UNIVERSITY OF AMSTERDAM

UvA-DARE (Digital Academic Repository)

Tying peptide ropes

van Maarseveen, J.H.

DOI
10.1038/s41557-021-00771-6
Publication date
2021
Document Version
Final published version
Published in
Nature Chemistry

License
Article 25fa Dutch Copyright Act (https://www.openaccess.nl/en/in-the-netherlands/you-share-we-take-care)
Link to publication

Citation for published version (APA):
van Maarseveen, J. H. (2021). Tying peptide ropes. *Nature Chemistry*, *13*(9), 822-823. https://doi.org/10.1038/s41557-021-00771-6

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: https://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.

UvA-DARE is a service provided by the library of the University of Amsterdam (https://dare.uva.nl)

Download date:06 Feb 2025

Check for updates

INTERLOCKED MOLECULES

Tying peptide ropes

Although the natural lasso peptide microcin J25 remains an elusive target for total chemical synthesis itself, this topologically non-trivial building block has now been used to construct a range of interlocked molecular architectures including rotaxanes, catenanes and daisy chains.

Jan H. van Maarseveen

Microcin J25 (MccJ25) is, in many ways, an ordinary peptide that is made up of 21 canonical amino acids stitched together with amide bonds. It was isolated almost 30 years ago[1], but why did it take more than a decade to unravel its structure? The answer lies in its unique lariat fold in which the tail of the peptide is threaded through a macrocyclic portion of the structure to form a three-dimensional arrangement reminiscent of a lasso (Fig. 1a). Based on extensive NMR spectroscopy and mass spectrometric analysis, its structure was independently reported[2–4] by three groups in the same issue of the *Journal of the American Chemical Society* in 2003.

The biosynthesis of MccJ25 involves several enzymes to facilitate synthesis of the linear ribosomally prepared leader-peptide, folding of the peptide chain into a proto-lasso and finally, locking of the lasso architecture

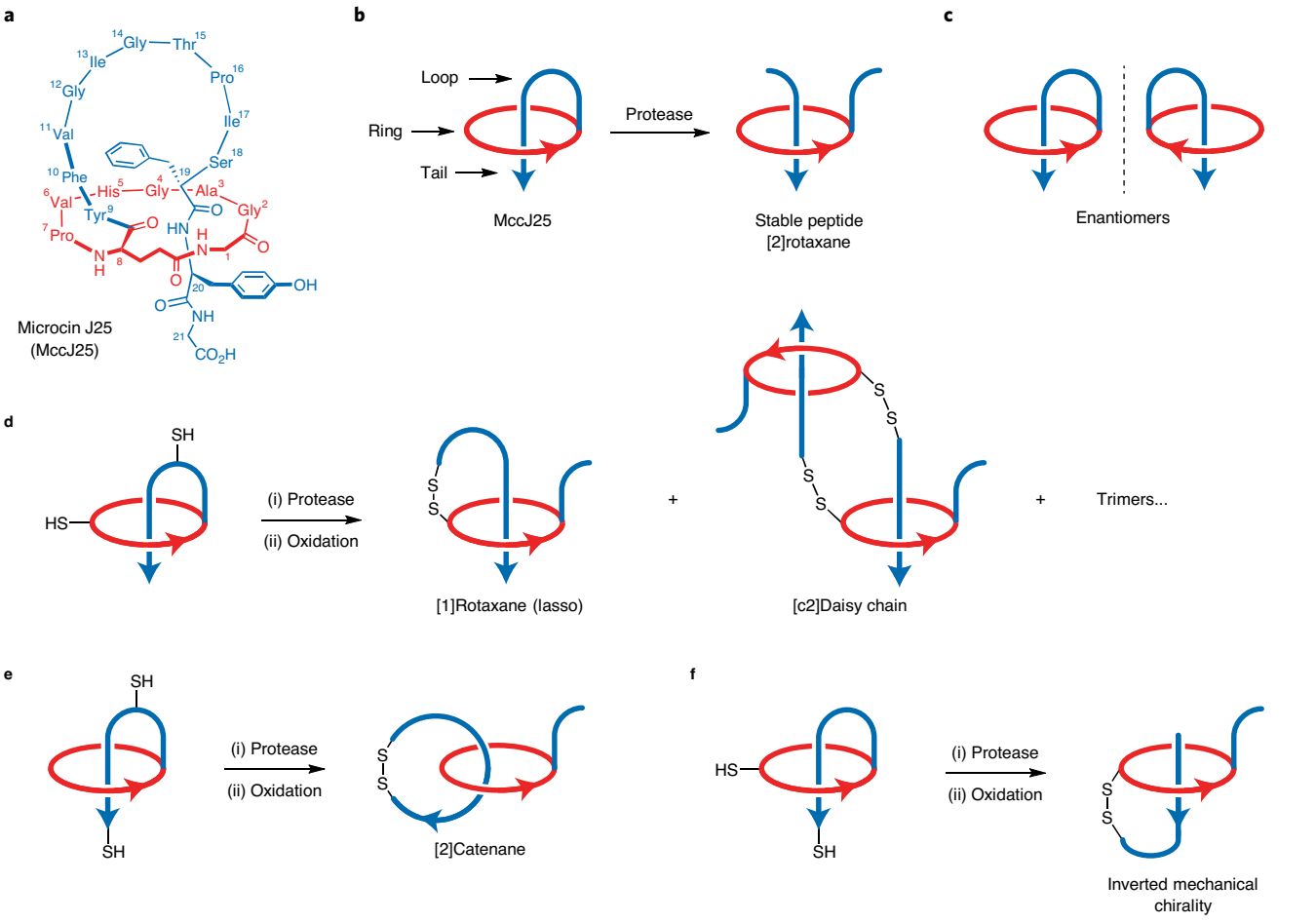

Fig. 1 | A lasso peptide and its conversion into other structures. a, Molecular structure of lasso peptide MccJ25. **b**, Biocatalytic loop cleavage within MccJ25 to give a [2]rotaxane. The arrows represent the backbone amide bond N→C directionality. **c**, Mechanical chirality arises from the backbone directionality. **d**, Transformation of a loop-and-ring cysteine-modified lasso peptide into a series of interlocked structures: [1]rotaxane (monomer), [c2]daisy chain (dimer) and trimer. The predominant product is the dimeric head-to-tail daisy chain. **e**, Transformation of a loop-and-tail cysteine-modified lasso peptide into a [2]catenane. **f**, Transformation of a ring-and-tail cysteine-modified lasso peptide into a [1]rotaxane with inverted mechanical stereochemistry as compared to the precursor lasso peptide.

by the formation of an amide bond between the glycine N terminus and the sidechain of a glutamic acid residue[5]. The resulting 26-membered macrocycle — encircling the C-terminal tail — is small enough that the steric encumberance exerted by the benzylic sidechains of the phenylalanine and tyrosine residues (Fig. 1a; 19 and 20) at each side of the macrocycle prevent the lasso from unthreading. The strength of this natural mechanical bond is exemplified by the survival of MccJ25 in boiling water, even with prolonged exposure[5]. Cleavage of an amide bond within the lasso loop fragment to afford a peptide [2]rotaxane (Fig. 1b) does not affect the extraordinary strength of this mechanical bond either. The tight binding of the thread into the relatively small ring gives a densely packed globular shape that not only resists thermal decomposition, but also proteolytic degradation. To date, over 80 lasso peptides have been isolated and pharmacological evaluation indicates their potential as antibiotics featuring new modes of action, but also as antiviral and anticancer drugs[5].

The directionality of the amide bonds in the backbone introduces another challenging feature that hampers the total synthesis of lasso peptides, namely mechanical chirality (Fig. 1c). The way in which the macrocycle forms around the linear portion of the peptide results in two pseudoenantiomeric lasso structures (they are not true enantiomers because the configuration of the individual amino acid building blocks remains the same in each compound, but from a topological point of view they are mirror images). The combination of the tight mechanical bond, unique stereochemistry and promise as future drugs makes the lasso peptide series an appealing target for total synthesis. Unfortunately, current methodologies used to prepare mechanically interlocked molecules typically fall short because lasso peptides lack the usual supramolecular or covalent templating features to pre-organize the ring and thread precursors for mechanical bond formation[6,7]. A total synthesis of lasso peptide BI-32169 has been disclosed[8] but whether this method will prove useful for making other lasso peptides is yet to be confirmed.

So, this begs the question of whether it is possible to use the natural biomachinery to make such compounds? In recent times, several groups have independently unpicked the biosynthetic sequence of the reactions used in the production of lasso peptides[5]. Meanwhile, progress in synthetic biology obviates a pure chemical synthesis approach — not only are native lasso peptides accessible, but also analogues that incorporate non-canonical amino acids[9] through recombinant tweaking. Now, writing in *Nature Chemistry*, a group of researchers led by A. James Link at Princeton University in the US has taken the synthesis of lasso peptides a step further[10] in a creative fashion.

Taking advantage of the 'unbreakable' lasso fold in MccJ25, analogues featuring two or three cysteine residues — introduced at specific positions in the respective ring, loop or tail fragments (Fig. 1d–f) — have been prepared biosynthetically via site-directed mutagenesis and expression in BL21 cells. Subsequent protease-mediated cleavage at a specific amide bond in the loop fragment yields a stable peptidic [2]rotaxane that acts as a scaffold amenable for further chemical diversification. Depending on the position of the cysteines, ring closure under dynamic covalent conditions by oxidative disulfide formation yields, as mainly determined by collision-induced dissocation tandem mass spectrometry, a range of unique mechanically interlocked peptides such as [c2]daisy chains, catenanes and even a lasso peptide with inverted mechanical chirality!

As with the parent MccJ25 compound, these unique mechanically interlocked peptidic architectures are inaccessible through current synthetic chemistry methodologies. However, the combination of biological and chemical synthesis opens up interesting horizons. For example, instead of oxidation, the cysteine sulfhydryl groups may also react with multivalent electrophilic scaffolds such as bis- or tris(bromobenzylic) benzenes to introduce robust covalent rigidification via macrocycle formation[11]. Such modifications will further increase the exceptional stability of lasso peptides against metabolic degradation. The inclusion of some non-canonical amino acids containing sidechains carrying functional groups with bio-orthogonal reactivities offers, next to cysteine-derived sulfhydryl groups, further selective handles for rigidification or decoration with additional pharmacophores or fluorophore tags. Genome mining has shown the existence of thousands of lasso peptides, so in combination with the biosynthetical and chemical techniques developed for MccJ25, the unique structural diversity shown by this pharmacologically promising class can be fully explored[12].

It may be expected that a practical and generally applicable chemical total synthesis of lasso peptides lies far in the future[6–8]. Nevertheless, lasso peptides provide extraordinary challenges for the synthetic chemistry community because the problems lying ahead are beyond those generally encountered for classical small-molecule secondary metabolites. The crux of the challenge does not lie on the development of novel methodology to make bonds, but on precise regio- and stereoselective folding of a linear precursor into the structurally dense and mechanically chiral lariat conformation using bio-inspired templates that are either supramolecular or covalent in nature. Instead of aiming at total syntheses, it may be even more useful to prepare structurally related analogues that mimic or even increase biological activity[6–8]. Considering the limitations of the biological toolbox, which is made up of the 20 canonical amino acids with just a few additional non-natural analogues that can be introduced by recombinant tweaking, chemical synthesis allows a far wider molecular diversity. Furthermore, unlike the current biological synthesis of lasso peptides, chemical synthesis is scalable.

Lasso peptides will most certainly remain a fruitful research target that encourages collaborations in many fields, including synthetic biology, chemical synthesis, computational chemistry and pharmacochemistry. The development of robust syntheses towards larger amounts of material is key in exploring the application of these unique motifs in materials science; for example, use of something like the peptidic [c2]daisy chain that was produced in this study in a device to mimic the contraction of natural muscles. ❐

Jan H. van Maarseveen ✉
Van 't Hoff Institute for Molecular Sciences, University of Amsterdam, Amsterdam, The Netherlands.
✉*e-mail: j.h.vanmaarseveen@uva.nl*

Published online: 23 August 2021
https://doi.org/10.1038/s41557-021-00771-6

References

1. Salomón, R. A. & Farías, R. N. *J. Bacteriol.* **174**, 7428–7435 (1992).
2. Bayro, M. J. et al. *J. Am. Chem. Soc.* **125**, 12382–12383 (2003).
3. Rosengren, K. J. et al. *J. Am. Chem. Soc.* **125**, 12464–12474 (2003).
4. Wilson, K.-A. et al. *J. Am. Chem. Soc.* **125**, 12475–12483 (2003).
5. Hegemann, J. D., Zimmerman, M., Xie, X. & Marahiel, M. A. *Acc. Chem. Res.* **48**, 1909–1919 (2015).
6. Martin-Gómez, H. & Tulla-Puche, J. *Org. Biomol. Chem.* **16**, 5065–5080 (2018).
7. Rowe, S. M. & Spring, D. R. *Chem. Soc. Rev.* **50**, 4245–4258 (2021).
8. Chen, M., Wang, S. & Yu, X. *Chem. Commun.* **55**, 3323–3326 (2019).
9. Piscotta, F. J., Tharp, J. M., Liu, W. R. & Link, A. J. *Chem. Commun.* **51**, 409–412 (2015).
10. Schröder, H. V., Zhang, Y. & Link, A. J. *Nat. Chem.* https://doi.org/10.1038/s41557-021-00770-7 (2021).
11. Heinis, C., Rutherford, T., Freund, S. & Winter, G. *Nat. Chem. Biol.* **5**, 502–507 (2009).
12. Si, Y., Kretsch, A. M., Daigh, L. M., Burk, M. J. & Mitchell, D. A. *J. Am. Chem. Soc.* **143**, 5917–5927 (2021).

Competing interests

The author declares no competing interests.