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FELLOWSHIP FINAL REPORT

Development of novel chemoselective ligation techniques for protein chemical synthesis

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REPORT INFO

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ABSTRACT

A novel strategy has been devised that allows a ligation of of thioacids and imidazolyl urea activated amines under aqueous conditions. This approach enables the traceless removal of imidazole and CO_2 to directly generate the desired amide bond without affecting the side chain reactive side chain functional groups on the peptide chain. Meanwhile, the novel synthesis of peptide thioacid is also reported.

Keywords :
(1) Chemical protein synthesis, (2) Innverse coupling, (3) Peptide thioacids, (4) Imidazolyl urea peptide

1- Introduction

Chemical protein synthesis is a useful tool to generate pure proteins which are otherwise difficult to obtain in sufficient amounts for structure and property analysis. Additionally, because of the precise and flexible nature of chemical synthesis, it allows for controllable variation of protein sequences, which is valuable for understanding the relationships between protein structure and function. Despite the usefulness of chemical protein synthesis, it has not been widely adopted as a tool for protein characterization, mainly because of the lack of general and efficient methods for the preparation and coupling of peptide fragments and for the folding of polypeptide chains. Native chemical ligation has revolutionised the field of chemical protein synthesis. This reaction enables the condensation of a peptide thioester with a cysteinyl peptide to generate a native amide through chemoselective *S*-to-*N* acyl transfer mechanism. In the mean time, Bode group developed the Keto Acid-Hydroxyl Amine (KAHA) ligation,[13] and Li laboratory has developed 'Ser/Thr Ligation' (STL, Figure 1).[9] However, each ligation methods have their own limitations, in terms of specific amino acids requried at the ligation junction, diffcult for the preparation of starting peptide precursors and several others. Although several mechanistically unique amide formations have emerged, none has yet proven to be readily applicable for protein synthesis by the combination of unprotected segments. Consequently, the development of a novel peptide ligation methods is of great significance and in great demand.

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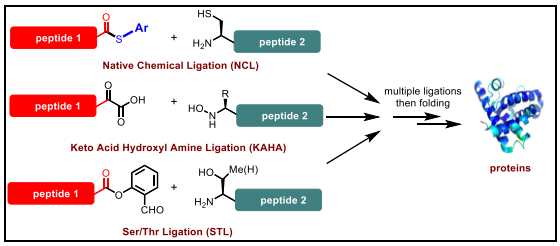
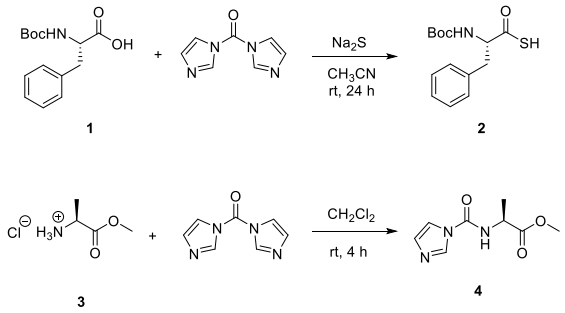


Figure 1. Site selective ligation methods developed

In an effort to streamline to develop novel ligation method, we sought to identify an activated amine (as imidazolyl urea) is capable of facilitating the rapid amide bond formation with the carboxylic acids so called inverse peptide bond formation. However, direct usage of this method as a ligation technique is severely hampered due to the presence of myriad reactive functional groups present in the unprotected peptides are known to react with N-terminally activated imidazolyl urea peptide. Herein we document our discovery of a novel ligation approach by the reaction of unprotected peptide thioacids with *N*-terminally activated imidazolyl urea peptide under aqueous conditions.

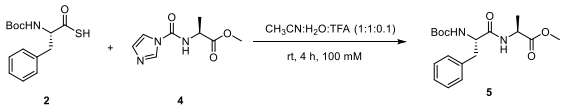
2- Results and discussion

In order to test our hypothesis for small dipeptide synthesis, we have choosen model amino acids **1** and **3** and were converted into respective thioacid **2** and imidazolyl urea **4** by the repored reaction conditions and were obtained in quantitative yields (Scheme 1).



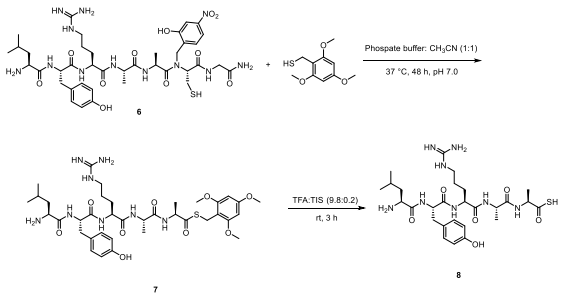
Scheme 1. Synthesis of thioacid **2** and imidazolyl urea **4**.

At the outset of our studies, Boc–Phe–SH **2** and imidazolyl urea derived from alanine methyl ester **4** were used as model substrates to investigate the whether amide bond formation could be performed in the presence of water at low concentrations which are required parameters to develop ligation methods. After extensive optimization of the reaction parameters such as concertation and reaction time, delightfully, the resulting amide product was obtained in good yield (82%) in 4 hour at 100 mM concentration. It is noted that under similar conditions reaction of Boc-Phe-OH with **2a** gave poor yield of amide product. Based on our initial result, a standard experimental protocol was developed in order to ascertain an initial scope for the reaction. These conditions included thioacid (1.0 equiv.) and imidazolyl urea (1.0 equiv.) in CH_3CN:H_2O:TFA (1:1:0.1), operating at room temperature in 100 mM concentration for 4 h hour standard time (Scheme 2).



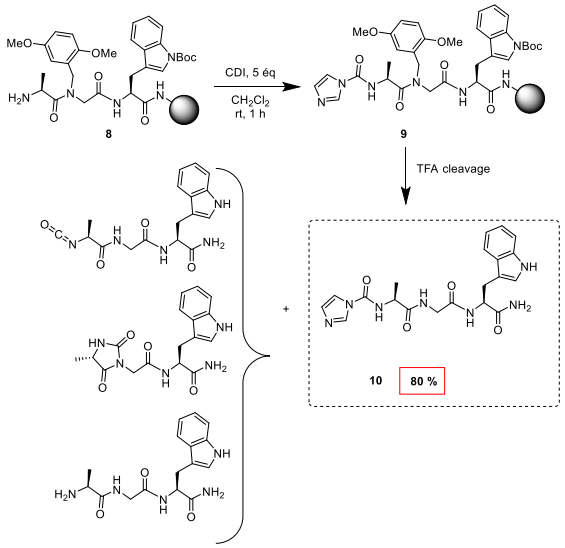
Scheme 2. Synthesis of dipeptide **5**

Encouraging with these results and attempts to extend this methodology as a chemoselective ligations, unprotected peptide building blocks were required. Thus, we first undertook the synthesis of unprotected peptide thioacid by the novel methodology based on the use of cyrptothioester device developed in the host laboratory for NCL applications. Here we examined the effectiveness of the Hnb-Cys cryptothioester for the synthesis of model peptide thioacid **8** by using Tmob-SH as a thiol source. Note that we used relatively dilute conditions for the peptide reactants (1 mM) and 20 mM of Tmb-SH. Relatively fast thioesterification was observed during the synthesis of model peptide thioester **7** as the reaction was completed in 48 h at 37 °C. The resulting Tmob-protected peptide thioester was purified by HPLC and lyophilized which could be stored for long time at 0 °C. The release of peptide thioacid from the Tmob-protected peptide thioester was carried out the by the treatment of trifluroacetic acid (TFA) and triisopropyl silane (TIS) combination. The resulting peptide thioacid was found be pure enough for the ligation reactions with imidazolyl urea peptide.



Scheme 3. Synthesis of peptide thioacid **8**

Another important building block was the synthesis of imidazolyl urea peptide. A model peptide **8** was chosen and the synthesis was carried out by Fmoc SPPS. In an attempt to synthesize peptide imidazolyl urea **9**, the resin peptide **8** was treated with 1,1 carbonyl dimidazole. The release of peptide from the resin was resulted in **10** along with side products as minor quantities. The most common side products are hydantion, isocyanate, and unreacted free amine. These side reactions could be further minimized by the suitable protecting group on the amide nitrogen atom of the (n-1) amino acid. Further, we also examined the stability of the imidazolyl urea peptide under aqueous conditions and was found to be stable up to 8 h which is sufficient to carryout out planned ligation reaction. With the required building blocks in hand we are currently investigating the ligation of unprotected peptide thioacids with unprotected imidazolyl urea peptide under physiological conditions.



Scheme 4. Synthesis of imidazolyl urea peptide **10**

Conclusion

In summary, we have developed a novel synthesis for the (a) unprotected peptide thioacids, and (b) unprotected imidazolyl urea activated peptides. Based on our proof of concept for the synthesis of dipeptide under semi aqueous conditions, we are investigating the ligation of peptide thioacids with imidazolyl urea activated peptides under physiological conditions.

3- Acknowledgements

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