Cyclotides and Macrocylic Peptides as Therapeutics

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Introduction

Cyclotides or macrocycles are small, disulfide-rich mini proteins or peptides found in plants. Their unique structure results in their high stability. Most proteins unfold when heated or exposed to stomach acid, however cyclotides are highly stabile. According to Deegala et al. (2025) more than 760 cyclotides have been identified in five major families, making them the largest known group of cyclic peptides.

In nature, plant cyclotides act like pesticides. When an insect or a caterpillar eats the plant, the cyclotides bind to the membranes in the insect's gut and create holes, eventually causing the cells to burst. Beyond plant defense, the indestructible nature of cyclotides makes them stable scaffolds for drug design.

The high stability of a cyclotide is a result of the Cyclic Cystine Knot (CCK) motif defined by two main features:

(1) **Head-to-Tail Cyclization** in which the protein backbone is a continuous, unbroken circle with no free "ends" for digestive enzymes to recognize, and
(2) the **Cystine Knot with six cysteine residues forming three disulfide bonds** in which two bonds form a ring, and the third passes through the middle, effectively "locking" the structure in place. Because of this, cyclotides can withstand boiling water and highly acidic environments without losing their shape or function.

Discovery and Studies of Cyclotides

During a Red Cross Relief Mission in Democratic Republic of Congo in the 1960s, Dr. Lorents Gran, observed that women in that region used '*kalata-kalata*', a medicinal tea made from the leaves of the plant *Oldenlandia affinis* to induce labour and facilitate smooth and painless deliveries. The purified active ingredient of *O. affinis* was identified as a peptide, named '*kalata B1*.' Saether et al. (1995) determined the structure of Kalata B1 as a cyclic cystine knot motif peptide now known as a cyclotide.

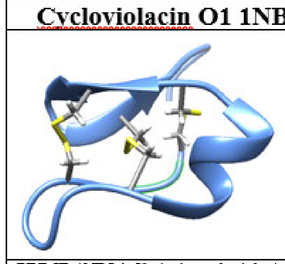
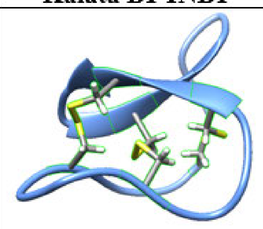
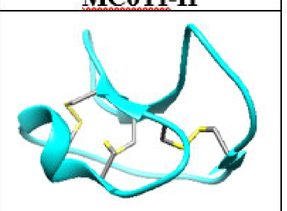
A few years later, Daly et al. (1999) reported the chemical synthesis of the cyclic peptide kalata B1. As reported by Craik et al. (1999), these cyclic peptides were first discovered in plants from the Rubiaceae (coffee) and Violaceae (violet) families but are also found in a range of other plants from the Cucurbitaceae (cucurbit) and Fabaceae (legume) families and appear to be widely distributed within the plant kingdom.

Plant sources of cyclotides

***C.ternatea*, *O. affinis*, *V. odorata*, *M. cochinchinensis*, *P. hybrida*.**

Cyclotide Peptide Structures

Solved Structures of Cyclotides

Cycloviolacin O1 1NBJ	Kalata B1 1NB1	MCoTI-II
		
PDB ID 1NBJ A Chain A, cycloviolacin O1	PDB ID 1NB1 A Chain A, kalata B1	PDB ID 4GUX D Chain D, Trypsin inhibitor 2

(Rosengren et al. 2003)

Chemical Synthesis Strategies

Several chemical strategies allow the synthesis of bicyclic peptides.

(a) **Head-to-tail cyclization**.

(b) Synthesis through an **on-resin intramolecular thioester ligation** and an **off-resin DMSO-mediated disulfide formation**.

(c) Synthesis by **ring-closing metathesis**.

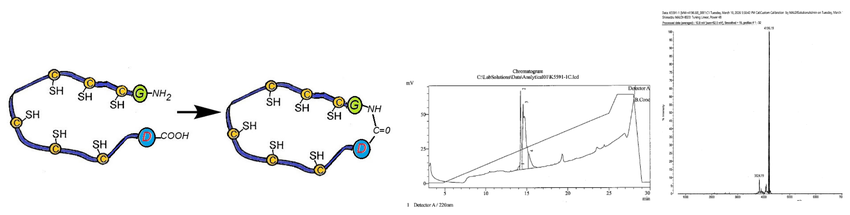
(d) Synthesis by **formation of C–C bond through an intramolecular palladium-catalyzed C–H activation process** between tryptophan and iodinated tyrosine or phenylalanine.

(e) Synthesis through **ring-closing alkyne metathesis**.

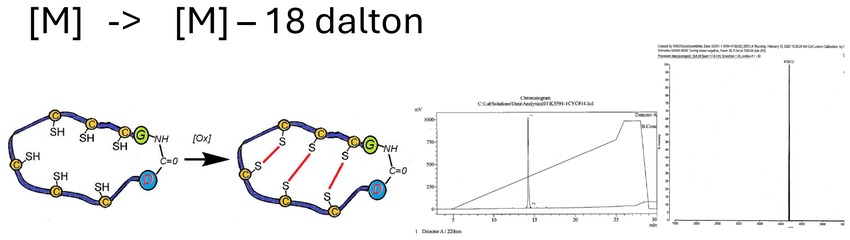
(f) Synthesis through the **formation of an amide bond between the α- or γ-carboxyl group of glutamate and the N-terminus**, then subjected to amide-bond formation conditions to perform the second cyclization.

Our Approach

- Synthesize linear peptide using SPPS.
- Cyclize using Head-to-Tail cyclization.
- Oxidize peptide to form cysteine bonds.



[M] -> [M] – 18 dalton



[M] – 18 - 6 dalton

Potential Use of Cyclotides

Use	Notes
Drug Delivery & Development	A **cyclotide scaffold** allows "grafting" of medicinal sequences into the scaffold for the design of drugs that can be taken orally without being destroyed by the stomach. Their rigid, circular structure allows them to act as scaffolds for grafting, making them ideal for developing stable, orally active drugs that target intracellular proteins.
Bioimaging	Design of molecular imaging agents for ultrasound, positron emission tomography (PET), or single-photon emission computed tomography (SPECT) imaging as well as others, as suggested by Gould & Camarero.
Anti-viral & Therapeutic, Anti-HIV or Cancer	Some cyclotides inhibit HIV entry or target cancer cell membranes. Potential treatments for HIV (CXCR4 antagonism), dengue virus (protease inhibition), and multiple sclerosis (immunomodulation).
Agriculture	Cyclotides allow the design and production of potentially eco-friendly, peptide-based sprays to protect crops from pests. Used as potent insecticides and anthelmintics (worm control) due to their natural role in protecting plants against pests.
Oncology	Investigated for anti-tumor and anti-metastatic properties, including inhibiting tumor angiogenesis and acting as CTLA-4 antagonists.
Other Uses	Evaluated for use in ultrasound and PET imaging of tumors, and for treating obesity, diabetes, and inflammation

Conclusions

Utilizing available synthesis strategies, we are able to synthesis a variety of cyclotides and macrocyclic peptides as well as peptide scaffolds.

The design and synthesis of conjugates or biotinylated cyclotides is also possible by tailoring scaffold sequences. We are also able to conjugate cyclotides and macrocyclic peptides to cargo molecule directly or though linkers.

References

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