# Intramolecular General Base Catalyzed Ester Hydrolysis. The Hydrolysis of 2-Aminobenzoate Esters

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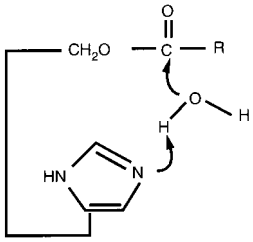
Rate constants have been obtained for the hydrolysis of the trifluoroethyl, phenyl, and *p*-nitrophenyl esters of 2-aminobenzoic acid at 50 °C in $H_2O$. The pseudo-first-order rate constants, $k_{obsd}$, are pH independent from pH 8 to pH 4 (the p$K_a$ of the amine group conjugate acid). The 2-aminobenzoate esters hydrolyze with similar rate constants in the pH-independent reactions, and these water reactions are ∼2-fold slower in $D_2O$ than in $H_2O$. The most likely mechanism involves intramolecular general base catalysis by the neighboring amine group. The rate enhancements in the pH-independent reaction in comparison with the pH-independent hydrolysis of the corresponding para substituted esters or the benzoate esters are 50−100-fold. In comparison with the hydroxide ion catalyzed reaction, the enhancement in $k_{obsd}$ at pH 4 with the phenyl ester is $10^5$-fold. Intramolecular general base catalyzed reactions are assessed in respect to their relative advantages and disadvantages in enzyme catalysis. A general base catalyzed reaction can be more rapid at low pH than a nucleophilic reaction that has a marked dependence on pH and the leaving group.

Classical general base catalysis, in which a base partially abstracts a proton from a water molecule in the transition state, has been suggested for a number of enzymatic reactions.[1−6] For example, the deacylation of acyl α-chymotrypsins has been considered to occur by a general base mechanism involving histidine-57 (**I**).[1,5]




**I**

General base catalyzed hydrolysis reactions involve restriction of at least one water molecule in the transition state and therefore should be less favorable than nucleophilic reactions that proceed by direct attack of a functional group at the reaction center. It is generally thought that nucleophilic reactions will occur when sterically possible. Current concepts can more easily explain the large rate constants in enzymatic reactions in terms of nucleophilic processes.[7] The reasons for a general base mechanism in enzymatic reactions are therefore not apparent.

Chemical intramolecular reactions resemble the intracomplex reactions of enzymes.[1] A general base mechanism has been found in ester and amide hydrolysis reactions in cases where a nucleophilic reaction is precluded, or difficult.[8−15] A direct comparison of intramolecular general base catalyzed reactions and intramolecular nucleophilic reactions of structurally similar esters would allow a rigorous evaluation of relative mechanistic advantages and disadvantages, which could in turn provide a chemical basis for the proposed mechanisms in enzymatic reactions.

2-Substituted benzoate esters are ideal compounds for the kinetic investigation of intramolecular reactions because of both the rigid steric relationship between the substituent group and the ester carbonyl and the systematic structural variations that are possible. A basic group in the 2-position of benzoate esters with which nucleophilic attack is sterically difficult might preferentially function in hydrolysis reactions as an intramolecular general base. We have therefore in this work determined rate constants for hydrolysis of the esters **II**−**IV** with which the neighboring amine group cannot easily act as a nucleophile because of the four-membered ring transition state that would be required; a tetrahedral intermediate resulting from nucleophilic attack by the neighboring amine would be highly strained.[16] It is important to determine the steric limitations of both

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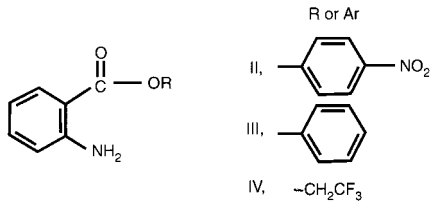
general base and nucleophilic reactions. The intramolecular nucleophilic reactions of 2-aminomethylbenzoate esters were reported in a preceding paper.[17]




There have been few previous examples of intramolecular general base catalysis by a primary amine group of the type in **II**−**IV**.[8] Capon and Ghosh[18] reported that the amine group of **III** did not participate in the hydrolysis reaction, but they only determined rate constants at high and low pH (>8.5 and <2). We have found pronounced participation by the neighboring group in the hydrolysis reactions at pH < 8.5.

## Experimental Section

**Materials.** The trifluoroethyl, phenyl, and *p*-nitrophenyl esters of anthranilic acid have been reported previously and were prepared by the literature procedures.[19,20] After recrystallization from chloroform, the hydrochloride of phenyl 2-aminobenzoate had an mp = 149 °C. Anal. Calcd for $C_{13}H_{12}ClNO_2$: C, 62.52; H, 4.81; N, 5.61. Found: C, 62.26; H, 4.44; N, 5.20.

Phenyl 4-aminobenzoate was prepared by reduction of phenyl 4-nitrobenzoate in absolute ethanol with a $PtO_2$ catalyst by the method of Berti et al.[20] The ester had an mp = 167 °C (lit.[20] 169−172 °C).

Infrared and NMR spectra were consistent in all cases with the expected ester structure. Buffers were prepared from reagent-grade material. Amine buffer components were freshly distilled or recrystallized prior to use.

**Kinetic Methods.** The hydrolysis of 2- and 4-aminobenzoate esters in water ($\mu$ = 0.1 M with KCl) was followed by monitoring the changes in absorbance at appropriate wavelengths between 245 and 400 nm with a Beckman DU-7000 spectrophotometer. The spectra at the conclusion of the reactions quantitatively matched those of 2- or 4-aminobenzoic acid and the alcohol or phenol. Repetitive scans during the course of the reactions gave no evidence for the formation of an accumulating intermediate. To initiate the reactions, 30 $\mu$L of an acetonitrile solution of the ester was added to the buffer in the cuvette. Buffers included chloroacetate, formate, acetate, cacodylate, imidazole, Tris, morpholine, trimethylamine, and carbonate. Significant buffer catalysis was not observed under conditions of the experiments (0.02 M buffer). The reactions were followed to completion, and infinity points were stable. The values of $k_{obsd}$, the pseudo-first-order rate constants, were computer calculated using a rigorous least-squares procedure. The reaction solution pH values were measured with either a Radiometer model 22 pH meter with a GK 2303 C combination electrode or a Beckman model 3500 pH meter with a combination electrode standardized against Mallinckrodt standard buffer solutions. The value of $K_w$ at 50 °C was taken to be $5.5 \times 10^{-14}$.

Computer modeling of the esters was carried out with a Silicon Graphics Indigo 2 workstation. Geometry was optimized with AMl semiempirical calculations. Energies were determined with Hartree−Fock 6-31G* ab initio and density functional pBP.DN* calculations.

(16) The instability of a tetrahedral intermediate was indicated by density functional and ab initio calculations. See Discussion.
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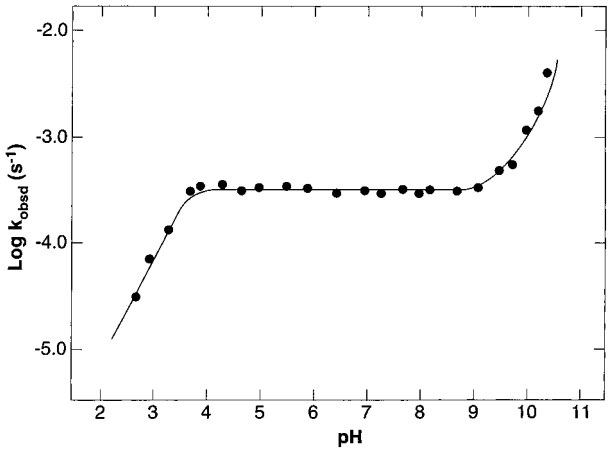



**Figure 1.** Plot of log $k_{obsd}$ vs pH for the hydrolysis of phenyl 2-aminobenzoate in $H_2O$ at 50 °C ($\mu$ = 0.1 M with KCl).

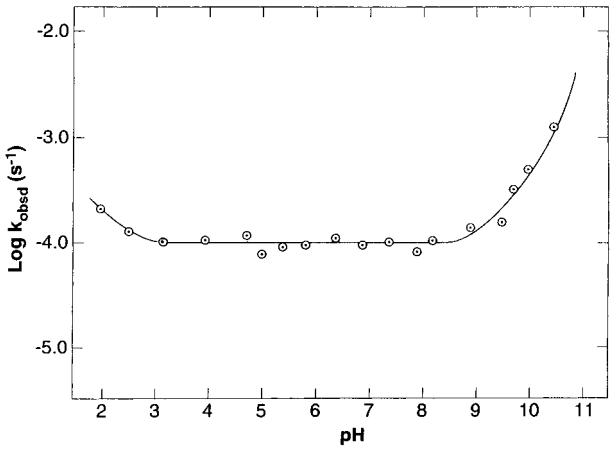



**Figure 2.** Plot of log $k_{obsd}$ vs pH for the hydrolysis of phenyl 4-aminobenzoate in $H_2O$ at 80 °C ($\mu$ = 0.1 M with KCl).

## Results

Figure 1 shows the plot of log $k_{obsd}$ vs pH for hydrolysis of phenyl 2-aminobenzoate (**III**) in $H_2O$ at 50 °C with $\mu$ = 0.1 M with KCl. The plot indicates $OH^-$ catalysis at pH > 9, and a reaction at pH < 9 that is dependent on the base species of the 2-amino group. The plot bends downward near pH 4 to give a slope of 1.0. The equation for $k_{obsd}$ is given in eq 1; $k_0$ has the

$$k_{obsd} = (k_0 + k_{OH}K_w/a_H)\left[\frac{K_a}{K_a + a_H}\right] \qquad (1)$$

value $3.2 \times 10^{-4}$ s$^{-1}$, $k_{OH}$ is 1.8 M$^{-1}$ s$^{-1}$, and p$K_{app}$ is 3.8. In $D_2O$, $k_0$ is $1.8 \times 10^{-4}$ s$^{-1}$ ($k_0^{H_2O}/k_0^{D_2O}$ = 1.8). The hydrolysis of the corresponding phenyl 4-aminobenzoate was too slow to measure conveniently at 50 °C. The reactions were, therefore, followed at 80 °C in $H_2O$. As seen in Figure 2, there is no apparent p$K_a$, and the pH-independent reaction from pH 3 to 8.5 has $k_{obsd}$ = $1 \times 10^{-4}$ s$^{-1}$.

Trifluoroethyl 2-aminobenzoate (**IV**) gives a plot of log $k_{obsd}$ for hydrolysis in $H_2O$ vs pH ($\mu$ = 0.1 M) that is similar at 50 °C to that for **III** in Figure 1. Again, the reaction is pH independent from pH 4 to 8.5. The value of $k_0$ from the pH-independent region of the profile is $4.7 \times 10^{-4}$ s$^{-1}$. Trifluoroethyl 4-aminobenzoate

**Table 1. Rate Constants for Hydrolysis of Esters II and IV at 50 °C and $\mu$ = 0.1 M with KCl**

| ester | $k_{obsd} \times 10^4$ s$^{-1}$ | pH | ester | $k_{obsd} \times 10^4$ s$^{-1}$ | pH |
|---|---|---|---|---|---|
| **II** | 4.04 | 2.2 | **IV** (contd) | 5.41 | 5.4 |
| | 5.61 | 2.5 | | 4.28 | 5.7 |
| | 8.13 | 3.0 | | 5.28 | 6.1 |
| | 24.5 | 3.3 | | 5.03 | 6.5 |
| | 26.1 | 3.7 | | 4.40 | 6.8 |
| | 24.6 | 4.0 | | 4.30 | 7.0 |
| | 27.4 | 4.25 | | 4.34 | 7.45 |
| | 27.1 | 4.4 | | 4.70 | 7.65 |
| | 28.6 | 4.7 | | 5.39 | 7.9 |
| | 24.2 | 4.9 | | 4.25 | 8.0 |
| | 29.9 | 5.3 | | 5.55 | 8.1 |
| | 43.1 | 5.5 | | 5.40 | 8.6 |
| | 58.5 | 5.75 | | 6.03 | 9.0 |
| **IV** | 4.52 | 3.45 | | 11.07 | 9.75 |
| | 5.21 | 4.0 | | 13.41 | 10.1 |
| | 3.32 | 4.3 | | 24.96 | 10.5 |
| | 4.91 | 4.6 | | 36.7 | 11.0 |
| | 3.98 | 4.9 | | | |

was prepared but was not of sufficient solubility in water for accurate kinetic measurements. Trifluoroethyl benzoate has $k_0 = 5 \times 10^{-6}$ s$^{-1}$ at 50 °C.[21]

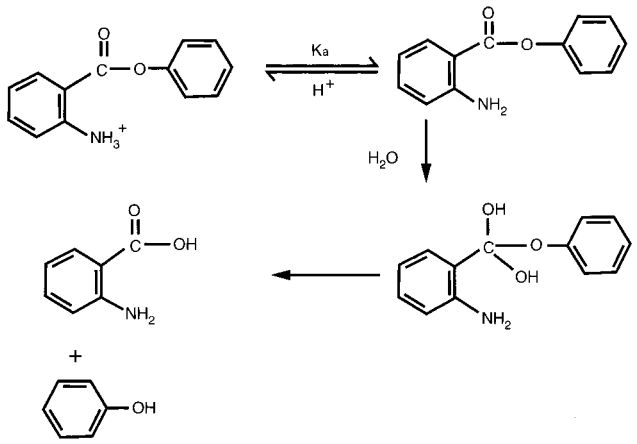
*p*-Nitrophenyl 2-aminobenzoate hydrolyzes in $H_2O$ at 50 °C ($\mu$ = 0.1 M with KCl) in a pH-independent reaction at pH > 4; p$K_{app}$ = 3.5. The value of the rate constant for the pH-independent reaction is $2 \times 10^{-3}$ s$^{-1}$. Again, the plot of log $k_{obsd}$ vs pH (not shown) bends downward at p$K_{app}$ to give a slope of 1.0. The observed rate constants for the reaction of **II** and **IV** are given in Table 1.

## Discussion

The most striking feature of the plot of log $k_{obsd}$ vs pH for the hydrolysis of phenyl 2-aminobenzoate (**III**) in Figure 1 is the large pH-independent region extending from pH 9 to 4. The reaction represented by the plateau in the profile is undoubtedly a water reaction. Substituted phenyl benzoate esters undergo a pH-independent hydrolysis reaction that is nearly independent of substitution in the acyl group.[22] However, the rate constants are considerably less than those for hydrolysis of **III**; the reported rate constant for pH-independent hydrolysis of phenyl benzoate in water at 90 °C is $3 \times 10^{-5}$ s$^{-1}$.[22] Near pH 4, the plot of Figure 1 bends downward to give a slope of 1.0 (p$K_{app}$ = 3.8). The downward bend corresponds with the expected p$K_a$ of the 2-amino group.[23] Therefore, protonation of the amino group retards the rate of the hydrolysis reaction. Rate retardation would not be expected on the basis of electronic effects; the increased electron withdrawal of the protonated amine should enhance the ease of attack of a water molecule at the carbonyl carbon. Thus, the neutral amine base is important in the water reaction of **III**.

That the amine base of **III** is directly participating in the pH-independent hydrolysis reaction is clear from the fast rate of hydrolysis in comparison with the corresponding 4-amino-substituted ester. A pH-independent water reaction of the latter ester also occurs in the neutral pH region, but at 50 °C, the rate constants are relatively small (**III** is more reactive than the 4-substituted ester by an estimated factor of at least 25−50-fold at 50 °C). At 80 °C, the 4-substituted ester hydrolyzes 3.2-fold more slowly in the pH-independent reaction than **III** at 50 °C. The pH-independent reaction of phenyl 4-aminobenzoate continues through the pH 4 region; as seen in Figure 2, there is no downward bend in the pH−rate constant profile.

**Scheme 1**




When the leaving group is made poorer with the trifluoroethyl ester **IV**, with which the p$K_a$ of the leaving group is 12.4,[24] participation by the neighboring amine group also occurs, and the rate constants for pH-independent hydrolysis are closely similar to those of **III**. Thus, the leaving group p$K_a$ has little effect in these intramolecular reactions. The $k_0$ for the para-nitro derivative is also similar to that for **IV**, even though the p$K_a$ of the leaving groups differ by 5.4 p$K_a$ units. A $\beta_{lg} = 0$ was found previously in the pH-independent water reactions of picolinate esters.[25,26] In those reactions, the rate constants did not vary appreciably as the leaving group was varied from dinitrophenol (p$K_a$ = 4.4) to trifluoroethanol (p$K_a$ = 12.4). A $\beta_{lg} \sim 0$ indicates an early transition state with little or no C−O bond breaking.

The pH-independent hydrolysis of **III** is characterized by a $D_2O$ solvent isotope effect ($k_0^{H_2O}/k_0^{D_2O}$) near 2, which is consistent with proton transfer in the transition state.[27] A slower reaction in $D_2O$ than $H_2O$ has characterized other reactions thought to involve intramolecular general base catalysis,[10−15] whereas a nucleophilic reaction should have a solvent isotope effect near unity. Thus, the amine base participation is most reasonably considered to occur via Scheme 1 and the general base mechanism (**V**) in which a proton is partially removed from a water molecule as it attacks the carbonyl group. There could be a hydrogen bonding interaction between the amine base and the attacking water molecule. That the intramolecular reactions of **II**−**IV** are not nucleophilic via sterically unfavorable four-membered ring transition states is also indicated by the very small leaving group dependence

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(22) Mollin, J.; Baisa, A. *Collect. Czech. Chem. Commun.* **1978**, *43*, 304.

(23) p$K_a$ values of anthranilic acid are 2.05 and 4.95 at 25 °C: Kortum, G.; Vogel, W.; Andrussow, K. *Dissociation Constants of Organic Acids in Aqueous Solution*; Butterworths: London, 1961; Vol. 476, p 380.

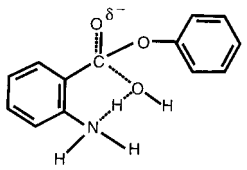
(24) Ballinger, P.; Long, F. A. *J. Am. Chem. Soc.* **1960**, *82*, 795.

(25) $\beta_{lg}$ is the slope of a plot of the log of the rate constant vs the p$K_a$ of the leaving group.

(26) Fife, T. H.; Przystas, T. J. *J. Am. Chem. Soc.* **1985**, *107*, 1041.

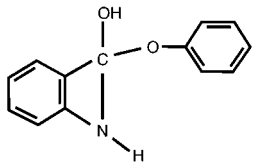
(27) Protons attached to the attacking water molecule and those hydrogen bonded to the developing negative charge on the carbonyl group could undergo changes that might account for the isotope effect of 1.8.

in the reaction, which is not typical of the nucleophilic reactions of weakly basic amines.[28] The effect of the leaving group is large in the nucleophilic reactions of the corresponding 2-aminomethylbenzoate esters ($\beta_{lg} \sim 1.0$)[17,29] and those of *o*-aminophenylacetate esters.[30]



V

The amine nitrogen of **II**−**IV** is held rigidly quite close to the carbonyl group carbon; AMl semiempirical calculations indicate with **III** and **IV** that the nonbonded distances are 2.99 and 3.08 Å, respectively. Unlike other apparent intramolecular general base catalyzed reactions,[10−15] there are no steric impediments to nucleophilic attack except the necessity of a four-membered ring transition state. The energy of the hypothetical tetrahedral intermediate (**VI**) from the density functional or ab initio calculations, is much higher than that of the isomeric reactant ($\Delta = +50.4$ kcal/mol).[31] The C−N−C bond angle is 88.82° in **VI**.



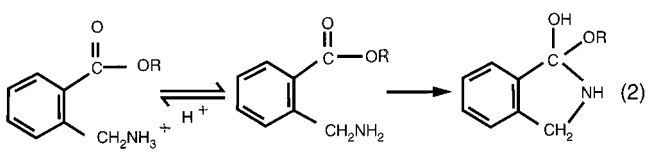
VI

Thus, the calculations indicate that nucleophilic attack in **II**−**IV** would be energetically unfavorable and therefore unlikely; the transition state for nucleophilic attack would necessarily lie much closer to the tetrahedral intermediate than to reactants. In this case, the calculations support and help explain the conclusions derived from the experimental data.

The rate enhancement provided by mechanism **V** can be calculated by comparison with the rate constant for the water reaction of the 4-substituted derivative or that of the unsubstituted benzoate ester. In those reactions, a water molecule very likely acts as a general base, partially abstracting a proton from another water molecule as it attacks at the carbonyl. In bimolecular general base catalyzed hydrolysis reactions, the Bronsted $\beta$ values usually range from 0.2 to 0.5.[32] A small $\beta$ implies only moderate proton transfer from the water molecule in the transition state. Thus, the moderate calculated rate enhancement for intramolecular general base catalysis in the hydrolysis of **III** (<100-fold) reflects the facility of the comparison reaction and a small $\beta$ coefficient. A small $\beta$ is consistent with a small rate enhancement due to the basic group. Note, however, that if the calculated $k_{obsd}$ values for the intramolecular general base and $OH^-$ catalyzed hydrolysis reactions are compared at the same pH, then the enhancement in $k_{obsd}$ due to general base catalysis will increase by a factor of 10 for each decrease in pH of one unit at pH values above the $pK_a$ of the general base. The enhancement of the observed rate constant can therefore become quite large ($10^5$-fold in the case of **III** at pH 4). This is because of the decrease in concentration of the fully ionized nucleophile ($OH^-$) with decreasing pH.

**General Base Catalysis.** The reactions of **II**−**IV** provide an excellent opportunity to assess the relative advantages and disadvantages of general base mechanisms in enzymatic reactions. General base catalysis gives relatively small rate enhancements, but a precise steric alignment is not required.[8] A nucleophilic mechanism, on the other hand, can give large rate constants at pH values near neutrality and is subject to further general acid−base catalysis.[17,30] At pH 7, phenyl 2-aminomethylbenzoate releases phenol 20 000-fold faster than **III**. However, the steric fit is highly important in the nucleophilic reactions. A steric situation comparable to the formation of a five- or six-membered ring transition state is very likely required in enzymatic nucleophilic reactions.[33] Consequently, if a variety of structurally dissimilar substrates are acted upon by a hydrolytic enzyme, then a mechanism without rigid steric constraints would be advantageous. It is now clear that a further point in favor of the general base mechanism is the lack of dependence of the rate constants on the leaving group ($\beta_{lg} \cong 0$). As a consequence, a large group of different substrates can be utilized without loss of catalytic efficiency.

The difference in pH dependence for nucleophilic and general base catalyzed reactions can lead to a significant advantage of the general base mechanism at pH values near or below neutrality. When the steric fit of an intramolecular nucleophile to the carbonyl is very good, the nucleophilic attack will be facile. The tetrahedral intermediate should then be present at high concentration. Breakdown of the intermediate to products can be rate determining and pH dependent; the cyclization of 2-aminomethylbenzoate esters proceeds with apparent $OH^-$ catalysis.[17,29] In contrast, a general base reaction involving attack of an incipient hydroxide ion will be pH independent at pH values greater than the $pK_a$ of the base catalyst. Consequently, at low pH values, a general base mechanism can in fact give the largest rate. With the present series of compounds, **IV** reacts faster than trifluoroethyl 2-aminomethylbenzoate at pH < 5. The mechanism for the latter ester cannot change to general base catalysis at pH < 5 because of amine protonation and the favorable nucleophilic attack that traps the functional group free base in a tetrahedral intermediate (eq 2).



(2)

(28) Jencks, W. P.; Gilchrist, M. *J. Am. Chem. Soc.* **1968**, *90*, 2622. Blackburn, G. M.; Jencks, W. P. *J. Am. Chem. Soc.* **1968**, *90*, 2638.

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(30) Fife, T. H.; Duddy, N. W. *J. Am. Chem. Soc.* **1983**, *105*, 74.

(31) The $\Delta$ values of the internal energies were similar employing density functional pBP.DN* or 6-31G* ab initio calculations.

(32) (a) Jencks, W. P.; Carriuolo, J. *J. Am. Chem. Soc.* **1961**, *83*, 1743. (b) Fife, T. H.; McMahon, D. M. *J. Org. Chem.* **1970**, *35*, 3699.

(33) In the deacylation of the *p*-nitrophenoxycarbonyl serine-195 acyl α-chymotrypsin, the leaving group is *p*-nitrophenol. Histidine-57 acts as a nucleophile, but the reaction is quite hindered, which is probably the result of poor steric fit: Hutchins, J. E. C.; Fife, T. H. *J. Am. Chem. Soc.* **1972**, *94*, 8848.

As the leaving group becomes poorer, the rate advantage of a general base mechanism near pH 7 would become larger,[34] in view of the large effect of the leaving group in the analogous intramolecular nucleophilic reactions.[17] Reduction of the p$K_a$ of the nucleophile would further increase the relative kinetic advantage of the general base reaction. A low p$K_a$ for a general base catalyst is advantageous when $\beta$ is small, since a high concentration of the base form is then present at moderate pH. Carboxyl groups have been implicated as general base catalysts with several enzymes.[2−4]

(34) This assumes that a general base catalyzed reaction can occur when the leaving group is poor. A pH-independent hydrolysis reaction of ethyl picolinate cannot be detected (ref 26).

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