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Studies on Pyrimidine Derivatives and Related Compounds. LXXXVII.[1] Reaction of Thiamine Analogues with Diethyl Benzoylphosphonate[2]

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Reaction of thiamine analogues lacking the 5-hydroxyethyl group (**6a—e**) with diethyl benzoylphosphonate (**7**) was carried out. Comparison of reactivity in 4'-substituted thiamine analogues and those lacking the hydroxyethyl group suggests the presence of an interaction in the thiamine molecule between the pyrimidine ring and the hydroxyethyl group in an aprotic solvent. Reactivity at the 2 position (the active center in enzymatic decarboxylation) in 4'-substituted thiamine analogues may be affected by this interaction.

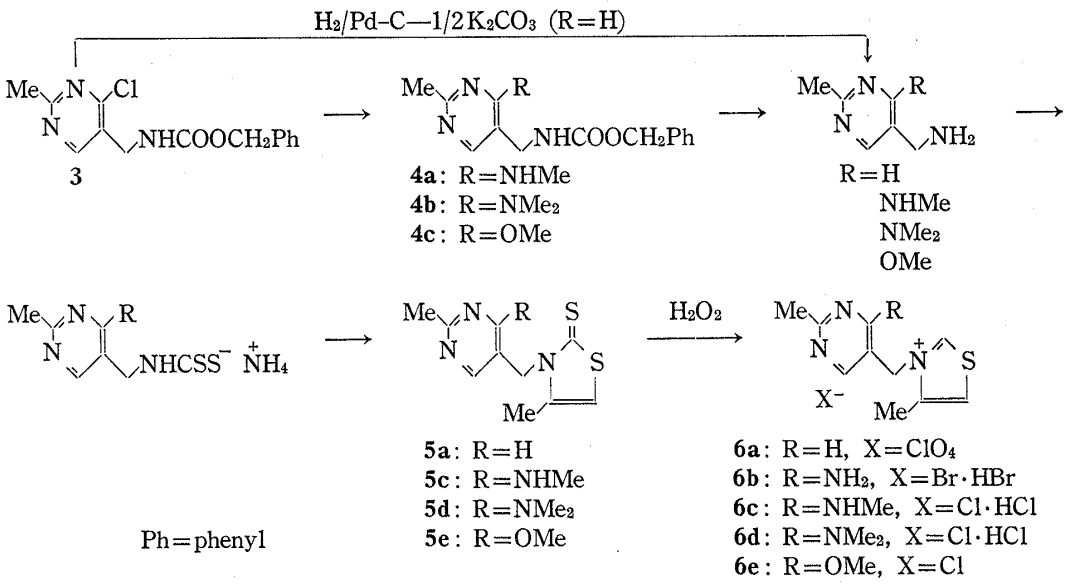
Previously,[2] we reported the reaction of various thiamine analogues with an electrophile, diethyl benzoylphosphonate. We found that in the reaction of 3-(2-methyl-4-substituted-5-pyrimidinyl)methyl-4-methyl-5-(2-hydroxyethyl)thiazolium salts (**1**) the yields of products in which the thiazolium 2 position was substituted by diethyl benzoylphosphonate varied markedly according to the 4'-substituent, the yield decreasing as the bulkiness of the 4'-substituent increased from H, NH_2, NHMe and OMe to NMe_2; while, in the reaction of 3-(2-substituted benzyl)-4-methyl-5-(2-hydroxyethyl)thiazolium salts (**2**) the 2'-substituent had no effect on the yield.

It is interesting to discover the cause of this difference in the reaction for an understanding of the role of the 4'-amino substituent on the pyrimidine ring in the decarboxylation of pyruvate by coenzyme thiamine pyrophosphate, since no relationship has been found between actual coenzyme activity and model experiments on the catalytic ability for acetoin formation of 4'-substituted thiamine analogues in water.[4,5] In addition, Lienhard and Crosby[6] have argued that catalysis in thiamine pyrophosphate dependent enzymatic reactions may be due in large part to binding of the thiazolium nucleus at a hydrophobic region of the enzymes, so it is interesting to observe the reaction behaviour of thiamine analogues in aprotic solvents, such as in the present reaction with diethyl benzoylphosphonate in dimethyl formamide (DMF).

In this paper we report an examination of the effect of the hydroxyethyl group at 5 position by studying the acylphosphonate reaction with 3-(2-methyl-4-substituted-5-pyrimidinyl)-methyl-4-methylthiazolium salts (**6**) lacking the hydroxyethyl group at the 5 position.

The thiamine analogues used were made according to the reaction scheme shown in Chart, starting from benzyl N-(2-methyl-4-chloro-5-pyrimidinylmethyl)carbamate (**3**) and using the SB_1 method. **6b** ($R=NH_2$) was synthesized according to Buchman.[7]

1) Part LXXXVI: A. Takamizawa and I. Makino, *Chem. Pharm. Bull.* (Tokyo), **22**, 1765 (1974).
2) A. Takamizawa and H. Harada, *Chem. Pharm. Bull.* (Tokyo), **21**, 770 (1973).
3) Location: *Fukushima-ku, Osaka, 553, Japan.*
4) C.D. May and P. Sykes, *J. Chem. Soc.* (*C*) **1966**, 649.
5) A. Schellenberger, *Angew. Chem. Internat. Edit.*, **6**, 1024 (1967).
6) J. Crosby and G.E. Lienhard, *J. Am. Chem. Soc.*, **92**, 5707 (1970); J. Crosby, R. Stone, and G.E. Lienhard, *ibid.*, **92**, 2891 (1970).
7) E.R. Buchman and E.M. Richardson, *J. Am. Chem. Soc.*, **67**, 395 (1945).



Chart

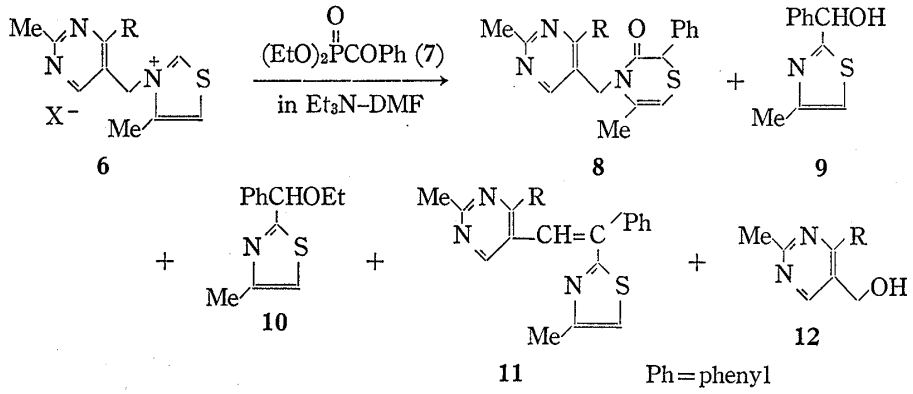
Reaction with benzoylphosphonate was carried out with 1 mmole of the thiamine analogue and 1.2 mmoles of diethyl benzoylphosphonate in dry DMF at room temperature using triethylamine as a base. The reaction mixture was treated with aqueous alkali after evaporation of DMF, then the chloroform extract was separated by preparative layer chromatography.

The structures of the products were elucidated by comparing their spectral data with those of known analogues. For example, the elemental analysis of **8a** agreed with $C_{17}H_{17}ON_3S$, and it had an amidocarbonyl band at 1668 cm^{-1} in its infrared (IR) spectrum. The nuclear magnetic resonance (NMR) spectrum of **8a** (in $CDCl_3$ sol.) showed signals at δ 1.95 (thiazine 5-Me) and δ 5.58 (thiazine 6-H) with a small coupling constant ($J\fallingdotseq1$ Hz), δ 2.72 (Pm 2-Me), 8.53 (Pm-4, 6H) as two singlets, δ 4.80, 5.13 (Pm 5-CH_2) as a AB quartet (J=16.0 Hz), δ 7.33 (C_6H_5) as a singlet, and δ 4.56 (thiazine 2-H), which is characteristic for 2-phenyl-3-oxo-2,3-dihydro-4H-1,4-thiazine,[2] as a doublet (J=1.7 Hz) coupled with thiazine 6H. Comparing this spectral data with that for the known 2-phenyl-3-oxo-4-(2-methyl-4-amino-5-pyrimidinyl)-methyl-5-methyl-6-(2-hydroxyethyl)-2,3-dihydro-4H-1,4-thiazine, the structure of **8a** can be written as 2-phenyl-3-oxo-4-(2-methyl-5-pyrimidinyl)methyl-5-methyl-2,3-dihydro-4H-1,4-thiazine. The structure of compound **9** was determined to be α-(4-methyl-2-thiazolyl)benzyl-alcohol as it had the composition $C_{11}H_{11}ONS$, and showed signals in its NMR spectrum at δ 2.33 (3H, d, $J\fallingdotseq1$ Hz, 4-Me), 4.05 (1H, OH), 5.98 (1H, s, C_6H_5ÇHOH), 6.77 (1H, m, 5-H), 7.20—7.60 (5H, m, C_6H_5). Compound **10** had the composition $C_{13}H_{15}ONS$, and as its NMR signals were the same as those of compound **9** except for replacement of the hydroxyl signal by an ethyl signal at δ 1.25 (3H, t, J=7.0) and 3.62 (2H, q, J=7.0 Hz), it was concluded that the structure of **10** was ethyl α-(4-methyl-2-thiazolyl)benzyl ether. This ethyl group might be derived from the ethyl ester of benzoylphosphonate, but details of the mechanism were not investigated.

Two geometric isomers of compound **11d** were obtained in 2:1 ratio. Both showed a parent peak at *m/e* 336 in their mass spectra. The NMR spectrum of the major product (**11d—a**) exhibited signals at δ 2.45 (3H, s, Pm 2-Me), 2.50 (3H, d, $J\fallingdotseq1$ Hz, Th 4-Me), 3.13 (6H, s, NMe_2), 6.87 (1H, m, Th 5-H), 7.33 (5H, s, C_6H_5), 7.60 and 7.70 (2×1H, 2×s, Pm 6-H and >=ĊH); and the minor product (**11d—b**) showed signals at δ 2.42 (3H, d, $J\fallingdotseq1$ Hz, Th 4-Me), 2.52 (3H, s, Pm 2-Me), 3.12 (6H, s, NMe_2), 6.92 (1H, m, Th 5-H), 6.97 and 7.97 (2×1H, 2×s, Pm 6-H and >=ĊH), 7.42 (5H, s, C_6H_5). From this data the structure of compound **11d** could be assigned as 2-methyl-4-dimethylamino-5-[β-(4-methyl-2-thiazolyl)stylyl]pyrimidine by comparison with the spectrum of 2-methyl-4-dimethylamino-5-{β-[4-methyl-5-

(2-hydroxyethyl)-2-thiazolyl]stylyl}-pyrimidine previously reported.[2] The 4′-dimethylamino compound (**6d**) showed the same tendency to cleave at the bond between the pyrimidinylmethyl carbon and the thiazolium nitrogen as the 4′-dimethylamino thiamine analogue reported previously.[2] Accordingly, the products were only substituted thiazole derivatives and no ring expanded product was obtained. Reaction products and their yields are shown on Table I.

Table I. Reaction Products and Their Yields in the Reaction of Benzoylphosphonate with 3-(2-Methyl-4-substituted-5-pyrimidinyl)methyl-4-methylthiazolium Halides



6 8 9 10 11 12

Starting material 6	R	Yields of products 8(%)	9(%)	10(%)	11(%)	Total 8—11(%)	12(%)
a	H	51			4	55	
b	NH_2	67				67	
c	NHMe	16	8	24		48	
d	NMe_2		14	20	10	44	12
e	OMe	71				71	

Table I shows the total yield of products substituted at 2 position was essentially independent of the 4′-substituent, though the kinds and yields of products varied in each series.

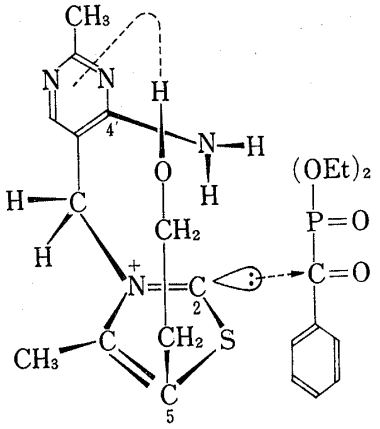
The total yields of the products substituted at the thiazolium 2 position are shown in Table II, compared with the results for 3-(2-methyl-4-substituted-5-pyrimidinyl)methyl-4-methyl-5-(2-hydroxyethyl)thiazolium salts (**1**) and 3-(2-substituted benzyl)-4-methyl-5-(2-hydroxyethyl)thiazolium salts (**2**) reported previously.[2] It is seen that the presence of both

Table II. Total Yields (%) of Products substituted at the 2 Position of Thiazolium in the Benzoylphosphonate Reaction

R	Compound 6	1	2
H	55	93	60
NH_2	67	90	77
NHMe	48	29	
NMe_2	44	11	88
OMe	71	13	63

substituents, the pyrimidine nucleus and the hydroxyethyl group at the 5 position, is necessary for appearance of a substituent effect by 4′-substituents.

Consideration of the role of these two substituents suggests the possibility of direct or indirect mutual intramolecular interaction between the substituents; the pyrimidine and thiazolium ring are fixed in a constant conformation where 4′-substituent R and 2-position of the thiazolium ring are in close proximity to each other and consequently yields of products substituted at the 2 position decrease through the steric repulsion between the 4′-substituent and the entering electrophile to an extent depending on the bulkiness of the 4′-substituent, as shown in **13**. Applying the conformation suggested above to the model proposed by Schellenberger,[5] we see that the 4′-substituent exists close to the thiazolium 2 position and that the 4′ amino group is located at a position where it can easily act to release an aldehyde molecule from hydroxyethylthiamine as intramolecular catalysis, a result which lends support to Schellenberger's hypothesis.



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In the coenzyme, however, this hydroxyethyl group exists as the pyrophosphate ester. The role of this pyrophosphate group has been thought to be that of a binding site for apoprotein, but it may be that it has rather a role of fixing the coenzyme molecule into a constant conformation by interaction with the pyrimidine nucleus. On this point, it is now necessary to see whether the substituent effects found with 3-(2-methyl-4-substituted-5-pyrimidinyl)methyl-4-methyl-5-(2-hydroxyethyl) thiazolium salts (**1**) are found with the pyrophosphate ester too.

Experimental[8]

3-(2-Methyl-5-pyrimidinyl)methyl-4-methylthiazoline-2-thione (5a)——To a solution of benzyl N-(2-methyl-4-chloro-5-pyrimidinylmethyl)carbamate (3)[2] (20 g) in dioxane (130 ml) was added a solution of aqueous K_2CO_3 solution (K_2CO_3 4.74 g, 100 ml) and the mixture was hydrogenated at atmospheric pressure and room temperature over palladium-black catalyst. The absorption vessel contained soda lime in order to take up carbon dioxide evolved. After absorption of 2 moles of hydrogen, catalyst was removed by filtration and the filtrate was evaporated *in vacuo*. The residue was dissolved in ether and dried over anhyd. K_2CO_3. The residue was dissolved in EtOH (60 ml) containing conc. NH_4OH (8 ml). To the stirred solution was added dropwise 10 ml of carbon disulfide and stirring was continued for an hour, then precipitate was collected by filtration. To a stirred suspension of the precipitate in EtOH (100 ml), chloracetone (6 g) was added at room temperature and stirring was continued for 4 hr, then the precipitate which formed was filtered off. The residue obtained by evaporation of the filtrate was dissolved in 5% HCl (50 ml) and the solution was warmed at 60° for 40 min then extracted with $CHCl_3$. Evaporation of the solvent and recrystallization from EtOAc gave colorless crystals (5.7 g, 31%), mp 142°. *Anal.* Calcd. for $C_{10}H_{11}N_3S_2$: C, 50.61; H, 4.67; N, 17.70; S, 27.02. Found: C, 50.37; H, 4.73; N, 17.47; S, 26.96.

3-(2-Methyl-4-methylamino-5-pyrimidinyl)methyl-4-methylthiazoline-2-thione (5c)——**4a**[2] (12 g) was dissolved in MeOH (60 ml) and acetic acid (6 g) and hydrogenated over palladium black using the same equipment as mentioned above. When an equimolar amount of hydrogen had been absorbed, the catalyst was filtered off and the filtrate was evaporated *in vacuo*. The residue was dissolved in a mixture of chloracetone (10 g), water (10 ml), conc. NH_4OH (30 ml), and EtOH (50 ml). Carbon disulfide (15 ml) was added dropwise to the solution and stirring was continued for 3 hr at room temperature. After overnight standing, the solvent was evaporated *in vacuo*, the residue was hydrolyzed with 5% HCl (80 ml) at 60° for 10 min, and the mixture was then neutralized with 40% NaOH. Collection by filtration and recrystallization from

8) All melting points are uncorrected. NMR spectra were obtained using a Varian A-60 Spectrometer with tetramethylsilane (TMS) as an internal standard. Signal multiplicities are represented by s (singlet), d (doublet), t (triplet), q (quartet), b (broad) and m (multiplet). Chemical shifts are expressed in δ values and coupling constants are in Herz.

EtOH using decolorizing charcoal gave 6.9 g (52%) of pale-yellow crystals, mp 206—207°. *Anal.* Calcd. for $C_{11}H_{14}N_4S_2$: C, 49.60; H, 5.30; N, 21.03; S, 24.07. Found: C, 49.38; H, 5.41; N, 20.81; S, 24.13.

3-(2-Methyl-4-dimethylamino-5-pyrimidinyl)methyl-4-methylthiazoline-2-thione (**5d**) was obtained as colorless crystals, mp 149—150°, by a work-up similar to that used for **5c**. Yield, 57.5%. *Anal.* Calcd. for $C_{12}H_{16}N_4S_2$: C, 51.40; H, 5.75; N, 19.98; S, 22.87. Found: C, 51.29; H, 5.92; N, 19.97; S, 22.73.

3-(2-Methyl-4-methoxy-5-pyrimidinyl)methyl-4-methylthiazoline-2-thione (**5e**) was obtained as colorless needles, mp 180°, by a work-up similar to that used for **5c**. Yield, 47%. *Anal.* Calcd. for $C_{11}H_{13}ON_3S_2$: C, 49.41; H, 4.90; N, 15.72; S, 23.98. Found: C, 49.17; H, 4.76; N, 15.84; S, 23.75.

3-(2-Methyl-5-pyrimidinyl)methyl-4-methylthiazolium Perchlorate (6a)——**5a** (2.37 g) was suspended in water (20 ml) containing 30% hydrogen peroxide (3.0 ml) and stirred until a clear solution was obtained. Barium hydroxide solution ($Ba(OH)_2 \cdot 8H_2O$, 1.56 g) and decolorizing charcoal were added and the mixture was filtered by suction. The filtrate was passed through a column of Amberlite IRA400 (ClO_4^- form). The eluate was neutralized to pH 6 with sodium bicarbonate solution then lyophilized. The crystalline residue was recrystallized from water to give colorless flakes (0.492 g), mp 198—200° (decomp.). *Anal.* Calcd. for $C_{10}H_{12}N_3S \cdot ClO_4$: C, 39.28; H, 3.96; N, 13.74; S, 10.49; Cl, 11.60. Found: C, 39.01; H, 3.99; N, 13.54; S, 10.50; Cl. 11.89.

3-(2-Methyl-4-methylamino-5-pyrimidinyl)methyl-4-methylthiazolium Chloride Hydrochloride (6c)——Thione (**5c**) (2.66 g) was suspended in water (25 ml) containing 30% hydrogen peroxide (3.5 ml) and stirred

Table III. Analytical Data (Mass Spectral Data) of the Products obtained in the Phosphonate Reaction

Compound No.	mp °C	Formula	Calcd.				Found			
			C	H	N	S	C	H	N	S
8a	181	$C_{17}H_{17}ON_3S$	65.57	5.50	13.49	10.30	65.55	5.40	13.73	10.52
8b	*a*)									
8c	amorph.	$C_{18}H_{20}ON_4S \cdot 1/2H_2O$	61.87	5.91	16.03	9.18	61.86	5.86	14.77	9.21
8e	oil	$C_{18}H_{19}O_2N_3S$	63.32	5.61	12.31	9.39	63.20	5.88	11.73	9.30
9	77—78	$C_{11}H_{11}ONS$	64.36	5.40	6.82	15.62	64.32	5.63	6.56	15.02
10	oil	$C_{13}H_{15}ONS$	66.92	6.48	6.00	13.74	66.91	6.67	5.88	13.34
11a	oil	$C_{17}H_{15}N_3S$		mol. wt. 293				M^+ 293		
11d—a	oil	$C_{19}H_{20}N_4S$		mol. wt. 336				M^+ 336		
11d—b	oil	$C_{19}H_{20}N_4S$		mol. wt. 336				M^+ 336		

a) A. Takamizawa and H. Sato, *Yakugaku Zasshi*, 92, 27 (1972)

Table IV. NMR Spectra of the Products obtained in the Phosphonate Reaction in $CDCl_3$ Solution

Compd. No.	NMR (δ) in ppm
8a	1.95^d (3H, J=1, Th-5-Me), 2.72^s (3H, Pm-2-Me), 4.56^d (1H, J=1.7, Th-2-H), 4.80, 5.13^{ABq} (2H, J=16.0, Pm-5-CH_2), 5.58^m (1H, Th-6-H), 7.33^s (5H, Ph), 8.53^s (2H, Pm-4,6-H)
8c	2.02^d (3H, J=1, Th-5-Me), 2.50^s (3H, Pm-2-Me), 3.01^d (3H, J=4.7, NHMe), 4.50^d (1H, J=2.0, Th-2-H), 4.67, 4.93^{ABq} (2H, J=16, Pm-5-CH_2), 5.57^m (1H, Th-6-H), 7.30^s (5H, Ph), 7.90^s (1H, Pm-6-H)
8e	1.88^d (3H, J=1, Th-5-Me), 2.58^s (3H, Pm-2-Me), 3.98^s (3H, OMe), 4.53^d (1H, J=1.7, Th-2-H), 4.73, 5.06^{ABq} (2H, J=17.0, Pm-5-CH_2), 5.51^m (1H, Th-6-H), 7.31^s (5H, Ph), 8.12^s (1H, Pm-6-H)
9	2.33^d (3H, J=1, Th-4-Me), 4.05^b (1H, OH), 5.98^s (1H, >CH–O), 6.77^m (1H, Th-5-H), 7.20—7.60^m (5H, Ph)
10	1.25^t (3H, J=7.0, Et), 2.38^d (3H, J=1, Th-4-Me), 3.62^q (2H, J=7.0, Et), 5.63^s (1H, >CH–O), 6.78^m (1H, Th-5-H), 7.15—7.65^m (5H, Ph)
11a	2.47^d (3H, J=1.0, Th-4-Me), 2.68^s (3H, Pm-2-Me), 6.95^{bs} (1H, =CH), 7.00^q (1H, J=1.0, Th-5-H), 7.38^s (5H, Ph), 8.37^s (2H, Pm-4,6-H)
11d—a	2.45^s (3H, Pm-2-Me), 2.50^d (3H, J=1, Th-4-Me), 3.13^s (6H, NMe_2), 6.87^m (1H, Th-5-H), 7.33^s (5H, Ph), 7.60^s (1H, =CH or Pm-6-H), 7.70^s (1H, Pm-6-H or =CH)
11d—b	2.42^d (3H, J=1, Th-4-Me), 2.52^s (3H, Pm-2-Me), 3.12^s (6H, NMe_2), 6.92^m (1H, Th-5-H), 6.97^s (1H, =CH, or Pm-6-H), 7.42^s (5H, Ph), 7.97^s (1H, Pm-6-H or =CH)

Ph=phenyl

until a clear solution was obtained at room temperature. Barium chloride solution ($BaCl_2 \cdot 2H_2O$ 2.44 g) was added and precipitate was removed by filtration. Evaporation of the filtrate and recrystallization from EtOH gave colorless crystals (1.72 g), mp 256° (decomp.). *Anal.* Calcd. for $C_{11}H_{16}N_4SCl_2 \cdot 1/2H_2O$: C, 41.78; H, 5.42; N, 17.72; S, 10.14; Cl, 22.42. Found: C, 42.15; H, 5.42; N, 17.71; S, 10.00; Cl, 21.84.

3-(2-Methyl-4-dimethylamino-5-pyrimidinyl)methyl-4-methylthiazolium Chloride Hydrochloride (6d)——According to the above procedure, hygroscopic crystals (0.79 g) of mp 190° (decomp.) were obtained from a reaction mixture of **5d** (1.40 g), 30% hydrogen peroxide (1.7 ml) and $BaCl_2 \cdot 2H_2O$ (1.22 g). *Anal.* Calcd. for $C_{12}H_{18}N_4SCl_2 \cdot H_2O$: C, 42.48; H, 5.94; N, 16.51; S, 9.45; Cl, 20.90. Found: C, 42.70; H, 6.03; N, 16.48; S, 9.74; Cl, 20.71.

3-(2-Methyl-4-methoxy-5-pyrimidinyl)methyl-4-methylthiazolium Chloride (6e)——**5e** (2.67 g) was suspended in water (30 ml) containing 30% hydrogen peroxide (3.5 ml) and stirred until a clear solution was obtained. Barium chloride ($BaCl_2 \cdot 2H_2O$, 2.44 g) and sodium bicarbonate (0.84 g) solutions were added and the precipitate which formed was removed by filtration. The filtrate was evaporated to dryness and recrystallization of the residue from EtOH gave colorless crystals (1.51 g) which decomposed gradually above 175°. *Anal.* Calcd. for $C_{11}H_{14}ON_3SCl$: C, 48.62; H, 5.19; N, 15.46; S, 11.80; Cl, 13.05. Found: C, 48.40; H, 5.14; N, 15.19; S, 11.94; Cl, 13.13.

General Procedure for the Phosphonate Reaction——To an ice-cooled suspension of thiazolium halide (1 mmole) and diethyl benzoylphosphonate (1.2 mmole) in dry dimethylformamide (5 ml) was added triethylamine (0.7 ml) with stirring in a nitrogen atmosphere. Stirring and ice-cooling were continued for 3 hr, then the mixture was allowed to stand overnight at room temperature. After evaporation of the dimethylformamide solution *in vacuo* at 60°, the resulting oil was dissolved in a mixture of EtOH (6 ml) and 10% NaOH (6 ml), warmed at 60° for 30 min, and the EtOH evaporated under reduced pressure. The residue was dissolved in $CHCl_3$ and washed with water. Then the $CHCl_3$ extract, after drying and evaporation, was separated using preparative layer chromatography (Kieselgel GF_{254} nach Stahl, E. Merck, developed with 4% MeOH–$CHCl_3$) (compound **11** was isolated by thin-layer chromatography using Al_2O_3–benzene system after Kieselgel separation). The elemental analyses and NMR spectra of the reaction products are listed in Tables III, and IV.