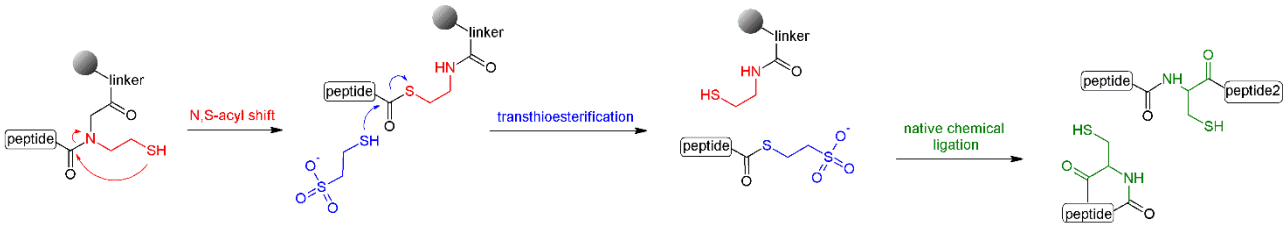
1. Research project goals

The aim of this project is the examination of N-(2-thioethyl)glycine analogues in orthogonal cleavage of peptides in solid-phase peptide synthesis (SPPS) and its optimization. The cleavage proceeds *via* N,S-acyl shift of N-(2-thioethyl)glycine residue incorporated to the solid support and further acyl transthioesterification with sodium 2-mercaptoethanesulphonate (MESNa) which results in the liberation of peptide thioester (Scheme 1). The library of active peptide thioesters can be hydrolyzed in mild conditions to a library of peptides or subjected to the native chemical ligation (NCL) with model N-terminal cysteine peptides in order to study the method applicability for combined peptide cleavage and native chemical ligation that yields in the formation of bigger peptides or small native proteins. This approach will also be used for the cyclization of N-terminal cysteine containing peptide thioesters in one-pot process.



Scheme 1. Peptide cleavage method via the N,S acyl shift of N-(2-thioethyl)glycine and further transthioesterification for the production of active peptide thioester feasible to native chemical ligation and self-ligation.

2. Description and motivation of research

Orthogonal techniques for peptide cleavage from solid support play an important role in combinatorial peptide chemistry, especially for combinatorial one-bead-one-compound (OBOC) peptide libraries that allow a fast search for new biologically active compounds by the split-and-mix technique resulting in a rapid synthesis of millions of compounds. Small peptide libraries will be synthesized according to SPPS strategy and cleaved quantitatively from resin in order to expand and optimize the applicability of investigated by us method.

Obtained by orthogonal cleavage peptide thioesters libraries will be subjected to the native chemical ligation with N-terminal cysteine (and or selenocysteine) peptides in mild water buffer conditions. NCL is a method of choice in the synthesis of big challenging peptides and proteins that cannot be synthetized by standard SPPS or their synthesis yield is unsatisfactory. Therefore we are going to apply our method in the synthesis of biologically active linear and branched peptides combined with their cleavage from solid-support. We pay special interest in lastly described in literature one-pot self-cleaving peptide cyclization, that we would apply for the synthesis of cyclic short peptides and bioactive challenging cyclic structures.

3. Expected results

It may be expected that new orthogonal peptide cleavage method will facilitate the synthesis and examination of peptide libraries for combinatorial peptide chemistry and overcome the drawbacks of currently used methods that limit the peptide sequence and require corrosive, toxic substrates. Furthermore the obtained active thioesters will be used for Native Chemical Ligation (NCL) to develop the new synthesis routes of biologically active peptides. Especially, this approach will result in one-pot on-resin peptide cyclization and further development of new synthetic routes for a broad family of cyclic peptides. This specific group of peptides, characterized by constrained structure resulting in numerous biological activities are found to be promising targets as medium sized therapeutics. We expect as well to obtain cyclic tetra- and penta-peptides which are still a synthetic challenge.