

## Selection of chemically-upgraded macrocyclic peptides by phage display

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Macrocyclic peptides (MPs) show great potential for the development of pharmaceuticals and chemical probes. Aided by in vitro selection strategies that enable the efficient culling of vast libraries, the number of approved MP drugs has steadily increased over the past decade.[1] However, man-made MPs are typically ill-suited for therapeutic intervention, as they cannot undergo the same chemogenetic optimization mechanisms that are key for ameliorating the properties of naturally-occurring MPs.[2] In nature, organisms take advantage of the evolutionary algorithm to fine-tune not only the amino acid sequence, but also posttranslational processes such as the introduction of non-peptidic moieties for peptide macrocyclization. As a result, mimicking such a chemogenetic optimization in the laboratory is desirable in order to improve the effectiveness (and pharmacological properties) of man-made MPs.[3]

To this end, we present an efficient two-step cyclization strategy to access chemically-upgraded macrocyclic peptides (CUMPs) via the programmed modification of a unique cysteine residue and an N-terminal amine. We demonstrated that this approach yields MPs featuring asymmetric cyclization units from both synthetic peptides and when linear precursors were appended onto a phage-coat protein. Finally, we showcased that our cyclization strategy is compatible with phage-display protocols and enables the selection of CUMP-binders against a model target protein from a naïve library.[4] We anticipate that the future selection of CUMPs by phage display for clinically relevant targets will enable a comprehensive exploration of a previously-unexplored chemical space and provide unique opportunities for drug discovery.

**OUR WORKFLOW:** (1) synthesis of privileged scaffolds featuring distinct cyclization handles; (2) evaluate CUMP formation with synthetic peptides and linear precursors displayed on the phage coat; (3-4) select CUMP binders against drug targets; (5) characterization and chemogenetic optimization of CUMPs.

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