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Merging Flow Synthesis and Enzymatic Maturation to Expand the Chemical Space of Lasso Peptides

Authors: Kevin Schiefelbein¹, Jakob Lang^{1,2}, Matthias Schuster¹, Claire E. Grigglesome¹, Robin Striga³, Laurent Bigler¹, Meredith C. Schuman^{1,2}, Oliver Zerbe¹, Yanyan Li³, Nina Hartrampf^{1,*}

Affiliations:

¹ Department of Chemistry, University of Zurich, Winterthurerstrasse 190, 8057 Zurich, Switzerland

² Department of Geography, University of Zurich, Winterthurerstrasse 190, 8057 Zurich, Switzerland

³ Laboratory Molecules of Communication and Adaptation of Microorganisms (MCAM). UMR7245, CNRS-Muséum National d'Histoire Naturelle (MNHN), Alliance Sorbonne Université, 57 rue Cuvier, 75005 Paris, France

* Correspondence to: nina.hartrampf@chem.uzh.ch

Abstract: *Many peptidic natural products—such as lasso peptides, cyclic peptides, and cyclotides—are conformationally constrained and show biological stability, making them attractive scaffolds for drug development. Although many peptides can be synthesized and modified through chemical methods, knot-like lasso peptides such as microcin J25 (MccJ25) and their analogs remain elusive. As the chemical space of MccJ25 analogs accessible through purely biological methods is also limited, we proposed a hybrid approach: flow-based chemical synthesis of non-natural precursor peptides followed by in vitro transformation with recombinant maturation enzymes to yield a more diverse array of lasso peptides. Herein, we established the rapid, flow-based synthesis of chemically modified MccJ25 precursor peptides (57 amino acids). Heterologous expression of enzymes MccB and MccC was extensively optimized to improve yields and facilitate the synthesis of multiple analogs of MccJ25, including the incorporation of non-canonical tyrosine and histidine derivatives into the lasso scaffold. Finally, using our chemoenzymatic strategy, we produced a biologically active analog containing three D-amino acids in the loop region and incorporated backbone N-methylations. Our method therefore provides rapid access to chemically modified lasso peptides that could be used to investigate structure-activity relationships, epitope grafting, and improvement of therapeutic properties.*

Introduction

Lasso peptides are a subclass of ribosomally synthesized and post-translationally modified peptides (RiPPs) classified by their mechanically constrained knot-like structure.¹ They are highly attractive starting points for drug discovery efforts as they combine remarkable enzymatic, thermal, and chemical stability²⁻⁴ with a variety of biological activities.^{5, 6} Rational design to increase the chemical space of the lasso scaffold would require methods for incorporating (multiple) non-canonical amino acids (ncAAs), currently inaccessible via biological methods. Chemical synthesis would enable the incorporation of ncAAs, however, unlike other cyclic and conformationally constrained peptides (**Fig. 1A**)⁷⁻¹⁰, the bioactive lasso peptide microcin J25 (MccJ25) has resisted numerous synthesis attempts.^{11, 12}

Nevertheless, biological methods were explored to derivatize MccJ25 with canonical amino acids to alter the activity and study its biogenesis.¹³⁻¹⁶ Biosynthetically, the lasso topology of MccJ25 (21 AA) is achieved through enzymatic maturation of the precursor peptide McjA (57 AA) by two enzymes: McjB removes the leader sequence, and McjC, a lasso cyclase, forms the isopeptide-linkage between the N-terminal amine and the Glu-8 side chain.¹⁷ Previous research has shown that McjB and McjC tolerate up to three simultaneous point mutations in McjA, with a higher acceptance in MccJ25's C-terminal tail with respect to lasso production and activity.¹⁸ Besides canonical amino acids, the incorporation of ncAAs could significantly modify and improve MccJ25's properties but remains challenging and underexplored.¹⁹⁻²³ Link and coworkers used genetic code expansion to incorporate four separate phenylalanine derivatives into MccJ25's lasso scaffold, but expression yields were significantly lower than for the wild-type peptide (**Fig. 1B**).²⁴ Thus far, post-isolation modifications of MccJ25 have included tyrosine nitration,²⁵ histidine carbethoxylation, or C-terminal amidation,²⁶ which are narrow in scope and not site-selective. A generally applicable chemical total synthesis strategy for lasso peptides, enabling more diverse modification, remains elusive.²⁷

An underexplored approach to incorporate a wider array of ncAAs and backbone modifications into lasso peptides would entail the chemical synthesis of modified precursor peptides followed by enzymatic transformation *in vitro*. However, this would require overcoming two key hurdles: synthesis of peptide precursors and enzyme expression. Whilst a chemical precursor synthesis approach would significantly increase the accessible chemical space, the prerequisite long sequences, like McjA (57 AA), can be challenging using standard SPPS protocols.²⁸ And, although the required enzymes McjB and McjC were previously obtained through recombinant expression in *Escherichia coli* (**Fig 1B**), the yield and purity were insufficient for a systematic study.^{17, 29}

Herein, we describe a method to generate chemically modified MccJ25 analogs containing various ncAAs and backbone *N*-methylations, many of which would be difficult or impossible to access by biological methods. First, we employed automated fast-flow peptide synthesis (AFPS), an optimized platform for single-shot synthesis of medium and long sequences, to produce modified precursor peptides.^{30, 31} Next, we established a reliable expression protocol for enzymes McjB and McjC. We enzymatically transformed over 20 MccJ25 precursor peptides, containing multiple ncAAs and core peptide substitutions, into lasso peptides to study the impact of these modifications on maturation and bioactivity. Our hybrid approach demonstrates the extensive promiscuity of McjB and McjC to ncAAs and could serve as a blueprint for exploring other lasso peptides and their application in mechanistic studies and drug discovery campaigns.

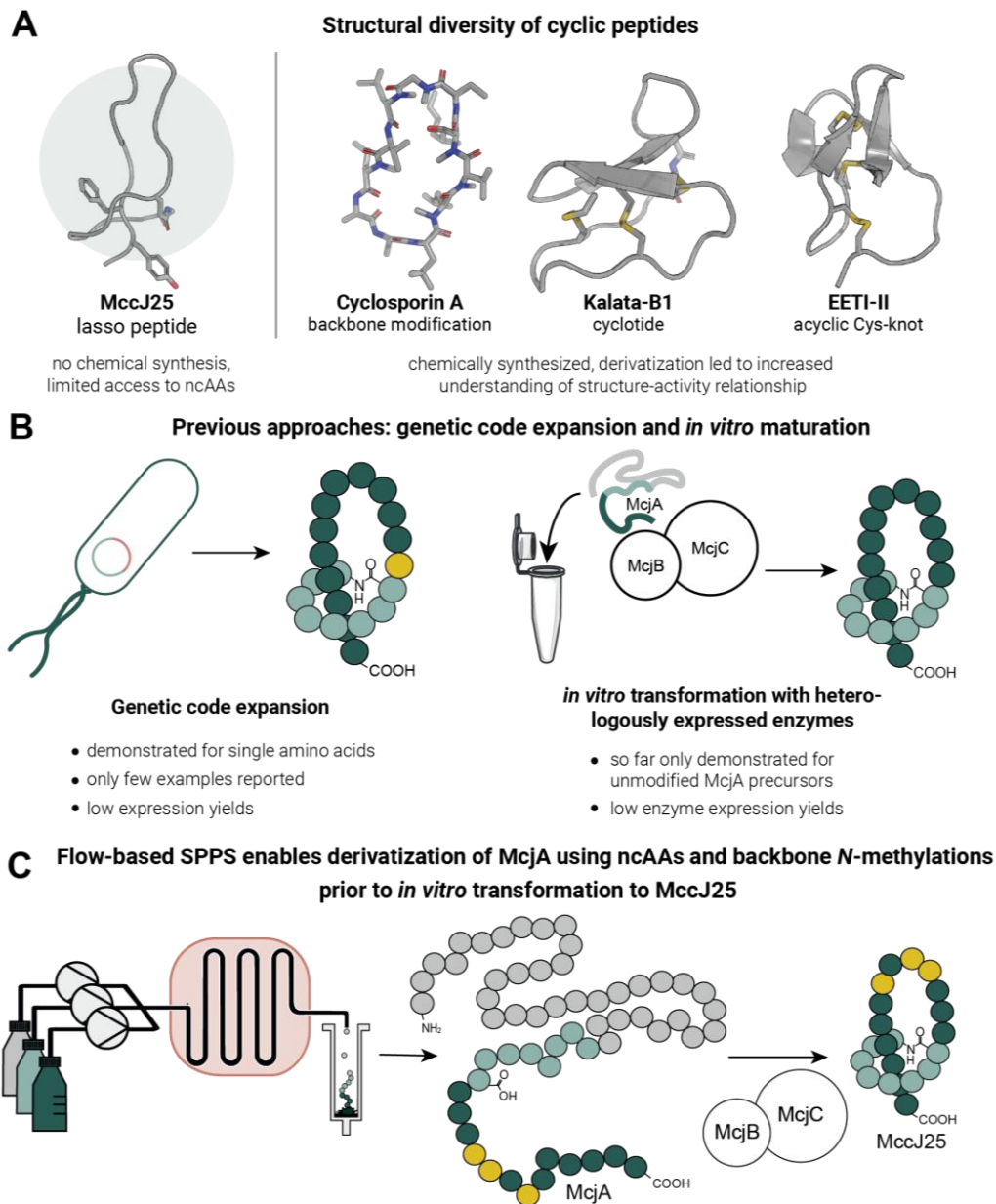


Figure 1. Microcin J25 (bio-)synthesis and derivatization. (A) Natural cyclic peptides show great diversity such as knot-like topology, cyclization, and backbone modifications. (B) Heterologous production of MccJ25 in *E. coli* enables the incorporation of a single nCAA via genetic code expansion. *In vitro* transformation of precursor McjA using heterologously expressed enzymes McjB and McjC yield MccJ25. (C) This approach: Synthesis of chemically modified precursor peptide McjA and subsequent *in vitro* transformation to obtain MccJ25 bearing nCAAs and backbone modifications.

Results

The low-yielding expression of lasso maturation enzymes McjB and McjC was optimized to establish a reproducible protocol.

The biosynthetic production of MccJ25 requires the simultaneous presence of the leader peptidase (McjB) and lasso cyclase (McjC) (**Fig. 2A**).¹⁷ To date, their molecular mechanism remains cryptic due to difficulties in obtaining enzymes in sufficient quantity and quality.¹² To further investigate enzyme promiscuity, we set out to develop a more robust expression protocol for McjB and McjC. In our hands, the expression of N-terminal His₆-tagged McjC in *E. coli* Rosetta(DE3)pLysS only yielded trace amounts, matching previous reports.^{12, 17} To improve expression yields, we performed a codon optimization of the McjC sequence before testing the expression in various *E. coli* strains.³² Expression in Rosetta(DE3)pLysS cells using the codon-optimized plasmid did not significantly increase enzyme production, but standard BL21(DE3) cells showed a clear improvement of the yields of His₆-McjC (**Fig. 2C**) as shown by SDS-PAGE. To further improve expression conditions, *E. coli* C41(DE3) and C43(DE3) were tested owing to their increased tolerance towards overexpression of toxic recombinant proteins,³³ and indeed showed superior yields of His₆-McjC. The resulting protein solutions were analyzed by LC-MS, confirming the presence of His₆-McjC (**Fig. S67**), which was further corroborated by standard trypsin digestion of the corresponding gel band (**Fig 2C**, highlighted in pink). Overall, C43(DE3) cells were found to be the best expression host for His₆-McjC due to the higher protein yield. Since McjB was active even as an MBP-fusion protein with a His₆-tag (**Fig. 2B**) for enhanced solubility, we tested its expression in *E. coli* BL21(DE3) following the reported procedure.¹⁷ Although a clear band for His₆-MBP-McjB was observed in cell lysates by SDS-PAGE, we were inspired by the results for McjC overexpression and investigated expression in C41(DE3) and C43(DE3) again.³³ Similarly, these strains proved to be superior expression hosts, and all subsequent recombinant expressions utilized C43(DE3) cells.

Cultivation medium can also have a significant influence on the yield of recombinant protein expression.³⁴ Changing the expression medium from LB to *terrific broth* (TB) medium gave greater amounts of cells and ultimately resulted in higher enzyme yields for both His₆-MBP-McjB and His₆-McjC. In summary, the optimized expression conditions featured C43(DE3) cells and TB medium, yielding the highest amounts of both processing enzymes. After a single step of gravity-flow affinity chromatography purification, both enzymes were used for the transformation of McjA to lasso peptide MccJ25.^{17, 29}

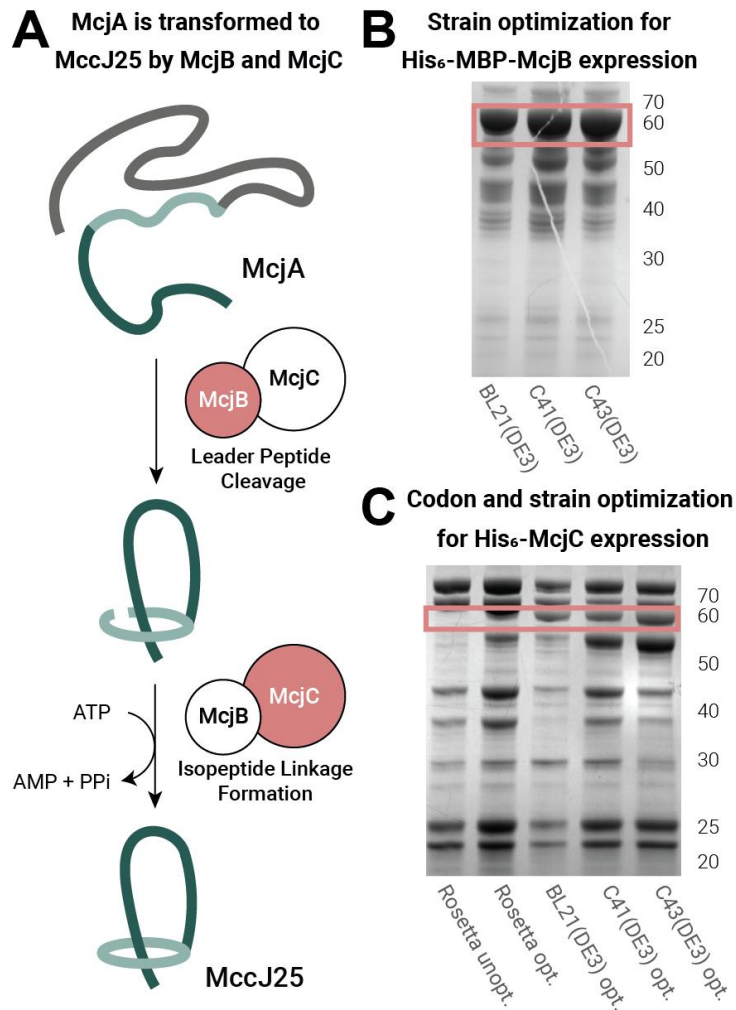


Figure 2. Production optimization of recombinant lasso enzymes McjB and McjC. (A) Mechanism of maturation from precursor peptide McjA to corresponding lasso peptide MccJ25 via cleavage of the leader peptide by McjB and ring closure by McjC. (B) SDS-PAGE gel analysis of His₆-MBP-McjB expression comparing expression outcome of different strains (*E. coli* BL21(DE3), C41(DE3) and C43(DE3)) after single Ni-NTA chromatography purification. *E. coli* C43(DE3) shows the highest protein yield. (C) SDS-PAGE gel analysis of optimized expression procedure of His₆-McjC in different strains (*E. coli* Rosetta(DE3)pLysS, BL21(DE3), C41(DE3) and C43(DE3)) before and after codon-optimization of the gene of interest (unopt. Vs. opt). Codon-optimization and expression in *E. coli* C43(DE3) yielded the best results.

Flow-based peptide synthesis enables the rapid production of lasso precursor peptides and their subsequent *in vitro* enzymatic maturation.

We next sought to establish a chemical synthesis of native McjA as a blueprint for chemically modified MccJ25 derivatives. The rapid, flow-based synthesis of wild-type McjA[2–58] (**Fig. 3A**) could be accomplished using standard AFPS conditions without further optimization.³⁰ The peptide was synthesized on HMPB-linked resin within 3.5 h (crude purity: 43%), then cleaved and purified twice by RP-HPLC to obtain McjA bearing the C-terminal carboxylic acid with a purity of 94% and an isolated yield of 8% (4.1 mg, 0.7 μmol) (**Fig. 3A**).

After successfully synthesizing McjA and expressing McjB and McjC, the *in vitro* maturation of synthetically produced MccJ25 precursor was performed.^{17, 29} The precursor McjA was mixed in a ratio of 2:1:1 with His₆-MBP-McjB and His₆-McjC, supplemented with ATP, MgCl₂, and TCEP (pH 8). The transformation was carried out on a 1.5 nmol scale (with respect to McjA) and tracked by LC-MS analysis (**Fig. 3B**). Lasso and branched-cyclic (unthreaded) peptide products were distinguished with LC-IM-MS measurements by comparing the mobilograms and the smallest collision cross section (CCS) peak (**Fig. 3C**) as previously established for lasso peptides.^{11, 12, 35} In addition, topology could be evaluated by thermolysin digestion combined with MS analysis as MccJ25 cleavage forms stable rotaxanes due to the steric plugs Phe-19 and Tyr-20 (**Fig. 3D**), while branched-cyclic peptides fully degrade.³⁶ For control samples, we used biologically produced MccJ25 and its chemically synthesized branched-cyclic analog, which was obtained via on-resin cyclization.³⁷ Comparison of MccJ25 obtained from *in vitro* maturation with the control samples confirmed the successful transformation of synthetic McjA to the corresponding lasso peptide. With an established *in vitro* maturation protocol in hand, we progressed to the chemical synthesis of lasso peptide precursors with non-canonical single point mutations and their enzymatic processing by McjB and McjC.

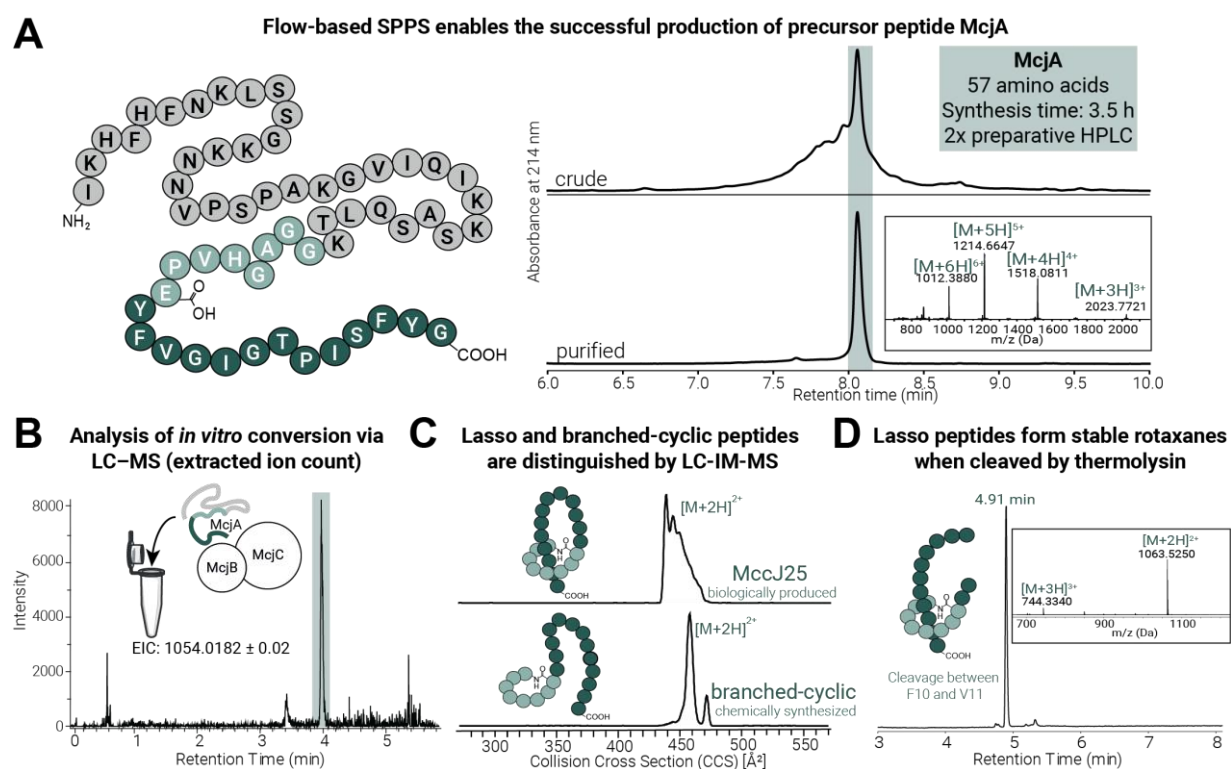


Figure 3. Establishing the chemical synthesis of precursor peptides and the analysis of lasso peptides after successful *in vitro* transformation. (A) Schematic representation of McjA and corresponding LC and MS analysis of synthetic McjA, using AFPS before and after purification by RP-HPLC. (B) LC-MS analysis (extracted ion chromatogram, EIC) of MccJ25 (highlighted in green) following the *in vitro* McjA transformation assay with McjB and McjC. (C) Peptide conformation analysis via LC-IM-MS to distinguish lasso and branched-cyclic peptides. (D) Digestion with thermolysin forms stable rotaxanes from MccJ25 due to Phe-19 and Tyr-20 residues, confirming lasso peptide topology.

Chemical synthesis allows for the rapid incorporation of non-canonical tyrosine and histidine derivatives as single point mutations.

The incorporation of ncAAs could yield additional insights into enzyme promiscuity and enable structure-activity relationship studies. As a first case study, we investigated the incorporation of ncAA derivatives of Tyr-9 in wild-type MccJ25 (**Fig. 4A**). Tyr-9 is reportedly essential for antimicrobial activity as it is involved in a key hydrogen bond to RNA polymerase (RNAP), inhibiting transcription by interfering with NTP binding (**Fig. 4C**).³⁸ Furthermore, it affects the membrane respiratory chain, resulting in superoxide production.³⁹ Therefore, we investigated derivatives of Tyr-9 that either lack the H-bond donor (-methoxy **2**, -fluoro **3**), contain a different donor moiety (aniline **5**), or are devoid of donor and acceptor attributes (Y9F, **8**). Notably, there are conflicting reports regarding the bioactivity of MccJ25 Y9F variants.^{13, 40} We additionally studied the effect of the position of the alcohol group (**7**) in creating different H-bonds in the RNA-binding pocket. A nitro (**4**) or *tert*-butyl group (**6**) was introduced in the meta-position of Tyr-9 to alter electronic and steric properties. The syntheses of all Tyr-9 modified analogs of MccJ25 were performed using AFPS technology, except for the ncAAs, which were incorporated via batch-SPPS using PyAOP and DIPEA (see SI). Aniline derivative Fmoc-Phe(4-NHTrt)-OH was incorporated as a crude mixture after side-chain protection of Fmoc-Phe(4-NH₂)-OH using trityl chloride (see SI). After synthesis, peptides were cleaved and purified by RP-HPLC to obtain all peptides in moderate-to-good isolated yields (2–6%) and high purity (87–>95%).

Next, we explored His-12 variants of MccJ25, inspired by previous reports of the improved antibacterial activity of MccJ25 after simultaneous installation of His-12, Phe-13, and Ile-15 into the loop region.¹⁸ The previously described His-12 MccJ25 mutant **9'** was further derivatized with ncAAs, including D-His (**10**) and four other five-membered heterocycles (compounds **11–14**). All His-12 derivatives (**9–14**) of MccJ25 were prepared by AFPS, as described for Tyr-9 derivatives **2–8**, but with lower isolated yields due to co-elution of target peptides with deletion side-products, and only RP-HPLC fractions with very high purity (>95%) were selected for folding and activity studies.

With MccJ25 peptides bearing either Tyr-9 or His-12 modifications in hand, *in vitro* transformations using His₆-MBP-MccJ25 and His₆-MccJ25 were performed and analyzed for conversion by LC-MS. To our delight, conversion of all MccJ25 derivatives was confirmed, demonstrating the capability of MccJ25 and MccJ25 to produce cyclic peptides with various ncAAs in the MccJ25 scaffold. To distinguish branched-cyclic from lasso topology, the products of the *in vitro* assays were analyzed by LC-IM-MS and compared to standards (**Fig. 3C**), evaluating mobilogram shape and the CCS of the peak with the smallest CCS value. The CCS value of the [M+2H]²⁺ charge state of expressed lasso standard MccJ25 (469.4 Å²) is significantly different from the branched-cyclic analog (**bc-1'**)(489.4 Å²). The results of the maturation assay of WT-MccJ25 (**1**) and Tyr-9 MccJ25 analogs, Phe(4-OMe) **2**, Phe(4-F) **3**, Tyr(3-NO₂) **4**, Phe(3-OH) **7**, and Phe (**8**), mimic the mobilogram of the native lasso standard, indicating successful formation of lasso topology. Tyr-9 derivative

6' (Tyr(3-*t*Bu)) showed a CCS value of 481.3 Å², which falls between the lasso and the branched-cyclic standard. To unambiguously assign the peptide topology, a branched-cyclic version (**bc-6'**) was synthesized, and its CCS value was measured via LC-IM-MS (497.3 Å²), demonstrating the formation of a lasso peptide after enzymatic maturation of compound **6**. For aniline derivative **5'**, the similar mobilograms of product and branched-cyclic peptide (**bc-5'**) from LC-IM-MS analysis of the [M+2H]²⁺ species (**Fig. S86**) precluded definitive assignment. However, the formation of a stable rotaxane upon thermolysin digestion indirectly confirmed successful maturation of aniline derivative **5'**. The digestion of the corresponding chemically synthesized branched-cyclic peptide resulted in complete degradation. For the His-12 derivatives, the presence of lasso peptide was confirmed via LC-IM-MS analysis. All histidine derivatives bearing different heterocycles were successfully transformed to the corresponding lasso peptides. Moreover, configurations with both L- and D-His were accepted and transformed, demonstrating the high versatility of this approach.

We next evaluated all lasso peptides and their corresponding branched-cyclic peptides for antimicrobial activity in spot-on-lawn assays against *Salmonella enterica* serotype Enteritidis.⁴¹ As expected, the tested branched-cyclic peptides bearing L-His or D-His did not show antimicrobial activity, while enzymatically matured His-12 lasso derivatives **9–14** were indeed active. The derivatives bearing azole side chains (**9**, **10**, and **11**) displayed slightly stronger activity (**Fig. S78**). For Tyr-9 derivatives, activities were only observed for matured WT-McjA (**1**) and aniline derivative **5**, indicating the importance of the H-bond donor at this position, while H-bond acceptor properties of Tyr-9 only play a minor role (**Fig. S79**). Moreover, steric constraints in the tight binding pocket are a limiting factor for Tyr-9 substitutions. To further confirm the importance of lasso topology, the activity of the branched-cyclic versions of (**1**) and (**5**) were evaluated (**Fig. S80**) and showed no activity. In conclusion, this study establishes the broad substrate scope of maturation enzymes McjB and McjC for single non-canonical mutations, even at a key residue of MccJ25, enabling the rapid production of non-natural lasso peptides.

***In vitro* maturation of chemically synthesized McjA precursor peptides enables the production of MccJ25 derivatives previously not accessible.**

After the successful incorporation of D-His, we considered simultaneously exchanging all three substitutions reported by Link and coworkers (His-12, Phe-13, and Ile-15)¹⁸ to their corresponding D-amino acids (**Fig. 4C**), which could give access to antimicrobial lasso peptides with improved proteolytic stability. The synthesis of the precursor peptide was performed on the AFPS system, coupling the three D-amino acids with the same synthesis procedure used for the native peptide. The resulting McjA derivative **15** was transformed *in vitro* following our standard protocol, which yielded the lasso peptide as indicated by the CCS values (compared to the corresponding branched-cyclic analog (**bc-15'**)). The antimicrobial activity

of **15'** tested by a spot-on-lawn assay gave comparable results to the lasso peptides bearing only L-amino acids or one D-histidine, while the branched-cyclic analog **bc-15'** was inactive. Having access to an active derivative bearing three D-amino acids in the tail portion provides an opportunity for further design of antimicrobial lasso peptides with enhanced stability against proteases in the loop region.

To probe the limits of McjB and McjC promiscuity, we attempted to transform even more modified precursor peptides. First, we employed a synthetic D-McjA precursor to establish whether McjB and McjC could recognize it and synthesize a D-lasso peptide MccJ25. However, the maturation assay did not yield the desired D-lasso peptide. We observed the same result when only the core peptide sequence was replaced by D-amino acids. Next, we explored if McjB and McjC are capable of transforming precursor peptides of other lasso peptides. To this end, the transformation of chemically produced precursor McyA to microcin Y (MccY)⁴²—a lasso peptide sharing key structural features with MccJ25 despite having eight canonical substitutions in the core peptide—was performed. Although LC-MS analysis indicated only low conversion, the enzymes' ability to produce mature lasso peptide MccY using a different leader peptide is remarkable. Notably, by synthesizing and transforming mixed derivatives between McjA and McyA, we determined that the MccY core with the McjA leader did not show conversion, while the leader peptide of McyA is accepted with the MccJ25 core sequence.

Introducing backbone modifications is a common strategy to further improve peptide stability.⁴³⁻⁴⁵ However, they are very challenging to incorporate by purely biochemical methods and have never been reported for lasso peptides. Therefore, we synthesized McjA derivatives in which either loop residue Gly-12 alone or in combination with Gly-14 was *N*-methylated. Due to reported difficulties in the production of *N*-methylated peptides,⁴³ synthesis conditions were slightly adapted for couplings following the *N*-methylated amino acids, giving both peptides in good yield (8% respectively 5%). The peptides underwent enzymatic transformation to the cyclized product as discerned by LC-MS. To draw conclusions regarding the topology, branched-cyclic analogs were synthesized in excellent yields (23% and 20% respectively) and LC-IM-MS measurements allowed for clear differentiation: CCS values after enzymatic transformations were 473.7 Å² for singly and 479.4 Å² for doubly methylated lasso peptides, while the branched-cyclic derivatives (**bc-16'** and **bc-17'**) showed much higher values (492.9 Å² and 497.5 Å²). In summary, the unmatched synthetic flexibility of SPPS, paired with the remarkable versatility of the maturation enzymes, facilitated the effective integration of numerous ncAAs and backbone *N*-methylations into MccJ25. This process yielded several novel lasso peptide derivatives that were previously unattainable.

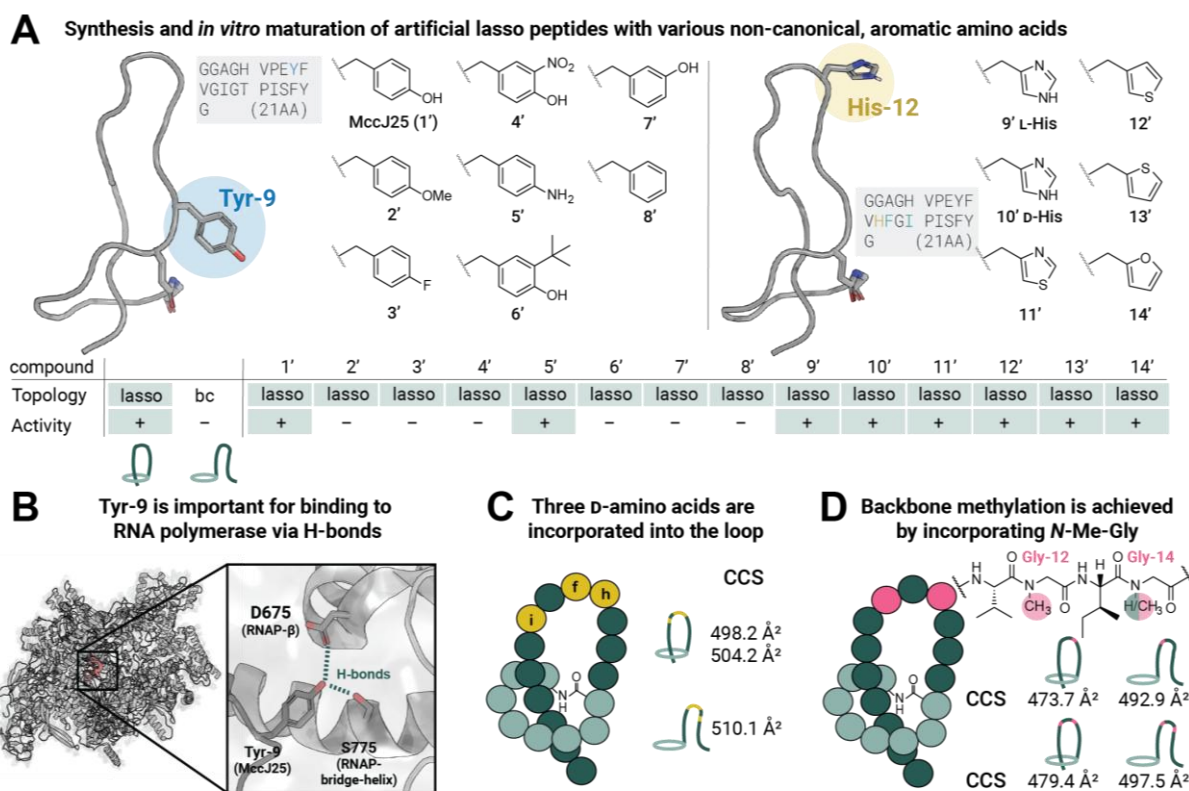


Figure 4. Investigation of non-canonical Tyr-9 and His-12 derivatives for their acceptance by enzymes and the resulting antimicrobial activity against *Salmonella enterica* serotype Enteritidis. (A) Structure of MccJ25 with Tyr-9 (PDB: 1Q71) and His-12 (mutated using PyMOL from PDB: 1Q71) side chain. The table indicates the transformation of all derivatives to lasso peptides with their corresponding CCS values and activity (+/green: active, -: inactive). (B) Binding of MccJ25 into the secondary channel of *E. coli* RNA polymerase sigma70-holoenzyme (PDB 6N60); highlighting the H-bonds of the Tyr-9 side chain inside the RNAP. (C) Schematic representation of MccJ25 with three substituted D-amino acids highlighted in yellow and CCS values of the corresponding lasso peptide and branched-cyclic peptide. (D) N-methylation of Gly-12 and additionally Gly-14 residue via incorporation of Fmoc-protected N-methyl glycine with CCS of the lasso peptide and the branched-cyclic peptide.

Discussion

In this study, we established the production of lasso peptides containing ncAAs via flow-based chemical synthesis of precursor peptide MccJ25 derivatives followed by enzymatic transformation to rapidly screen enzyme promiscuity and antimicrobial activity of target peptides. The production of the enzymes MccJ25 and MccJ26 was optimized to obtain a reproducible and robust protocol with improved yields. We demonstrated that these enzymes are capable of transforming all synthesized non-natural precursors into the lasso topology *in vitro*, enabling studies of their antimicrobial activity. While all His-12 derivatives were active, only the Tyr-9 and its aniline analog 5' retained activity, underscoring the importance of an H-bond donor at this position for biological activity. Inspired by a single point mutation with D-His in the lasso scaffold, we incorporated three D-amino acids into the loop region, which could lead to enhanced peptide stability.

Remarkably, installing potentially conformation-altering *N*-methylations in the loop region did not disrupt the enzymes' ability to mature the precursor peptide into the lariat knot topology.⁴³⁻⁴⁵ Overall, the incorporation of His-12 and Tyr-9 derivatives as well as several D-amino acids and backbone modifications demonstrate the potential of combining enzymatic maturation with chemical synthesis to obtain biologically active modified lasso peptides unattainable with current biological methods.⁴⁶ Furthermore, investigations of the related lasso peptide MccY gave insights into enzyme promiscuity regarding precursor recognition. McjB/McjC transformed native precursor McyA (albeit with low conversion), but not the mixed species of McjA leader and McyA core. This is in contrast to previous implications of the McjA leader peptide in the binding of peptidase McjB, and could indicate more nuanced interactions between leader peptide, core peptide and McjB/McjC lasso synthetase complex.^{47, 48} The successful conversion of McjA's core with McyA's leader into MccJ25, in turn, can be rationalized: the first six amino acids are identical, and McjA variants with minimal leader sequences are still (inefficiently) transformed.⁴⁹⁻⁵¹ These synthetic hybrid precursor peptides could complement computational tools to further study lasso biogenesis.^{52, 53}

The main advantage of our *in vitro* approach lies in the rapid production of a series of ncAA-containing McjA precursors for processing by maturation enzymes McjB and McjC. This permits the rapid progression to biological screening, circumventing potentially cumbersome genetic code expansion for each derivative. We also demonstrate the first incorporation of multiple ncAAs (three D-amino acids) and unnatural backbone modifications into MccJ25, a significant advancement for epitope grafting onto the lasso scaffold.¹⁵ On the backdrop of MccJ25's antimicrobial activity, this opens new avenues for discovering antibiotics with enhanced activity and stability.

The technology of flow-based chemical synthesis of precursor peptides could be further expanded to other *in vitro* lasso peptide maturation systems such as fusilassin⁵⁴ and fuscanodin⁵⁵ or cell-free biosynthesis procedures.⁵⁶ With over 80 discovered lasso peptides,¹² and more to be identified by genome mining,^{57, 58} there is significant room for future exploration. The combination of reliable chemical peptide synthesis and reproducible enzymatic expression creates a screening platform to investigate enzyme promiscuity and mechanism. This adds lasso peptides to the RiPP enzyme toolbox and could shed light on transformation mechanisms as well as offer new possibilities to add novel functionalities, paving the way for RiPP-based therapeutics with improved drug-like properties.⁵⁹

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Author contributions

The project was conceptualized by K.S. and N.H.; K.S. synthesized all peptides with assistance of C.E.G.; enzyme expression was performed by K.S. with assistance of M.S. and R.S.; transformation of synthetic peptides was performed by K.S., experimental data for LC-IM-MS was collected by J.L. and analyzed by J.L. and K.S., the antimicrobial assays were performed by Y.L., the manuscript was written by K.S. and N.H. with input from all co-authors.

Competing interests

The authors have no competing interests to declare.