

## N to S Acyl Shift Reaction in Peptide Ligation

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### Introduction

Peptide thioester is a key building block in contemporary ligation strategy for polypeptide synthesis [1]. In the thioester method, partially protected peptide thioesters are used as building blocks and are condensed in the presence of silver ion as an activating reagent for the thioester [2]. Native chemical ligation permits chemoselective ligation, in which an unprotected peptide thioester is ligated with a cysteinyl peptide in an aqueous buffer solution [3]. In the extended chemical ligation strategy, a thiol auxiliary, attached to the N-terminal amino group, is used instead of a cysteine residue and side-chain non-protected peptide thioesters are used as building blocks, thus maintaining the advantageous features of the native chemical ligation reaction [4]. During the course of our studies related to new ligation methodology, we focused on an *N* to *S* acyl shift reaction of a cysteine-containing peptide **1**, resulting an *S*-peptide (peptide thioester) **2** (Fig. 1) [5]. In this paper, two kinds of applications of the *N*-*S* acyl shift reaction for the peptide thioester preparation are described.

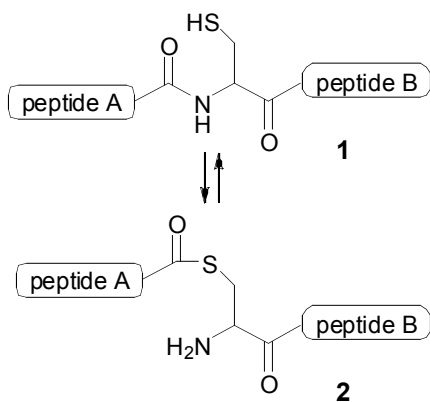


Fig. 1. *S*-Peptide formation via an *N* to *S* acyl shift reaction

### Results and Discussion

When Fmoc-Ile-Ala-Gly(1-<sup>13</sup>C)-Cys-Arg-NH<sub>2</sub> (**3**) was dissolved in a trifluoroacetic acid (TFA) solution containing chloroform-*d* and tris(2-carboxylethyl)phosphine hydrochloride, and analyzed by <sup>13</sup>C NMR, the signal corresponding to *S*-peptide, Cys[Fmoc-Ile-Ala-Gly(1-<sup>13</sup>C)]-Arg-NH<sub>2</sub> (**4**) was observed [5]. The ratio of **3** and **4** reached a nearly constant value of 1 to 4 after one month. The thioester **4** easily reproduced the original peptide **3** even in acidic aqueous solutions containing 0.1% TFA (HPLC conditions).

*N*-4,5-Dimethoxy-2-mercaptobenzyl (Dmmb) group was reported as an auxiliary in the extended chemical ligation strategy [4c]. After the ligation, it can be removed by acid treatment such as trifluoromethanesulfonic acid. However, under weak acidic conditions such as TFA, unexpected *N* to *S* acyl shift reaction occurred [5,6]. When a peptide, Fmoc-Gly(1-<sup>13</sup>C)-D,L-(Dmmb)Ala-OCH<sub>3</sub> (**5**) was treated in the TFA solution, the corresponding peptide thioester, D,L-{*S*-[Fmoc-Gly(1-<sup>13</sup>C)]Dmmb}Ala-OCH<sub>3</sub> (**6**), was observed. After 48 h, <sup>13</sup>C signal of peptide **3** had nearly disappeared. The thioester **6** was more stable than the *S*-peptide **4**, and was able to be observed on the HPLC. By using the Dmmb group, preparation of peptide thioester was examined according to Fig. 2 [5,7,8].

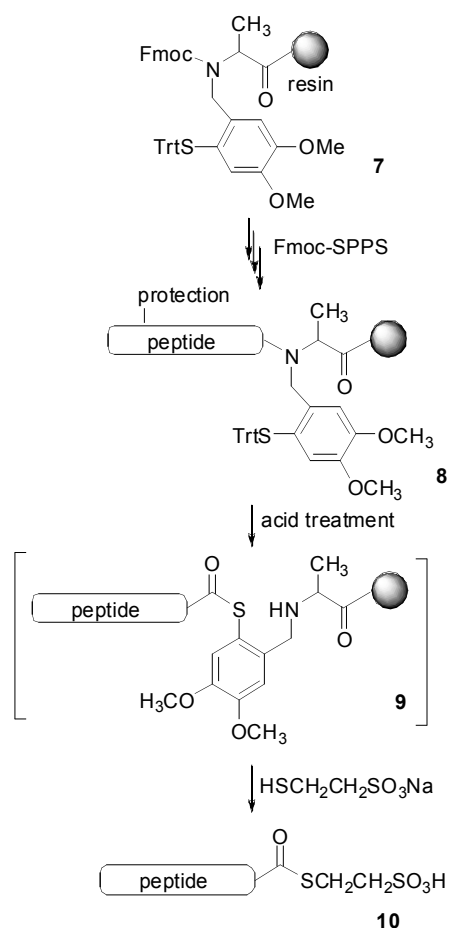


Fig. 2. Peptide thioester preparation via *N* to *S* acyl shift reaction on the Dmmb group

A protected peptide resin, Fmoc-His(Trt)-Pro-Ile-Arg(Pbf)-Gly-D,L-[Dmmb(Trt)]Ala-Ala-OCH<sub>2</sub>-Pam resin (**8**), was prepared from Fmoc-Gly-D,L-[Dmmb(Trt)]Ala-Ala-OCH<sub>2</sub>-Pam resin (**7**) by standard Fmoc solid phase peptide synthesis, and treated with a TFA solution containing water, phenol, and triisopropylsilane (88:5:5:2) to remove the protecting groups and to promote the *N-S* acyl shift reaction, providing the resin-bound thioester intermediate **9**. Then, the resin was treated with 2-mercaptoethanesulfonic acid, followed by *N,N*-diisopropylethylamine. The corresponding peptide thioester, Fmoc-His-Pro-Ile-Arg-Gly-SCH<sub>2</sub>CH<sub>2</sub>SO<sub>3</sub>H (**10**), was released from the resin and purified by RP-HPLC in 31% yield. The *N-S* acyl shift reaction was found to proceed efficiently under the aqueous acid conditions such as aqueous TFA and hydrochloric acid. In 1 M hydrochloric acid in aqueous acetonitrile, the *N-S* acyl shift reaction on ChemMatrix resin [9] was carried out to give the peptide thioester, BPTI(1-29)-SCH<sub>2</sub>CH<sub>2</sub>SO<sub>3</sub>H, containing a partial sequence of bovine pancreatic trypsin inhibitor (BPTI), in 12% yield.

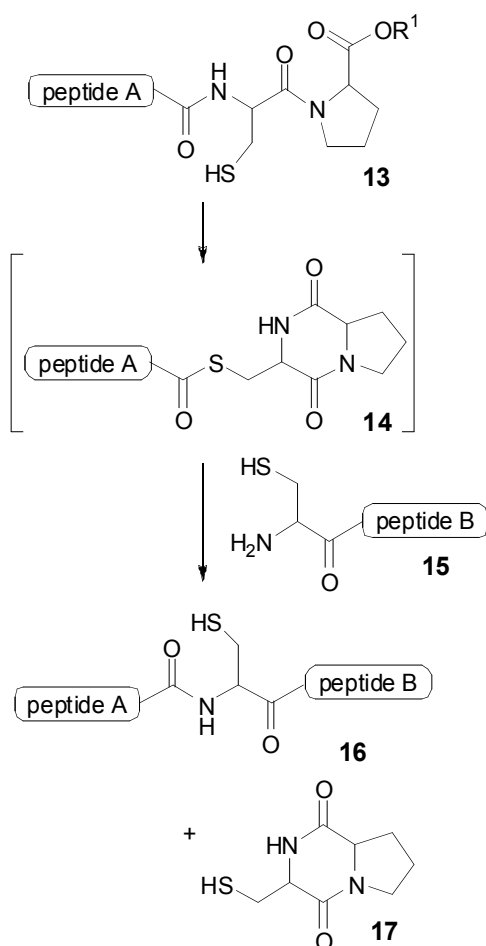


Fig. 3. CPE Ligation

On the other hand, in 1985, Zanotti *et al.* reported that a diketopiperazine thioester, *cyclo*(-Cys(COCH<sub>2</sub>Ph)-Pro-) (**11**) was formed when a *p*-nitrophenyl (Np) ester, PhCH<sub>2</sub>CO-Cys(S<sup>t</sup>Bu)-Pro-ONp (**12**), was treated with

tributylphosphine under aqueous conditions [10]. The thioester **11** would be formed *via* the intramolecular *N-S* acyl shift reaction followed by diketopiperazine formation. We applied this cysteinyl prolyl ester (CPE) unit to a peptide ligation reaction (Fig. 3). When a peptide, Fmoc-His-Pro-Ile-Arg-Gly-Cys-Pro-OCH<sub>2</sub>CONH<sub>2</sub> (**13**), which was transformed automatically into a peptide thioester **14**, was reacted with Cys-Asp-Ile-Leu-Leu-Gly-NH<sub>2</sub> (**15**) in a tricine buffer (pH 8.2) containing 20 mM tris(hydroxypropyl)phosphine and 6 M guanidine for 24 h, the ligated product, Fmoc-His-Pro-Ile-Arg-Gly-Cys-Asp-Ile-Leu-Leu-Gly-NH<sub>2</sub> (**16**), was formed and purified by RP-HPLC in 60% yield [11]. The autoactivating function of the CPE unit can be utilized to prepare the peptide thioester. A peptide thioester of the partial sequence of histone H3 (44-95) was prepared by the reaction of the corresponding peptide-CPE and sodium 2-mercaptoethylsulfonate in a sodium phosphate buffer (pH 7.5).

In summary, we have shown that the *N-S* acyl shift can be mediated by both a thiol auxiliary residue and a cysteine residue, which was applied to peptide thioester synthesis. We also found that the CPE unit mediates the peptide ligation. The observation provides an important lead to open a new methodology for polypeptide synthesis.

#### Acknowledgments

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