JOC Note

pK_a-Dependent Formation of Amides in Water from an Acyl Phosphate Monoester and Amines

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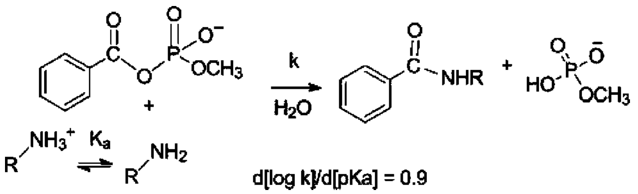

Acyl phosphate monoesters are readily prepared biomimetically activated anionic derivatives of carboxylic acids that react rapidly with amines in water to form amides. A plot of the logarithms of the rate constants for the reactions of a series of primary amines with benzoyl methyl phosphate depends on the pK_a of the conjugate acids of the amines ($\beta_{nuc} \approx 0.9$). This provides a simple and quantitative basis for regioselective acylation with these reagents.

Acyl phosphate monoesters are readily prepared biomimetically activated derivatives of carboxylic acids that can be used to produce amides from amines in water.[1–3] Their anionic character, due to the phosphate monoester leaving group, causes them to be electrostatically selective anionic electrophiles.[3–5] On the basis of these properties, we had developed acyl phosphate monoesters as charge-directed reagents for modifying and cross-linking proteins at cationic sites.[5,6] Recently, Kozarich and co-workers showed a dramatic extension of functionalized acyl phosphate and pyrophosphate[7] monoesters derived from adenosine in proteomics: derivatives of biotin and AMP (as well as ADP) specifically acylate lysines in ATP-binding sites of kinases, providing a novel approach for determining the site-specificity of drugs.[8] We have now addressed the specificity of acyl phosphate esters with respect to reactions with amines of varying basicity.

TABLE 1. Second-Order Rate Constants for the Formation of Substituted Benzamides from Benzoyl Methyl Phosphate and Primary Amines

amine	pK_a[a]	k_{nuc}, M^{-1} s^{-1} (25 °C)
2,2,2-trifluoroethylamine	5.40	2.5×10^{-5}
2,2-difluoroethylamine	7.45	1.7×10^{-3}
2-fluoroethylamine	9.19	5.0×10^{-2}
2-methoxyethylamine	9.68	2.0×10^{-1}
n-propylamine	10.58	1.1
n-butylamine	10.61	1.2
6-amino-caproic acid	10.75	2.7

[a] pK_a values are for the conjugate acids of the amines. Values for the first five entries are from: Kluger, R.; Hunt, J. C. *J. Am. Chem. Soc.* **1984**; *106*, 5667−5670. Entries 6 and 7 were determined by titration as described in the cited reference.

The first systematic observations of the aminolysis reactions of acyl phosphate monoesters were reported by Di Sabato and Jencks. They elucidated the contrasting reactivity patterns of acetyl phosphate and acetyl phenyl phosphate.[1,9] The latter material, an acyl phosphate monoester, was prepared in situ from acetic anhydride and dilithium phenyl phosphate,[10] a method that does not provide material suitable for use as a reagent. These workers observed that reactions of acetyl phenyl phosphate with amines produce substituted acetamides with little competition from hydrolysis and that the rate of the reaction correlates with the basicity of the amine reactant.

With a reliable method of producing acyl phosphate monoesters in sufficient quantities and purity to be suitable as reagents, we have determined their reactivity with respect to a set of related amines. We find that, as expected, there is a direct correlation between the pK_a of the conjugate acid of the amine and the rate constant for amide formation. By logical extension, this can serve as a basis for regioselectivity of this class of reagents with multifunctional substrates.

Second-order rate constants, derived from plots of observed first-order rate coefficients vs amine concentrations for aminolysis, are presented in Table 1. The values for k_{obs} were derived from the slope of plots of observed first-order rate coefficients (measured under pseudo-first-order conditions) vs amine concentration

A plot of the logarithms of the derived second-order rate coefficients as a function of the pK_a of the conjugate acid of the amine nucleophiles is linear with a slope (β_{nuc}) = 0.91 ± 0.02 (Figure 1). This slope is within the range of values (0.8−1.0) established for carboxyl substitution reactions where the rate-determining step is decomposition of the tetrahedral addition intermediate[11] and similar to the early value reported for the aminolysis of acetyl phenyl phosphate (0.83).[9] In addition to the rate coefficient and concentrations of the reactants, the relative amount of free vs protonated amine determines the overall rate of amide formation.

We also analyzed the dependence of the rate of the competing hydrolysis reaction of benzoyl methyl phosphate on the pH of

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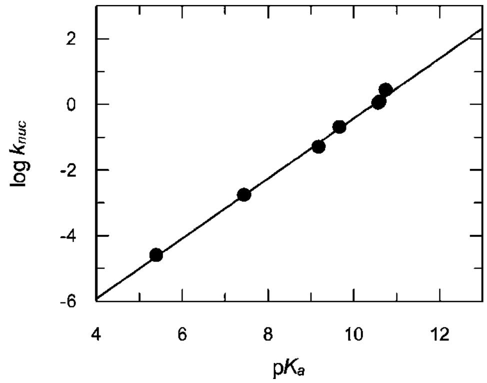
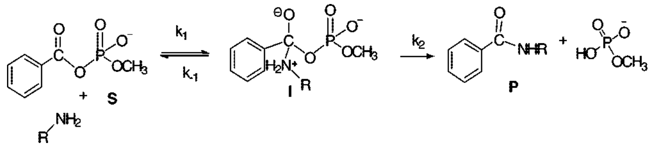


FIGURE 1. Brønsted-type plot of the data in Table 1 for the reaction of benzoyl methyl phosphate with primary amines. The term "pK_a" refers to that of the conjugate acid of the amine.

SCHEME 1



the solution. The competing hydrolysis is independent of pH from pH 3 to 6 and is base-catalyzed above pH 6. At 58 °C, the observed first-order rate coefficient for the water-catalyzed reaction, k_w, is only 6.5×10^{-7} s^{-1} and the second-order rate constant for the hydroxide-catalyzed process, k_{OH}, is 2.4 M^{-1} s^{-1}. Therefore, hydrolysis is not a significant competitor under normal conditions.

The mechanism of aminolysis of benzoyl methyl phosphate that we show in Scheme 1 follows the established pattern of carboxyl substitution reactions,[11] where k_2 represents the rate constant for the decomposition of the intermediate toward the amide product.

The rate law associated with the mechanism in Scheme 1, with I as a steady state intermediate, is given by eq 1:

$$-d[S]/dt = (k_1[RNH_2]k_2/(k_{-1} + k_2))[S] = k_{nuc}[S][RNH_2] \tag{1}$$

The relationship of "k_{nuc}" to the rate law in eq 1 is:

$$k_{nuc} = k_1k_2/(k_{-1} + k_2) \tag{2}$$

On the basis of the observed dependence of rate on amine basicity, the most basic amino group will react fastest in a competitive situation as long as the pH of the reaction solution is above the pK_a of that of the amine.

These results show that acyl phosphate monoesters are a class of useful reagents for producing amides from amines in water and that their reactivity toward amines of differing basicity permits reaction distinctions within molecules, where amino groups of differing basicity coexist. In addition, the choice of acyl group substituents and phosphate ester group permit reagents to be developed based on their specific affinity for enzyme binding sites and properties that can be used for separation based on recognition.[8]

Experimental Section

Commercial reagents were utilized without further purification. Solvents were dried prior to use. Benzoyl methyl phosphate was prepared according to the published procedure.[12]

All amines except 2,2-difluoroethylamine were commercially available and were purified prior to use. 2,2-Difluoroethylamine was synthesized by diborane reduction of the 2,2-difluoroacetamide.[13] Benzamide and N-substituted benzamides resulting from the aminolysis reaction were isolated and characterized in preparative scale experiments prior to kinetic analysis.

Ionic strength was maintained at 1.0 by addition of potassium chloride. Reactions were initiated by injection of 10−20 μL of a freshly prepared aqueous solution of benzoyl methyl phosphate (nominally 0.01 M). The concentration of benzoyl methyl phosphate in the reaction solution was about 5×10^{-5} M. Each solution was mixed by inverting the cell three times. The cells were placed in the temperature-controlled jacketed cell holder in the sample compartment of the spectrometer. Data were collected with an interfaced computer for four to five half-times. Rate constants were calculated fitting the experimental data to the integrated first-order rate expression, using a least-squares algorithm. All determinations were conducted at 25 °C. Five concentrations of the amine in 0.1 M pH 9 sodium carbonate buffer were used to determine the dependence of rate on amine concentration. Plots of the observed first-order rate coefficients vs concentrations of the free amines (calculated with the Henderson−Hasselbalch equation) gave the second-order rate constants with correlation coefficients of 0.998 or better.

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