Accelerated Microfluidic Native Chemical Ligation at Difficult Amino Acids Toward Cyclic Peptides

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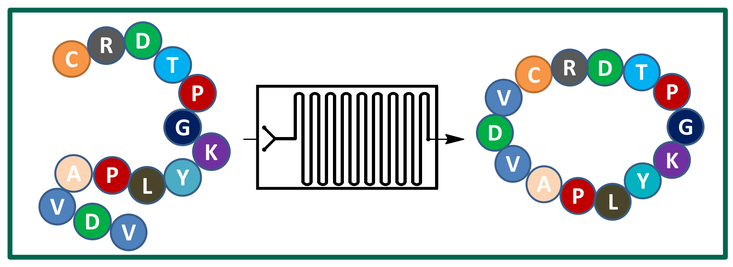
1 | Introduction

Synthetic peptide-based therapeutics have a bright forecast

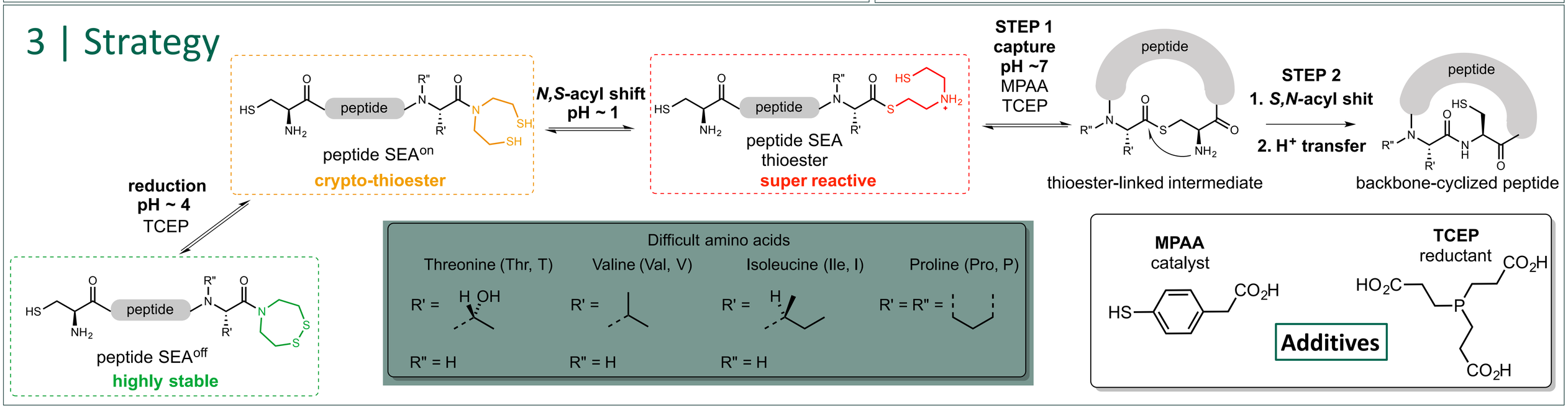
- 70+ approved peptide APIs (+140 clinical trials; 500+ preclinical development)
- Pressing demand for developing new synthetic technologies compatible with increasing regulatory constraints, versatility and fast time-to-market
- In phase with the actual transitioning toward flow and microfluidic technologies for pharmaceutical production, the combination of microfluidics and peptide production has gained significant attention over the last decade

2 | Aim

The aim was to develop and **implement** a **telescoped** continuous flow strategy for the generation and usage of a **highly unstable/super reactive thioester species** toward the preparation of **cyclic peptides** under **microfluidic conditions**.

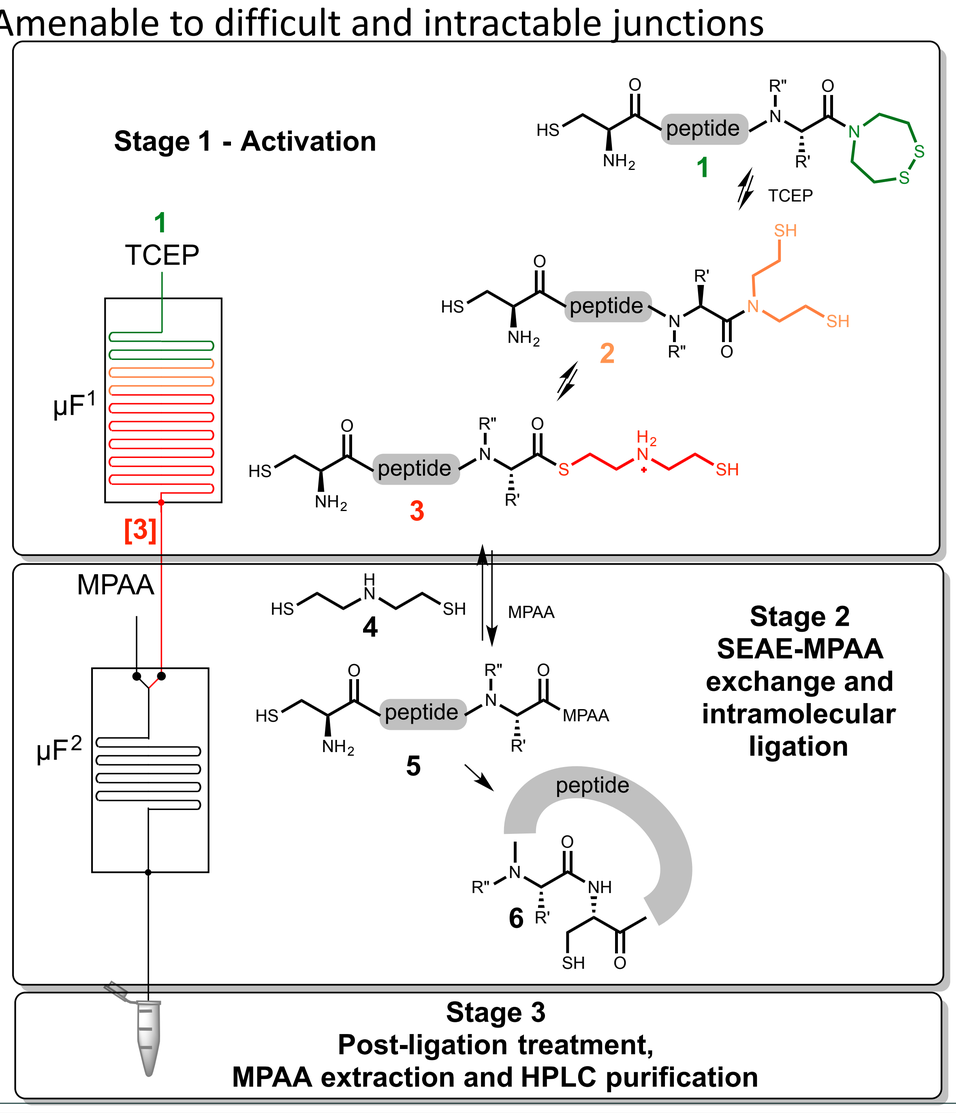


3 | Strategy

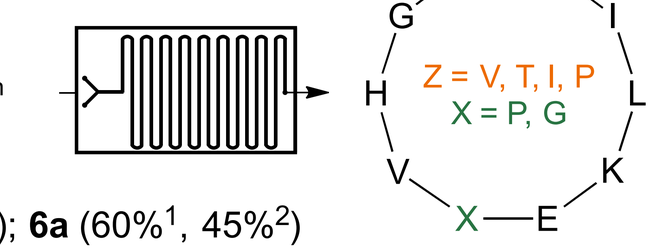


4 | Microreactor

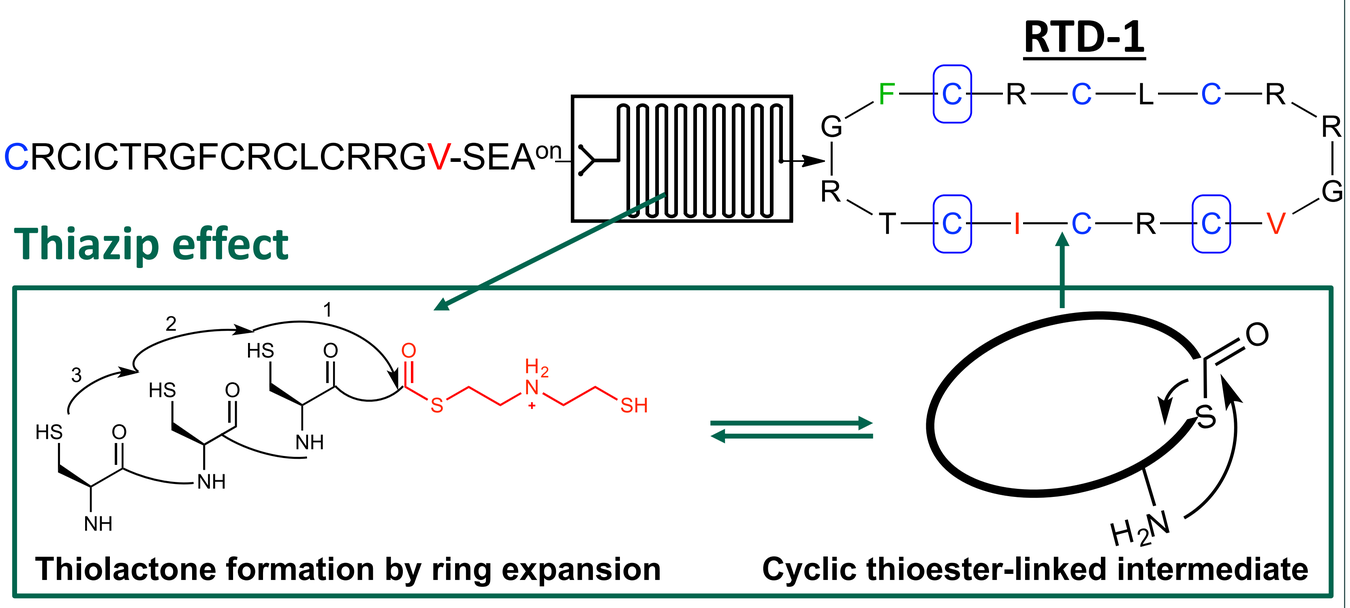
- ✓ Precise control of pH and residence time
- ✓ Theoretically unlimited production
- ✓ Generation and use of unstable/highly reactive thioester species from stable amides
- ✓ Extremely fast cyclative ligations (down to 2 min *vs* 48 h under conventional conditions)
- ✓ Amenable to difficult and intractable junctions



5 | Results



CILKEPVHGV-SEA^{on} (**2a**); **3a** (94%); **6a** (60%[1], 45%[2])
CILKEGVHGV-SEA^{on} (**2b**); **3b** (91%); **6b** (85%[1], 46%[2])
CILKEGVHGT-SEA^{on} (**2c**); **3c** (90%); **6c** (90%[1], 50%[2])
CILKEGVHGI-SEA^{on} (**2d**); **3d** (88%); **6d** (96%[1], 51%[2])
CILKEGVHGP-SEA^{on} (**2e**); **3e** (74%); **6e** (84%[1], 38%[2])
[1]HPLC Conversion, [2]Overall Yield



CRCICTRGFCRCLCRRGV-SEA^{on} (**2f**); T^1 = 90 °C, T^2 = 45 °C, t^2 = 15 min, **6f** (71%[1])
CRC(-S*t*Bu)ICTRGFCRCLCRRGV-SEA^{on} (**2f'**); T^1 = 90 °C, T^2 = 45 °C, t^2 = 15 min, **6f'** (82%[1])
CRAIATRGFARALARRGV-SEA^{on} (**2g**); T^1 = 90 °C, T^2 = 37 °C, t^2 = 4 min, **6g** (71%[1])
CTRGFCRCLCRRGVCRCI-SEA^{on} (**2h**); T^1 = 90 °C, T^2 = 45 °C, t^2 = 15 min, **6h** (69%[1])
CRCLCRRGVCRCICTRGF-SEA^{on} (**2i**); T^1 = 65 °C, T^2 = 45 °C, t^2 = 15 min, **6i** (83%[1])
[1]HPLC Conversion

6 | Conclusion

The development of a **telescoped continuous flow** strategy for the preparation of **cyclic peptides** of various sizes was successfully implemented in a **microreactor** setup. Upon optimization of the entire flow process, three type of cyclic peptides (POL, 10 residues; RTD-1, 18 residues and F_2-K_1, 28 residues) were synthesized in a **compact** microfluidic setup**. Unprecedented short cyclative ligation rates** (< 5 min) were obtained even for **difficult** and **intractable** junctions in conversions ranging from 60 to 96% depending on the peptide sequence and the ligation site. The microfluidic system is **flexible** and **versatile**, and could be easily adapted to the inherent specificities of various cyclic peptides.

7 | Acknowledgements

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