

Single Atom Contraction of Macrocycles via Cornforth Rearrangement

2023.08.05. Literature Seminar
M1 Takahiro Migita

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1. Introduction

2. Main Article



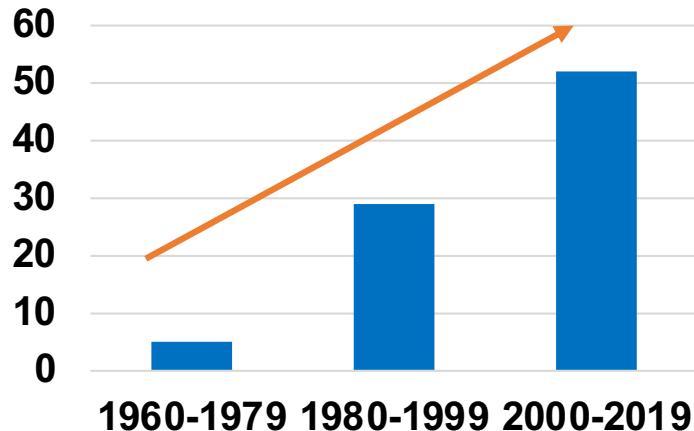
Research Article | Full Access

Single Atom Ring Contraction of Peptide Macrocycles Using Cornforth Rearrangement

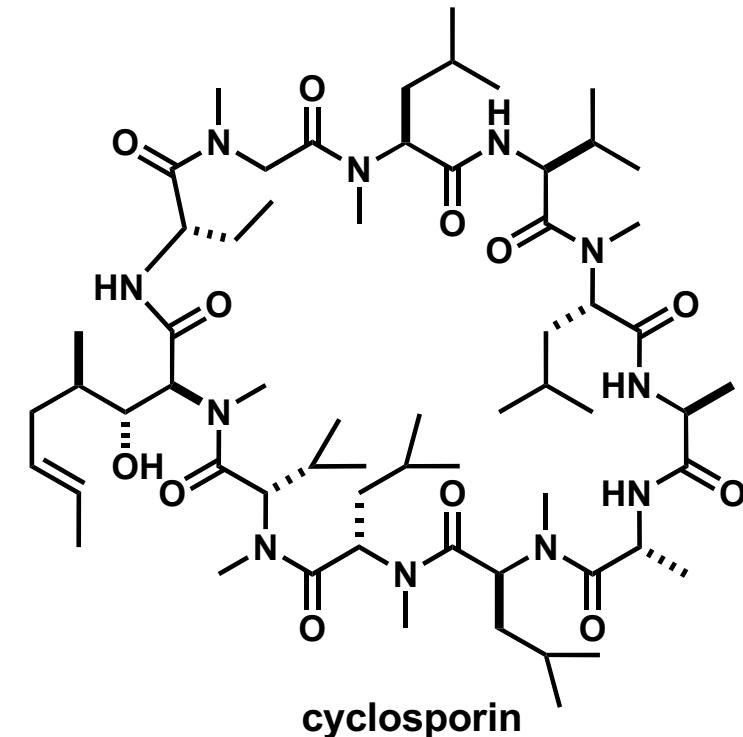
Dr. Sungjoon Huh, Dr. George J. Saunders, Prof. Andrei K. Yudin

Peptides in Drug Development

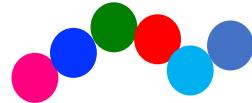
- *Peptides are potent modality.*



- >50 in the market
- >170 in clinical development
- >200 at preclinical studies

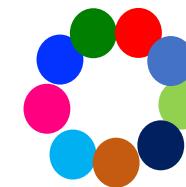


- ***linear peptides vs. cyclic peptides***



linear peptide

- hormone (e.g., insulin)
 - usually, impermeable
 - subject to proteolytic degradation



cyclic peptide

- some of them show **cell permeability**
 - **resistant** against proteolytic degradation
 - **affinity** with target protein

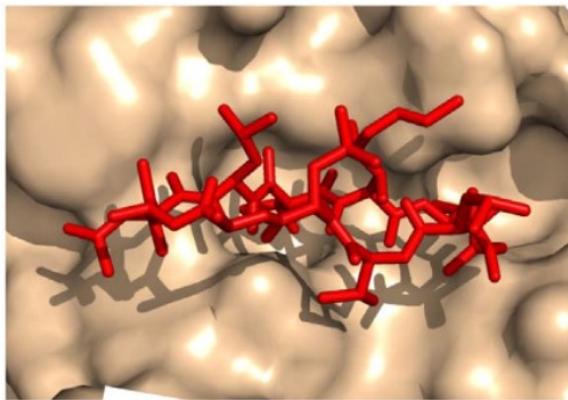
Cyclic peptides are prominent drug seeds to inner cell targets

1) Henninot A.; Collins, J. C. ; Nuss, J. M. *J. Med. Chem.* **2018**, *61*, 1382.

2) Muttenthaler, M.; King, G.F.; Adams, D.J.; Alewood, P. F. *Nat. Rev. Drug. Discov.* **2021**, *20*, 309

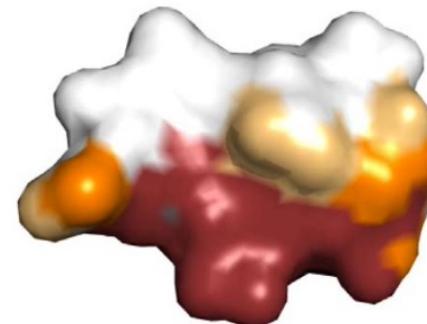
Importance of the Conformation of Macrocycles

- Interaction with target protein



cyclosporin with cyclophilin

Side view

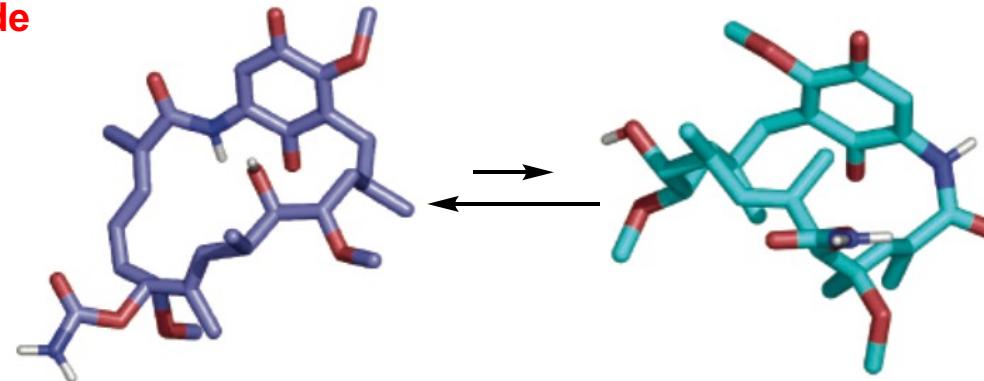
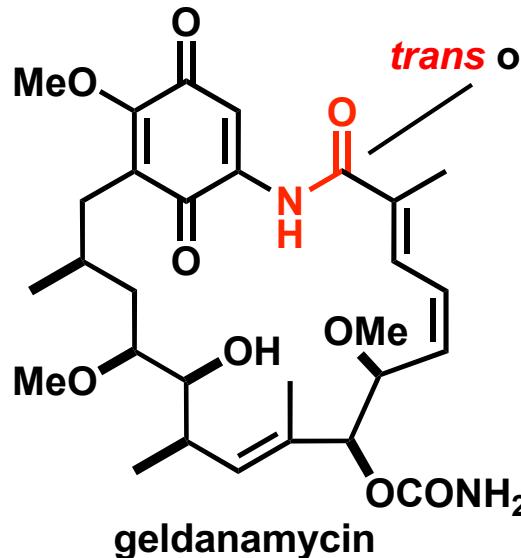


The redder,
the more buried.

flattened and elongated ring

→ solvent-exposed substituents can contact with the protein

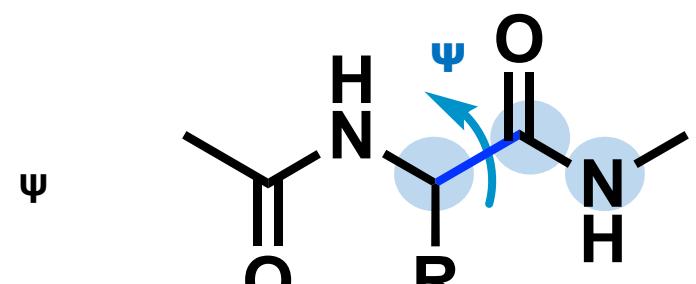
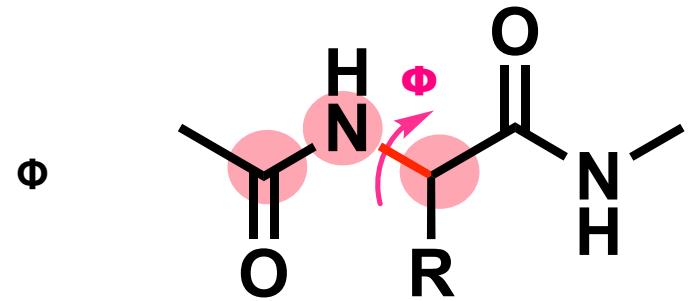
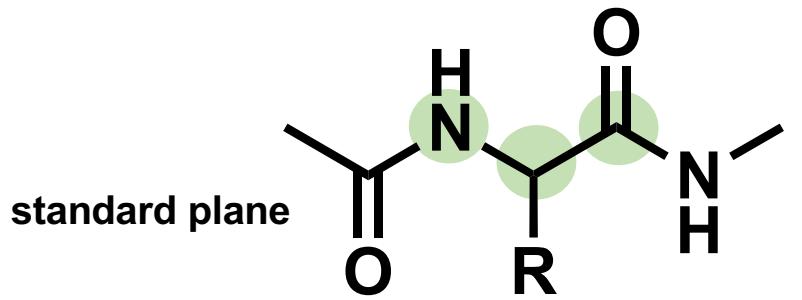
- Bioactive conformation is not the major state.



bioactive conformation
only 4% of the population

Controlling the conformation is needed for pursuing new bioactive compounds.

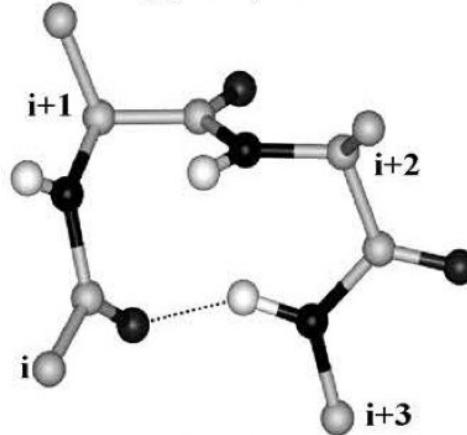
β -Turn



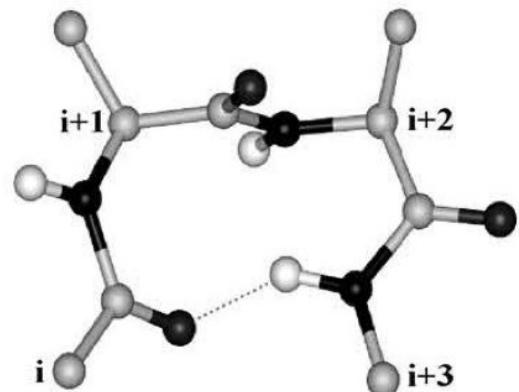
dihedral angles

	Φ_{i+1}	Ψ_{i+1}	Φ_{i+2}	Ψ_{i+2}
I	-60°	-30°	-90°	0°
II	-60°	120°	80°	0°
I'	60°	30°	90°	0°
II'	60°	-120°	-80°	0°

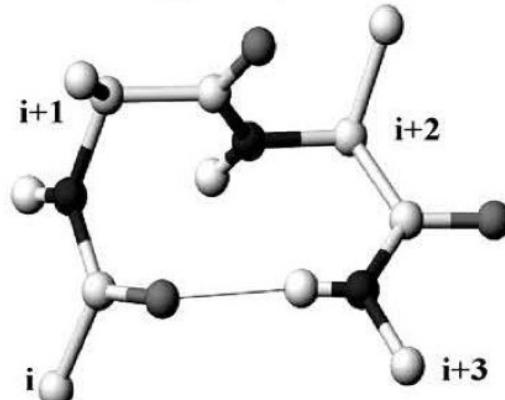
Type I β -turn



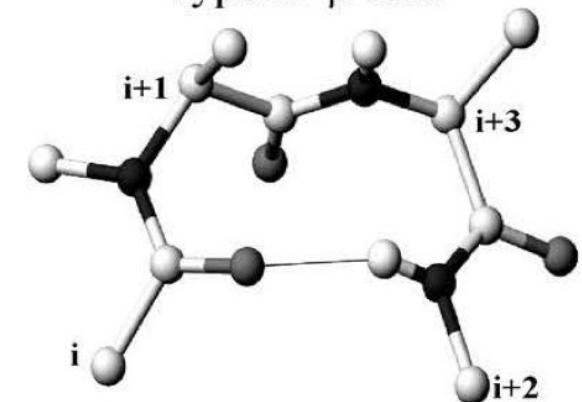
Type II β -turn



Type I' β -turn



Type II' β -turn

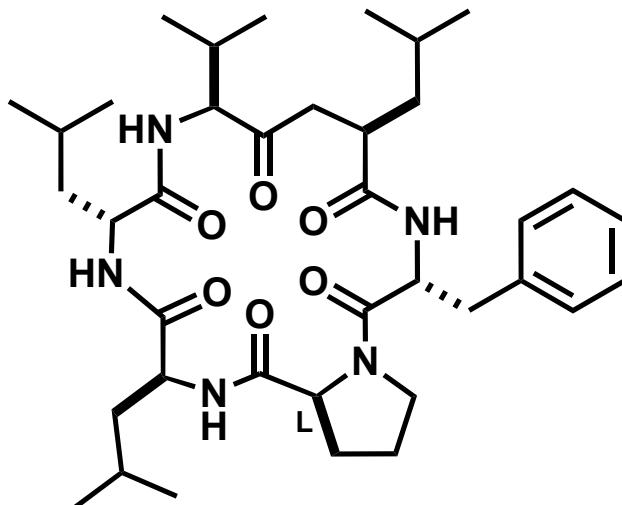


1) Appavu, R.; Mohan, D.; Kakumanu, R.; Munisamy, G. *Transcriptomics*. 2016, 4, 131.

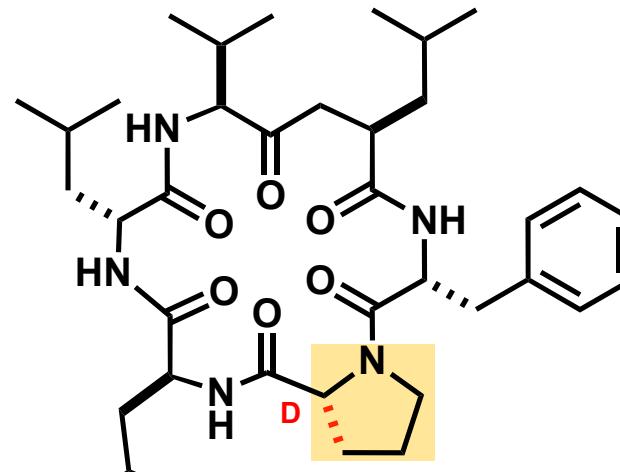
2) Zhou, A. Q.; O'Hern, C. S.; Regan, L. *Protein Sci.* 2011, 20, 1166.

Structural Modification of Macrocycles

- **D-amino acids:** D-amino acid can change the type of β -turn



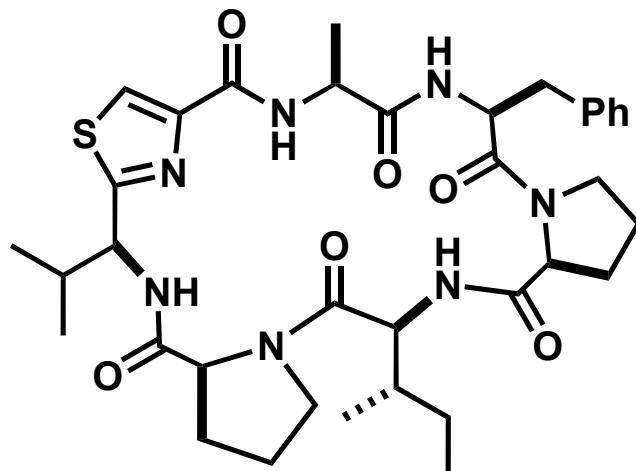
Phepropeptin A (natural product)



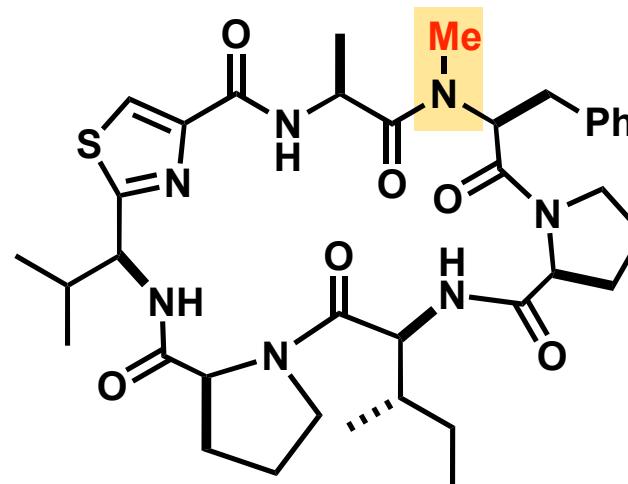
epi-Phepropeptin A

- comparable aqueous solubility
- 4-fold lower cell permeability

- **N-alkylation:** blocking hydrogen bonding and facilitating cis amides



Sanguinamide A (natural product)

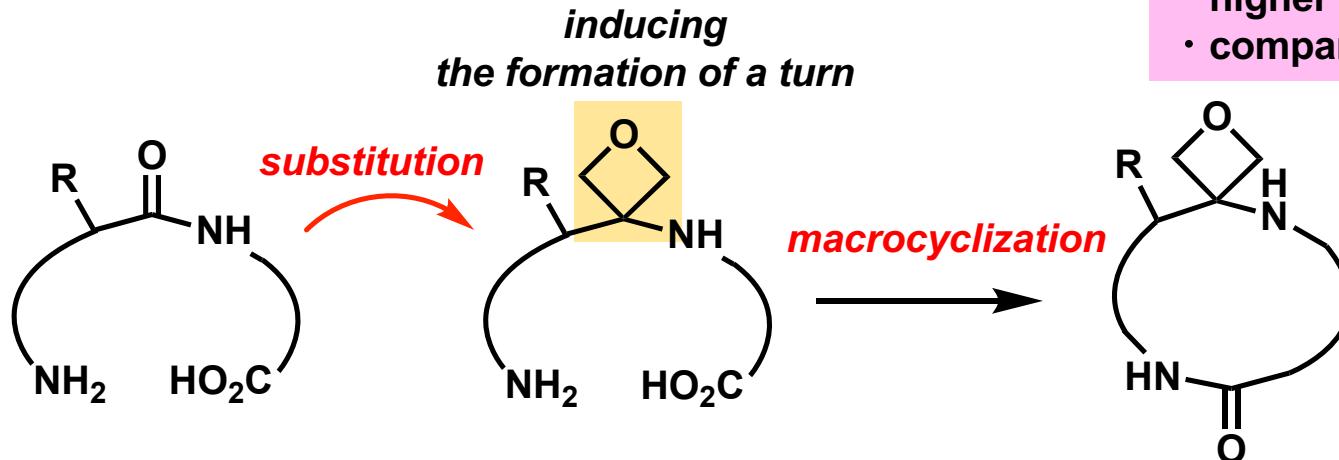


analogue (6-fold higher cell permeability)

1) Jwad, R.; Weissberger, D.; Hunter, L. *Chem. Rev.* **2020**, 120, 9743. 2) Schwochert, J.; Lao, Y.; Pye, C. R.; Naylor, M. R.; Desai, P. V.; Gonzalez Valcarcel, I. C.; Barrett, J. A.; Sawada, G.; Blanco, M-J.; Lokey, R. S. *ACS. Med. Chem. Lett.* **2016**, 7, 757.
3) Bockus, A. T.; Schwochert, J. A.; Pye, C. R.; Townsend, C. E.; Sok, V., Bednarek; M. A.; Lokey, R. S. *J. Med. Chem.* **2015**, 58, 7409.

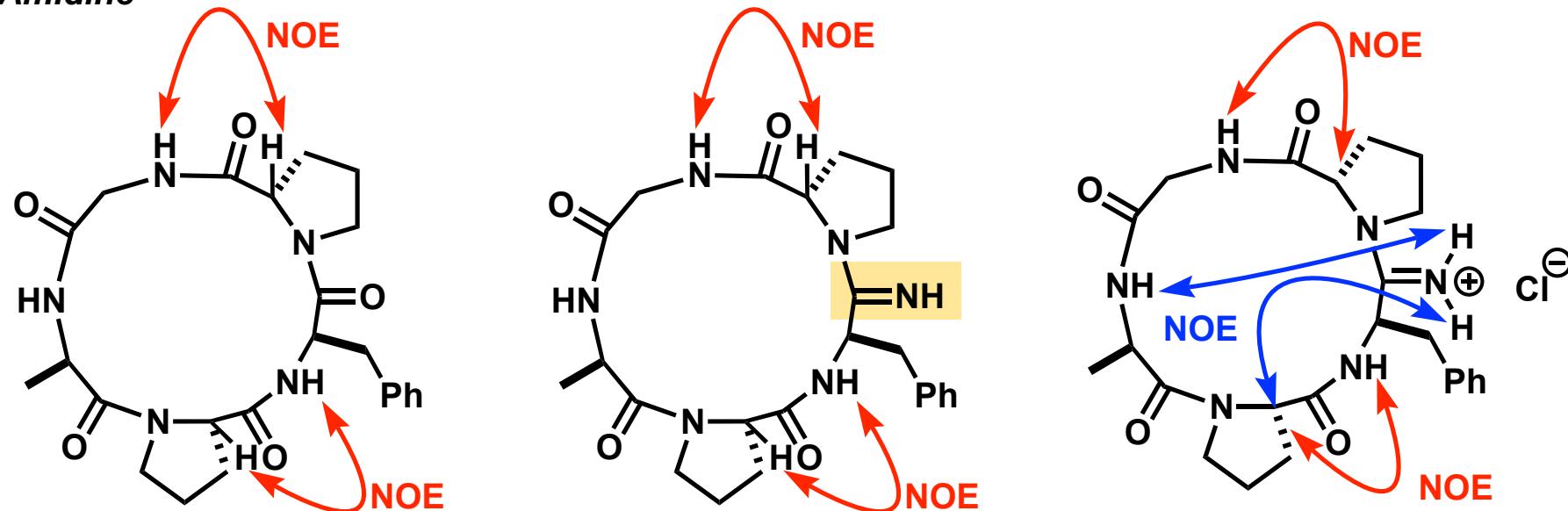
Introduction of Bioisosteres into Macrocycles

- Oxetane



- higher yields
- comparable bioactivity

- Amidine



Prof. Andrei K. Yudin



Career

1992 :B.Sc @ Moscow State University (Prof. N. S. Zefirov)
1996 :Ph.D. @ University of Southern California
(Profs. G. K. Surya Prakash and George A. Olah)
1996- :Postdoctoral Fellow @ the Scripps Research Institute
(Prof. K. Barry Sharpless)
1998- :Assistant professor @ University of Toronto
2002- :Associate Professor @ University of Toronto
2007- :Full Professor @ University of Toronto

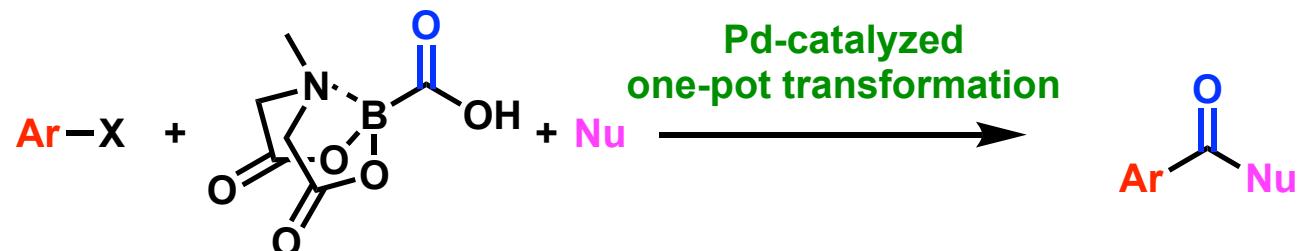
Research Field

1. Macrocycles and Peptides
-Today's session

4. Amphoteric Molecules
-compounds working as both a nucleophile and an electrophile

2. Reaction Discovery

3. Chemical Biology



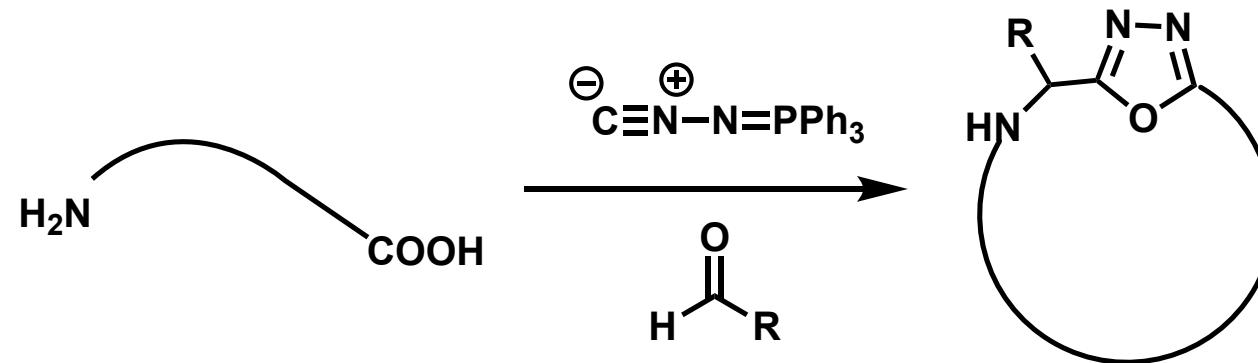
1) <https://sites.chem.utoronto.ca/yudinlab/content/andrei-yudin>

2) Tien, C. H.; Trofimova, A.; Holownia, A.; Kwak, B. S.; Larson, R. T.; Yudin, A. K.
Angew. Chem. Int. Ed. 2021, 60, 4342.

Heterocycles in Macrocycles – Prof. Yudin's works

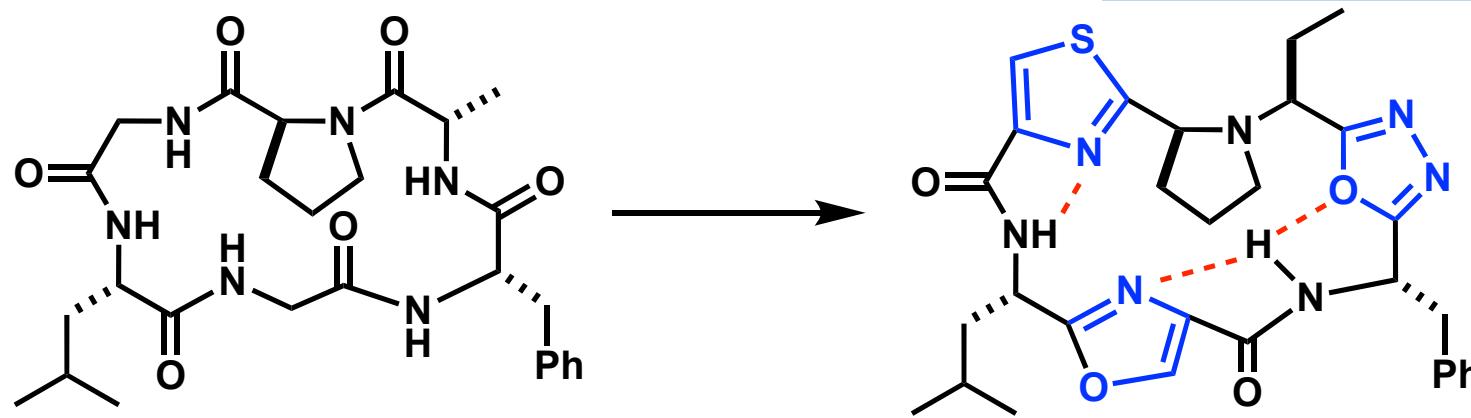
Heterocycles contribute to improving the **synthesis** and the **property** of macrocycles.

- **Oxadiazole grafts**



Oxadiazole stabilizes the conformation by hydrogen bonds.
High passive membrane permeability

- **Replacement of amide with azole heterocycles**



poor permeability
multiple conformer

higher permeability than cyclosporin A
rigid structure

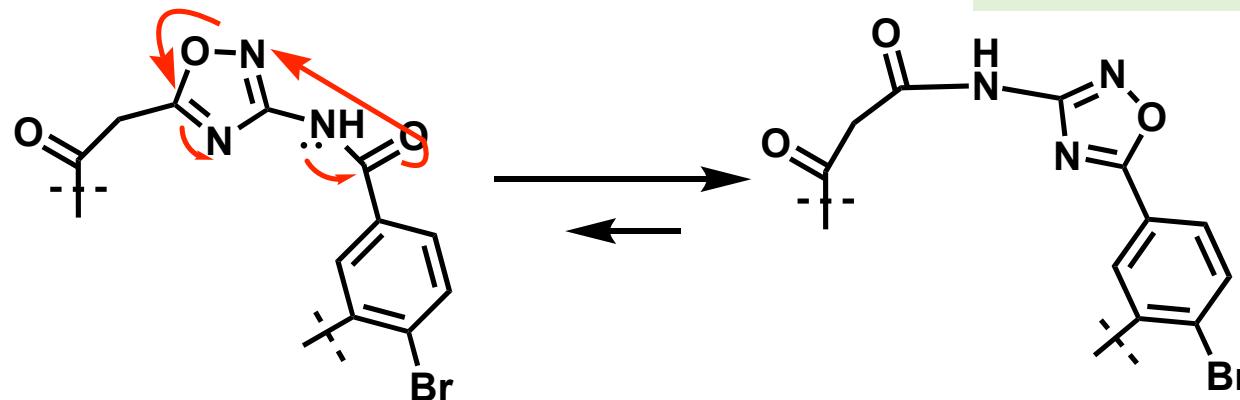
1) Frost, J. R.; Scully, C. C.; Yudin, A. K. *Nat. Chem.* **2016**, 8, 1105.

2) Saunders, G. J.; Yudin, A. K.. *Angew. Chem. Int. Ed.* **2022**, 134, e202206866.

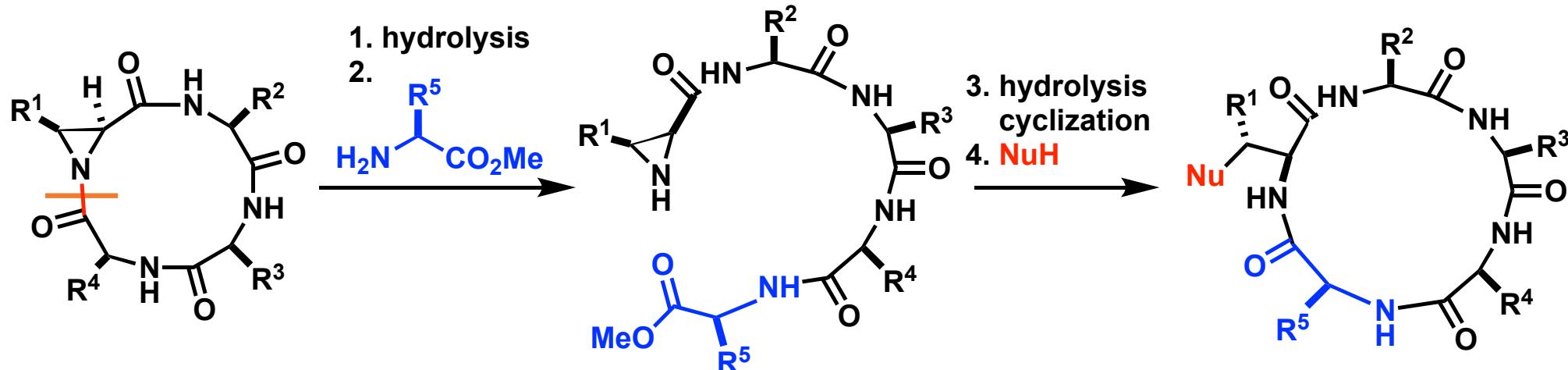
Late-Stage Modification – Prof. Yudin's works

Late-stage sequence modification of macrocycles has been achieved by utilizing heterocycles.

- Conformational Shifts via Boulton-Katritzky reaction



- Integration of Amino Acid Fragments



Aziridine amide controls site-selective cleavage and regioselective addition.

1) Kobori, S.; Huh, S.; Appavoo, S. D.; Yudin, A. K. *J. Am. Chem. Soc.* **2021**, *143*, 5166.

2) White, C. J.; Hickey, J. L.; Scully, C. C.; Yudin, A. K. *J. Am. Chem. Soc.* **2014**, *136*, 3728.

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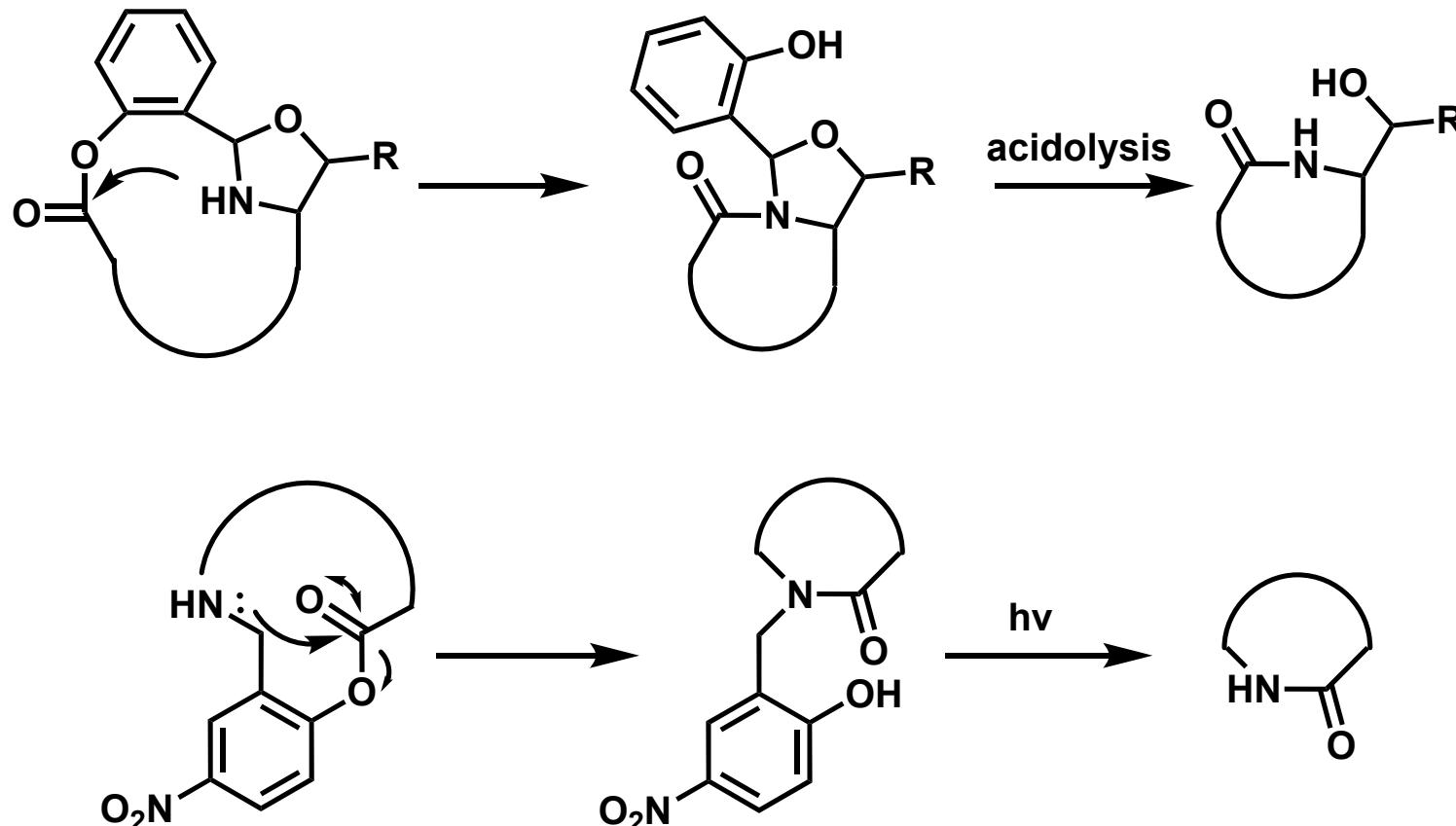


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Single Atom Ring Contraction of Peptide Macrocycles Using Cornforth Rearrangement

Dr. Sungjoon Huh, Dr. George J. Saunders, Prof. Andrei K. Yudin

Traditional Methods for Ring Contraction

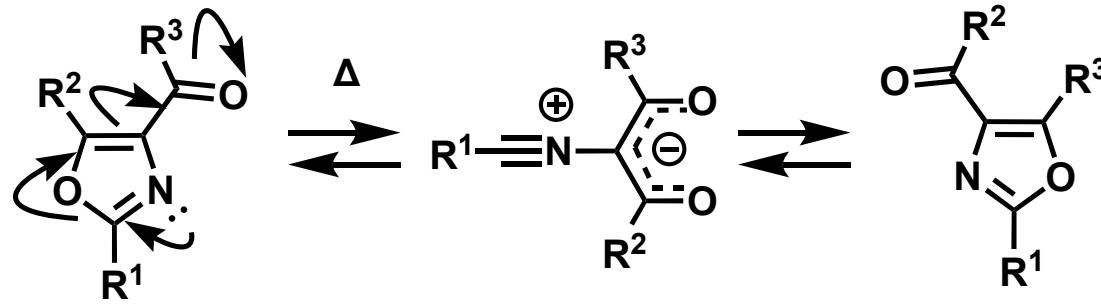


Single atom contraction was not achieved.

- 1) Wong, C. T.; Lam, H. Y.; Song, T.; Chen, G.; Li, X. *Angew. Chem. Int. Ed.* **2013**, *52*, 10212. 2) Meutermans, W. D.; Golding, S. W.; Bourne, G. T.; Miranda, L. P.; Dooley, M. J.; Alewood, P. F.; Smythe, M. L. *J. Am. Chem. Soc.* **1999**, *121*, 9790.

Cornforth Rearrangement

- reported in 1949
- rarely used reaction appeared in 140 papers/books (Google Scholar, 7/31/2023)



rate-determining step



J. W. Cornforth
(Nobel Prize, 1975)

Hammett plot was plotted on electron-donating ability of R¹

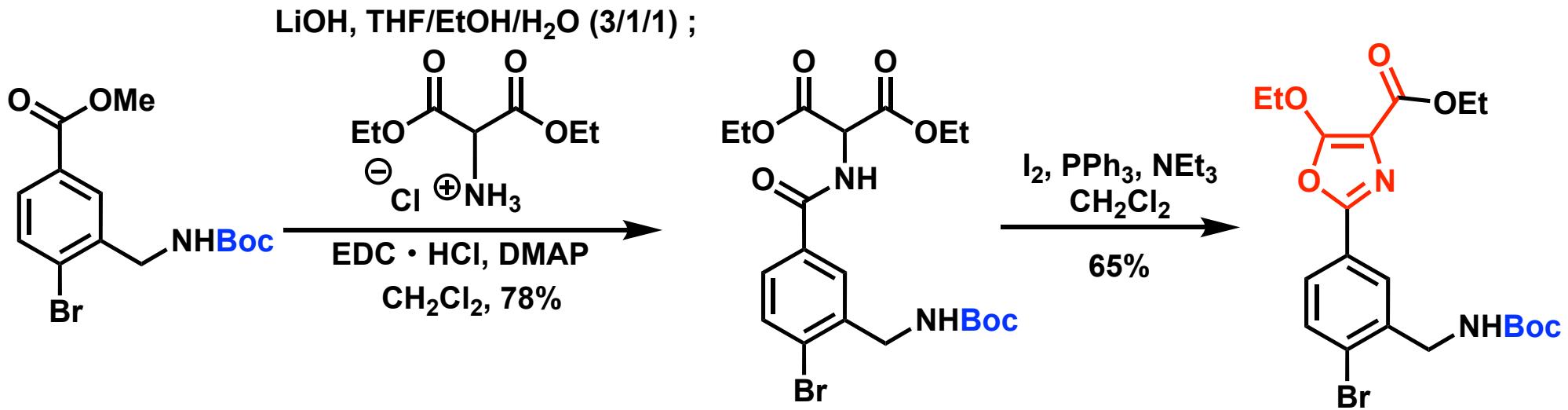
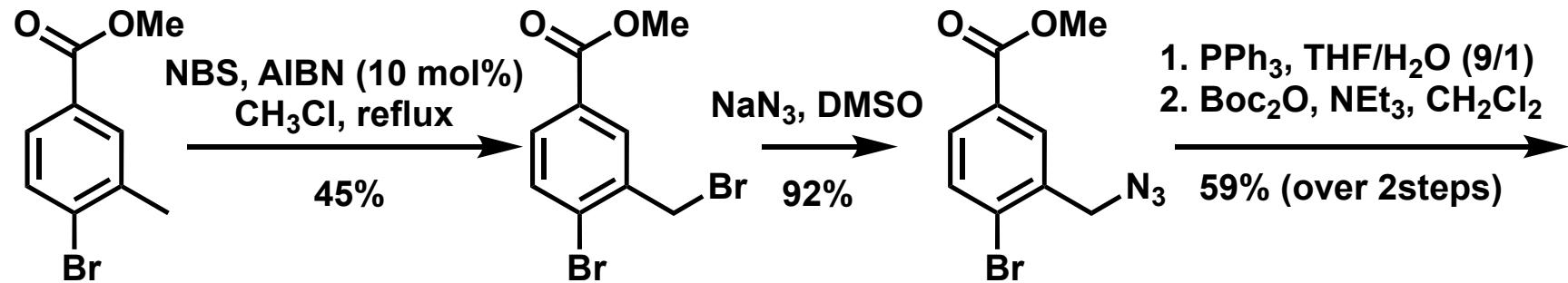
→ ρ -value of the reaction was **negative** (-1.16)

If R¹ is electron-donating group, the reaction is accelerated.

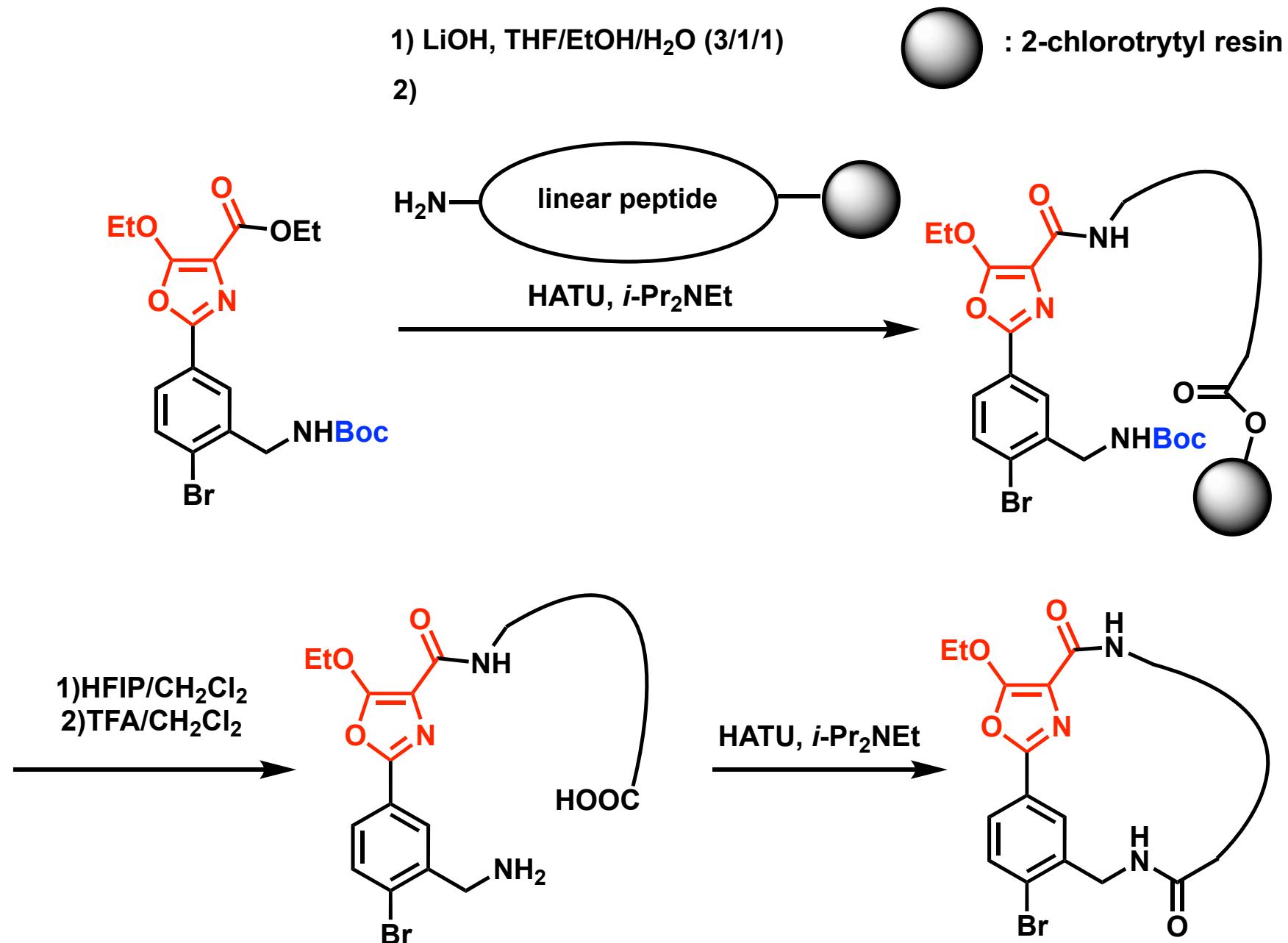
1) Dewar, M. J. S. *J. Am. Chem. Soc.* **1974**, 96, 6148. 2) Hanson, J. *Nature*. **2014**, 506, 35.

3) Huh, S.; Saunders, G. J.; Yudin, A. K. *Angew. Chem. Int. Ed.*, **2023**, 135, e202214729.

Synthesis of Oxazole Moiety

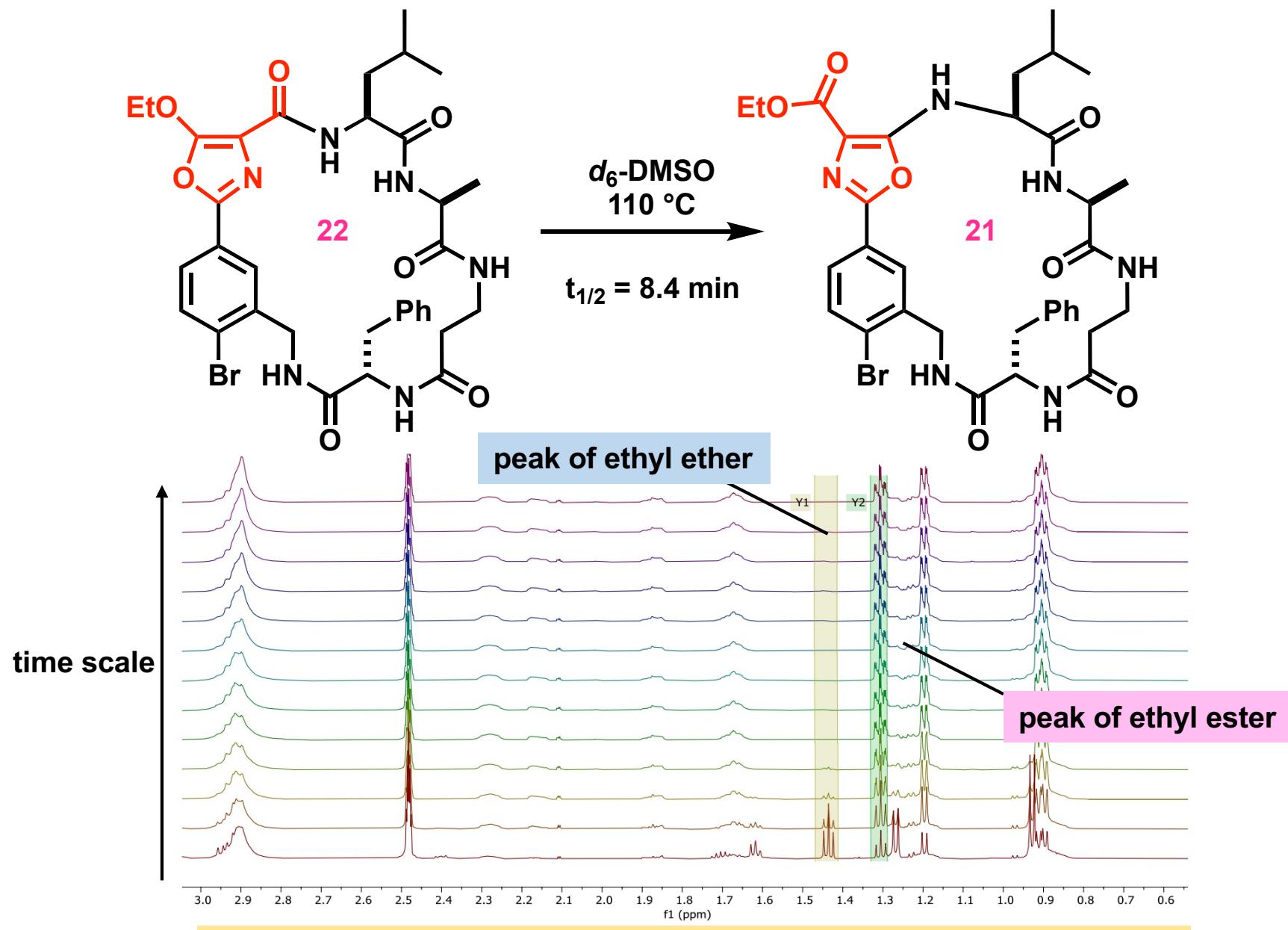


Synthesis of Subjected Macrocycles



Monitoring Rearrangement

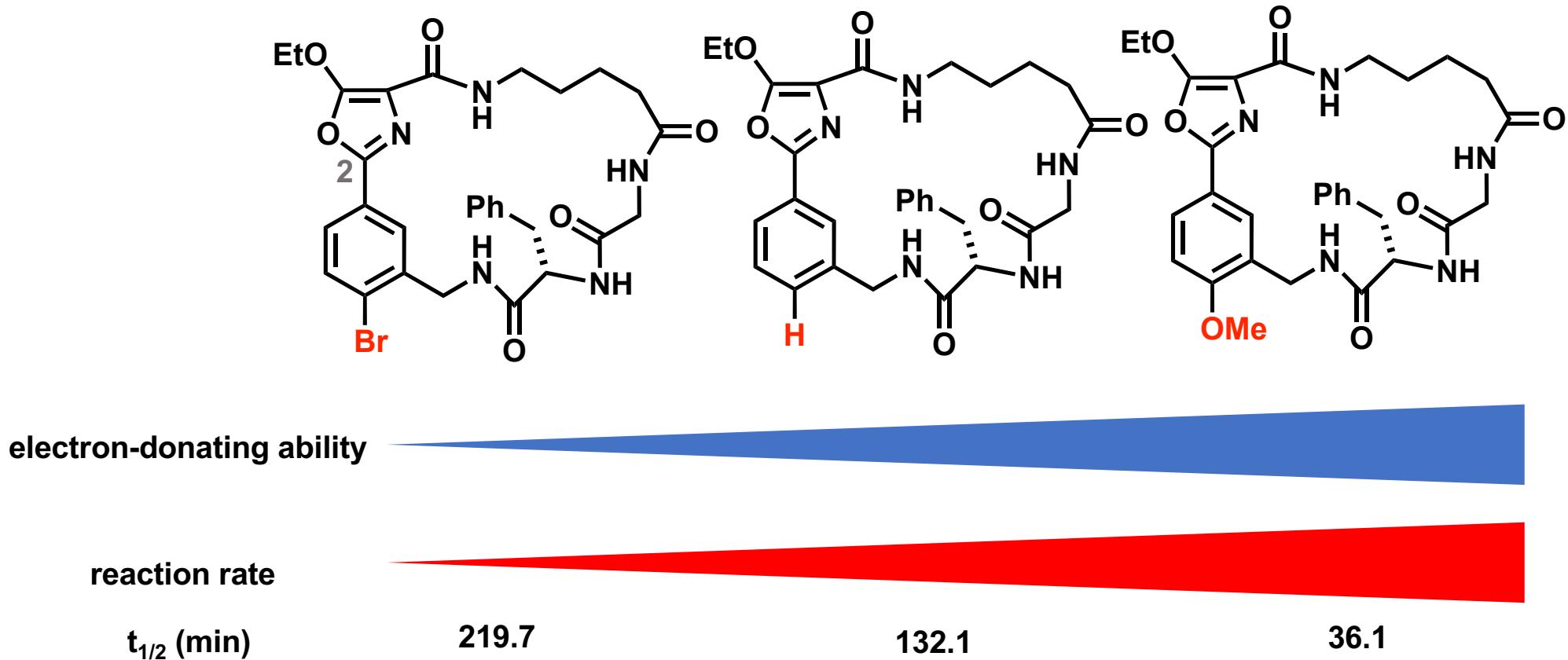
$t_{1/2}$: needed time for SM concentration to decrease to half of its initial concentration



To fully understand the reactivity, mechanistic studies were conducted.

Effect of Substitution Groups

Question: Does the mechanism change in the case of macrocycles?

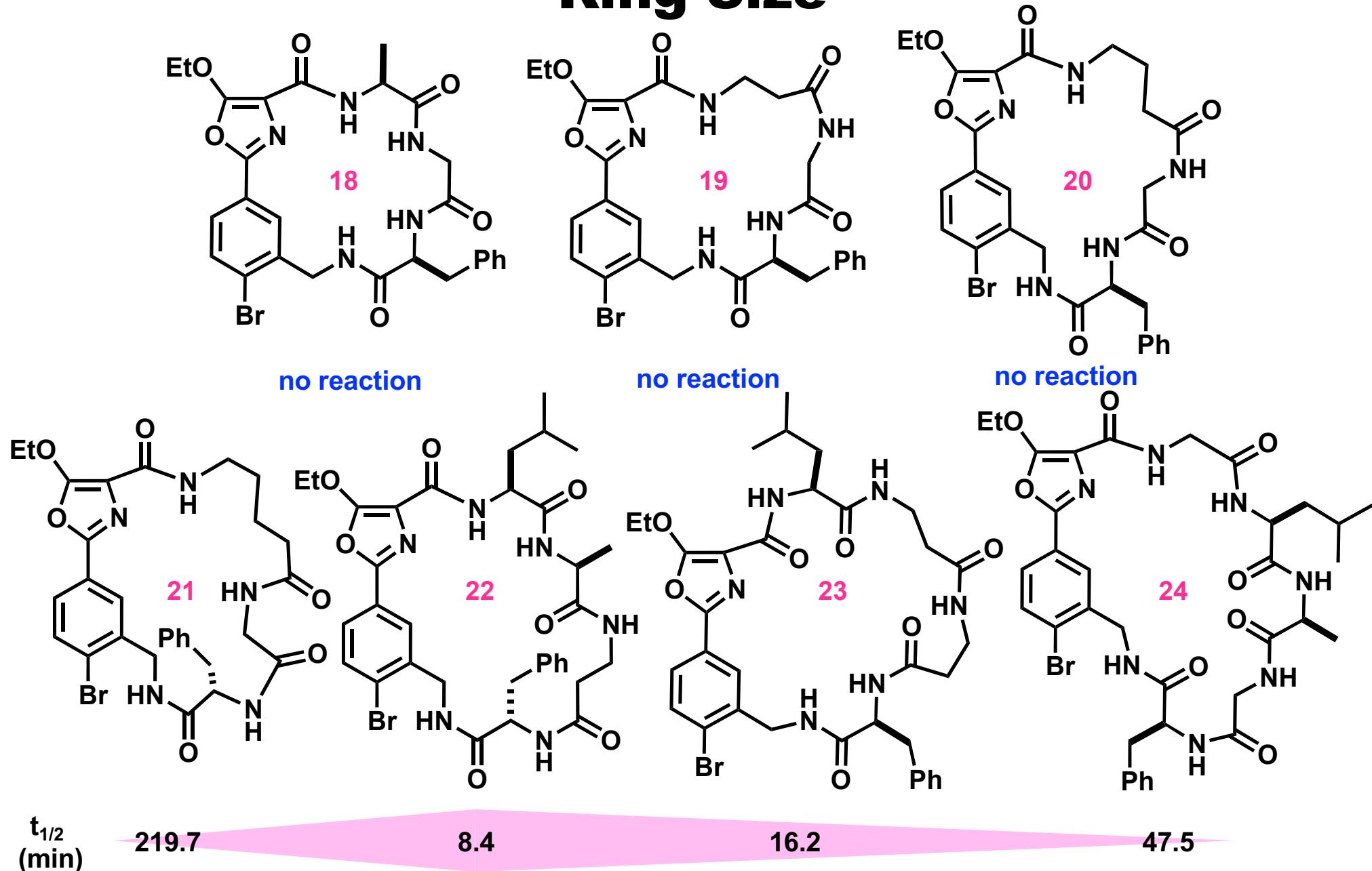


Answer: Following the similar mechanism (rate determining step is the ring opening)

1) Huh, S.; Saunders, G. J.; Yudin, A. K. *Angew. Chem. Int. Ed.*, 2023, 135, e202214729.

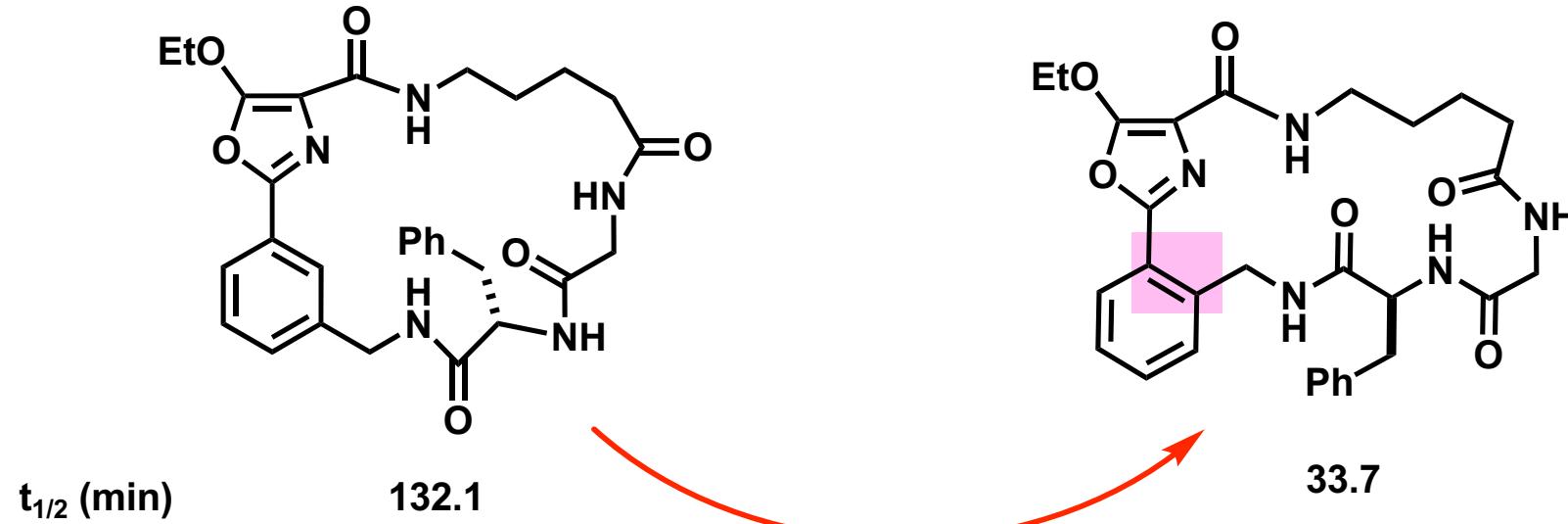
2) Dewar, M. J. S. *J. Am. Chem. Soc.* 1974, 96, 6148.

Ring Size



Ring size is not the main factor of the rearrangement.
→the backbone is the key to the reactivity.

Backbone is the Key; *m*- vs. *o*- substitution



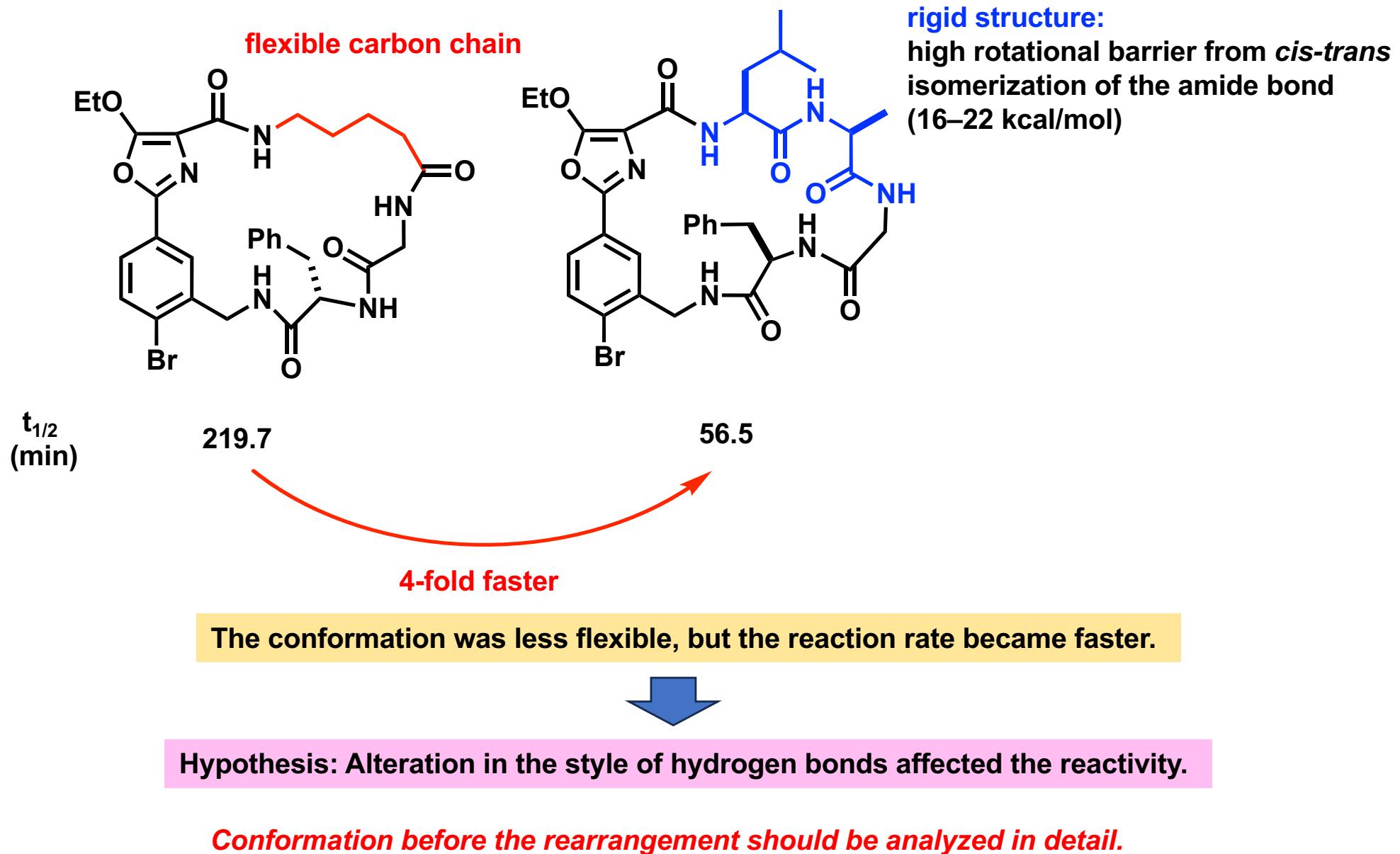
4-fold faster

Composition of the backbone is the same, but the reaction rate became faster.

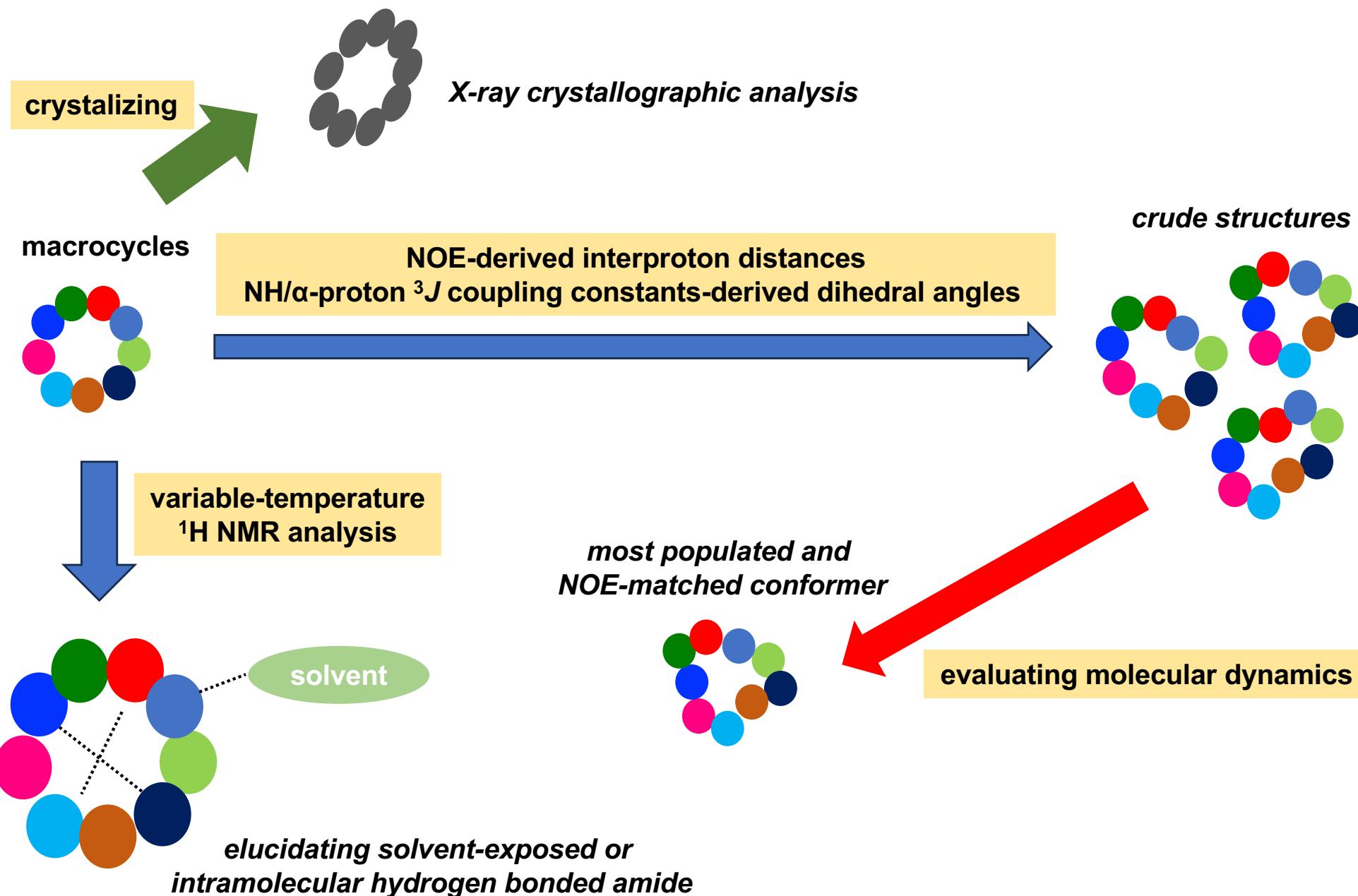


Hypothesis: Conformational differences by the style of substitution affected the reactivity.

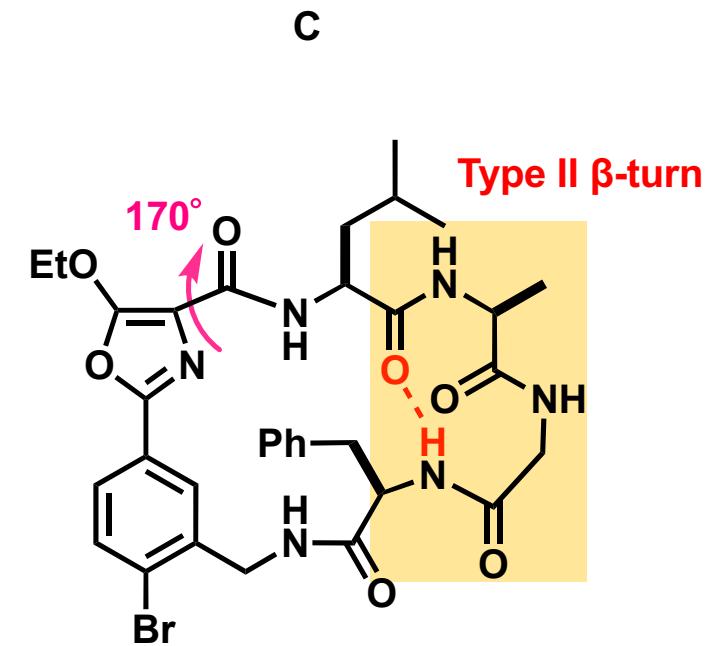
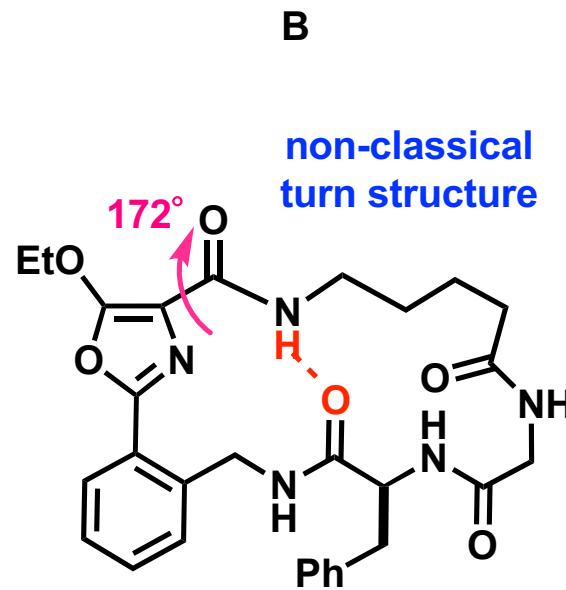
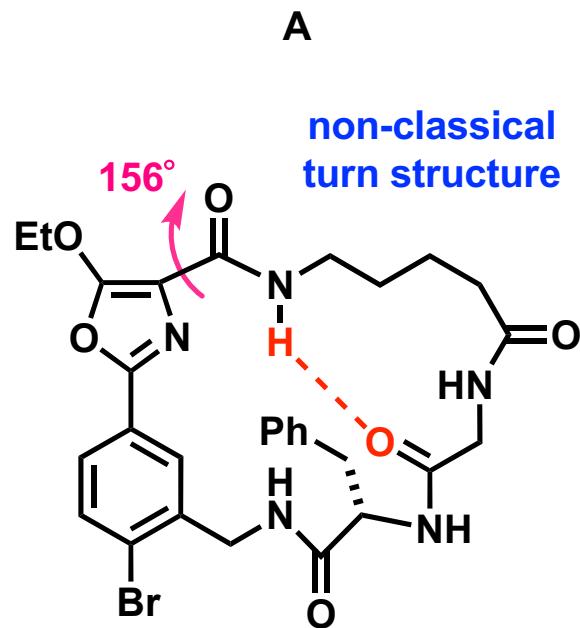
Backbone is the Key; Amide Bond



Analyzing Conformations



Analyzed Conformation



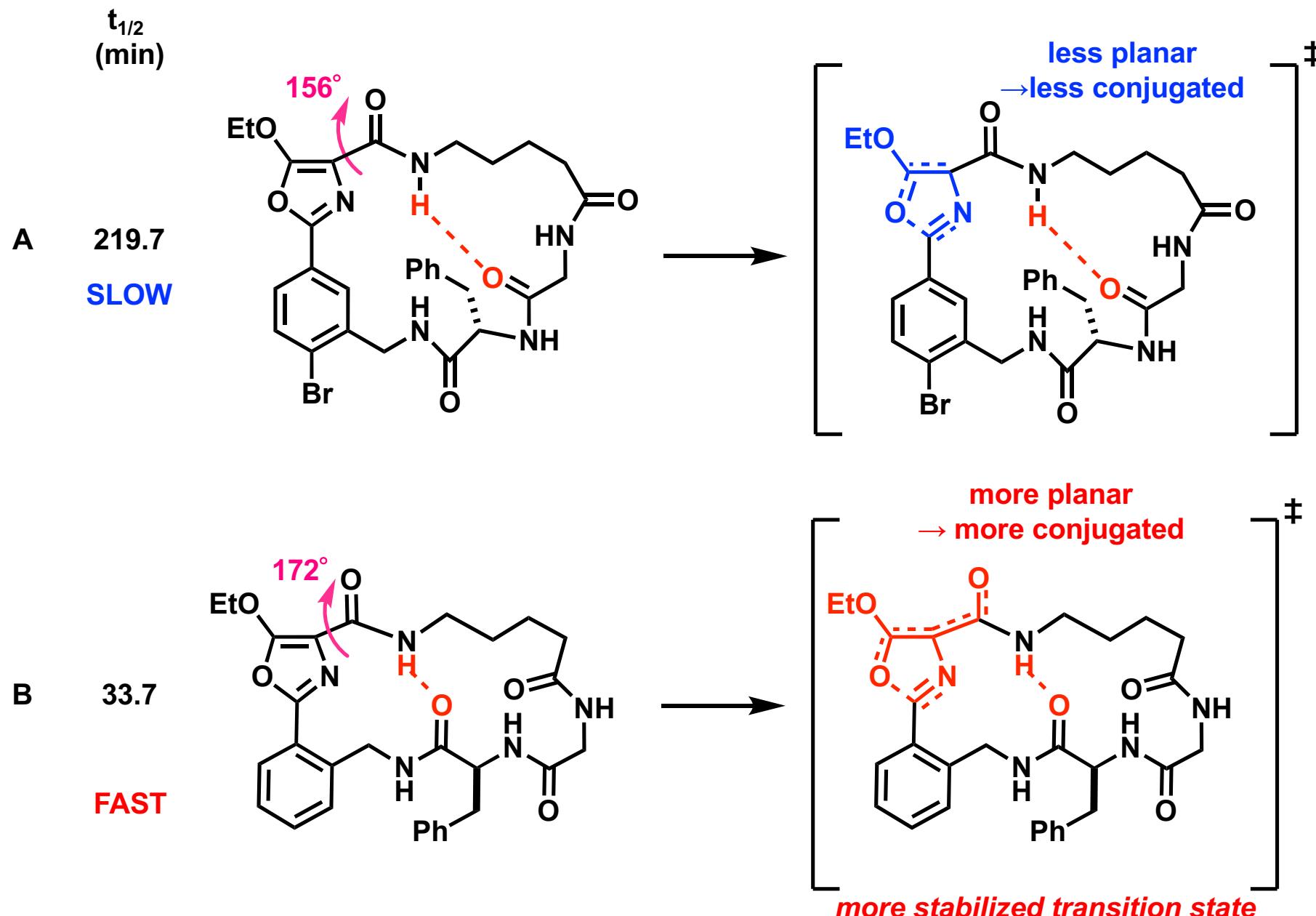
$t_{1/2}$
(min)

219.7

33.7

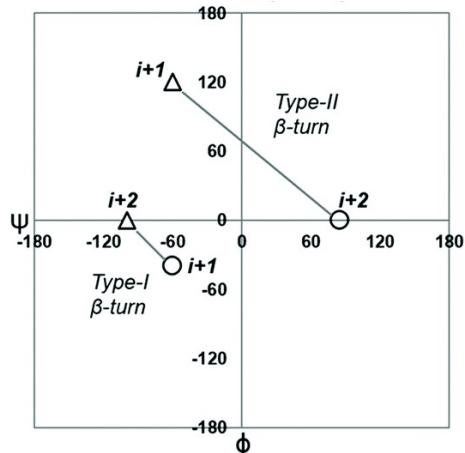
56.5

TS-Stabilizing Conformation



Macrocyclic Conformational Mapping

Question: How does the conformation change after the rearrangement?

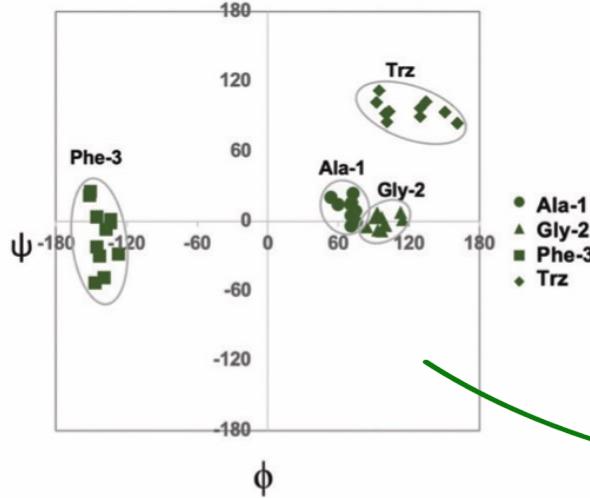


traditional conformational map
Ramachandran plot

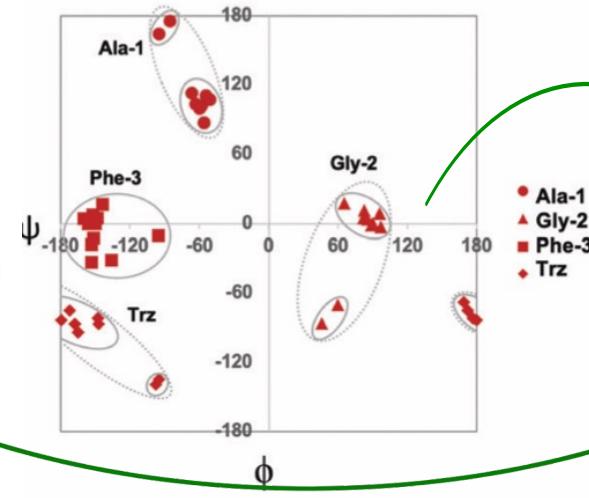
specialized for analysis of untraditional macrocycles

plotted by top 10 conformers
in each diastereomer

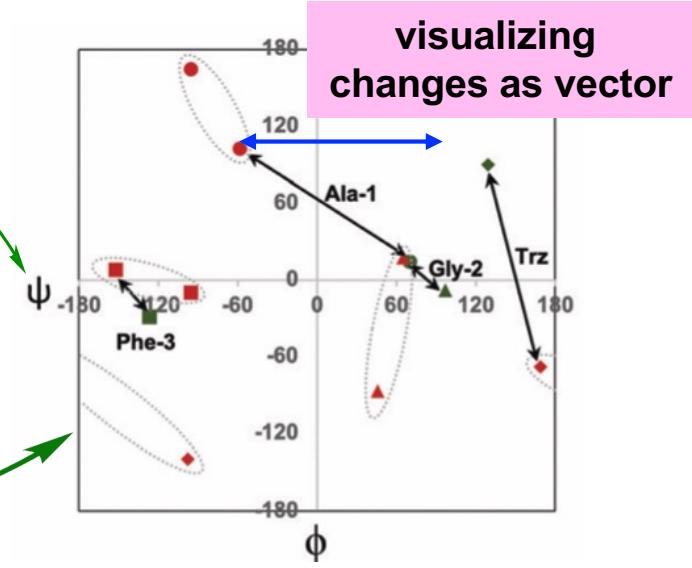
MCM of
conformer A



MCM of
conformer B



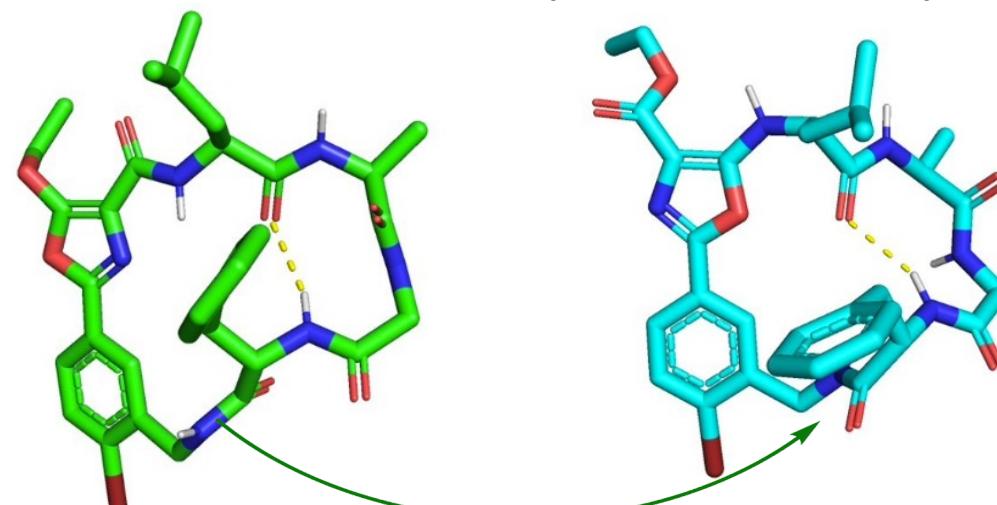
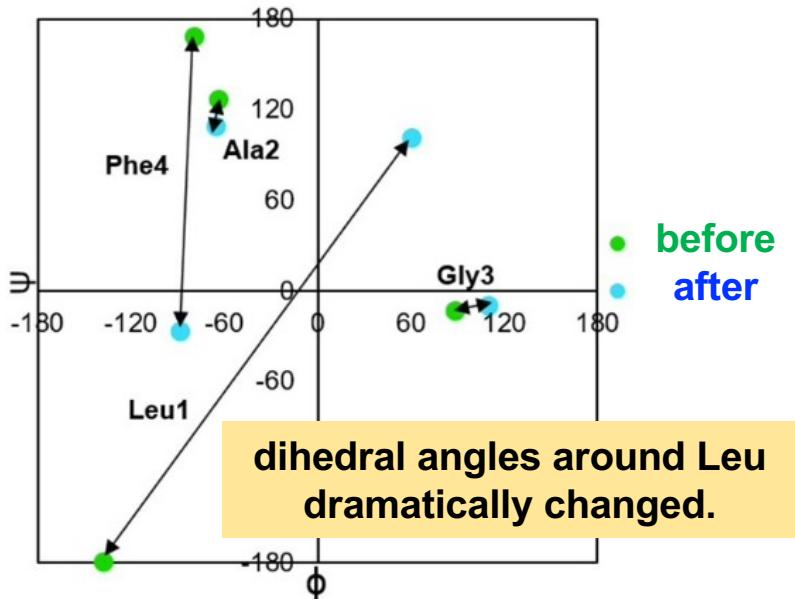
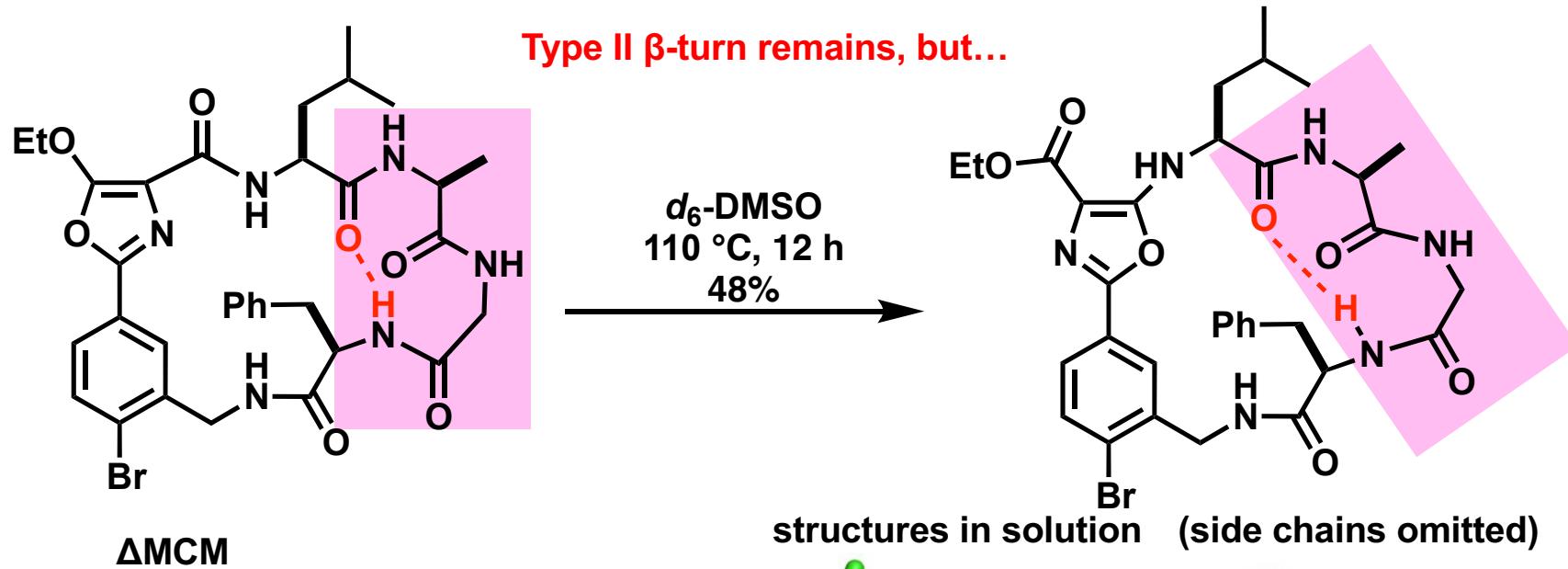
Δ MCM



1) McTiernan, T. J.; Diaz, D. B.; Saunders, G. J.; Sprang, F.; Yudin, A. K. *RSC Chem. Biol.* **2022**, 3, 739.

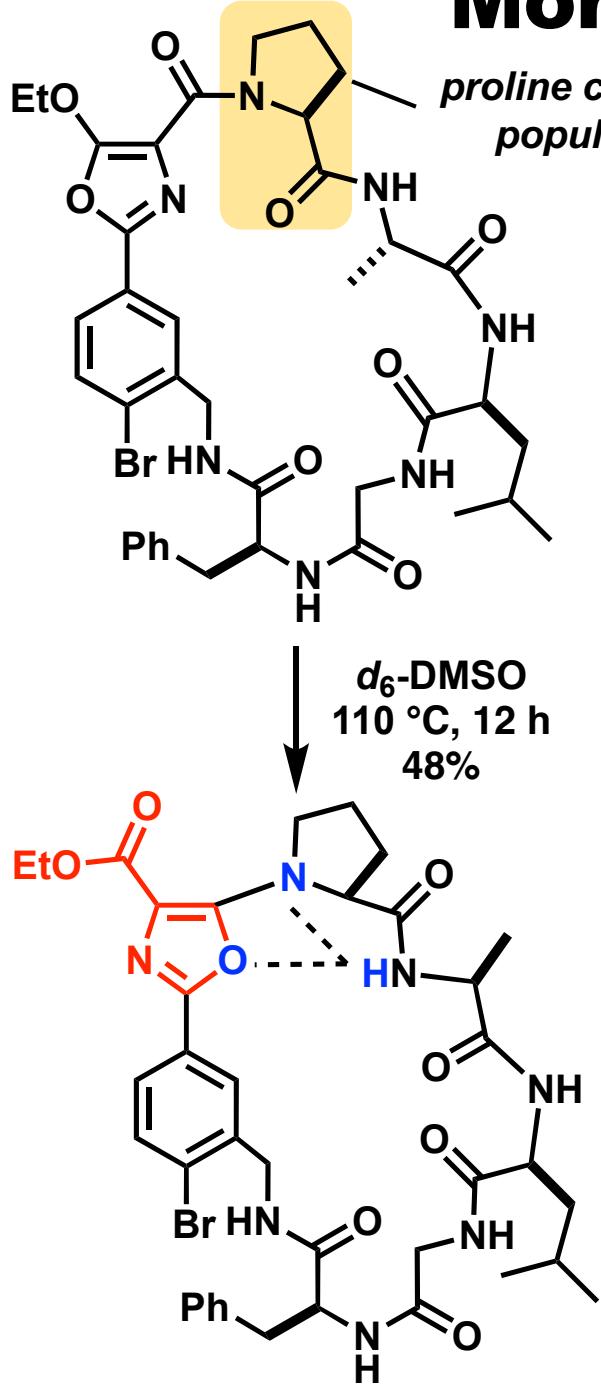
2) Huh, S.; Saunders, G. J.; Yudin, A. K. *Angew. Chem. Int. Ed.*, **2023**, 135, e202214729.

Reorganized Conformation



Ring contraction caused local dihedral change, which provoked drastic overall conformational change.

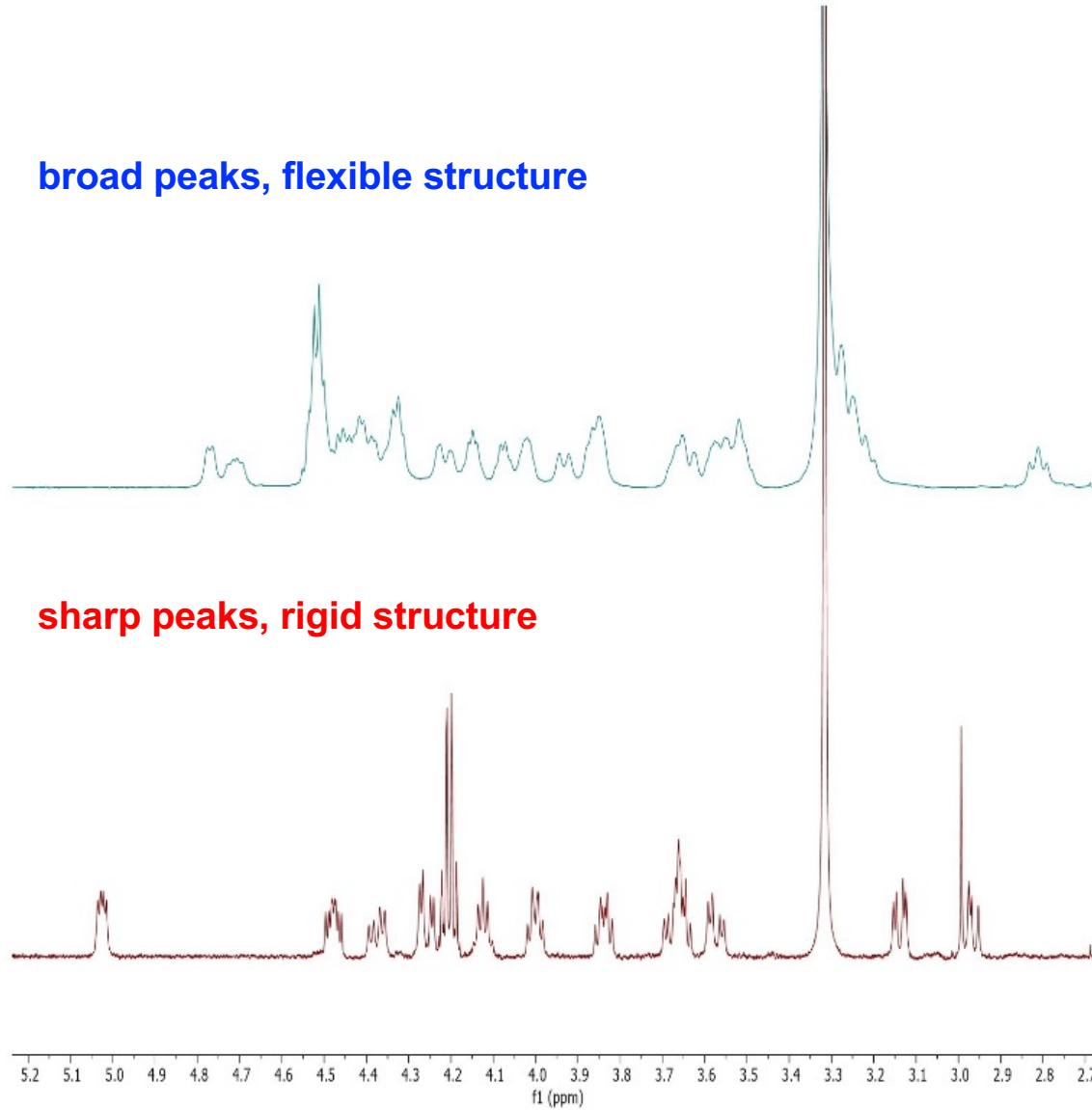
More Rigid Conformation



proline can maintain cis-amide in higher population than other amino acids.

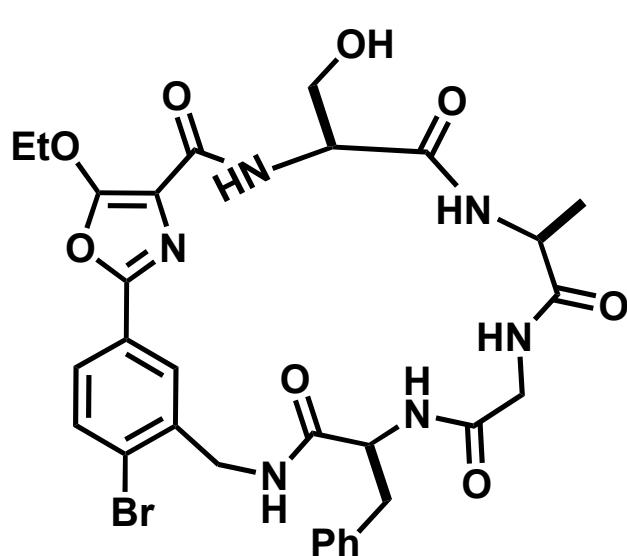
broad peaks, flexible structure

sharp peaks, rigid structure



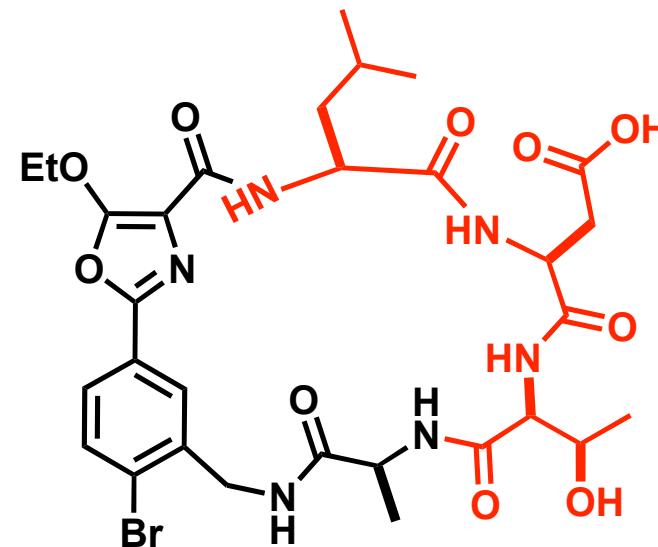
Scope of the Reaction

The reaction proceeded with unprotected nucleophilic residues (Ser, Thr, Glu)



$t_{1/2}$
(min)

58.0

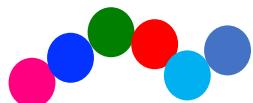


26.4

L-D-T motif can inhibits interactions of
the integrin $\alpha 4\beta 7$ with MAdCAM-1

This strategy can apply to broad composition of residues and potentially bioactive macrocycles.

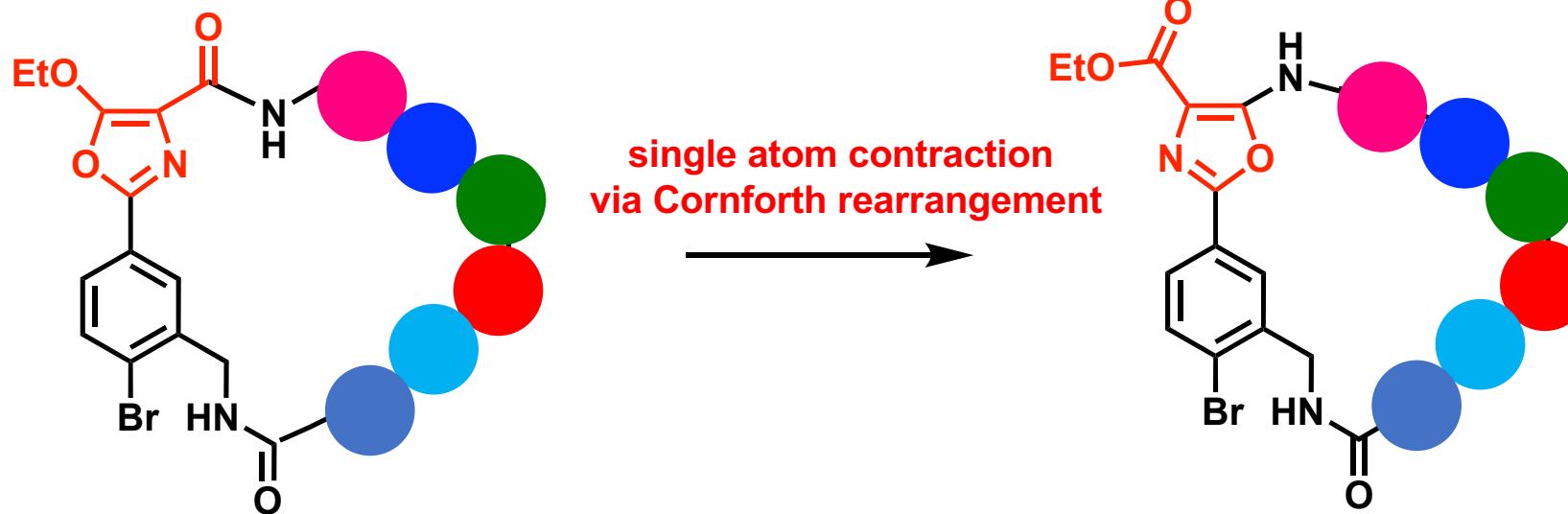
Future Perspective



linear bioactive peptide motif: easily degraded by enzymes, predictable structure



introducing the motif into the rearrangement precursor

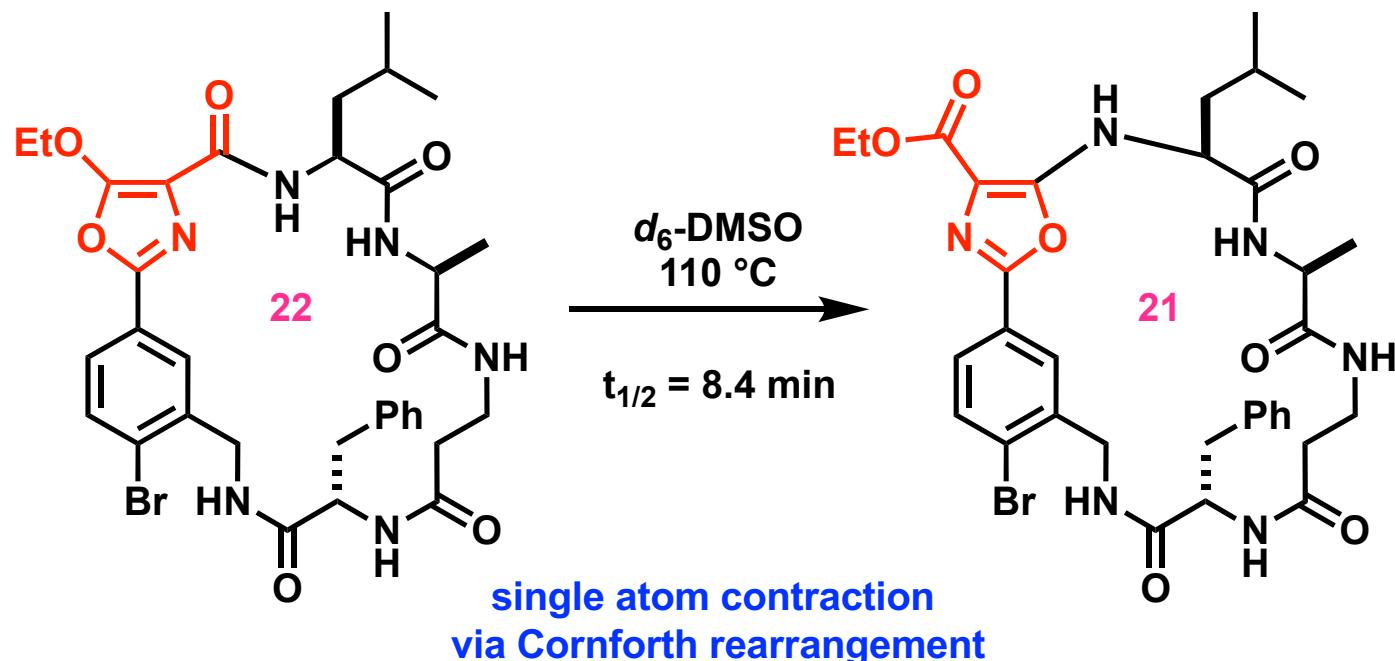


- difficult to be degraded by enzymes
- investigating two chemical spaces from one macrocycle
- uncommon formation of bioactive structure → **modification** of the activity

Summary

peptide macrocycle: potent drug seeds

controlling its conformation is essential for finding new drug seeds



single atom contraction
via Cornforth rearrangement



uncommon conformation

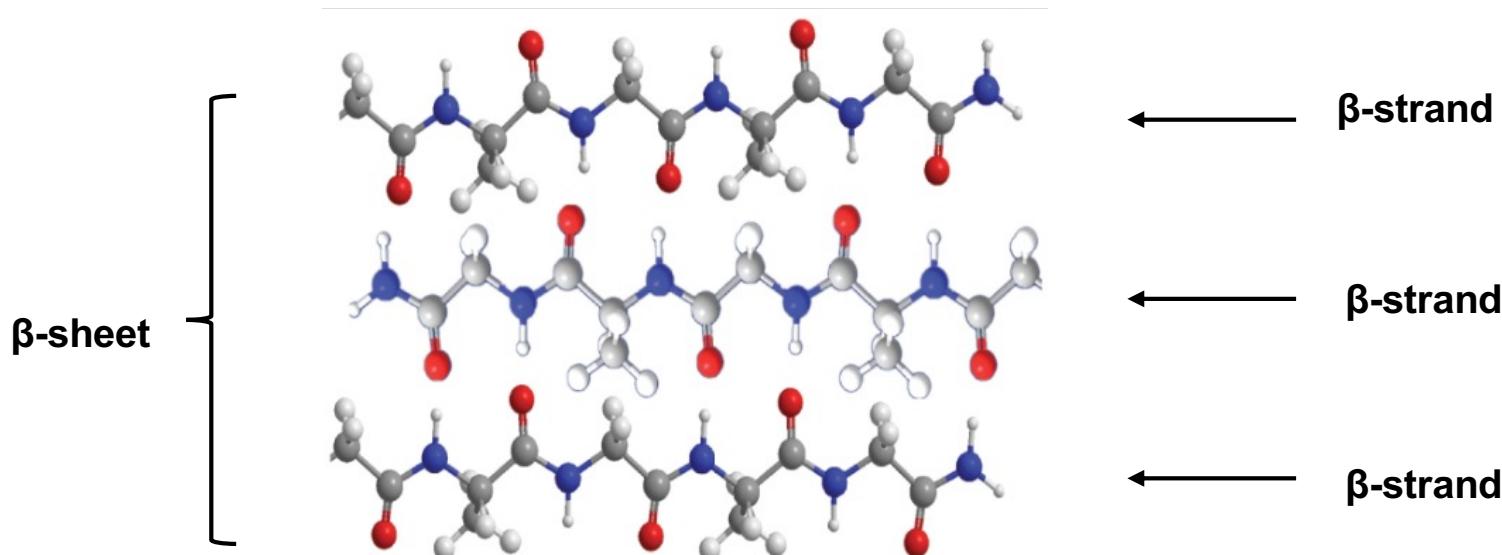
drastic changes, rigid structure, unusual intramolecular hydrogen bonds

Late-stage conformational diversification broadens our horizons to unexplored chemical space.

Appendix

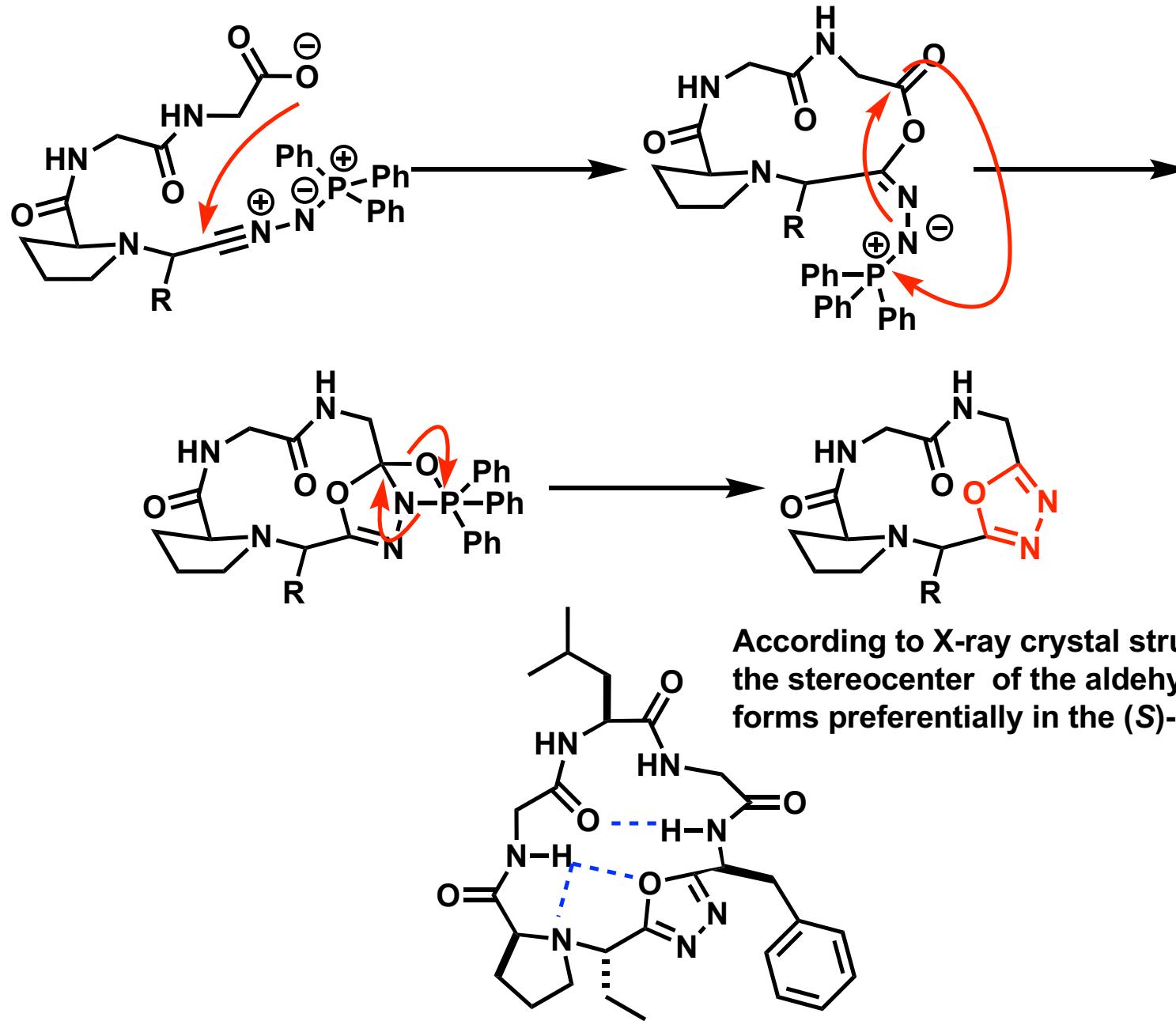
Resistance against Hydrolysis

Proteases recognize β -strand in their active sites.
Macrocycles cannot form β -strand, which gives them resistance.



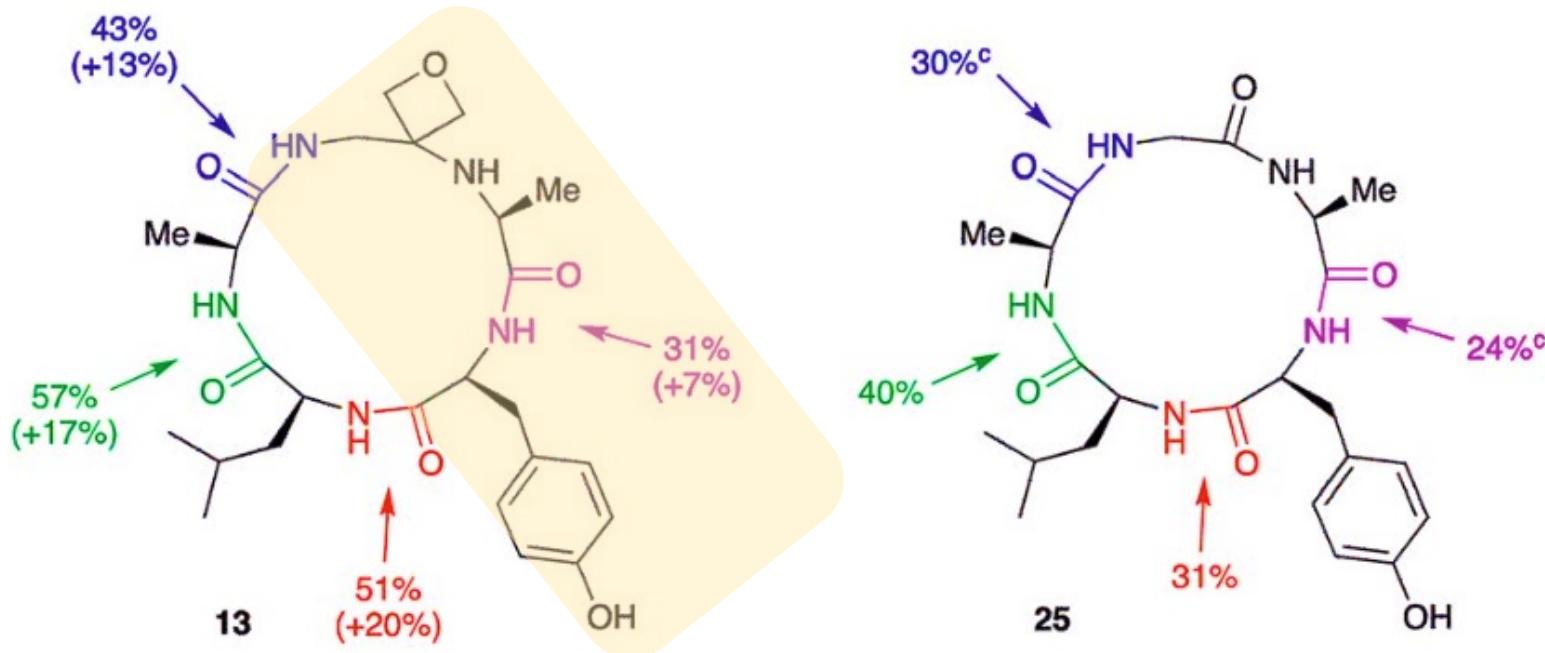
1) Tyndall, J. D.; Nall, T.; Fairlie, D. P. *Chem. Rev.* **2005**, *105*, 973. 2) Cebe, P.; Hu, X.; Kaplan, D. L.; Zhuravlev, E.; Wurm, A.; Arbeiter, D.; Schick, C. *Sci. Rep.* **2013**, *3*, 1130.

Proposed Mechanism of Oxadiazole Grafts



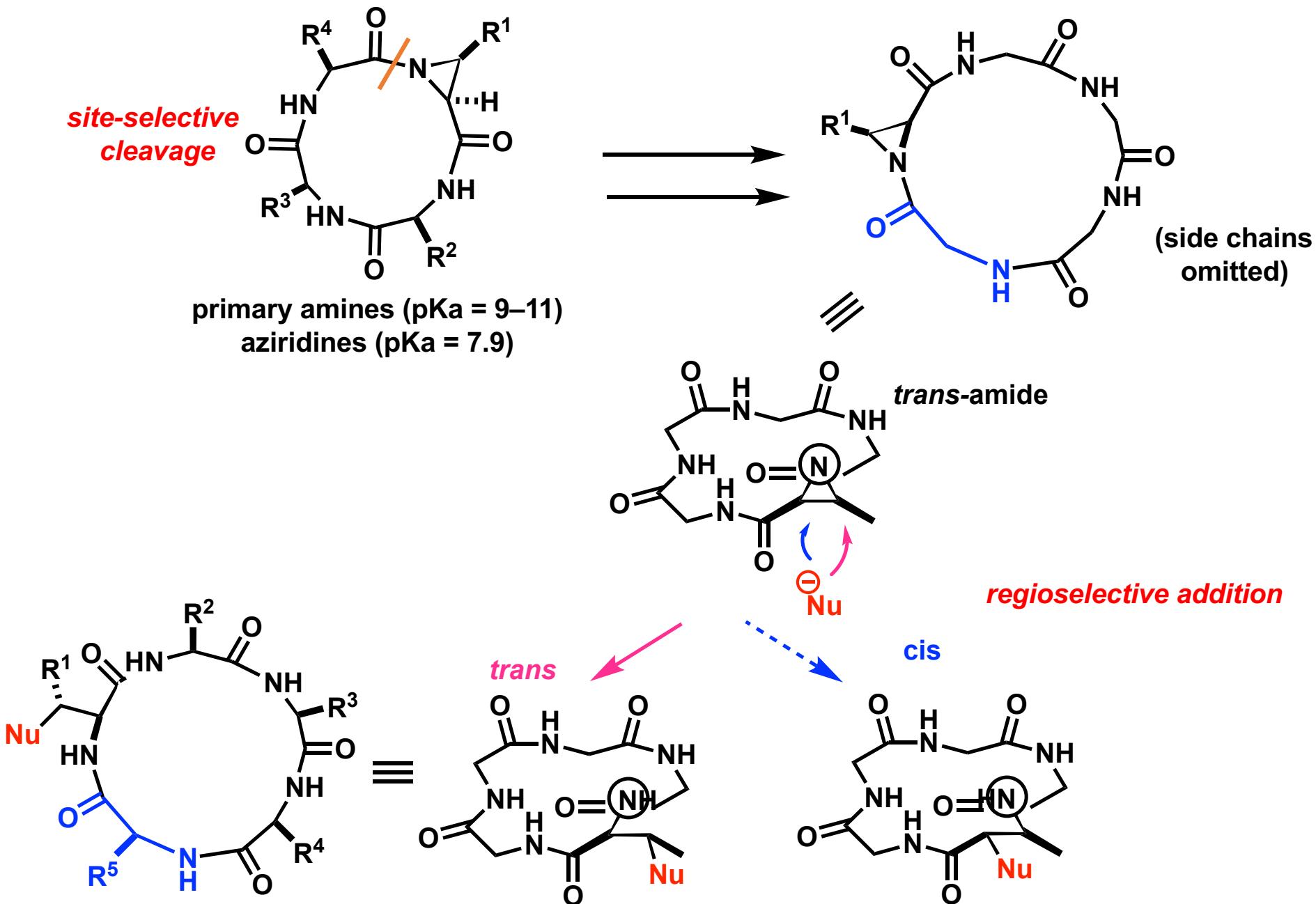
Replacement with Oxetane

turn formation
(according to NOE data)

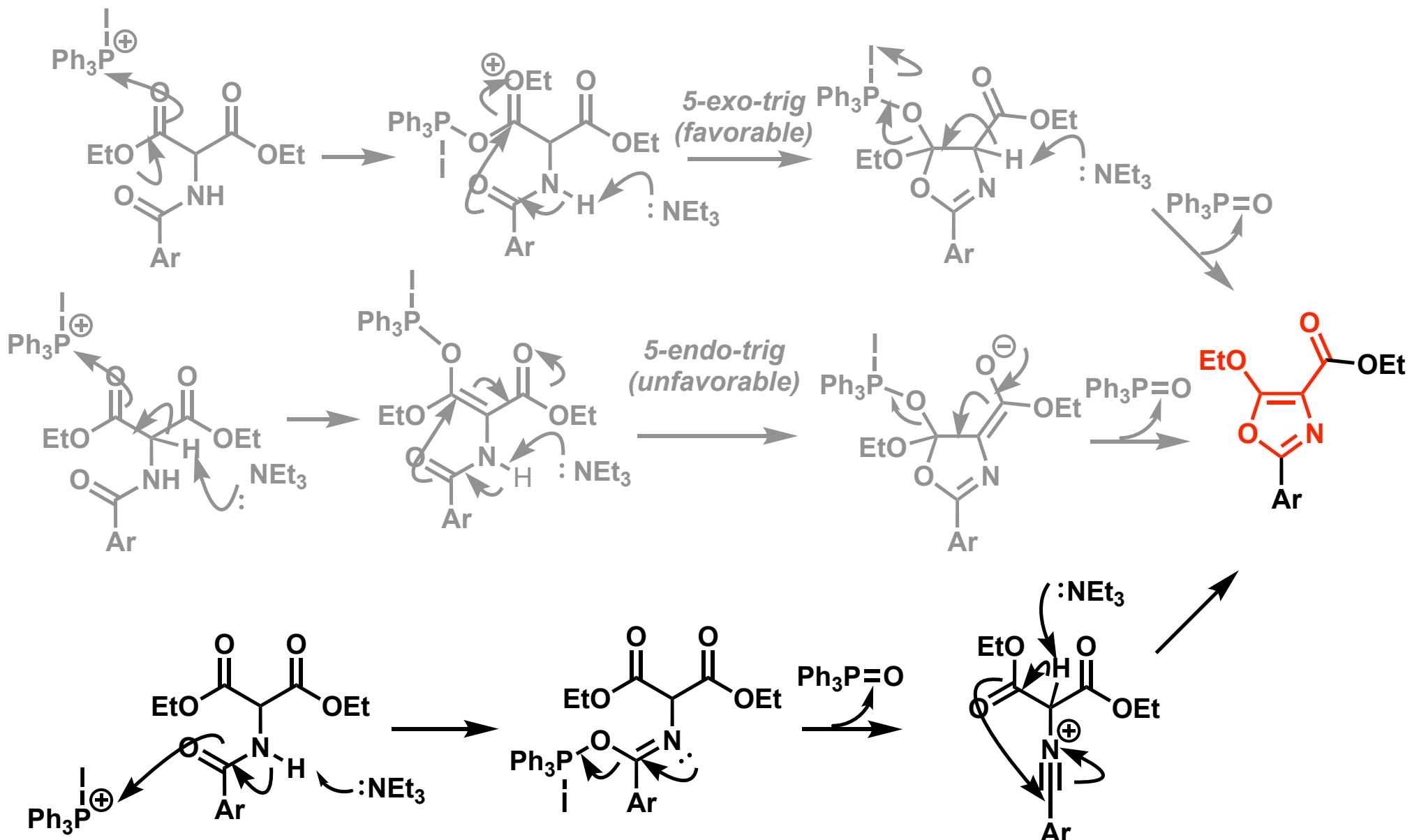


The yields were improved no matter where macrocyclization took place.

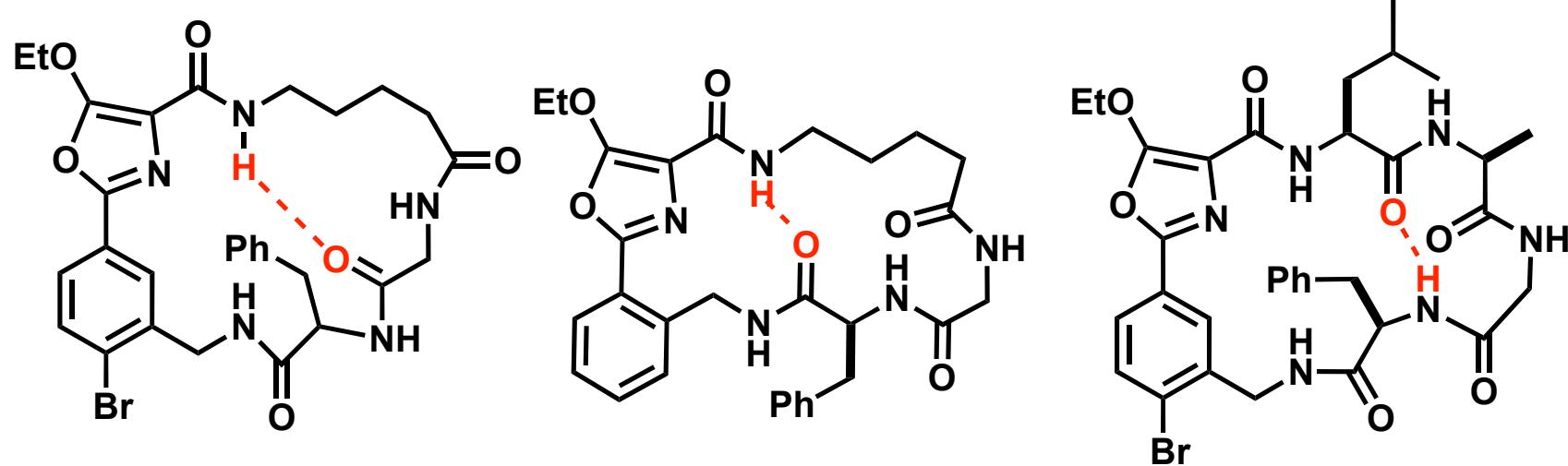
Selectivity of Aziridine Macrocycle



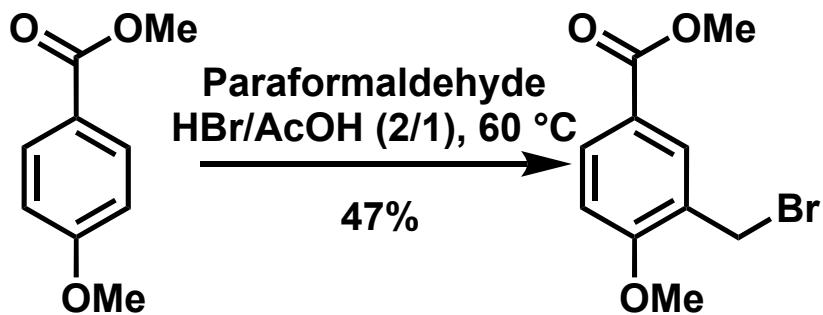
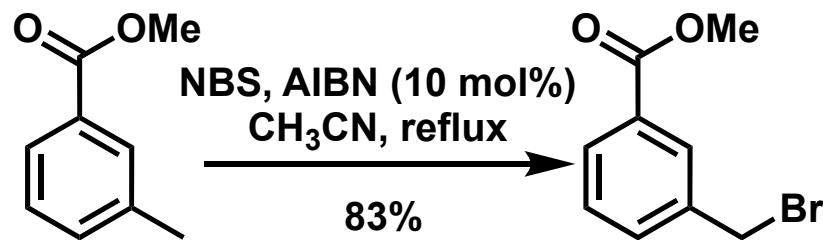
Formation of Oxazole



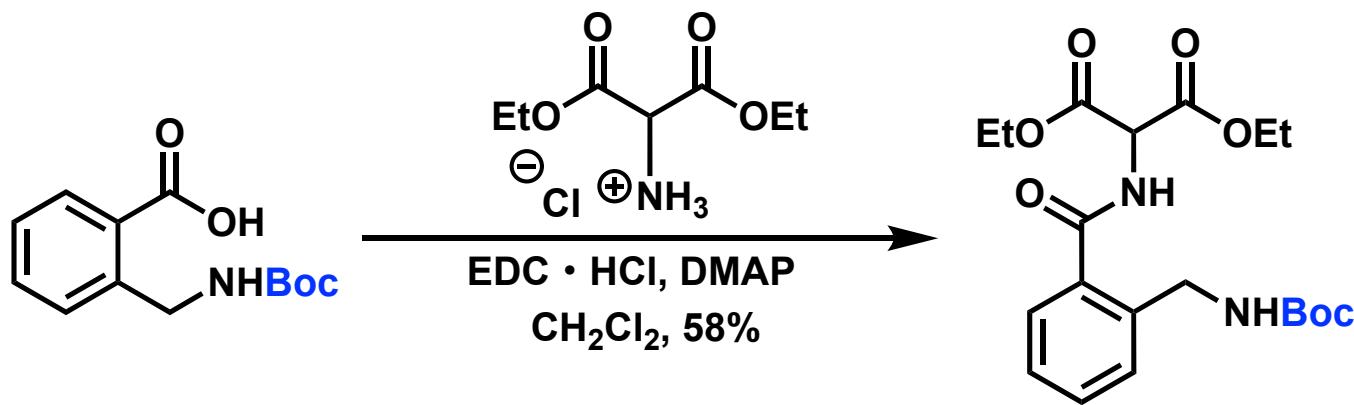
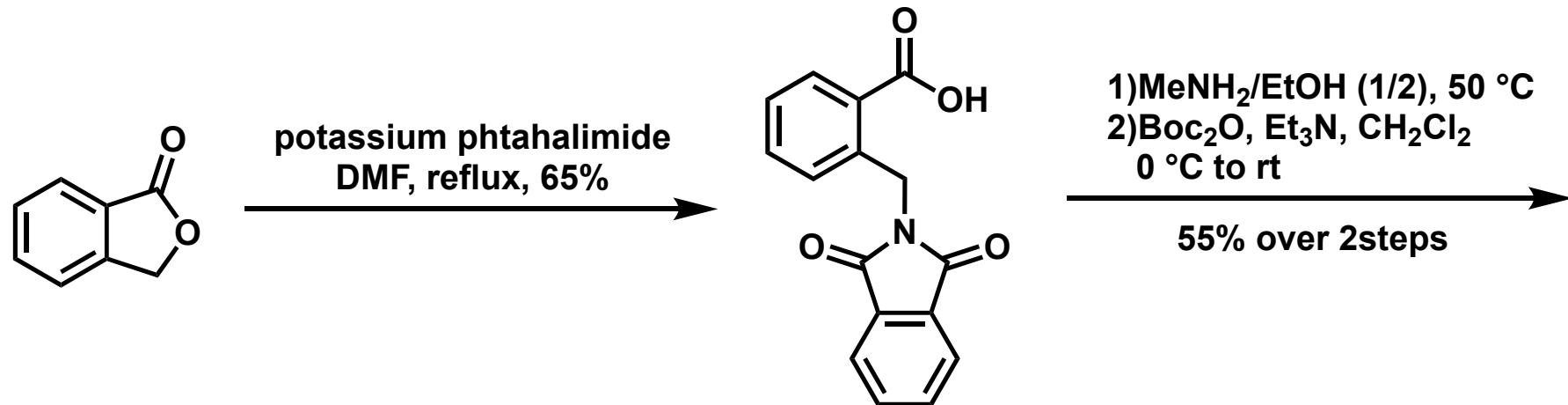
Structure



H or OMe Substituted Compound



Ortho Substituted Compound

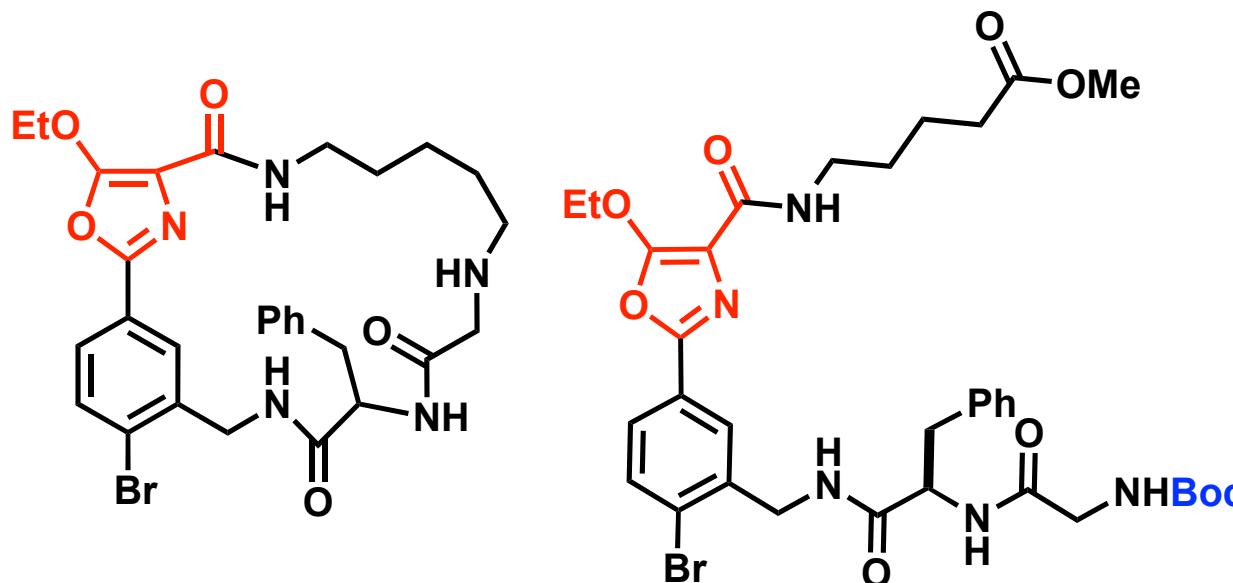


Comparison with Linear Peptide

Question: Does the mechanism change in case of the macrocycles?

$$\text{Eyring equation: } k = \frac{\kappa k_B T}{h} \exp\left(\frac{\Delta G^\ddagger}{R}\right)$$

k: rate constant, *κ*: transmission coefficient, *k_B*: Boltzmann constant, *h*: Planck constant

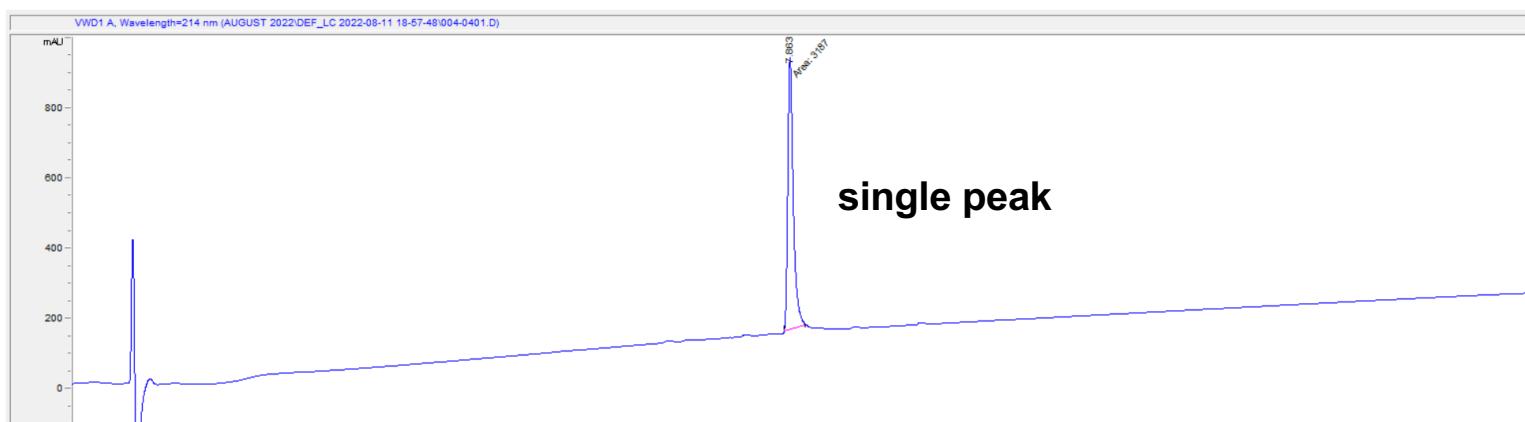
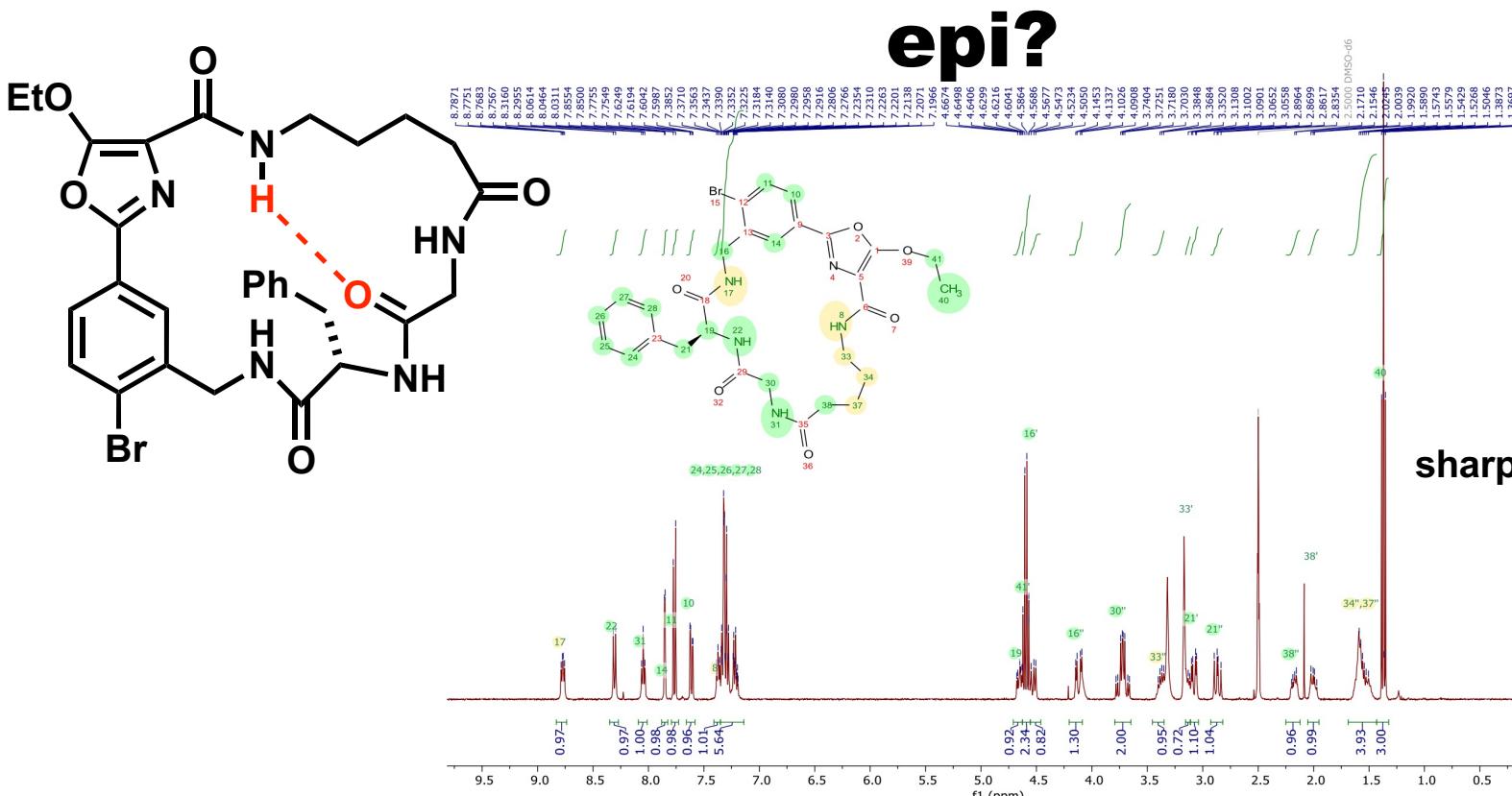


	macrocycle	linear peptide
ΔG^\ddagger at 110 °C	30.1 kcal/mol	29.3 kcal/mol

There are no obvious changes in Gibbs activation energy.

1) Huh, S.; Saunders, G. J.; Yudin, A. K. *Angew. Chem. Int. Ed.*, **2023**, 135, e202214729.

2) Dewar, M. J. S. *J. Am. Chem. Soc.* **1974**, 96, 6148.



A linear gradient starting from 5% of B to 95% over 15 min at a flow rate of 1.0 mL/min.
Stays constant at 95% for 1 min and then returns to 5% over 0.5 min.
eluent A (0.1% formic acid in water) and B (0.1% formic acid in acetonitrile).

ROESY-Derived Distance Restraint

Integrated volumes of ROESY crosspeaks were converted to proton interatomic distances by using an inverse sixth power relationship.



A reference integral was calculated as the average integral between sets of geminal protons which was then set to the calculated geminal interproton distance of 1.78 Å.

For example,

the list of intensity of geminal protons: 0.3, 0.4, 0.35



average intensity: 0.35 defined as 1.78 Å



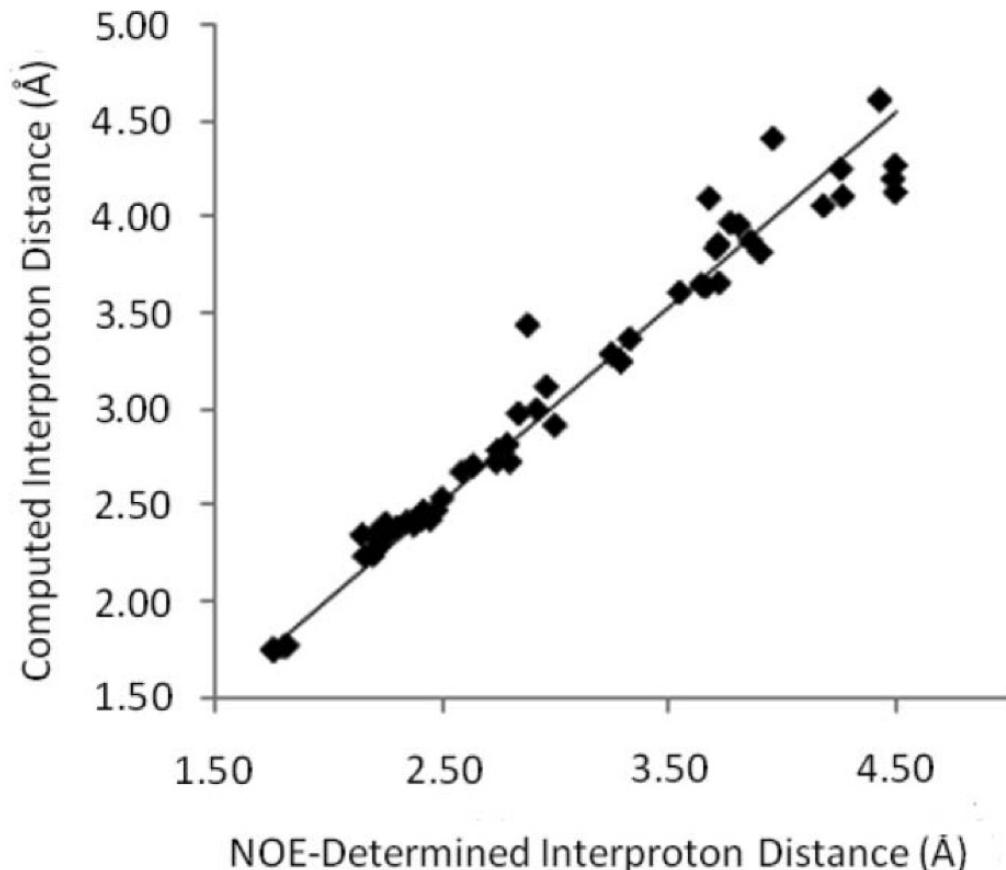
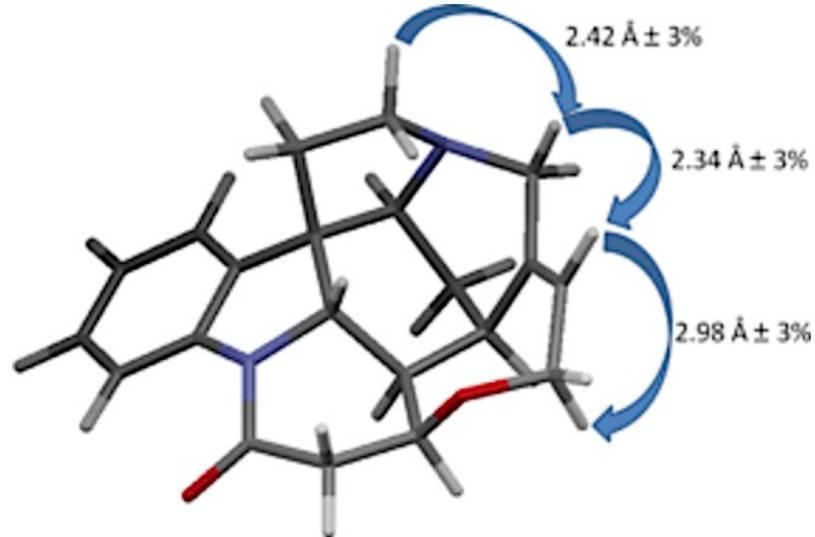
If the intensity was 0.1, the distance (X Å) between the two protons is

$$X = 1.78 \times \sqrt[6]{\frac{0.35}{0.1}} = 2.19$$

Considering uncertainty, the distance X used for calculation was 0.9 X to 1.1 X

Accuracy of NOE-Derived Distance

NOE-derived distance is consistent with computed distance
in both case of flexible and rigid molecules.

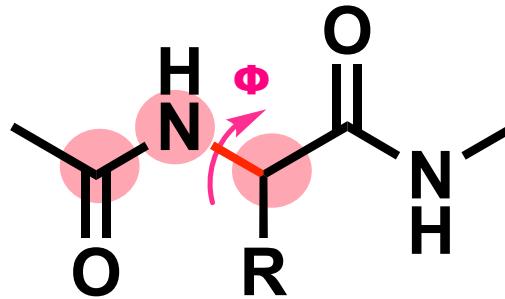


- 1) Butts, C. P.; Jones, C. R.; Towers, E. C.; Flynn, J. L.; Appleby, L.; Barron, N. *J. Org. Biomol. Chem.* **2011**, 9, 177.
2) Jones, C. R.; Butts, C. P.; Harvey, J. N. *Beilstein J. Org. Chem.* **2011**, 7, 145.

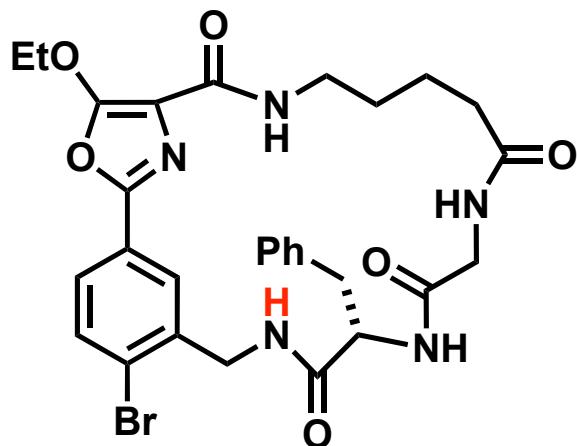
\j -Coupling Constants-Derived Dihedral Angles

3J coupling constants were recorded from the ^1H NMR spectrum.

NH-C α H 3J coupling constants of < 6 Hz $\rightarrow \Phi : -60^\circ \pm 25^\circ$
NH-C α H 3J coupling constants of > 8 Hz $\rightarrow \Phi : -120^\circ \pm 25^\circ$



Temperature Coefficient

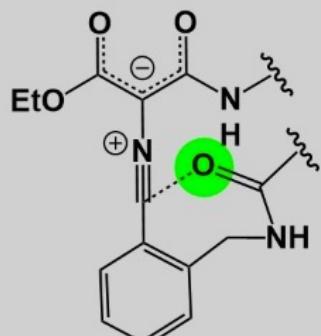


Temp (K)	Linker ¹ -NH	Gly ² -NH	Phe ³ -NH	Bn ⁴ -NH
298	7.37	8.05	8.31	8.77
303	7.37	8.02	8.27	8.74
308	7.37	8.00	8.23	8.72
313	7.37	7.97	8.20	8.69
318	7.38	7.94	8.16	8.66
T _{coeff}	0.26	-5.12	-7.13	-5.32

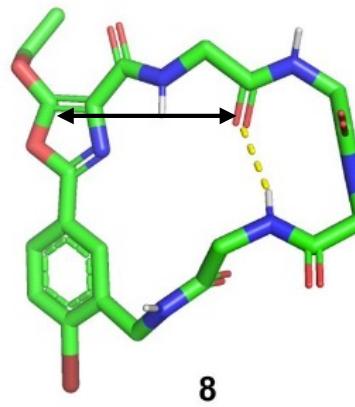
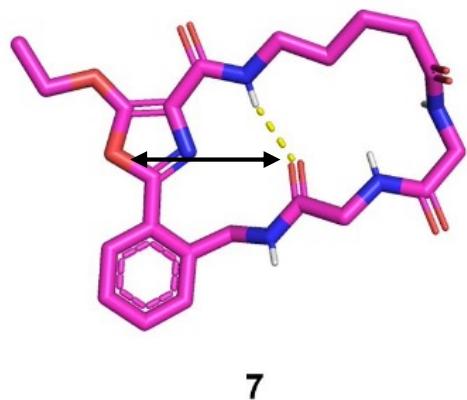
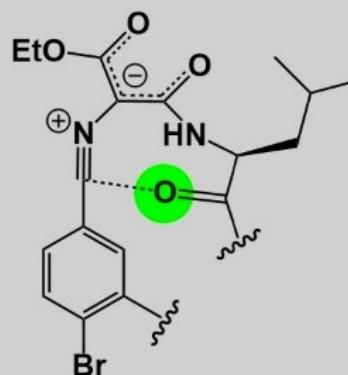
- 1) Huh, S.; Saunders, G. J.; Yudin, A. K. *Angew. Chem. Int. Ed.*, **2023**, 135, e202214729.
2) Kessler, H. *Angew. Chem. Int. Ed.* **1982**, 21, 512.

Authors' opinion

7-membered stabilization

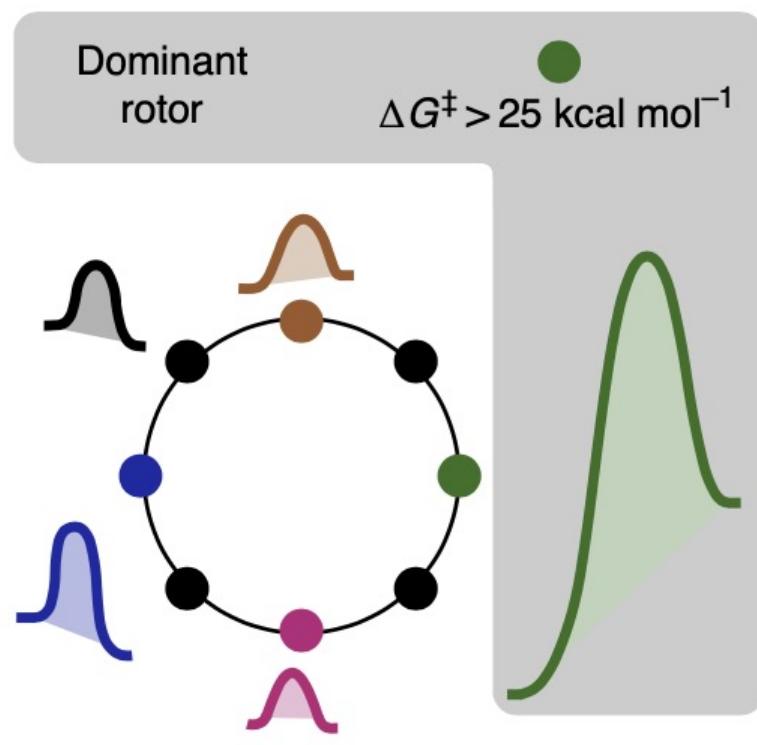


8-membered stabilization



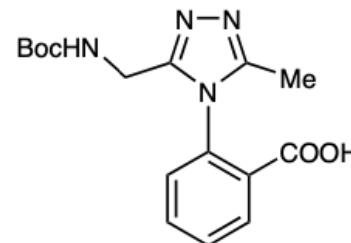
my opinion: too far to interact with

“Dominant Rotor”

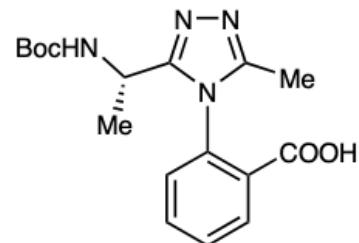


cis/trans isomerization ~20 kcal/mol

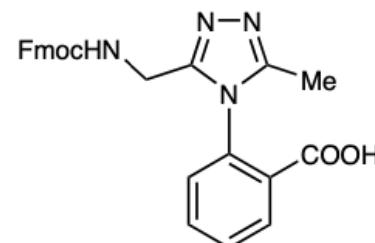
biaryl linker



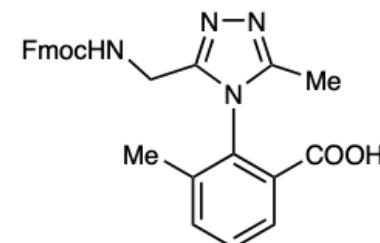
3 (29.6)



4 (29.7)



5 (33.2)



6 (48.4)