

# **Late Stage C–H Functionalization Modification of Peptides**

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Scripps Research**

# Content

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- Modifications on aliphatic side-chains**
- Modifications on aromatic side-chains**
- Modifications on polar, non-ionizable side chains**
- Modifications on polar, ionizable side chains**
- Modifications on C-/N-terminus and peptide backbone**
- Synthesis of stapled peptides**

# Content

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## ☐ Background

### ☐ Modifications on aliphatic side-chains

### ☐ Modifications on aromatic side-chains

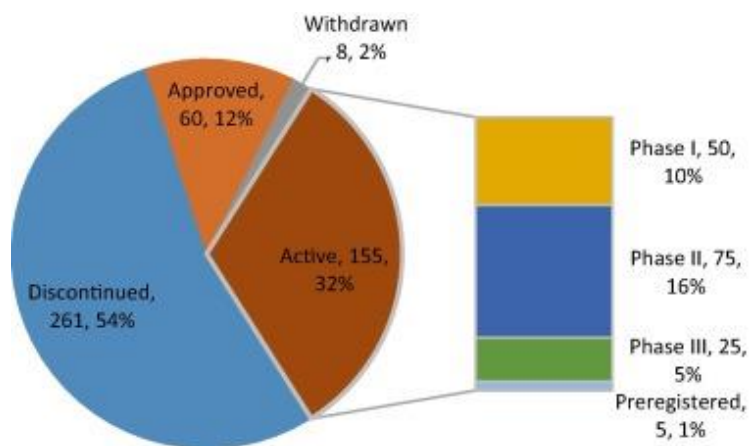
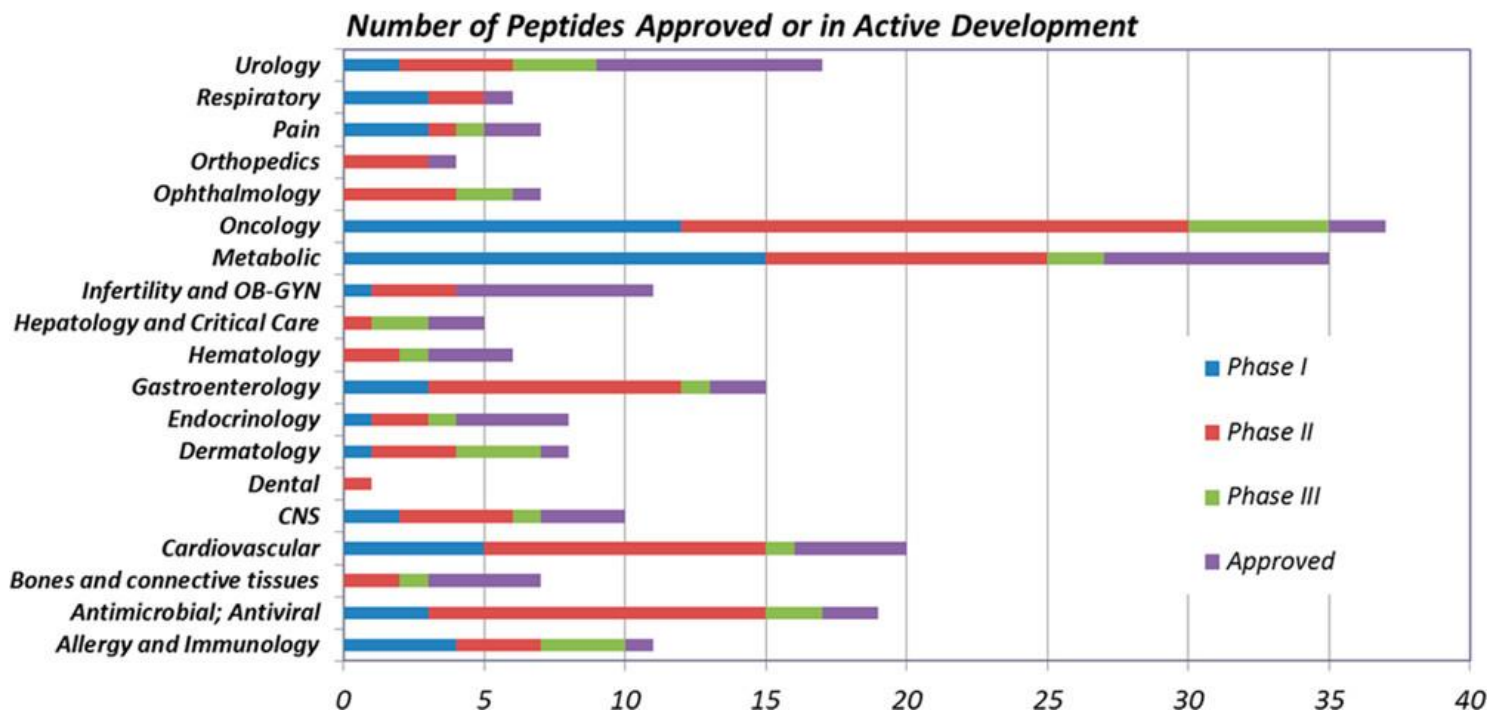
### ☐ Modifications on polar, non-ionizable side chains

### ☐ Modifications on polar, ionizable side chains

### ☐ Modifications on C-/N-terminus and peptide backbone

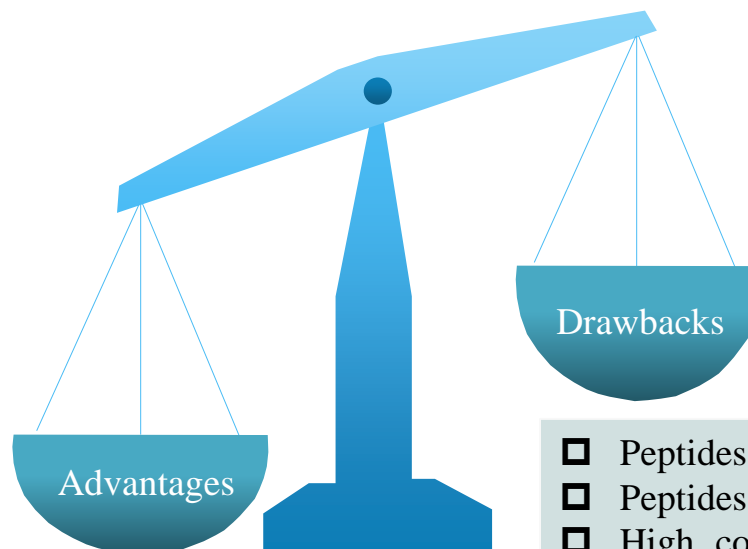
### ☐ Synthesis of stapled peptides

# Background



Peptides have profoundly impacted the development of the modern pharmaceutical industry and have contributed significantly to the advancement of biological and chemical science. With a projected annual growth rate of 9–10%, peptide drug sales are likely to exceed \$70 billion in 2019 (insulins included).

# Background



- ❑ Peptides are the natural ligands for many cell surface receptors.
- ❑ Peptides are good choices for protein-protein-interaction modulators design.
- ❑ Peptides do not accumulate in tissues.
- ❑ Drug–drug interactions and nonmechanistic-based toxicology of peptides are rarely observed.
- ❑ Peptide discovery optimization has a significant resource advantage over small molecules.

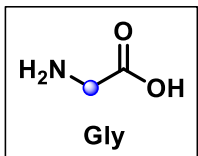
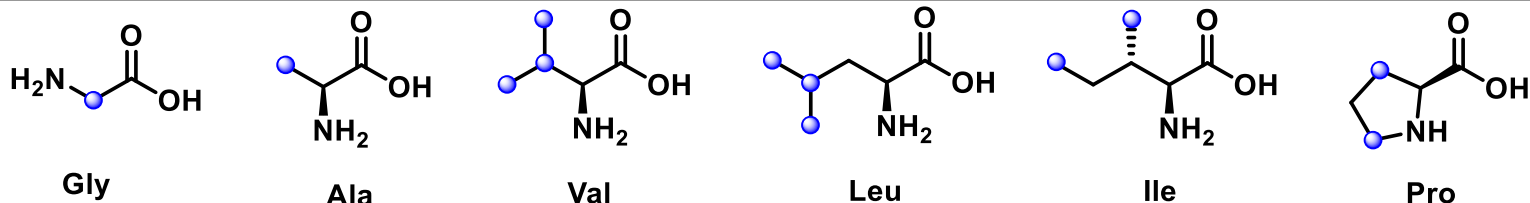
- ❑ Peptides are membrane impermeable.
- ❑ Peptides are biologically unstable.
- ❑ High cost of peptide production and the necessary HPLC purification.
- ❑ Peptides are also generally unable to cross the blood–brain barrier.
- ❑ Detriment to patient convenience and compliance via subcutaneous or intravenous injection of peptide drugs.

# Content

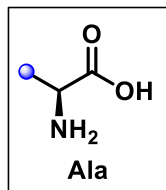
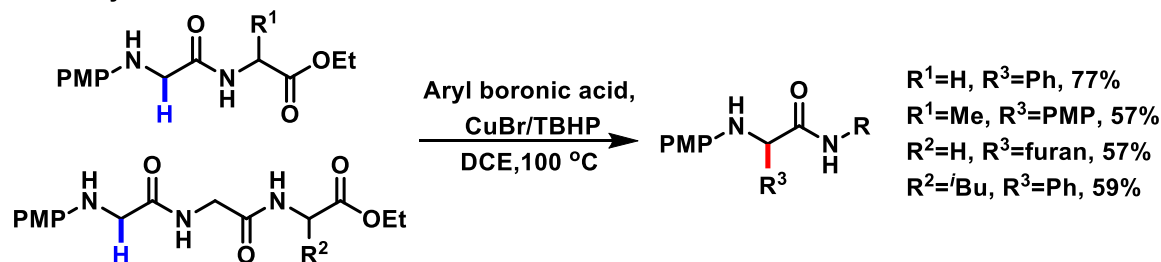
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- **Modifications on aliphatic side-chains**
- Modifications on aromatic side-chains
- Modifications on polar, non-ionizable side chains
- Modifications on polar, ionizable side chains
- Modifications on C-/N-terminus and peptide backbone
- Synthesis of stapled peptides

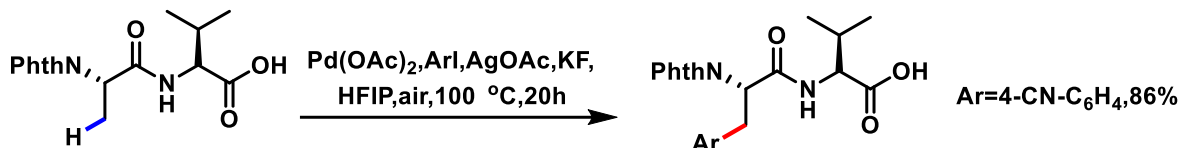
# Modifications on aliphatic side-chains



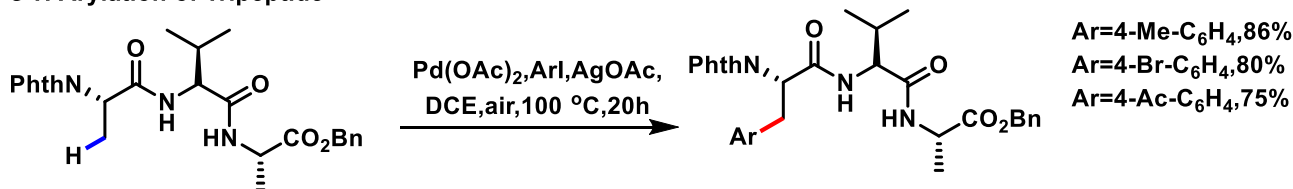
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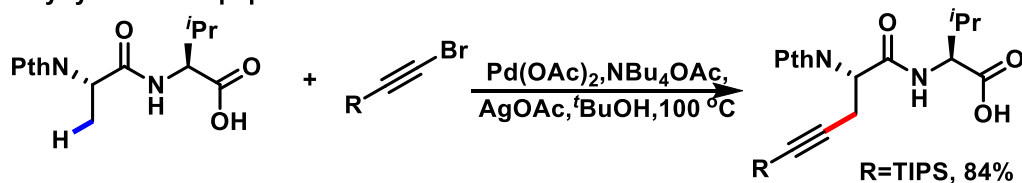
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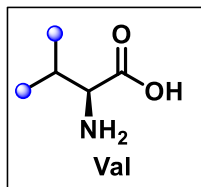
C-H Arylation of Tripeptide



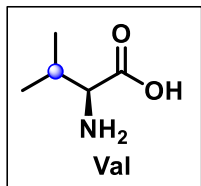
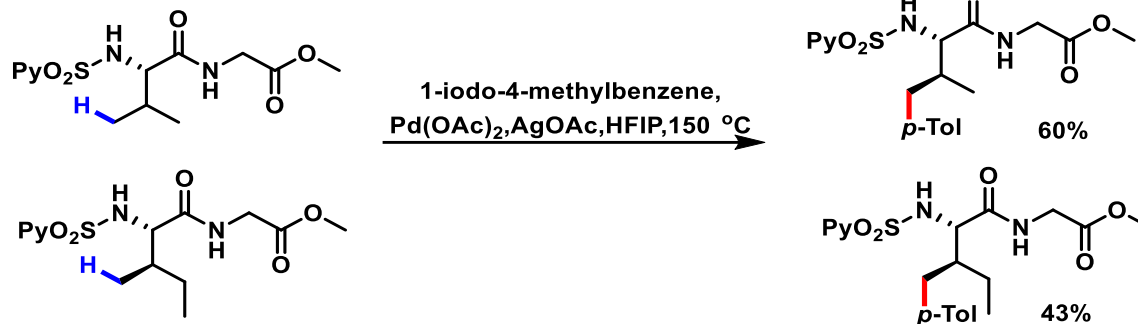
Alkynylation of dipeptide



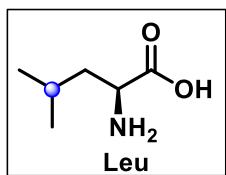
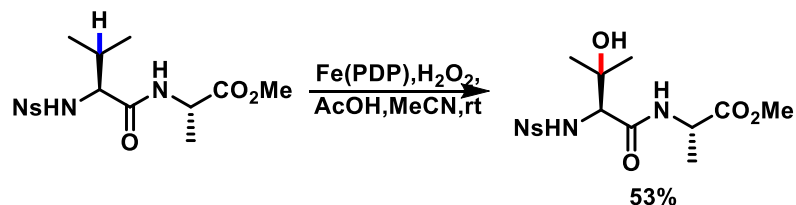
# Modifications on aliphatic side-chains



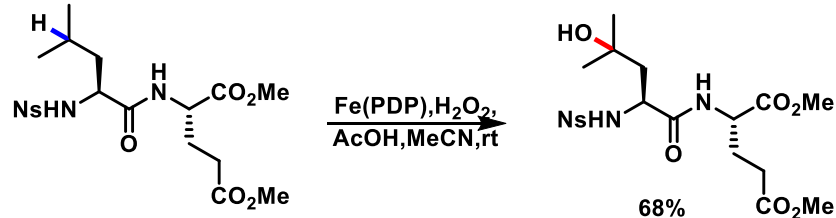
C-H arylation:



Hydroxylation of Val:

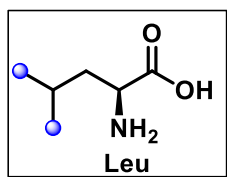


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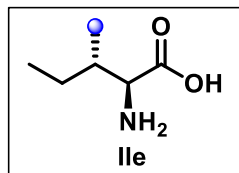
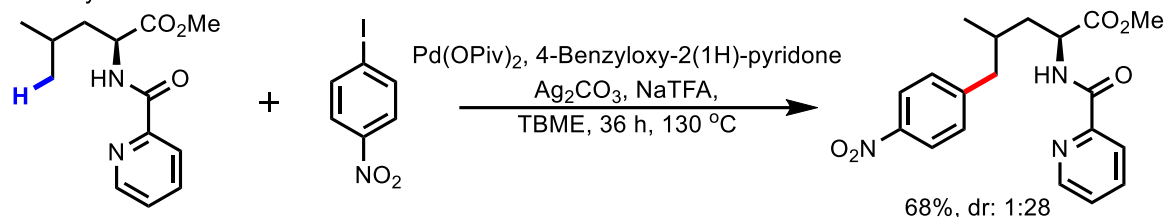




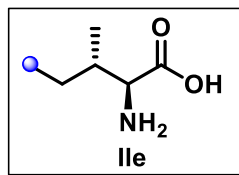
# Modifications on aliphatic side-chains



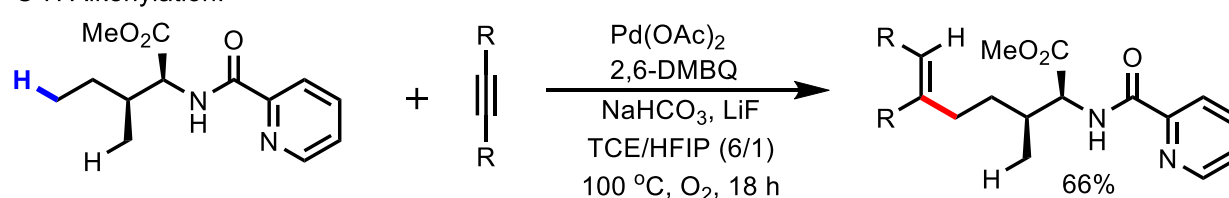
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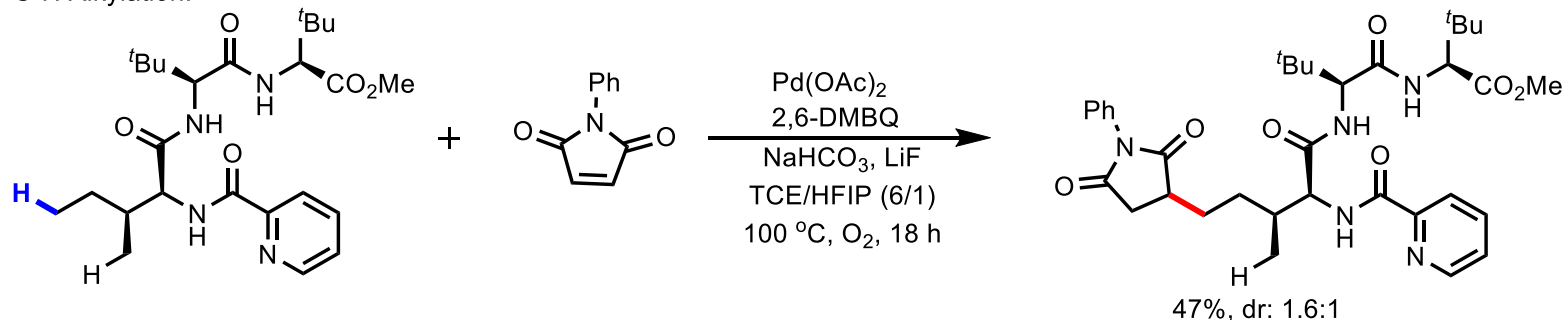
C-H Chalcogenation:



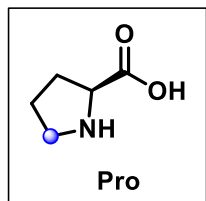
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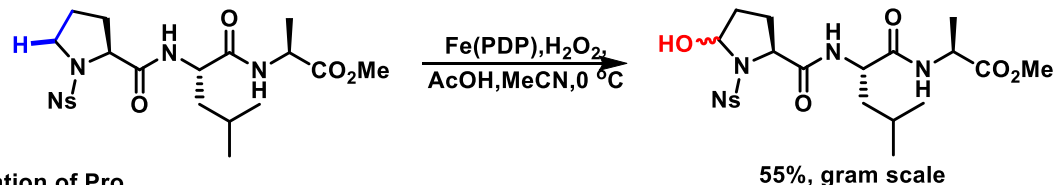
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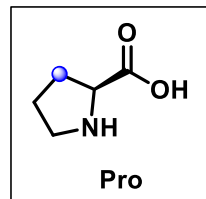
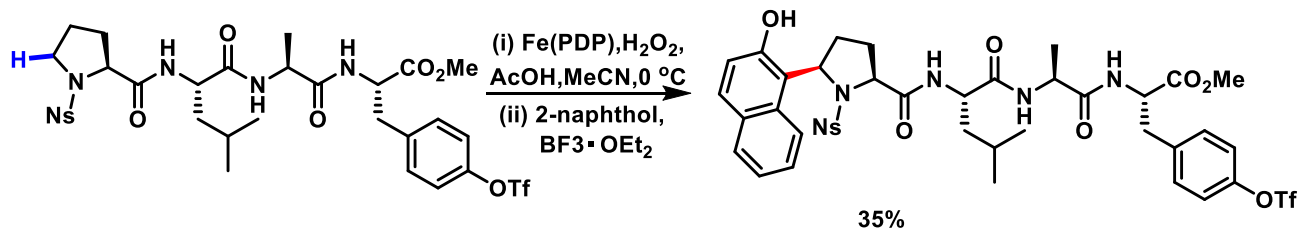
# Modifications on aliphatic side-chains



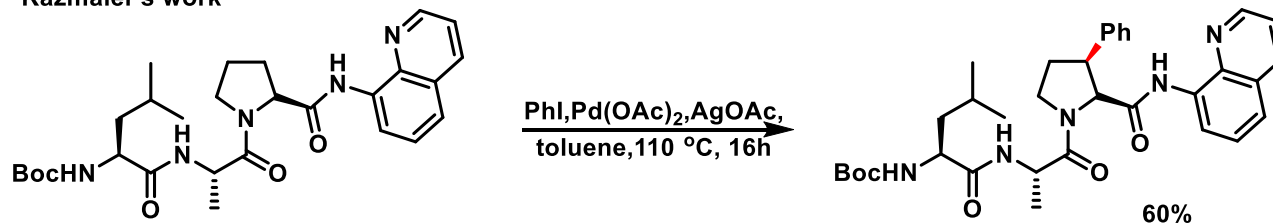
Hydroxylation of Pro



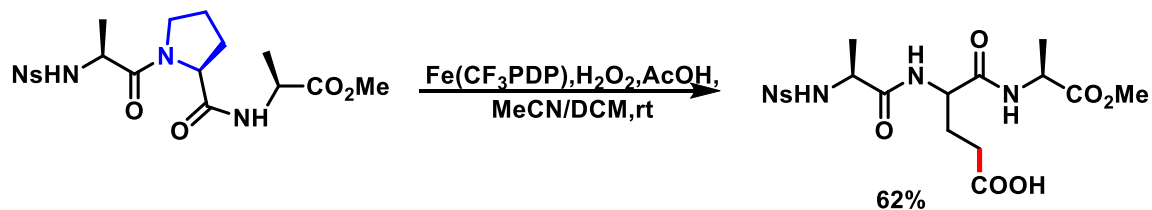
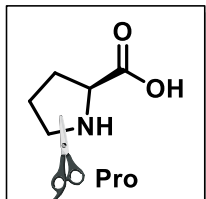
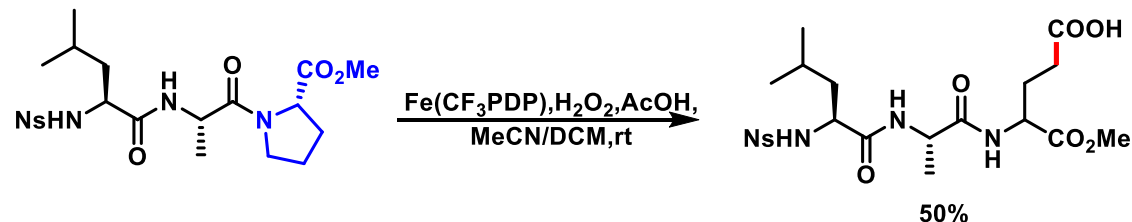
Arylation of Pro



Kazmaier's work



Oxidative modification of C-terminal Pro



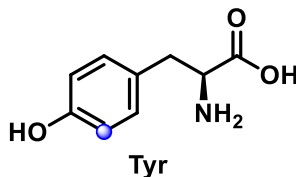
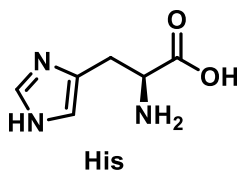
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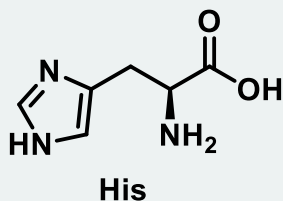
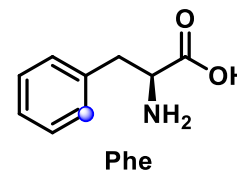
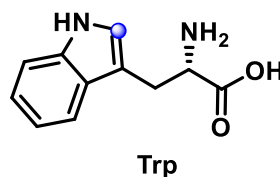
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# Modifications on aromatic side-chains

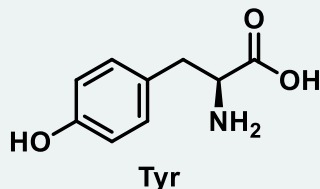
ionized



non-ionized

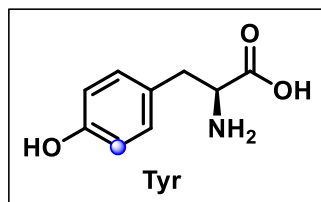


- ❑ Under physiological conditions, the protonation state of His is split between the imidazole and imidazolium forms, leading to the epithet, “Nature’s proton shuttle”.
- ❑ In metalloprotein, His serves as a common coordinating ligand.
- ❑ Relatively few methods exist for the functionalization of His.

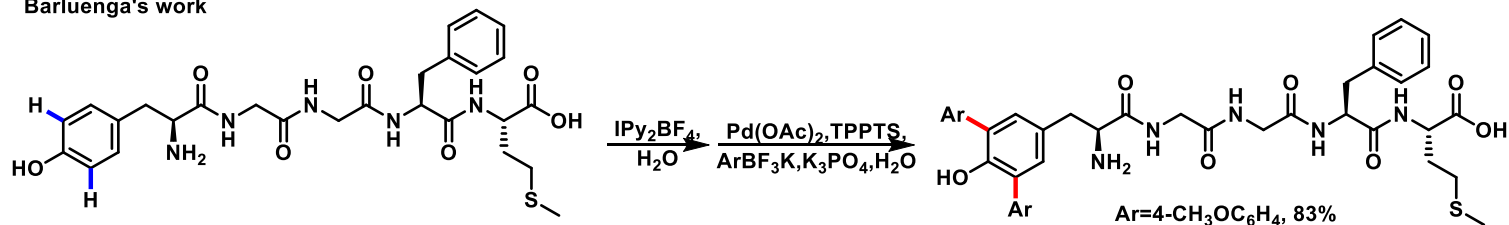


- ❑ The tyrosine side-chain may exist in either the phenol or phenolate form; while the latter is more reactive.
- ❑ The electron rich aromatic ring participates in a number of transformation, including O- and C-alkylation and arylation, diazonium couplings and ene-like reactions.
- ❑ In cases of C-targeted modification, the vast majority of methods afford either mono- or bis-functionalization at the ortho position to the hydroxyl group.

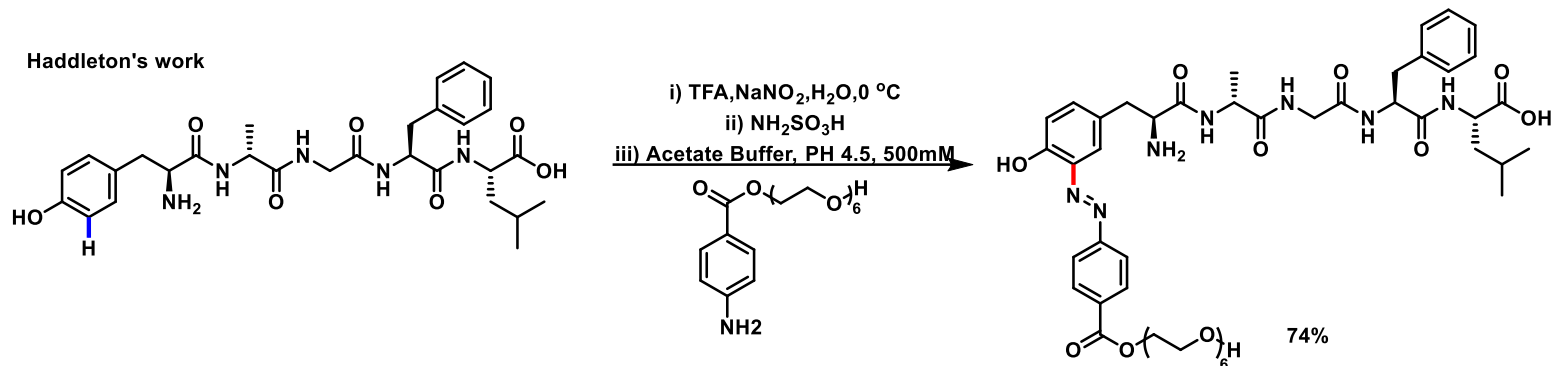
# Modifications on aromatic side-chains



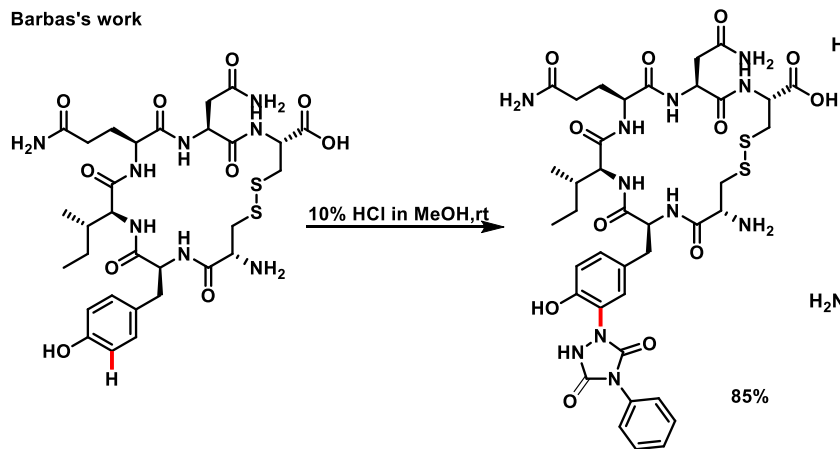
Barluenga's work



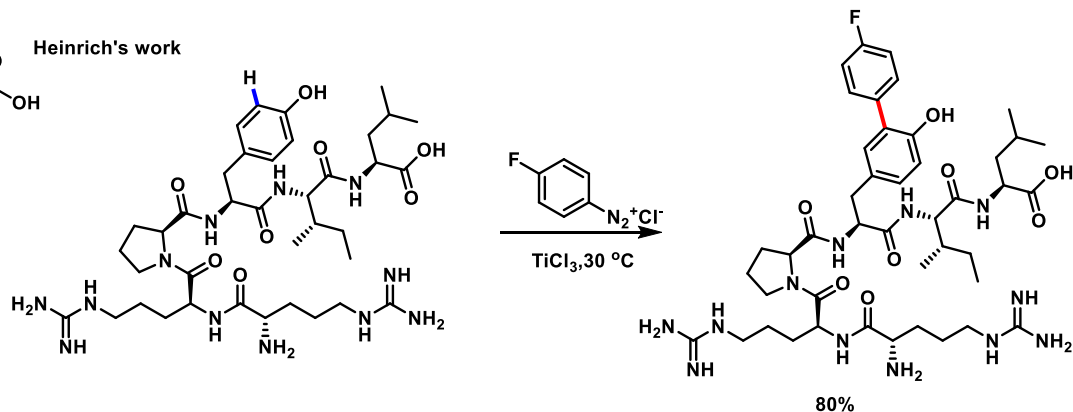
Haddleton's work



Barbas's work

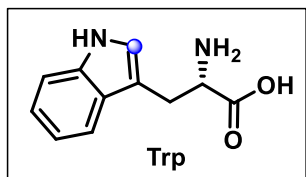


Heinrich's work



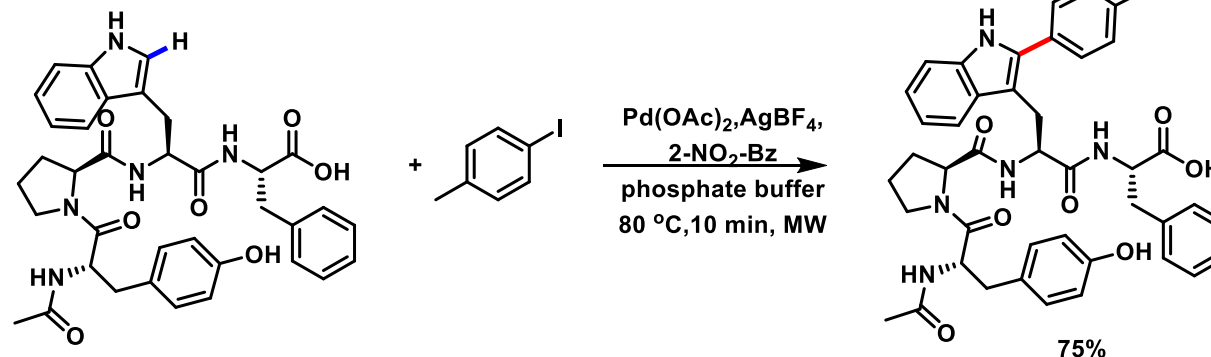
*Org Lett.* **2008**, 10, 3243; *J. Am. Chem. Soc.* **2010**, 132, 1523; *J. Am. Chem. Soc.* **2012**, 134, 7406; *Tetrahedron* **2016**, 72, 7888

# Modifications on aromatic side-chains

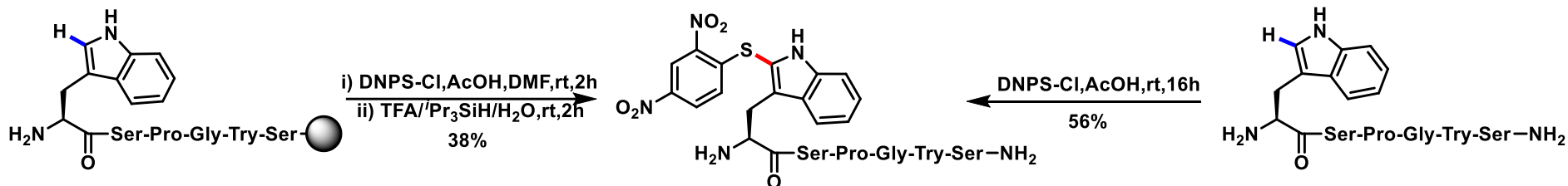


- Trp indole moiety offers unique opportunities for targeted functionalization.
- C-2 modifications have proven the most facile- and the most explored-avenue of derivatization.

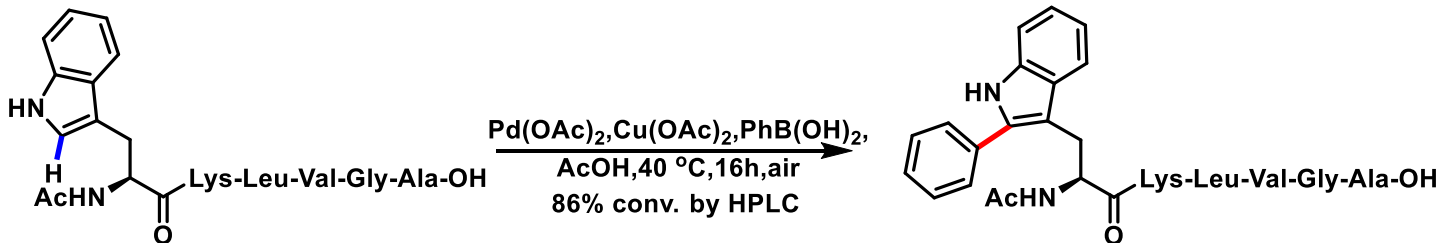
Lavilla's work



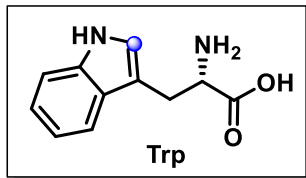
Payne's work



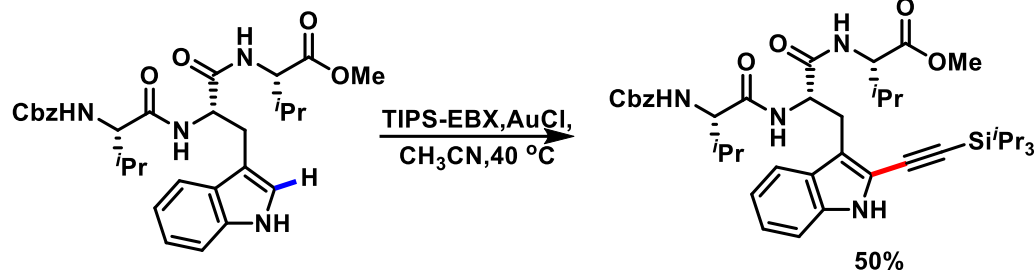
Fairlamb's work



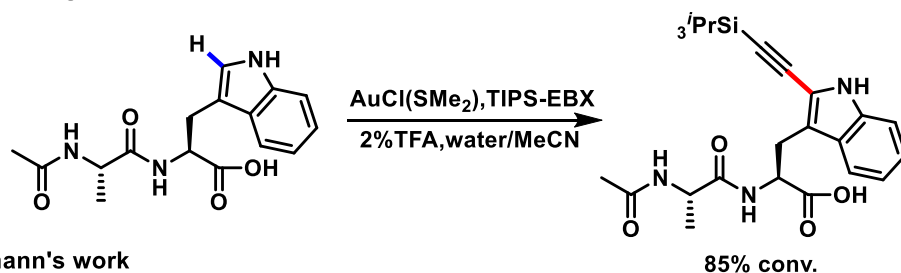
# Modifications on aromatic side-chains



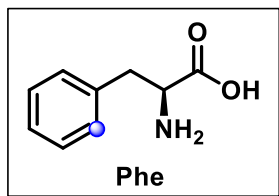
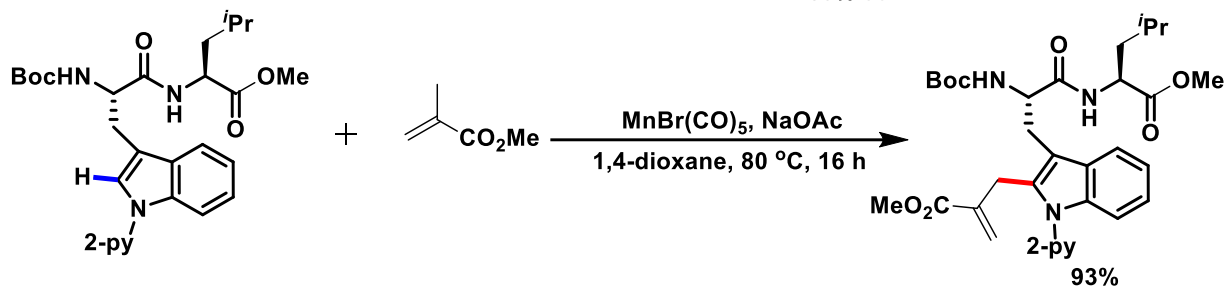
Waser's work



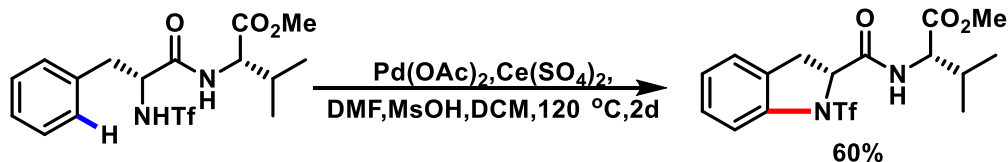
HoegJensen's work



Ackermann's work



Shi's work



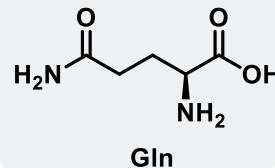
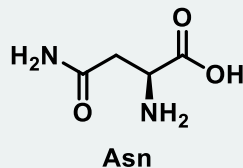
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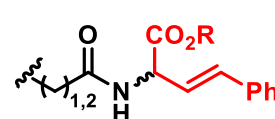
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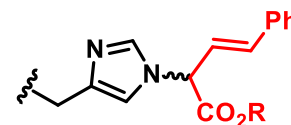
## Modifications on polar, non-ionizable side chains



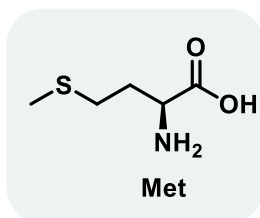
- ❑ Targeted modifications of Asn and Gln residues within peptides and proteins are exceedingly rare.
- ❑ Enzyme-mediated post-translational modifications targeting these residues are common.



**Asn,Gln**



His



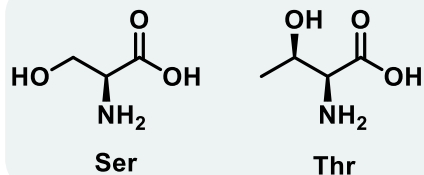
- ❑ Met is the only native residue that can be alkylated under acidic conditions.
- ❑ The methylthioether provides a platform for a number of modifications, including oxidation to the corresponding sulfoxide/sulfone or sulfimide.

# Content

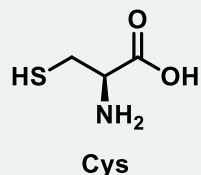
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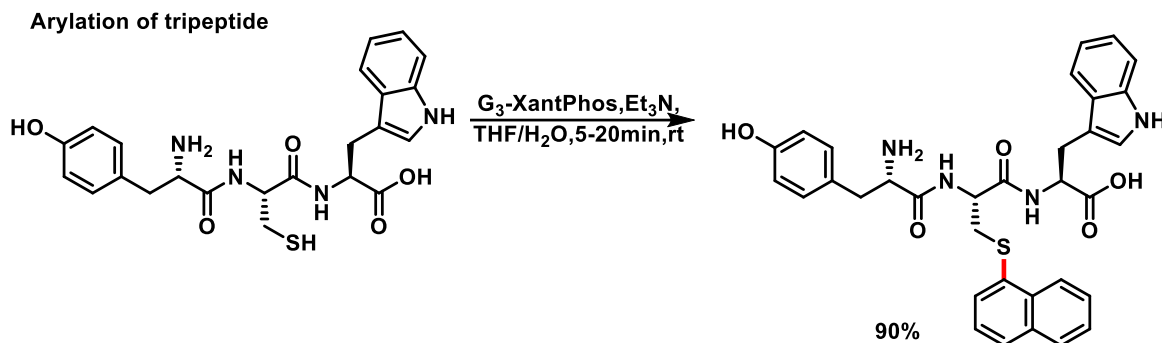
# Modifications on polar, ionizable side chains



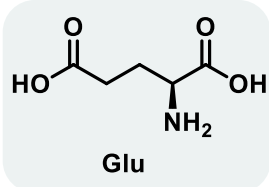
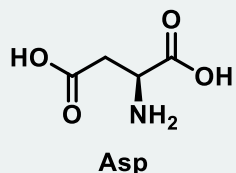
- Of the few existing methods, most are proximity-driven, or are highly sequence-dependent, relying on the local peptide environment to enhance the reactivity of the target hydroxyl side-chain.



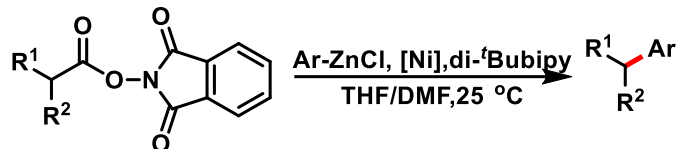
- The inherently low pKa (~8.3) and substantial nucleophilic character provide a convenient handle for site-selective derivatization.
- Historically, cysteine–maleimide conjugation has been the most common manner of thiol modification, but the low stability of the adducts has rendered this particular reaction outmoded.



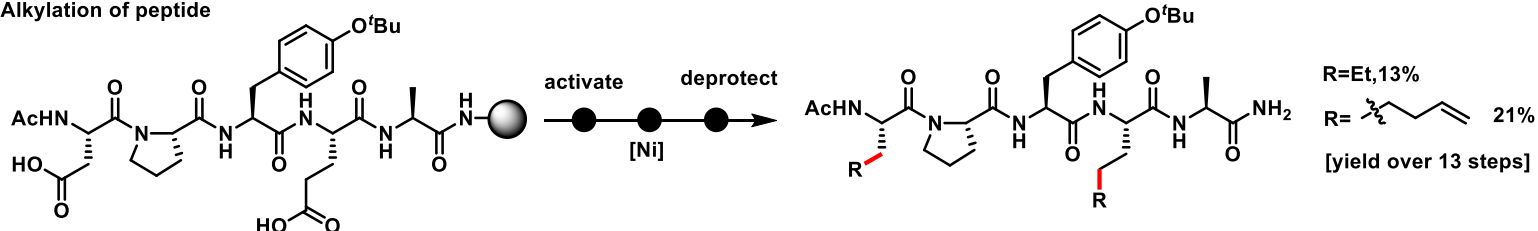
# Modifications on polar, ionizable side chains



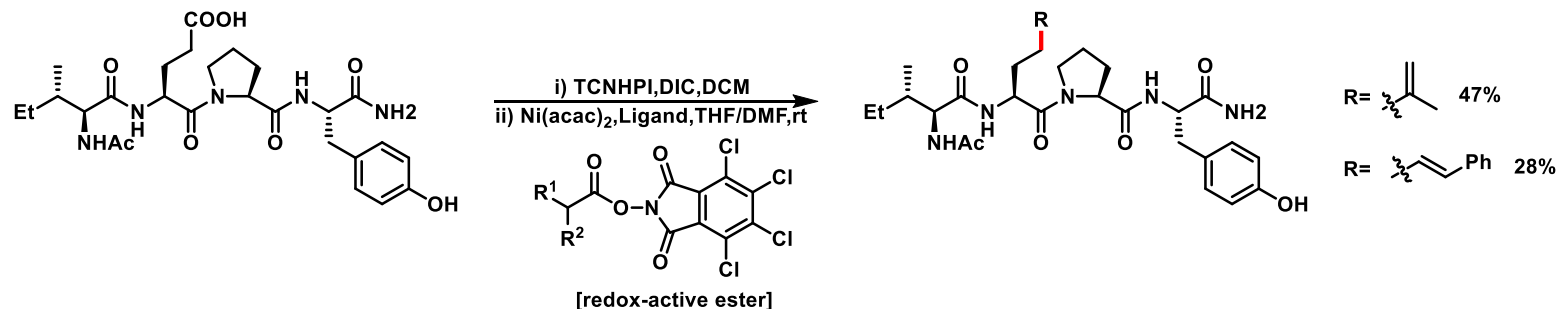
- Despite the vast array of carboxylic acid transformations available, site-selective modification of the Asp/Glu side-chain is often hampered by the presence of an unprotected C-terminal carboxylic acid.
- Common approaches include (a) orthogonal protection strategies and (b) reliance on the resin-linker as a masking group which reveals the desired C-terminal functionality only on cleavage.



Alkylation of peptide

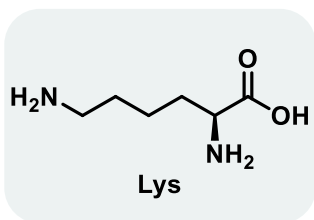


Alkenylation of peptide

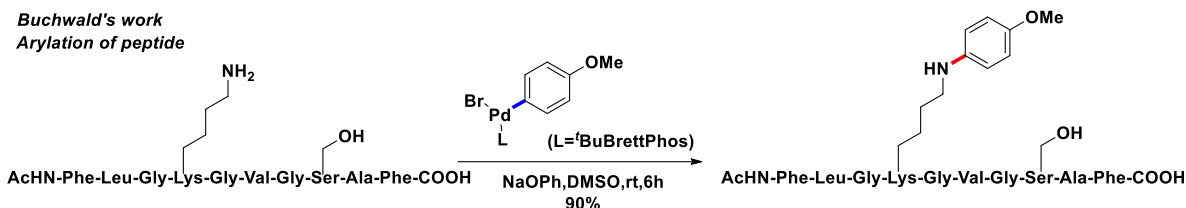


*J. Am. Chem. Soc.* **2016**, 138, 2174; *Science* **2016**, 352, 801; *Nature* **2017**, 545, 213

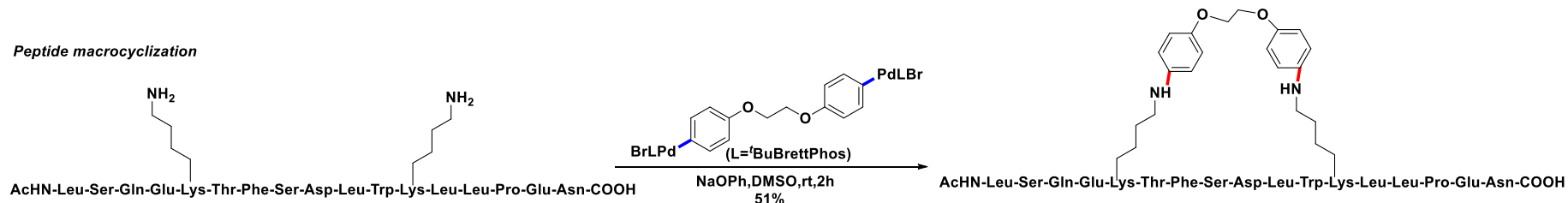
# Modifications on polar, ionizable side chains



- Lys has served as a convenient nucleophilic handle for the introduction of diverse functionalities.
- Common challenges include (a) differentiation between the  $\epsilon$ -amine of Lys and the N-terminal  $\alpha$ -amine and (b) prevention of unwanted side reactions with the notably more nucleophilic Cys.



*Peptide macrocyclization*



# Content

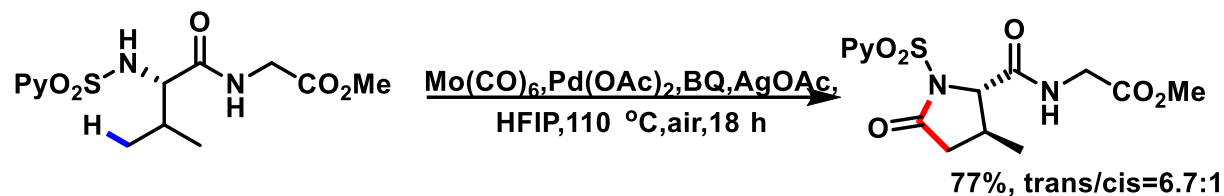
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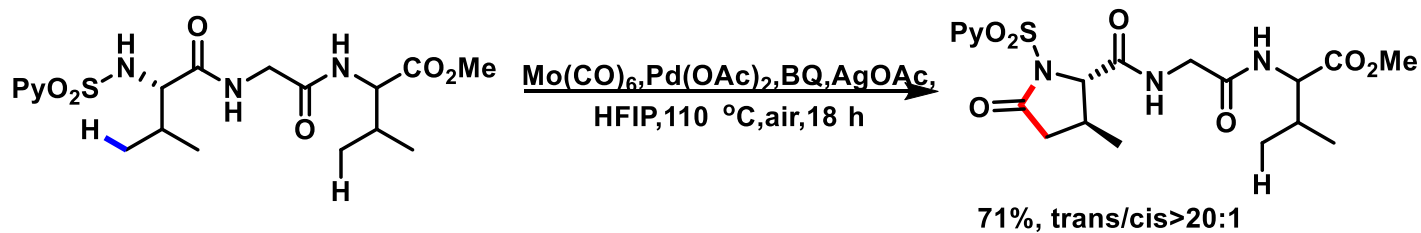
# Modifications on C-/N-terminus and peptide backbone

Carretero's work

Late-stage carbonylation of dipeptide



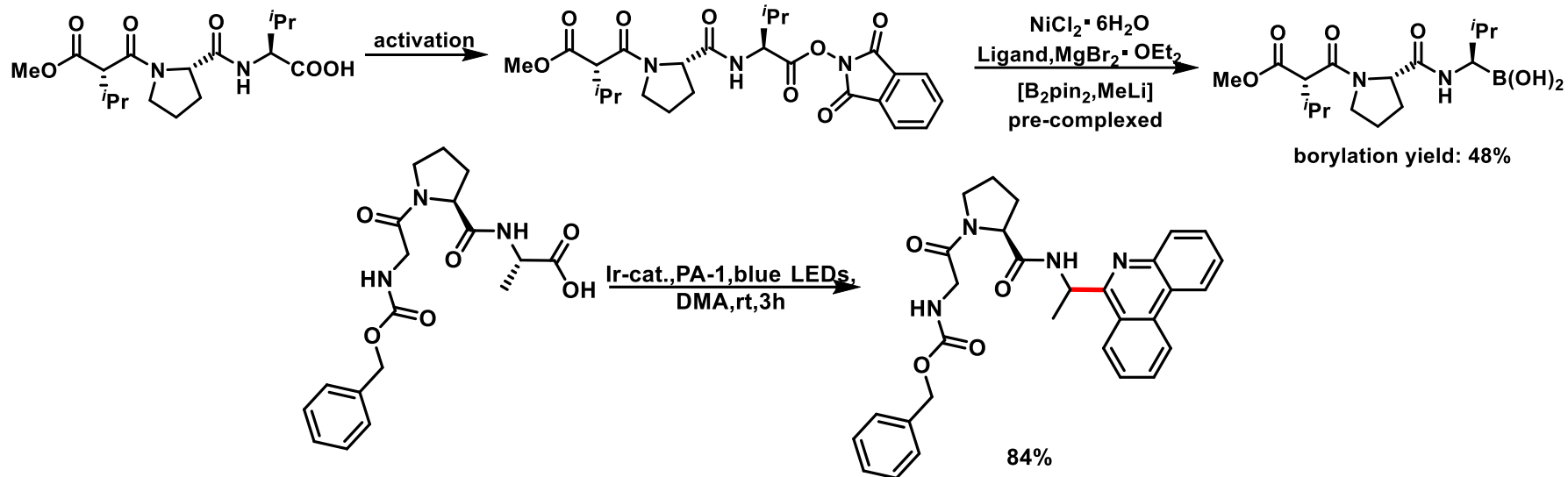
Late-stage carbonylation of tripeptide



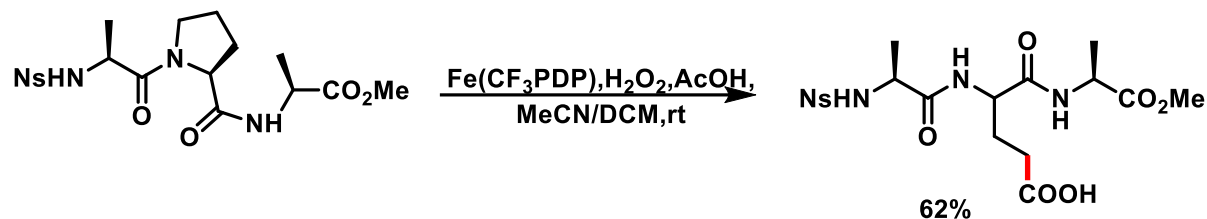
*ACS Catal.* **2016**, 6, 6868; *J. Org. Chem.* **2016**, 81, 9122

# Modifications on C-/N-terminus and peptide backbone

## Baran's work



## White's work: Oxidative modification of internal Pro





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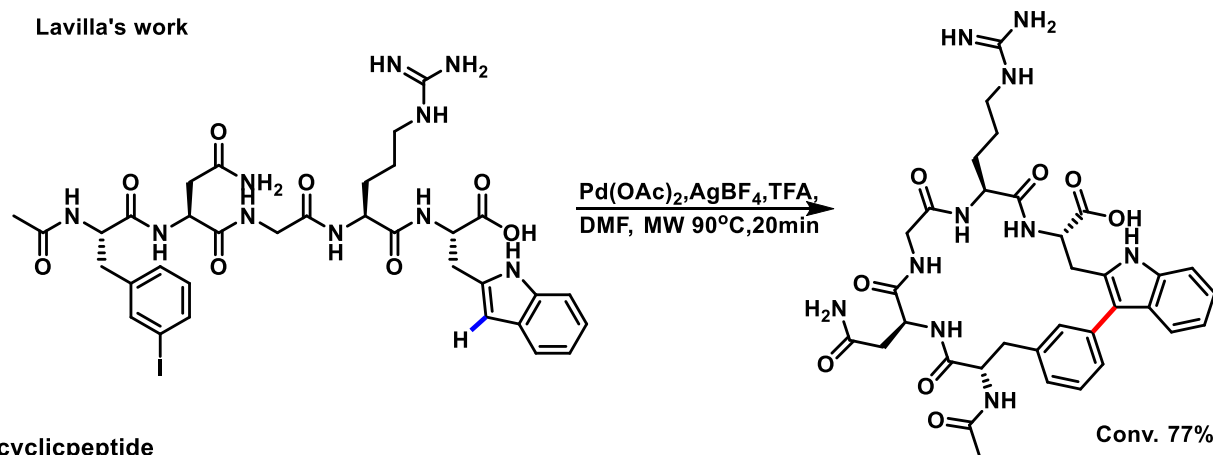
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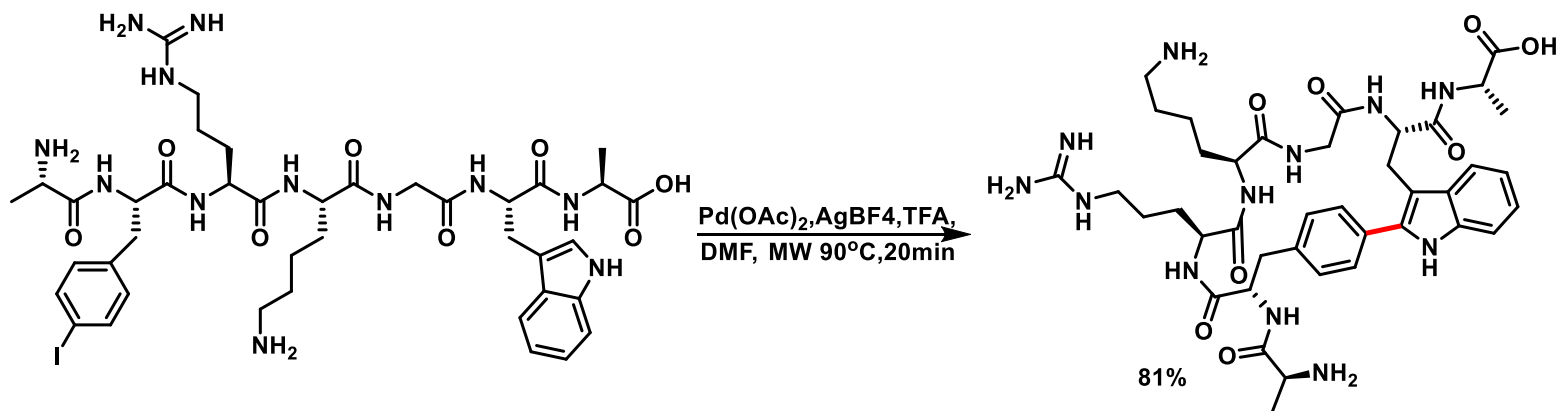
# Synthesis of stapled peptides

- Cyclic peptide and staple peptide could improve the stability and membrane permeability compared with liner peptide.

Lavilla's work



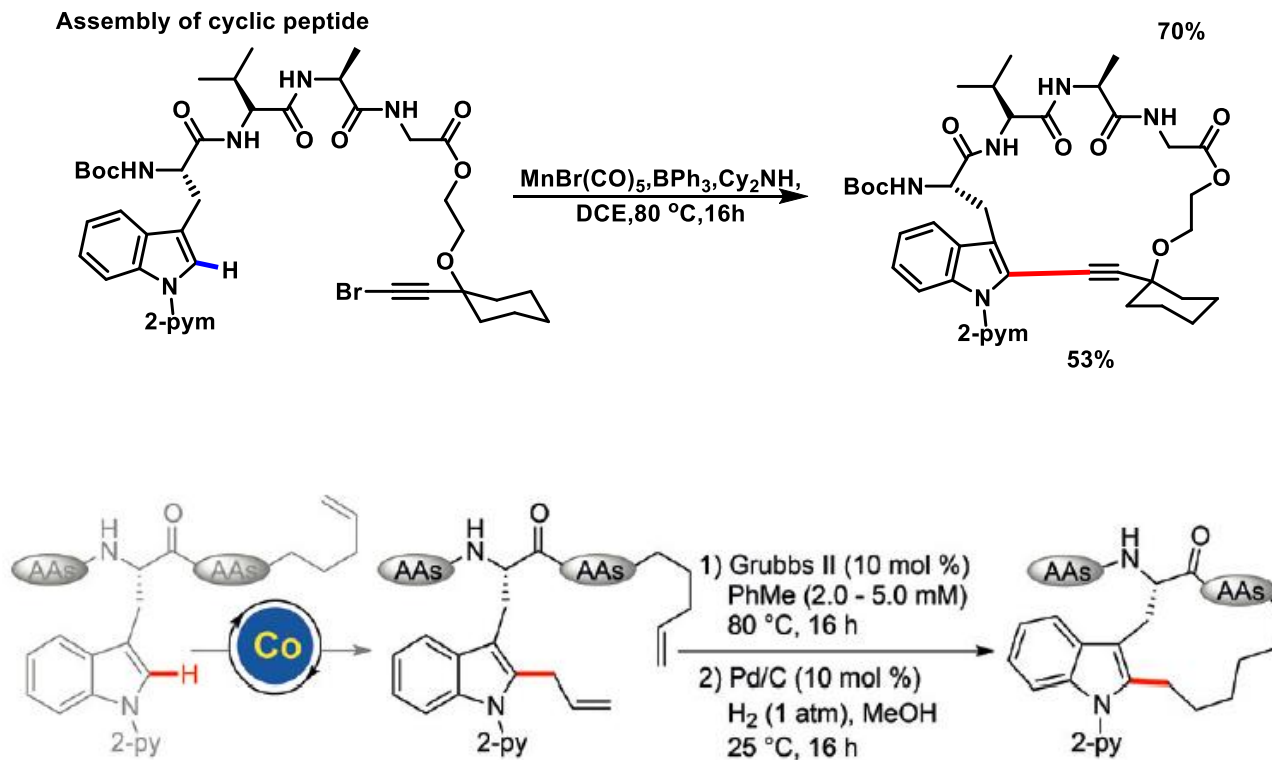
Formation of cyclicpeptide



*Nat. Commun.* **2015**, 6, 7160; *Chem. Eur. J.* 2016, 22, 13114

# Synthesis of stapled peptides

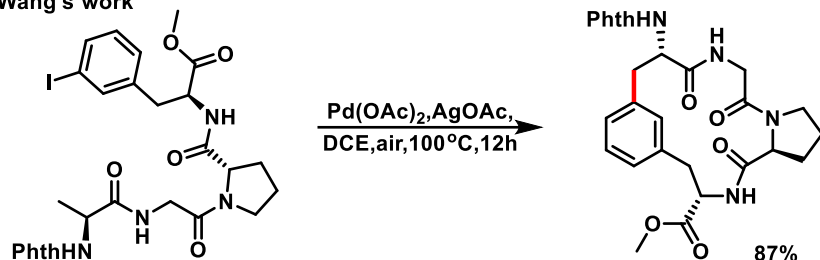
## Ackermann's work



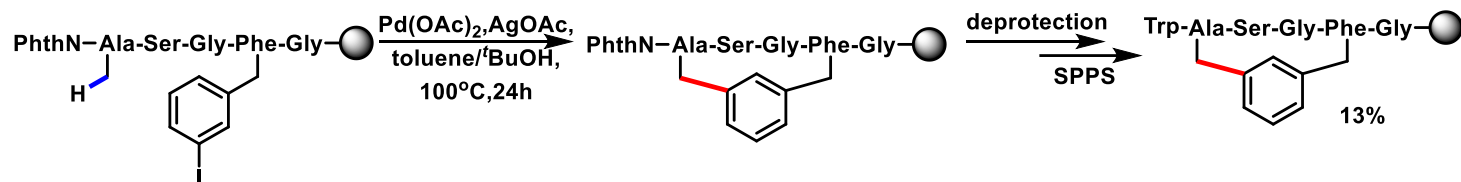
*Angew.Chem.Int.Ed.* **2017**, 56, 3172; *Angew. Chem. Int. Ed.* **2019**, 58, 1684

# Synthesis of stapled peptides

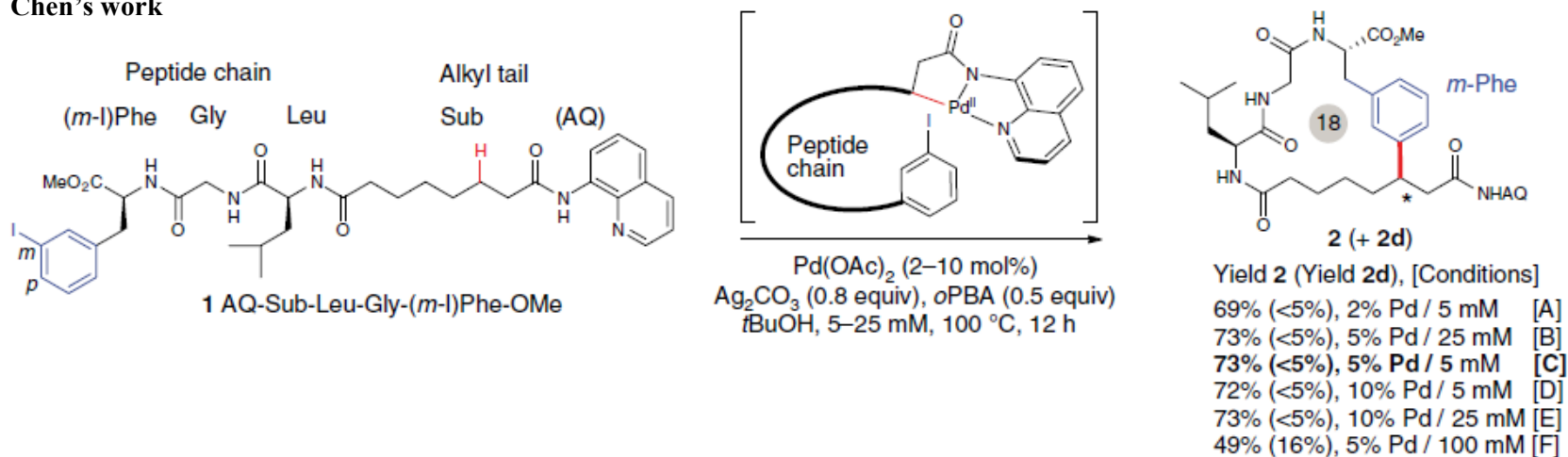
Wang's work



Albericio's work



Chen's work



**Thank you for your kind attention**