https://doi.org/10.17952/37EPS.2024.P1297

Three-dimensional structure and membrane permeability relationship of cyclopropane-restricted cyclic peptides

Yuki Yamazaki[1], Hiroyuki Kumeta[2], Satoshi Shuto[1] and Mizuki Watanabe[1]

[1]Faculty of Pharmaceutical Sciences and [2]Faculty of Advanced Life Science, Hokkaido University, Sapporo, Hokkaido 060-0812, Japan

Background

Cyclic peptides improve

- ✓ the stability against proteolytic degradation.
- ✓ bioactivity and selectivity for the target protein.

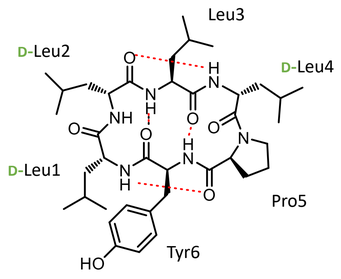
Cyclic peptides are expected to be effective for regulating "undruggable" targets.

But

The control of cell membrane permeability is a major issue.

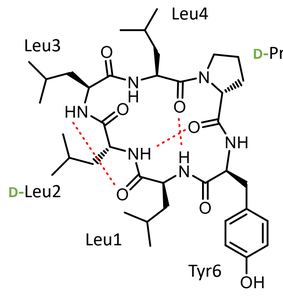
Membrane permeability of cyclic peptides is strongly related to the 3D structure.

cyclo[D-Leu-D-Leu-Leu-D-Leu-Pro-Tyr]



High permeability
Pe : 0.63×10^{-6} cm/s
(PAMPA)

cyclo[Leu-D-Leu-Leu-Leu-D-Pro-Tyr]

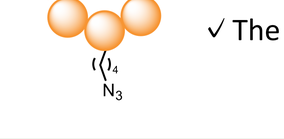


Low permeability
Pe : $<0.01 \times 10^{-6}$ cm/s
(PAMPA)

Lokey, R. S. *et al. J. Am. Chem. Soc.* **2006**, *128*, 2510.

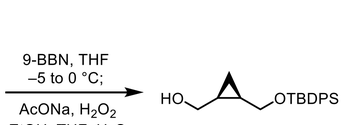
Previous work

α,δ-Disubstituted cyclopropane δ-amino acid (CPA)

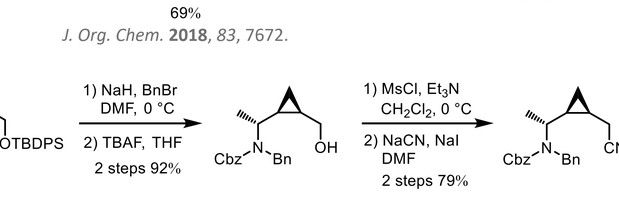


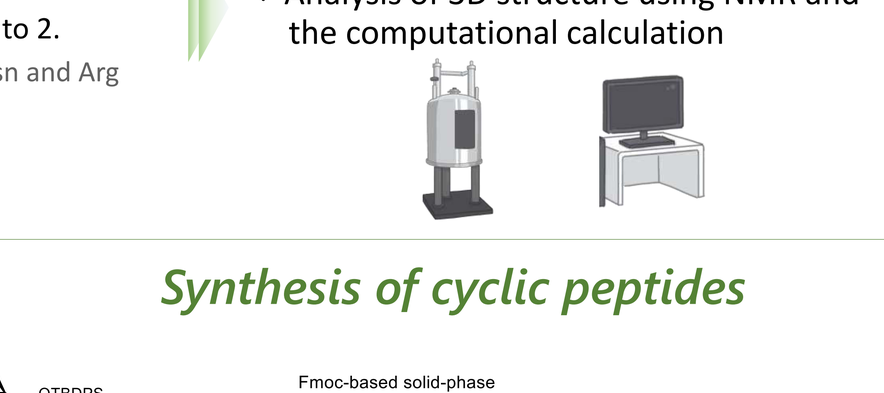
The conformation of CPA is strongly restricted by the properties of cyclopropane.

cis/trans restriction



Cyclopropylic strain



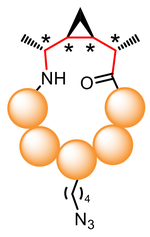


Chem. Eur. J. **2017**, *23*, 3034.

- ✓ CPAs can regulate the conformation of the peptide backbone.
- ✓ The cyclic peptide that contains a particular stereoisomer of CPAs showed remarkably high membrane permeability.

This work

Design and synthesis of cyclic hexapeptides introducing a ***cis*-NfCf**, the specific stereoisomer of CPA.



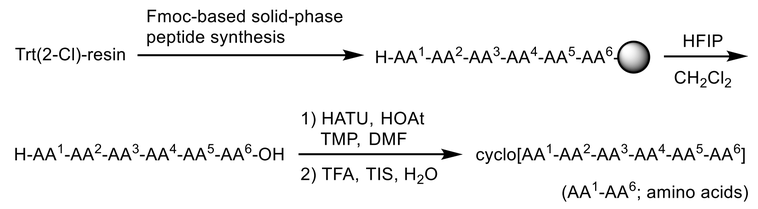
- ✓ The number of polar amino acids is 0 to 2.
  → Ser, Tyr, Thr, Gln, Asn and Arg
- ✓ The clogP range is 4.4 to 7.4.

- ✓ Evaluation of membrane permeability

- ✓ Analysis of 3D structure using NMR and the computational calculation

Synthesis of the cyclopropane δ-amino acid

Synthesis of cyclic peptides

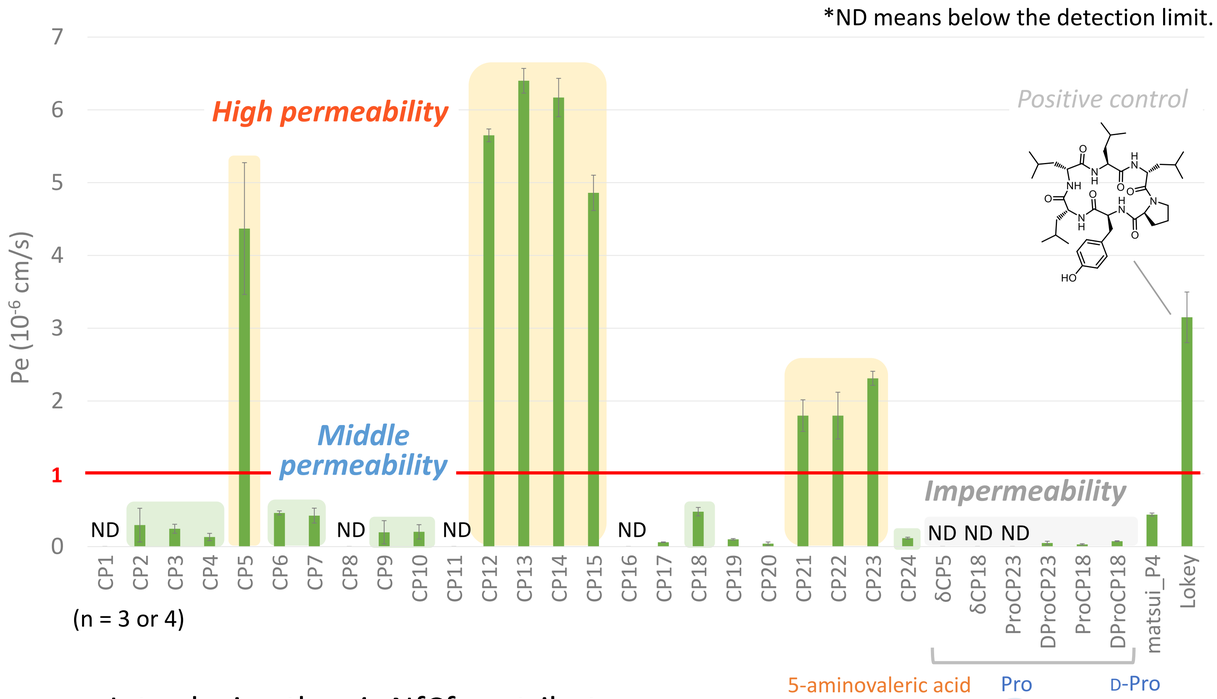


1) HATU, HOAt

Thirty cyclic peptides with different amino acid sequences and azido group positions were synthesized.

Evaluation of membrane permeability using PAMPA

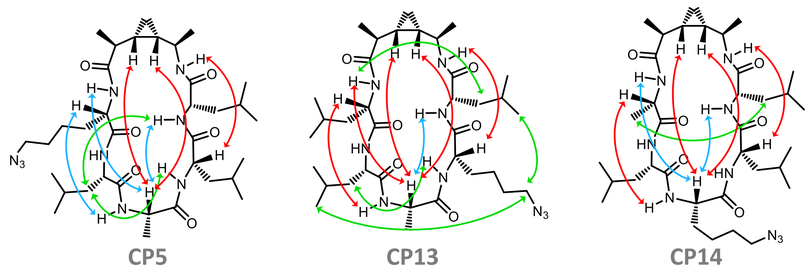
*ND means below the detection limit.



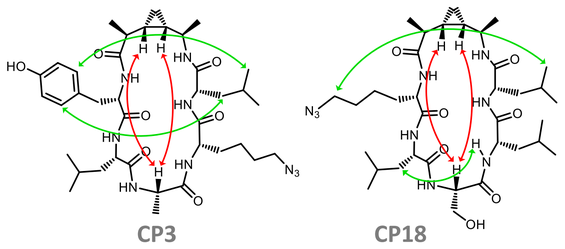
Introducing the *cis*-NfCf contributes significantly to improving the membrane permeability of the peptides.

NMR Analysis of 3D structure *(DMSO-d_6 or $CDCl_3$, 600 MHz)*

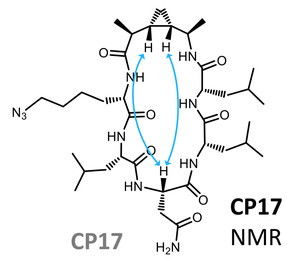
High permeability



Middle permeability



Low permeability



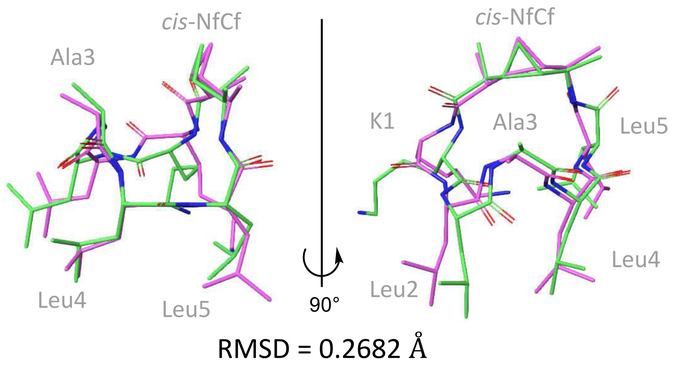
CP17 gave no assignable NMR peaks in $CDCl_3$.

Long-range ROE correlation
- both in DMSO-d_6 & $CDCl_3$
- only in DMSO-d_6
- only in $CDCl_3$

The backbone conformation of cyclic peptides would be similar in DMSO-d_6 and $CDCl_3$ despite passive permeability.

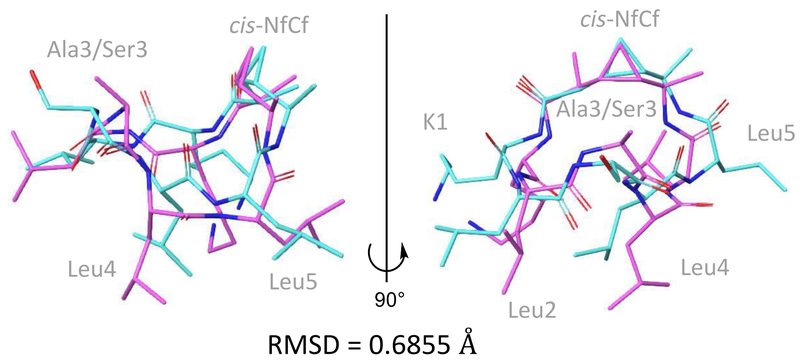
3D structural calculations based on NMR

CP5 in DMSO-d_6 vs $CDCl_3$



RMSD = 0.2682 Å

CP5 vs CP18 in DMSO-d_6



RMSD = 0.6855 Å

(Calculation: CYANA ver 2.1, Conditions: distance constraints of ROE signals; < 5Å, View: MacroModel 13.2)

3D structures of the cyclic peptide's main chains had a **similar "closed" conformation** regardless of the amino acid sequences and environments.

- ✓ We succeeded in controlling the 3D structure in a sequence-independent manner by introducing the *cis*-NfCf into cyclic peptides.
- ✓ These results indicate that cyclic peptides don't have sufficient passive permeability even when controlled to a "closed" conformation.