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# Chemoselective Ligation of Multifunctional Peptides to Topological Templates via Thioether Formation for TASP Synthesis

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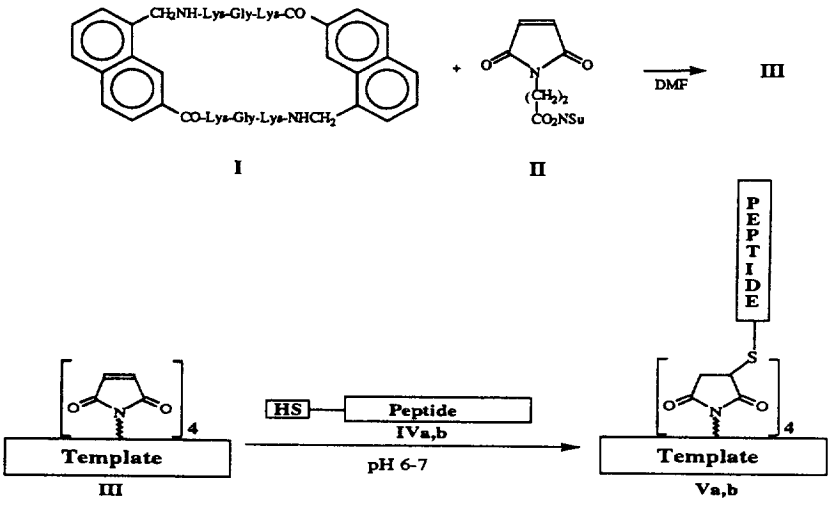
***Abstract:*** *Covalent attachment of multifunctional peptides to a topological template containing four maleimide functions was achieved via thioether formation; the resulting TASP (Template Assembled Synthetic Protein) molecules are obtained in high yield and purity.*

The concept of Template Assembled Synthetic Proteins (TASP) has been introduced some years ago[1] to bypass the so-called protein folding problem[2]. In this approach, peptide blocks with inherent secondary structure are attached to a topologically defined template, which promotes their folding into a distinct tertiary structure[3]. Although convergent strategies, i. e. the condensation of protected peptide fragments to a given template molecule in solution have led to the successful preparation of a number of 4-α-helical bundle TASPs[4], the low solubility of fully protected peptide fragments often exerts an adverse effect on coupling kinetics, resulting in low yields and extensive purification protocols of the final products. For this reason, recently proposed methods of chemoselective ligation open a more efficient way for the synthesis of TASP molecules[5].

We describe here a new method of chemoselective ligation for TASP synthesis using the maleimide function, which is known to react rapidly and rather selectively with thiols[6]. This should allow for the condensation of peptides containing a thiol group to template molecules containing the maleimide function. As a first example, we have chosen the multifunctional tripeptide **γ–Glu-Cys-Gly (IVa)** for chemoselective ligation to the β-turn mimetic containing template molecule **I** as depicted in Scheme 1. To this end, template **I**[7] was treated with equimolar amounts ( with respect to the ε- amino functions of Lys in **I**) of N-maleoyl-β-alanine-N-hydroxysuccinimide ester **II** in DMF at pH 7. As followed by analytical HPLC, complete disappearance of the starting material and formation of the functionalized template **III** was observed within 1 hour. The mixture was then treated *in situ* with equimolar amounts of **IVa**; within less then 10 minutes, the reaction proceeded in high yield even in the abscence of an excess of peptide thiol **IVa**. After HPLC purification, the target molecule **Va** was obtained in an overall yield of 85%. The versatility of this chemoselective ligation method in TASP synthesis was further evaluated by the condensation of the potentially β-sheet forming oligopeptide **Ac-(Ala-Ser)5-Cys (IVb)**[8] using serine-oxazolidine derivatives (`pseudo-prolines')[9] as temporary protection technique for Ser at position 4 and 8 to disrupt any secondary structure during the ligation step; the 11 mer-peptide could be attached quantitatively to template **III** according to Scheme 1 within less than 10 min. Most notably, this β-meander TASP molecule has not been accessible so far using conventional strategies. The integrity of the TASP molecules **V** was proved by analytical HPLC, amino acid analysis and LDI-mass spectrometry.

In conclusion, with alternative chemoselective ligation methods and **R**egioselectively **A**ddressable **F**unctionalized **T**emplates (RAFTs), the present procedure opens the way for approaching TASP molecules of higher structural or functional complexity.




Scheme 1: Chemoselective ligation of thiopeptides to a maleimide functionalized template (see text).

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7. Typical procedure for the chemoselective ligation (Scheme 1): **I** (0.352 mmol) was dissolved in anhydrous DMF (0.25 ml), 4 equivalents (1.408 mmol) of **II** were added and the reaction mixture was stirred at r.t. The formation of **III** was followed by analytical HPLC and was complete after 1 hour. A solution of the thiopeptide **IV** (1.408 mmol) in $H_2O$, pH 6-7 was added. The formation of the corresponding TASP molecule **V** occurred within less than 10 min. The reaction mixture was lyophilized and the target TASP **V** as the major component was isolated by preparative HPLC. The chemical integrity was confirmed by LDI-MS.
8. The oligopeptide **IVb** was synthesized by Solid Phase Synthesis on a Rink MBHA resin (0.4mmol/g; Novabiochem) Using Fmoc chemistry. Side-chain deprotection of the peptide and simultaneous cleavage from the resin was performed in a mixture of TFA/ethanedithiol/thioanisole/water (40,1,1,1) for 5 hrs. After filtration, the peptide was precipitated with cold diethyl ether, washed three times with ether and dried in vacuo. **IVb** was purified by preparative HPLC on a Vydac RP-$C_{18}$ column and its integrity confirmed by $^1H$ NMR and ES-MS.
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