Purification-free synthesis and screening of thousands of cyclic peptides

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Macrocycles are an attractive class of therapeutics. Their ability to bind to challenging targets while retaining cell permeability enables a large target spectrum previously rendered "undruggable". One major holdback for the development of macrocyclic therapeutics on large scale are the time and resource intensive ways to produce compound libraries of such. This project is presenting a way to generate thousands of cyclic peptides in a parallel approach. Di-thiol peptides, carrying one thiol on their N- and one on their C-terminus, are synthesized via solid-phase peptide synthesis (SPPS) as linear precursors for the cyclization reaction. The C-terminal thiol is implemented in form of a cysteamine derivative, that can be immobilized on solid support via disulfide-bridging and hence facilitates SPPS from its amino group. The N-terminal thiol of the peptide is introduced by coupling of *S*-trityl-3-mercaptopropionic acid under regular SPPS conditions. After synthesis and deprotection, the peptide is cleaved from the resin with 1,4-butanedithiol, a reducing agent which is volatile and therefore easy to remove. Cyclization is achieved by utilizing a wide range of bis-electrophilic linker yielding highly diverse peptidic macrocycles with excellent purity ready for screening. The immense increase of efficiency lies in the application of powerful reactions and reaction conditions, facilitating a purification-free process. Additionally, automated liquid handling and acoustic liquid ejection enables high-throughput processing and screening in 384- or 1536-well microtiter plates. Implementing this strategy to synthesise a focused library against the trypsin-like serine proteases thrombin produced multiple highly potent nanomolar inhibitors. Conclusively, this method can be used to generate large libraries of cyclic peptides in a purification-free way enabling identification of highly potent binders to model targets and to ultimately take on more challenging targets in the future.