An Observation of a Series of *S*→*N*→*N* Acyl Transfers in 3-Mercaptoproline

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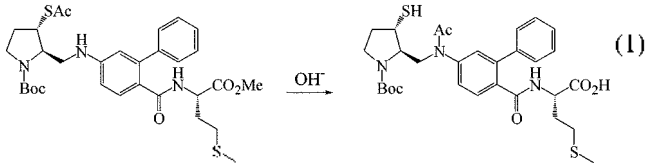
Since small peptides do not tend to exist as a single conformation in solution and possess a large amount of flexibility, determination of the preferred peptide structure should be approached by chemical modifications designed to decrease conformational flexibility. The flexibility of a peptide can be limited by utilization of local constraints and cyclization of peptide strand. Also, the incorporation of conformationally constrained amino acids into peptides has been extensively used in the design of conformationally restricted peptides. In order to retain biological activity of parent peptides, the constrained analogs should accommodate the peptide backbone conformation and frequently sustain the crucial functionalities.[1]

As a part of our program for targeting zinc-containing enzyme, we designed a series of cysteine-incorporated small peptidomimetic analogs. It is well known that cysteine is the most frequent residue in the catalytic zinc sites of metalloenzymes.[2] In general, zinc ion is likely to coordinate with the cysteine sulfur on the protein/peptide substrate and participate in catalytic and structural sites.[3] This strongly suggested that the cysteine thiol group is one of key elements for enzyme activity.

The incorporation of conformationally constrained amino acids into peptides is a powerful approach for generating structurally defined peptides as conformational probes and bioactive agents. Particularly, we were interested in prolines as conformationally constrained cysteine residues. During the synthesis of 3-mercaptoprolines, we found a serious of acyl transfers. Here, we wish to report the observation of a series of *S*→*N*→*N* acyl transfer in the deprotection of the *S*-acetate in 3-mercaptoproline systems.

Results and Discussion

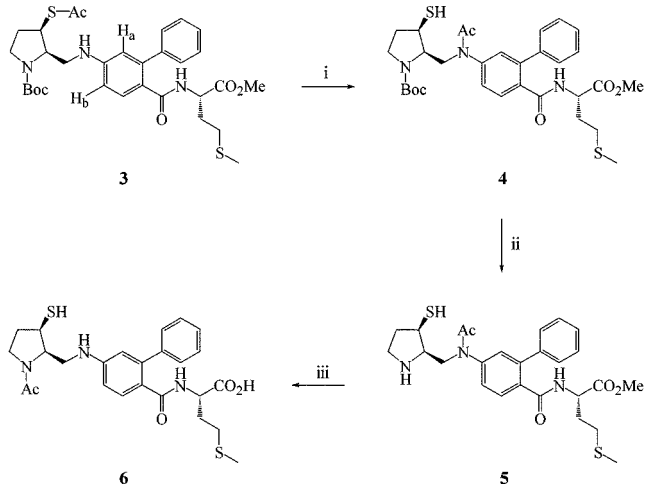
Synthesis of *cis*-3-TBSO-L-prolinal is well-described in the literature.[4] Thus, the thioacetate **1** was prepared from the reductive amination of prolinal with 2-phenyl-4-aminobenzoyl-Met-OMe[5] ($NaBH_3CN$, 73%), followed by the deprotection of TBS group (TBAF, 77%) and the inversion of the resulting hydroxyl group to the thioacetate (AcSH, TPP, DIAD, 87%). A problem was encountered in the final deprotection of *S*-acetate in **1** under 1 *N* LiOH in MeOH. The reaction mainly gave the *S*→*N* transacetylation into aniline amine along with the methyl ester hydrolysis (**2** in eq. 1). This was not surprising in retrospect, because base-catalyzed acyl transfer may be facilitated through a cyclic intermediate. The phenomenon of *S*→*N*, *S*→*O*, *N*→*O*, and *O*→*N* acyl migration is known in a few cases and several detailed investigations have clarified the course of the reactions with amino acid, peptide, and pyrrolidine system.[6] In turn, it was very difficult to deprotect the *N*-acetate in **2**, even though some efforts were made.



(1)

1 **2**

In order to block the facile transacetylation, it seemed that protection of the aniline amine is necessary. This amine resisted protection with $(Boc)_2O$, TMS-Cl, MOM-Br using standard conditions, respectively. Presumably, the secondary amine is embedded in a bulky hydrophobic environment. Next, we attempted the deprotection of the *S*-acetate under acidic conditions. Although boron tribromide was reported to facilitate the cleavage of amino acid protective groups such as *N*-Boc, ester, and ether groups,[7] in our case, it only deprotected Boc at -78 °C, and Boc and methyl ester at room

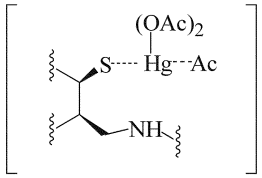


3 **4** **6** **5**

Scheme 1. A series of *S*→*N*→*N* Acyl transfers. *Reagents and Conditions*: i, $Hg(OAc)_2$, MeOH, rt, 2 hr; H_2S, 2 hr, 64%; ii, TFA, Et_3SiH, CH_2Cl_2, 1 hr; Prep-HPLC, 78%; iii, 1*N* LiOH, MeOH, 3 hr; Prep-HPLC, 34%.

temperature leaving the *S*-acetate untouched. Notably, we did not observed a facile *S*→*N* acyl transfer in the acidic conditions.

The failure of the removal of *S*-acetate led us to try heavy metal salts. The similarly prepared **3** was allowed to react with mercuric acetate in MeOH at room temperature, followed by treatment with hydrogen disulfide. Surprisingly, this reaction yielded the *S*→*N* transacetylated acetanilide **4** (Scheme 1), as determined by ^{1}H NMR. The reason is not clear. But we guess the *S*→*N* acyl transfer may be facilitated through a mercury-sulfide complex, such as **7**, within a proper orientation. The next question was another possibility of acyl migration from aniline to proline nitrogen in basic conditions. After Boc group deprotection (50% TFA, CH_2Cl_2, Et_3SiH), further treatment with 1 *N* LiOH of **5**



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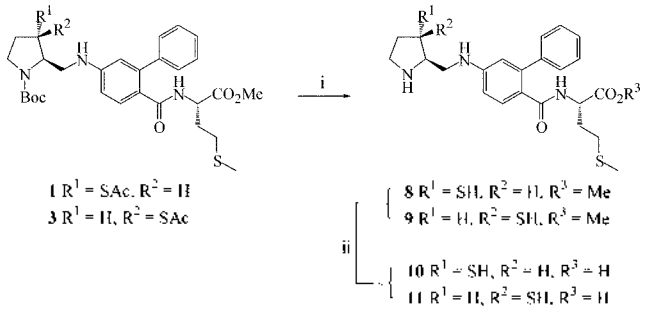
showed the *N*→*N* transacetylated derivative **6**. We believe that the *N*→*N* acyl transfer is greatly influenced by the puckered conformation of the pyrrolidine ring.[6c] The confirmation of the assigned structures was obtained by the chemical shifts of the two *ortho* hydrogens and acetyl groups, shown in Table 1. Because chemical shifts of the two *ortho* protons are diagnostic for ring substitution patterns,[8] and the acetyl group transfer greatly affected these resonances; they relied upon for the correct assignment and monitoring of the position of the acetyl group.

We then reasoned that a use of acidic conditions in the deprotection of the *S*-acetate might substantially block acetyl transfer due to the decreased nucleophilicity of the protonated amine species. The *S*-Ac proline derivatives, **1** and **3**, were dissolved in cold TFA and allowed to react with mercuric acetate.[9] The reaction mixtures, after precipitation of the mercury-sulfide complexes and further treatment with mercaptoethanol, were subjected to Prep-HPLC isolation to give the *S*-Ac, *N*-Boc-deprotected methyl esters, **8** and **9**, without any acetyl transfer. Further basic hydrolysis of methyl esters furnished the desired final compounds, **10** and **11**. We found that the *S*→*N* acyl transfer was substantially suppressed in the presence of the acidic media during mercury-assisted deprotection of the *S*-Ac, as shown in

Table 1. Chemical shifts of the two *ortho* hydrogens and acetyl groups

Compound	H_a	H_b	Ac
3[a]	δ6.37 (s)	δ6.55 (d, J = 7.7 Hz)	δ2.32 (s)
4[b]	–[c]	–[c]	δ1.89 (s)
5[b]	–[c]	–[c]	δ1.87 (s)
6[b]	δ6.63 (s)	δ6.73 (d, J = 5.0 Hz)	δ1.92 (s)

[a]$CDCl_3$ used. [b]CD_3OD used. [c]merged at δ 7.60-7.36.



Scheme 2. *Reagents and Conditions*: i. $Hg(OAc)_2$, TFA, rt, 2 hr; $HSCH_2CH_2OH$, MeOH, rt, 3 hr; Prep-HPLC, 34%; ii. 1*N* LiOH, MeOH, 4 hr; Prep-HPLC, 41%.

Scheme 2. Generally, the precaution for selecting protecting groups is necessary for the manipulation of *S*-containing amino acids. However, in a latter introduction of *S*-groups into a molecular entity, the selection is quite limited. Among them, the most frequently used reagents are thiolacetic acid[10] or potassium thioacetate.[6b] In this case, the acidic deprotection conditions (mercuric acetate, TFA; H_2S or mercaptoethanol) might be applicable to avoid a possible acyl transfer.

In summary, we observed a series of *S*→*N*→*N* acyl transfers in 3-mercaptoproline, when it was treated with basic conditions. Within our knowledge, no precedent for a series of *S*→*N*→*N* acyl transfers has been reported. It is also noteworthy that the pathway of *S*→*N*→*N* acyl transfers bears a resemblance to that of polypeptide ligation and protein splicing in many aspects.[11]

Experimental Section

Deprotection of 1 (10). To a solution of **1** (106 mg, 0.17 mmol) in TFA (2 mL) was added mercuric acetate (216 mg, 0.67 mmol) at 0 °C under argon. The reaction mixture was allowed to be stirred for 2 hr at room temperature. To this mixture, ether was added and the resulting powder was collected by filtration and then suspended in methanol (10 mL). To this was added β-mercaptoethanol (330 μL) and the reaction mixture was stirred for 3 hr at room temperature. The black precipitate was filtered off and the filtrate was concentrated to dryness. The residue was taken up with a 1 : 1 solution (1 mL) of water and THF, and purified by Prep-HPLC to give the methyl ester **8** (34%) as a white power. After the resulting ester was subjected to hydrolysis (1 *N* LiOH, MeOH, 4 hr), the reaction mixture was neutralized with TFA and then evaporated. The residue was taken up with a 1 : 1 solution (1 mL) of water and THF, and purified by Prep-HPLC to afford the final product **10** (41%) as a white power. ^{1}H NMR (CD_3OD): δ 7.45-7.39 (m, 6H), 6.74 (br s, 1H), 6.70 (br s, 1H), 4.44 (br s, 1H), 3.72-3.30 (m, 7H), 2.56 (br s, 1H), 2.18 (m, 1H), 2.02-1.96 (m, 2H), 2.01 (s, 3H), 1.80 (m, 1H); HRMS (FAB): *m/z* Calcd for $C_{23}H_{29}N_3O_3S_2$: 460.1728. Found: 460.1728.

Deprotection of 3 (11). ^{1}H NMR (CD_3OD): δ 7.46-7.31 (m, 6H), 6.67 (s, 1H), 6.65 (s, 1H), 4.48 (dd, 1H, J = 9.0,

4.1), 3.98 (s, 1H), 3.75 (m, 1H), 3.61 (m, 1H), 3.43-3.29 (m, 4H), 2.45 (m, 1H), 2.25 (m, 1H), 2.14 (m, 1H), 2.01 (s, 3H + 1H), 1.80 (m, 1H); HRMS (FAB): *m z* Calcd for $C_{23}H_{29}N_3O_3S_2$: 460.1728. Found: 460.1728.

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