Expanding the Chemical Space of Lasso Peptides: Enzymatic Maturation of Synthetic Peptide Precursors

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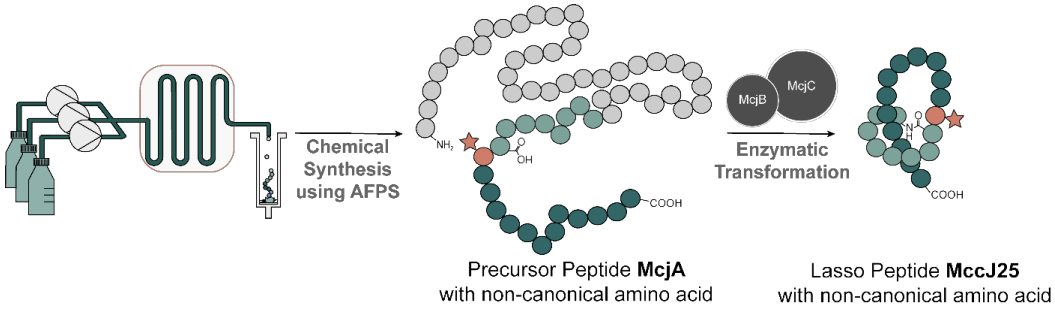
Lasso peptides are a class of ribosomally synthesized and post-translationally modified peptides (RiPPs), and many display antimicrobial, antiviral, and antitumor activity.[1] Their biological activities and their excellent stability against heat treatment and enzymatic digestion make them potential therapeutic agents, and chemical modifications would be desirable to explore this potential.[2] A chemical synthesis, however, is challenging because of their unique knot-like structure, and therefore, the most prominent member of their class – Microcin J25 (MccJ25), which shows activity against Gram-negative bacteria[3] – has not been chemically synthesized to date.

Here, **we use flow-based peptide synthesis in combination with *in vitro* enzymatic maturation to investigate the promiscuity of the processing enzymes and give access to several chemically modified MccJ25 derivatives including non-canonical amino acids.** We confirm lasso-formation by ion-mobility mass spectrometry, and perform antimicrobial assays to obtain additional information about the influence of these chemical modifications. Incorporating non-canonical amino acids will expand the chemical space; this allows for rational drug design and enables grafting onto this scaffold to synthesize lasso peptide libraries.



[1] J. D. Hegemann, M. A. Marahiel, *Cyclic Peptides*, **2017**; 206-224
[2] F. J. Piscotta, J. M. Tharp, W. R. Liu, A. J. Link, *Chemical Communications* **2015**, 51 (2), 409-412.
[3] K. P. Yan, Y. Li, S. Zirah, C. Goulard, T. A. Knappe, M. A. Marahiel, S. Rebuffat,*ChemBioChem* **2012**, 13 (7), 1046-1052.