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Highly Selective Acylations using a Biomimetic Strategy#

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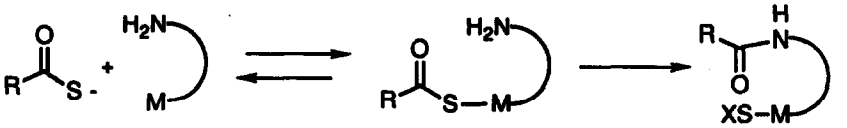
Key Words: selective; acylation; thioacid; silver; peptide.

Abstract: *Rapid, high yield acylation of thiol-bearing amines by one equivalent of thioacid may be carried out at 1 mM in the presence of large excesses of other primary amines. Silver ion, known to activate thioacids toward nucleophilic attack, simultaneously also serves as a binding site to deliver a thiol-tethered amine. An interesting dependence of yields and selectivity on [Ag+] was found, as well as a significant effect of chloride.*

Chemical selectivity and rate accelerations are the hallmarks of enzymatic catalysis.[1] Enzymatic rate accelerations result both from proximity of groups "effective molarities",[2] and the chemical catalysis by pendant acid, base, or other prosthetic groups. We wish to report an extremely simple model which mediates a bimolecular condensation using both of these enzyme-like features.

Our goal is to demonstrate simultaneous binding and activation of a substrate to facilitate a bimolecular reaction. We have chosen to facilitate the reaction between thiocarboxylic acids and amines because successful development of a highly selective and efficient process would be very useful for peptide fragment condensations. Many recent approaches to the problem of peptide fragment coupling, still generally unsolved, attest to the desirability of such a process. The main difficulty is the low concentration of reactive groups that must rapidly couple with one another, without side reactions characteristic of highly reactive acylating agents. Kemp's elegant solution[3] involves rapid trapping by disulfide exchange, followed by *intramolecular* acyl transfer of a moderately active ester. In certain cases, proteolytic enzymes[4,5,6] may be induced to mediate such couplings. Other approaches focus on ready preparation of fragments and fine tuning of standard approaches to their coupling[7]. A particularly useful attribute would be the ability to selectively couple without intereference by unprotected amine or acid groups.

We propose a general scheme in which a thiocarboxylate ion ligates a metal to which an amine is tethered. Ligation chemically activates the thiocarboxylate toward nucleophilic attack *only* when it is in the presence of the amine tethered to the metal complex. Thus, binding and activation are the same process. Our initial efforts[8] to realize this scheme using aryl mercury templates suggested the scheme was plausible, but instability and insolubility of the aryl mercury template led us to investigate other metals.

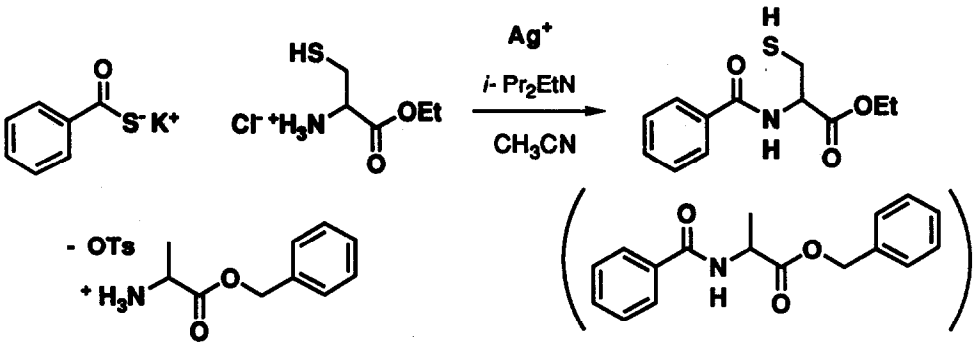


Scheme 1. General scheme for metal-activated intracomplex acyl transfer.

#This paper is dedicated to Professor Ronald Breslow on the occasion of his 60th birthday

Silver ion has been shown to activate thiocarboxylate ions, themselves poor electrophiles, toward attack by amines to form amides. C-terminal peptidal thiocarboxylates can acylate peptidal amines.[9] Increases in efficiency and selectivity would be particularly useful. We reasoned that an amine bearing a thiol substituent might be anchored to the activating silver, and thus be intramolecularly delivered to the reactive site. In the case of an N-terminal cysteine peptide, this would constitute a useful procedure,[3] and if sufficiently selective for the aminothiol, would allow coupling of peptides bearing unprotected amino substituents.

We studied the reaction of potassium thiobenzoate[10] with cysteine ethyl ester to produce N-benzoyl cysteine ethyl ester. Alanine benzyl ester, used as a competing nucleophile to determine the selectivity for cysteine, was present in a ten-fold excess over cysteine. Thiobenzoate efficiently acylates the cysteine even in the presence of large excesses of alanine.



Scheme 2. Benzoylation of cysteine ethyl ester is carried out in the presence of a 10-fold excess of alanine benzyl ester. Under certain conditions, very little of the benzoylated alanine is formed as a side product.

Standard Coupling Procedure: L- cysteine ethyl ester hydrochloride[11] (11.1 mg, 0.060 mmol) and alanine benzyl ester tosylate (21.1 mg, 0.600 mmol) were dissolved in freshly distilled acetonitrile (58 mL). Silver nitrate (0.880 mL of 191 mM in acetonitrile, 0.168 mmol) was added, followed by diisopropylethylamine (DIEA, 150 microliters, 0.864 mmol) After a necessary 15 minute stirred incubation, 0.054 mol of potassium thiobenzoate was added as a 0.0555 M solution in CH_3CN. After stirring under nitrogen for 135 minutes, the reaction was quenched with 20 mL of pH 7 0.1M mercaptoacetate containing 0.3 M glycine and 0.1 M phosphate. After stirring 30 minutes, the volume was reduced to 20 mL by rotary evaporation, and filtered. The filtrate and solids were extracted 3x with ethyl acetate. The combined extracts were washed 2x with mercaptoacetate solution, 3x with 0.1 M sodium bisulfate, 2x with 0.025 M pH 7 phosphate buffer, then 1x with 50% saturated sodium chloride solution. The ethyl acetate solution was dried over sodium sulfate, filtered and the solvent removed by rotary evaporation. After drying under vacuum, 1,3,5- trimethoxybenzene was added as an integration standard for ^1H NMR analysis of the product mixture.

As expected for a stoichiometric reaction, the yield of benzoylated product increases with increasing silver ion concentration. The selectivity for benzoylation of cysteine ethyl ester in competition with alanine benzyl ester is also dependent on silver ion concentration. As shown by the open symbols in Figure 1, in the absence of chloride,[11] increasing [Ag^+] causes increasing acylation yield, but decreasing selectivity for cysteine. A maximum benzoylated cysteine yield occurs at a silver ion concentration of 1.8 mM, which corresponds to 1.8 equivalents as [CysOEt] = 1.0 mM. The yield is low (21% based on thiobenzoate) and the selectivity is only 40 to 1. Surprisingly, though the selectivity ratio is better at lower [Ag^+], and after the coupling the silver is probably inaccesible as AgS, syringe pump addition of the silver ion does not improve the ratio.

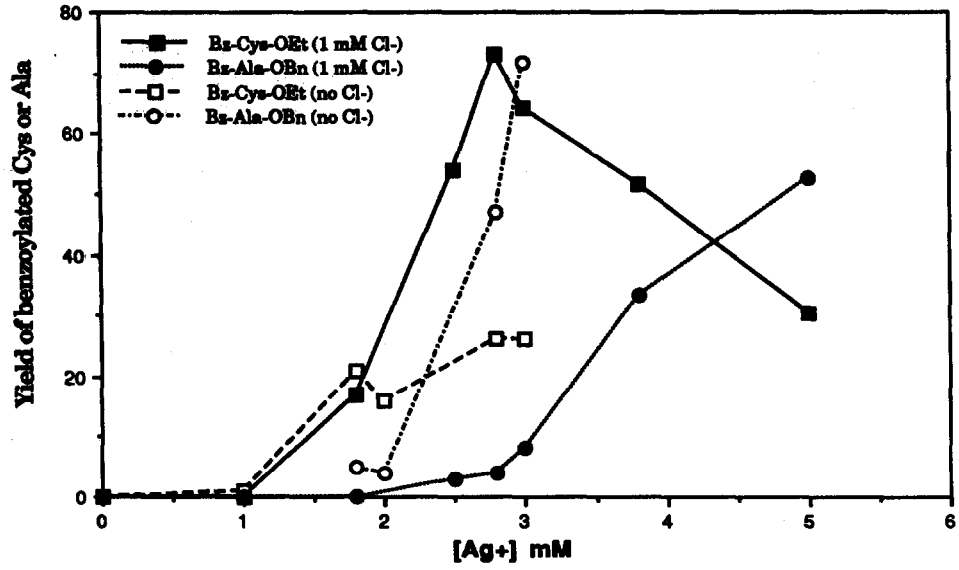


Figure 1. Plot of benzoylated cysteine or alanine product yields vs. [Ag^+]. Reactions were run 135 min. in CH_3CN with a 1: 10: 0.9 Cys: Ala: Thiobenzoate ratio and 14.4 mM DIEA . [Cys] = 1.0 mM .

The yield and selectivity of the acylation are improved dramatically by the presence of 1 mM chloride ion, as indicated by the solid symbols in Figure 1. Under optimum conditions, the desired product, N-benzoyl cysteine ethyl ester, was isolated in 73% yield. This represents a 190-fold selectivity for cysteine over alanine, though the relative rates of their reactions must be higher than this as the cysteine is largely consumed during the reaction. The extraction yield of authentic N-benzoyl cysteine ethyl ester from a control reaction mixture was sensitive to the concentration of silver ion, and at 2.8 mM was only 65%. Therefore, the yield of product is probably higher than 73%.

Table 1. Acylation of Various Nucleophiles by Thiobenzoate / Ag+

	% Yield	
Nucleophile	Benzoylated Nucleophile	Benzoylated AlaOBn
CysteineOEt.HCl	40.0	<0.3
S-Me-Cysteine.HCl	<0.3	<0.3
SerineOEt.HCl	<0.3	5.0
Ethyl 2-Mercaptoacetate	0.4	<0.3

Reactions were run 135 min. in CH_3CN with 4.8 mM DIEA. Cys: Ala: Thiobenzoate: Ag+ ratio is 1: 1: 0.9: 2.8; with [Cys] = 1.0 mM.

Having observed the proposed selectivity, we needed to verify its origin. We did this by studying related competitive acylations as shown in Table 1. The pKa of cysteine is lower than that of alanine; under acidic conditions cysteine might be expected to be more readily acylated. While our conditions are not acidic,[12] we nonetheless studied the acylation of S-methyl cysteine ethyl ester, which lacks the thiol, but retains the pKa of

cysteine. This compound was much less efficiently acylated than was cysteine ester, and after prolonged reaction times, was seen to react less rapidly even than alanine. This demonstrates that the pKa of the amine nucleophile is not the crucial variable. Initial acylation at the thiol, followed by S-N transfer[13] was also ruled out as the reason for the thiol requirement by noting that a simple thiol is not significantly acylated under our conditions, and that the side chain alcohol of serine is not effective for rapid acylation.

We have successfully incorporated both enzymatic strategies: activation by proximity, and by chemical reactivity enhancement, in a very simple scheme. Binding and activation events are one and the same as activation of the acyl takes place only in the presence of thiol-bound amine.

Our reaction constitutes a method for the activation of a specific amine for acylation, in contrast to the virtually universal strategy of protecting other nucleophilic groups.

The reaction works rapidly, in high yield at low concentration, exactly the attributes needed for an effective peptide fragment condensation. Several other requirements must be met,[3] however, before this reaction may be considered a solution to the long-standing problem of fragment coupling.

While it appears that intracomplex reaction within a silver thiolate/chloride cluster[14] takes place, we do not yet know the composition of the active cluster, or whether other ligands may substitute for chloride, or other metals for silver. Experiments to probe these questions are planned.

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(11) The cysteine ethyl ester was added as the trifluoroacetate salt for the chloride-free experiments. Trifluoroacetate has no significant effect on product yields.
(12) The reason we use such a basic buffer as DIEA is that we have observed a very slow ($t_{1/2}$ ~ 1 day) acylation in the absence of silver ion. This buffer-catalyzed process causes quite selective acylation of cysteine over alanine in the presence of pyridine or N-methyl morpholine trifluoroacetate buffers. We believe that this takes place by S acylation, followed by S-N transfer. Diisopropylethylamine removes the cysteine rate preference, and slows down the acylation, so we are certain that the rapid silver-mediated acylations are mechanistically distinct.
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