# Kinetics and Mechanisms of the Aminolysis of *N*-Hydroxysuccinimide Esters in Aqueous Buffers

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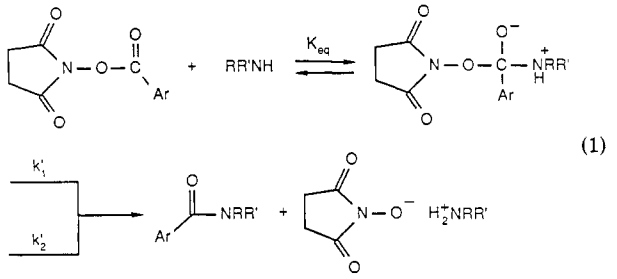
Rate constants for the aminolysis of the *N*-hydroxysuccinimide (NHS) ester of *p*-methoxybenzoic acid, in aqueous buffer systems (20% dioxane), have been determined under pseudo-first-order conditions. For the amines studied ($pK_a$ = 7.60–11.1), the data fit the rate expression $k_{obsd} - k_{OH^-} \times [OH^-] = k_1[amine]_{free}$. This rate equation is in contrast to the two-term rate equation ($k_{obsd} = k_1[amine] + k_2[amine]^2$) obtained for this reaction in anhydrous dioxane (Cline, G. W.; Hanna, S. B. *J. Am. Chem. Soc.* **1987**, *109*, 3087) and is suggestive of a disproportionate decrease in the catalyzed vs the uncatalyzed reaction path upon changing from a nonaqueous to an aqueous solvent system. The correlation of amine basicity with the nucleophilic rate constant, $k_1$, yields a slope $\beta_{nuc}$ = 1.0. The magnitude of $\beta_{nuc}$, in terms of a reaction mechanism where a tetrahedral intermediate is formed in a fast preequilibrium followed by rate-determining breakdown to products, reflects the sensitivity to changes in charge accumulation in the formation of the tetrahedral intermediate. The resultant increased rate constants, with increased basicity, are due to the effect of an increased concentration of the tetrahedral intermediate. A qualitative evaluation of the literature and current data concerning the leaving ability of *N*-hydroxy esters, in comparison to phenyl esters (equivalent acyl groups and nucleophiles), reveals that, with leaving groups of comparable basicity, the nucleophilic rate constants for *N*-hydroxy esters are about 2 orders of magnitude greater than that for phenyl esters.

## Introduction

*N*-Hydroxysuccinimide esters (NHS) have proved to be useful acylation agents of amino acids, peptides, proteins, and other compounds of biochemical interest.[1] This versatility stems from the relative stability of NHS esters in weakly basic buffer solutions (in contrast to the high reactivity of acyl halides in such media) and the quick and, reportedly, selective acylation of free amino groups, in particular, that of the $\epsilon$-amino group of lysine.[2] Despite the wide acceptance and multiple applications that NHS esters have enjoyed, there have been few methodical studies with regard to the factors influencing the reactivity and selectivity of these esters.

We have recently reported the results of a study designed to address this question.[3] Because many syntheses utilizing NHS esters are conducted in aprotic solvents (e.g., solid-phase peptide synthesis[4]), we first investigated the mechanism of the aminolysis of NHS esters in 1,4-dioxane. We found that the reactivity was increased by a decrease of electron density at the acyl carbon ($\rho \sim 1$). With regard to the nucleophilic amine, three observations were noted. The rate was found to be (1) proportional to basicity ($\beta \sim 0.7$), (2) significantly decreased by steric hindrance, and (3) subject to general-base catalysis ($k_{obsd} = k_1[amine] + k_2[amine]^2$). From these observations, we proposed that the mechanism (eq 1) for the aminolysis of NHS esters is one in which the preequilibrium formation of tetrahedral intermediate, $K_{eq}$, is followed by rate-determining breakdown to products by two concurrent paths, a noncatalyzed route, $k_1'$, and a general-base-catalyzed route, $k_2'$ (eq 1).

In light of the significant contributions that have resulted from the application of NHS esters to evaluate the proximity and interaction of various integral membrane proteins,[5] to label specific receptor sites,[6] and to modify

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specific amino acid residues within proteins and polypeptides,[1b] we felt it essential that our study be extended to include aqueous solvent systems. Here we report the kinetic parameters, with regard to the basicity of the amine, governing the aminolysis of anisoyl-NHS ester in aqueous dioxane media. The application of these results can provide a systematic means of planning experimental protocol and of interpreting results from those studies in which NHS esters are employed for the acylation of amino groups in aqueous solvent systems.

## Experimental Section

**General Methods.** $^1H$ NMR spectra were recorded on a Varian EM 360 or a JEOL JNM-FX100 instrument, with $CDCl_3$ (Aldrich 99.8% D, 1% v/v $Me_4Si$) as solvent. IR spectra were recorded on a Perkin-Elmer 1750 FTIR instrument (KBr pellets). UV spectra were recorded on a Perkin-Elmer 552 UV–vis spectrophotometer. TLC (Whatman KC18, developed with MeOH/$H_2O$, 70:30) and HPLC (Supelco LC-18 column (25 cm × 6.4 mm); solvent system, aqueous acetate buffer (0.2 M, pH 4.8)/$CH_3CN$, 65:35)) analyses were performed as previously described.[3] pH measurements were obtained with a Fisher 825 pH meter and electrode 13-639-252 (Ag/AgCl reference).

**Reagents.** The solvents, 1,4-dioxane (Aldrich Gold Label), acetonitrile (Aldrich HPLC grade), methylene chloride, methanol, and ethanol (Fisher ACS certified grade) were used as obtained. Water was double distilled (all-glass still) over permanganate. Methylamine hydrochloride (Matheson, Coleman, and Bell), glycylglycine hydrochloride (ICN), glycine hydrochloride, and glycine ethyl ester (Sigma) were reagent grade. Allylamine hydrochloride and *n*-butylamine hydrochloride were prepared by the addition of cold, concentrated HCl to a solution of the amine

(1) (a) Anderson, G. W.; Zimmerman, J. E.; Callahan, F. M. *J. Am. Chem. Soc.* **1964**, *86*, 1839. (b) Kowalski, D.; Laskowski, M., Jr. *Biochemistry* **1976**, *15*, 1300. (c) Van Brussel, W.; Van Sumere, C. F. *Bull. Soc. Chim. Belg.* **1978**, *87*, 791.
(2) Becker, J. M.; Wilchek, M. *Biochim. Biophys. Acta* **1972**, *264*, 165.
(3) Cline, G. W.; Hanna, S. B. *J. Am. Chem. Soc.* **1987**, *109*, 3087.
(4) Stewart, J. M.; Young, J. D. *Solid Phase Peptide Synthesis*; W. H. Freeman: San Francisco, 1969; p 26.
(5) For a review, see: Gaffney, B. J. *Biochim. Biophys. Acta* **1985**, *822*, 289.
(6) Henderson, G. B.; Montague-Wilkie, B. *Biochim. Biophys. Acta* **1983**, *735*, 123.

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Table I. Aminolysis of Anisoyl-NHS Ester[a]

| amine | $pK_a$[b] | concn range | pH range | $k_1$,[c] $M^{-1}$ $s^{-1}$ |
|---|---|---|---|---|
| glycine ethyl ester | 7.75 | 0.0313–0.500 | 6.0–8.4 | 0.125 ± 0.023 |
| glycylglycine | 8.25 | 0.0178–0.453 | 7.6–9.2 | 0.191 ± 0.027 |
| glycine | 9.60 | 0.0029–0.457 | 8.6–10.3 | 5.30 ± 0.67 |
| allylamine | 9.49 | 0.0312–0.499 | 9.4–10.1 | 6.61 ± 0.35 |
| *n*-butylamine | 10.60 | 0.0156–0.250 | 10.2–10.8 | 50.9 ± 3.7 |
| methylamine | 10.63 | 0.0313–0.500 | 9.7–11.0 | 95.8 ± 9.8 |

[a] [Anisoyl-NHS] = $5 \times 10^{-5}$ M; aqueous dioxane, 20% v/v; $T$ = 25.0 ± 0.3 °C. [b] *Beilsteins Handbuch der Organischen Chemie*; Springer-Verlag: Berlin, Heidelberg, New York. [c] $k_1$ (calculated from eq 2) ± 95% confidence limit.

(Aldrich reagent grade) in ethanol. The solvent was removed by rotary evaporation, and the precipitated salts were crystallized from ethanol; the purity of the products was confirmed by titration with standard base. All amine hydrochloride salts and inorganic salts were dried in either a vacuum oven or an Abderhalden drying pistol and stored in vacuo (over Drierite) until needed. All inorganic salts were potassium salts.

Anisoyl-NHS was synthesized by the condensation of anisic (*p*-methoxybenzoic) acid and NHS with dicyclohexylcarbodiimide and crystallized from 2-propanol, mp 141–143 °C (lit.[7] mp 141–142 °C).[3]

**Kinetics.** Buffer solutions for aminolysis reactions were prepared daily by dissolving the amine hydrochloride salt in water, adding the appropriate volumes of a standardized KOH solution and an aliquot of dioxane calculated to yield a final concentration of 20% (v/v), and bringing the solution to volume with distilled, deionized water. Buffers were prepared such that the ionic strength, $\mu$, was equal to 1.0. Ionic strength was maintained at $\mu$ = 1.0, and the dioxane concentration was maintained at 20% (v/v) by serial dilutions of the buffers with a stock solution of 1.0 M $(CH_3)_4NCl$ in aqueous dioxane, 20% (v/v). The pH was maintained constant at that of the undiluted buffer by the addition of standardized KOH solution.

Phosphate (pH 7.6–8.4), borate (pH 8.9–9.6), and carbonate (pH 9.9–10.9) buffers ($\mu$ = 1.0, aqueous dioxane, 20% (v/v)) were utilized in the evaluation of hydrolysis rate constants. Buffer catalysis was evaluated by dilution of the buffer, 1:10, as described above for the dilution of amine buffers.

The anisoyl-NHS solution was prepared daily by diluting, 1:25, a stock solution of anisoyl-NHS, $2.5 \times 10^{-3}$ M in dioxane, with 1.0 M $(CH_3)_4NCl$ in aqueous dioxane, 20% (v/v). The stock solution of anisoyl-NHS was kept frozen until needed. Periodical measurements of UV absorbance ($\lambda_{max}$ = 266 nm, $\epsilon = 2.05 \times 10^4$) showed no decomposition of anisoyl-NHS during the course of this study. The presence of peroxides in the dioxane solutions was checked by an iodide test.[8] Solutions testing positive were discarded.

The kinetic experiments were performed on a Durrum–Gibson stopped-flow apparatus, as previously described,[3] and the data were evaluated by the Guggenheim method.[9] Rate constants for each amine were determined at three or more pH values, with five concentrations at each pH value. Each value of $k_{obsd}$ represents the average of four or more independent runs.

The hydrolytic rate constant was evaluated as the slope of the line in a plot of $k_{obsd}$ vs $[OH^-]$.

Rate constants for aminolysis were determined from plots of $k_{obsd} - k_{OH^-}[OH^-]$ vs $[amine]_{free}$, where $[amine]_{free}$ was calculated from eq 2.

$$[amine]_{free} = \frac{K_a[amine]_{total}}{[H^+] + K_a} \quad (2)$$

**Product Analysis.** Reaction products, obtained under the conditions of the kinetic runs, were determined qualitatively by TLC and for *n*-butylamine, quantitatively, by HPLC (>95% yield). Amides synthesized from the NHS ester and amine in aqueous buffers were compared with amides obtained by Schotten–Baumann acylation of amines.

Table II. Effect of Salt Concentration on Observed Rate Constants for Aminolysis of Anisoyl-NHS[a]

| $[(CH_3)_4NCl]$, M | $k_{obsd}$,[b] $s^{-1}$ |
|---|---|
| 0.50 | 1.74 ± 0.37 |
| 0.60 | 1.63 ± 0.29 |
| 0.70 | 1.64 ± 0.28 |
| 0.80 | 1.64 ± 0.26 |
| 0.96 | 1.62 ± 0.18 |

[a] [Anisoyl-NHS] = $5 \times 10^{-5}$ M, [methylamine] = $4.0 \times 10^{-2}$ M, aqueous dioxane (20% v/v), $T$ = 24.8 ± 0.1 °C, pH 10.5. [b] $k_{obsd}$ ± 95% confidence limit.

## Results

The hydrolysis of anisoyl-NHS is first-order in $[OH^-]$, pH 7.6–11.1, and is independent of buffer concentration; $k_{OH^-}$ = 87 $M^{-1}$ $s^{-1}$.

The aminolysis of anisoyl-NHS is first-order in $[amine]_{free}$, and $k_1$ is independent of pH. Under pseudo-first-order conditions in [amine], the aminolysis was found to be adequately described by eq 3. Rate constants for

$$k_{obsd} - k_{OH^-} \times [OH^-] = k_1[amine]_{free} \quad (3)$$

the aminolysis of anisoyl-NHS in aqueous buffers are collected in Table I.

There is a notable increase in the rate constant, $k_1$, on going from nonaqueous dioxane[3] to aqueous buffer systems (e.g., for the reaction of anisoyl-NHS with *n*-butylamine, pseudo-first-order in amine; $k_1 = 3.9 \times 10^{-2}$ $M^{-1}$ $s^{-1}$ in dioxane; $k_1$ = 50.9 $M^{-1}$ $s^{-1}$ in aqueous dioxane, 20% (v/v)).

An assessment of variations in the rate, due to changes in $[(CH_3)_4NCl]$, resulting from serial dilutions of the buffer solutions, revealed no significant salt effects upon $k_{obsd}$ (Table II).

## Discussion

The aminolysis of anisoyl-NHS, with amines ranging in basicity from $pK_a$ = 7.75 to $pK_a$ = 10.63, in aqueous dioxane solutions, shows only first-order dependence upon the concentration of free amine (eq 3) and does not reflect any contributions from specific or general-base catalysis. The correlation of amine nucleophilicity with basicity[10] can be described by the equation (coefficient of correlation 0.993)

$$\log k_1 = 1.0\ pK_a - 8.8 \quad (4)$$

A value of 1.0 for $\beta_{nuc}$ reflects a direct proportionality between the concentration of the tetrahedral intermediate and the basicity of the nucleophile, if it is recognized that $k_1'$ is virtually independent of the identity of the amine,

(7) Horner, L.; Jordan, M. *Justus Liebigs Ann. Chem.* **1978**, *No. 9*, 1518.

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(9) Guggenheim, D. A. *Philos. Mag.* **1926**, *2*, 538.

(10) The $pK_a$ values used are published values for the amines in water. Potentiometric determinations of base strength in aprotic solvents[11] have shown that relative basicity remains constant for either aqueous or nonaqueous solvents. Thus, while the intercept may vary, the slope of $pK_a$ vs $\log k/k_0$ will remain constant.

(11) Hall, H. K., Jr. *J. Phys. Chem.* **1956**, *60*, 63.

and is determined only by the leaving group. This is, in fact, the restriction imposed by Ritchie[12] in which a generalized equation (eq 5) was derived to correlate nucleophilicity with basicity for the uncatalyzed reaction of nucleophiles with esters, where the preequilibrium formation of a tetrahedral intermediate is followed by rate-determining breakdown to products. In eq 5, $k_0$ is dependent only upon the ester; $N_+$ is dependent upon the nucleophile and solvent; $k_{-x}$ corresponds to the rate constant for the reverse step of the preequilibrium, and $k_{-y}$ corresponds to $k_1'$ (eq 1). The surprisingly widespread correlation, obtained by the application of eq 5 to the aminolysis of esters, led to the conclusion that the arbitrarily imposed restriction on the independence of $k_{-x}$ and $k_{-y}$ may, in fact, be a good description of the system under investigation.

$$\log k_{obsd} = \log k_0 + N_+ - \log [1 + k_{-x}/k_{-y}] \quad (5)$$

The possibility that $k_1'$ is independent of the nucleophile is borne out in experimental work on the partitioning of the zwitterionic intermediate as a function of the $pK_a$ of amine nucleophiles and of oxyanion leaving groups, and by an evaluation of the factors governing kinetic vs thermodynamic partitioning of the intermediate, i.e., those factors that stabilize the transition states and the products.[13a] With the amine as the leaving group, resonance contributions from the alkoxy oxygen can stabilize both the transition state and the product. However, when the C–O bond is broken, no resonance contribution is available from the protonated amino nitrogen in either the transition state or the intermediate protonated product. From this, it follows that the scission of the C–O bond, in the aminolysis of esters, is determined primarily by the inherent leaving ability of the departing oxyanion. This interpretation implies that the correlation of basicity and nucleophilicity is, in actuality, a correlation between the equilibrium protonation of an amine with the equilibrium formation of the tetrahedral intermediate, and that the sensitivity to change in charge accumulation at the amino nitrogen is the same for both equilibria. Therefore, an increase in $k_{obsd}$, with an increase in the $pK_a$ of the nucleophile, is due to the effect of mass action on the rate-determining step, $k_1'$.

The absence of an observable catalytic contribution, $k_2'$ (eq 1), to the rate of aminolysis is indicative of a disproportionate increase of $k_1'$ over $k_2'$ upon going to aqueous solvent systems, where solvent-mediated proton transfer and stabilization of anionic charges can both contribute to facilitate breakdown via $k_1'$. a decisive factor in determining the relative magnitude of $k_1'$ to that of $k_2'$ is the inherent leaving ability of the amine vs oxyanion from the tetrahedral intermediate. The partitioning of the intermediate, between amide and ester, will be determined, to a large extent, by the relative $pK_a$'s of the leaving groups. It is expected that the more acidic group (lower $pK_a$) will be the better leaving group (complicated, somewhat, in the case of amine vs oxyanion leaving groups, where the amine seems to have an inherent advantage, as the leaving group, by 4–5 $pK$ units[13b]). An increase in the leaving ability of the oxyanion should make the breakdown of the intermediate, to the amide, less dependent upon catalysis and be reflected in a decrease in the ratio $k_2'/k_1'$ with a decrease of the $pK_a$. An excellent example is afforded by the aminolysis of phenyl esters, in aqueous buffers, where it is seen that general-base catalysis becomes less dominant as the leaving ability of the phenyl group is increased (Table III).[13b,14] On the basis of $pK_a$'s, NHS ($pK_a$ = 6.0)[15] should be similar to or a better leaving group than *p*-nitrophenol ($pK_a$ = 7.4) and the loss of an observable catalytic term, upon changing the medium from anhydrous dioxane to an aqueous dioxane system, is expected for our NHS ester or for any other ester with a leaving group having a $pK_a$ smaller than the $pK_a$ for *p*-nitrophenol.

If we examine the leaving ability of the oxyanion, with regard to its $pK_a$ (constant nucleophile), a decrease in $pK_a$ is reflected in an increase in the rate for alkyl,[13b] phenyl,[13b,c,14,16] and *N*-hydroxy[17] esters (Figures 1 and 2).

**Table III. Aminolysis of Substituted Phenyl Acetates in Aqueous Buffers**

| amine | substituent | $pK_{a(lg)}$ | $k_2/k_1$[a] |
|---|---|---|---|
| $NH_2NH_2$[b] | *p*-$OCH_3$ | 10.2 | 57.5 |
| | *p*-$CH_3$ | 10.2 | 34.7 |
| | H | 9.95 | 29.1 |
| | *p*-Cl | 9.38 | 17.8 |
| | *m*-$NO_2$ | 8.35 | 2.6 |
| | *p*-$NO_2$ | 7.14 | not obsd |
| | 2,4-$(NO_2)_2$ | 4.02 | not obsd |
| $NH_3$[c] | *p*-$CH_3$ | 10.2 | 3.9 |
| | H | 9.95 | 2.9 |
| | *p*-Cl | 9.38 | 0.78 |
| | *m*-$NO_2$ | 8.35 | not obsd |
| | *p*-$NO_2$ | 7.14 | not obsd |

[a] $k_2$ is the rate constant for general-base-catalyzed aminolysis; $k_1$ is the rate constant for noncatalyzed aminolysis. [b] Reference 13b. [c] Reference 14.

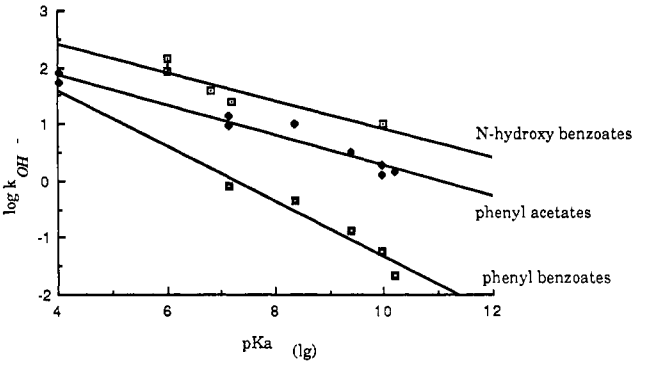


**Figure 1.** Rates of alkaline hydrolysis of esters as a function of leaving group. Kinetic data are taken from ref 17, 3, and this work for *N*-hydroxy benzoates, ref 13b and 13c for phenyl acetates, and ref 16 for phenyl benzoates.

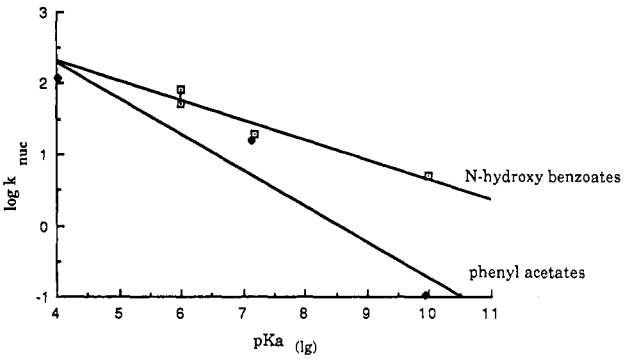


**Figure 2.** Rates of aminolysis with ethylamine (butylamine for *N*-hydroxysuccinimide ester) as a function of leaving group. Kinetic data are taken from ref 17, 3, and this work for *N*-hydroxy benzoates and ref 13c for phenyl acetates.

(12) Ritchie, C. D. *J. Am. Chem. Soc.* **1975**, *97*, 1170.
(13) (a) Gresser, M. J.; Jencks, W. P. *J. Am. Chem. Soc.* **1977**, *99*, 6963. (b) Satterthwait, A. C.; Jencks, W. P. *J. Am. Chem. Soc.* **1974**, *96*, 7018. (c) Jencks, W. P.; Gilchrist, M. *J. Am. Chem. Soc.* **1968**, *90*, 2622.
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(16) Kirsch, J. F.; Clewell, W.; Simon, A. *J. Org. Chem.* **1968**, *33*, 127.

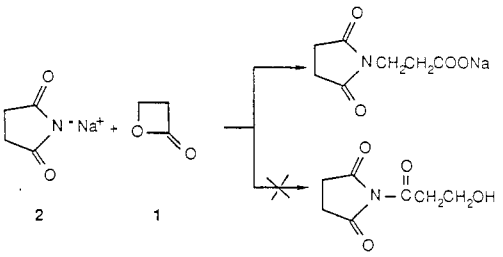
Attempts to derive mechanistic details, by the derivation of $\beta_{lg}$ for *N*-hydroxy compounds, are hampered by the limited data available for correlation purposes. An appropriate correlation requires that the acyl group, nucleophile, and solvent systems be identical. However, a qualitative assessment can be obtained from the work of McCarthy and Hegarty[17] for which the rates of aminolysis and hydrolysis of benzoate esters of *N*-hydroxy compounds were determined in aqueous dioxane (25% v/v) buffer solutions. For comparison purposes, the kinetic constants of *p*-methoxybenzoyl-NHS have been extrapolated to that expected for benzoyl-NHS by the use of the value of $\rho$ obtained for aminolysis in anhydrous dioxane ($\rho \sim 1$).[3] This can be considered to be the limiting value of $\rho$[18] and is indicated in Figures 1 and 2 with a bar encompassing the limits of $k_{nuc}$ ($\rho = 0 \rightarrow 1$). For the sake of simplicity and to avoid placing undue significance (due to the limited data sets) on any apparent curvature of the plots, the rate constants for each group of esters have been fit by a simple linear-least-squares analysis.

An inspection of the rates of hydrolysis, as a function of the $pK_a$ of the leaving group (Figure 1), reveals that the rate constants for the *N*-hydroxy benzoate esters are about 2 orders of magnitude greater than those of phenyl benzoates with leaving groups of comparable basicity. In relation to phenyl acetates, the rate differential is decreased to a factor of ~5 and is commonly attributed to steric factors.[16,17] However, a rate differential, comparable to that seen with phenyl benzoates, is expected to apply for the acetate esters of *N*-hydroxy compounds in comparison to the acetate esters of phenols.

Although no suitable data are available for a direct comparison of the rate constants for the aminolysis of benzoate esters, an inspection of *N*-hydroxy benzoates and phenyl acetates (Figure 2) is helpful in assessing relative reactivities. For the *N*-hydroxy benzoates and phenyl acetates, the same rate differential appears to hold for aminolysis with ethylamine (for NHS esters, the kinetic constant for butylamine was used) as that seen for hydrolysis. It is probable that this would also be true for *N*-hydroxy benzoates and phenyl benzoates (i.e., for leaving groups of comparable $pK_a$, $k_1$ for *N*-hydroxy benzoate is $\sim 100 \times k_1$ for phenyl benzoates). In general, for equivalent acyl groups and leaving groups of comparable basicity, acyl transfer from *N*-hydroxy esters is about 2 orders of magnitude faster than that from phenyl esters.

The unusually high reactivity of NHS esters, compared to *p*-nitrophenyl esters, may be explained in terms of the HSAB principle.[19] The aminolysis of NHS esters involves the interaction of a hard Lewis acid, the acyl carbon, with a hard Lewis base, $R_2NH$. For hard–hard interactions, the basicity of the attacking amine will be a decisive factor in determining the rate of the reaction if the acyl carbon behaves as a proton (i.e., an increase in positive charge at the electrophilic center causes an increase in the rate of the reaction). On the basis of HSAB principles, it is expected that the rate of acyl transfer from NHS esters to amines will increase both with the basicity of the amine and with a decrease in electron density at the acyl carbon.

An additional factor in determining the partitioning of products between amide and ester is the relative hardness of the amino N of the amine vs that of the alkoxy O of NHS, as highly reactive or labile species are oftentimes due to the union of a hard base with a soft acid. By an analysis of bimolecular reactions, Saville has developed rules for determining optimum conditions of multicenter organic reactions.[20] For displacement reactions, optimum conditions are achieved when the hard–soft dissymmetry of the labile bond of the substrate is maximized. Even though both $R_2NH$ and $RO^-$ are hard bases, the hardness of the reacting center can be modified by "symbiotic" interactions of neighboring groups (e.g., the presence of soft ligands on an acid center will tend to make it softer).[21] In the case of NHS, the succinimidyl anion is a soft base, as evidenced by (1) opening of the $\beta$-propiolactone ring, **1**, by nucleophilic attack of the succinimidyl anion, **2**, at the $\beta$-carbon,[22] as opposed to ring opening at the harder acyl carbon (eq 6), and (2) alkylation of enamides at the amino N by the

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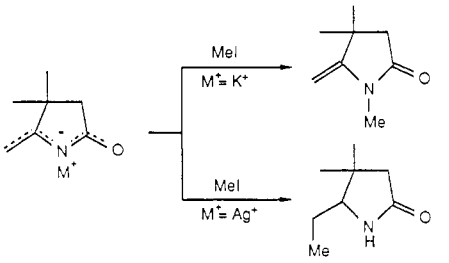
soft electrophile $CH_3I$ when the counterion is hard (e.g., $K^+$), but at the vinylidene carbon when the counterion is soft (eq 7), due to the close association of the soft amino

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N with the soft counterion, $Ag^+$, in the latter instance.[21] Thus the proximity of succinimidyl anion to the hard alkoxy O will soften its nucleophilic character, and the displacement of NHS by amines will be a facile reaction due to the replacement of a hard–soft interaction by a hard–hard Lewis acid–base interaction.

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(23) Reference 19, p 41.