crude allylic alcohol (580 mg). A mixture of the allylic alcohol, manganese dioxide (4.0 g), and CH_2Cl_2 (20 mL) was stirred at 25 °C for 2 h and filtered. The filtrate was concentrated in vacuo to give **39a** (540 mg, 88% yield): R_f 0.46 (AcOEt-benzene, 1:2); IR (film) 1690 (CHO), 1600 (phenyl), 975 (trans olefin); NMR (CCl_4) δ 10.0-9.66 (1 H, m, CHO), 7.35-6.52 (5 H, m, aromatic H and C*H*=CC=O), 6.32-5.34 (3 H, m, olefin in the ω chain and C=C*H*C=O).

16-(3-Chlorophenoxy)-17,18,19,20-tetranor-2,3-*trans*,4,5-*trans*,6,7-*trans*-hexadehydroprostaglandin $F_{1\alpha}$ Methyl Ester (40a). A mixture of **39a** (150 mg, 0.24 mmol), (3-carbomethoxy-2-propenyl)triphenylphosphorane (360 mg, 1.0 mmol), and CH_2Cl_2 (5 mL) was refluxed for 12 h and concentrated in vacuo. The residue was column chromatographed on silica gel (Merck, 10 g) with AcOEt-benzene (1:7) to give the tris(THP) conjugated ester. A mixture of this ester, THF (2 mL), and 1 N HCl (2 mL) was stirred at 45 °C for 1 h, diluted with AcOEt (5 mL), washed with saturated aqueous $NaHCO_3$ and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was column chromatographed on silica gel (Mallinckrodt, 15 g) with AcOEt-benzene (1:1) to afford **40a** [28 mg, 26%; R_f 0.22 (AcOEt)], the C_{15S}-hydroxy isomer [26 mg, 24%; R_f 0.26 (AcOEt)], and their mixture (14 mg, 13%). **40a**: IR (film) 3400 (OH), 1702 (ester), 1615 (conjugated olefin), 1593 (phenyl), 978 (trans olefin) cm^{-1}; NMR ($CDCl_3$) δ 7.72-5.92 (9 H, m, aromatic H and conjugated olefinic H, except for C_2 H), 5.97-5.53 (3 H, m, C_2, C_{13}, and C_{14} H), 4.43 (1 H, m, C_{15} H), 3.73 (3 H, s, COOMe); UV (EtOH) λ_{max} 306 nm. High-resolution MS for $C_{23}H_{25}O_5Cl$ (dehydration peak from molecular ion): calcd, *m/e* 416.13904; found, 416.13996.

16-Phenoxy-17,18,19,20-tetranor-2,3-*trans*,4,5-*trans*,6,7-*trans*-hexadehydroprostaglandin $F_{1\alpha}$ Methyl Ester (40b). **40b** was prepared by starting with the Wittig reaction of **33b** (=**1b**) by the same six steps as described for **40a**. **40b**: R_f 0.20 (AcOEt, R_f of the C_{15S}-hydroxy isomer 0.26); IR 3380 (OH), 1710 (ester), 1615 (conjugated olefin), 1600 (phenyl), 972 (trans olefin) cm^{-1}; NMR ($CDCl_3$) δ 7.44-5.23 (13 H, m, aromatic H and olefinic H), 4.67-4.31 (1 H, m, C_{15} H), 4.34-3.71 (4 H, m, C_9, C_{11}, and C_{16} H), 3.73 (3 H, s, COOMe); UV (EtOH) λ_{max} 306 nm. High-resolution MS for $C_{23}H_{28}O_6$ (molecular ion peak): calcd, *m/e* 400.18858; found, 400.19194.

Biological Procedure. Antinidatory Effect. By the same biological procedure as described in the preceding paper in this issue,[20] the antinidatory effects of prostaglandin analogues were examined.

Acknowledgment. We are grateful to Professor Hisashi Yamamoto of the University of Hawaii for his continuing advice and stimulating discussions.

2,4-Diamino-5-benzylpyrimidines as Antibacterial Agents. 4. 6-Substituted Trimethoprim Derivatives from Phenolic Mannich Intermediates. Application to the Synthesis of Trimethoprim and 3,5-Dialkylbenzyl Analogues[1]

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The preparation of a wide variety of 6-substituted trimethoprim analogues was readily accomplished by the reaction of 2,4-diamino-6-substituted-pyrimidines with 2,6-dimethoxy-4-[(*N*,*N*-dimethylamino)methyl]phenol at 120-160 °C. The less reactive 2,6-dialkyl-4-[(*N*,*N*-dimethylamino)methyl]phenols reacted successfully with 2,4-diamino-6-(alkylthio)pyrimidines to give 5-(substituted benzyl)pyrimidines. The phenolic groups of the products were alkylated in high yield when a nonreactive 6-substituent was present in the pyrimidine ring. 6-(Alkylthio) groups were easily removed with Raney nickel. Trimethoprim was thus obtained in high yield from its 6-(methylthio) counterpart. The 6-substituted trimethoprim analogues all had low activity as inhibitors of *Escherichia coli* dihydrofolate reductase and as antibacterial agents.

A previous paper in this series[2] described the synthesis of trimethoprim (**18**), a broad-spectrum antibacterial agent,[3] from 2,4-diaminopyrimidine and phenolic Mannich bases. This route had limited applicability to the preparation of benzylpyrimidine analogues; it was not useful with 3,5-dialkylphenolic Mannich bases. However, the highly successful condensation of 2,4-diaminopyrimidin-6(1*H*)-one and 2,4-diamino-6-methylpyrimidine with Mannich bases[2] suggested that a wide gamut of 6-substituted derivatives might be prepared by this approach. This paper describes such analogues and their biological activity. Furthermore, it presents a synthesis of 3,5-dialkyl-4-hydroxy- and 3,5-dialkyl-4-methoxybenzyl analogues of trimethoprim which utilizes the readily reactive 2,4-diamino-6-(methylthio)pyrimidine as an intermediate.[1,4]

A study of the effect of 6-substitution on the biological activity of trimethoprim was considered of importance. Its 6-methyl derivative[2] had less antibacterial activity and dihydrofolate reductase (DHFR) inhibitory activity (*E. coli* and *P. berghei*) than did the parent. However, related 6-alkyl-2,4-diamino-5-benzylpyrimidines[5] had high antimalarial activity.[6] It seemed plausible that appropriate 6-modification, such as lengthening the chain and introducing an aromatic or conceivably a polar substituent, would provide useful activity and perhaps yield information about the interaction of these compounds with DHFR derived from different species.

(1) (a) A portion of this paper dealing with trimethoprim geometry and 6-substitution was presented at the 164th American Chemical Society Meeting. See "Abstracts of Papers", 164th National Meeting of the American Chemical Society, New York, N.Y., Aug 1972, American Chemical Society, Washington, D.C., Abstr MEDI 23. (b) B. Roth, U.S. Patent 3 772 289 (1973). (c) B. Roth, U.S. Patent 3 822 264 (1974).
(2) For paper 2 in this series, see B. Roth, J. Z. Strelitz, and B. S. Rauckman, *J. Med. Chem.*, **23**, 379 (1980).
(3) (a) B. Roth, E. A. Falco, G. H. Hitchings, and S. R. M. Bushby, *J. Med. Pharm. Chem.*, **5**, 1103 (1962). (b) *Drugs*, **1**, 7 (1971), and references therein. (c) Symposium on Trimethoprim-Sulfamethoxazole, *J. Infect. Dis.*, **128**, supplement (Nov, 1973).
(4) G. D. Daves, Jr., C. W. Noell, R. K. Robins, H. C. Koppel, and A. G. Beaman, *J. Am. Chem. Soc.*, **82**, 2633 (1960).
(5) E. A. Falco, S. DuBreuil, and G. H. Hitchings, *J. Am. Chem. Soc.*, **73**, 3758 (1951).
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Table I. 6-Substituted 2,4-Diamino-5-(4-hydroxybenzyl)pyrimidines via Mannich Condensations

no.	pyrimidine 6-substituent	benzene substituents 3	benzene substituents 5	% yield[a]	recrystn solvent[b]	mp, °C	emp formula	anal.
3a[c]	C_6H_5	OCH_3	OCH_3	98	A	275-276	$C_{19}H_{20}N_4O_3$	C, H, N
3b[d]	OC_6H_5	OCH_3	OCH_3	74		229-230.5	$C_{19}H_{20}N_4O_4$	N[e]
3c[f]	OCH_3	OCH_3	OCH_3	49	B	203-205	$C_{14}H_{18}N_4O_4$	C, H, N
3d	NH_2	OCH_3	OCH_3	92	A	274 dec	$C_{13}H_{17}N_5O_3$	C, H, N
3e	C_3H_7-*n*	OCH_3	OCH_3	46	B	225-227	$C_{16}H_{22}N_4O_3$	C, H, N
3f	C_3H_7-*i*	OCH_3	OCH_3	16				*g*
3g	Cl	OCH_3	OCH_3	6.5	G	270-280	$C_{13}H_{15}ClN_4O_3$	C, H, N[h]
3h[i]	SCH_3	OCH_3	OCH_3	89	A	225-227	$C_{14}H_{18}N_4O_4$	C, H, N
3i	SC_2H_5	OCH_3	OCH_3	83	B	185.5-187.5	$C_{15}H_{20}N_4O_3S$	C, H, N
3j	SC_4H_9-*n*	OCH_3	OCH_3	83	C	154-156	$C_{17}H_{24}N_4O_3S \cdot H_2O$	*j*
3k	SC_6H_{13}-*n*	OCH_3	OCH_3	35	C	150-152	$C_{19}H_{28}N_4O_3S$	C, H, N
4	SCH_3	Br	Br	95	B	269-271	$C_{12}H_{12}Br_2N_4OS \cdot HCl$	C, H, N
5	SCH_3	CH_3	CH_3	35				*g*
6	SCH_3	CH_3	C_4H_9-*t*	38	B	164-165	$C_{17}H_{24}N_4OS$	C, H, N
7	SCH_3	C_2H_5	C_2H_5	60	D	210-216 dec	$C_{16}H_{22}N_4OS \cdot HCl$	C, H, N
8	SCH_3	C_3H_7-*i*	C_3H_7-*i*	85	E	281 dec	$C_{18}H_{26}N_4OS \cdot HCl$	C, H, N
9	SCH_3	C_4H_9-*t*	C_4H_9-*t*	67	E	218-221.5	$C_{20}H_{30}N_4OS \cdot HCl$	C, H[k]
10	NH_2	C_4H_9-*t*	C_4H_9-*t*	20	F	240 dec	$C_{19}H_{29}N_5O \cdot HCl \cdot H_2O$	C, H, N, Cl

[a] Yield of semipure product, after extraction of nonheterocyclic products with Et_2O or Me_2CO. [b] A, β-methoxyethanol; B, EtOH; C, MeOH; D, Me_2CO-MeOH; E, EtOH-Et_2O; F, MeOH-Bz; G, extracted repeatedly with hot EtOH. [c] 1a, P. B. Russell, *J. Chem. Soc.*, 2951 (1954). [d] 1b, ref 7. [e] Calcd: C, 62.28; H, 4.95. Found: C, 61.80; H, 5.53. [f] 1c, B. Roth, J. M. Smith, and M. E. Hultquist, *J. Am. Chem. Soc.*, **73**, 2869 (1951). [g] Crude product used directly in the following reaction. [h] Calcd: Cl, 11.41. Found: 10.87. [i] 1h, ref 4. [j] Calcd: C, 53.39; H, 6.84; N, 14.65. Found: C, 53.89; H, 7.16; N, 14.15. [k] NMR showed the presence of $^2/_3$ mol of EtOH. Anal. Calcd for $C_{20}H_{30}N_4OS \cdot HCl \cdot {}^2/_3$ EtOH: C, 58.01; H, 7.99; N, 12.68. Found: C, 58.25; H, 7.85; N, 12.65.

A range of easily available 6-substituted 2,4-diaminopyrimidines (Scheme I) was then treated with various phenolic Mannich bases to determine the scope of this reaction. Table I lists the results. In practically all cases except those involving reactive or bulky 6-substituents, high yields of 5-benzylpyrimidines were obtained. The 6-(dimethylamino)- and 6-piperidino-2,4-diaminopyrimidines (**1l,m**) produced mixtures of tarry decomposition products with **2a**; this was an exceptional result.

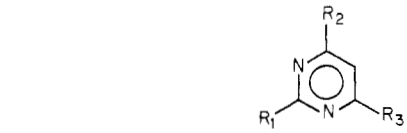
The facile reaction of **2a** with 6-(alkylthio)pyrimidines (**1h–k**) was of particular interest, because of the possibility of subsequent dethiation. Several 2,6-dialkylphenolic Mannich bases had previously been found to give unsatisfactory condensations with 2,4-diaminopyrimidine.[2] The results suggested that the reaction rate to produce 5-substituted pyrimidines was slower than the self-condensation of the Mannich bases to yield diarylmethanes, since such products were formed in high yield in the presence of 2,4-diaminopyrimidine (see, for example, **30** under Experimental Section). However, it was found that **2c–g** reacted well with **1h** to produce **5–9** (Table I). A 2,6-dibromophenolic analogue (**2b**) reacted almost quantitatively with **1h** to give **4**.

The 6-substituent effect of markedly facilitating electrophilic attack on the pyrimidine carbon-5 would strongly suggest electron donation by these added functions. Actually, a 6-substituent on 2,4-diaminopyrimidine has an almost purely inductive effect, as determined by a study of the dissociation constants of such compounds.[7] (2,4,6-Triaminopyrimidine, for example, is a weaker base than is 2,4-diaminopyrimidine.[7,8]) Electron donation could then only apply to the case of the 6-alkylpyrimidines. Another explanation must be sought for the other substituents.

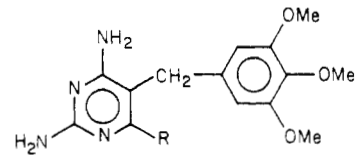
2,4-Diamino-6-substituted-pyrimidines, such as 2,4,6-triaminopyrimidine, may exist to a slight extent in the 4-imino-4,5-dihydro form and be capable of producing a 5-anion, which would react easily with **2**. The addition of a small amount of sodium methylate to the reaction of **2a** with 2,4,6-triaminopyrimidine increased the yield from 70 to over 90%. Furthermore, this condensation was found not to occur in acid;[2] the milieu of the reaction as described is basic, without additional sodium methylate. The Mannich base generates dimethylamine and the reactive electrophile, a methene quinone, upon heating.

Other experiments which would favor this conclusion are (1) H exchange with NaOD. An NMR spectrum in D_2O plus NaOD showed rapid exchange of the 5-proton. (2) Nonreaction of a 2,4,6-tris(*tert*-amino)pyrimidine, 2,4,6-tripyrrolidinopyrimidine (**27**; Chart I). No benzylpyrimidine was obtained on treating **27** with **2a**. Attempts to brominate **27** were likewise unsuccessful. Compounds such as **1l**, on the other hand, undergo bromination extremely readily.[9] (3) Nonreaction of a 1,3-disubstituted uracil. 1,3-Dimethyluracil does not react with **2a** under

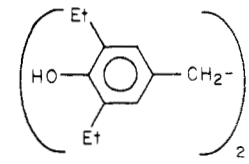
Chart I



23, R_1, $R_2 = NH_2$; R_3 = Pr-*n*
24, $R_1 = NH_2$; $R_2 = OH$; R_3 = Pr-*i*
25, $R_1 = NH_2$; R_2 = Cl; R_3 = Pr-*i*
26, R_1, $R_2 = NH_2$; R_3 = Pr-*i*
27, R_1, R_2, R_3 = c-NC_4H_8



28, R = Cl
29, R = NMe_2



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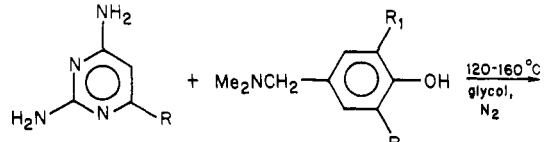
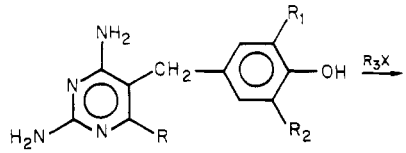
(8) A. Albert, R. Goldacre, and J. Phillips, *J. Chem. Soc.*, 2240 (1948).
(9) B. Roth, unpublished results.

Scheme I[a]

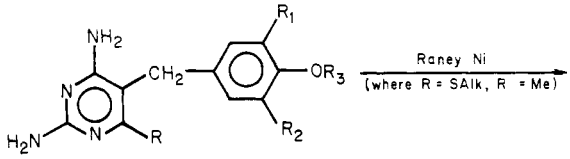


1a, R = Ph
b, R = OPh
c, R = OMe
d, R = NH_2
e, R = Pr-*n*
f, R = Pr-*i*
g, R = Cl
h, R = SMe
i, R = SEt
j, R = SBu-*n*
k, R = SHex-*n*
l, R = NMe_2
m, R = c-NC_5H_{10}

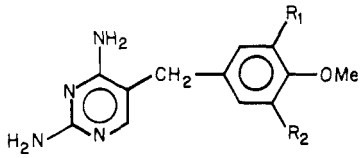
2a, R_1, R_2 = OMe
b, R_1, R_2 = Br
c, R_1, R_2 = Me
d, R_1 = Me; R_2 = Bu-*t*
e, R_1, R_2 = Et
f, R_1, R_2 = Pr-*i*
g, R_1, R_2 = Bu-*t*



3a-k, R_1, R_2 = OMe
4, R = SMe; R_1, R_2 = Br
5, R = SMe; R_1, R_2 = Me
6, R = SMe; R_1 = Me; R_2 = Bu-*t*
7, R = SMe; R_1, R_2 = Et
8, R = SMe; R_1, R_2 = Pr-*i*
9, R = SMe; R_1, R_2 = Bu-*t*
10, R = NH_2; R_1, R_2 = Bu-*t*



11a-f,h-k, R_1, R_2 = OMe; R_3 = Me
12, R = SMe; R_1, R_2 = Me; R_3 = Me
13, R = SMe; R_1, R_2 = Et; R_3 = Me
14, R = SMe; R_1, R_2 = Pr-*i*; R_3 = Me
15, R = SMe; R_1, R_2 = Bu-*t*; R_3 = Me
16, R = NH_2; R_1, R_2 = Bu-*t*; R_3 = Me
17, R = NH_2; R_1, R_2 = OMe; R_3 = Hex-*n*



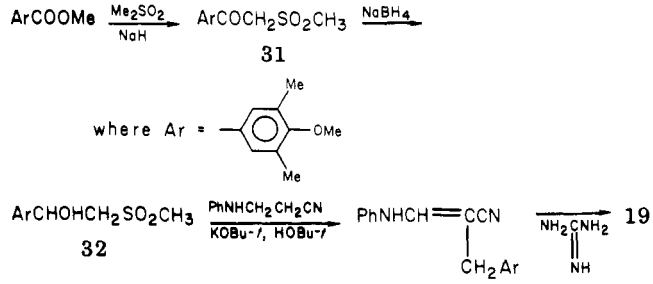
18, R_1, R_2 = OMe (trimethoprim)
19, R_1, R_2 = Me
20, R_1, R_2 = Et
21, R_1, R_2 = Pr-*i*
22, R_1, R_2 = Bu-*t*

[a] For R substituent of 3a-k, 11a-f, and 11h-k, see compound 1 substituents.

the conditions described here; however, uracil may exist to a slight extent as a 4-oxo-4,5-dihydro tautomer.

Alkylation of the phenolic benzylpyrimidines 3–10 (Scheme I) was carried out prior to removal of 6-(alkylthio) groups to produce 18–22. Yields of 4′-ethers were observed to be markedly higher when 6-substituents were present in the pyrimidine ring. A possible cause of this long-distance effect is competitive alkylation at N-1 with the 6-unsubstituted derivatives. A 6-substituent would decrease this possibility on both steric and electronic grounds, since the effect is inductive. The 4′-ethers generated are described in Table II.

Scheme II



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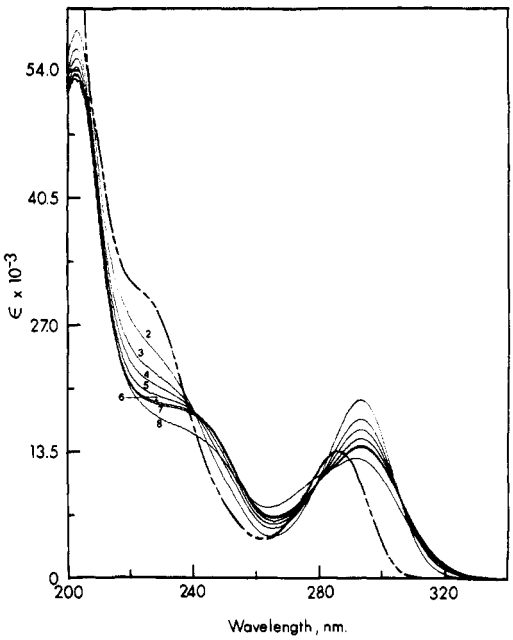

Figure 1. Ultraviolet absorption spectra of **29**: (1) in 0.01 N NaOH; (2) initial spectrum (20 °C) in 0.001 N HCl; (3–7) spectra in 0.001 N HCl after 15, 35, 70, 130, and 230 min, respectively; (8) spectrum in 0.001 N HCl after 20 h, 20 °C. Note that a second reaction has occurred, with loss of isosbestic points.

Dethiation of **11h** to produce trimethoprim (**18**) proceeded in greater than 80% yield using freshly prepared W-5 Raney nickel in refluxing β-methoxyethanol. Considerably lower yields were obtained at lower temperatures and longer times in ethanol (the conditions used to prepare Table III compounds). This synthesis of trimethoprim, in three steps from 2,4-diamino-6-(methylthio)pyrimidine, gave high yields at each stage and utilized readily available starting materials.

2,4,6-Triamino-5-(3,4,5-trimethoxybenzyl)pyrimidine (**11d**) was also prepared by another route, from 3,4,5-trimethoxybenzylmalononitrile and guanidine. Likewise, 2,4-diamino-5-(4-methoxy-3,5-dimethylbenzyl)pyrimidine (**19**) was prepared by an alternative route from 4-methoxy-3,5-dimethylbenzoic acid (Scheme II). In both cases the products were identical by the two procedures used.

2,4-Diamino-6-(dimethylamino)-5-(3,4,5-trimethoxybenzyl)pyrimidine (**29**) was prepared from its 6-chloro analogue (**28**), which in turn was prepared by selective amination of one halogen of 2-amino-4,6-dichloro-5-(3,4,5-trimethoxybenzyl)pyrimidine.[10] The UV spectra of **29** in neutral to mildly acidic aqueous solutions were remarkable in that they changed with time in a manner

(10) This compound was prepared by John Mentha in this laboratory by chlorination of the corresponding 4,6-pyrimidinedione and used directly in the reactions which followed.

Table II. 6-Substituted 2,4-Diamino-5-(4-alkoxybenzyl)pyrimidines by Alkylation of Corresponding Phenols

no.	pyrimidine 6-substituent	benzene substituents 3	4	5	% yield[a]	recrystn solvent[b]	mp, °C	emp formula	anal.
11a	C_6H_5	OCH_3	OCH_3	OCH_3	72	A	160-161	$C_{20}H_{22}N_4O_3$	C, H, N
11b	OC_6H_5	OCH_3	OCH_3	OCH_3	95	A	177-179	$C_{26}H_{22}N_4O_4$	C, H, N
11c	OCH_3	OCH_3	OCH_3	OCH_3	76	B	166	$C_{15}H_{20}N_4O_4$	C, H, N
11d[c]	NH_2	OCH_3	OCH_3	OCH_3	80	A	244-246 dec	$C_{14}H_{19}N_5O_3$	C, H, N
11e	C_3H_7-*n*	OCH_3	OCH_3	OCH_3	60	A	161-163	$C_{17}H_{24}N_4O_3$	C, H, N
11f	C_3H_7-*i*	OCH_3	OCH_3	OCH_3	31	C	172-174	$C_{17}H_{24}N_4O_3$	C, H, N
11h	SCH_3	OCH_3	OCH_3	OCH_3	91	D	178	$C_{15}H_{20}N_4O_3S$	C, H, N; S[d]
11i	SC_2H_5	OCH_3	OCH_3	OCH_3	53	E	177-179	$C_{16}H_{22}N_4O_3S$	C, H, N
11j	SC_4H_9-*n*	OCH_3	OCH_3	OCH_3	19	E	119.5-120.5	$C_{18}H_{26}N_4O_3S$	C, H, N
11k	SC_6H_{13}-*n*	OCH_3	OCH_3	OCH_3	71	E	120-122	$C_{20}H_{30}N_4O_3S$	C, H, N
12[e]	SCH_3	CH_3	OCH_3	CH_3	48	A	152-153	$C_{15}H_{20}N_4OS$	C, H, N
13	SCH_3	C_2H_5	OCH_3	C_2H_5	84	A	215-218 dec	$C_{17}H_{24}N_4OS \cdot HCl$	C, H, N
14	SCH_3	C_3H_7-*i*	OCH_3	C_3H_7-*i*	45	A		$C_{19}H_{28}N_4OS \cdot HCl$	C, H, N
15[f]	SCH_3	C_4H_9-*t*	OCH_3	C_4H_9-*t*	69	C	215-216	$C_{21}H_{32}N_4OS \cdot HCl$	C, H, N
16	NH_2	C_4H_9-*t*	OCH_3	C_4H_9-*t*	78	F	210-212	$C_{20}H_{31}N_5O$	H, N; C[g]
17	NH_2	OCH_3	OC_6H_{13}-*n*	OCH_3	97	D	155-156	$C_{19}H_{29}N_5O_3$	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, MeOH; C, absolute EtOH-EtOAc; D, EtOAc; E, absolute EtOH; F, 60:40 methyl cellosolve-H_2O. [c] This compound was also prepared by condensation of 3,4,5-trimethoxybenzylmalononitrile (0.02 mol) with guanidine (0.02 mol) in EtOH (50 mL) by refluxing for 3 h, concentrating, and chilling: yield 77%; mp 245-247 °C dec. [d] Calcd: S, 9.53; found, 10.08. [e] NMR (Me_2SO-d_6): δ 2.16 (s, 6, Me_2), 2.38 (s, 3, SMe), 3.61 (s, 5, OMe, CH_2), 5.81 (br s, 2, NH_2), 5.97 (br s, 2, NH_2), 6.83 (s, 2, Ar). [f] UV, neutral species (0.01 N NaOH), sh 232 nm (ε 20 300), sh 248 (9 500), λ_{max} 296 (9900); UV, cation (0.01 N HCl), sh 222 nm (ε 21 500), sh 241 (14 300), λ_{max} 308 (11 900). [g] Calcd: C, 67.19; found, 66.73.

Table III. 2,4-Diamino-5-(3,5-dialkyl-4-methoxybenzyl)pyrimidines by Dethiation of 6-(Methylthio) Derivatives

no.	benzene substituents 3	5	% yield[a]	recrystn solvent[b]	mp, °C	emp formula	anal.
19[c]	CH_3	CH_3	35	A	207[c]	$C_{14}H_{18}N_4O$	H, N; C[d]
20	C_2H_5	C_2H_5	31	A	153-154	$C_{16}H_{22}N_4O$	C, H, N
21[e]	C_3H_7-*i*	C_3H_7-*i*	43	B	205-207	$C_{18}H_{26}N_4O$	C, H, N
22	C_4H_9-*t*	C_4H_9-*t*	56	B	272-275	$C_{20}H_{30}N_4O \cdot HCl$	C, H, N

[a] After one recrystallization. [b] A, dilute EtOH; B, 95% EtOH. [c] This compound was also prepared by another route; see Experimental Section. [d] Calcd: C, 65.10; found, 65.70. [e] UV, neutral species (0.01 N NaOH), sh 241 nm (ε 9400), λ_{max} 288 (6280); UV, cation (0.01 N HCl), sh 220 (31 400), λ_{max} 272 (4700).

Table IV. Inhibition of Dihydrofolate Reductases by 6-Substituted 2,4-Diamino-5-(substituted-benzyl)pyrimidines[a]

no.	pyrimidine 6-subst	benzene substituents 3	4	5	$I_{50} \times 10^8$ vs. DHFR, M *E. coli*	rat liver	*P. berghei*
18	H	OCH_3	OCH_3	OCH_3	0.5	26 000	12
a	CH_3	OCH_3	OCH_3	OCH_3	10	>40 000	34
a	CH_3	OCH_3	OH	OCH_3	30		48
11e	C_3H_7-*n*	OCH_3	OCH_3	OCH_3	150	150 000	
3e	C_3H_7-*n*	OCH_3	OH	OCH_3	80		150
11f	C_3H_7-*i*	OCH_3	OCH_3	OCH_3	200-400		
11a	C_6H_5	OCH_3	OCH_3	OCH_3	300	>40 000	
3a	C_6H_5	OCH_3	OH	OCH_3	200	>4 500	
11b	OC_6H_5	OCH_3	OCH_3	OCH_3	>4 500	>4 500	
11c	OCH_3	OCH_3	OCH_3	OCH_3	>45 000	>10 000	
11h	SCH_3	OCH_3	OCH_3	OCH_3	>4 500	>4 500	~2000
13	SCH_3	C_2H_5	OCH_3	C_2H_5	>4 000	>4 000	
28	Cl	OCH_3	OCH_3	OCH_3	0% ~ 400		
11d	NH_2	OCH_3	OCH_3	OCH_3	1 000	>90 000	
16	NH_2	C_4H_9-*t*	OCH_3	C_4H_9-*t*	6 000		
29	$N(CH_3)_2$	OCH_3	OCH_3	OCH_3	450 000		

[a] See ref 2.

suggestive of covalent hydration. Figure 1 illustrates the spectral changes with time at pH 3; upon treatment with alkali, the spectrum rapidly reverted to its precise original character in 0.01 N NaOH, shown also in Figure 1. At pH 7, the spectrum in Tris buffer gradually changed over 90 min to show absorption which was almost the same as that obtained immediately in 0.0067 M phosphate. In 1 N HCl additional spectral changes occurred upon heating the solution, to give absorption values practically identical with those of 2,4-diamino-5-(3,4,5-trimethoxybenzyl)pyrimidin-6(1*H*)-one in the same medium. The latter compound did not exhibit the above-described phenomena. This reaction and its implications will be discussed in detail in a forthcoming publication.

Biological Activity. Table IV lists the I_{50} activities of the 6-substituted trimethoprim analogues against *E. coli* and rat liver DHFR, which in all cases are considerably less than that of trimethoprim. With alkyl and aryl substituents, the activity decreased directly with increasing substituent size. The case of the OPh, OMe, and SMe derivatives is complicated by the fact that all of these substituents lower the dissociation constants of the pyri-

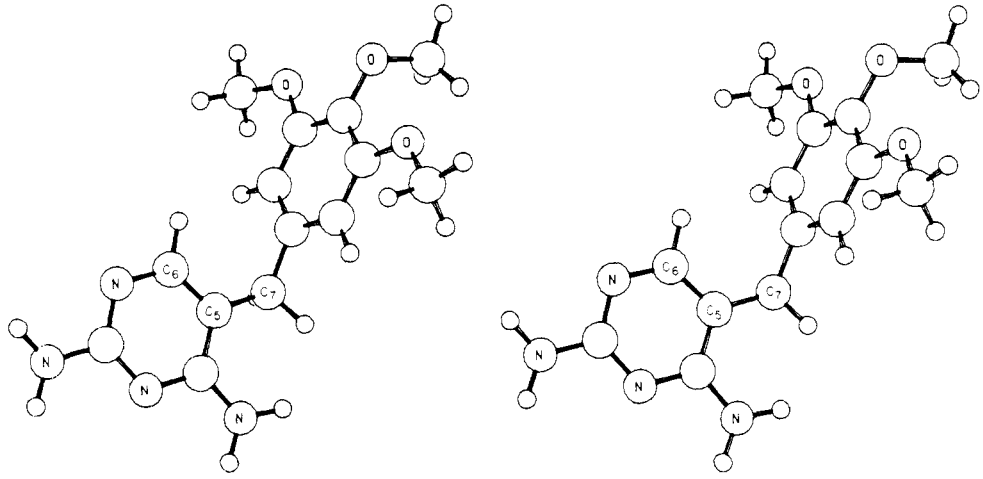

Figure 2. Stereo view of trimethoprim in the conformation of its crystalline hydrobromide salt.

Table V. Antibacterial Activity of 6-Substituted 5-(Substituted-benzyl)pyrimidines

no.	pyrimidine 6-subst	benzene substituents	MIC, μg/mL, microorganisms[a]					
			St. pyogenes	*S. aureus*	*Sal. typhosa*	*E. coli*	*Shig. dys.*	*P. vulgaris*
11a	C_6H_5	3,4,5-$(OCH_3)_3$	16	62	250	250	125	>500
11b	OC_6H_5	3,4,5-$(OCH_3)_3$	>500	>500	>500	>500	>500	>500
11c	OCH_3	3,4,5-$(OCH_3)_3$	8	125	>500	>500	>500	>500
15	SCH_3	3,5-$(C_4H_9$-$t)_2$-4-OCH_3	>500	>500	>500	>500	>500	>500

[a] Organisms, respectively, are: *Streptococcus pyogenes* CN10, *Staphylococcus aureus* CN491, *Salmonella typhosa* CN512, *Escherichia coli* CN314, *Shigella dysenteriae* CN1513, *Proteus vulgaris* CN329.

midines, to make them weaker bases by 1 to 2 units. The pK_a value for **11h**, for example, is 5.09 (20 °C), compared to 7.12 for trimethoprim.[7] In all cases, the activities of these derivatives were too low to measure accurately, because of solubility problems at the high concentration required for inhibition. In general, diaminopyrimidine derivatives (other than direct analogues of folic acid) have not exhibited high inhibitory activity for bacterial DHFR enzymes unless they are appreciably protonated at physiological pH. This physical property then obscures any other effect which such substituents might have on binding.

The one polar substituent useful for a test of its effect on binding is 6-NH_2. Although the dissociation constant remains near 7, the activity is lowered 1000-fold. The 6-(dimethylamino) derivative (**29**) was thought originally to represent an unusual case in that the site of protonation was very likely changed from N-1 to N-3, which would change the orientation of the compound with reference to an anionic site on the enzyme, known now to be Asp-27 for *E. coli* DHFR.[11] However, the above-described UV data (Figure 1) indicate a more complex phenomenon which destroys the analogy of **29** to the remainder of the series. It will be noted that the compound is almost totally inactive.

Figure 2 depicts a stereo view of trimethoprim showing the conformation of its hydrobromide salt as determined by X-ray crystallography.[12] The acetate salt was found to have another conformation in the crystal state, with an ortho proton of the benzene ring aimed toward the π cloud of the pyrimidine.[13] Cayley et al.[14] have studied the conformation of trimethoprim in interaction with *E. coli* DHFR by NMR spectroscopy and found two possible conformations, both of which have torsional angles about the C_5–C_7 bond similar to that shown in Figure 2. It is evident that any but a very small 6-substituent would of necessity change the conformation of trimethoprim from that of Figure 2, due to the proximity of this position to the benzene ring. The polar 6-amino group would be expected to create a repulsion from such a conformation. The lack of activity of all of the 6-substituted trimethoprim derivatives is then consistent with an enforced conformational change of the inhibitor, although it is certainly possible that other enzymatic restraints could be responsible for this result.

Table V illustrates the antibacterial activity of a few of the derivatives, which show only feeble activity against the Gram-positive organisms.

The biological activity of the compounds described in Table III will be discussed in a future paper of this series which gathers the data from all alkylbenzyl derivatives prepared by a variety of routes.

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. The analyses were carried out by Drs. Samuel Blackman and Stuart Hurlbert and their staffs or by Atlantic Microlabs, Inc., Atlanta, Ga. Nuclear magnetic resonance (NMR) spectra were recorded on Varian A-60, XL-100, and T-60 spectrophotometers; chemical shifts are reported in parts per million (δ) from internal tetramethylsilane. Ultraviolet spectra were obtained using a Cary 118 spectrophotometer.

General Method for the Preparation of 5-(4-Hydroxy-

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benzyl)pyrimidines from Phenolic Mannich Bases and 2,4-Diamino-6-substituted-pyrimidines. The method used was that described in paper 2 of this series.[2] It was found that in most cases it was not necessary to add sodium methylate in excess of what was required to neutralize salts of starting materials. The isolation and purification were normally conducted as previously described. Compounds prepared, as well as their yields and physical properties, are listed in Table I.

Alkylation of 4-Hydroxybenzylpyrimidine Derivatives. Alkylations were carried out using 1 equiv of alkyl halide per mole of the phenol as its sodium or potassium salt, by adding NaOMe or KO-*t*-Bu in Me_2SO, following the procedure of paper 2.[2] The compounds prepared and their properties are listed in Table II.

Desulfurization of 6-(Alkylthio)pyrimidines. The compounds listed in Table III were dethiated with a large excess of freshly activated Raney nickel (W-4 or W-5) in ethanol, usually over a 6-h period. Yields were not high by this procedure; usually, a considerable portion of the product was adsorbed on the nickel. A better procedure is described below for the preparation of **18**.

2,4-Diamino-5-(3,4,5-trimethoxybenzyl)pyrimidine (18). To 0.88 g of **11h** in 70 mL of 2-methoxyethanol plus 5 mL of H_2O was added 11 g of freshly prepared Raney nickel paste in EtOH. The nickel was prepared by the W-5 process.[15] The mixture was heated for 1 h at 120 °C with stirring and then filtered hot. The nickel was extracted with more hot solvent. The filtrates were combined and evaporated to dryness, yielding 0.63 g (83%) of **18**, mp 196–199 °C (EtOH), which gave a single spot on TLC. Anal. ($C_{14}H_{18}N_4O_3$) C, H, N. The spectral properties were identical with those of previously prepared samples.[2]

2,4-Diamino-6-*n*-propylpyrimidine Hydrochloride (23).[16] Ten grams of 6-*n*-propyluracil[17] was refluxed with 50 g of $POCl_3$ for 90 min, followed by concentration to a syrup, drowning on ice, and neutralization with NH_4OH. The resultant oil was extracted with ether, dried, and stripped of ether. The oil was then mixed with 75 mL of MeOH saturated at 0 °C with NH_3 and heated for 7 h at 150 °C in a stainless-steel bomb with glass liner. The resultant mixture was filtered and the filtrate taken to dryness. The solid was extracted with 30:1 Me_2CO–*i*-PrOH to remove a brown insoluble impurity. The solution was concentrated and treated with HCl in Et_2O, followed by crystallization from EtOH–Et_2O and then from water: yield 5 g; mp 190–194 °C. Anal. ($C_7H_{12}N_4$·HCl) C, H, N.

2-Amino-6-isopropyl-4(3*H*)-pyrimidinone (24).[16] A mixture of 30 g (0.19 mol) of ethyl isobutyrylacetate, 20.1 g (0.21 mol) of guanidine hydrochloride, 22.6 g (0.42 mol) of NaOMe, and 180 mL of EtOH was refluxed for 2 h, filtered hot, concentrated to a thick syrup, dissolved in water, and neutralized with HCl, yielding 20.8 g of **24** (71.5%), mp 246–248 °C (90% EtOH). Anal. ($C_7H_{11}N_3O$) C, H, N.

2-Amino-4-chloro-6-isopropylpyrimidine (25).[16] Compound **24** (15 g) was refluxed for 2 h with 67 g of $POCl_3$, concentrated, treated with ice and NH_4OH, allowed to stand for 1 h at −10 °C, and filtered: yield 7.5 g (45%); mp 99–102 °C. Anal. ($C_7H_{10}ClN_3$) C, H, N.

2,4-Diamino-6-isopropylpyrimidine Hydrochloride (26).[16] Seven grams (0.0407 mol) of **25** was heated in a bomb in saturated methanolic NH_3 for 6 h at 160 °C. After concentration to remove most of the excess NH_3, the solution was acidified with HCl, taken to dryness, and recrystallized from *i*-PrOH followed by H_2O, with charcoal treatment. Trituration with 75:25 Me_2CO–Et_2O removed a slight impurity, mp 181–184 °C (H_2O). Anal. ($C_7H_{12}N_4$·HCl) C, H, N.

2,4,6-Tri-*N*-pyrrolidinopyrimidine (27).[18] A solution of 9.17 g (50 mmol) of 2,4,6-trichloropyrimidine in 100 mL of absolute EtOH was chilled in an ice bath and 8 mL (100 mmol) of pyrrolidine was added dropwise. The mixture was then allowed to warm to room temperature and another 16 mL of pyrrolidine was added, followed by heating under reflux for 2 h. The solvent was removed, and the residue was washed with dilute NaOH, followed by water, yield 14.4 g. Recrystallization produced a product which analyzed as a monochlorodipyrrolidinopyrimidine. This substance (5.96 g, 0.024 mol) was heated in a sealed glass tube with 2.2 mL (0.026 mol) of pyrrolidine at 140 °C for 6 h. The product was washed with dilute NaOH and water, followed by recrystallization from absolute EtOH: mp 165–166.5 °C; UV (neutral species in 0.01 N NaOH) λ_{max} 285 nm (ϵ 18 500), 230.5 (46 000). Anal. ($C_{16}H_{25}N_5$·$0.1H_2O$) C, H, N.

Attempts were made to react this product with Mannich base **2a** using between 0.1 and 1.0 mol of NaOMe. After 4 h at 150–170 °C, the starting material (**27**) was recovered in all cases, along with Mannich decomposition products.

2,4-Diamino-5-(3,4,5-trimethoxybenzyl)-6-chloropyrimidine (28). A mixture of 1.25 g of 2-amino-4,6-dichloro-5-(3,4,5-trimethoxybenzyl)pyrimidine[10] and 20 mL of EtOH saturated with NH_3 at 0 °C was heated in a sealed tube at 117 °C for 7 h. After the mixture cooled, the crystalline product was filtered: yield 0.8 g; mp 222–224 °C (EtOAc). Anal. ($C_{14}H_{17}ClN_4O_3$) C, H, N.

2,4-Diamino-5-(3,4,5-trimethoxybenzyl)-6-(*N*,*N*-dimethylamino)pyrimidine (29). A mixture of 0.5 g of **28** and 5 mL of a 25% ethanolic solution of Me_2NH was heated in a sealed tube at 132 °C for 4 h. After the mixture was chilled, the white product was filtered, washed with EtOH, and dried, yield 375 mg. This was slurried in dilute NaOH, filtered, and recrystallized from EtOH: mp 211–214 °C dec; UV (neutral species, 0.01 N NaOH) λ_{max} 286 nm (13 400), sh 225 (29 300). (See also Figure 1 for additional spectral results.) Anal. ($C_{16}H_{23}N_5O_3$) C, H, N.

Bis(3,5-diethyl-4-hydroxyphenyl)methane (30). A mixture of 4.4 g (0.04 mol) of 2,4-diaminopyrimidine, 8.4 g (0.04 mol) of 2,6-diethyl-4-[(*N*,*N*-dimethylamino)methyl]phenol,[2] 0.22 g (0.004 mol) of NaOMe, and 40 mL of ethylene glycol was heated for 4 h at 140 °C, cooled, and neutralized with HOAc. The ethylene glycol was removed in vacuo, and the residue was extracted with H_2O. There remained 5.8 g of **30** (93.5%), mp 99–100 °C (dilute EtOH). Anal. ($C_{21}H_{28}O_2$) C, H.

α-(Methylsulfonyl)-4-methoxy-3,5-dimethylacetophenone (31). 4-Methoxy-3,5-dimethylbenzoic acid was converted to its methyl ester [bp 79–84 °C (1 mm)] and treated with dimethyl sulfone in the presence of NaH according to the procedure of Cresswell, Mentha, and Seaman:[19] mp 105.5–106.5 °C (absolute EtOH); NMR (Me_2SO-d_6) δ 2.30 (s, 6, Me_2), 3.14 (s, 3, SO_2Me), 3.74 (s, 3, OMe), 5.03 (s, 2, CH_2), 7.78 (s, 2, Ar); UV (EtOH) λ_{max} 209 nm (ϵ 18 700), 271 (12 900); MS 256, 163, 105, 91. Anal. ($C_{12}H_{16}O_4S$) C, H; S: calcd, 12.51; found, 11.80.

Methyl 2-Hydroxy-2-(3,4,5-trimethoxyphenyl)ethyl Sulfone (32). Compound **31** was reduced with $NaBH_4$ as described in ref 19 to produce **32**: mp 142–143.5 °C (absolute EtOH); NMR (Me_2SO-d_6) δ 2.20 (s, 6, Me_2), 3.00 (s, 3, SO_2Me), 3.26 (t, 2, CH_2SO_2), 3.62 (s, 3, OMe), 4.97 (m, 1, C*H*OH), 5.75 (d, 1, CHO*H*), 7.04 (s, 2, Ar); UV (EtOH) λ_{max} 266 nm (ϵ 500), sh 215 (10 300). Anal. ($C_{12}H_{18}O_4S$) C, H, S.

β-Anilino-α-(4-methoxy-3,5-dimethylbenzyl)acrylonitrile (33). Compound **32** was treated with β-anilinopropionitrile as described in ref 19 to give **33**: mp 186–188 °C (EtOH); NMR (Me_2SO-d_6) δ 2.21 (s, 6, Me_2), 3.51 (s, 2, CH_2), 3.64 (s, 3, OMe), 6.91 (m, 3, Ar), 7.23 (s, 2, Ar), 7.3 (m, 2, Ar), 7.64 (d, 1, CH, J = 13 Hz), 9.08 (d, 1, NH, J = 13 Hz); UV (95% EtOH) sh 210 nm (ϵ 22 800), λ_{max} 285 (26 100), 306 (27 400); MS 292, 277, 261, 200, 155, 149, 136. TLC showed two spots (two isomers), R_f 0.3, 0.5 (hexane–EtOAc, 4:1, on silica gel). Anal. ($C_{19}H_{20}N_2O$) C, H, N.

2,4-Diamino-5-(4-methoxy-3,5-dimethylbenzyl)pyrimidine (19). Sodium methylate (8.0 g, 0.15 mol) was dissolved in 200 mL of absolute EtOH and then stirred with 10.5 g (0.11 mol) of guanidine hydrochloride for several minutes, after which it was filtered from salt and mixed with 10.3 g (0.036 mol) of **33**. The resultant solution was heated under reflux for 24 h and chilled. No precipitate separated. The solvent was then removed, and the residue was extracted with H_2O and isolated, yield 9.5 g. This was extracted with Et_2O, followed by crystallization twice from EtOH: yield 3.24 g (39%); mp 206–207 °C; UV (neutral species, 0.01 N NaOH) sh 240 nm (ϵ 10 500), λ_{max} 286 (7400); UV (cation,

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0.01 N HCl) sh 220 (30900), λ_{max} 271 (5400). (See also Table III.) Anal. ($C_{14}H_{18}N_4O$) C, H, N.

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Synthesis, Biological Activity, and Structure–Activity/Toxicity Relationships of a Series of Terphenyl Analogues of Hemicholinium-3 and Acetyl-*seco*-hemicholinium-3.[1] 3[2]

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Further work on the development and investigation of activity and site of action of inhibitors which act presynaptically on neuromuscular function is reported. Terphenyl HC-3 (**5c**) and a series of six new terphenyl analogues of hemicholinium-3 (HC-3, **1**) and acetyl-*seco*-hemicholinium-3 (AcHC-3, **3a**) all having a common terphenyl central nucleus were synthesized. The seco form of terphenyl HC-3 (**5c**) was altered at the choline moieties' oxo terminal to give the acetate **6a**, ether **6b**, ketone **6c**, alkane **6d**, thioacetate **6e**, and thiol **6f** analogues which, along with **5c**, are stable in slightly acidic H_2O. Ester hydrolysis of **6a** and enolization of **6c** slowly occurs at pH 7.4, with subsequent cyclization to form **5c** and a hemiacetalene **8**, respectively. Reaction or decomposition at pH 7.4 is insignificant for all seven terphenyl compounds for 4 to 5 h, but at pH 9.4 greater than 10% decomposes. In the presence of acetyl- or butyrylcholinesterase in H_2O at pH 7.4, contrary to its biphenyl analogue, **6e** does not hydrolyze; like their biphenyl analogues, all the other compounds are stable except **6a**, which reacts within seconds, apparently by an irreversible binding to the esterase, without hydrolysis and subsequent cyclization to **5c**. Compared to their respective biphenyl analogues, mouse toxicity studies (LD_{50}) show comparable lethalities of the terphenyl compounds, except for **6b** and **6e**, which are 9 and 23 times less toxic, respectively. Choline and neostigmine only slightly altered the toxicity of all compounds except **6a**, whose toxicity was effectively antagonized. Structure–activity/toxicity relationships of **5c** and **6a–f** are discussed relative to each other and their biphenyl analogues.

The synthesis of hemicholinium-3 (HC-3, **1**), a prototypical prejunctional neuromuscular inhibitor, was reported in 1954 by Long and Schueler.[3] HC-3 (**1**) is synthesized as the seco form **2**, but rapidly undergoes intramolecular cyclization in H_2O to form the hemiacetal (**1**).[3,4] The pharmacological significance of cyclization was first evaluated with acetyl-*seco*-hemicholinium-3 (AcHC-3, **3a**), which, however, slowly undergoes hydrolysis in H_2O with subsequent cyclization to form HC-3 (**1**).[5–10] Alteration of the choline or acetylcholine moiety of **1** or **3a**, respectively, and further analysis of cyclization vs. noncyclization were studied in our laboratory with the analogs **3b–f**[2,6,7,11,12] and **4**.[2,11] The type and potency of pharmacological activity and toxicity varied with R group and cyclization. Replacement of the aromatic nucleus with an aliphatic (hexamethylene) chain, compound **5a**,[13] was reported in 1962, while substitution of a norphenyl nucleus, compound **5b**, was studied in 1966 in our laboratory.[14] Most of the

3a, R = $OOCCH_3$ **3c**, R = CH_2COCH_3 **3e**, R = $SOCCH_3$
b, R = OC_2H_5 **d**, R = n-C_3H_7 **f**, R = SH

4

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