

Macrocyclization Using Selective Triazination Reaction (STaR)

**2024.01.20. Literature Seminar
B4 Yuto Torigoe**

Contents

1. Introduction

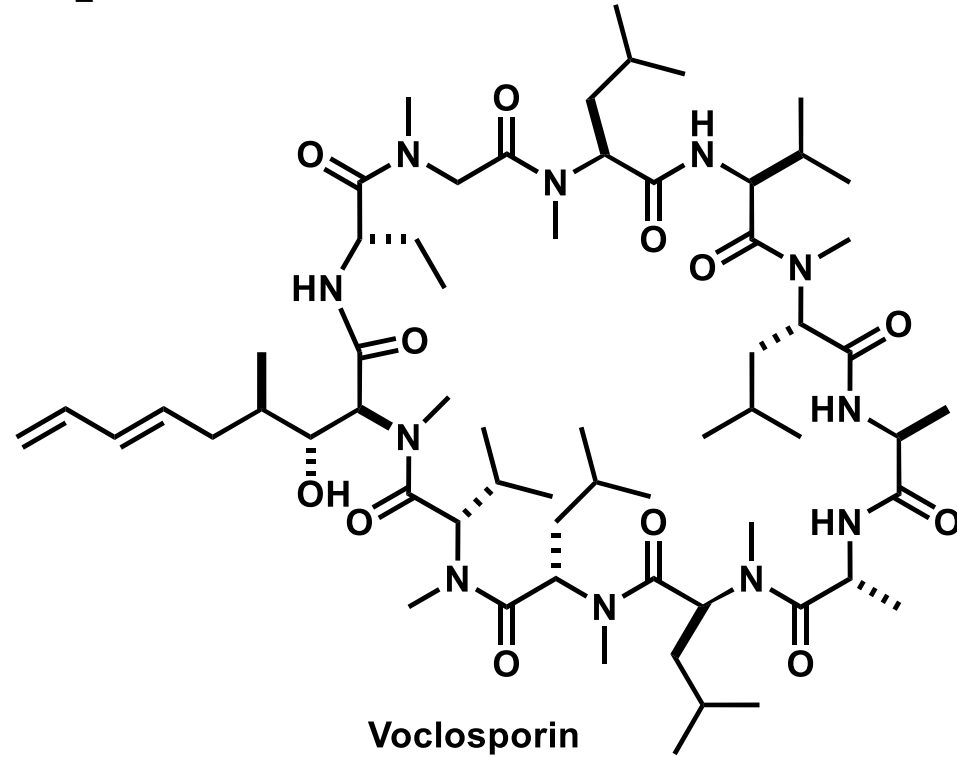
2. Selective Triazination Reaction (STaR) of Secondary Amine

3. Rapid Arene Triazene Chemistry for Macrocyclization

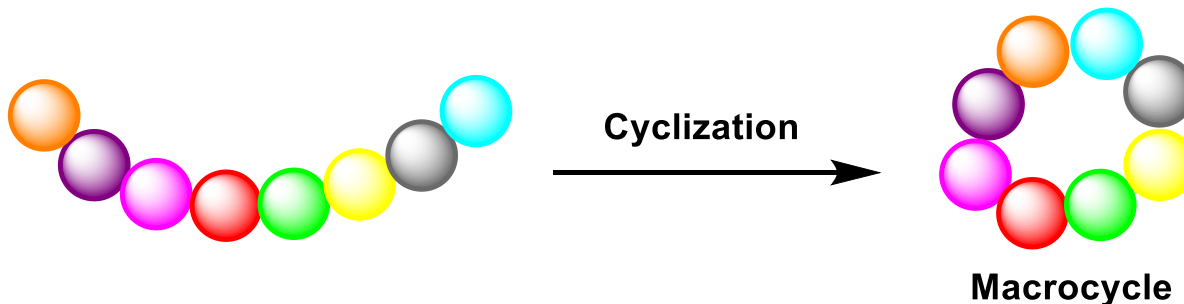
Cyclic Peptides

Favorable properties

1. **Binding affinity and specificity**
Adopting fewer conformations
→ Binding site is more probable)
2. **Stability**
less flexible
→ often hinder active site of proteases)
3. **Membrane permeability**
formation of intramolecular hydrogen bond within peptides
→ reducing solvation and burying polar surface

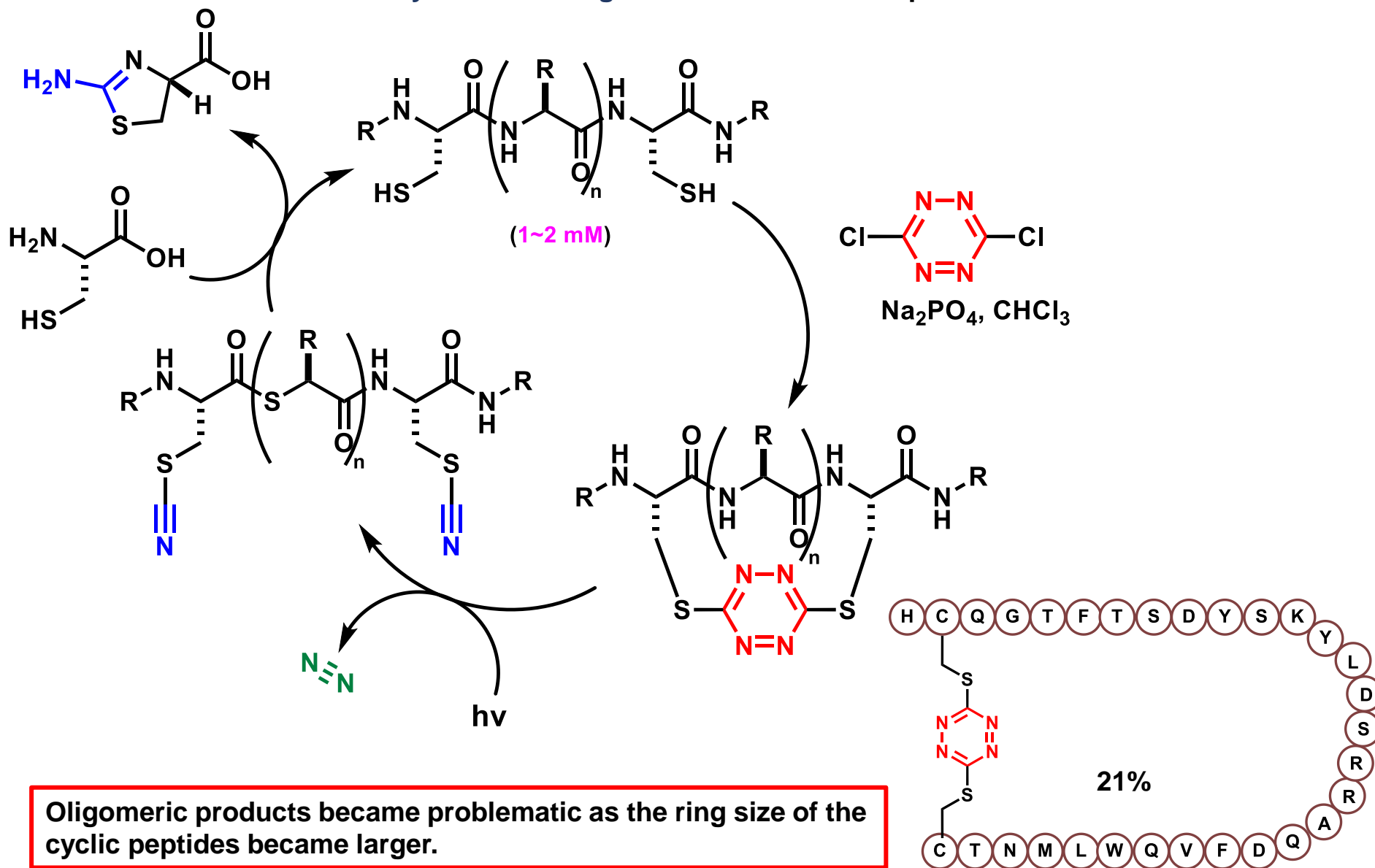


Target: Calcineurin
Approval: 2021



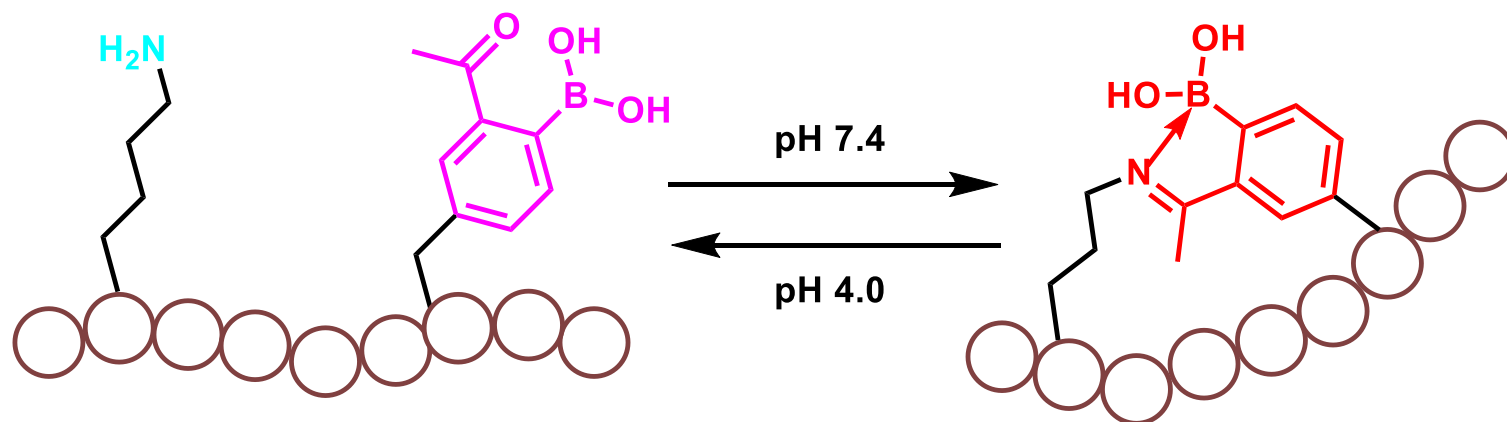
Macrocyclization Strategies (1)

Macrocyclization using thio-etherification: respond to UV

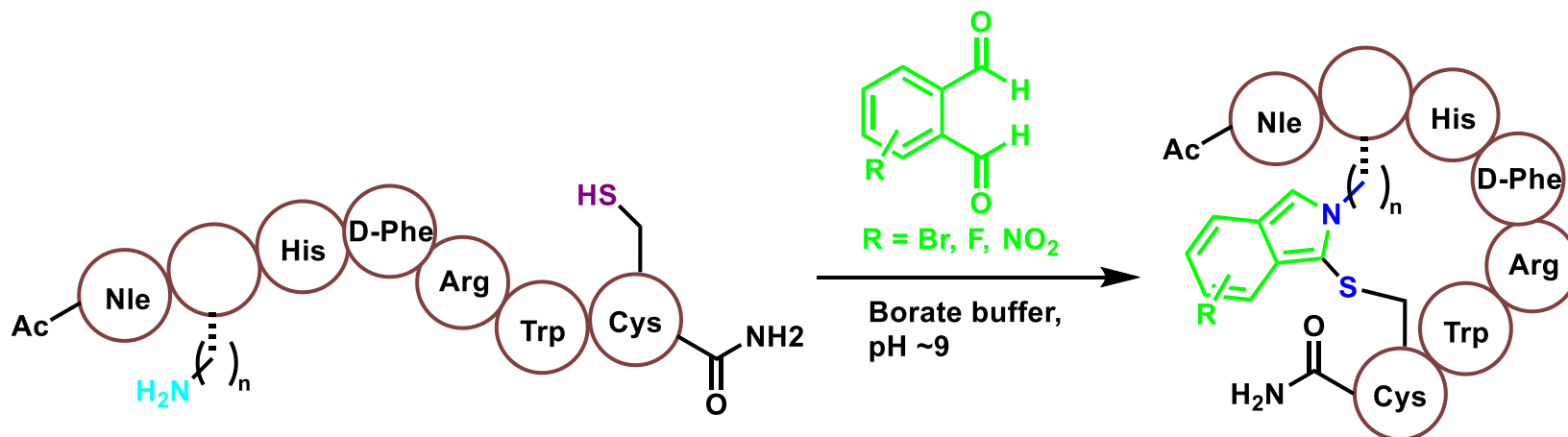


Macrocyclization Strategies (2)

Macrocyclization using intramolecular iminoboronate formation: respond to pH



Macrocyclization using fluorescent isoindole crosslinking (FIICk): fluorescent



These cyclization can be applied to the field of chemical biology

1) Bandyopadhyay, A; Gao, J. *J. Am. Chem. Soc.* **2016**, *138*, 2098–2101.

2) Todorovic, M.; Perrin, D. M. *Methods in Enzymology.* **2020**, 313-332

Contents

1. Introduction

**2. Selective Triazene Reaction (STaR)
of Secondary Amine**

**3. Rapid Arene Triazene Chemistry
for Macrocyclization**

Prof. Monika Raj



Career

- 2009 :Ph.D. @ Indian Institute of Technology, Kanpur
(Prof. Vinod K. Singh)
- 2009-: Postdoctoral Fellow @ University of Pennsylvania
(Prof. Barry S. Cooperman)
- 2010-: Postdoctoral Fellow @ New York University
(Prof. Paramjit S. Arora)
- 2014-: Assistant Professor @ Seton Hall University
- 2017-: Assistant Professor @ Auburn University
- 2020-: Associate Professor @ Emory University

Research Interest

- Utilizing organic chemistry tools
- Development of new chemical reactions, catalysis, and ligation methodologies
- Synthesis of chemical probes

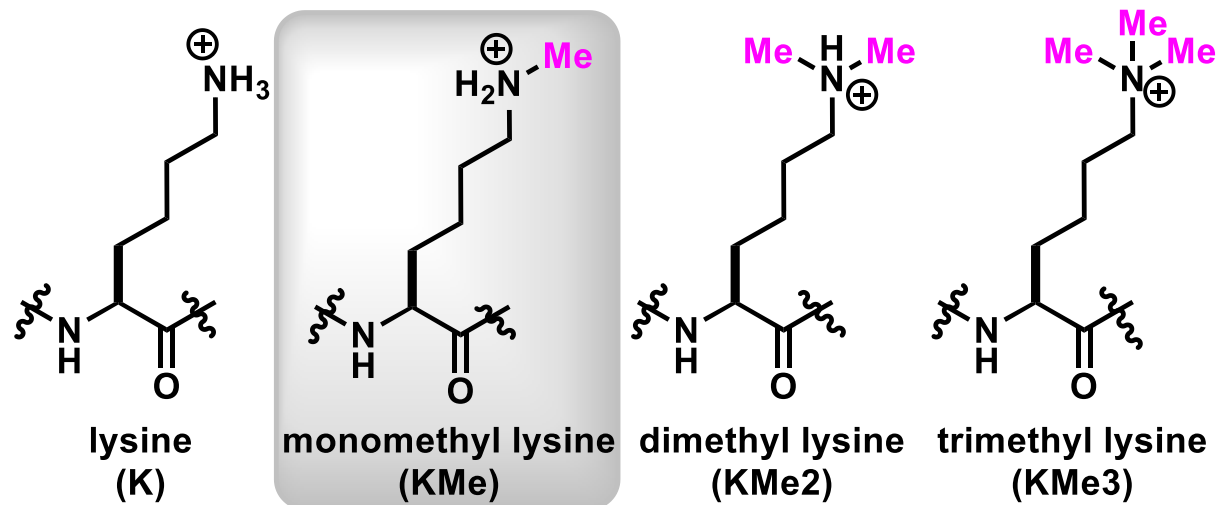
Monomethyl Lysine (Kme)

Lysine methylation (post-translational modification (PTM))
: regulate cell growth, gene expression, and DNA/RNA binding

Especially, Kme PTM is related to heart disease, cancer, and diabetes.

Current method to detect Kme PTMs

- antibody & methyl binding domain
→ unable to distinguish different methylation state (mono, di, tri)
- MS
→ change in the mass by monomethylation is identical to substitution of some amino acids

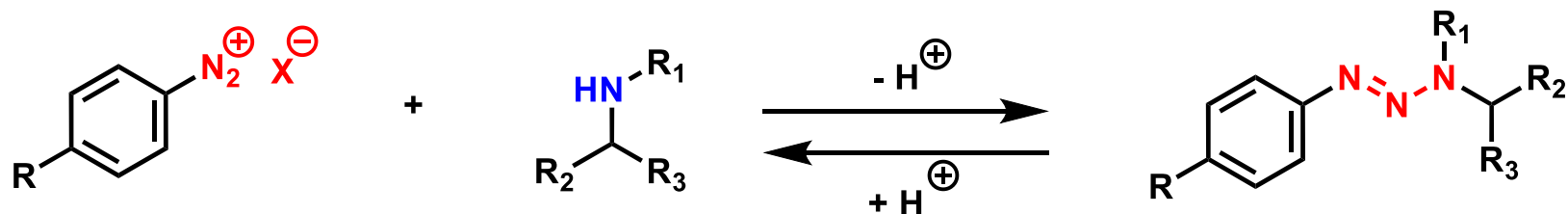


• Negligible change in charge, bulk, and hydrophobicity

Discovery of chemoselective reaction for secondary amine is needed.

Selective triaznation using arene diazonium ion is adopted.

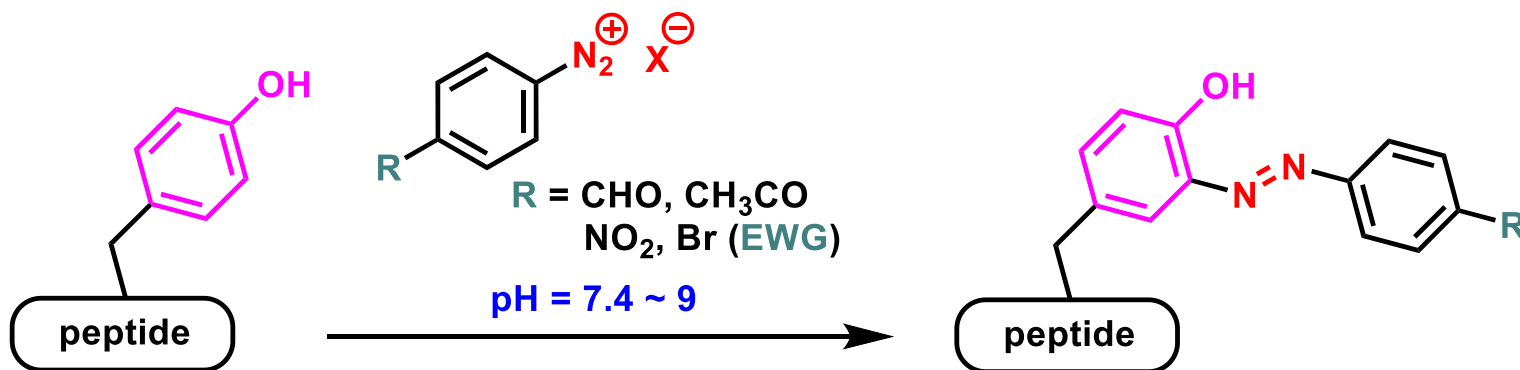
Triazenation Using Arene Diazonium Ion



primary amine: **unstable**
secondary amine: **stable**

Arene diazonium ion selectively forms stable triazene with secondary amine.

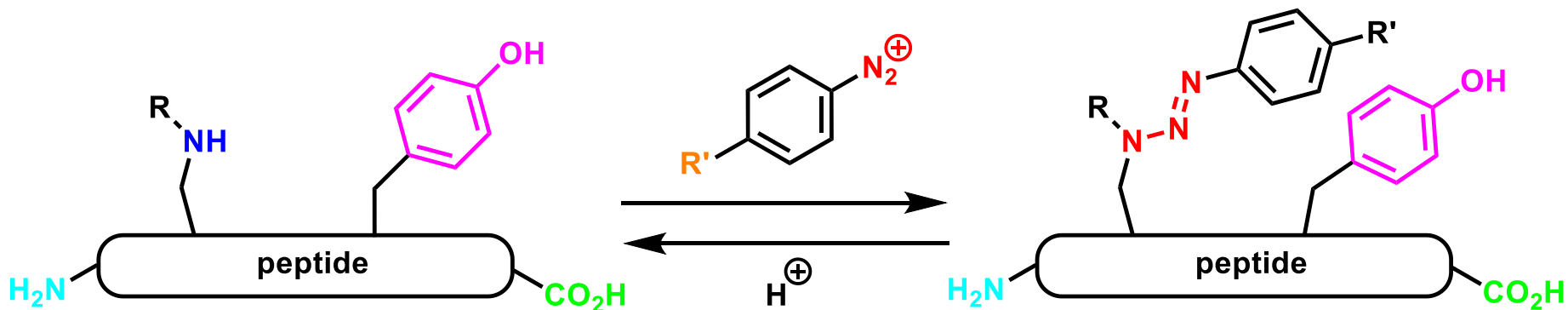
Side reaction with phenol of Tyr: more electrophilic arene is needed



Highly electrophilic arene diazonium ion possibly reacts with phenol.

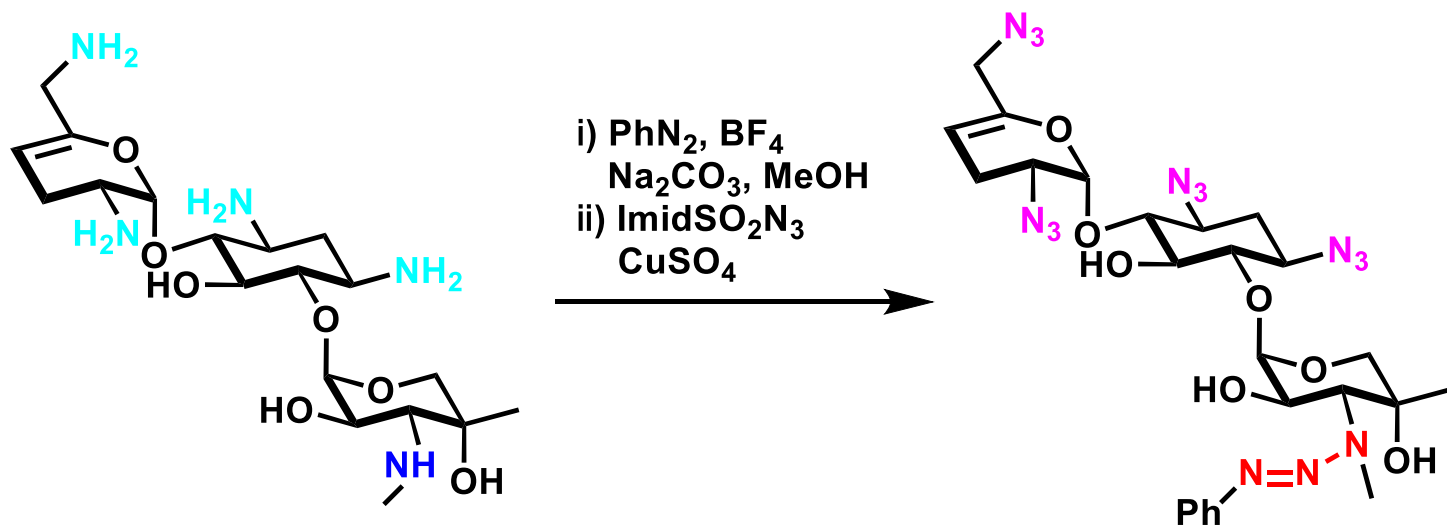
- 1) Schlick, T. L.; Ding, Z.; Kovacs, E. W.; Francis, M. B. *J. Am. Chem. Soc.* **2005**, *127*, 3718–3723.
- 2) Gavriluk, J.; Ban, H.; Nagano, M.; Hakamata, W.; Barbas, C. F. *Bioconjugate Chem.* **2012**, *23*, 2321–2328.

Selective Triazeneation Reaction (STaR)

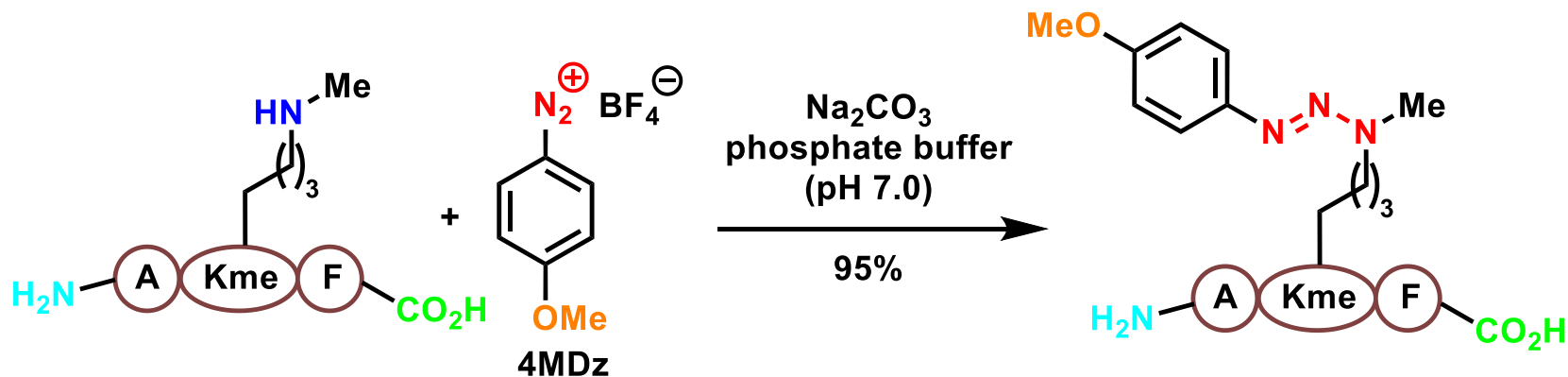
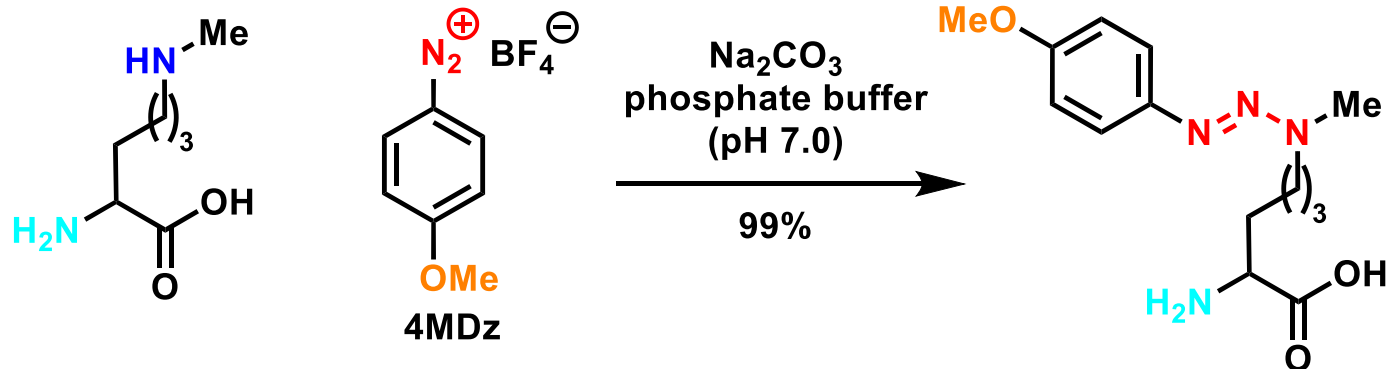


Selective triazeneation of secondary amine would be achieved by controlling electrophilicity of arene diazonium ion.

Ex.)

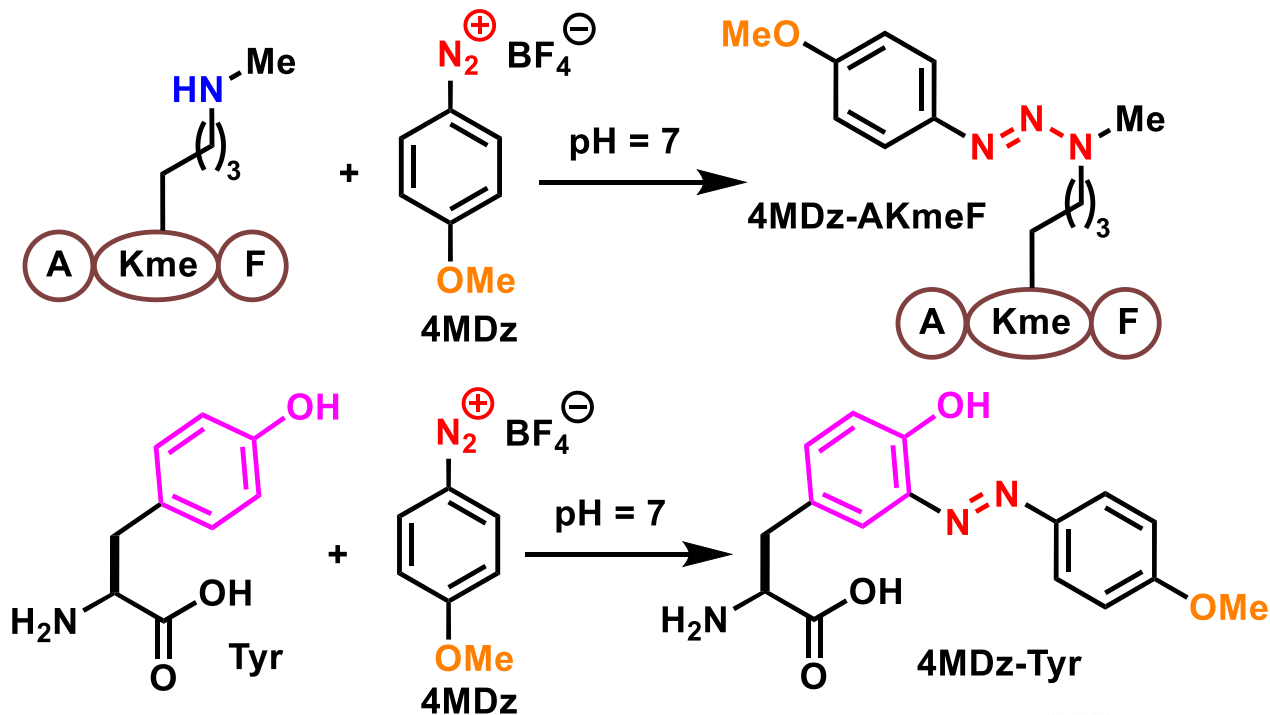


STaR of Monomethyl Lysine

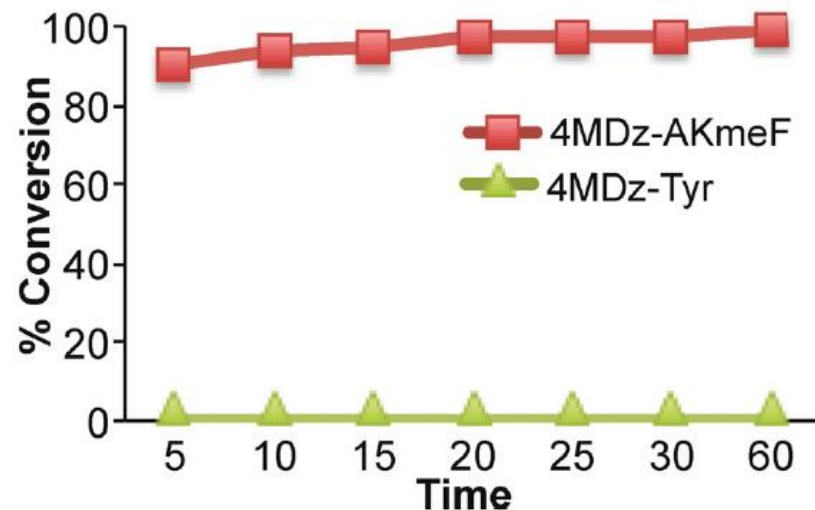


4MDz reacts selectively with secondary amine of Kme monomer and Kme containing peptide.

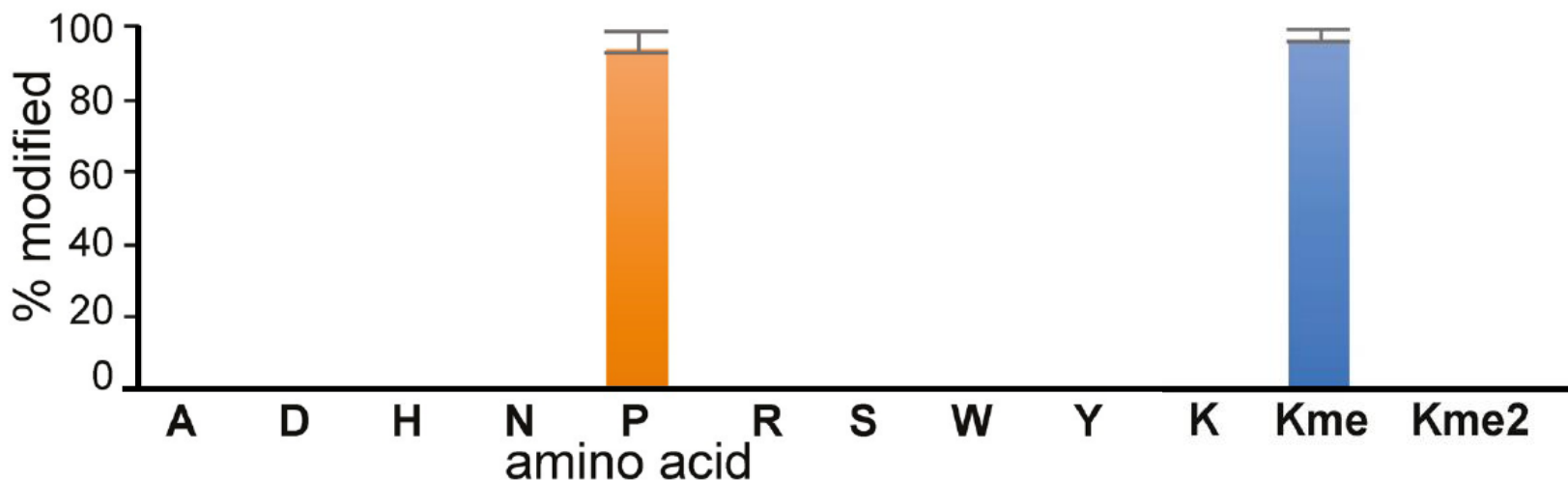
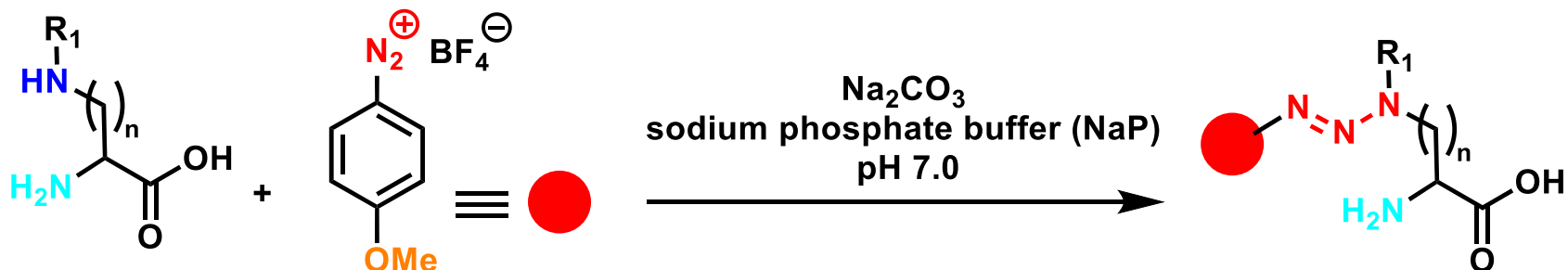
Reactivity of 4MDz



While 4MDz reacts secondary amine rapidly, it does not react with phenol of Tyr.

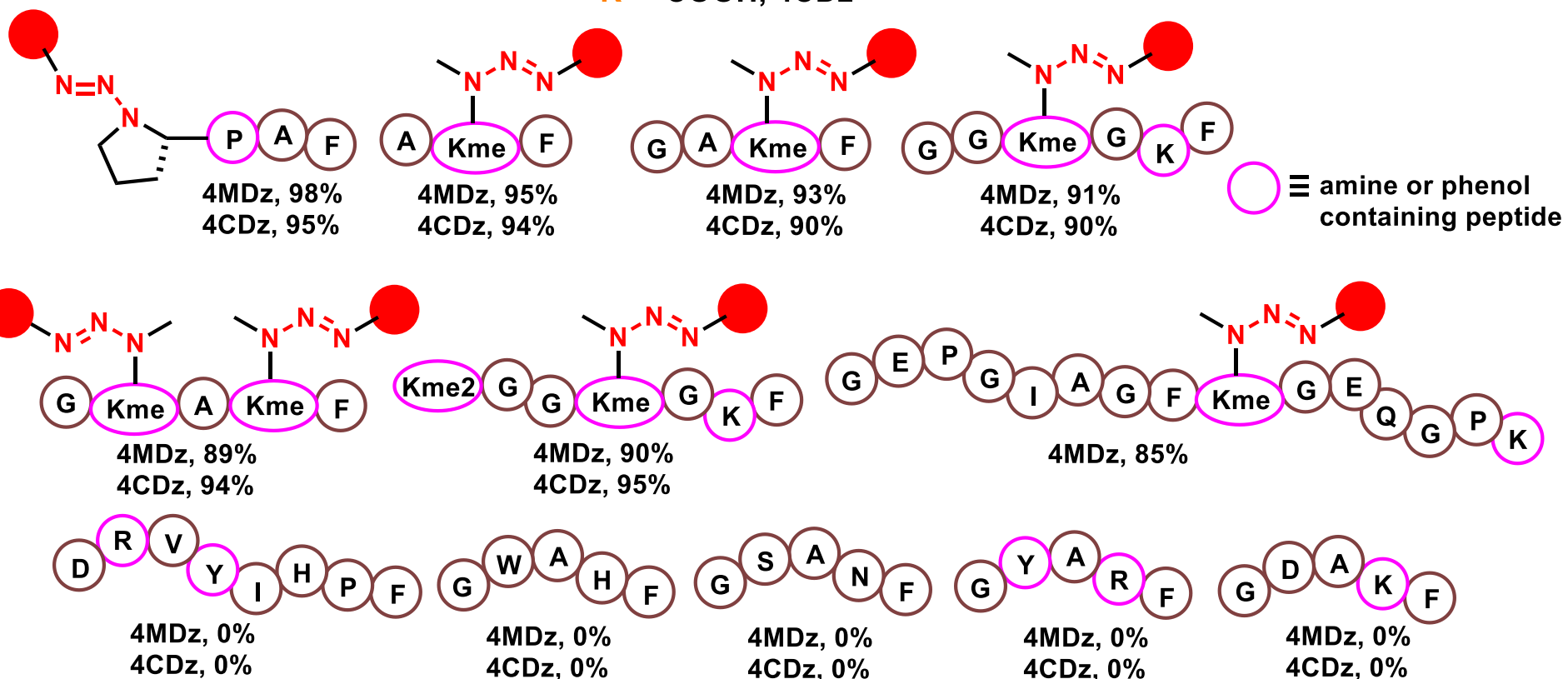
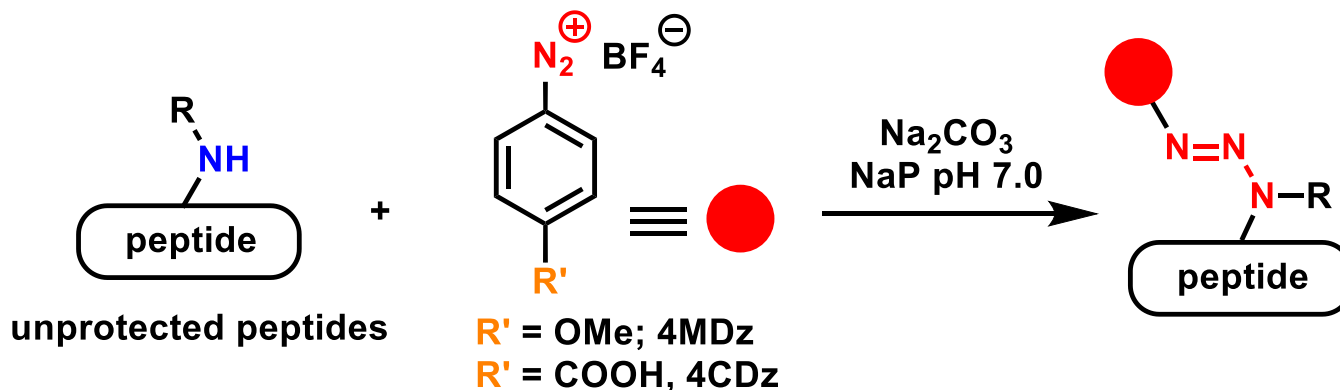


Reactivity of 4MDz with amino acid monomer



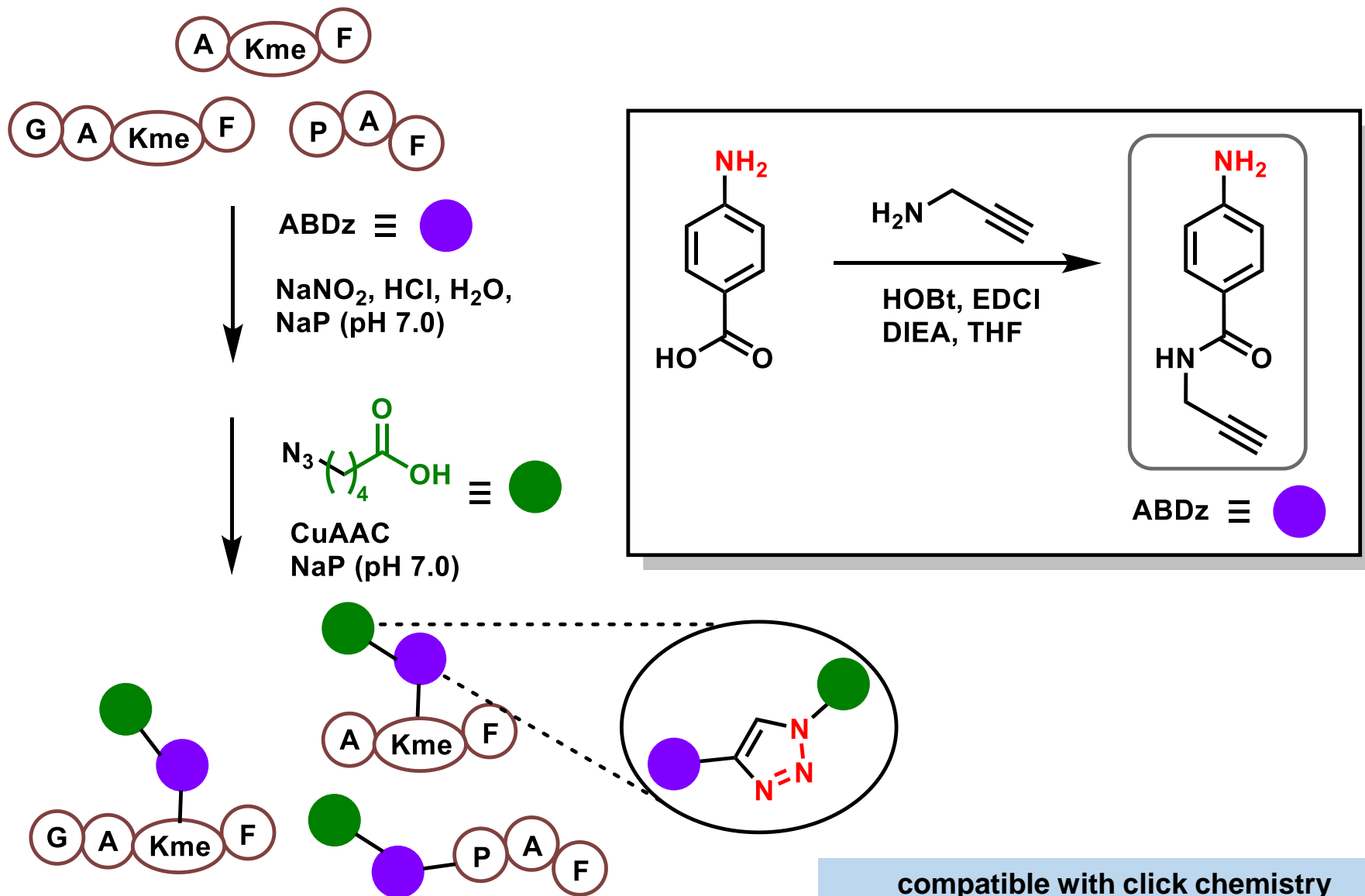
- 4MDz reacts only with amino acids bearing secondary amine (P, Kme)
- 4MDz does not react with phenol of Tyr

STaR of peptides

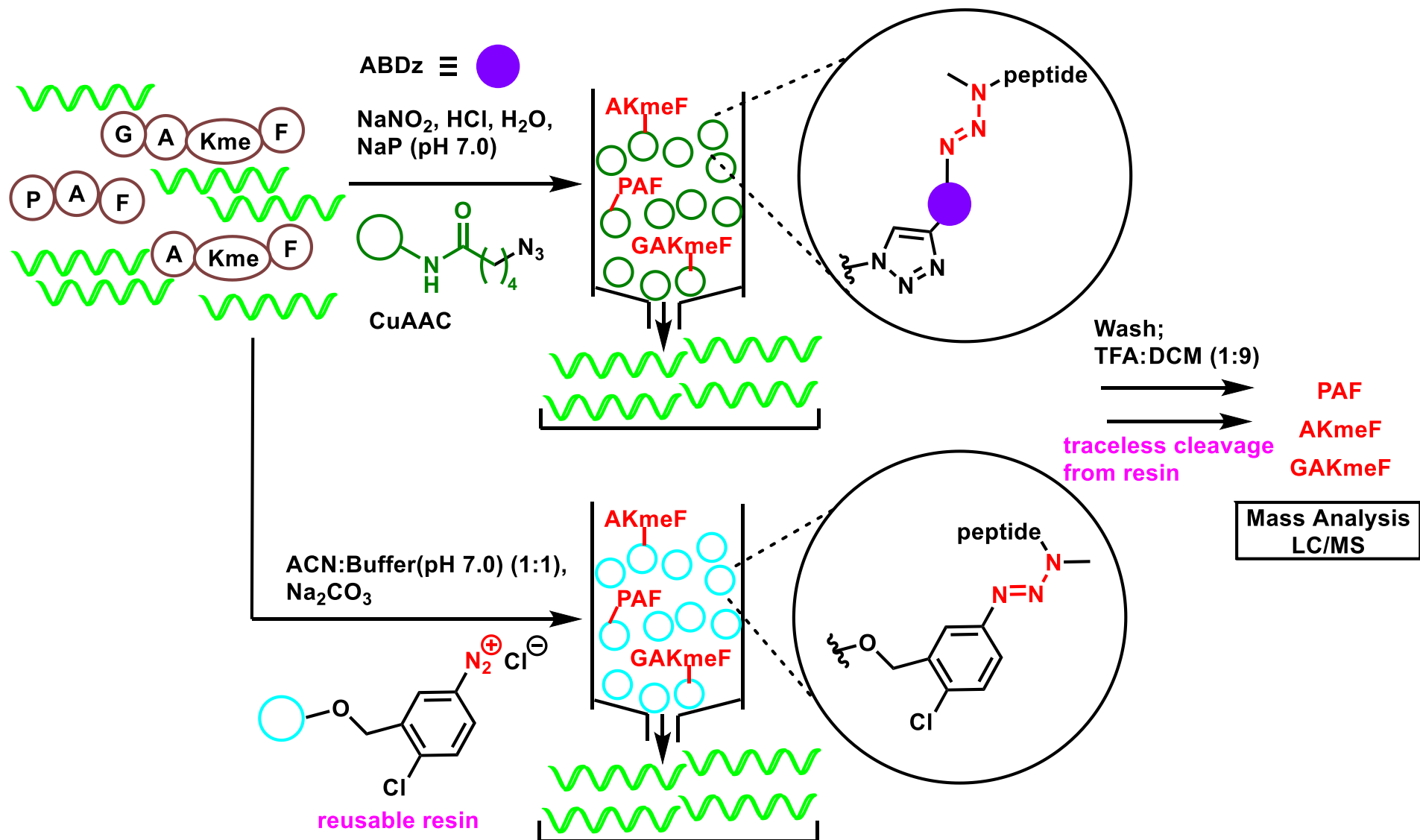


4MDz and 4CDz selectively reacted with peptides bearing P or Kme.

Selective Tagging of secondary amine (1)



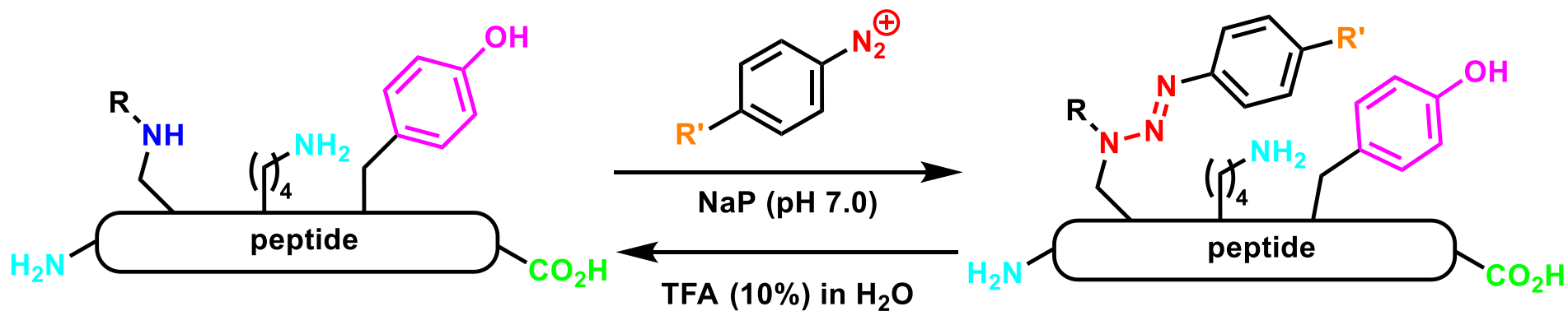
Selective Tagging of peptides bearing secondary amine (2)



STaR enables selective Kme or N-terminal P tagging and from a complex mixture.

Short Summary

STaR of Kme or N-terminal P containing peptide



- Chemoselective for secondary amines
- Respond to pH
- Reversible
- Utilizing for traceless tagging

Contents

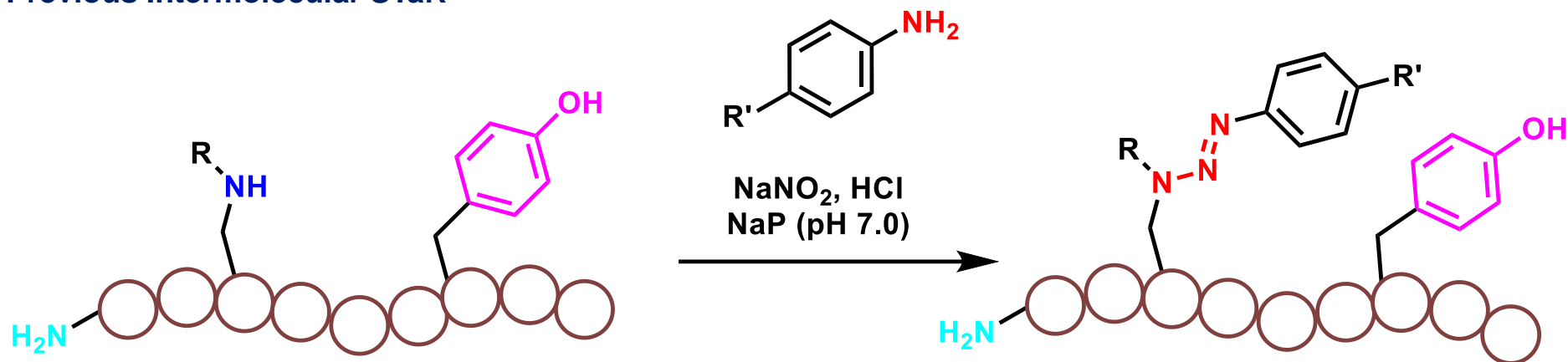
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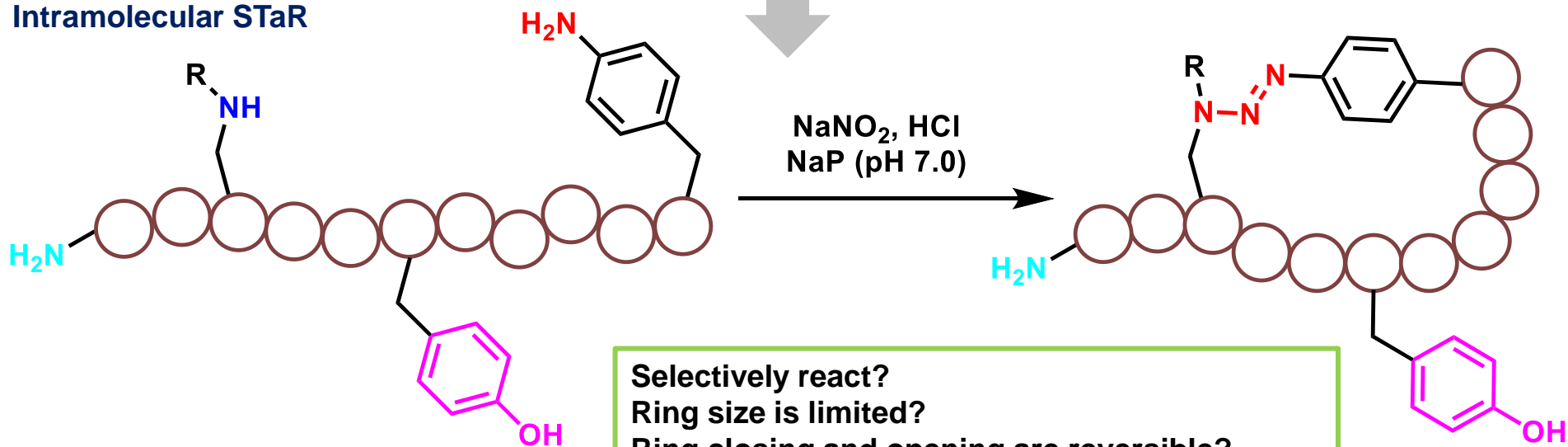
**3. Rapid Arene Triazene Chemistry
for Macrocyclization**

Application of STaR to Macrocyclization

Previous Intermolecular STaR

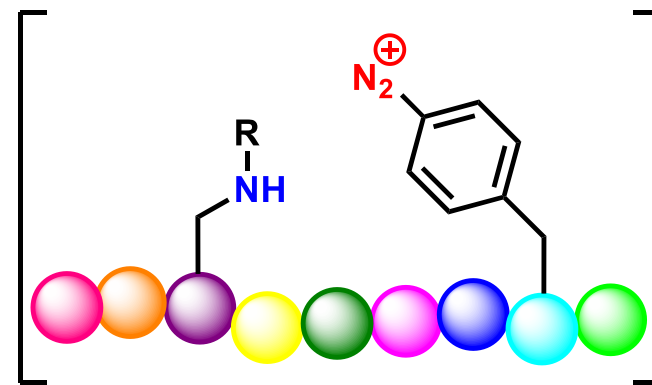
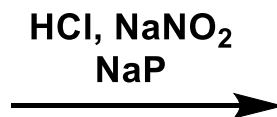
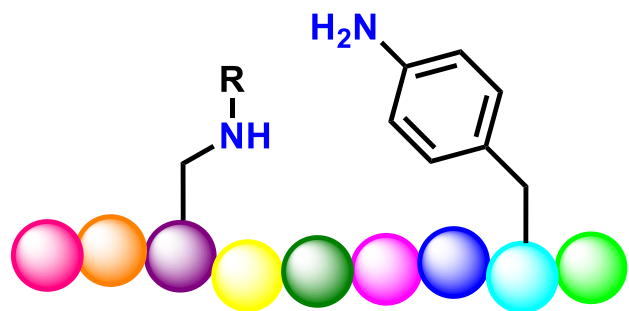


Intramolecular STaR



Selectively react?
Ring size is limited?
Ring closing and opening are reversible?
Side reaction occurs? (azo-coupling, oligomer)

New Macrocyclization Strategy

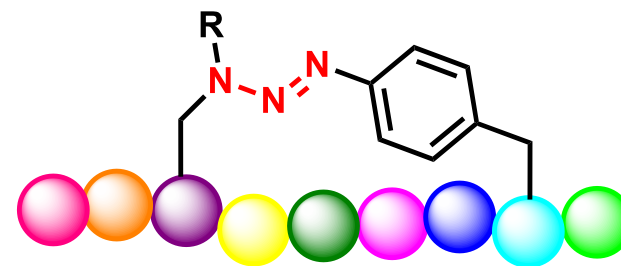


Diazonium ion

 ≡ peptide with secondary amine
ex.) N-terminal proline
monomethyl lysine

 ≡ *p*-amino phenylalanine (pAF)

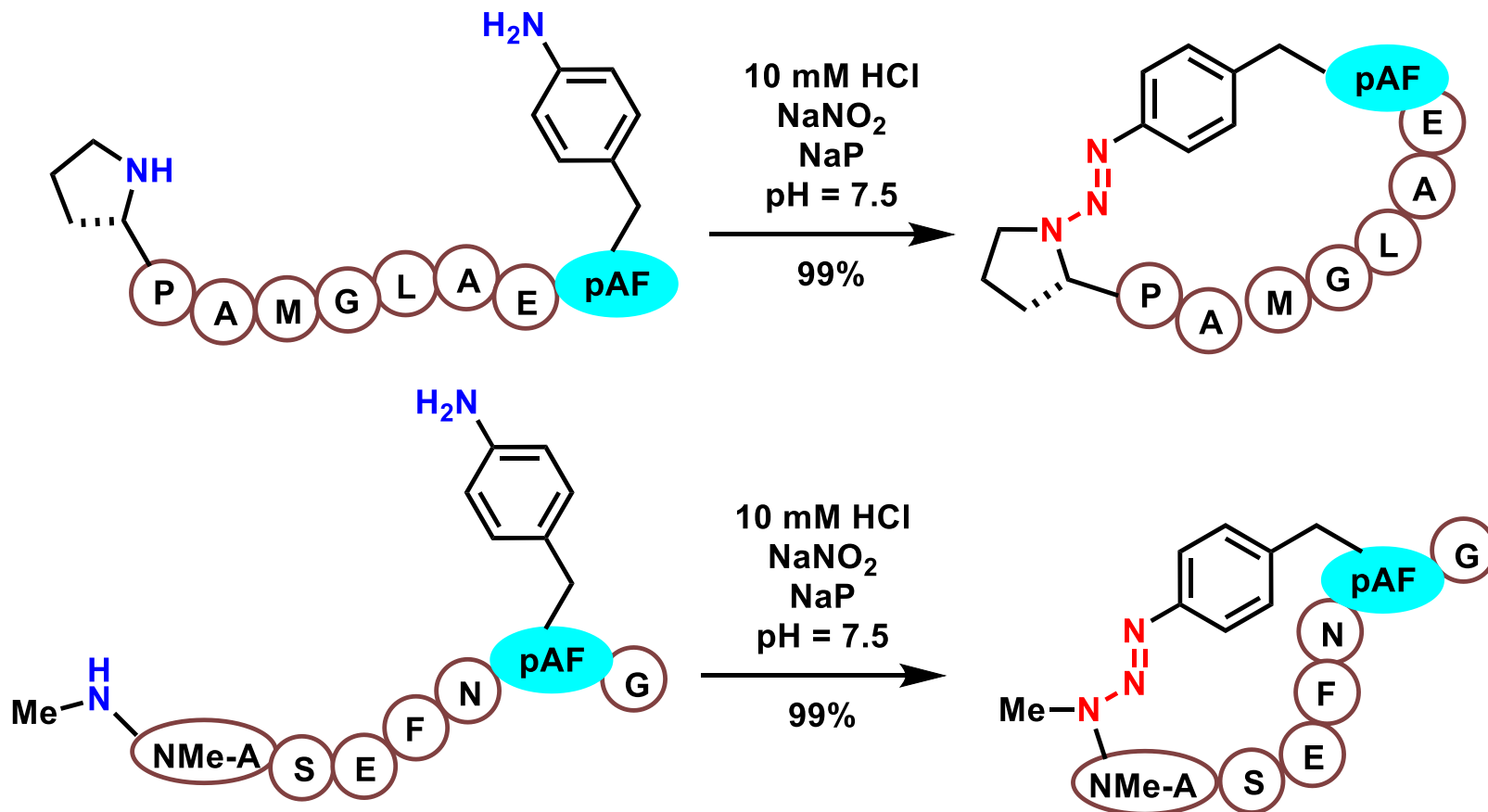
Ring opening
(pH 1.5) ↑
↓ Ring closing
(pH 7.5)



Triazene macrocycle

- *p*-Amino phenylalanine is incorporated.
- Arene diazonium ion is easily introduced inside the peptide.
- Triazene macrocycle is generated under neutral pH condition.
- Ring opening and closing are reversible (**respond to pH**).
- Triazene macrocycle becomes chromophore.

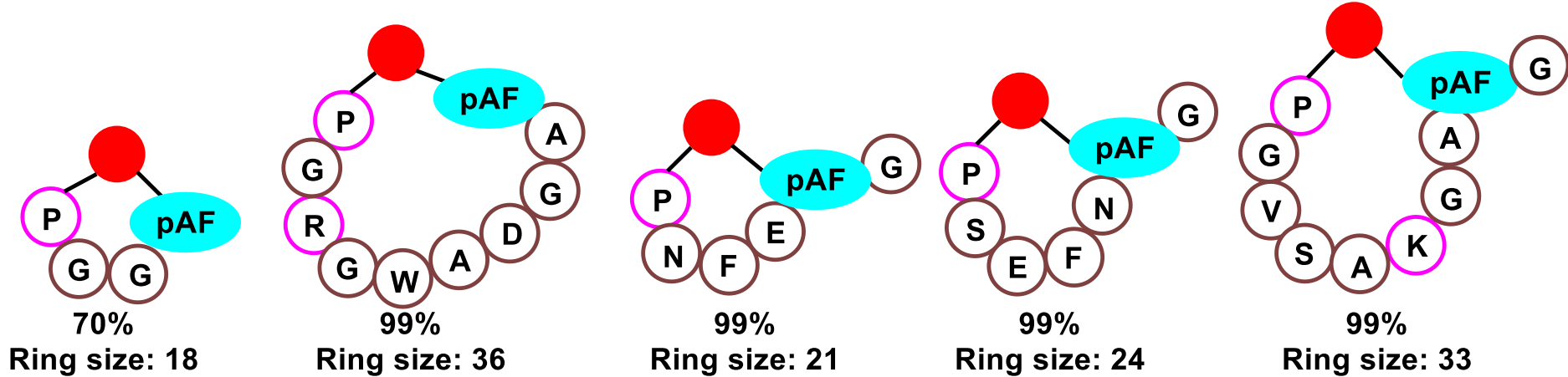
Triazene Cyclization of peptides (1)



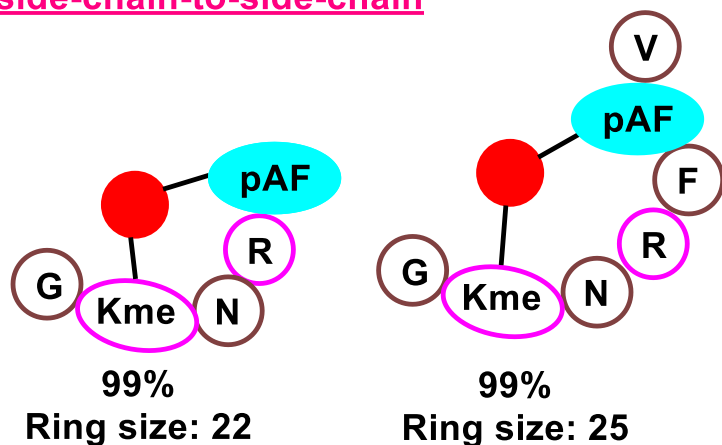
Triazene-mediated macrocyclization was carried out in high yield.

Triazene Cyclization of peptides (2)

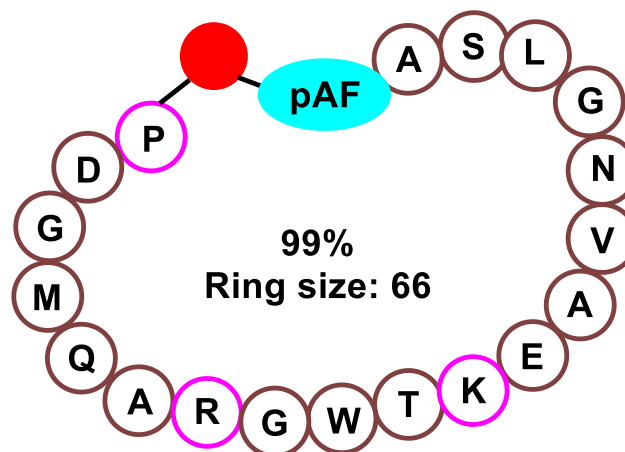
head-to-side-chain



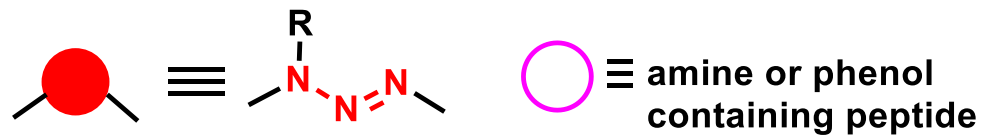
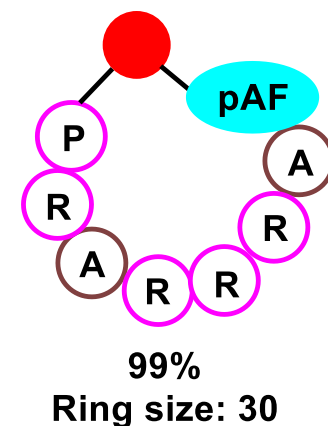
side-chain-to-side-chain



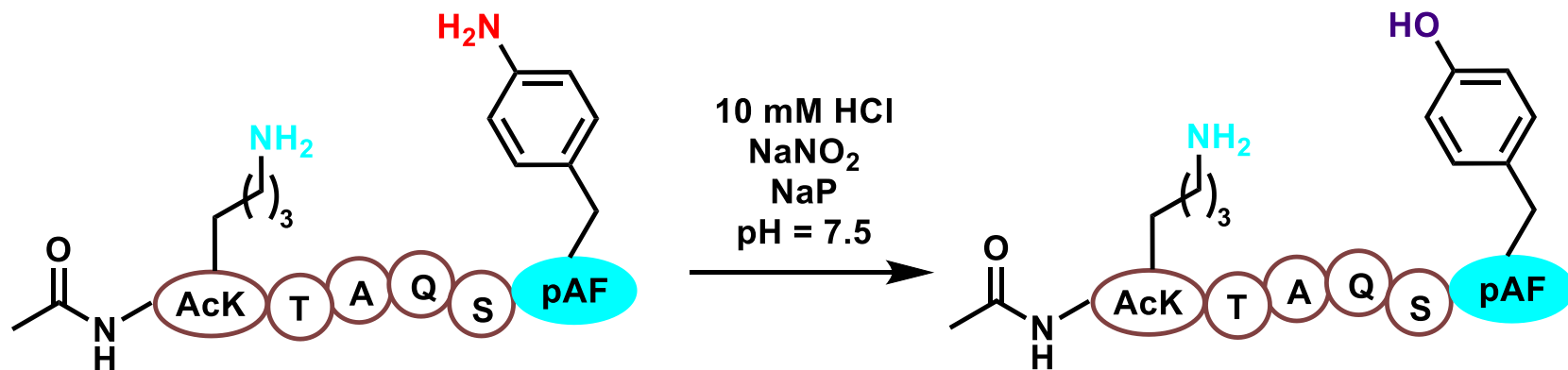
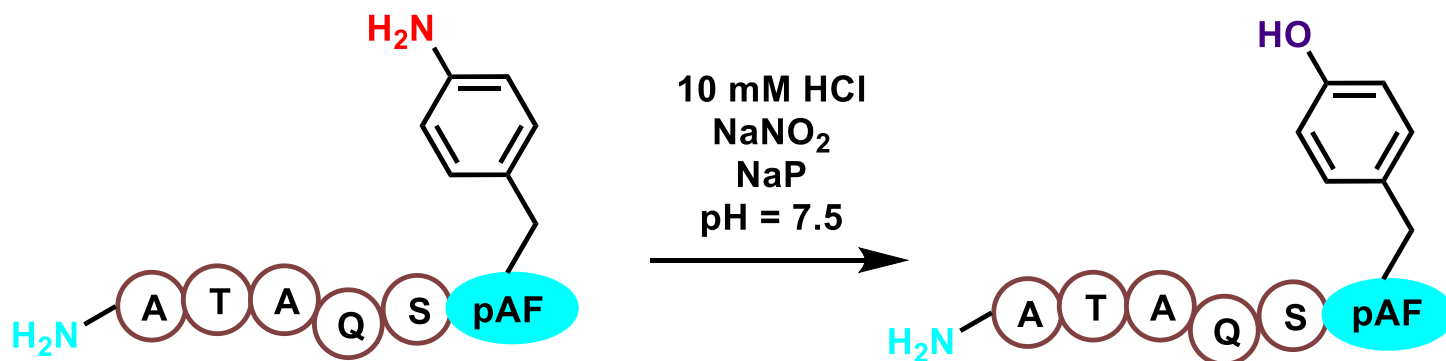
long peptides



multiple arginines containing peptides

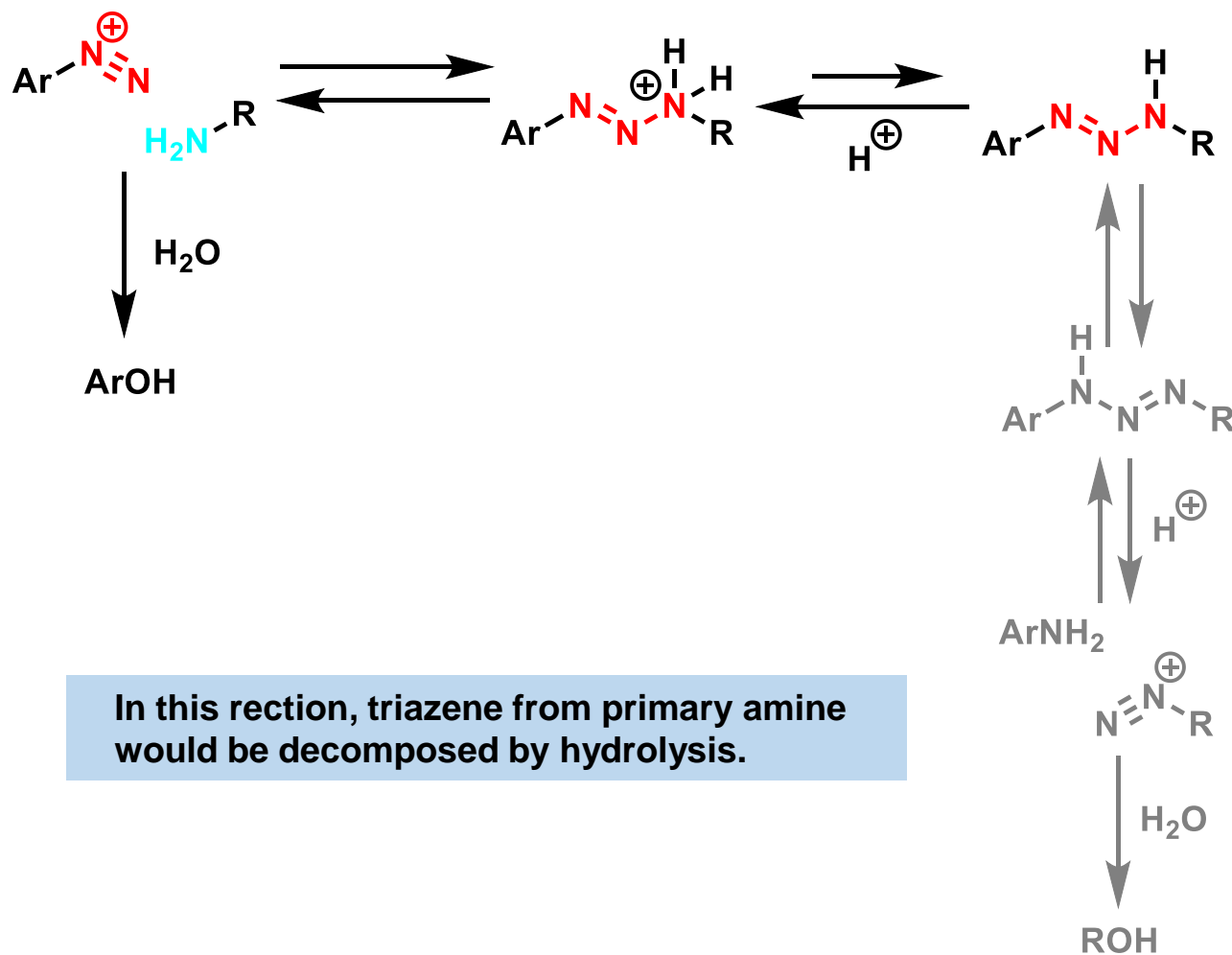


STaR of Peptides without Secondary Amine



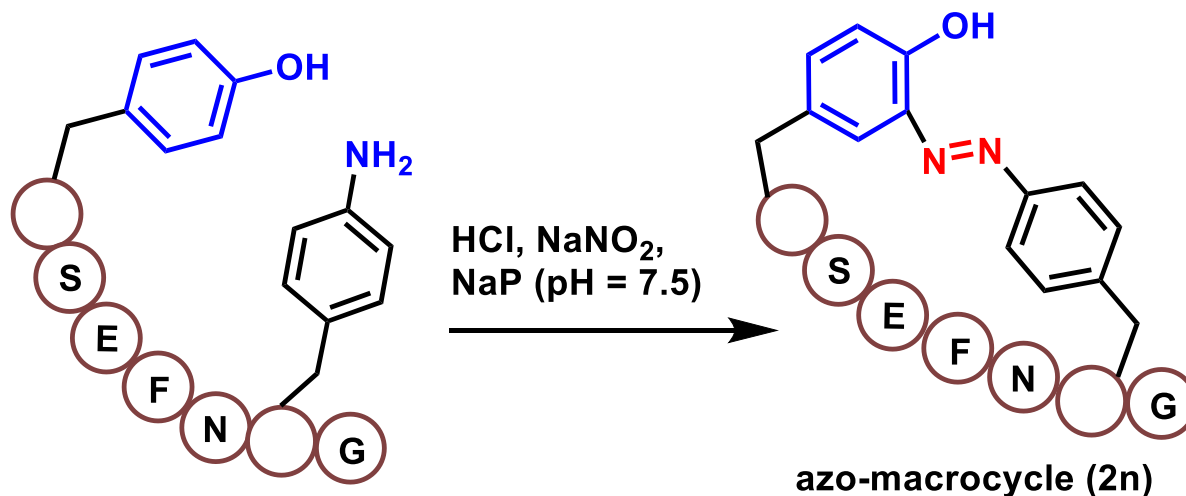
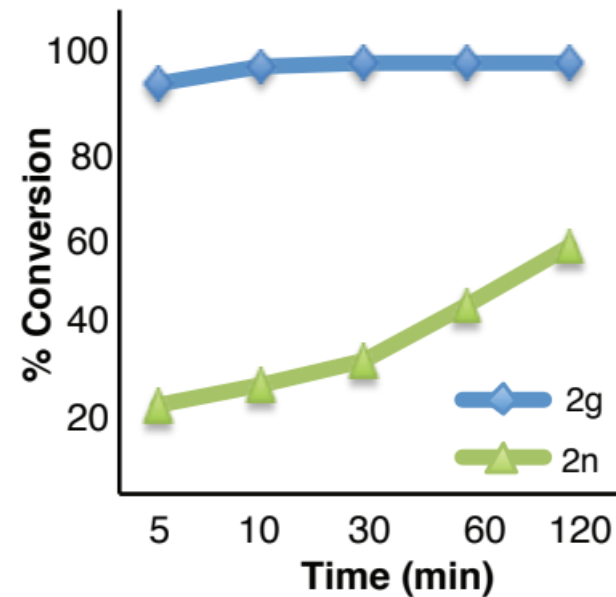
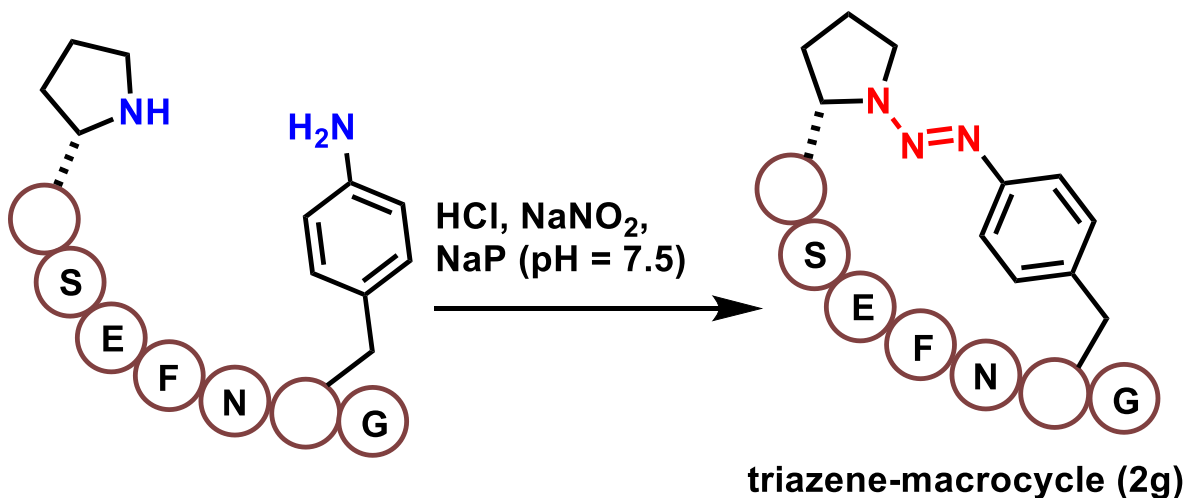
Triazene derived from primary amine was decomposed due to its instability. (pAF→Tyr)

Decomposition of Triazene from Primary Amine



In this reaction, triazene from primary amine would be decomposed by hydrolysis.

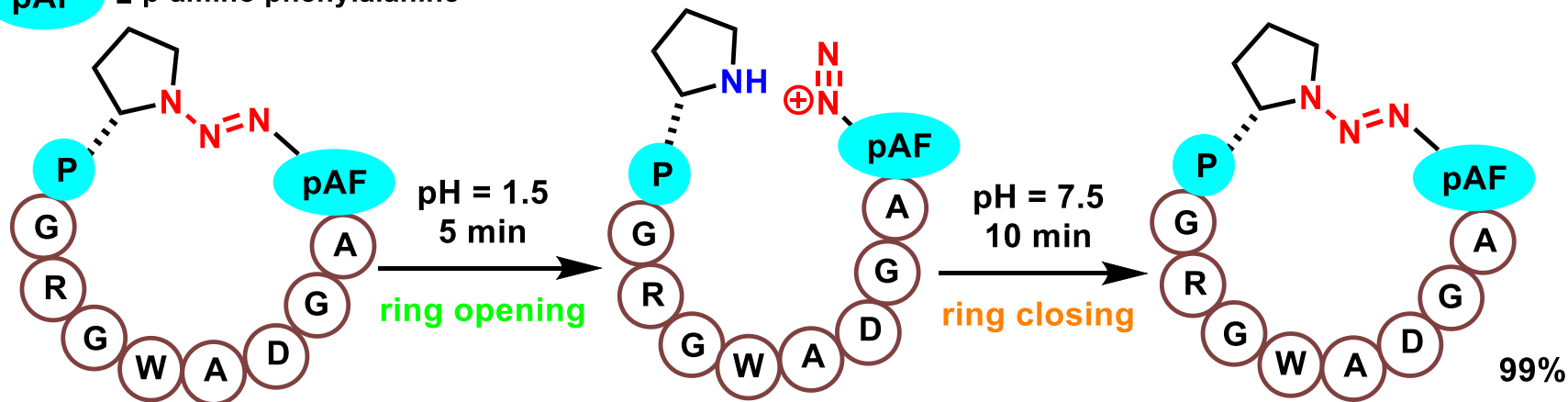
Comparison with Triazene vs Azo Cyclization



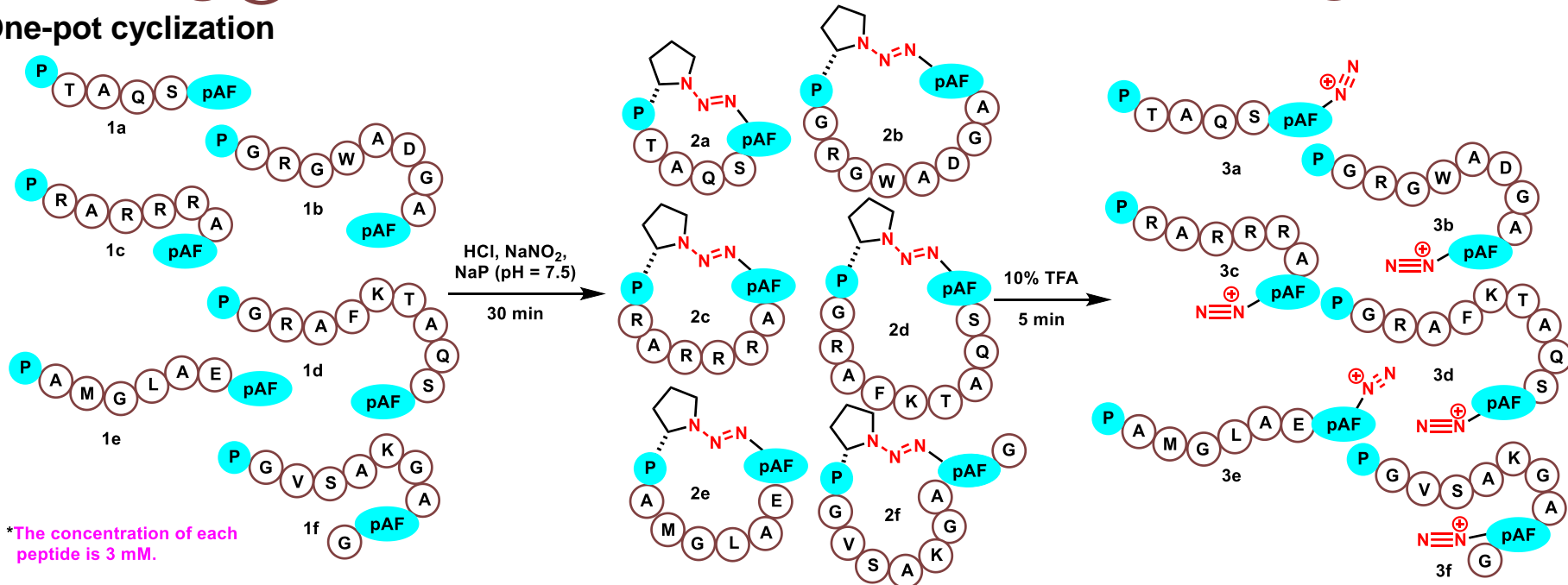
Triazene-macrocycle formed much faster than azo-macrocycle.

Reversibility of Ring Closing & Opening; Response to pH

pAF \equiv p-amino phenylalanine

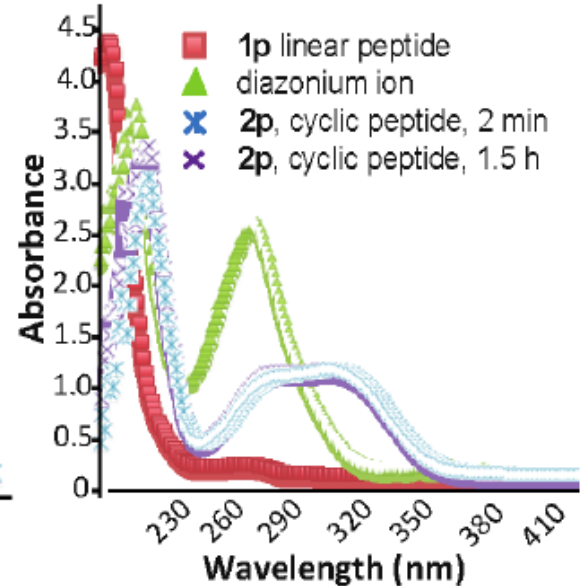
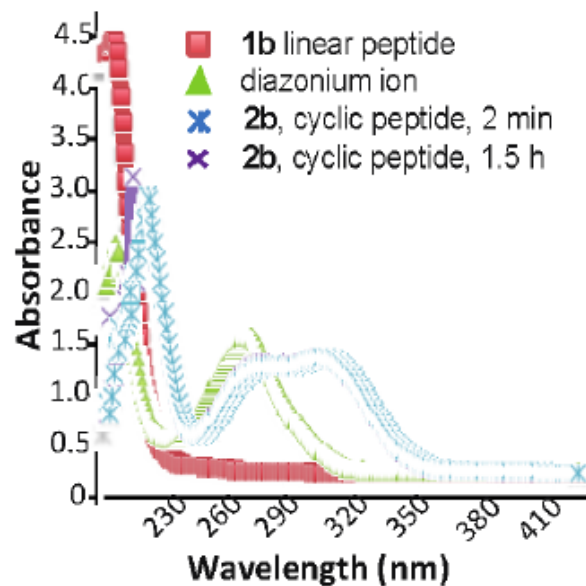
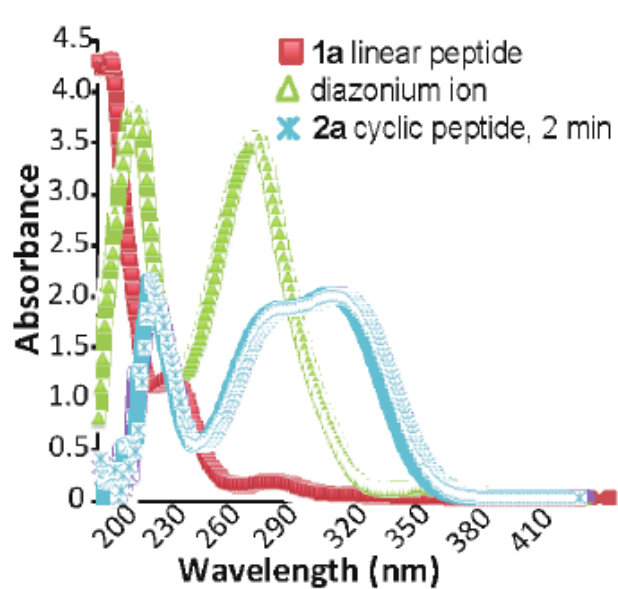


One-pot cyclization



Oligomers are not generated in one-pot cyclization of various peptides. → suitable for library screening

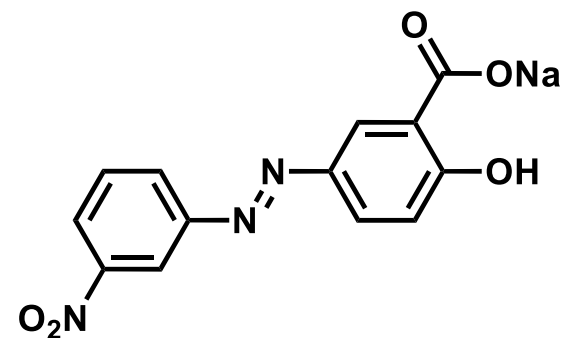
Chromophoric Property



Generally chromophoric groups are bulky.

→ Since triazene macrocycle is chromophore itself, there is lower influence of peptides' binding affinity and solubility.

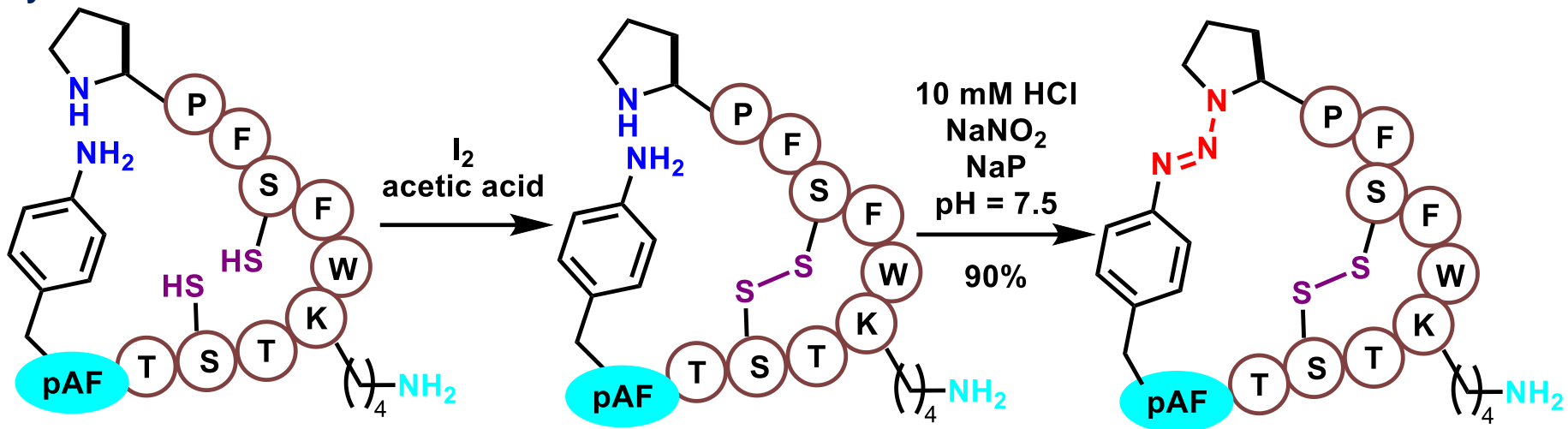
Ex.) big bulky chromophoric group



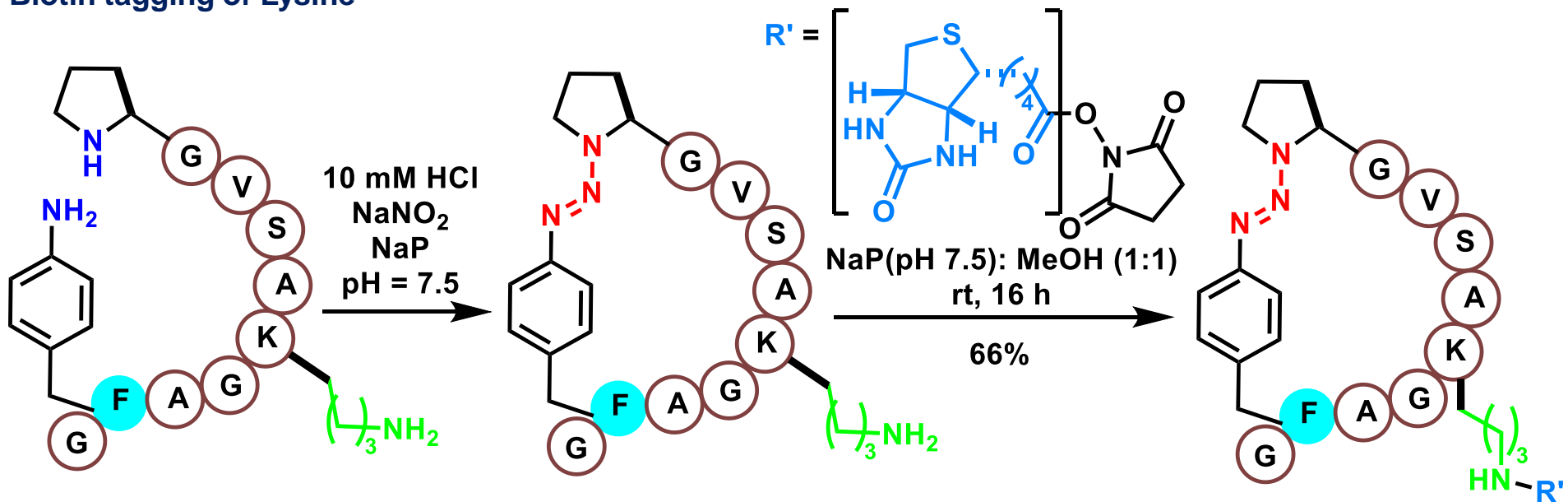
Alizarin yellow GG: $\lambda_{\max} = 367 \text{ nm}$
(used as an azo dye)

Postcyclization Modification

Bicyclization

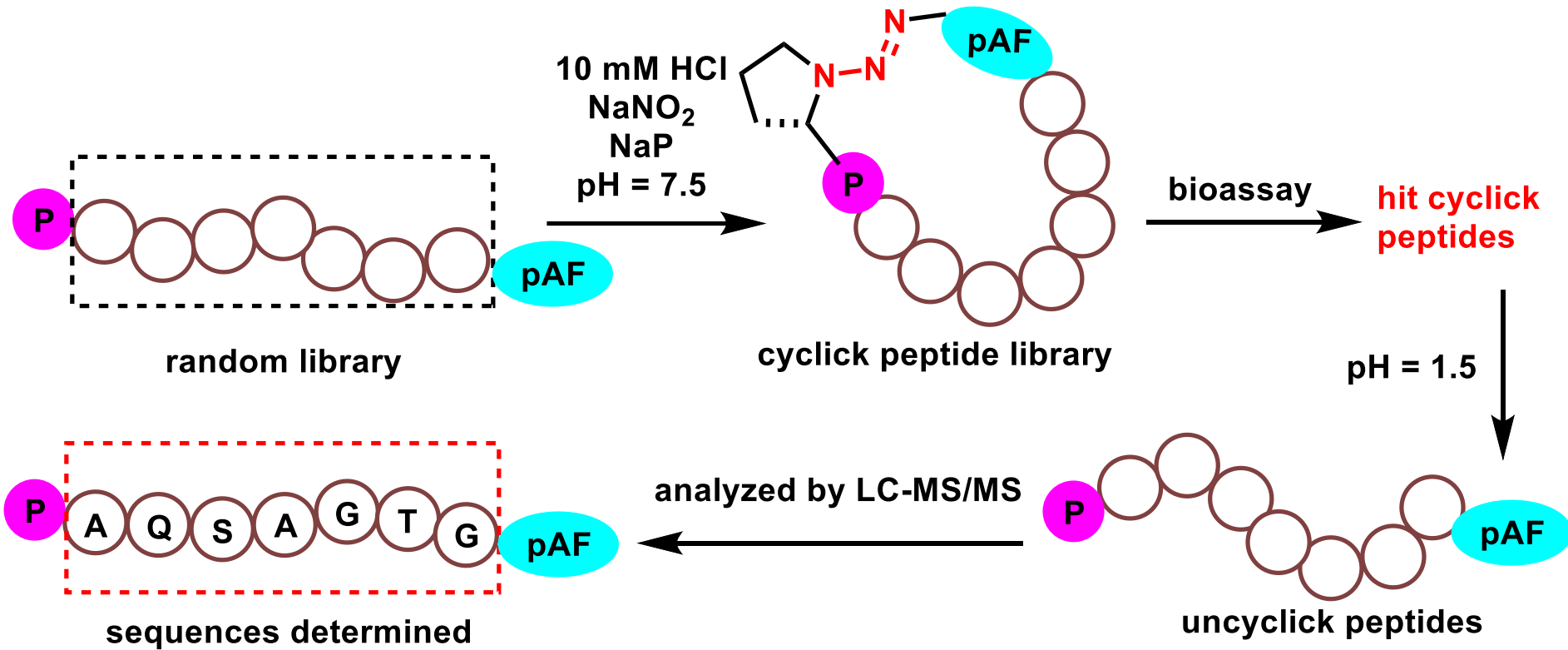


Biotin tagging of Lysine



Since triazene macrocycle is stable, various late-stage modification can be carried out.

Future Perspective

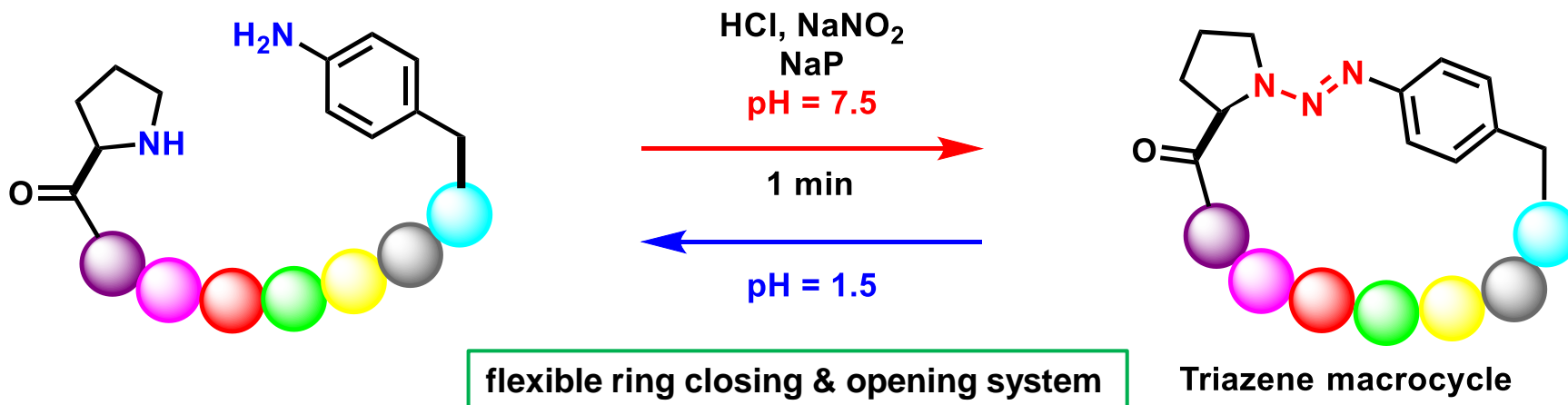


Triazene macrocycle: stable, perfectly reversible ring opening and closing

This cyclization system can be incorporated into cyclick peptides library screening.

Summary

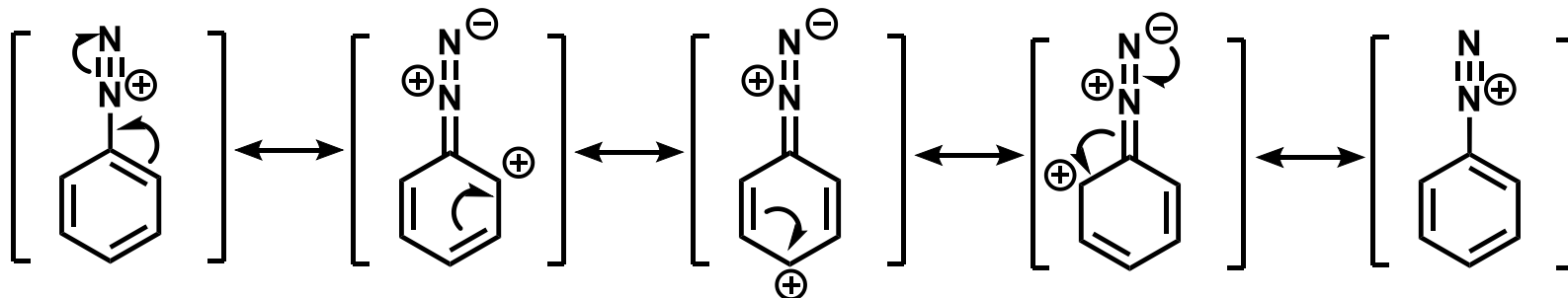
Macrocyclization using STaR



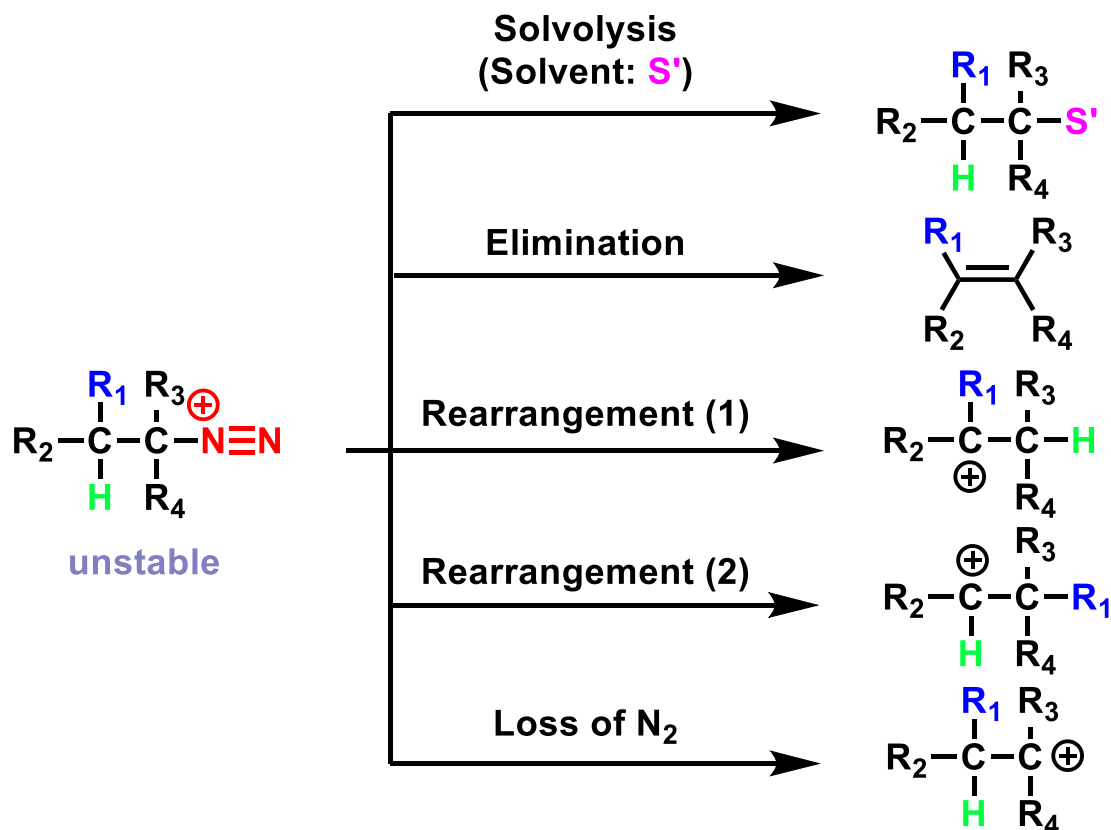
- Chemoselective for secondary amines
- Fast reaction kinetics
- Inbuilt chromophore
- Reversible
- Respond to external stimuli
- Broad substrate scope
- Various macrocyclization
(Head-to-side-chain, Side-chain-to-side-chain, Bicyclization, Postcyclization modification)

Appendix

Comparison with Arene vs Alkane Diazonium



Arene diazonium ion is stable by the effect of conjugation

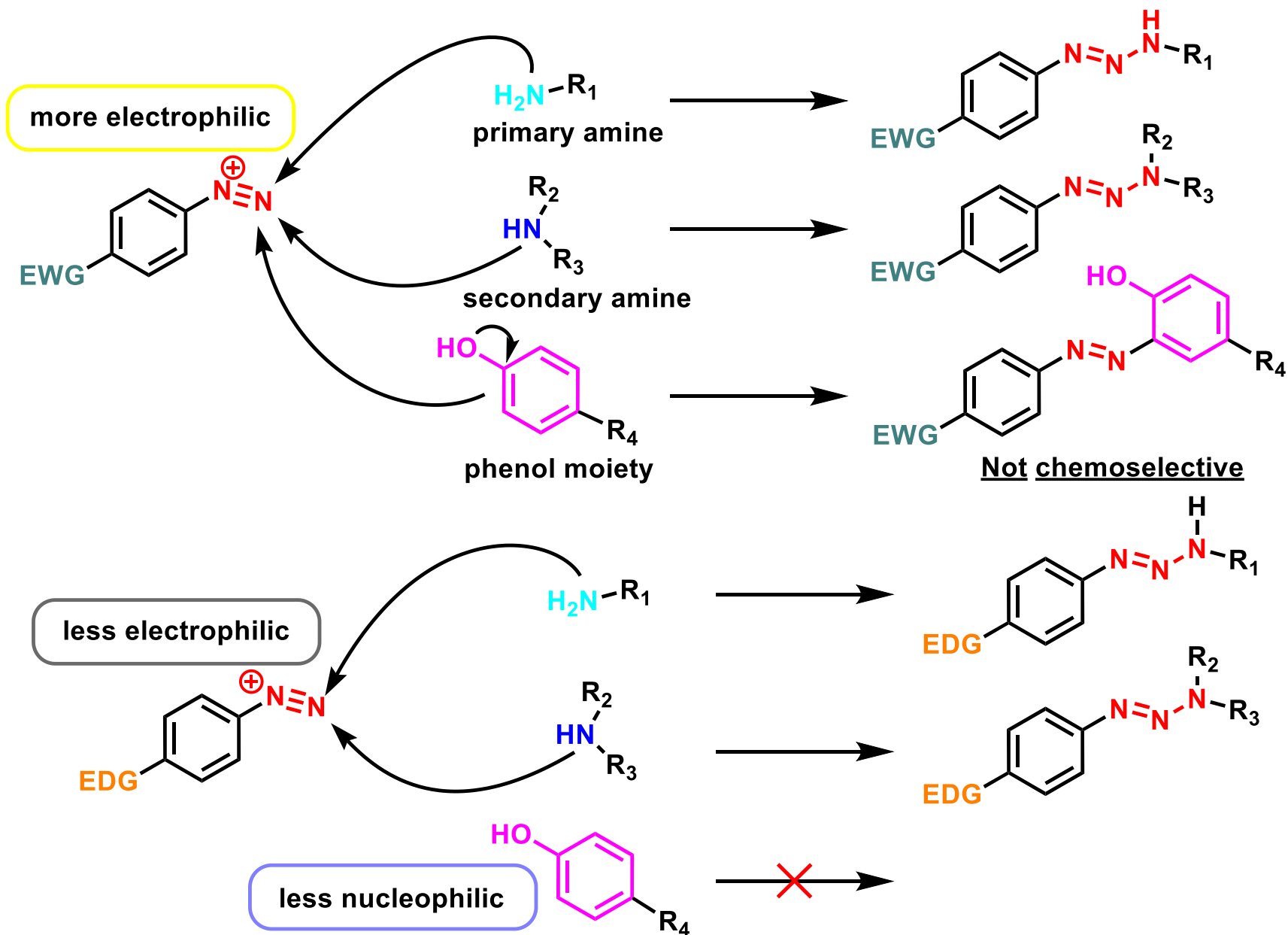


Stability of nitrogen molecule (driving force)



Decomposition of alkane diazonium ion into nitrogen and carbonium ion easily occurs. Also, various reactions can compete.

Effect of Electron Donating Group

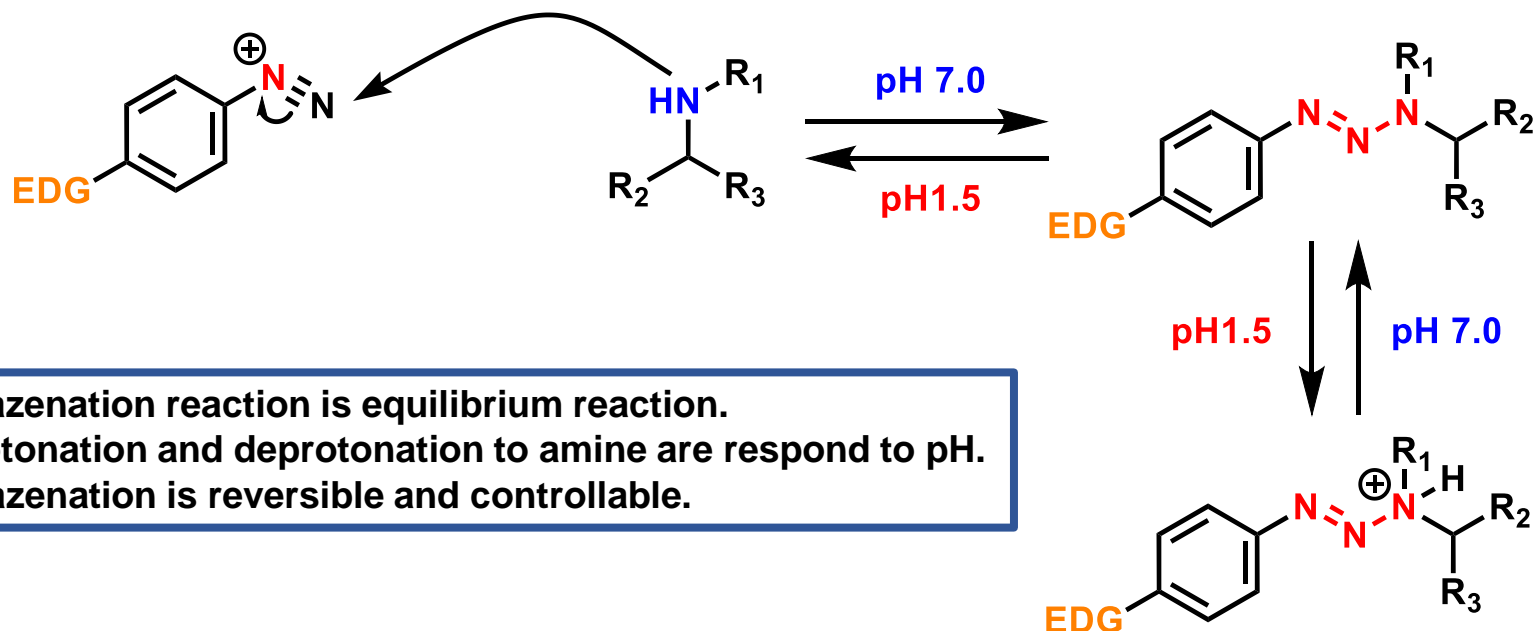


1) Henderson, Jr, W. A.; Schultz, C. J. *J. Org. Chem.* **1962**, *27*, 4643–4646

2) Schlick, T. L.; Ding, Z.; Kovacs, E. W.; Francis, M. B. *J. Am. Chem. Soc.* **2005**, *127*, 3718–3723.

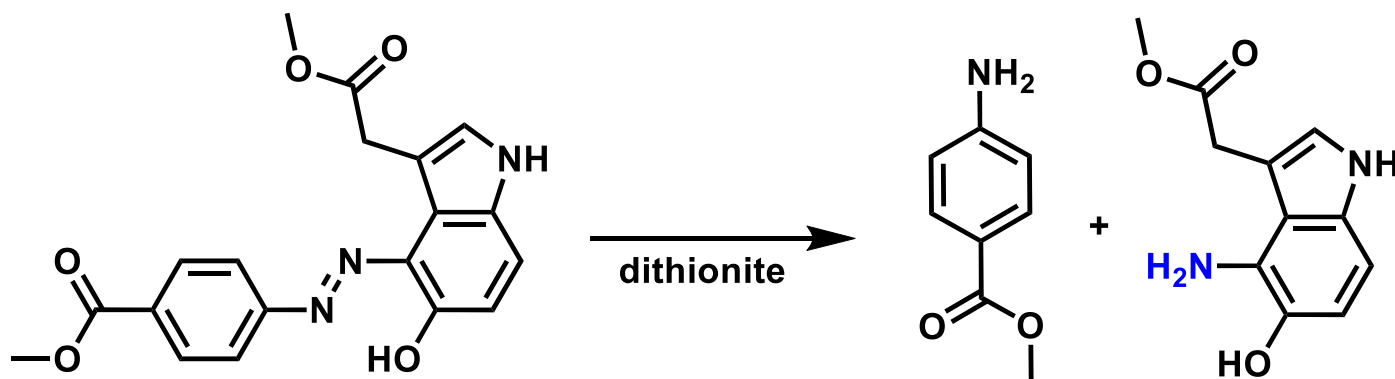
3) Gavriluk, J.; Ban, H.; Nagano, M.; Hakamata, W.; Barbas, C. F. *Bioconjugate Chem.* **2012**, *23*, 2321–2328

Riversibility of Triazene and Diazonium Ion



- Triazene reaction is equilibrium reaction.
- Protonation and deprotonation to amine are respond to pH.
- Triazene is reversible and controllable.

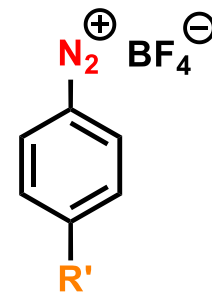
Reversible coupling reaction



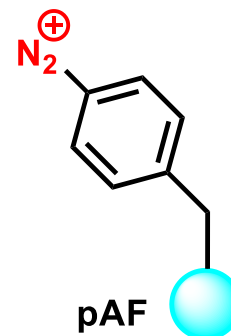
Azo-linkage was cleaved, but the decoupled products were modified.

σ value: Discussion about Selectivity

Group	σ value*
-OMe	- 0.268
-COOH	0.45
-CO ₂ ⁻	0.0
-CH ₂ CH ₃	- 0.151



$\text{R}' = \text{OMe}; 4\text{MDz}$
 $\text{R}' = \text{COOH}, 4\text{CDz}$



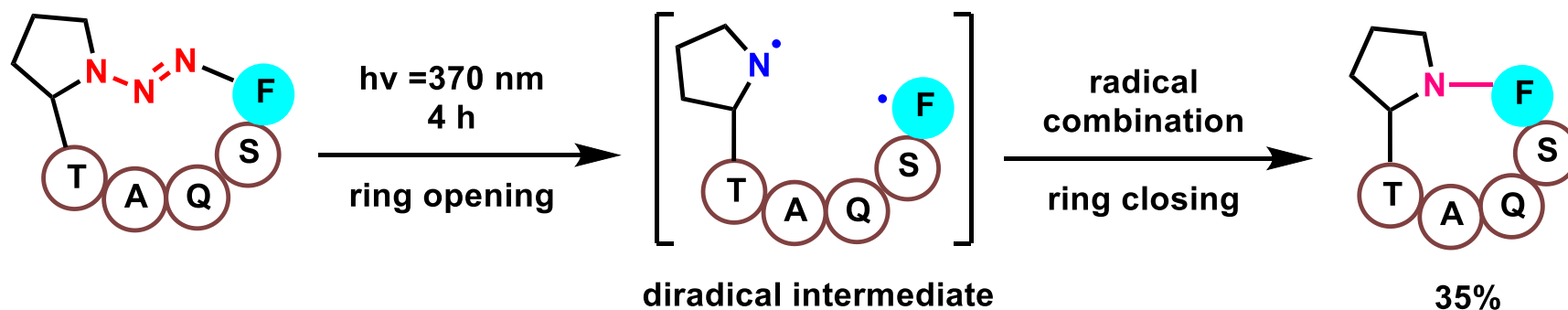
* σ values about para substituent group

4MDz with OMe group and 4CDz with COOH group selectively reacted to secondary amine.



Judging from σ value, STaR occurs about diazonium ion from pAF.

Sensitive to UV Radiation



- C-N bond between proline and *p*-amino phenylalanine was generated.
- It might be possible that UV radiation mediated cyclization becomes another macrocyclization strategy.