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General Acid Catalysis of the Cleavage of 2-(1-Hydroxybenzyl)thiamin by a Preassociation Mechanism[1]

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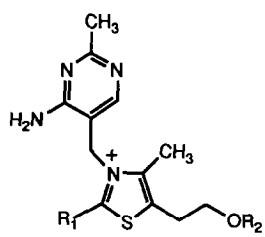
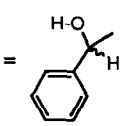
Cleavage of racemic 2-(1-hydroxybenzyl)thiamin (HBT) to benzaldehyde and thiamin in aqueous solution, a retrograde aldol-type reaction, is catalyzed by substituted acetate ions and other oxygen-containing buffer bases at 40°C and ionic strength 1.0 M (KCl). The Brønsted β value is 0.61 for N(1′)-protonated HBT, but there is no significant solvent deuterium isotope effect for catalysis by acetate ion. The water and buffer base-catalyzed reactions are formulated as general acid catalysis of the departure of thiamin from the alcoholate anion ($pK_a'^{ROH}$ = 10.7) of HBT (general base catalysis of thiamin attack in the reverse direction). It is concluded that this reaction proceeds by a concerted mechanism in aqueous solution that is determined by the short lifetime of the thiazolium C(2)-ylide even though the carbanion has a significant lifetime in aqueous solution and a stepwise pathway for the aldol-type addition reaction of the C(2)-ylide must exist. It is suggested that thiamin diphosphate-dependent enzymes could also use the lower energy, preassociation pathway.

INTRODUCTION

Aldol-type addition reactions between thiamin diphosphate (TDP, **1**)[3] and carbonyl compounds are catalyzed by several TDP-dependent enzymes (Scheme 1) (*1*). It is not known whether the thiazolium C(2)-ylide (**2**) exists as a discrete carbanion intermediate on TDP-dependent enzymes. A pK_a' value of ≤ 14 is required for enzyme-bound thiamin C(2)-H if the C(2)-ylide exists as an intermediate



1, R_1 = H, R_2 = $P_2O_6^{3-}$

3, R_1 =  , R_2 = H

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[3] Abbreviations used: TDP, thiamin diphosphate; HBT, 2-(1-hydroxybenzyl)thiamin; Tris, tris(hydroxymethyl)aminomethane.

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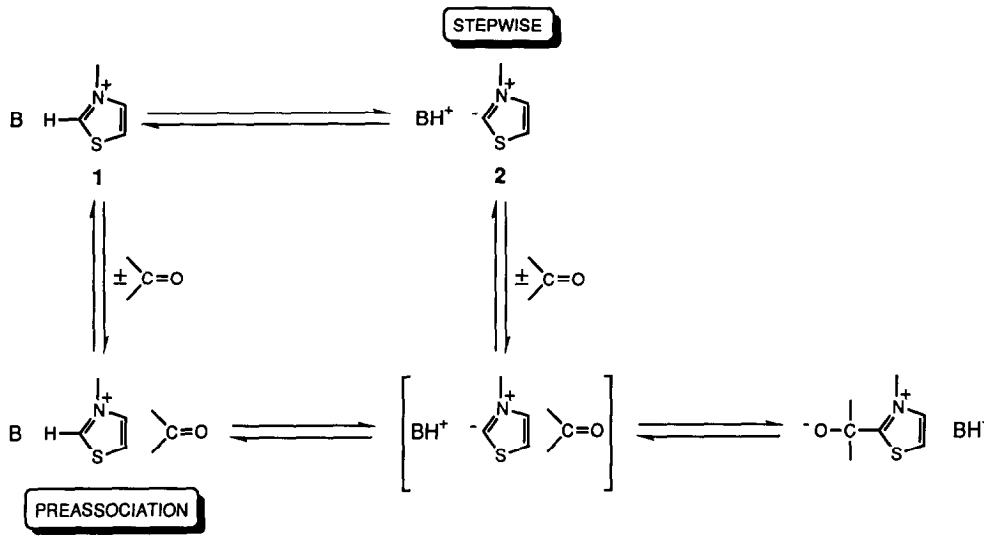


SCHEME 1

in a stepwise enzymic addition reaction (Scheme 1) (*2*), which is much lower than the pK_a' value of 17.7–18.0 for thiamin C(2)-H in aqueous solution (*3*). Therefore, because the C(2)-ylide has a significant lifetime in aqueous solution (*4*), these enzymes either change the relative thermodynamic stabilities of thiamin and its C(2)-ylide for a stepwise pathway or they provide a preassociation pathway (Scheme 1) involving a concerted or hydrogen-bonding mechanism that avoids such an unstable carbanion intermediate (*5*). The stepwise pathway must exist, but it might be bypassed by a nonenforced ("free choice") preassociation pathway because of the relative instability of the carbanion.

In order to determine whether stepwise and preassociation mechanisms coexist for the relatively unstable C(2)-ylide carbanion in aqueous solution we have examined a retrograde aldol-type reaction involving thiamin, the cleavage of 2-(1-hydroxybenzyl)thiamin (HBT, **3**) to form thiamin and benzaldehyde. Our attention was directed to this reaction by the suggestion that carbon–carbon bond cleavage is the rate-limiting step for aldehyde release from HBT and related compounds (*6*) in aqueous solution. In this paper we describe evidence that is inconsistent with a stepwise mechanism involving the free C(2)-ylide as a discrete intermediate, which is commonly assumed for this reaction (*1*), and suggests that a nonenforced preassociation pathway that avoids the unstable C(2)-ylide is available for aldol-type addition reactions catalyzed by TDP-dependent enzymes.

EXPERIMENTAL PROCEDURES

Materials. All organic chemicals were reagent-grade and were purified by recrystallization or distillation. Reagent-grade inorganic chemicals were used as re-

ceived. All water was prepared on a four-bowl Milli-Q water system including an Organex-Q cartridge (Millipore). All deuterated compounds were ≥99 atom% D. Thiamin was dried *in vacuo* at 80°C against P_2O_5 for 5 h before use: mp 242–243°C dec. The synthesis of racemic 2-(1-hydroxybenzyl)thiamin chloride hydrochloride has been described (*7*): mp 182–184°C (lit. 181–183°C) (*8*); the product is unhydrated and free of NaCl on the basis of the calculated molecular weight obtained from a mercurimetric titration of chloride using diphenylcarbazone (*9*). Stock solutions of reagents in D_2O were prepared by first exchanging exchangeable protium from the reagents with D_2O to give ≤2 atom% exchangeable protium in the stock solution. Solutions containing acetate ion in D_2O were prepared by the partial neutralization of acetic acid-*d* with KOD.

Methods. Solution pH was measured at 40°C with an Orion Model SA 720 pH meter and a Radiometer GK2321C combination electrode standardized at pH 6.97 and 4.03 or 9.87. The value of pD was obtained by adding 0.40 to the observed pH of solutions in D_2O (*10*). Based on measurements of pH at known concentrations of hydroxide ion at 40°C and ionic strength 1.0 M (KCl), Eq. [1] was used to calculate the concentration of the lyoxide ion. Equation [1] includes the ion product of H_2O (pK_w = 13.535) or D_2O (pK_w = 14.385) at 40°C (*11*).[4]

$$[LO^-] = 1.22 \times 10^{pL - pK_w} \qquad [1]$$

The pK_a' values of the buffer catalysts in aqueous solution at 40°C and 1.0 M ionic strength, maintained with potassium chloride, were determined from the pL values of gravimetrically prepared buffer solutions containing the acid and base form at a 1 : 1 ratio or by potentiometric titration according to the method of Albert and Serjeant (*12*).

A value of pK_a' = 5.3 in H_2O at 40°C and ionic strength 1.0 M (KCl) for the aminopyrimidinyl group on thiamin and HBT was determined by potentiometric titration under a nitrogen atmosphere (*12*) and a pK_a' value of 5.8 in D_2O was estimated on the basis of $\Delta pK_a = 0.5$ for the solvent isotope effect on the ionization of weak acids (*13*); these pK_a' values are similar to previously reported values at 25°C and ionic strength 2.0 M (*3*).

Kinetics. Rate constants for decomposition of HBT were determined from initial rate measurements, in which the reaction had proceeded ≤2%. The reactions were followed spectrophotometrically in L_2O at 40°C and ionic strength 1.0 M, maintained with KCl, by trapping the benzaldehyde formed with hydrazine derivatives and following the increase in absorbance at 295 nm for reactions at pH ≤ 8.5, which is the wavelength where the difference in the molar extinction coefficients for the hydrazone and HBT is greatest. The trapping reagents used were semicarbazide (0.02–0.04 M, pH 2.5–6.1) and hydrazine (0.01–0.04 M, pH 6.1–8.5). The extinction coefficients for benzaldehyde semicarbazone and hydrazone at 295 nm are 1.40×10^4 M^{-1} cm^{-1} and 4.45×10^3 M^{-1} cm^{-1}, respectively, under the reaction conditions in dilute aqueous solution, acetic acid/acetate, and $H_2PO_4^-/HPO_4^{2-}$ buffers. The

[4] The term "hydron" refers to the hydrogen cation (L^+) without regard to nuclear mass. The specific names "proton" (^1H) and "deuteron" (^2H) refer to the specific isotopes (Commission on Physical Organic Chemistry, IUPAC *Pure Appl. Chem.*, **60**, 1115–1116.) and are abbreviated here as $^1H^+$, H; $^2H^+$, D.

concentrations of trapping reagents were such that there was no induction period in the initial rate of appearance of hydrazone and control experiments with double the concentration of trapping reagent were shown to give the same rate constants. Stock solutions of the trapping reagents were adjusted to the pH of the buffer before use. A dual wavelength assay was used at 295 and 300 nm to account for the significant contribution to the observed rate by hydrolysis of thiamin in reactions at pH $\geq$ 8.5 (*14*). Kinetic runs were initiated by injecting 100–200 μl of a 3.0 mM HBT solution in L_2O into 800–900 μl of reaction mixture to give a final substrate concentration of 0.3–0.6 mM. The observed pseudo-first-order rate constant, k_{obsd}, was obtained by dividing the initial rate of appearance of benzaldehyde by the initial concentration of HBT. When duplicate determinations of k_{obsd} were made, they agreed with ±5% of the average value.

Second-order rate constants for general-base-catalyzed cleavage of N(1′)-protonated HBT (k_B) were obtained from the slope of plots of $\geq$4 values of k'_{obsd} (corrected for the fraction of the substrate present in the N(1′)-protonated form (*15*)) against [B], where B is the catalytic general base, as described previously (*4a*).[5] Values of k'_{obsd} were calculated from k_{obsd} by subtracting the rate of catalysis by HO^- and H_2O, according to $k'_{obsd} = k_B[B] = k_{obsd} - k_{HO^-}[HO^-] - k_{H_2O}$. This treatment corrects for small changes in $[HO^-]$ with increasing buffer concentration that had the effect of decreasing the dependence of k_{obsd} on buffer concentration (*4a*). The catalyst concentration, [B], at the pH of the reaction was calculated from its apparent pK_a (see above). We estimate error limits of ±15% in the second-order rate constants for general base catalysis based on the maximum and minimum slopes that could be drawn in plots of k'_{obsd} against [B] assuming an error of ±12% in k'_{obsd} (*4a*). The second-order rate constant for catalysis by lyoxide ion was obtained from the slope of a plot of k_{obsd}, extrapolated to zero buffer concentration, against lyoxide ion concentration; the rate constant of the pL-independent "water" reaction was obtained from the intercept of this plot at zero lyoxide ion concentration.

RESULTS

The kinetics of the breakdown of racemic HBT to benzaldehyde and thiamin in aqueous solution in the pH range 2–11 at 40°C and ionic strength 1.0 M (KCl) were followed by the appearance of benzaldehyde, the benzaldehyde hydrazone, or benzaldehyde semicarbazone at 295 nm under initial rate conditions. Identical initial rates for the release of benzaldehyde were measured using ^1H NMR as described by Gallo and Sable (*6*).

Figure 1 shows the dependence on pH of the observed pseudo-first-order rate constants, extrapolated to zero buffer concentration, for cleavage of HBT in the pH range 2–11. The pH-log rate profile for cleavage of HBT is described by Scheme

[5] The fact that identical values of k_B were obtained with the N(1′)-methyl substrate demonstrates that this treatment of the data gives the rate constant for the N(1′)-protonated substrate (data not shown).

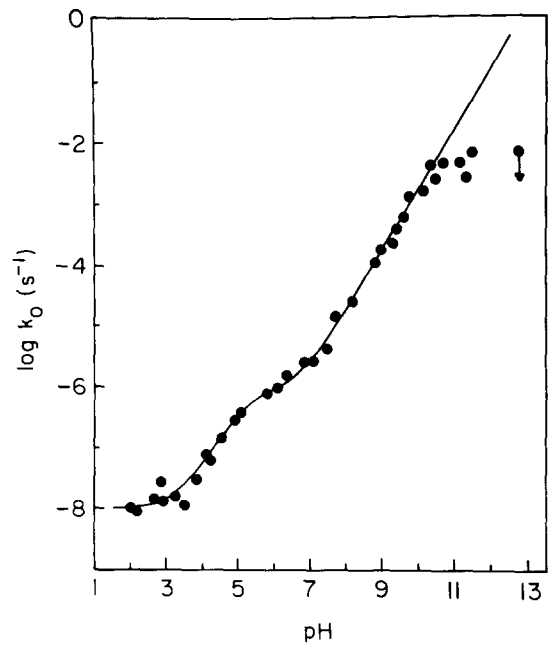
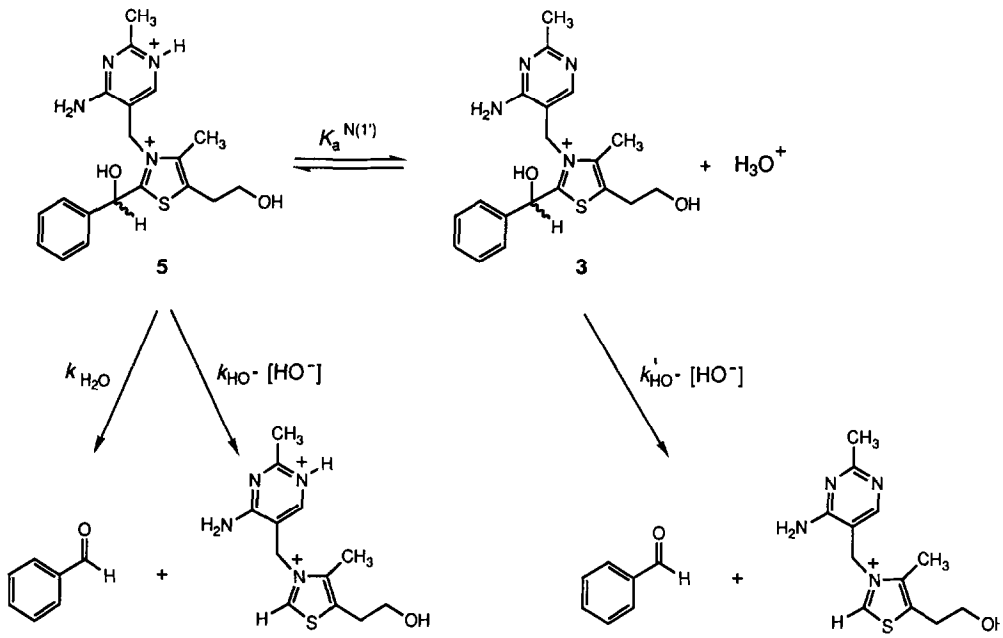


FIG. 1. Buffer-independent rate constants as a function of pH for cleavage of 2-(1-hydroxybenzyl)thiamin at 40°C and I = 1.0 M (KCl) in H_2O. Reactions above pH 8.5 were performed in unbuffered solution and the observed first-order rate constants were extrapolated to zero buffer concentration below pH 8.5. The solid line is based on Scheme 2 using the rate law given in Eq. [2] with $k_{HO^-} = 1.1 \times 10^2\ M^{-1}\ s^{-1}$, $k'_{HO^-} = 4.1\ M^{-1}\ s^{-1}$, $k_{H_2O} = 1.0 \times 10^{-8}\ s^{-1}$, and $pK_a'^{N(1')} = 5.3$ (see text); this line shows the rate increase that would be expected if there were no ionization of the free substrate to the alcoholate anion with a pK'_a value of 10.7. The upper limit for the rate constant at pH 12.8 is indicated.

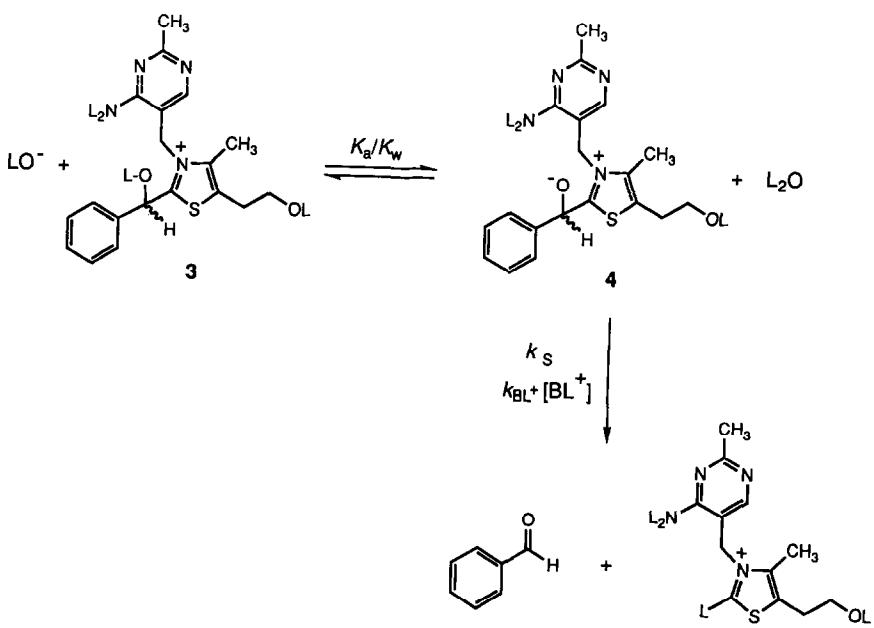
2 and the rate law given in Eq. [2] for the formation of free or N(1')-protonated thiamin using $k_{HO^-} = 1.1 \times 10^2\ M^{-1}\ s^{-1}$, $k'_{HO^-} = 4.1\ M^{-1}\ s^{-1}$, $k_{H_2O} = 1.0 \times 10^{-8}\ s^{-1}$, and $pK_a'^{N(1')} = 5.3$.

$$k_0 = (k'_{HO^-}[HO^-]K_a^{N(1')}/a_{H^+} + k_{HO^-}[HO^-] + k_{H_2O})/(1 + K_a^{N(1')}/a_{H^+}) \quad [2]$$

The pH-log rate profile for HBT (Fig 1) indicates a change in the pathway for breakdown by the upward deviation in the profile at pH ≈ 3 from pH-independent water to hydroxide ion-catalyzed breakdown. The downward deviation in the profile near pH 5 represents the decrease in the rate for hydroxide ion-catalyzed breakdown upon deprotonation of N(1')-protonated HBT (**5**), with $pK_a'^{N(1')} = 5.3$, to form the less reactive free HBT (**3**). In more concentrated solutions of hydroxide ion the rate constants show a negative deviation from linearity that is attributed to conversion of a significant fraction of the free substrate (**3**) to the alcoholate anion (**4**, Scheme 3). The pH-log rate profile for the hydrolysis of 2-acetyl-TDP has been explained similarly, where the cleavage intermediates are the anions of the hydrates at the two aminopyrimidine protonation levels (*16*).



SCHEME 2



SCHEME 3

It is difficult to obtain an accurate rate constant in the pH region in which **3** is entirely converted to the alcoholate anion (**4**) because of rapid hydrolysis of the thiazolium ion product (*14*), so it was necessary to obtain the pK_a' of the carbinol by an extrapolation procedure. A plot of $1/k_0$ against the hydrogen ion activity according to Eq. [3] gives $-K_a$ as the abscissa intercept, which gives $pK_a'^{ROH} = 10.7 \pm 0.2$ for the ionization of free HBT to the alcoholate anion (**4**), and the first-order rate constant for breakdown of the alcoholate anion, $k_S^{H_2O} = (5.4 \pm 2.1) \times 10^{-3}\ s^{-1}$, as the ordinate intercept (*15*).

$$1/k_0 = 1/k_S + a_{H^+}/(K_a k_S). \qquad [3]$$

The value of $pK_a'^{ROH} = 10.7$ in H_2O at 40°C and ionic strength 1.0 M for the carbinol in free HBT agrees reasonably well with the value of 10.3 that was estimated from a Hammett correlation for the ionization of R'R"CH(OH) (*17*) using $\sigma^* = 0.75$ for R = phenyl (*18*) and 3.2 for R = free thiamin.[6] A $pK_a'^{ROD}$ value of 11.2 was estimated on the basis of $\Delta pK_a = 0.5$ for the solvent isotope effect on the ionization of similar weak acids (*13*).

The cleavage reaction is catalyzed by oxygen-containing buffers and follows the rate law described by Eq. [4].

$$v/[ROH] = k_{obsd} = k_{HO^-}[HO^-] + k_B[B] + k_{H_2O} \qquad [4]$$

The rate constants for catalysis of the breakdown reaction by the basic species of buffers were determined as described under Experimental Procedures; no general acid catalysis was detected. Representative data are shown in Fig. 2 for catalysis of the cleavage of N(1')-protonated HBT (**5**) by acetate ion. Rate constants for a series of different general base catalysts are summarized in Table 1.

The amount of buffer catalysis for cleavage of HBT was typically moderate and ≈100%. Experiments to measure general base catalysis were generally performed at relatively high total buffer concentrations of ≤0.4 M and buffer ratios of $A^-/A_{total} \leq 0.4$ in order to obtain maximal rate increases from general base catalysis (*4a*). When working with high concentrations of buffer components, it is important to assess the effects of varying $[M^+B^-]$ and [BH] even when ionic strength is kept constant. The following points suggest that medium effects are small: (i) No significant curvature at high buffer concentration was observed in plots of k'_{obsd} against [buffer base] (see Fig. 2). (ii) The rate constant for catalysis of the cleavage of **5** by acetate ion or HPO_4^{2-} is not sensitive to variations in the nature or concentration of salts used to maintain the ionic strength at 1.0 M; there is no significant change in k_B (≤5%) upon substituting potassium trifluoroacetate for potassium chloride to maintain the ionic strength at 1.0 M. (iii) Values of k_B do not change significantly (≤15%) with the fraction of buffer base, which indicates that there is no significant effect of the acid component of the buffer on the rate constants for general-base-catalyzed cleavage of **5**. (iv) We examined the effect of adding small organic molecules to the reaction medium with 0–5.5 vol% acetonitrile, which corresponds to 0–1.0 M organic solvent in the reaction. There is no

[6] The value of $\sigma^* = 3.2$ for a thiazolium ion was calculated from $pK_a' = 15.9 - 1.42\ \Sigma\sigma^*$ with $pK_a' = 11.4$ for 2-(1-hydroxyethyl)-3,4-dimethylthiazolium ion (*2a*) and $\sigma^* = 0$ for R = CH_3 (*18*).

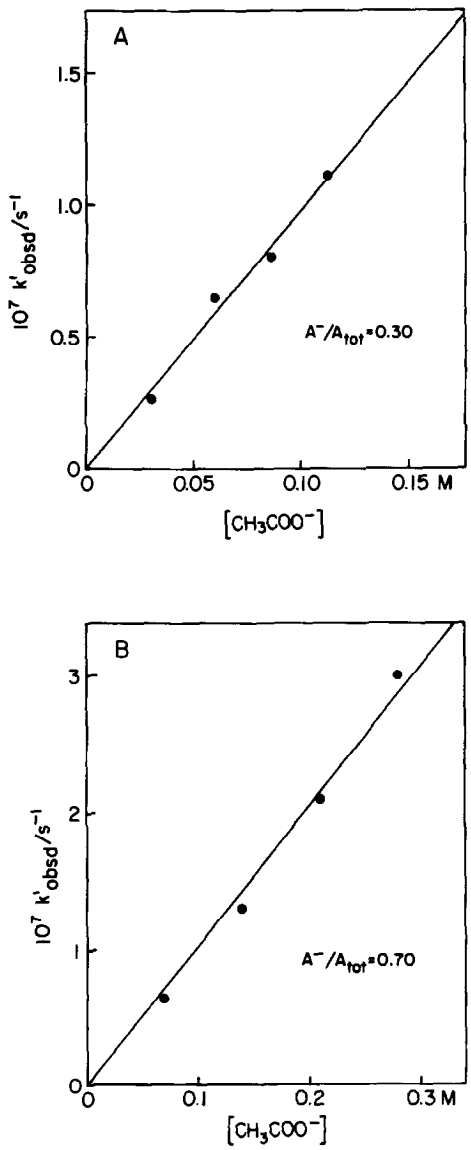


FIG. 2. Dependence of the rate constant for cleavage of 2-(1-hydroxybenzyl)thiamin on the concentration of acetate ion at 40°C and I = 1.0 M (KCl) in H_2O. Values of k'_{obsd} (= k_B[buffer base]) were calculated from k_{obsd} after correcting for catalysis by hydroxide ion and water (see text). The slope of both lines ($k_B = 1.0 \times 10^{-6}$ M^{-1} s^{-1}) is the rate constant for general base catalysis by acetate ion and is independent of the fraction of buffer base. This general base catalysis represents a 110% rate increase at 0.28 M acetate for A^-/A_{total} = 0.70 and a 150% rate increase at 0.11 M acetate for A^-/A_{total} = 0.30.

TABLE 1

Rate Constants for General Base Catalysis of the Cleavage of N(1′)-Protonated 2-(1-Hydroxybenzyl)thiamin[a]

Catalyst	pK_a'[b]	k_B (M^{-1} s^{-1})
H_2O	−1.74	1.8×10^{-10}
HO^-	15.3[c]	110
$NCCH_2COO^-$	2.35	4.0×10^{-8}
$CH_3OCH_2COO^-$	3.50	1.1×10^{-7}
$HCOO^-$	3.65	3.9×10^{-7}
CH_3COO^-	4.63	1.0×10^{-6}
$(CH_3)_2AsO_2^-$	6.23	$\leq 9.1 \times 10^{-6}$
HPO_4^{2-}	6.47	2.0×10^{-5}

[a] At 40°C and ionic strength 1.0 M (KCl) in H_2O. The rate constant k_B is defined in Eq. [4].

[b] Apparent pK_a of the conjugate acid at 40°C and ionic strength 1.0 M (KCl) in H_2O (see text).

[c] Calculated from the ion product of H_2O based on a standard state of 55.1 M for pure H_2O at 40°C, with $K_a^{H_2O} = K_w/[H_2O] = 10^{-13.535}/55.1$ (*11*).

significant change (≤5%) in the value of k_B for catalysis by acetate ion with increasing organic solvent in the reaction medium.

Even though the ionic strength was kept constant at 1 M with potassium chloride there was usually a small decrease in the observed pH of ≤0.1 unit for carboxylate catalysts and ≤0.05 for the other oxygen-containing catalysts between the lowest and the highest buffer concentrations examined. This variation in pH with [buffer] had the effect of decreasing the dependence of k_{obsd} on [buffer]; in some reactions it resulted in decreases in k_{obsd} with increasing buffer for several carboxylate buffers which could be mistaken for a specific salt effect on the cleavage reaction. Consequently, a correction of k_{obsd} was made for the rate resulting from the variation in $[HO^-]$ as described under Experimental Procedures. There is no significant effect of errors in pH and catalyst pK_a' on k_B because, for the buffer ratios of $A^-/A_{total} \leq 0.4$ that were used to determine k_B, the fractional rate increase resulting from general base catalysis is constant—$[HO^-]$ changes in parallel with $[M^+B^-]$ (*4a, 19*).

In summary, the following provide evidence that this treatment of k_{obsd} is valid and provides a reliable measure of general base catalysis:

(i) No medium effects were observed upon addition of an organic solvent or change in the nature of salts at constant ionic strength.

(ii) Changing the concentration of the acid component of the buffer has no effect on k_B (Fig. 2), although it changes pH.

(iii) The amounts of buffer catalysis observed are different for several 2-(1-

hydroxybenzyl)-3-R-4-methylthiazolium ions (*20*). A medium effect would be expected to cause similar or identical changes in the rate constants for the different substrates.

We observed general base catalysis of the cleavage of HBT only for relatively small catalysts, which suggests that there are unfavorable steric effects that must be overcome to observe buffer catalysis with this substrate. Cacodylate is significantly more bulky than the relatively effective phosphate and carboxylate catalysts and no significant catalysis by cacodylate was observed. Gallo and Sable reported no significant general base catalysis by a bulky primary amine, Tris, of the cleavage of HBT (*6*). Several bulky primary amines, including Tris, show negative deviations in their proton transfer reactions with thiazolium C(α)-H, presumably because of an unfavorable steric effect on the diffusional encounter step of thermodynamically unfavorable proton transfers (*21*). There is no significant general base catalysis by phosphate dianion or imidazole of a similar addition–elimination reaction, the carbon–carbon bond cleavage in the hydrolysis of 2-acetyl-3,4-dimethylthiazolium ion (*22*).

There is no detectable solvent deuterium isotope effect on the acetate-catalyzed cleavage of **5**. Experiments with acetic acid–acetate buffers (A^-/A_{total} = 0.2 and 0.5), in which >98% of the exchangeable solvent hydrons were deuterium, gave a rate constant for acetate of $(k_B)_{D_2O}$ = (8.0 ± 1.2) × 10^{-7} M^{-1} s^{-1}, which is independent of the acid component of the buffer. This rate constant does not differ significantly from the value of (1.0 ± 0.15) × 10^{-6} M^{-1} s^{-1} for the reaction in H_2O (Table 1).

DISCUSSION

A preassociation mechanism. The observation of general base catalysis means that the transition state for the addition and expulsion of thiamin at a carbonyl group is stabilized by either simple hydrogen bonding and "solvation" or by active proton transfer driving the reaction or both. A Brønsted plot with a slope of β = 0.61 for general base catalysis of the cleavage of N(1′)-protonated HBT (**5**), including the "water" reaction, is presented in Fig. 3. The fact that the point for water fits on the Brønsted plot of Fig. 3 suggests that the mechanism of the water reaction is the same as that for the other catalysts on the plot. Though the observed general base catalysis of the cleavage of **5** is unmistakable and has most of the characteristics that are expected for classical general base catalysis, it is also relatively weak. Statistical correction of the catalyst pK_a' values and the rate constants for base catalysis (*23*) improves the fit of the data to the Brønsted plot, but does not change β significantly.

The mechanism of the buffer-catalyzed reaction may be assigned to general acid catalysis of the breakdown of the alcoholate anion (**4**) in a class *n* mechanism (Scheme 4) rather than to the kinetically equivalent general base catalysis of proton transfer between the catalyst and the oxygen atom in a class *e* mechanism.

A class *e* mechanism is excluded because: (i) there is no thermodynamic advantage for such catalysis with $BH^+ = H_2O$ (*24*); (ii) addition–elimination reactions

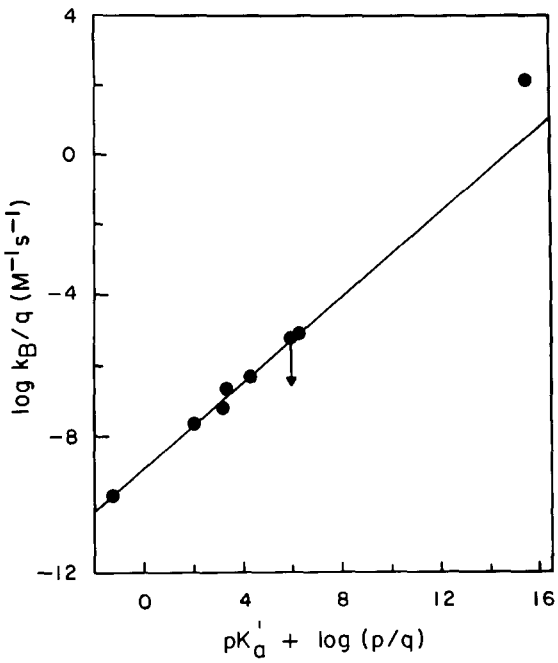
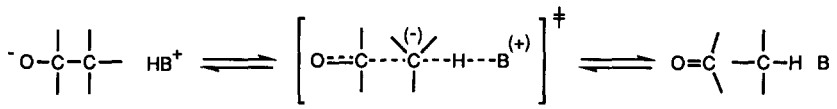


FIG. 3. Brønsted plot for general base catalysis of the cleavage of N(1')-protonated 2-(1-hydroxybenzyl)thiamin at 40°C and I = 1.0 M (KCl) in H_2O. Statistical corrections were made according to Bell and Evans (*23*). The upper limit for catalysis by cacodylate is indicated. The line of slope β = 0.61 is the best fit through the data, including water but omitting the point for hydroxide ion.

of the carbonyl group and strongly basic nucleophiles are not subject to general acid–base catalysis at the carbonyl oxygen atom (*25*); (iii) the decrease in β with increasing basicity and decreasing leaving ability of the C(2)-ylide, in the cleavage of 2-(1-hydroxybenzyl)-3,4-dimethylthiazolium ion compared to **5** (Scheme 2),[7] is in the opposite direction from that expected for a class *e* mechanism (*5, 25*); and (iv) a thermodynamically unfavorable rate-limiting proton transfer to form the alcoholate anion should give a Brønsted slope of 1.0 for general base catalysis rather than the observed slope of β = 0.61 (Fig. 3), which shows that the alcoholate anion (**4**, Scheme 3) is at equilibrium with the carbinol (**5**) and the formation of **4** cannot be rate determining. In addition, it is reasonable that catalysis would occur in a manner that would avoid the most unstable intermediate, as in the class *n* mechanism of Scheme 4.

Consequently, the rate constants reported in Table 1 have been interpreted according to the rate law described by Eqs. [5]–[8] for the kinetically equivalent mechanism for the buffer-catalyzed reactions (Scheme 3) involving the preequilibrium removal of a proton from the substrate (specific basic catalysis) accompanied

[7] A Brønsted β value of $\leq$0.1 was obtained for general base catalysis of the cleavage of 2-(1-hydroxybenzyl)-3,4-dimethylthiazolium ion (*20*).



SCHEME 4

by general acid catalysis by the acidic species of the buffer (*5*). Rate constants for general acid catalysis of the cleavage of N(1′)-protonated HBT were calculated with Eqs. [5]–[8] using the rate constants in Table 1 and are summarized in Table 2.

$$v/[RO^-] = k_{obsd} = k_{BH^+}[BH^+] + k_{H_3O^+}[H^+] + k_S \quad [5]$$

$$k_{BH^+} = k_B K_a^{BH^+}/K_a^{ROH} \quad [6]$$

$$k_{H_3O^+} = k_{H_2O}/K_a^{ROH} \quad [7]$$

$$k_S = k_{HO^-}K_w/K_a^{ROH}. \quad [8]$$

TABLE 2

Calculated Rate Constants for General Acid Catalysis of the Cleavage of the Alcoholate Anion of N(1′)-Protonated 2-(1-Hydroxybenzyl)thiamin[a]

Catalyst	pK_a'[b]	k_{BH^+} (M^{-1} s^{-1})
H_3O^+[c]	−1.74	500
H_2O[d]	15.3[e]	2.8×10^{-3}
$NCCH_2COOH$	2.35	6.5
CH_3OCH_2COOH	3.50	1.7
HCOOH	3.65	4.4
CH_3COOH	4.63	1.2
$(CH_3)_2AsO_2^-$	6.23	≤0.27
HPO_4^{2-}	6.47	0.34

[a] At 40°C and ionic strength 1.0 M (KCl) in H_2O. The rate constant k_{BH^+} is defined in Eq. [5] and was calculated using Eq. [6] with the values in Table 1 and $pK_a'^{ROH} = 10.7$.

[b] Apparent pK_a at 40°C and ionic strength 1.0 M (KCl) in H_2O (see text).

[c] Calculated using Eq. [7] with $k_{H_2O} = 1.0 \times 10^{-8}$ s^{-1}.

[d] Calculated using Eq. [8] with $k_{HO^-} = 110$ M^{-1} s^{-1} for N(1′)-protonated HBT.

[e] Calculated from the ion product of H_2O based on a standard state of 55.1 M for pure H_2O at 40°C, with $K_a^{H_2O} = K_w/[H_2O] = 10^{-13.535}/55.1$ (*11*).

It has been suggested that the cleavage of the carbon–carbon bond in the hydrates formed during the hydrolysis of 2-acetyl-TDP (*16*) and 2-acetyl-3,4-dimethylthiazolium ion (*22*) also occurs by decomposition of the alcoholate anion which is derived from and in equilibrium with the hydrate.

The class *n* mechanism is also expected for hydroxide ion catalysis because there is a thermodynamic advantage to protonation of the C(2)-ylide by water acting as a general acid catalyst. The 10-fold positive deviation of the rate constant for catalysis by hydroxide ion from the Brønsted plot for general base catalysis (Fig. 3) will be examined further in a subsequent paper.

The data are consistent with a mechanism for the cleavage of HBT in which a general acid assists carbon–carbon bond cleavage by transferring a proton to the leaving C(2)-ylide. The reverse, aldol-type condensation reaction involves the removal of the C(2)-proton from the attacking thiazolium C(2)-position by the conjugate base of the catalyst, as it attacks the carbonyl group. The data are inconsistent with the commonly assumed stepwise mechanism (*1*) involving addition and expulsion at a carbonyl group of the C(2)-ylide as a discrete intermediate. The class *n* mechanism of Scheme 4 is supported by the observed Brønsted slope of $\beta = 0.61$ (Fig. 3), which is equivalent to a value of $\alpha = 1 - \beta \approx 0.36$ for catalysis by BH^+ (Fig. 4); a stepwise mechanism with a rate-limiting thermodynamically unfavorable proton transfer from thiazolium C(2)-H would give an α value approaching zero for rate-limiting diffusion-controlled protonation of the C(2)-ylide in the cleavage direction (*26*).

There is no detectable solvent deuterium isotope effect on the buffer-catalyzed cleavage of **5**. The rate constants for catalysis by acetate ion in H_2O and D_2O correspond to a value of $(k_{HOH}/k_{DOD})_{BH^+} = 1.3 \pm 0.3$ for general acid catalysis by acetic acid; this isotope effect was calculated using Eq. [6] with the values in Table 1, $pK_a' = 5.14$ for CH_3COOD (*13*), $pK_a'^{ROD} = 11.2$, and $(k_B)_{D_2O} = 8.0 \times 10^{-7}$ M^{-1} s^{-1}. This small isotope effect suggests (i) that the proton has not lost a significant amount of zero-point energy as a consequence of being "in flight" in the transition state and (ii) that the catalysis involves stabilization of the transition state by hydrogen bonding to the buffer acid, which would be consistent with the suggestion that the proton is in a potential well in the transition state and the catalysis arises by hydrogen bonding, or "solvation," with a catalyst (*27*).

However, the observed catalysis is much larger than can be accounted for by simple hydrogen bonding, which suggests that proton transfer is actively involved in facilitating the cleavage reaction and not simply passively stabilizing the developing negative charge on the C(2)-ylide by hydrogen bonding. The calculated maximum value of β is approximately 0.23 for hydrogen bonding between electronegative atoms (*28*), which is much smaller than the observed value of $\beta = 0.61$ (Fig. 3).[8] Also, the observed value of $\beta = 0.61$ is far outside of the range of values for α and β (in the range 0.06–0.26) that has been attributed to stabilization of the

[8] A value of the Hine interaction coefficient greater than the value of $\tau = \partial\alpha/\partial pK_{BH} = -\partial\beta/\partial pK_{AH} = 0.013$ observed for oxygen and nitrogen as the hydrogen bond donor–acceptors will account for the data using a hydrogen-bonding preassociation mechanism. However, this is unlikely for reactions involving carbon as a hydrogen bond donor–acceptor because carbon is less electronegative than oxygen or nitrogen and the value of τ decreases with decreasing electronegativity of the donor–acceptor (*28*).

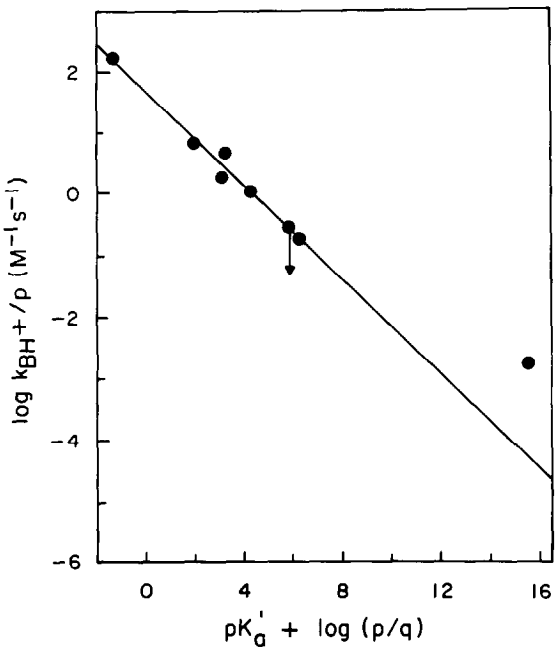


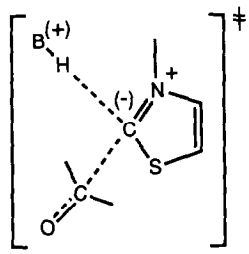
FIG. 4. Brønsted plot for general acid catalysis of the cleavage of the alcoholate anion derived from N(1')-protonated 2-(1-hydroxybenzyl)thiamin at 40°C and I = 1.0 M (KCl) in H_2O. The rate constant k_{BH^+} is defined in Eq. [5] and was calculated using Eq. [6] with the values in Table 1 and $pK_a'^{ROH}$ = 10.7. Statistical corrections were made according to Bell and Evans (*23*). The upper limit for catalysis by cacodylic acid is indicated. The line of slope α = 0.36 is the best fit through the data, including H_3O^+ but omitting the point for water.

transition state in general acid–base catalysis by hydrogen bonding to the catalyst (*29*). Accordingly, only a very small fraction of the catalysis can be explained by simple hydrogen bonding and the observed catalysis must be concerted. The small isotope effect does not exclude a concerted mechanism for the cleavage of **5** because isotope effects may also be decreased by coupling of the motions of the proton and heavy atoms in the transition state (*30*).

Isotope discrimination experiments (*5*), which would also provide a measure of the primary kinetic isotope effect for proton transfer in a concerted mechanism, are not possible for thiamin C(2)-H because of facile C(2)-H → D transfer in the pH range 2–11 (*3*, *4*).

A remaining problem is the assignment of the developing lone pair of electrons formed upon carbon–carbon bond cleavage in the transition state of Scheme 4. Clearly, the observed buffer catalysis requires that proton donation to the leaving C(2)-ylide withdraws electrons from carbon and provides the driving force for cleavage of the C–C bond. In the reverse, addition direction concerted proton transfer to the base in the transition state increases the nucleophilicity of the attacking thiazolium C(2)-ylide. A concerted mechanism is supported by the large amount of observed catalysis, but what is protonated and what is the structure of the transition state?

Proton transfer to the sp^2-hybridized orbital that is involved in the carbon–carbon bond that undergoes cleavage is unlikely because there is no clear precedent for such electrophilic assistance to C–C bond cleavage. A mechanism for acid-catalyzed cleavage involving electrophilic displacement at carbon with a transition state in which the leaving carbon and entering proton both interact weakly with the sp^2-hybridized orbital of the C(2)-ylide (**6**) is improbable because steric requirements would require



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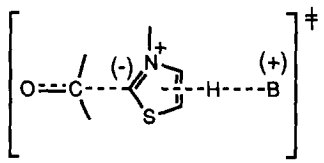
unfavorable frontside electrophilic attack by the carbonyl group on the C(2)-H bond of thiamin in the addition direction (*4a*).

We suggest that the site of proton donation involves the thiazolium ring itself, as shown in **7**. This type of interaction at the C(2)-ylide has precedent: the modest amount of acid inhibition of C(2)-H



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ionization in 3-(cyanomethyl)-4-methylthiazolium ion provides evidence for some component of proton donation stabilizing the C(2)-ylide (*4a*), perhaps by interactions of the proton with antibonding σ^* orbitals or the sulfur atom of the thiazolium ion,[9] or formation of an "Hπ bond" (*32*) between the proton and the π electrons of the thiazolium ring. This protonation is not so different from protonation of any aromatic system, which normally is believed to form a cationic intermediate species (*33*). A species related to **7** may or may not exist as an unstable intermediate, but this species would not be as unlikely as a transition state as might appear on first glance.

Comparison with related reactions. The mechanisms of general acid–base catalysis of carbonyl additions and related reactions are determined by the lifetime of reaction intermediates and follow a sequence of solvent-catalyzed, trapping, preassociation, and concerted mechanisms as the intermediate becomes progres-

[9] The reason(s) that sulfur stabilizes a vicinal carbanion remain uncertain (*4a*). Hogg has suggested that the thiazolium sulfur 3*d* orbitals might stabilize the negative charge on the alcoholate anion (**4**) (*31*).

sively less stable (*34*). A reaction must follow a one-step, concerted mechanism if no intermediate exists, but it has been less clear whether a reaction involving a carbanion will follow a concerted mechanism when the intermediate of a stepwise mechanism has a lifetime. Concurrent concerted and stepwise pathways have been observed only for reactions in which one of the steps involves proton transfer between electronegative atoms, which has a small barrier but can cause a large stabilization of the transition state (*35*). This has not been observed for similar reactions involving ordinary carbanions in which both processes have larger barriers (*36*).

These results demonstrate that concerted catalysis can occur in general acid–base-catalyzed reactions that involve proton transfer between electronegative atoms and carbon even when the intermediates for the stepwise reactions have a significant lifetime. This is probably because the barrier for proton transfer between electronegative atoms and thiazolium C(2)-H is small (*4b*), so that the disadvantage from concerted proton transfer is less than the disadvantage from including an additional process in the transition state of a stepwise reaction involving a relatively unstable carbanion (*35d*). To our knowledge this is the first demonstration of a reaction involving a carbanion following a concerted mechanism when the intermediate of a stepwise mechanism has a significant lifetime.[10]

Implications for enzyme-catalyzed reactions. These results are inconsistent with a stepwise mechanism for aldol-type addition–elimination reactions involving the free C(2)-ylide in aqueous solution and show that a nonenforced pathway that avoids the relatively unstable C(2)-ylide is available for aldol-type reactions catalyzed by benzoylformate decarboxylase (*37*), benzaldehyde lyase (*38*), and other TDP-dependent enzymes (*1a*). If these enzymes use a similar concerted mechanism, they might catalyze the aldol-type reactions by assistance to carbon–carbon bond formation and cleavage by a significant amount of proton transfer in the transition state, which would serve to avoid the formation of the unstable C(2)-ylide and the transition states leading to its formation. Enzymes may stabilize the C(2)-ylide (or destabilize TDP) (*39*) to facilitate proton transfer in a concerted mechanism rather than to increase the lifetime of the C(2)-ylide for a stepwise mechanism. Enzyme catalysis by electrophilic assistance to C(2)-proton removal in the transition state for C–C bond formation would be minor if C–C bond formation was largely uncoupled from C(2)-proton removal.

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[10] The rate constant for reprotonation of the C(2)-ylide (k_{-p}) was estimated from a curved plot of log k_{-p} against ΔpK_a based on an intrinsic barrier of 1.3 kcal mol^{-1} for C(2)-T $\rightarrow$ L exchange and is $\leq 10^{13}$ s^{-1} (the lifetime of the intermediate is greater than a bond vibration frequency) (*36*) for $\Delta pK_a \leq 19.5$ (*4b*). This means (i) that the C(2)-ylide ($pK_a' = 18$) (*3*) has a short, but significant, lifetime in aqueous solution in the presence of buffer acids (*4a*) and (ii) that the mechanism is not enforced concerted in the presence of buffer acids.

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