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Chemoselective Peptidomimetic Ligation Using Thioacid Peptides and Aziridine Templates

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Abstract: Chemoselective peptidomimetic ligation has been made possible using thioacid peptides and NH aziridine-terminated amino acids and peptides. In the course of this reaction, a reduced amide bond is incorporated into the backbone of a peptide. This process enables incorporation of reduced cysteine, reduced substituted cysteine, reduced phenylalanine, and reduced alanine. Our method should be adaptable to other unnatural amino acid residues at the ligation site. Experiments aimed at evaluating the chemoselectivity of this process in the presence of competing thiol nucleophiles suggest high specificity at micromolar concentrations. This holds even in the presence of glutathione, which neutralizes xenobiotic electrophiles in cells.

Structural modification of peptide backbones has been widely used in the study of biological processes.[1,2] Reduced peptide bonds are present in a number of potent protease inhibitors. These linkages display interesting conformational preferences, inducing folds that are similar to γ- and β-turns. The secondary amine of the reduced amide bond is protonated at physiological pH, which enables formation of a hydrogen bond between the protonated nitrogen center and carbonyl oxygen, similar to $(i + 2)$ or $(i + 3)$ interactions in peptides.[3] Chemoselective strategies for linking two peptide fragments by a peptide-bond mimetic hold significant potential for discovery of novel peptide and protein scaffolds. Our contribution describes efforts to develop peptidomimetic ligation during which a reduced amide bond is incorporated into the backbone of a peptide. This process enables incorporation of reduced cysteine, reduced substituted cysteine, reduced phenylalanine, and reduced alanine and relies on unprotected aziridine aldehydes.[4] Side-chain variation at the ligation site is a function of substituents present in the aziridine aldehyde reagent. Experiments aimed at evaluating the chemoselectivity of this reaction in the presence of competing thiol nucleophiles suggest high specificity. This holds even in the presence of glutathione, which neutralizes xenobiotic electrophiles in cells.

In order to demonstrate peptidomimetic ligation, we employed the recently developed unprotected aziridine aldehydes.[4] The peptide aziridines **1**−**8** (Figure 1) were prepared via reductive amination between aziridine aldehyde dimers and peptides. We tested the feasibility of peptidomimetic ligation between templates **1**−**8** and thioacid-terminated peptides prepared using sodium sulfide and carbonyldiimidazole (CDI) (Figure 2).[5] Mechanistically, the aziridine ring opening was projected to trigger S-to-N acyl group transfer. We found that thioamino acids and the corresponding COSH-containing peptides underwent clean conjugation with templates **1**−**8**, delivering a single addition/rearrangement product in every attempted reaction (Scheme 1 and Table 1). Reduced cysteine and its substituted derivatives are readily accessible using

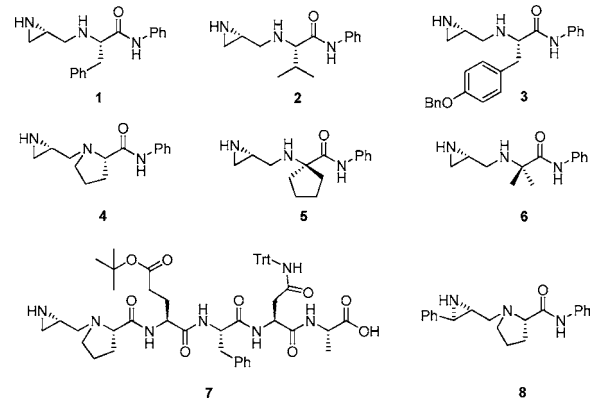

Figure 1. Strained peptide diamines used in this study.

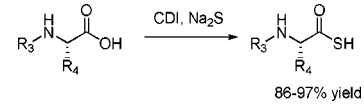

Figure 2. Preparation of thioacid starting materials.

this method.[6] Subsequent treatment with Raney Ni produces the corresponding desulfurized residues, opening the possibility of introducing a desired side chain next to the reduced amide bond by choosing an appropriate aziridine aldehyde reagent.[7]

Several control elements are responsible for the observed chemoselectivity. One of the factors is the attenuated reactivity of the thioamino acids, which contrasts with the double acylation product observed with the more reactive thiobenzoic acid.[8] We were particularly pleased to note that the reactions with thioacids remain effective at low molarity. A thousandfold dilution did not diminish the efficiency of the process.[9] Mechanistically, the thioester generated upon aziridine ring opening is trapped by the primary amine. The acyl transfer proceeds via a five-membered cyclic intermediate.[10] A balance of relative rates results in the peptidomimetic rather than peptidic backbone (Scheme 1).[11] We did not observe competition from the aza-Payne rearrangement.[12] The reaction was also found to proceed without epimerization. The aziridine ring opening was regioselective. We posit that this type of regioselectivity is unique to the unsubstituted NH aziridine-containing templates that do not have electron-withdrawing substituents at the carbon adjacent to the three-membered ring.[13] The ring opening was also regioselective for the phenyl-substituted aziridine ring (see the Supporting Information). We were also happy to note that unprotected acid-terminated peptides readily participate in this chemistry. We ran experiments aimed at further evaluating the chemoselectivity of our protocol. Because of the ubiquity of thiol nucleophiles in biological systems, it was important to perform

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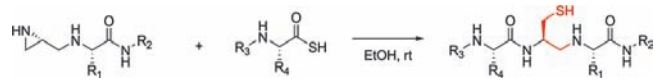
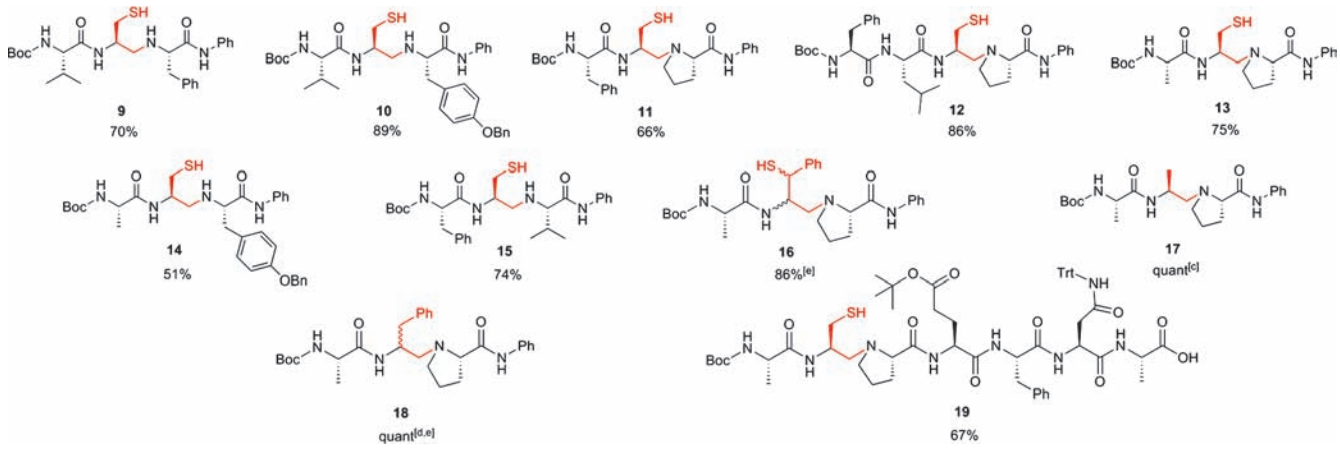
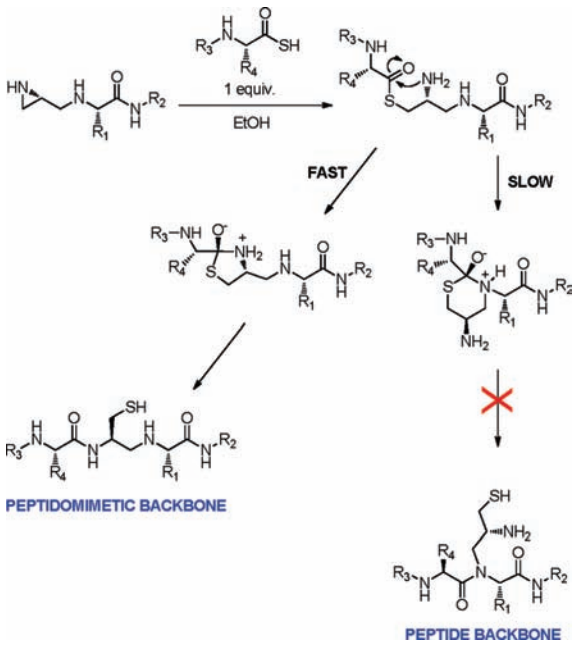
Table 1. Scope of Peptidomimetic Ligation[a]





[a] The peptide diamine concentration was 0.1 M in ethanol, and 1 equiv of thioamino acid was used in each case. [b] Isolated yields. [c] Desulfurization reaction run with Raney Ni on entry **13**. [d] Desulfurization reaction run with Raney Ni on entry **16**. [e] 1:1 mixture of diastereoisomers.

Scheme 1. Mechanism of Peptidomimetic Ligation



control experiments with glutathione present in the reaction mixture. Pleasingly, the ligation remained thioacid-specific even under these conditions.[14]

In summary, the first example of chemoselective peptidomimetic ligation has been made possible using thioamino acids and NH aziridine-terminated amino acids and peptides. This method enables incorporation of a peptidomimetic linkage at the site of ligation. The technique is not accompanied by epimerization and appears to perform chemoselectively in the presence of competing thiol nucleophiles known to react with aziridines. In view of the accessibility of aziridine aldehydes, it should be possible to generate a wide range of peptidomimetics and backbone-modified proteins under mild conditions. With the recently developed solid-phase protocols for long-chain thioacid synthesis,[15] it should be possible to extend this methodology to the solid phase. Equally attractive is the possibility of interfacing this reaction with the subtiligase-catalyzed thioacid synthesis.[16] We also note that the sequence of bonds that are installed as a result of our method matches the repeating structure present in protein nucleic acids (PNAs), which should facilitate construction of substituted PNAs under mild conditions.

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Supporting Information Available: Complete experimental details and characterization data for new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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