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<td>Wang, Yingqi</td>
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Chemical Synthesis of Cyclotide from the Medicinal Plant

*Clitoria ternatea*

Wang Yingqi, Nguyen Thi Kim Ngan, and James P Tam

**Introduction**

Cyclotides are a special class of ultrastable peptides which possess a cyclized backbone and three disulfide bridges. They display many bioactivities and are attractive targets for drug discovery. The medicinal plant *Clitoria ternatea* contains a high abundance cyclotides, named as cliotides (cT), which have been shown to constitute the active ingredients of *C. ternatea*. cT19 is one of the cliotides that showed strong antibacterial and immunomodulatory activities. Native cT19 yield from conventional extracting methods is relatively low and it is difficult to separate cT19 from its deaminated form cT19a. With the recent understanding of cT19’s structure and amino acid sequence, chemical synthesis of cT19 is made possible.

**Methodology**

- Purify folded cT19
- Check by co-elution with native cT19
- Fold with best folding condition
- Test folding conditions

**Results**

Images generated by High-performance liquid chromatography

**I. Cyclization**

<table>
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<tr>
<th>cT19-hydrazide</th>
<th>Cyclized cT19 4mM</th>
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<tr>
<td>cT19-hydrazide</td>
<td>after PREP-HPLC purification</td>
</tr>
<tr>
<td>Cyclized cT19 1mM (Adjusted concentration)</td>
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</table>

Cyclized cT19 was obtained for further folding.

**II. Folding: Test Conditions: 0.1mg/ml cT19, cT19:GSH:GSSG=1:150:5, RT with different co-solvent**

- 50% PrOH
- H2O
- 50% TFE (24hr)
- 50% TFE (46hr)

Thus, 50% TFE was chosen as the co-solvent, the incubation time was 46hr.

**III. Co-elution & Yield**

0.1mg/ml cT19 Co-Elution with Native cT19

The sample co-eluted with native cT19.

**Calculating Yield:**

Starting material:

- cyclized cT19 0.1mg/ml

Folded cT19

| Area=2330444 | Area=488035 |

Yield = \( \frac{488035}{2330444} = 20.9\% \)

**Conclusion**

The peptide cT19 was successfully synthesized. The folding condition provides information for further study on cyclotide folding. Further studies can be done on better folding conditions to achieve a higher yield.

**Acknowledgement**

I want to express my heart-felt thanks to Prof James P. Tam, my mentor Miss Kim and other lab members for their help on the project.

**Reference**


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**Project Title:** Chemical Synthesis of Cyclotide from the Medicinal Plant *Clitoria ternatea*

**Supervisor:** A/P Sze Siu Kwan

**Co-supervisor:** Prof James P. Tam

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**Project:** Cyclized cT19 was obtained for further folding.

The sample co-eluted with native cT19.

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**Category:** 6

**School of Biological Sciences**

**Student:** Wang Yingqi

**Project ID:** SBS12050

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**URECA Undergraduate Research Experience on Campus**