2,4-Diamino-5-benzylpyrimidines and Analogues as Antibacterial Agents. 5. 3',5'-Dimethoxy-4'-substituted-benzyl Analogues of Trimethoprim[1-3]

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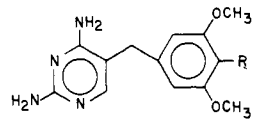
Forty trimethoprim analogues in which the para substituent in the benzene ring was varied were prepared for antibacterial evaluation. All were very potent inhibitors of *Escherichia coli* dihydrofolate reductase. The similarity of their inhibitory activities strongly suggested that the side chains beyond the first two atoms were not in contact with the enzyme. However, among 38 ether derivatives which varied widely in their bulk and lipophilicity, very few approached trimethoprim in their broad-spectrum in vitro antibacterial activity. The 4'-methyl and 4'-ethyl analogues and the allyloxy and γ-chloropropoxy ethers had activities fairly close to that of trimethoprim. The two ethers were chosen for further evaluation in vivo. Neither compound quite matched trimethoprim in efficacy in mice, and their half-lives, as well as that of the β-methoxyethoxy analogue, were found to be shorter in dogs.

The synthesis of trimethoprim (1),[4] a broad-spectrum

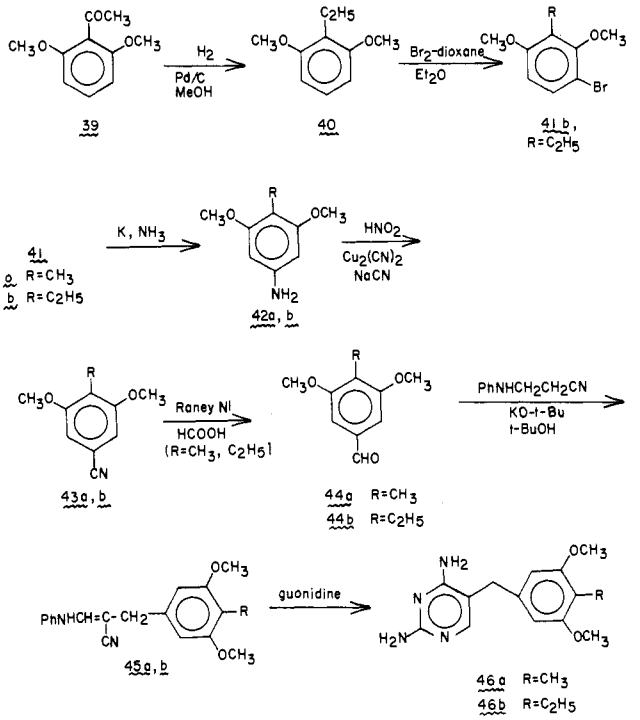


1, R = OCH_3 (trimethoprim)
2, R = OH

antibacterial agent,[5-7] by a Mannich route, which produces 2,4-diamino-5-(3',5'-dimethoxy-4'-hydroxybenzyl)pyrimidine (2) as an intermediate, is described in part 2 of this series.[8] Compound 2 was found to be almost as potent an inhibitor of *E. coli* dihydrofolate reductase (DHFR) as is trimethoprim; however, it lacked the high degree of specificity for bacterial, as opposed to mammalian, DHFR.[8,9] Furthermore, it was rapidly metabolized.[10]

The effect of the 4'-methoxy group of trimethoprim of apparently increasing activity slightly against a bacterial enzyme and decreasing it considerably against a mammalian congener strongly invited the preparation of other 4'-substituted derivatives. The availability of 2 as described above, as well as by demethylation of trimethoprim,[11] made the preparation of a series of 4'-ethers particularly attractive. This paper describes the synthesis and

Scheme I



biological properties of such derivatives, as well as two 4'-alkyl analogues.

It was of interest to prepare derivatives of varying size, polarity, and lipophilicity for both practical and theoretical studies. Such changes might alter the species specificity against various DHFR enzymes, for example by increasing antimalarial activity relative to the antibacterial activity.[9] They might also alter the absorption or the metabolism of the drug, which could give improved pharmacokinetic properties in some mammalian species.[12,13]

Chemistry. The ethers prepared here (compounds 3-38) are described in Table I. Most of these compounds

(1) Roth, B.; Strelitz, J. Z. U.S. Patent 3692787, 1972.
(2) Roth, B.; Strelitz, J. Z. "Abstracts of Papers", 154th National Meeting of the American Chemical Society, Chicago, IL, 1967; American Chemical Society: Washington, DC, 1967, Abstr MEDI 18.
(3) Roth, B.; Aig, E.; Lane, K.; Rauckman, B. S. *J. Med. Chem.* **1980**, *23*, 535.
(4) Roth, B.; Falco, E. A.; Hitchings, G. H.; Bushby, S. R. M. *J. Med. Pharm. Chem.* **1962**, *5*, 1103.
(5) Bushby, S. R. M.; Hitchings, G. H. *Br. J. Pharmacol. Chemother.* **1968**, *33*, 72.
(6) "Evaluations on New Drugs: Trimethoprim-Sulfamethoxazole" **1971**, *1*, 7, and references therein.
(7) "Symposium on Trimethoprim-Sulfamethoxazole", *J. Infect. Dis.* **1973**, *128*, supplement (Nov).
(8) Roth, B.; Strelitz, J. Z.; Rauckman, B. S. *J. Med. Chem.* **1980**, *23*, 379.
(9) Burchall, J. J.; Hitchings, G. H. *Mol. Pharmacol.* **1965**, *1*, 126.
(10) Schwartz, D. E.; Vetter, W.; Englert, G. *Arzneim.-Forsch.* **1970**, *20*, 1867. U.S. Patent 3684810, 1972.
(11) Brossi, A.; Grunberg, E.; Hoffer, M.; Teitel, S. *J. Med. Chem.* **1971**, *14*, 58.
(12) White, G. *Chem. Ind. (London)* **1975**, *14*, 589.
(13) (a) Nielsen, P.; Rasmussen, F. *Acta Pharmacol. Toxicol.* **1975**, *37*, 309. (b) Nielsen, P.; Rasmussen, F. *Zentralbl. Veterinaermal., Reihe A*, **1975**, *22*, 562.

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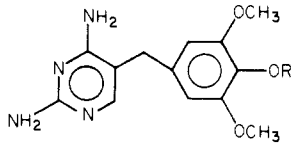
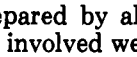
Table I. 2,4-Diamino-5-(3',5'-dimethoxy-4'-substituted-benzyl)pyrimidines



no.	benzene 4'-substituent (OR)	yield,[a] %	recrystn solvent[b]	mp, °C	formula	anal.
3	OC_2H_5	69	A	185-187	$C_{15}H_{20}N_4O_3$	C, H, N
4	OC_3H_7-*n*		B	171-172	$C_{16}H_{22}N_4O_3$	C, H, N
5	OC_3H_7-*i*	58	C	195-196	$C_{16}H_{22}N_4O_3$	C, H, N
6	$OCH_2CH{=}CH_2$	67	D	193-194	$C_{16}H_{20}N_4O_3$	C, H, N
7	OC_4H_9-*i*	28	E	185-186	$C_{17}H_{24}N_4O_3$	C, H, N
8	OC_4H_9-*s*	30	D	176-177	$C_{17}H_{24}N_4O_3$	C, H, N
9[c]	OC_5H_{11}-*n*		F	162-165	$C_{18}H_{26}N_4O_3$	C, H, N
10	OC_6H_{13}-*n*	69	D	158-159	$C_{19}H_{28}N_4O_3$	C, H, N
11	OC_8H_{17}-*n*	35	A	163	$C_{21}H_{32}N_4O_3$	C, H, N
12	OCH_2CH_2OH	4	F	197-198	$C_{15}H_{20}N_4O_4$	C, H, N
13[d]	$OCH_2CH_2OCH_3$	90	G	151-153	$C_{16}H_{22}N_4O_4$	C, H, N
14	OCH_2CH_2Cl	25	D	193-194	$C_{15}H_{19}ClN_4O_3$	C, H, N
15	$OCH_2CH_2N(CO)_2C_6H_4(1,2)$	13	F[e]	197-198	$C_{23}H_{23}N_5O_5$	C, H, N
16[f]	$OCH_2CH_2NH_2$	31	F	215.5-217	$C_{15}H_{21}N_5O_3 \cdot {}^1/_3H_2O$	C, H, N
17	$OCH_2CH_2N(CH_2CH_2)_2O$	39	C	173-174	$C_{19}H_{27}N_5O_4$	C, H, N
18	$OCH_2COOC_2H_5$	34	C	153-154	$C_{17}H_{22}N_4O_5$	C, H, N
19[g]	OCH_2COOH	85[g]	H	288-289	$C_{15}H_{18}N_4O_5 \cdot H_2O$	C, H, N
20	$OCH_2CH_2CH_2OH$		F	199-200	$C_{16}H_{22}N_4O_4$	C, H, N
21	$OCH_2CH_2CH_2Cl$		A	163-164	$C_{16}H_{21}ClN_4O_3$	C, H, N
22	$OCH_2CH_2CH_2N(CO)_2C_6H_4(1,2)$	56	I	260-264	$C_{24}H_{25}N_5O_5 \cdot HCl$	C, H, N
23[f]	$OCH_2CH_2CH_2NH_2$	79	A	276-279	$C_{16}H_{23}N_5O_3 \cdot 2HCl$	C, H, N, Cl
24	$OCH_2CH_2CH_2N$-c-$(CH_2)_5$	47	C	164-165	$C_{21}H_{31}N_5O_3$	C, H, N
25	$O(CH_2)_3N(CH_2CH_2)_2NCH_3$	23	E	176-177	$C_{21}H_{32}N_6O_3$	C, H, N
26	$OC_6H_4NO_2(4)$	83	J	251.5-252.5	$C_{19}H_{19}N_5O_5$	C, H, N
27[h]	$OC_6H_4NH_2(4)$	84	I	304-308 dec	$C_{19}H_{21}N_5O_3 \cdot 2HCl$	C, H, N, Cl
28[i]	$OC_6H_4NHCOCH_2Br(4)$	67	I	276-278 dec	$C_{21}H_{22}BrN_5O_4 \cdot HBr$	C, H, N, Br
29	$OCH_2C_6H_5$	33	D	162-164	$C_{20}H_{22}N_4O_3$	C, H, N
30	$OCH_2C_6H_4NO_2(4)$	68	C	219-221	$C_{20}H_{21}N_5O_5$	C, H, N
31	$OCH_2C_6H_2(OCH_3)_3(3,4,5)$	40	A	164-165	$C_{23}H_{28}N_4O_6$	C, H, N
32	$O(CH_2)_4N(CO)_2C_6H_4(1,2)$	51	C	182-185	$C_{25}H_{27}N_5O_5$	C, H, N
33[j,k]	$O(CH_2)_6N(CO)_2C_6H_4(1,2)$	33	E	135-136	$C_{27}H_{31}N_5O_5 \cdot 0.25C_4H_8O_2$[k]	C, H, N
34[j,f]	$O(CH_2)_6NH_2$	13	A	260-265 dec	$C_{19}H_{29}N_5O_3 \cdot 2HCl \cdot 1.5H_2O$	C, H, N
35[j,l]	$O(CH_2)_5COOCH_3$	24	K	125-126	$C_{20}H_{28}N_4O_5 \cdot 0.5C_3H_6O$[l]	C, H, N
36[j,g]	$O(CH_2)_5COOH$	64	G	95-100	$C_{19}H_{26}N_4O_5 \cdot 0.5H_2O$	C, H, N
37[m]	$OCH_2CHOHCH_2OH$		F	158-159	$C_{16}H_{22}N_4O_5$	C, H, N
38[n]		26	A	170-171	$C_{16}H_{20}N_4O_4$	C, H, N

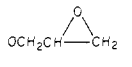
[a] Yield of semipurified product, after extraction of residual phenol; usually the yield of a single run. [b] A, 95% EtOH; B, 25% Me_2CO; C, absolute EtOH; D, 50% EtOH; E, EtOAc; F, MeOH; G, H_2O; H, reprecipitated from alkali; I, 90% EtOH; J, $MeOCH_2CH_2OH/H_2O$; K, Me_2CO. [c] This compound was prepared by Paul Stenbuck in this laboratory. [d] Belgian Patent 812 375 (1974). [e] Chromatographed on silica gel, $CHCl_3$/MeOH, 9:1, before recrystallization. [f] From the phthalimide by the method of Sheehan, C.; Chapman, D. W.; Roth, R. W. *J. Am. Chem. Soc.* 1952, *74*, 3822. [g] This compound was prepared by the hydrolysis of the corresponding ester. [h] By reduction of 26 with Pd/C, NH_2NH_2, according to Balcom, D.; Furst, A.; *J. Am. Chem. Soc.* 1953, *75*, 4334; Mosby, W. L.; *J. Org. Chem.* 1959, *24*, 421. [i] From 27 by the method of Baker, B. R.; Santi, D. V.; Howard, J. K.; Shapiro, H. S.; Jordaan, J. H. *J. Heterocycl. Chem.* 1966, *3*, 425. [j] This compound was prepared by Dr. Lee Kuyper in this laboratory. [k] NMR (Me_2SO-d_6) δ 1.44 (m, 8 H), ~3.65 (m, 4 H), 3.51 (s, 2 H), 3.68 (s, 6 H), 5.63 (s, 2 H), 6.02 (s, 2 H), 6.51 (s, 2 H), 7.50 (s, 1 H), 7.83 (s, 4 H); 0.25EtOAc [1.15 (t, 3 H), 1.95 (s, 3 H), 3.98 (q, 2 H)]. [l] NMR (Me_2SO-d_6) δ 1.48 (m, 6 H), 2.31 (m, 2 H), 3.52 (s, 2 H), 3.58 (s, 3 H), 3.70 (s, 6 H), 3.77 (m, 2 H), 5.74 (s, 2 H), 6.14 (s, 2 H), 6.54 (s, 2 H), 7.50 (s, 1 H); 0.5Me_2CO[2.09 (s, 6 H)]. [m] This compound was prepared by Dr. Susan Daluge in this laboratory, from **6**, using OsO_4-catalyzed oxidation by the method of van Rheenen, V.; Kelly, R. C.; Cha, D. Y.; *Tetrahedron Lett.* 1976, 1973. [n] This compound was prepared by Paul Skonezny by alkylation of the 4'-phenol with epibromohydrin in DMF, in the presence of K_2CO_3 and Et_4NBr. The product was purified by column chromatography (silica gel) to give 26% of **38**.

were prepared by alkylation or arylation of **2**. Other methods involved well-known procedures, which are indicated in the footnotes of the table. The steric hindrance offered by the flanking methoxy groups, the ready oxidation of **2** in alkali, and competition for alkylation offered by the N-1 function often resulted in somewhat low yields of alkylation product.[8] The large-scale preparation of any given member of the series was sometimes better accomplished by alkylation of 3,5-dimethoxy-4-hydroxybenzaldehyde, followed by condensation with β-anilinopropionitrile or similar derivative and then guanidine.[14] Such a procedure is described for the 4'-(allyloxy) derivative (**6**).

The 4'-alkyl analogues (**46a**,[15] **46b**) were prepared by a route shown in Scheme I. The key reaction in this synthesis of the 4'-methyl and 4'-ethyl derivatives is a "benzyne-type" reaction (**41** and **42**) in which **41**, a *m*-bromoalkylbenzene, is converted to a para-substituted

(14) Cresswell, R. M.; Mentha, J. W.; Seaman, R. L. U.S. Patent 3956327, 1976.
(15) Grunberg, E.; Hoffer, M. German Patent 2303838, 1973; *Chem. Abstr.* 1973, *79*, 115 620a.

Table II. Comparison of Dihydrofolate Reductase Inhibition and in Vitro Antibacterial Activity of 2,4-Diamino-5-(3′,5′-dimethoxy-4′-substituted-benzyl)pyrimidines

		$I_{50} \times 10^8$ vs. DHFR, M		ratio, MIC of compd/MIC of TMP[a] vs. bacteria[b]							
no.	benzene 4′-substituent	*E. coli*	rat liver	*E. coli*	*Staph. aur.*	*Kleb. pneum.*	*Sal. typh.*	*Shig. dys.*	*Entero. aer.*	*Pr. mir.*	*Pr. vulg.*
1	OCH_3	0.5-0.7[c]	26 000-43 000[c]	1	1	1	1	1	1	1	1
2	OH	1.1	9 200	1	1	1	1	1	0.3	3	1
3	OC_2H_5	0.75	62 000	10	3	3	10	10	10	30	10
4	OC_3H_7-*n*	1.3	25 000	10	3	10	3	10	10	10	30
5	OC_3H_7-*i*	2.4	9% @ 40 000	30	30	10	10	10	30	30	10
6	$OCH_2CH{=}CH_2$	0.52	48 000	10	3	3	10	10	10	10	10
7	OC_4H_9-*i*	2.0		100	10	30	30	30	100	>30	30
8	OC_4H_9-*s*	1.6		30	10	30	30	10	30	>30	30
9	OC_5H_{11}-*n*	1.5	27 000	30	10	30	30	30	30	100	100
10	OC_6H_{13}-*n*	0.42	20 000-30 000	30	3	10	100	30	30	30	100
11	OC_8H_{17}-*n*	0.4, 0.8		1000	30	100	300	300	300	30	100
12	OCH_2CH_2OH	2.2	>40 000	3	3	10	3	3	10	10	10
13	$OCH_2CH_2OCH_3$	1.8	16% @ 40 000	10	3	10	10	10	3	10	10
14	OCH_2CH_2Cl	1.5	60 000	3	10	10	10	10	10	10	10
15	$OCH_2CH_2N(CO)_2C_6H_4(1,2)$	1.8		100	3	300	300	100	1000	>30	>30
16	$OCH_2CH_2NH_2$	2.2	38% @ 40 000	100	300	300	300	100	300	>30	>30
17	$OCH_2CH_2N(CH_2CH_2)_2O$	1.4	>44 000	10	3	3	10	10	10	>30	>30
18	$OCH_2COOC_2H_5$	1.7	12% @ 40 000	100	100	30	100	100	100	>30	>30
19	OCH_2COOH	32, 16	20% @ 22 400	100	100	100	100	300	300	>30	>30
20	$OCH_2CH_2CH_2OH$	0.42	24% @ 10 000	10	3	10	10	3	30	10	10
21	$OCH_2CH_2CH_2Cl$	0.5	25 000	3	1	3	3	3	1	1	3
22	$O(CH_2)_3N(CO)_2C_6H_4(1,2)$	1.8	6 900	300	1	>100	100	300	300	>30	>100
23	$OCH_2CH_2CH_2NH_2$	4.6	39% @ 10 000	10	30	10	10	10	10	>30	30
24	$OCH_2CH_2CH_2N$-c-$(CH_2)_5$	4.6	20% @ 40 000	30	30	10	10	10	30	30	30
25	$O(CH_2)_3N(CH_2CH_2)_2NCH_3$	2.1	36% @ 40 000	100	100	30	30	30	100	>30	>30
26	$OC_6H_4NO_2(4)$	0.78	5 000	100	10	100	300	100	300	>30	>100
27	$OC_6H_4NH_2(4)$	0.63	8% @ 1 000	30	10	30	30	100	100	30	3
28	$OC_6H_4NHCOCH_2Br(4)$	0.4,[d] 0.7	37% @ 29 000[d]	100	10	100	100	300	100	>30	>30
29	$OCH_2C_6H_5$	1.4	4 700	30	10	100	100	100	100	30	30
30	$OCH_2C_6H_4NO_2(4)$	0.8	23% @ 2 000	30	10	30	30	30	30	>30	30
31	$OCH_2C_6H_2(OCH_3)_3(3,4,5)$	2.0	1 500	300	30	30	300	100	300	>30	100
32	$O(CH_2)_4N(CO)_2C_6H_4(1,2)$	2.8	5 400	500	1	>100	200	500[h]	100	>10	>100
33	$O(CH_2)_6N(CO)_2C_6H_4(1,2)$	4.0	25% @ 2 400	300	10	>300	300	1000	1000	>30	>100
34	$O(CH_2)_6NH_2$	1.5	56 000	30	0.1	100	10	30	300	30	300
35	$O(CH_2)_5COOCH_3$	6.2	110 000	1000	10	>1000	300	1000	1000	>100	>100
36	$O(CH_2)_5COOH$	2.0	29 600	30	10	100	100	30	100	30	300
37	$OCH_2CHOHCH_2OH$	1.4	32% @ 27 400	30	10	100	100		100	>30	>100
38		1.4	23% @ 16 000	10	3	30	30	30[h]	30	30	30
46a	CH_3	0.74	12 000	3	1	3	3	3	3	3	3
46b	C_2H_5	0.99[e]	20 000[f]	3	1	3	3	3	3	3	3
47[g]	H	5.6	9 000	3	10	10	1	3[h]	10	3	3
48[g]	OC_4H_9-*n*	1.0	58 000	30	3	10	30	30	3	10	30

[a] MIC = minimum inhibitory concentration, determined as micrograms per milliliter. TMP = trimethoprim. Ratios greater than 1 indicate an activity less than that of trimethoprim. Typical MIC values (μg/mL) for TMP with the organisms listed are 0.1-0.3, 0.3-1, 0.03-0.1, 0.03-0.1, 0.1, 0.3-1.0, 3-10, and 1-10, respectively. [b] *Escherichia coli* CN 314, *Staphylococcus aureus* CN 491, *Klebsiella pneumoniae* CN 3632, *Salmonella typhi* CN 512, *Shigella dysenteriae* CN 1513, *Enterobacter aerogenes* 2200/86, *Proteus mirabilis* S 2409, and *Proteus vulgaris* CN 329. [c] Range of six determinations over a period of time. [d] This compound, a potential irreversible inhibitor, exhibited no irreversible inhibition of this enzyme. [e] Average of three determinations. [f] Average of two determinations. [g] Reference 4. [h] *Shigella flexneri*.

aniline. The use of potassium, rather than sodium,[16] in this reaction is required for high yields (80 vs. 20%, approximately). We previously had made many attempts to prepare the related intermediate, 3,5-dimethoxy-4-methylbenzoic acid, by disulfonation of *p*-methylbenzoic acid, followed by KOH fusion and alkylation. This reaction is extremely capricious. Oxidation of the methyl group often occurs; in our hands, only about one out of ten reactions was successful.

Biological Activity and Discussion

Comparative Inhibition of DHFR Enzymes. Table II depicts the inhibition shown by these compounds against DHFR from *E. coli* and rat liver and the in vitro antibacterial activities against certain bacterial species. Data on the 3′,5′-dimethoxy-4′-unsubstituted-benzyl analogue of TMP (**47**) are also included for comparison in evaluating the role of the 4′-substituents.

All of the ethers of Table II are very potent inhibitors of *E. coli* DHFR. Furthermore, with few exceptions, the compounds all have I_{50} values between 0.5 and 2×10^{-8} M, despite the fact that they vary widely in bulk and polarity. The only ether with significantly lower activity is **19**. The fact that such a diversity in side chains results in such similarity in effect leads one to conclude that the side chain beyond the 4′-methoxy functionality lies outside the hydrophobic cleft of DHFR and makes very little useful contact with the enzyme.

To date no report has appeared on the X-ray crystallography of trimethoprim in complex with a DHFR species.[17] Matthews and co-workers have established the

(16) Doyle, F. P.; Naylor, J. H. C.; Waddington, H. R. J.; Hanson, J. C.; Thomas, G. R. *J. Chem. Soc.* **1963**, 497.

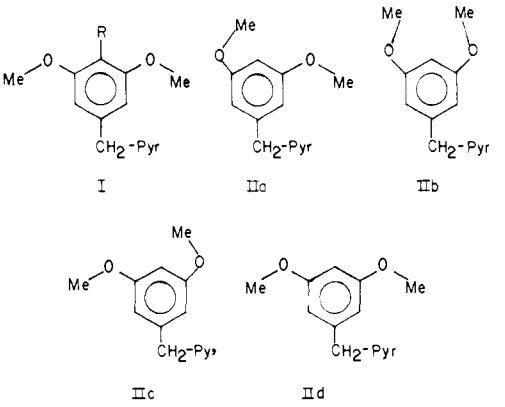
structures of the *E. coli* DHFR–methotrexate binary complex[18] and the *Lactobacillus casei* DHFR–methotrexate–NADPH ternary complex[19a,b] by X-ray crystallography. Cayley et al.[20] have investigated the conformation of TMP in complex with DHFR by some elegant NMR techniques. By making the assumption that the pyrimidine ring of TMP is bound at the same site as the pyrimidine ring of methotrexate in DHFR, they were able to deduce two possible conformations for TMP. It is possible by use of their deductions to place a model of TMP in the cleft of a DHFR model so that a chain attached to the 4′-methoxy function of TMP would actually extend out into solution.

Compound **19**, which has an anionic carboxy function attached directly to the 4′-methoxy group, is less active than is TMP as an inhibitor of the *E. coli* enzyme by a factor of 30 or more. The hydration sheath surrounding its ionized center could interfere with hydrophobic bonding between inhibitor and protein, due to its proximity to the benzene ring. In comparison, compound **36**, a carboxypentyloxy derivative, has its charged center sufficiently removed from the ring that it does not cause this interference to any appreciable extent. Compounds **16** and **23–25**, which have positively charged centers two to three atoms away from the ether oxygen, are slightly less active than the other compounds of the table, probably because of hydration interference again.

A recent report by Kompis et al.[21] on a series of 4′-carbon-substituted TMP analogues provides additional supporting data for these conclusions. Their 4′-carboxy and 4′-(aminomethyl) derivatives, for example, both of which have their charged centers within two atoms of the benzene ring, were found to be considerably less active than **19** or **16** ($I_{50} \times 10^8$ = 150 and 60, respectively, vs. *E. coli* DHFR).

These findings suggest that the 4′-methoxy substituent of TMP lies near the edge of a hydrophobic cleft and that it may assist in close packing near the surface. However, the role of this substituent may be more complex. We prepared the 4′-alkyl analogues **46a** and **46b** for comparison with TMP and its demethyl and demethoxy derivatives **2** and **47**.[4] Data on the 4′-halo analogues have also been reported.[22] These substituent effects, listed in decreasing order of activity against *E. coli* DHFR ($I_{50} \times 10^8$) are as follows: Cl, 0.3; OCH_3, 0.5; CH_3, 0.74; Br, 0.9; C_2H_5, 0.99; OH, 1.1; F, 1.9; I, 2.4; H, 5.6. Differences in I_{50} values less than ±50% are, in general, not significant. However, the differences which do occur are apparently related to the size of the substituent, rather than to the polarity or lipophilicity. The 4′-unsubstituted derivative (**47**) is significantly less active than the others (one-tenth that of TMP); the bulky 4′-iodo analogue, at the other end of the spectrum of size, is also significantly less active than the rest. This information suggests that one important role of the 4′-substituent may be to force the two *m*-methoxy groups into the two outward positions in the plane of the ring, as indicated by I, compared to IIa–d, in Chart I. In the absence of such a substituent, each *m*-methoxy group might be expected to bend toward the 4′ position half of the time, from a statistical point of view, and thus decrease the probability of important meta-space interactions effected by conformer IId.[23] The activity of the compound might be lessened by a factor of four just for this reason. On the other hand, a large 4′-substituent might distort the orientation of the methoxy substituents and thus decrease binding energy.

Chart I. Possible In-Plane Conformations of Methoxy Groups for 4′-Substituted 3′,5′-Dimethoxybenzylpyrimidines (I) and 4′-Unsubstituted Analogues (IIa–d)



That interactions occur between the 3′,4′, and 5′ vicinal substituents is suggested by a determination of log *P* values of various derivatives, which were found in some instances to be quite different from calculated values.[24] The log *P* of TMP (measured in octanol/0.01 N NaOH) was discussed in part 2 of this series.[8] The low value obtained (0.89, compared to 1.60 for the unsubstituted benzyl derivative) can be ascribed to the effect of the out of plane 4′-ether, which can permit hydrogen bonding. Such results have been previously documented and discussed by Leo et al.[25]

The 4′-methyl and 4′-ethyl analogues (**46a** and **46b**) gave measured log *P* values (octanol/0.01 N NaOH) of 2.14 (calcd 2.12) and 2.72 (calcd 2.70), in excellent agreement with the expected values. However, compound **27**, the *p*-aminophenoxy derivative, produced a remarkably low value of 0.90 (calcd 2.4), very similar to that of TMP itself. This result suggests that the 3′,5′-dimethoxy groups both lie out of plane, as with the 4′-methoxy group of TMP, with resultant π values of approximately –0.7. This would bring the calculated log *P* down to around 1.0. The enzyme binding seems not to have been affected by this postulated conformational change. Attempts are being made to relate results such as this to minimum-energy conformations

(17) **Note added in proof:** A report on the X-ray crystallography of TMP in binary complex with *E. coli* DHFR has just appeared (Baker, D. J.; Beddell, C. R.; Champness, J. N.; Goodford, P. J.; Norrington, F. E. A.; Smith, D. R.; Stammers, D. K. *FEBS Lett.* **1981**, *126*, 49–52). The hypotheses presented here are confirmed.

(18) Matthews, D. A.; Alden, R. A.; Bolin, J. T.; Freer, S. T.; Hamlin, R.; Xuong, N.; Kraut, J.; Poe, M.; Williams, M.; Hoogsteen, K. *Science* **1977**, *197*, 452.

(19) (a) Matthews, D. A.; Alden, R. A.; Bolin, J. T.; Filman, D. J.; Freer, S. T.; Hamlin, R.; Hol, W. G. J.; Kisliuk, R. L.; Pastore, E. J.; Plante, L. T.; Xuong, N.; Kraut, J. *J. Biol. Chem.* **1978**, *253*, 6946. (b) Matthews, D. A.; Alden, R. A.; Freer, S. T.; Xuong, N.; Kraut, J. *J. Biol. Chem.* **1979**, *254*, 4144.

(20) Cayley, P. J.; Albrand, J. P.; Feeney, J.; Roberts, G. C. K.; Piper, E. A.; Burgen, A. S. V. *Biochemistry* **1979**, *18*, 3887.

(21) Kompis, I.; Then, R.; Boehni, E.; Rey-Bellet, G.; Zanetti, G.; Montavon, M. *Eur. J. Med. Chem.* **1980**, *15*, 17.

(22) Kompis, I.; Wick, A. *Helv. Chim. Acta* **1977**, *60*, 3025.

(23) Conformational properties and QSAR analyses of these compounds have been studied in depth by Drs. J. G. Vinter and Richard Hyde, The Wellcome Research Laboratories, Beckenham, Kent, England. A publication is in preparation.

(24) The calculations used here were based on use of the aliphatic "Fr" and aromatic π constants listed in Tables VI-1 and -2 of Hansch, C.; Leo, A. in "Substituent Constants for Correlation Analysis in Chemistry and Biology"; Wiley: New York, 1979. These were added to the measured value for TMP or the unsubstituted benzyl derivative, as required, and corrected for H.

(25) Leo, A.; Hansch, C.; Elkins, D. *Chem. Rev.* **1971**, *71*, 525.

calculated by molecular mechanics. Additional log P data and conclusions will be reported elsewhere.

The data from rat liver DHFR give a measure of the species specificity of the various inhibitors for a bacterial, as opposed to a mammalian, DHFR. Although the I_{50} values generated from the two enzymes are not directly comparable, since the Michaelis constants (K_M) are different, they nevertheless depict the relative degree of specificity of inhibition.[26]

Most of the ethers have I_{50} values in the range of 10–50 $\times 10^{-5}$ M for inhibition of the rat liver DHFR. Those with greater activity all have aromatic functions in the side chain (e.g., **26**, **29**, and **31**). Side-chain interactions are then possible between 4′-substituents and this eucaryotic protein. Leu-28 of *E. coli* DHFR lies near the surface and is replaced by Phe or Tyr in the known mammalian and avian sequences.[27-30] Conceivably this favors an aromatic interaction.

The aliphatic ether derivatives all have less activity against rat liver DHFR than do the three compounds lacking the ether chain, i.e., **47**, **2**, and **46a** (4′-H, -OH, and -CH_3, respectively). This suggests that the out of plane α-carbon of the ethers may interfere with binding to the mammalian, but not bacterial, DHFR,[8] and thus contribute to antibacterial specificity. Compound **46b**, in which the 4′-methoxy group is replaced by ethyl, provides marginal evidence for the same trend. In repeated simultaneous assays, **46b** was found to be about half as active against rat liver DHFR as was **46a**, its methyl counterpart.

Kompis et al.[21] prepared several 4′-substituted analogues in which the 4′-function extends out of plane in *both* directions from the benzene ring. Their 4′-isopropenyl-3′,5′-dimethoxy derivative was reported to have tenfold greater specificity, as well as higher *E. coli* DHFR activity, than does TMP ($I_{50} \times 10^8 = 0.28$ for *E. coli* DHFR and 150 000 for the rat liver enzyme). Similarly, their corresponding 4′-carbomethoxy, -acetyl, and -isopropyl analogues gave values of 0.9/70 000, 2.0/70 000, and 2.0/>100 000 ($I_{50} \times 10^8$ M), respectively. The binding interference may well occur only at one face of the benzene ring. Our 4′-methoxy and 4′-ethyl analogues may conceivably have somewhat different degrees of influence to bind in one direction or the other with the bacterial and mammalian enzymes.

In Vitro Antibacterial Activity. Most of the compounds listed in Table II do not exhibit the high broad-spectrum antibacterial activity of TMP. A representative selection of 8 organisms out of 24 tested is shown in the table. Of these organisms, the least sensitive to TMP were the *Proteus* species, which were affected by only a handful of analogues. Even very minor changes in structure caused some loss in activity. The most active compound of the group appeared to be **21**, the γ-chloropropoxy derivative, which was also very active as an inhibitor of *E. coli* dihydrofolate reductase. This compound, as well as **6**, the allyloxy derivative, and the two alkyl analogues, **46a** and **46b**, were selected for further screening against a series of bacterial strains.

Table III. Relative in Vitro Antibacterial Activities of 21 and 46a against Various Strains of Microorganisms, Compared to Trimethoprim (1)

organism	strain	MIC of compd/MIC of trimethoprim[a] 21	46a
Streptococcus pyogenes	S3640	1, 3	1, 3
	S6019	3	1
	S6046	3	1
	S6058	1	1
Staphylococcus aureus	CN491	3, 10	1, 3
	S6023	3	1
	S5970	3	1
	S5532	3	1
Enterobacter aerogenes	2200/86	10	3
	S6029a	3	3
	S5759	10	3
	S5639	10	10
Escherichia coli	CN314	3, 10	3, 3
	S6203	3	3
	S6132	10	3
	S6146	3	1
Proteus mirabilis	S2409	10	3
	S6168	10	3
	S6105	10	3
	S5854	10	3
Proteus vulgaris	CN329	10	3
	S5958	10	3
	S4906	10	3
	S4774	10	3
Nocardia species	S5525	3	1
	S5505	3	1
	S5112	3	1
	S5692	1	1
Neisseria gonorrhoea	S5311	3	0.3
	S3130	3	0.3
	S6720	3	1
	S6272	1	0.3
	S6280	3	1
	S6285	3	0.3
Brucella abortus	S2578	10	3
	S2594	10	10
	S2607	10	3
	S2008	10	3
Haemophilus influenzae	S5880	3	3
	S5656	30	3
	S5658	3	1
	S4007	3	1
	S5494	10	1
	S5390	10	1

[a] Numbers greater than 1 indicate less activity for the test compound than for trimethoprim.

Table III shows the results of testing **21** and **46a** in comparison with TMP (1) against a battery of organisms. In general, both analogues were slightly less active than was TMP. Table IV compares **46b** and **6** with TMP against some Gram-positive strains of bacteria. Again, both compounds are slightly less active than the standard. Table V lists a comparison of two closely related compounds, **13** and **48**, which differ only in the third atom of a four-atom chain beyond the ether oxygen, i.e., O vs. CH_2

(26) The K_M of dihydrofolate for the rat liver DHFR is very low ($1-2 \times 10^{-7}$) [Jarabak, J.; Bachur, N. R. *Arch. Biochim. Biophys.* **1971**, *142*, 417; Wang, D.; Werkheiser, W. C. *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **1964**, *23*, 324] compared to the value of 8.9×10^{-6} M for the *E. coli* DHFR [Baccanari, D. P.; Averett, D.; Briggs, C.; Burchall, J. *Biochemistry* **1977**, *16*, 3566]. From the relationship $K_i = I_{50}/(1 + S/K_M)$, the apparent K_i for TMP for rat liver DHFR is calculated to be $0.66-1.3 \times 10^{-6}$ M, and the value for the *E. coli* enzyme is 1.3×10^{-9} M. Thus, the differential binding affinities of TMP to the DHFR from these two species is 500–1000 based on K_i values, and not the ≃60 000 value calculated from the observed 50% inhibitory concentrations.

(27) Stone, D.; Paterson, S. J.; Raper, J. H.; Phillips, A. W. *J. Biol. Chem.* **1979**, *254*, 480.

(28) Smith, S. L.; Patrick, P.; Stone, D.; Phillips, A. W.; Burchall, J. J. *J. Biol. Chem.* **1979**, *254*, 11 475.

(29) Freisheim, J. H.; Kumar, A. A.; Blankenship, D. T.; Kaufman, B. T. in "Chemistry and Biology of Pteridines", Kisliuk, R. L.; Brown, G. M., Eds.; Elsevier/North Holland: New York, 1979; p 419.

(30) Lai, P.; Pan, Y.; Gleisner, J. M.; Peterson, D. L.; Blakley, R. L. Ref 29, p 437.

Table IV. Relative in Vitro Antibacterial Activities of 6 and 46b against Various Strains of Gram-Positive Organisms Compared to Trimethoprim (1)[a]

organism	strain	MIC of compd/ MIC of trimethoprim	
		6	46b
Streptococcus pyogenes	CN10	3	3
	S3640	3	3
	S5519	3	3
	S5525	3	10
	S5526	1	3
	S6019	3	10
	S6046	3	10
	S6058	3	3
Streptococcus faecalis	CN478	3	3
	S5867	3	3
	S6084	1	3
	S6085	3	10
Staphylococcus aureus	CN491	3	3
	S5435	10	3
	S5532	10	3
	S5638	3	1
	S5862	3	3
	S5970	10	3
	S5981	10	3
	S6016	3	1
	S6023	3	1

[a] Numbers greater than 1 indicate less activity for the test compound than for the standard, trimethoprim.

Table V. Relative in Vitro Antibacterial Activities of 13 and 48 against a Range of Organisms Compared to Trimethoprim (1)

organism	strain	MIC of compd/ MIC of trimethoprim	
		48[a]	13[a]
St. pyogenes	CN10	2	10
St. pyogenes	S3640	2.5	7.5
St. faecalis	CN478	1	5
Staph. aureus	CN491	10	10
Vibrio cholerae	ATCC14035	5	5
Past. multocida	ATCC6587	75	50
Myco. smegmatis	S3254	20	5
Sal. typhimurium (LT-2)	S8587	50	10
Sal. typhi	CN512	100	20
Shig. flexneri	CN6007	75	50
E. coli	CN314	50	7.5
Serr. marcescens	UNC18	100	20
Kleb. pneumoniae	CN3632	100	10
Entero. aerogenes	2200/86	100	10
Citro. freundii	2200/77	100	10
Proteus vulgaris	CN329	50	50

[a] Average of two determinations. Numbers greater than 1 indicate less activity for the test compound than for trimethoprim.

in the β-methoxyethoxy and *n*-butoxy derivatives, respectively. The former compound has recently been introduced abroad for human medicine as an antibacterial agent.[31] Neither derivative is as active as TMP. However, it will be noted that **48**, which contains the more lipophilic substituent, is more active against the Gram-positive organisms, whereas the reverse is true of **13**. Hansch[32] has previously noted such an effect.

In a series such as this, where the enzyme inhibition constants are very much the same in most instances, it would appear attractive to try to relate the in vitro antibacterial activities to the physical properties of the compounds, including log *P* and solubility properties. However, it would only seem appropriate to attempt this with *Escherichia coli*, since one cannot assume that the other bacterial DHFR enzymes behave the same. By qualitative assessment, we see no obvious correlation of log *P* with the MIC data. It would appear that more finely tuned methodology, coupled with more physical data and more information on cell-wall penetration, will be required to solve this problem. A selected series of compounds is currently being subjected to such scrutiny.

Table VI. Comparison of in Vivo Antibacterial Activities of 6, 21, and 1 in Mice

organism	expt	ED_{50}, mg/mouse, of TMP analogues		
		6	21	1
Streptococcus pyogenes CN 10	1	12		6.1
	2	4.3	3.3	3.7
	3	5.1	7.2	8.6
Staphylococcus aureus CN 491	1	2.5	2.1	1.3
	2	>10	>10	7.3
	3	8.1	7.1	6.1
Escherichia coli CN 348	1	7.5	6.2	1.4
	2	11	9.4	1.8
	3	>10	>10	1.9
Proteus vulgaris CN 329	1	~10	8.2	1.5
	2	>10	>10	2.6
Klebsiella pneumoniae CN 3632	1	4.6	1	1.4
	2	10		1.3
	3	>10	>10	8.1

Table VII. Comparisons of Half-lives and Concentrations of Trimethoprim Analogues (6 and 21) in Serum after Oral Administration in Mice[a]

time after dosing, min	dose (mouse), mg	concn, μg/mL		
		6	21	1
10	10	84	41	170
	2	18	7	20
30	10	59	31	74
	2	46	2.9	10
90	10	51	26	54
	2	1.0	1.3	1.7
180	10	27	15	18
	2	0.26	0.7	0.8
360	10	2.8	7	0.94
	2	<0.1	0.24	0.5
		calcd half-life, h		
	10	1.1	2.4	0.76
	2	0.35	1.9	2.7

[a] Pooled samples were from groups of three mice (18–20 g).

In Vivo Studies. Two compounds, **6** and **21**, were selected for in vivo antibacterial studies in mice. The protocol is described under Experimental Section. The protective ED_{50} values of these compounds, compared with that of TMP, are shown in Table VI. The results show that both **6** and **21** afforded less protection to mice, on a weight basis, than did TMP.

Comparison of the concentrations of **1** and its analogues in serum of mice is shown in Table VII. The calculated half-lives are shown in Table VII, based on the slopes of the curves from the time when the compounds had apparently become evenly distributed. The standard TMP (**1**) gave higher peak concentrations in the blood, and although the calculated half-lives appear to vary with the size of the dose, they do not suggest that shorter duration of action may be a contributing factor to the lower effi-

(31) Wise, R.; Reeves, D. S., Eds. *J. Antimicrob. Chemother.* **1979**, *5*, suppl B (Nov).
(32) (a) Lien, E. J.; Hansch, C.; Anderson, S. M. *J. Med. Chem.* **1968**, *11*, 430. (b) Dietrich, S. W.; Smith, R. N.; Brendler, S.; Hansch, C. *Arch. Biochem. Biophys.* **1979**, *194*, 612.

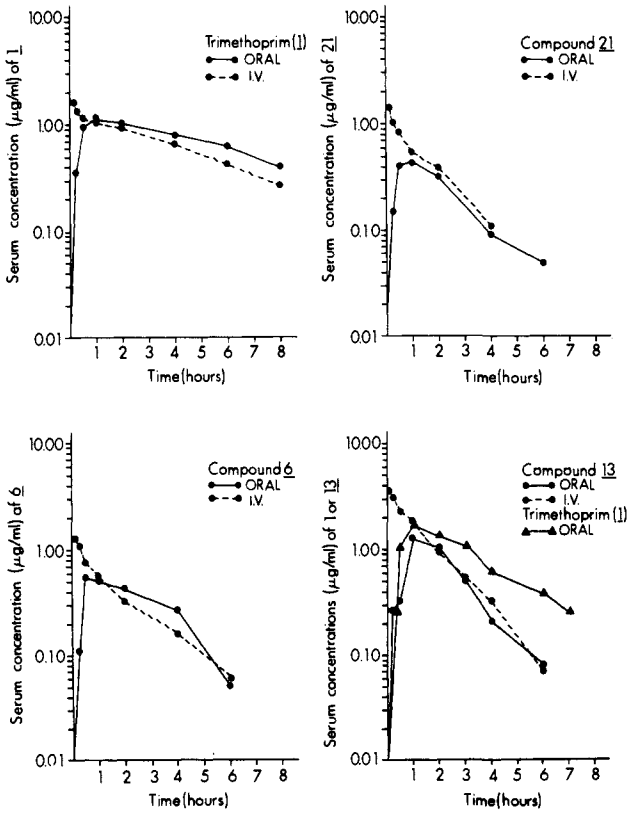


Figure 1. Comparison of serum concentrations of trimethoprim and analogues in the dog as a function of time after oral and intravenous administration.

cacies of the analogues; the cause may be due to lower intrinsic activity or to differences in disposition, including distribution, or to metabolism of the drugs.

Three compounds (**6**, **21**, and **13**) were selected for pharmacokinetic investigation in the dog. These compounds were of interest for human or veterinary medicine either because of their spectrum of antibacterial activity or because of their physicochemical properties, which were expected to alter their disposition relative to that of TMP.

The pharmacokinetic profile of TMP administered to dogs was described in detail previously by Kaplan et al.[33] Blood level data (iv administration) to two dogs was fitted to a biexponential equation; the slow disposition rate constants (β phase) corresponded to half-lives ($t_{1/2}$) of 2.5 and 3.7 h. The mean absolute bioavailability was 103%, which was indicative that the drug was absorbed completely.

In the current work, the pharmacokinetic profile of TMP in the dog was compared directly with those of the related benzylpyrimidines.[34] Serial blood and cumulative urine samples were obtained during the first 24 h after each dose. The concentrations were measured by making appropriate modifications to the sensitive and specific quantitative TLC methods developed in this laboratory for TMP and sulfonamides.[35,36]

In the first study, male beagle dogs (n = 3) received a single oral dose (5 mg/kg) of TMP in a gelatin capsule on one day and 2 weeks later received (iv) the same quantity in a propylene glycol solution. The average β-phase $t_{1/2}$ was 4.2 h (Figure 1), and the absolute bioavailability was 107%, which compares well with the data of Kaplan.[33] An average of 7.6% of the dose was recovered as TMP in the 24-h cumulative urine.

In the second study, two groups of male beagle dogs (n = 3) received a single oral dose (5 mg/kg) of compound **6** or **21** on one day and the same quantity iv 2 weeks later. The mean serum $t_{1/2}$ (iv dose) values were 1.43 h for **6** and 0.91 h for **21**. Peak serum concentrations occurred within 1 h and were approximately 0.5 μg/mL (Figure 1); the absolute bioavailabilities were 95% for **6** and 68% for **21**. Both compounds were extensively metabolized. On the average, 2% of the dose was found as intact drug in the 24-h cumulative urine sample.

The third compound, **13**, was tested as follows: three dogs received TMP (5 mg/kg) orally on day 1, compound **13** orally on day 7, and **13** (5 mg/kg) as an iv dose on day 15. Peak serum concentrations of **13** and TMP were reached within 1 h (oral dose) and were 1–2 μg/mL (Figure 1). Compound **13** was eliminated from serum more rapidly than was TMP; the average $t_{1/2}$ was 0.98 h for **13** and 2.01 h for TMP. The absolute bioavailability for **13** was 61%, which was suggestive either of incomplete absorption or of extensive first pass metabolism. An average of 10.6 ± 1.23% of the dose of **13** was recovered as intact drug in the 24-h cumulative urine.

Thus, these studies in dogs indicated that TMP and the three related compounds were rapidly absorbed but extensively metabolized, with less than 10% of the dose excreted in the urine as intact drug in 24 h. TMP had a longer plasma $t_{1/2}$ than any of the analogues and the greatest bioavailability. Extending the 4′-ether chain of TMP with three types of substituents has apparently created greater opportunity for metabolism, causing more rapid elimination and decreased bioavailability, in all three cases.

The compounds of this paper were also screened against *P. berghei* DHFR, with quite different results than those described here for *E. coli* and rat liver reductases. This material will form the subject of another publication.

Experimental Section

Melting points were determined in open capillaries on a Mel-Temp or Thomas-Hoover apparatus and are uncorrected. Elemental analyses were performed by Drs. Samuel Blackman and Stuart Hurlbert and their staffs and by Atlantic Microlab, Inc., Atlanta, GA. Where indicated by symbols of the elements, the analytical results obtained were within ±0.4% of the calculated values. The NMR spectra were measured on a Varian T-60, A-60, or HA-100 spectrometer in Me_2SO-d_6 unless otherwise indicated. Chemical shifts are expressed in parts per million (ppm) on the δ scale from internal Me_4Si. UV spectra were determined on a Cary 13 or 118 spectrophotometer. IR spectra were obtained on a Beckman IR4 spectrophotometer and are reported in reciprocal centimeters. The spectroscopic data for all new compounds were consistent with the assigned structures. DHFR enzyme assays were conducted by methods described in part 2 of this series.[8] Dissociation constants were measured as described by Roth and Strelitz.[37]

General Procedure for Alkylation or Arylation of 2,4-Diamino-5-(3′,5′-dimethoxy-4′-hydroxybenzyl)pyrimidine (2). Procedures used and general precautions are described in parts 2 and 4 of this series.[8,3] Compounds prepared are described in Table I.

(33) Kaplan, S. A.; Weinfeld, R. E.; Cotler, S.; Abruzzo, C. W.; Alexander, K. *J. Pharm. Sci.* **1970**, *59*, 358.

(34) Preslar, D.; Grace, M. E.; Sigel, C. W. in "Current Chemotherapy and Infectious Disease", Proceedings of the 11th International Congress of Chemotherapy and the 19th Interscience Conference on Antimicrobial Agents and Chemotherapy, Boston, MA, 1979; Nelson, J. D.; Grassi, C., Eds.; American Society for Microbiology: Washington, DC, 1980; p 428.

(35) De Angelis, 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.

(36) Sigel, C. W.; Woolley, J. L.; Ref 35, pp 677–694.

(37) Roth, B.; Strelitz, J. Z. *J. Org. Chem.* **1969**, *34*, 821.

4-(Allyloxy)-3,5-dimethoxybenzaldehyde (49). To 18.2 g (0.1 mol) of syringic aldehyde in 40 mL of MeOH was added a solution of 6 g (0.11 mol) of NaOMe in 35 mL of MeOH. A clear yellow solution was formed. Allyl bromide (15.4 g, 0.13 mol) was added, and the mixture was refluxed for 1.5 h. A clear solution was formed within about 15 min. The solvent was removed in vacuo and the residue recrystallized twice from hexane: yield 18 g (81%); mp 45–46 °C. Anal. ($C_{12}H_{14}O_4$) C, H.

2-[4-(Allyloxy)-3,5-dimethoxybenzyl]-3-morpholinoacrylonitrile (50). To a mixture of 15.5 g (0.11 mol) of β-morpholinopropionitrile, 1 g of NaOMe, and 35 mL of Me_2SO was slowly added a solution of 22.2 g (0.1 mol) of **49** in 35 mL of Me_2SO while heating the first mixture on the steam bath. After heating for a total of about 20 min, the solution was cooled and an excess of cold water was added. The mixture was cooled and scratched; a viscous oil separated, which was washed thoroughly with cold water, taken up in Et_2O, and dried over Na_2SO_4, followed by removal of the solvent. The resultant oil was washed thoroughly with hexane. A thick glassy product was obtained, which could not be induced to crystallize: yield 29.4 g (85%). Anal. ($C_{19}H_{24}N_2O_4 \cdot 0.5H_2O$) C, H, N.

2,4-Diamino-5-[4′-(allyloxy)-3′,5′-dimethoxybenzyl]pyrimidine (6). Compound **50** was treated with guanidine base in the manner described for **46a** below, which resulted in the separation of **6** in 70% yield, identical in properties with the product described in Table I.

3,5-Dimethoxy-4-methylaniline (42a).[16] A 500-mL flask equipped with magnetic stirrer, dry ice condenser, and gas inlet tube was thoroughly flame dried under N_2, after which approximately 250 mL of anhydrous liquid NH_3 was added. Potassium (0.5 g) was added to the NH_3, followed by 0.25 g of ferric nitrate. Then 7.3 g (0.2 mol) of K was added in small pieces at a rate such that the blue color was discharged prior to each addition. The mixture was then stirred for 15 min, followed by the slow addition of 23.1 g (0.1 mol) of 2,6-dimethoxy-3-bromotoluene (**41a**).[16] The mixture was stirred for 3 h after the addition was complete, followed by the slow addition of 8.0 g (0.15 mol) of NH_4Cl, and then 50 mL of benzene. The NH_3 was evaporated in a stream of N_2, and 150 mL of H_2O and 150 mL of benzene were then added. The mixture was warmed to 50 °C and stirred until all was dissolved, after which it was cooled and the layers were separated. The benzene layer was washed well with H_2O and poured with stirring into 100 mL of 6 N HCl. The resultant precipitate was isolated, slurried for 1 h in 10 N NaOH, filtered, washed with H_2O, and dried: yield 13.7 g (82%) of crude **42a**; mp 119–120 °C. The NMR spectrum indicated that only this isomer was present: NMR (Me_2SO-d_6) δ 1.83 (s, 3, Me), 3.65 [s, 6, $(OMe)_2$], 4.83 (br s, 2, NH_2), 5.87 (s, 2, Ar). For comparison, **3,4,5-trimethoxyaniline** gave the following NMR spectrum (Me_2SO-d_6): δ 3.50 (s, 3, 4-OMe), 3.65 [s, 6, 3,5-$(OMe)_2$], 5.85 (s, 2, Ar).

4-Cyano-2,6-dimethoxytoluene (43a). Compound **42a** (12.5 g, 0.075 mol) was diazotized by the usual procedure and then neutralized with Na_2CO_3 at 0 °C. A solution of 9.0 g (0.10 mol) of $Cu_2(CN)_2$ and 12.2 g (0.25 mol) of NaCN in 100 mL of H_2O was prepared and cooled to 0 °C. Cold toluene (200 mL) was added to this solution, and the diazonium solution was then poured slowly into the cyanide mixture with stirring. The mixture was stirred at 0 °C for 30 min and then allowed to warm to room temperature, after which it was heated to 60 °C and stirred for 1 h. After the mixture cooled, the organic layer was separated and steam distilled to yield 4.8 g (36%) of **43a**: mp 129–130 °C; IR (KBr) 2232 cm^{-1} (CN). Anal. ($C_{10}H_{11}NO_2$) C, H, N.

3,5-Dimethoxy-4-methylbenzaldehyde (44a).[15,38] A mixture of 4.8 g (0.027 mol) of **43a**, 82 mL of H_2O, 109 mL of 90% formic acid, and 4.8 g of activated Raney Ni (50:50) was heated under reflux with stirring for 6 h. The hot mixture was then filtered and chilled. A cream precipitate separated, yielding 2.2 g (45%) of **44a**: mp 93–94 °C; NMR (Me_2SO-d_6) δ 2.07 (s, 3, Me), 3.87 [s, 6, $(OMe)_2$], 7.17 (s, 2, Ar), 9.25 (s, 1, CHO). Anal. ($C_{10}H_{12}O_3$) C, H.

2,4-Diamino-5-(3′,5′-dimethoxy-4′-methylbenzyl)pyrimidine Hydrochloride (46a).[15] A mixture of 1.8 g (0.010 mol) of **44a** and 1.6 g (0.011 mol) of β-anilinopropionitrile in 5 mL of dry Me_2SO was heated to 40 °C under N_2, and a solution of 1.3 g (0.011 mol) of *t*-BuOK in 8 mL of *t*-BuOH was added dropwise. The reaction was then heated at 55–60 °C for 1.5 h. The solvent was removed in vacuo and the residue added to 200 mL of ice water. The resultant crystalline solid was isolated, yielding 3 g (97%) of crude α-(3,5-dimethoxy-4-methylbenzyl)-β-anilinoacrylonitrile (**45a**), which was used directly in the next reaction without characterization. This product (2.9 g, ca. 0.0094 mol) was added to a solution of 2.2 g (0.040 mol) of NaOMe and 2.9 g (0.030 mol) of guanidine hydrochloride in 60 mL of absolute EtOH and heated under reflux for 24 h. The solvent was removed and the residue was slurried in water: yield of precipitate 2.4 g (93% of crude **46a**). This was crystallized from 175 mL of EtOH plus 1 mL of concentrated HCl to yield the hydrochloride: mp 303–306 °C dec; UV λ_{max} (cation, 0.1 N HCl) 271 nm (ε 5750), sh 279 (4950); UV λ_{max} (neutral species, 0.1 N NaOH) sh 278 nm (ε 5950), 287 (6500). Treatment with 0.1 N NaOH yielded the free base, mp 220 °C (EtOH). Anal. ($C_{14}H_{18}N_4O_2 \cdot HCl$) C, H, N, Cl.

1-Ethyl-2,6-dimethoxybenzene (40).[38] 2,6-Dimethoxyacetophenone (**39**)[39] was reduced in a Parr apparatus with 10% Pd on C in MeOH to produce **40** (92%), mp 58–59 °C (MeOH).

1-Bromo-3-ethyl-2,4-dimethoxybenzene (41b). To a solution of 16.6 g (0.1 mol) of **40** in 150 mL of Et_2O was slowly added a freshly prepared solution of dioxane dibromide [from 32 g (0.2 mol) of Br_2 plus 17.6 g (0.2 mol) of dioxane] in 300 mL of Et_2O during a 30-min period. After an additional 30 min, the solution was extracted well with H_2O, $NaHCO_3$, and H_2O again and then dried over $CaCl_2$. The solvent was removed, and the residue was fractionally distilled in vacuo: bp 95 °C (1 mm); yield 19.2 g (78%). Anal. ($C_{10}H_{13}BrO_2$) C, H.

4-Ethyl-3,5-dimethoxyaniline (42b). A 24.5-g (0.1 mol) sample of **41b** was treated with K in liquid NH_3 in the manner of **42a** to produce **42b**: crude yield 15.3 g (70%). The product was stirred with 10 N NaOH for 1 h, followed by isolation and then thorough washing with H_2O: mp 154.5–157.5 °C (EtOH). Anal. ($C_{10}H_{15}NO_2$) C, H, N.

3,5-Dimethoxy-4-ethylbenzonitrile (43b). Compound **42b** (18.1 g, 0.1 mol) was diazotized and treated with $Cu_2(CN)_2$ and NaCN in the manner of **43a** to produce 9.5 g (50%) of **43b**: mp 110–113 °C (EtOH). Anal. ($C_{11}H_{13}NO_2$) C, H, N.

4-Ethyl-3,5-dimethoxybenzaldehyde (44b). This compound was prepared from **43b** by the method of **44a**. From 9.5 g (0.05 mol) of **43b** there was obtained 6.8 g (70%) of **44b**, mp 49–53 °C. Anal. ($C_{11}H_{14}O_3 \cdot 0.5H_2O$) H; C: calcd, 65.01; found, 65.71.

3-Anilino-2-(4-ethyl-3,5-dimethoxybenzyl)acrylonitrile (45b). Compound **44b** (8.5 g, 0.044 mol) was treated with β-anilinopropionitrile in the manner described for **45a**: yield of crude product 9.5 g (67%); mp 175.5–179.5 °C (EtOH). Anal. ($C_{20}H_{22}N_2O_2$) C, H, N.

2,4-Diamino-5-(4′-ethyl-3′,5′-dimethoxybenzyl)pyrimidine (46b). An 8.7-g (0.09 mol) portion of guanidine hydrochloride was added to a solution of 6.0 g (0.12 mol) of NaOMe in 150 mL of absolute EtOH, filtered from salt, and mixed with 9.0 g (0.028 mol) of **45b**. The mixture was refluxed for 24 h. After chilling, the precipitate (**46b**) was isolated, washed with water, and dried: yield 4.8 g; mp 223–226 °C (from 225 mL of 95% EtOH). The pK_a value of **46b** was 7.16 (24 °C). Anal. ($C_{15}H_{20}N_4O_2$) C, H, N. An additional 1.7 g was obtained from the filtrate, which was purified by conversion to the **hydrochloride (46b·HCl)**, mp 308–310 °C (absolute EtOH). Anal. ($C_{15}H_{20}N_4O_2 \cdot HCl$) C, H, N, Cl.

Methods. In Vitro Antibacterial Assays. The compounds were sterilized by dissolving in dimethylformamide. After 30 min the solutions were diluted into sterile distilled water, and the compounds which precipitated were dissolved as the isethionate salts by slowly adding 1 N isethionic acid. Two-third serial dilutions were prepared at 10 times the desired final strength in sterile distilled water, and 1–5 mL quantities were added to 13–15 mL of molten Wellcotest Sensitivity Test Agar (or to nutrient agar of low thymidine content), containing 5% lysed horse blood at 50 °C. After pouring into 90-mm petri dishes, each plate was

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inoculated with 24 test organisms by means of a multiple inoculation consisting of 1-mm diameter loops. The inocula, which consisted of 18-h nutrient broth cultures, suitably diluted to yield approximately 500 colonies, were streaked along 1 cm. After 18 h of incubation at 37 °C, the MIC was read as the lowest concentration which caused 80% inhibition, as judged by eye, of the control growth on the medium containing no test compound. As a standard, trimethoprim (1) was included in each daily series of tests.

In Vivo Antibacterial Assays. (1) Infections. Groups of six mice (18–20 g) were infected ip with 10–100 lethal doses of the infecting organisms. The test substances, suspended in 0.5% carboxymethylcellulose, were administered po immediately after infection and 6-h later; on the following days the number of doses depended on the acuteness of the infection. In the case of *Klebsiella pneumoniae* and *Proteus vulgaris*, the drugs were given once on the following day, and in the case of *Escherichia coli*, twice on that day. With the staphylococcal infections, the drugs were given twice on each of the following two days, and with the streptococcal infections, the dosing was twice on the 2nd day and once on the 3rd.

The test substances were given in graded doses, differing in 2/3 intervals, and the ED_{50} was determined by the method of Reed and Muench.[40]

(2) Blood Concentrations. A single dose of the test substance was given po to groups of three mice (18–20 g), and then at intervals ~0.2 mL of blood was drawn from the supra orbital plexus of each mouse and pooled. The serum was separated and the concentration of the test substance was determined microbiologically using *Bacillus pumilus* as the test organism.

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Synthesis and Antiviral Activity of Certain 9-β-D-Ribofuranosylpurine-6-carboxamides

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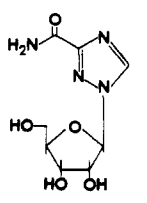
To examine the structural parameters necessary for antiviral efficacy of certain purine nucleosides, several 9-β-D-ribofuranosylpurine-6-carboxamides have been synthesized. Glycosylation of the Me_3Si derivative of purine-6-carboxamide with protected ribofuranose in the presence of a Lewis acid gave the blocked nucleoside which on deprotection furnished 9-β-D-ribofuranosylpurine-6-carboxamide (**6a**). Alternatively, **6a** was synthesized via the nucleophilic displacement of 9-β-D-ribofuranosyl-6-iodopurine with cyanide ion. Certain 2-amino- and 2-methyl-9-β-D-ribofuranosylpurine-6-carboxamides have also been prepared. 8-Carbamoylguanosine (**16**) has been prepared by homolytic acylation of the parent nucleoside. These compounds were tested against several RNA and DNA viruses in cell culture. 9-β-D-Ribofuranosylpurine-6-carboxamide (**6a**), the corresponding 6-thiocarboxamide (**7b**), and 4-amino-8-(β-D-ribofuranosylamino)pyrimido[5,4-*d*]pyrimidine (**8**) showed significant in vitro antiviral activity at nontoxic dosage levels. **6a** employed in the treatment of Rift Valley fever virus infected mice at 50 (mg/kg)/day gave a 55% survival rate on day 21 compared to a 30% survival in the controls.

The role of viruses in chronic and degenerative diseases is a subject of considerable interest. A tremendous amount of evidence is accumulating which implicates viral etiology in diseases such as arthritis, diabetes, multiple sclerosis, mental retardation, and infectious mononucleosis.[2] The progress made in the synthesis and development of antiviral agents has recently been reviewed.[3-5] Approval of 9-β-D-arabinofuranosyladenine (*ara*-A) for use against herpes infection is indeed a step forward.[6] The broad-spectrum activity of synthetic 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide (1, ribavirin) against influenza,



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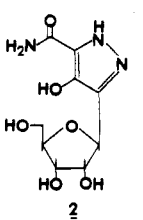

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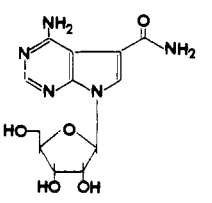

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