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Mechanism of Nucleophilic Substitution of Thiamine and Its Analogs: Methanol and Water Solvents[1]

JOHN A. ZOLTEWICZ,[2] GEORGE URAY, THOMAS D. BAUGH, AND HANNA SCHULTZ

Department of Chemistry, University of Florida, Gainesville, Florida 32611

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4-Amino-1,2-dimethyl-5-(substituted methyl)pyrimidinium ions undergo lyate ion-catalyzed nucleophilic substitution in methanol and in water. The substituents at position 5 of these thiamine analogs include phenols and pyridinium ions. The observation of separate rate- and product-determining steps indicates a multistep mechanism of substitution. Linear free energy relationships suggest that addition of lyate ion to the pyrimidinium ring is rate-limiting when phenoxide ions act as leaving groups and that a change occurs when pyridines depart. Elimination of the pyridine becomes the rate-limiting step following rapid addition of the lyate ion. Reactivities of phenol substrates in methanol and in water are similar but, when pyridine departs, changing from water to methanol gives rise to a large rate acceleration. An increase in ground state electrostatic destabilization of the dicationic substrate in the less polar alcohol provides the rate enhancement.

Thiamine (vitamin B_1) has long been known to undergo rapid nucleophilic substitution by aqueous sulfite ion (*1*, *2*). The mechanism for this reaction only recently has been established (*3*). The pathway is multistep and has separate rate- and product-determining steps. More recently, *N*-methylated thiamin analogs, **I**, have been shown to react with sulfite ion by the same mechanism (*4–6*).

Here we report that substrates **I** also undergo facile substitution in methanol and in water solvents. A mechanism similar to that for sulfite ion is followed. Methoxide and hydroxide ions act as nucleophiles in place of sulfite ion prior to the product-forming step.

RESULTS

Methanol

Rate constants for the second-order substitution reactions of thiamine analogs having a phenoxide ion (**Ia**) or a pyridine (**Ib**) leaving group (L) were obtained by a standard spectrophotometric method. Amine buffers used under pseudo-first-or-

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[2] To whom correspondence should be addressed.

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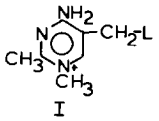
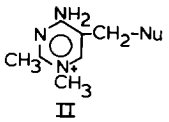


I
a, L = OAr
b, L = a Pyridine



II
a, Nu = OCH_3
b, Nu = an amine
c, Nu = OCO_2^-
d, Nu = OH

der conditions include piperidine, 4-aminopyridine, and diazabicyclo[2.2.2]octane. The less reactive phenol derivatives were examined at 40.0°C, and most pyridinium ion substrates at 25.0°C. That compound having 4-aminopyridine as a leaving group required 40°C in order to achieve convenient rates. Buffer concentrations were varied in order to establish the identity of the reacting base. The ionic strength was maintained in the range of 0.001 to 0.01 M by controlling the concentration of the ionic conjugate acid of the amine buffer. Methoxide ion concentrations were calculated from a knowledge of the buffer ratio, the pK_a of the amine, and the ion product of methanol. Rates of substitution for both classes of substrates were first order in methoxide ion. No evidence was found to indicate that rates depended on the concentration of the amine. Moreover, different amines gave the same second-order rate constant calculated using methoxide ion rather than the amine as a reactant (Table 1). Thus, under our conditions the reactions are specific rather than general based catalyzed,

$$\text{Rate} = k\psi[\text{S}] = k_2[\text{S}][\text{CH}_3\text{O}^-], \qquad [1]$$

where $k\psi$ is the pseudo-first-order rate constant, S is the substrate, and k_2 is the second order constant.

Second-order rate constants for phenolic reactants increase when electron-attracting groups are added to the phenol ring. Rate constant ratios for substrates **Ia** having phenol, *p*-nitrophenol, *p*-cyanophenol, and *m*-chlorophenol substituents are 1 : 100 : 42.6 : 8.52, respectively. The effects of substituents on the reactivity of the leaving group are not large.

Electron-donating groups attached to the pyridine ring decrease the reactivity of this class of substrates. Thus, when substituent L of **Ib** is pyridine, 4-methylpyridine, or 3,4-dimethylpyridine the second-order rate constant ratio is 1 : 0.47 : 0.0930, respectively. If the rate constant for the 4-amino compound was reduced by a factor of about three upon lowering the temperature from the experimental value of 40°C to the 25°C employed for the other pyridines, then the rate constant ratio for this substrate would be about 7×10^{-5}. Clearly, the 4-amino group markedly deactivates the substrate.

Although the rates of substitution depend on the concentration of methoxide ion, the observed major substitution product does not contain this base. Instead, the buffer base (Nu) is bonded to the site formerly occupied by the leaving group to give **IIB**, not **IIA**. This conclusion is based on several observations: (i) We have synthesized in good yield a wide variety of thiamine analogs, including those

TABLE 1

RATE CONSTANTS AND CONDITIONS FOR THE REACTIONS OF 4-AMINO-1,2-DIMETHYL-5-(SUBSTITUTED METHYL)PYRIMIDINIUM IONS IN METHANOL

Substituent	Number of runs	Buffer	$[CH_3O^-]$ ($\times 10^5$ M)	Avg k_2 (M^{-1} s^{-1})[a]
Phenol[b]	3	P[c]	1.39–5.74	1.07 ± 0.07
P-NO_2[b]	19	P[c]	0.143–5.74	111 ± 7
P-NO_2[b]	9	AP[d]	0.00994–0.0103	103 ± 7
				Avg 107 ± 7
p-CN[b]	12	P[c]	0.143–5.74	45.6 ± 1.2
m-Cl[b]	5	P[c]	0.708–5.74	9.12 ± 1.3
Pyridine[e]	3	D[f]	0.0174–0.000955	$3.15 \pm 0.27 \times 10^4$
4-CH_3[e]	1	P[c]	0.0126	8.73×10^3
4-CH_3[e]	2	D[f]	0.0174–0.000955	$7.31 \pm 1.21 \times 10^3$
				Avg $7.78 \pm 1.19 \times 10^3$
3,4-$(CH_3)_2$[e]	1	P[c]	0.126	2.52×10^3
3,4-$(CH_3)_2$	1	D[f]	0.0174	3.33×10^3
				Avg $2.93 \pm 0.57 \times 10^3$
4-NH_2[b]	5	P[c]	1.43	6.41 ± 0.35

[a] Including standard deviation.
[b] 40.0 ± 0.1°C.
[c] Piperidine.
[d] 4-Aminopyridine.
[e] 25.0 ± 0.1°C.
[f] Diazabicyclo[2.2.2]octane.

containing phenols, pyridines, and amines as the substituent L in **I** under preparative conditions. 1′-Methylthiaminium ion[7] [**I**, L is 4-methyl-5-(2-hydroxyethyl) thiazole] was the substrate reacting in methanol in the presence of various nucleophiles (*7, 8*). These experiments differ from the kinetic runs chiefly in having a higher concentration of substrate and a thiazole as a leaving group. They clearly demonstrate that a nucleophile other than methoxide ion is incorporated into the product. (ii) Analysis by NMR of reaction mixtures containing an analog having *p*-nitrophenoxide ion as a leaving group and an amine nucleophile such as piperidine or 4-aminopyridine shows that the major substitution product is the amine.

Owing to poor reproducibility, kinetic studies on 1′-methylthiaminium ion were abandoned.

Water

Substrates **Ia** having phenoxide ion leaving groups were subjected to nucleophilic substitution in aqueous buffers at 25.0°C under pseudo-first-order conditions. Again, rates depended on the concentration of lyate ion and were independent of the buffer. The *p*-nitro substrate was examined carefully to determine whether carbonate ion might act as a general base; no significant catalysis (<15%) was detected even when the concentration of carbonate ion was as high as 0.17 M.

TABLE 2

RATE CONSTANTS AND CONDITIONS FOR THE HYDROLYSIS OF 4-AMINO-5-ARYLOXYMETHYL-1,2-DIMETHYLPYRIMIDINIUM IONS IN CARBONATE BUFFERS AT 25.0°C AND 1.0 M IONIC STRENGTH

Substituent	Number of runs	pH		k_2 (M^{-1} s^{-1})
H	6	10.62–11.16		4.63 to 6.43 × 10^{-2} [a]
	1	11.04 [b]		6.23 × 10^{-2}
	1	12.54 [c]		5.76 × 10^{-2}
	1	12.89 [d]		5.49 × 10^{-2}
			Avg.	5.61 ± 0.63 [e] × 10^{-2}
p-NO_2	13	9.90–11.28		3.88 to 5.51 × 10^{-1} [a]
	1	10.94 [f]		5.62 × 10^{-1}
	1	12.54 [c]		4.06 × 10^{-1}
	1	12.89 [d]		3.37 × 10^{-1}
			Avg.	4.63 ± 0.66 [e] × 10^{-1}
p-CN	2	10.76	Avg.	3.00 ± 0.42 [e] × 10^{-1}
m-Cl	2	10.38–10.75		2.06 to 2.22 × 10^{-1} [a]
	1 [c]	12.54		2.37 × 10^{-1}
	1 [d]	12.89		1.76 × 10^{-1}
			Avg.	2.10 ± 0.26 [e] × 10^{-1}
p-Cl	5	10.07–10.64		1.27 to 1.56 × 10^{-1} [a]
			Avg.	1.40 ± 0.14 [e] × 10^{-1}
m-CH_3O	4	10.03–10.75		9.28 to 10.6 × 10^{-2} [a]
	1	11.04 [b]		9.33 × 10^{-2}
	1	12.54 [c]		1.16 × 10^{-1}
			Avg.	1.00 ± 0.09 [e] × 10^{-1}

[a] Range observed.
[b] Phosphate buffer.
[c] Saturated $Ca(OH)_2$.
[d] KOH.
[e] Standard deviation.
[f] pH-stat.

As found for reactions in methanol, electron-withdrawing groups on the phenol facilitate substitution (Table 2). Relative rate constants for the phenol, *p*-nitro, *p*-cyano, *m*-chloro, *p*-chloro, and *m*-methoxy substrates are 1 : 8.25 : 5.35 : 3.74 : 2.50 : 1.78, respectively. Substituent effects are small. The redetermined rate constant for the *p*-cyano substrate is in excellent agreement with our value reported earlier (*9*).

Our reported product studies show that buffer base is incorporated into the observed major substitution product when this base is phosphate ion, **II** (Nu is OPO_3^{-2}) (*9*). Moreover, a sulfide substitution product can be isolated in high yield in the presence of an arenethiolate ion trapping agent under conditions where hydroxide ion is the kinetically important base (*9*).

In the present kinetic experiments addition of carbonate ion to the methylene side chain is likely to give an unstable carbonate monoester, **IIc**, that is expected

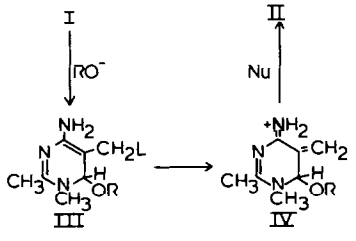
to decarboxylate to the corresponding alcohol, **IId**. This alcohol also could arise from a reaction with hydroxide ion. Hence, it is not possible to determine what is the major pathway for the formation of alcohol substitution product. The alcohol is highly water soluble and difficult to isolate (*10*). It is not readily degraded in alkaline solution as our control experiment reveals.

DISCUSSION

Our kinetic and product studies provide a most interesting conclusion. While methoxide ion is the kinetically important base, it is the buffer base that is incorporated into the major product, i.e., there are separate rate and product determining steps. An intermediate must be formed. Second-order substitution cannot proceed by an S_N2 mechanism.

The multistep mechanism of substitution taking place in methanol and in water solvents is a variation of that reported for thiamine (*3, 11, 12*), its *N*-methylated analogs, **I** (*4, 5, 13*), and sulfite ion acting as a nucleophile in water. Our reported scheme only needs to be modified to change the identity of the kinetically active nucleophile, now either methoxide or hydroxide ion. Thus, substitution commences when the nucleophile adds to the unsubstituted position of **I** to give sigma adduct, **III**. Intermediate **III** then expels the leaving group to give resonance-stabilized cation **IV**. Capture of this intermediate by a second nucleophile followed by expulsion of the first gives the observed substitution product **II**, Scheme I. The first and second nucleophiles need not be the same.

Another mechanism is consistent with our kinetics. Instead of lyate ion adding to the pyrimidinium ion ring, it deprotonates the amino group. The resultant iminopyrimidine then undergoes elimination to give a cationic intermediate that is captured by a second nucleophile. Protonation of the amine in the final step gives product. We disfavor this process for two reasons: (i) Such a pathway is not consistent with the kinetics for the sulfite ion reactions of these analogs (lyate ion does not appear in the kinetic equation for the substitution reactions by sulfite ion); and (ii) the ρ values for the linear free energy correlations involving the sulfite ion- and lyate ion-catalyzed reactions are sufficiently similar to suggest a common pathway. We therefore believe the route to substitution products is given correctly in Scheme 1.



SCHEME I.

Hammett correlations may be constructed using the second-order rate constants for the phenol substrates. The four-point plot for methanolysis has a ρ equal to 2.58 (correlation coefficient; $r = 0.999$) and the six-point plot for hydrolysis shows that ρ is 1.08 ($r = 0.980$). These values are not unlike the ρ value of 1.35 ($r = 0.983$) for substitution on the same six substrates by aqueous sulfite ion (*13*). Standard sigma values for *meta* and *para* substituents (*14*) are employed in the construction of the linear free energy plots.[3]

A Brønsted plot may be constructed using the pK_a values for the conjugate acids of the phenoxide ion leaving groups. However, this plot is curved. We reject this relationship as being mechanistically significant because a plot of σ versus pK_a for the same substituents also has the same curved shape. Curvature reflects the fact that σ and σ^- values must be used to correlate the pK_a values for phenols, the σ^- values reflecting the need to express the conjugation with an electron-withdrawing *para* substituent. An implication of such a curved plot is that the identity of the rate-limiting step changes with the nature of the substituents. Our preferred linear Hammett correlation does not require such an interpretation.

A Hammett plot may also be constructed for the pyridine substrates reacting in methanol. However, there is some question about the selection of σ values. Controversy surrounds the problem of correlating the effects of substituents on rates and equilibria associated with the formation of pyridinium ions from pyridines (*16*). In our case using a σ^+ value for the *para*-amino substituent and σ values for the other groups gives rise to a ρ value of 3.59 ($r = 0.994$). However, the question dealing with the choice of σ constants may be avoided by using pK_a values (*17*) for the conjugate acids of the pyridine leaving groups. The resultant Brønsted plot has a β of -1.12 ($r = 0.997$). This value may be compared with a β value of -0.86 for similar pyridine analogs of thiamine reacting with sulfite ion (*6*).

The linear free energy plots show that the identity of the rate-limiting step in Scheme 1 varies with the nature of the leaving group. For the phenols (a) linear plots are obtained with σ rather than with σ^- values, and (b) small values are observed for ρ. Therefore, departure of the phenoxide ion does not take place in the rate-limiting step. Rather, addition of the nucleophile to starting material to give **III** is rate limiting; subsequent departure of the phenoxide ion is fast.

Our failure to detect buffer base catalysis in the reactions of the phenol substrates is not evidence against our postulate concerning the identity of the rate-limiting step. A similar absence of buffer catalysis has also been reported for uracils that react with hydroxide ion at an unsubstituted ring position in water (*18*). The presence of such catalysis is consistent with a mechanism in which buffer base assists the addition of solvent to the pyrimidinium ion ring. This process does not compete kinetically with lyate ion addition in our studies.

The large Brønsted slope found for pyridine substrates suggests that loss of the pyridine leaving group to give **IV** is the rate-limiting step. Addition of the nucleophile to the ring to form **III** must be reversible. A similar conclusion was reached for reactions involving these substrates and sulfite ion (*6*).

A major difference between the phenol and pyridine substrates is found in the

[3] A correlation using a single substituent constant to represent the inductive effect of the entire OAr group would be interesting. Unfortunately, very few values are available (*15*).

extra positive charge present on the quaternized pyridine compounds. This gives rise to considerable ground state electrostatic destabilization in the dications. The electrostatic effect is removed when hydroxide or methoxide ion adds to the pyrimidinium ring to give **III**. Charge neutralization that removes the destabilizating interaction facilitates the addition of the nucleophile. This sharp contrast between the two classes of substrates is revealed on comparing the influence of solvent on reactivity.

The reactivity of the phenols in methanol is only a little greater than that in water, in part due to the 15°C higher temperature. Without making a temperature correction, for example, the unsubstituted phenol is 23 times more reactive in methanol than in water.

Substitution is a remarkable 1.2×10^4 times faster in methanol than in water for that substrate having pyridine as a leaving group. This comparison is based on the value of 2.69 M^{-1} s^{-1} for a reaction with hydroxide ion in water (*9*) and the value of 3.15×10^4 M^{-1} s^{-1} from Table 1 for the same substrate in methanol, also at 25°C. This rate acceleration reflects the additional ground state electrostatic destabilization that is removed upon addition of methoxide ion to the ring in the less polar solvent methanol.

Of the three nucleophiles reacting with thiamine analogs, sulfite ion is the least nucleophilic. It is approximately five to nine times less reactive than hydroxide ion toward the phenol and pyridine analogs.

Significantly, starting material and intermediate must have different affinities for the nucleophiles present. The less abundant methoxide ion, ranging from about 1×10^{-8} to 6×10^{-5} M, prefers to react with starting material, while the more abundant amine nucleophile, typically from 1×10^{-3} to 1×10^{-1} M, selectively reacts with the intermediate after the rate-limiting step. In comparison, in nucleophilic addition to a carbon atom that is part of an aromatic ring (S_NAr substitution) methoxide ion and piperidine are about equally reactive (*19*). Our pattern is not likely to be a consequence of steric effects because piperidine is known to add to hindered carbon atoms in S_NAr reactions (*20*).

Our remarkable results are understandable in terms of a multistep mechanism of substitution. In order for a reactant to function catalytically it must be more than just a good nucleophile; it must also be a poor leaving group. It must remain attached to the ring until the side chain group departs. Thus, methoxide ion is a poorer leaving group than piperidine (*20, 21*) and so methoxide ion functions as the catalyst. In other words, the equilibrium affinity of carbon is greater for methoxide ion than for piperidine. A similar example of kinetic versus thermodynamic control involving methoxide ion is available (*22*).

Electrostatic destabilization may play a role in the enzyme-catalyzed substitution reactions of thiamine and its analogs. We have suggested that reactions of thiaminase I and II may proceed according to Scheme 1 with a cysteine unit of the enzyme acting as a nucleophile (*6, 9*). Such reactions might take place in a region of low polarity within the enzyme, thereby taking advantage of enhanced electrostatic destabilization present in the ground state dication under such condition.

Our unusual multistep mechanism is not limited to sulfite ion. The present results and conclusions indicate that it is a common pathway for substrates **I** undergoing nucleophilic substitution under a variety of conditions.

EXPERIMENTAL PROCEDURES

Syntheses

Preparation of 4-amino-1,2-dimethyl-5-phenoxymethylpyrimidinium perchlorate. To a suspension of 1′-methylthiaminium diperchlorate (2.52 g, 5.28 mmol) and phenol (1.17 g, 12.5 mmol) in methanol (30 ml) was added methanolic sodium hydroxide (0.211 g, 5.28 mmol in 2.57 ml). Following heating at reflux for 8 h and ice cooling for 30 min, the mixture was filtered. The crude product was washed first with ethyl acetate (2 × 5 ml) and then with ethyl ether (2 × 5 ml) to give 1.10 g of product, mp 247–250°C (dec.). Recrystallization from a 1/3 (v/v) mixture of dimethylformamide and 0.1 M perchloric acid gave 0.70 g (2.12 mmol, 40%) of a white solid, mp 252–255°C (dec.). An analytical sample was prepared by further recrystallization from 20% aqueous dimethylformamide followed by vacuum drying at 100°C over magnesium perchlorate for 2 h, mp 255.5–257°C (dec): ^{1}H NMR (c. 0.76 M, Me_2SO-d_6, Me_4Si) δ2.59 (2-CH_3), 3.82 (NCH_3), 4.94 (CH_2), 6.9–7.4 (aromatic mult.), 8.43 (H_6), 8.51, 9.10 (NH_2); ^{13}C NMR (c. 0.76 M, Me_2SO-d_6, Me_4Si) δ21.5 (2-CH_3), 41.7 (NCH_3), 62.3 (CH_2), 111.6 (C_5), 114.7 (C_2'), 121.3 (C_4'), 129.5 (C_3'), 146.8 (C_6), 157.6 (C_1'), 161.6, 162.2 (C_2, C_4).

Anal. Calcd for $C_{13}H_{10}ClN_3O_5$ (329.7): C, 47.35; H, 4.89; N, 12.74. Found: C, 47.45; H, 4.92; N, 12.76.

Preparation of 4-amino-1,2-dimethyl-5-[3-methoxyphenoxy]methylpyrimidinium perchlorate. To a suspension of 1′-methylthiaminium diperchlorate (5.00 g, 10.4 mmol) and 3-methoxyphenol (3.00 g, 24.2 mmol) in methanol (50 ml) heated to reflux was added methanolic sodium hydroxide (0.516 g, 12.9 mmol in 3.00 ml). Heating was continued for 2 h followed by evaporation of the solvent under reduced pressure. The residue was triturated with ethyl acetate (20 ml); the resulting solid was filtered and washed first with ethyl acetate (3 × 10 ml) and then with acetone (10 ml), giving 2.37 g of a slightly yellow solid, mp 270–275°C (dec.). Recrystallization from a 2/3 (v/v) mixture of dimethylformamide and 0.1 M perchloric acid gave 1.68 g (4.67 mmol, 45%) of a white solid, mp 286–288°C (dec.). A sample was prepared for analysis by three further recrystallizations from 50% aqueous dimethylformamide followed by vacuum drying at 100°C over magnesium perchlorate for 12 h, mp 289–290.5°C (dec.): ^{1}H NMR (c. 0.70 M, Me_2SO-d_6, 80°C, Me_4Si) δ2.60 (2-CH_3), 3.76 (OCH_3), 3.83 (NCH_3), 4.97 (CH_2), 6.5–7.3 (aromatic mult.), 8.36 (H_6), 8.57 (NH_2, broad); ^{13}C NMR (c. 0.70 M, Me_2SO-d_6, 80°C, Me_4Si) δ21.5 (2-CH_3), 41.9 (NCH_3), 55.4 (OCH_3), 62.9 (CH_2), 101.8 (C_2'), 107.5 (C_4'), 112.0 (C_5), 130.0 (C_5'), 146.8 (C_6), 159.0, 160.8 (C_1', C_3'), 161.7, 162.2 (C_2, C_4).

Anal. Calcd for $C_{14}H_{18}ClN_3O_6$ (359.8): C, 46.74; H, 5.04; N, 11.68. Found: C, 46.78; H, 5.04; N, 11.69.

Preparation of 4-amino-1,2-dimethyl-5-[3-chlorophenoxy]methylpyrimidinium perchlorate. 3-Chlorophenol (4.01 g, 31.2 mmol) in methanol (10 ml), partially converted to the phenolate by addition of sodium methoxide (7.00 ml of 1.67 M, 11.7 mmol), was added by drops to a stirred suspension of 1′-methylthiaminium diperchlorate (5.00 g, 10.4 mmol) in methanol (50 ml). The suspension was heated at reflux for 8 h followed by ice cooling (30 min) and filtration. The resulting solid

was washed first with ice-cold methanol (2 × 10 ml), then with ethyl acetate (2 × 10 ml), and finally with ethyl ether (2 × 10 ml) to give 3.40 g of product, mp 285–287°C (dec.). Recrystallization from a 1/1 (v/v) mixture of dimethylformamide and 0.1 M perchloric acid yielded 3.20 g (8.79 mmol, 85%) of product, mp 297–300°C (dec.). An analytical sample was prepared by further recrystallization from 50% aqueous dimethylformamide followed by heating a suspension of the compound in ethanol at 45°C for 30 min and then vacuum drying at 100°C over magnesium perchlorate for 3 h, mp 298–299°C (dec.): ^{1}H NMR (c. 0.70 M, Me_2SO-d_6, 45°C, Me_4Si) δ2.61 (2-CH_3), 3.83 (NCH_3), 4.98 (CH_2), 7.01–7.46 (aromatic mult.), 8.43 (H_6), 8.43, 9.06 (NH_2); ^{13}C NMR (c. 0.70 M, Me_2SO-d_6, 45°C, Me_4Si) δ21.6 (2-CH_3), 41.8 (NCH_3), 62.9 (CH_2), 111.3 (C_5),113.9 (C_6'), 114.9 (C_2'), 121.3 (C_4'), 130.9 (C_5'), 133.8 (C_3'), 147.0 (C_6), 158.6 (C_1'), 161.6, 162.3 (C_2, C_4).

Anal. Calcd for $C_{13}H_{15}Cl_2N_3O_5$ (364.2): C, 42.87; H, 4.15; N, 11.54. Found: C, 42.86; H, 4.16; N, 11.53.

Preparation of 4-amino-1,2-dimethyl-5-[4-methylphenoxy]methylpyrimidinium perchlorate. 1′-Methylthiaminium diperchlorate (5.00 g, 10.4 mmol) was suspended in a solution of 4-methylphenol (3.00 g, 27.7 mmol) in methanol (50 ml) and heated to reflux. Methanolic sodium hydroxide (0.516 g, 12.9 mmol in 3.00 ml) was added to the suspension and heating was continued for 2 h. The solvent was removed under reduced pressure and the residue was triturated with ethyl acetate (20 ml). The resulting solid was washed first with ethyl acetate (3 × 10 ml) and then with acetone to give 1.68 g of a light tan material, mp 235–245°C (dec.). Recrystallization from 20% aqueous dimethylformamide and charcoal produced 1.35 g (4.01 mmol, 38%) of slightly off-white needles, mp 243–245°C (dec.). An analytical sample was prepared by three further recrystallizations from 25% aqueous dimethylformamide, resulting in white crystals which were vacuum dried at 100°C over magnesium perchlorate, mp 245.5–247°C (dec.): ^{1}H NMR (c. 0.70 M, Me_2SO-d_6, Me_4Si) δ2.25 (4′-CH_3), 2.59 (2-CH_3), 3.82 (NCH_3), 4.90 (CH_2), 6.95 (H_2', H_6', apparent AB, $J_{2',3'} \cong 8$ Hz), 7.15 (H_3', H_5', apparent AB, $J_{2',3'}$, ≅ 8 Hz), 8.40 (H_6), 8.47, 9.08 (NH_2); ^{13}C NMR (c. 0.70 M, Me_2SO-d_6, Me_4Si) δ20.0 (4′-CH_3), 21.5 (2-CH_3), 41.7 (NCH_3), 62.5 (CH_2), 111.8 (C_5), 114.6 (C_2'), 129.8 (C_3'), 130.2 (C_4'), 146.6 (C_6), 155.5 (C_1'), 161.5, 162.2 (C_2, C_4).

Anal. Calcd for $C_{14}H_{18}ClN_3O_5$ (343.8): C, 48.91; H, 5.28; N, 12.22. Found: C, 48.95; H, 5.29; N, 12.22.

Preparation of 1-[*(4-amino-1,2-dimethyl-5-pyrimidinio)methyl*]*pyridinium diperchlorate.* A suspension of 1′-methylthiaminium diperchlorate (4.79 g, 10.0 mmol) and pyridine (2.53 g, 32.0 mmol) in methanol (50 ml) was heated at reflux for 48 h. The resulting mixture was cooled in ice for 30 min and filtered. The crude material was washed with methanol (2 × 5 ml), giving 3.83 g of product, mp 259–261°C (dec.). Recrystallization from 0.1 M perchloric acid gave 3.43 g (8.26 mmol, 83%) of white crystals, mp 261.5–263.5°C (dec.). An analytical sample was prepared by three recrystallizations from 0.1 M perchloric acid followed by vacuum drying at 100°C over magnesium perchlorate for 2 h, mp 262–263.5°C (dec.): ^{1}H NMR (c. 0.36 M, Me_2SO-d_6, Me_4Si) δ2.63 (2′-CH_3), 3.82 (NCH_3), 5.75 (CH_2), 8.19 (H_3, H_5, dd, $J_{2,3} = J_{5,6} = 7$ Hz, $J_{3,4} = J_{4,5} = 9$ Hz), 8.49 (H_6'), 8.68 (H_4, t, $J = 9$ Hz),

9.03 (H_2, H_6, d, J = 7 Hz), 8.80, 9.28 (NH_2); ^{13}C NMR (c. 0.36 M, Me_2SO-d_6, Me_4Si) δ21.6 (2′-CH_3), 42.0 (NCH_3), 56.0 (CH_2), 106.8 (C_5'), 128.1 (C_3, C_5), 144.5, 146.4 (C_2, C_6, C_4), 151.4 (C_6'), 161.8, 163.1 (C_2', C_4').

Anal. Calcd for $C_{12}H_{16}Cl_2N_4O_8$ (415.2): C, 34.71; H, 3.88; N, 13.49. Found: C, 34.67; H, 3.90; N, 13.49.

Preparation of 1-[(4-amino-1,2-dimethyl-5-pyrimidino)methyl]-4-aminopyridinium diperchlorate. A suspension of 1′-methylthiaminium diperchlorate (2.50 g, 5.22 mmol) and 4-aminopyridine (0.540 g, 5.74 mmol) in methanol (30 ml) was stirred at ambient temperature for 16 h. The mixture was filtered and the product was washed with ethyl acetate (2 × 5 ml), giving 1.92 g of a white solid, mp 198–201°C. Recrystallization from 0.1 M perchloric acid gave 1.58 g (3.67 mmol, 70%) of white crystals, mp 198–201°C. An analytical sample was prepared by recrystallization, twice from 0.1 M perchloric acid followed by recrystallization from water and twice from 0.1 M perchloric acid with vacuum drying at 100°C over magnesium perchlorate for 12 h. On heating there was slight melting at 198–200°C with some resolidification above 205°C and final melting at 215.5–217.5°C.

Anal. Calcd for $C_{12}H_{17}Cl_2N_5O_8$ (430.2): C, 33.50; H, 3.98; N, 16.28. Found: C, 33.47; H, 4.02; N, 16.28.

Over a period of months the melting point of the analytical sample changes such that the slight melting at 198–200°C becomes predominant, although not complete; a small amount of material remains solid to 213°C. Upon drying under vacuum at 100°C over magnesium prechlorate for 48 h the melting at 198–200°C again becomes only slight although not negligible. A sample recrystallized from water and air-dried melts completely between 196 and 199°C. The presence of small amounts of water either results in hydrate formation or alters the crystal form of the product. Proton and carbon NMR samples prepared from material with a melting point of 198–200°C are consistent with the proposed structure. ^1H NMR (c. 0.35 M, Me_2SO-d_6, Me_4Si) δ2.60 (2′-CH_3), 3.80 (NCH_3), 5.23 (CH_2), 6.85 (H_3, H_5, apparent AB, $J_{2,3} \cong 7$ Hz), 8.15 (H_2, H_6, apparent AB, $J_{2,3} \cong 7$ Hz), 8.18 (4-NH_2), 8.31 (H_6'), 8.67, 9.08 (4′-NH_2); ^{13}C NMR (c. 0.35 M, Me_2SO-d_6, Me_4Si) δ21.5 (2′-CH_3), 41.9 (NCH_3), 52.3 (CH_2), 109.0, 109.5 (C_5', C_3, C_5), 142.6 (C_2, C_6), 149.5 (C_6'), 158.9 (C_4), 161.6, 162.7 (C_2', C_4').

Preparation of 4-amino-1,2-dimethyl-5-[1,4-triethylenediammonio]methylpyrimidinium triperchlorate. A suspension of 1′-methylthiaminium diperchlorate (5.00 g, 10.4 mmol) and 1,4-triethylenediamine hydrate (3.51 g, 27.0 mmol) in methanol (50 ml) was stirred at ambient temperature for 4 h. The resulting mixture was cooled in ice for 30 min and filtered, followed by washing first with methanol (2 × 10 ml), then with ethyl acetate (2 × 10 ml), and finally with ethyl ether (2 × 10 ml). The hygroscopic solid was dissolved in hot 3.9 M perchloric acid (15 ml). The recrystallized protonated product was filtered, washed with ice-cold water (2 × 10 ml), and suspended in absolute ethanol (30 ml) for 30 min. The product was collected and air-dried to give 4.60 g (8.38 mmol, 81%) of white crystals, mp 264.5–266.5°C (dec.) with prior elimination of water at 150°C. A sample for analysis was prepared by recrystallization from 0.1 M perchloric acid, then from 1.2 M perchloric acid, followed by vacuum drying at 100°C over anhydrous magnesium perchlorate for 3 h and ambient temperature for 15 h, mp 268–270°C (dec.) with

elimination of water at 150°C. A small portion of the analytical sample was not vacuum-dried but was air-dried for 18 h, mp 265–268°C (dec.): ^{1}H NMR (Me_2SO-d_6, Me_4Si) δ2.63 (2-CH_3), 3.70 (2′-CH_2, 3′-CH_2, broad), 3.83 (NCH_3), 4.67 (5-CH_2), 8.47 (H_6), 8.93, 9.40 (NH_2); ^{13}C NMR (c. 0.35 M, Me_2SO-d_6, Me_4Si) δ21.6 (2-CH_3), 42.0 (NCH_3), 43.2, 49.8, 59.4 (2′-CH_2, 3′-CH_2, 5-CH_2), 101.9 (C_5), 153.8 (C_6), 163.1, 163.2 (C_2, C_4).

Anal. Calcd for $C_{13}H_{24}N_5Cl_3O_{12} \cdot H_2O$ (566.7): C, 27.55; H, 4.62; N, 12.36. Found: C, 27.62; H, 4.66; N, 12.40.

Preparation of 4-amino-1,2-dimethyl-5-[1-piperidino]methylpyrimidinium perchlorate. A suspension of 1′-methylthiaminium diperchlorate (5.00 g, 10.4 mmol) and piperidine (2.66 g, 31.2 mmol) in methanol (50 ml) was stirred at ambient temperature for 90 min. The solvent was removed under reduced pressure, leaving a slightly oily residue. Trituration of the residue with ethyl acetate (5 ml) followed by filtration gave a slightly yellow solid. Washing with ethyl acetate (5 × 3 ml) produced 2.02 g of an off-white solid, mp 157–162°C. Recrystallization from a 4/1 (v/v) mixture of ethyl acetate and methanol with charcoal gave 1.69 g (5.27 mmol, 51%) of colorless hexagonal platelets, mp 161–163°C. A sample for analysis was prepared by recrystallization from ethyl acetate/methanol followed by vacuum-drying at ambient temperature over magnesium perchlorate for 16 h, mp 161–162.5°C: ^{1}H NMR (c. 0.69 M, Me_2SO-d_6, Me_4Si) δ1.45 (3′, 5′-CH_2, 4′-CH_2), 2.36 (2′, 6′-CH_2), 2.55 (2-CH_3), 3.36 (5-CH_2), 3.78 (NCH_3), 8.15 (H_6), 8.2–9.2 (NH_2, broad); ^{13}C NMR (c. 0.69 M, Me_2SO-d_6, Me_4Si), δ21.4 (2-CH_3), 23.7, 24.5 (C_3', C_5', C_4'), 41.6 (NCH_3), 53.4 (C_2', C_6'), 55.6 (5-CH_2), 112.3 (C_5), 146.4 (C_6), 161.5, 162.9 (C_2, C_4).

Anal. Calcd for $C_{12}H_{21}ClN_4O_4$ (320.8): C, 44.93; H, 6.60; N, 17.47. Found: C, 45.03; H, 6.65; N, 17.49.

pH Measurements in Methanol

Materials. 4-Picoline and piperidine were purified by distillation from sodium. 4-Aminopyridine was recrystallized from acetonitrile. 1,4-Diazabicyclo[2.2.2]octane (Dabco) was used as received. 4-Picolinium perchlorate (mp 100–101°C) and 4-aminopyridinium perchlorate (mp 271.5–273°C) were recrystallized from isobutyl alcohol and dried at 25 and 100°C, respectively under vacuum over magnesium perchlorate. Dabco monoperchlorate (mp 300°C, dec.) was recrystallized from a 5/3 (v/v) mixture of ethanol and methanol and vacuum-dried at 100°C over magnesium perchlorate. Piperidinium perchlorate (mp 157.5–158.5°C) was recrystallized from chloroform and vacuum-dried at 25°C over magnesium perchlorate. Salicylic acid was recrystallized from acetonitrile and sublimed at 100°C, 1 Torr. Benzyltriethylammonium perchlorate (BTAP) was recrystallized from water and vacuum-dried at 25°C over magnesium perchlorate (mp 148–150°C). All solid materials were kept in a dessicator over calcium sulfate.

Instrumentation. pH measurements were made with a Radiometer PHM64 Research pH meter in conjunction with a Radiometer GK2321C or GK2401B combination electrode. Electrodes were prepared for use in methanol by rinsing the electrode cavity repeatedly with methanol and then filling with methanol saturated

with KCl. The electrodes were equilibrated for an hour in methanol prior to use. Prolonged use and storage of an electrode in methanol tends to dehydrate the glass electrode membrane, leading to unstable and erratic measurements. When not in use electrodes were stored in water.

Methanolic buffer preparation. Buffer concentrations were chosen such that the conjugate acid concentration was fixed at 1.00×10^{-2} M while varying the base concentration. These stock solutions were then diluted with methanol to an ionic strength of 1.00×10^{-3} M or diluted with the ionic strength maintained at 1.00×10^{-2} M by the further addition of sodium perchlorate or BTAP.

Salicylic acid buffers were made by half neutralizing salicylic acid with methanolic sodium methoxide standardized with potassium hydrogen phthalate.

pH measurements were made by equilibrating buffer solutions, pH standards, and the electrode at the appropriate temperature at least 30 min prior to measurement. The pH meter was calibrated by adjusting the calibration control with a 1/1 Dabco buffer to pH 8.99 (*23*); this value was set as the iso-pH point on the meter. A 1/1 4-picoline buffer was set to read 6.09 (*24*) with the sensitivity control. The temperature control was set at 25°C. The same procedure was followed at 40°C using pH values of 5.82 (*24*) and 8.75 for 4-picoline and Dabco buffers, respectively. The temperature dependence of the Dabco buffer in methanol was assumed to be the same as that in water (*25*). The ionic strength of the calibrating buffers was maintained at 1.00×10^{-3} M based on the concentration of the respective conjugate acids. As a check on the calibration, the pH of a 1/1 salicylic acid buffer (0.01 molal) was 7.50; this is to be compared with the literature value of 7.53 (*26*).

Determination of pK_a values for piperidine and 4-aminopyridine in methanol. The pH values of a series of piperidine/piperidinium perchlorate and 4-aminopyridine/4-aminopyridinium perchlorate buffers of known concentration were measured at 25.0 and 40.0°C at ionic strengths of 1.00×10^{-2} and 1.00×10^{-3} M. The ionic strength was determined either by the concentration of the conjugate acid or by added 1.00×10^{-2} M sodium perchlorate or BTAP. Several pH readings of each solution were made. After converting pH to hydrogen ion concentration, K_a values were calculated and then averaged. Solvolysis corrections of buffer acid and base concentrations are insignificant.

The pK_a values for piperidine and 4-aminopyridine at 25.0 and 40.0°C and ionic strengths of 1.00×10^{-2} and 1.00×10^{-3} M buffer acid and 9.48×10^{-3} M [BTAP] were corrected for ionic strength dependence with the Debye–Huckel treatment. At 25°C corrections amount to 0.15 (0.01 M) and 0.06 (0.001 M). The ion size parameter used in calculating the correction was taken to be 5 Å.

Even after making the activity corrections, pK_a values for 10^{-2} and 10^{-3} M ionic strengths differ by about 0.1–0.2. The pK_a values determined at both ionic strengths were averaged to produce operational pK_a values: piperidine, 11.02 (25°C) and 10.73 (40°C); and 4-aminopyridine, 9.82 (25°) and 9.58 (40°). These operational values were employed to calculate methoxide ion concentrations for the buffered kinetic runs using the stoichiometric buffer ratios and the ion product (pK_s) of methanol. The pK_s of methanol is 16.92 and 16.56 at 25.0 and 40.0°C, respectively (*27, 28*). The use of the operational pK_a value is apparently justified by the fact that the kinetic studies using 10^{-2} and 10^{-3} M ionic strength buffers give

observed rate constants that are the same within experimental error. A value of 11.1 has recently been reported for piperidine in methanol at 25°C using solutions said to be 0.001 M (*29*).

Kinetics

Substitution reactions of phenol and pyridine derivatives of 1'-methylthiaminium diperchlorate(7) in methanolic buffers. Substrates usually were prepared from 1'-methylthiaminium ion (*7, 8*). Absorbance measurements were made with a Cary 17-D or a Perkin–Elmer 330 spectrophotometer maintained at 40.0 or 25.0°C with a Lauda K-2/R circulating temperature bath. The bath temperature was determined with an NBS-certified thermometer and the cell temperature with a thermister coupled to a digital readout. Reported kinetic temperatures are cell temperatures. For long kinetic runs, cuvettes were designed with a length of quartz tubing that could be sealed with a torch.

Buffer solution was temperature equilibrated for a minimum of 30 min. The reaction was initiated by addition of substrate solution with a microliter syringe. The wavelengths (in nanometers) for the phenols were *p*-nitro, 390 or 400; *p*-cyano, 272; *m*-chloro, 285; and the unsubstituted phenol, 280. For the pyridines they were 4-methyl, 250 or 270; 3,4-dimethyl, 270 and 280; 4-amino, 275; and the unsubstituted pyridine, 280.

Some instability was observed in the infinity values for the phenolic substrates. Some of the drift was due to evaporation of the methanol from the cuvette during long runs. Drift was reduced markedly but often not completely by using flame-sealed cuvettes. Drift rates appear to be highest for runs with high piperidine concentrations and are linear with time. These changes were compensated for by extrapolating the linear region back to zero time and measuring absorbance changes with respect to this line. Thus, the "infinity" value is not a constant. The resultant first-order plots were linear.

Product studies: (a) Piperidine a nucleophile. A methanolic solution of piperidine (0.20 M, 10 ml) was thermostated in a bath at 41.0°C. To it was added the *p*-nitrophenol substrate, **Ia** (50 mg, 1.3×10^{-4} mol) giving a 0.013 M substrate solution. After approximately five half-lives (3 min) the mixture was cooled in a dry ice/acetone bath and methanol was removed under vacuum. The NMR spectrum of the products was taken in DMSO-d_6. The chemical shifts of the products agreed with those of authentic samples.

(b) 4-Aminopyridine as nucleophile. To 4-aminopyridine (0.071 g, 0.75 mmol) and the *p*-nitrophenol substrate, **Ia** (0.051 g, 0.14 mmol) in an NMR tube was added methanol to give a total volume of 0.5 ml. The tube was sealed with a torch and heated at 55°C. The reaction was monitored by following the appearance of the aromatic signals assigned to the phenoxide ion and by the disappearance of those assigned to the substrate. The reaction reached completion after 60 min. The methanol was then removed under reduced pressure and replaced with D_2O, and the contents were gently warmed to effect solution. The NMR of the reaction mixture clearly showed a single resonance for the N-CH_3 and CH_2 groups of product but the C-CH_3 group had undergone H/D exchange. The signals of prod-

uct were verified as belonging to the 4-aminopyridine substitution product by addition of authentic compound.

Kinetics

Substitution reactions of phenol derivatives of 1'-methylthiaminium diperchlorate(*7*) in water at 25.0°*C*. To thermally equilibrated buffer was added a few microliters of substrate solution. The wave lengths (in nanometers) for the substituted phenol substrates, **Ia,** are *p*-nitro, 400; *p*-cyano, 273 and 290; and all others, 290. The pH was measured at the start and at the end of each run; if values differed by more than about 0.05 pH, then the run was discarded. Pseudo-first-order rate constants were obtained from a computer fit of absorbance changes to the standard logarithmic equation, using an iteration method to obtain the best infinity value. Plots generally were linear over three to six half-lives; correlation coefficients usually were at least 0.999. The hydroxide ion concentration was calculated from the measured pH and pK_w of 14.00. Kinetic plots for *p*-nitro and *p*-cyano substrates were linear over six to seven half-lives, showing that liberated phenoxide ion does not inhibit the reaction by a common ion effect.

Possible deprotonation of the 4-amino group. The ultraviolet absorption spectra of several substrates **I** in water were recorded at room temperature. When L of **I** was *m*-chlorophenol the spectra of pH 1 ($HClO_4$), 9.1 (borate), and 10.0 (carbonate) buffers had a peak at 249 nm that did not shift. At pH 11.7 (KOH) the spectra were time dependent due to hydrolysis. When L of **I** was 4-aminopyridine the spectra at pH 1 and 9.1 had the same absorption maximum; at pH 10.0 this peak was slightly broadened at lower wavelengths, and a shoulder appeared about 310 nm. A shift in the absorption maximum from 272 to 266 nm was clearly evident at pH 11.7. When L of **I** was piperidine; the spectra of pH 10.0 and 11.7 solutions had the same maximum absorption at 252 nm.

These results are in keeping with the observation that the related compound, 1-methyl-4-aminopyrimidine, has a pK_a of 12.1 (*30*).

Stability checks of compounds involved in hydrolysis reactions: (a) p-Cyanophenoxide substrate. A suspension of 90 mg of substrate **Ia** having *p*-cyanophenoxide ion as a leaving group was stirred at room temperature for 20 h with 15 ml of 0.13 M Na_2CO_3. Unreacted starting material was removed; the pH was 10.6. Following acidification of the pale yellow solution and evaporation to dryness, the residue was dissolved in about 0.5 ml of D_2O. NMR analysis showed the expected hydroxymethylpyrimidine, **IId**, and phenoxide ion products. Integration confirms the expected 1 : 1 ratio. Only trace impurities are seen in the methyl region, suggesting that degradation of the pyrimidine ring is insignificant.

(*b*) *Hydroxymethyl substitution product* ***IId.*** 4-Amino-1,2-dimethyl-5-hydroxymethylpyrimidinium perchlorate (0.106 mmol) was added to water raised to pH 12.0 with a pH-stat at 25°C. In the first few minutes following addition of substrate, 30% of one equivalent of base was consumed, with less than an additional 10% being consumed over the next 135 min. Following acidification with HCl to pH 2.1 and evaporation to dryness under reduced pressure, NMR analysis of the residue dissolved in D_2O showed the presence of starting material and a very small

peak 8 Hz downfield from CCH_3. No significant degradation took place. Perhaps the initial consumption of base is due to ionization of the amino group.

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