl) C, H, N.

2,6-Dimethyl-4-[(*N*,*N*-dimethylamino)methyl]phenol Hydrochloride (14). The method of **1a** produced **14**: yield 92%; mp 230 °C dec (absolute EtOH). Anal. ($C_{11}H_{17}NO$·HCl) C, H, N.

2,6-Diethyl-4-[(*N*,*N*-dimethylamino)methyl]phenol (15). The method of **1a** yielded **15**: 93%; mp 62–63.5 °C (hexane). Anal. ($C_{13}H_{21}NO$) C, H, N.

5-[(Dimethylamino)methyl]uracil Hydrochloride (11). A mixture of uracil (**2c**; 11.2 g), formalin (13 g), 20% aqueous Me_2NH (42 mL), and 2 N HCl (100 mL) was heated on the steam bath until dissolved (2 days), evaporated to dryness, extracted with MeOH, and dried: yield 19 g of white product. Anal. ($C_7H_{11}N_3O_2$·HCl) C, H, N. This was used directly in the following reaction with 2,6-dimethoxyphenol (**12**; see Table II). The Mannich base could also be prepared in the absence of HCl by allowing a solution of 1 equiv each of uracil and formalin and 2 equiv of aqueous Me_2NH to stand at room temperature for a day, followed by evaporation and extraction with MeOH. The crude product was used directly in the next reaction.

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(26) B. Roth and J. Z. Strelitz, *J. Org. Chem.*, 34, 821 (1969).
(27) D. A. Matthews, R. A. Alden, J. T. Bolin, S. T. Freer, R. Hamlin, N. Xuong, J. Kraut, M. Poe, M. Williams, and K. Hoogsteen, *Science*, **197**, 452 (1977).
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General Method for the Preparation of 5-(*p*-Hydroxybenzyl)pyrimidines from Phenolic Mannich Bases and a 5-Unsubstituted Pyrimidine. The pyrimidine was dissolved in approximately 10 volumes of ethylene glycol and an equivalent amount of **1a** or its corresponding hydrochloride was added, along with NaOMe in 0.01–0.1 molar excess of the amount needed to neutralize the hydrochloride. The mixture was heated with stirring under N_2 to an internal temperature of approximately 140–160 °C for 3–4 h. Me_2NH was evolved, which was normally trapped in aliquots of standard H_2SO_4 in order to follow the course of the reaction. Some products crystallized upon cooling the reaction mixture. Others were isolated by stripping the glycol from the mixture under vacuum or by pouring the glycol solution into water. The latter method occasionally produced gummy products, where stripping the glycol did not. Extraction of the residue with ether or acetone removed bis(3,5-dimethoxy-4-hydroxyphenyl)methane (**10**) and related byproducts formed by self-condensation of the Mannich base. Further purification of the product was accomplished by recrystallization. Conversion to the hydrochloride salts prevented oxidation and discoloration of the phenolic product. Data on pyrimidines prepared by this method are listed in Table II. An example follows.

Method B. Method A. To a solution of 22 g (0.2 mol) of 2,4-diaminopyrimidine (**2a**) and 49.5 g (0.2 mol) of **1a**·HCl in 300 mL of ethylene glycol was added 11 g (0.203 mol) of NaOMe. This mixture was heated under N_2 with stirring to 150–160 °C, at which point Me_2NH was evolved. This was collected in standard sulfuric acid. After 3 h, 80% of the theoretical amount had been collected. The solvent was stripped off under high vacuum, and the residual oil was then washed well with water, followed by acetone. A tan precipitate was obtained, yield 23 g (42%). This was purified by recrystallizing twice from DMF, which yielded white plates, mp 265–267 °C (Table II). This material darkened on standing in a brown bottle; conversion to the hydrochloride obviated this problem: NMR (free base) (Me_2SO-d_6) δ 3.50 (s, 2, CH_2), 3.72 [s, 6, $(OMe)_2$], 5.68 (br s, 2, NH_2), 6.05 (br s, 2, NH_2), 6.53 (s, 2, Ar), 7.51 (s, 1, Pyr 6 H), 8.0 (br s, 1, Ar OH); UV (monoanion, 2 N NaOH) λ_{max} 288 nm (ϵ 10450); UV (monocation, 0.01 N HCl) λ_{max} 272.5 nm (ϵ 6170). The compound had two overlapping dissociation constants, so the UV spectrum of the neutral species was not obtained. The dissociation constants were calculated from spectra at pH 2, 7.31, 8.98, 10.08, and 14 by use of the Thamer equation,[29,30] which gave pK_a values of 7.36 and 10.17 using 0.1 N buffer solutions at 20 °C.

Method B. Compound **5** (1.0 g, 0.036 mol) was refluxed with 25 mL of $POCl_3$ for 2 h, followed by removal of excess of $POCl_3$ and treatment of the residue with ice–water and alkali. The precipitated chloro derivative weighed 0.42 g. This crude product (0.35 g) was heated with 15 mL of EtOH saturated with NH_3 in an autoclave at 160 °C for 9 h. The solvent was removed, and the residue was slurried in 40 mL of water. The product was extracted with $CHCl_3$–MeOH (3:1), which upon evaporation left a residue of 0.17 g. Recrystallization from EtOH plus an equivalent of hydrochloric acid produced white crystals which were identical in all respects with the product of method A (analysis, NMR, mass spectrum).

Bis(4-hydroxy-3,5-dimethoxyphenyl)methane (10). A mixture of 7.43 g (0.03 mol) of **1a**·HCl, 1.78 g (0.033 mol) of NaOMe, and 40 mL of ethylene glycol was heated at 140 °C for 1 h under a stream of N_2. The escaping gases were trapped in standard sulfuric acid in order to follow the rate of Me_2NH evolution. Within 1 h, 85% of the calculated amine had been evolved. The mixture was heated for another 2 h, and the glycol was removed in vacuo. The residual oil was triturated with water containing a few drops of HCl, which yielded a brown precipitate: 6 g; mp 108.5–110 °C (colorless after EtOH–H_2O); NMR (Me_2SO-d_6) δ 3.75 [s, 14, $(OMe)_4$, CH_2], 6.54 (s, 4, Ar), 8.05 (s, 2, Ar OH). Anal. ($C_{17}H_{20}O_6$) C, H.

3,4,5-Trimethoxybenzyltrimethylammonium Iodide (16). 3,4,5-Trimethoxybenzyldimethylamine[31] was treated with an excess of MeI in MeOH to produce the methiodide, mp 228–232 °C (Me_2CO–MeOH). Anal. ($C_{13}H_{22}INO_3$) C, H, N, I.

3,4,5-Triethoxybenzyltrimethylammonium Tosylate (17). To 4.8 g (0.02 mol) of 3,4,5-triethoxybenzyl alcohol in 100 mL of dry Et_2O was added 3.8 g (0.02 mol) of tosyl chloride. The mixture was cooled to −40 °C and a suspension of approximately 3 g of powdered KOH in Et_2O was added in portions. The temperature was allowed to rise to −10 °C during this process, after which the mixture was stirred for 5 h. A solution of 15 g of trimethylamine in 100 mL of Et_2O was then added, which resulted in a rapid precipitation. After 1 day, the solid was isolated and washed with Et_2O: yield 5.5 g; mp 196–197 °C (EtOH–Et_2O). Anal. ($C_{23}H_{35}NO_6S$) C, H.

2,4-Diamino-5-(3,4,5-trimethoxybenzyl)pyrimidine (Trimethoprim, 13).[2] Compound **3** (3.5 g, 0.0127 mol) was dissolved in 50 mL of dry Me_2SO by heating to 50 °C with stirring. The mixture was then cooled to room temperature, and 0.69 g (0.0127 mol) of NaOMe was added, under an atmosphere of N_2. When this was dissolved, 1.80 g (0.0127 mol) of MeI was added. The mixture was allowed to stand at room temperature under N_2 for 4 h and then neutralized with AcOH. The Me_2SO was removed under vacuum, and the residue was extracted with NaOH to remove residual starting material. The insoluble product weighed 2.44 g (66%): mp 197–199 °C (EtOH); NMR (Me_2SO-d_6) δ 3.57 (s, 2, CH_2), 3.64 (s, 3, 4-OMe), 3.74 [s, 6, 3,5-$(OMe)_2$], 5.73 (br s, 2, NH_2), 6.10 (br s, 2, NH_2), 6.58 (s, 2, Ar), 7.57 (s, 1, Pyr 6 H). This product was identical in all respects with that of ref 2. Anal. ($C_{14}H_{18}N_4O_3$) C, H, N.

2,4-Diamino-5-(3,4,5-trimethoxybenzyl)-6-methylpyrimidine (18). Compound **8** was methylated by the procedure used to prepare **13**: yield 44%; mp 190–192 °C (EtOH). Anal. ($C_{15}H_{20}N_4O_3$) C, H, N.

2,4-Diamino-5-(3,4,5-trimethoxybenzyl)-1-methylpyrimidinium Iodide (19). A solution of 5.8 g (0.02 mol) of **13** and 2.86 g (0.02 mol) of MeI in 25 mL of dry Me_2SO was allowed to stand at room temperature in a stoppered flask for 3 days. The solvent was removed in vacuo, and the residue was purified by recrystallization from EtOH: mp 234–235 °C; UV (0.01 N HCl) λ_{max} 223 nm (ϵ 43600), 273 (6950). This spectrum is similar to that of **13** cation,[19] with a 4-nm bathochromic shift of the long-wavelength peak. The UV was essentially unchanged at pH 12; at pH 13, λ_{max} at 273 nm decreased in intensity by 33%, indicating proton loss and probable decomposition in the pH 13–14 region. NMR (Me_2SO-d_6) δ 3.50 (s, 3, N_1-Me), 3.63 (s, 5, CH_2, 4-OMe), 3.77 [s, 6, 3,5-$(OMe)_2$], 6.67 (s, 2, Ar), 7.70 (s, 1, Pyr 6 H), 7.5–8.3 [v br, 4, $(NH_2)_2$]. Anal. ($C_{15}H_{21}IN_4O_3$) C, H, N.

2,4-Diamino-5-(4-hydroxy-3,5-dimethoxybenzyl)-1-methylpyrimidinium Iodide (20). A solution of **3** (2.76 g, 0.01 mol) and MeI (1.42 g, 0.01 mol) in 40 mL of Me_2SO was allowed to stand at room temperature for 1 h, at which time completion of the reaction was indicated by TLC. The solvent was removed, and the product was crystallized from EtOH: mp 238–239 °C dec; UV (0.1 N HCl) λ_{max} 222 nm (ϵ 35700), 273 (6110); UV (0.01 N NaOH) sh 274 nm (ϵ 8800). The increase in λ_{max} at pH 12 indicates ionization of the 4-OH in the benzene ring: NMR (Me_2SO-d_6) δ 3.50 (s, 3, N_1-Me), 3.60 (s, 2, CH_2), 3.76 [s, 6, 3,5-$(OMe)_2$], 6.62 (s, 2, Ar), 7.53 (br s, 1, NH), 7.67 (s, 1, Pyr 6 H), 7.77 (br s, 2, NH_2), 8.08 (br s, 1, NH), 8.17 (s, 1, Ar OH). Anal. ($C_{14}H_{19}IN_4O_3$) C, H, N.

2,4-Diamino-5-(3,4,5-trimethoxybenzyl)-6(1*H*)-pyrimidinone (21). Compound **9** was methylated with 1 equiv of MeI, cf. **13**, which produced 30% of **21** plus recovered starting material. No other methylated products were isolated: mp 272–273 °C (EtOH–Et_2O); NMR (Me_2SO-d_6) δ 3.47 (s, 2, CH_2), 3.60 (s, 3, 4-OMe), 3.70 [s, 6, 3,5-$(OMe)_2$], 5.70 (br s, 2, NH_2), 6.00 (br s, 2, NH_2), 6.63 (s, 2, Ar), 9.90 (br s, 1, NH). The IR spectrum was identical with that of the product made from cyanoacetic ester plus trimethoxybenzaldehyde, followed by reduction and condensation with guanidine.[32] Anal. ($C_{14}H_{18}N_4O_4$) C, H, N.

Methylation of 2-Amino-5-(4-hydroxy-3,5-dimethoxybenzyl)-4(3*H*)-pyrimidinone (4). Methylation of **4** with 1 equiv of MeI and 1 equiv of NaOMe in Me_2SO produced a mixture of methylation products, as determined by TLC, NMR, and mass

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(31) A. Uffer and E. Schlittler, *Helv. Chim. Acta*, **31**, 1397 (1948).
(32) The latter reaction was carried out by R. Cresswell and J. Mentha in these laboratories.

spectrometry. Analysis of the crude product by mass spectrometry at 70 eV showed m/e 277, 291, 305, and 319, which are molecular ions for **4** and mono-, di-, and trimethylated **4**. A spectrum at 20 eV showed the ratio of molecular ions to be 1.7:8.2:10.0:2.8, respectively. The spectrum of trimethoprim (**13**) has prominent peaks at m/e 290 (M^+), 275 ($M^+ - CH_3$), 259 ($M^+ - OCH_3$), and 123. A high-resolution mass spectrum showed m/e 123 to have the compositon $C_5H_7N_4$, corresponding to the pyrimidomethylene portion of the molecule. The use of defocused metastable ions in the first field-free region showed that the m/e 290, 275, and 259 all yielded m/e 123. This information can be used to analyze how much of **4** is monomethylated in the benzene vs. pyrimidine rings, since m/e 291 should yield m/e 124 or m/e 138, respectively. A DADI metastable spectrum of m/e 291 produced a peak from the transition m/e 291 → 138, but none for m/e 291 → 124. The accurate mass of m/e 291 and 138 was that expected for $C_{14}H_{17}N_3O$ and $C_6H_8N_3O$, indicating that, within the limits of detection, monomethylation occurs on the pyrimidine portion of the molecule.

Biological Assays. Antibacterial assays were carried out using methods described in ref 3. Assays of dihydrofolate reductase inhibition were carried out using partially purified enzyme from rat liver[33] and affinity-column-purified enzyme from *E. coli*.[34] The assay method was that of Baccanari et al.,[34] in phosphate buffer. Compounds were incubated for 5 min with enzyme and buffer at 37 °C, followed by the addition of NADPH and then FH_2 to initiate the reactions. The inhibitory potencies are reported as the concentration required to reduce the reaction rate by 50% (IC_{50}), as calculated from titrations of three to six concentrations of compound, or as the percent inhibition observed at the highest concentration tested. Examination of data collected over a period of 16 years by several operators showed the 90% tolerance limits for values reported to be ±52.5%.

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2,4-Diamino-5-benzylpyrimidines and Analogues as Antibacterial Agents. 3. C-Benzylation of Aminopyridines with Phenolic Mannich Bases. Synthesis of 1- and 3-Deaza Analogues of Trimethoprim

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Electrophilic substitution of 2,4-diaminopyridine by 2,6-disubstituted-4-[(*N*,*N*-dimethylamino)methyl]phenols and by halogens (bromine and fluorine) produces 3-benzyl and 3-halo derivatives, plus a small amount of disubstitution at the 3,5 positions. Treatment of a 2,4-diamino-3-halopyridine with phenolic Mannich bases gives 5- and N-benzylation. 2,4-Diamino-3-bromo-5-(4-hydroxy-3,5-dimethoxybenzyl)pyridine was methylated on the phenolic group in good yield and dehalogenated to produce 3-deazatrimethoprim [2,4-diamino-5-(3,4,5-trimethoxybenzyl)pyridine]. This compound is about 300-fold less active as an inhibitor of *Escherichia coli* dihydrofolate reductase than is trimethoprim. 2,6-Diaminopyridine is very readily dibenzylated at the 3,5 positions, as well as on an amino group, by a phenolic Mannich base; use of a fourfold excess of the pyridine provided a 3-benzylated 2,6-diaminopyridine in 50% yield; this was inactive as an inhibitor of dihydrofolate reductase at 10^{-4} M. 2-Amino- and 4-aminopyridines do not produce C-benzylated products under the conditions reported here.

The significance of trimethoprim (**1**)[1] as a broad-spectrum antibacterial agent[2] made it of importance to investigate related heterocycles. This paper describes the synthesis and biological activities of the 3-deaza- (**31d**) and 1-deaza-4′-demethyl (**4a**) analogues of trimethoprim and some derivatives.

Chemistry. One synthetic route to trimethoprim involves the condensation of a phenolic Mannich base with 2,4-diaminopyrimidine to produce a 5-(substituted-benzyl)pyrimidine.[3] It seemed plausible that such a condensation might succeed with aminopyridines, although a variety of products were possible. Since 2,6-diaminopyridine (**2**) and a dicarbomethoxy intermediate to 2,4-diaminopyridine (**17**) are commercially available, investigation of this route seemed particularly desirable.

Scheme I illustrates the reactions of 2,6-disubstituted pyridines with the Mannich bases from two phenols. When heated in a solvent such as ethylene glycol to about 120–150 °C, such Mannich bases lose dimethylamine to produce an intermediate methene quinone, which is polarized positively at the methene carbon. This then can attack an electron-rich center, as is expected to be present at the 3 and 5 positions of **2**. Use of molar equivalents of **2** and **3a** actually produced 3,5-disubstitution chiefly; introduction of one benzylic moiety apparently aids materially in facilitating 5-substitution. The desired monosubstituted benzyl derivative, **4a**, was produced by using an excess of the pyridine. Small amounts of an N-benzylated product, **6a**, were also obtained.

Somewhat surprisingly, it was found that the Mannich base from a 2,6-dialkylphenol (**3b**) reacted about as successfully with 2,6-diaminopyridine as did the 2,6-dimethoxy analogue. This is in marked contrast to the pyrimidine series, where this was not the case.[3] In fact, by using equivalent amounts of **2** and **3b**, a reasonable yield of **4b**

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