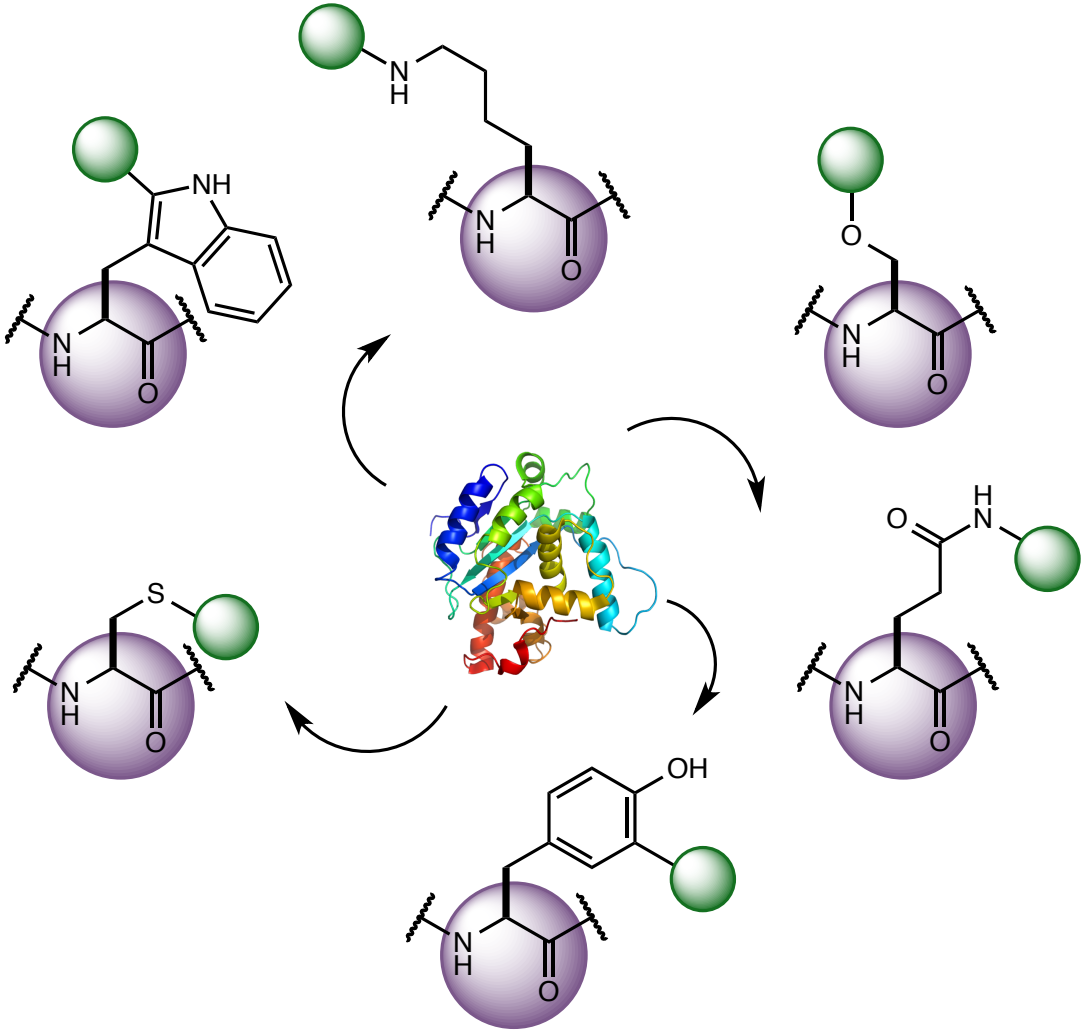
Click Reactions For Post-Translational Protein Modification



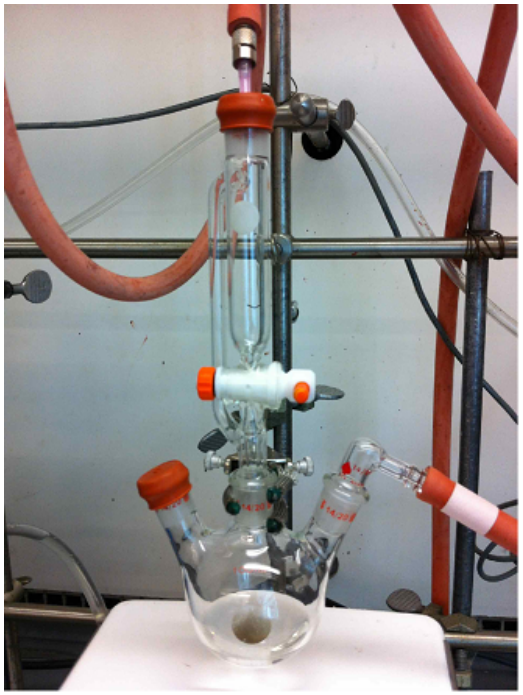
Steven Bloom

Group Meeting

12/2/2015

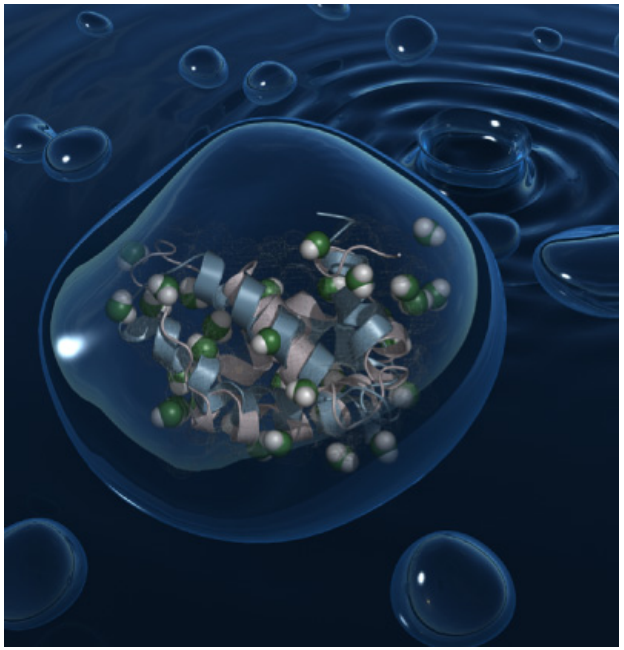
Click Reactions For Post-Translational Protein Modification

- Reactions to an organic chemist



total control of reaction parameters

vs



environment controlled

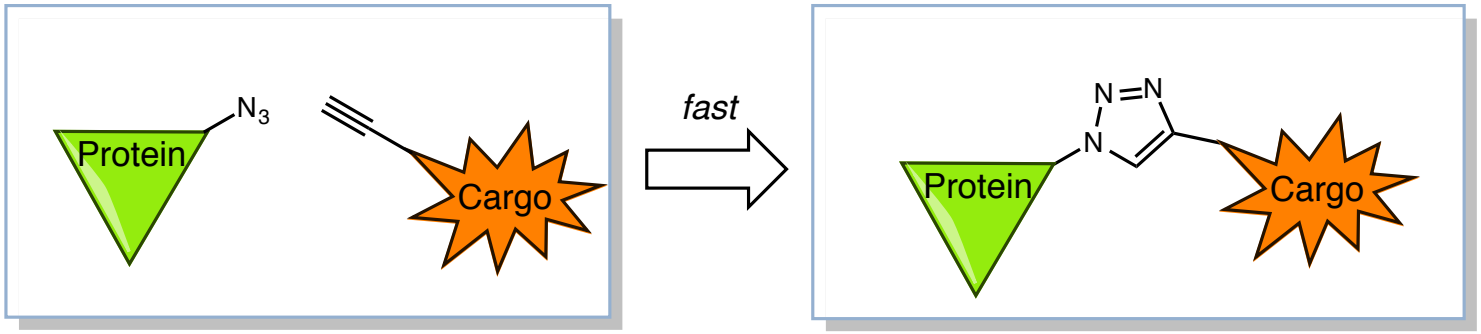
Baathula, K.; Xu, Y.; Quian, X. *Nature Protocols* **2011**, *6*, 1990-1997.
http://papoian.chem.umd.edu

Click Reactions For Post-Translational Protein Modification

■ Definition: "Click" Chemistry

Coined by K. Barry Sharpless in 1998

- Reactions that generate substances rapidly and reliably by joining small molecules together

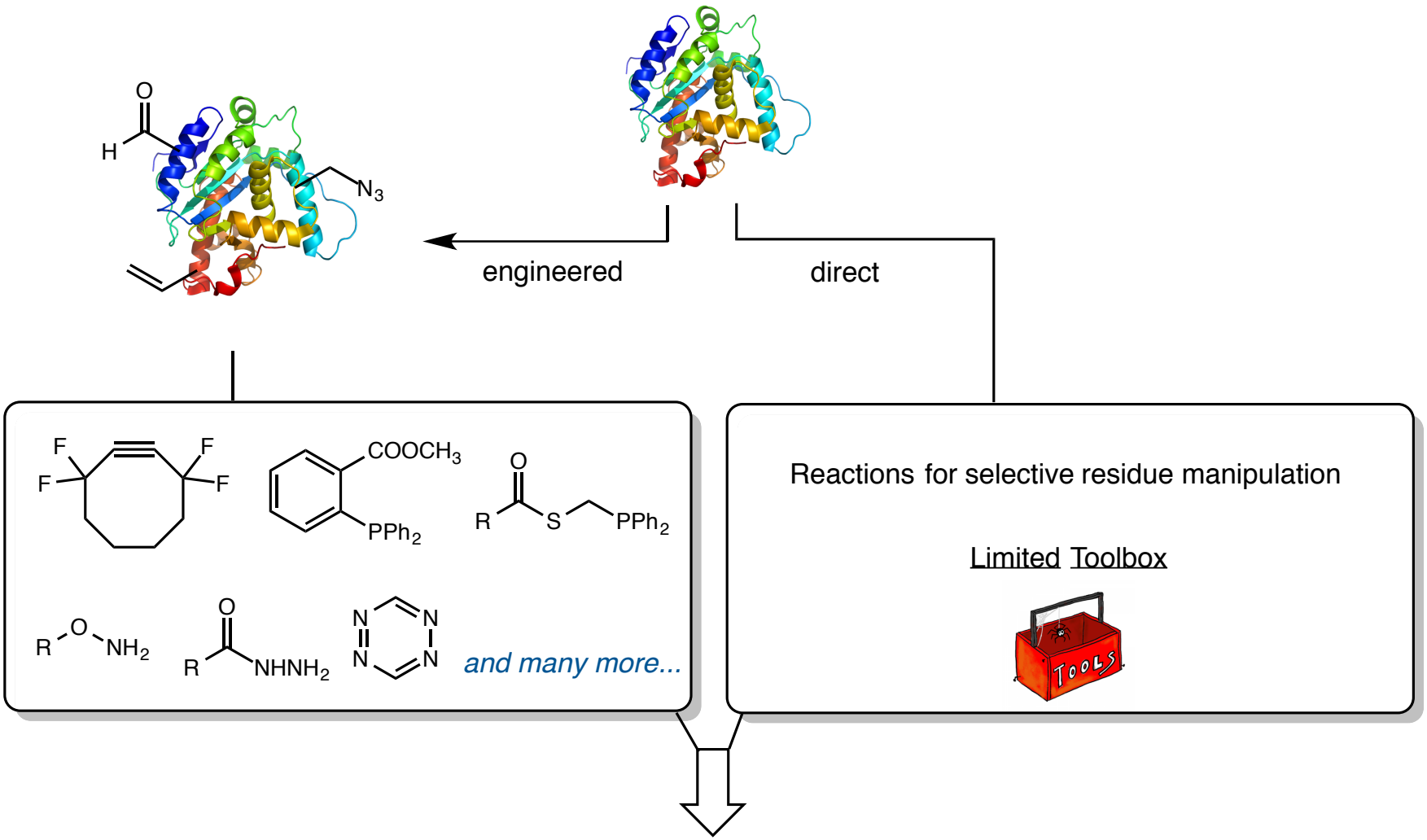


- *simple (minimal components)*
- *readily available reagents*
- *use benign solvents (H_2O)*
- *simple product isolation (non-chromatographic methods)*
- *irreversible*

Kolb, H. C.; Finn, M. G.; Sharpless, K. B. *Agnew Chem. Int. Ed.* **2001**, 2004-2021
Jewett, J. C.; Bertozzi, C. R. *Chem. Soc . Rev.* **2010**, *39*, 1272-1279.

Click Reactions For Post-Translational Protein Modification

■ Two Approaches: Protein Engineering or Direct Functionalization



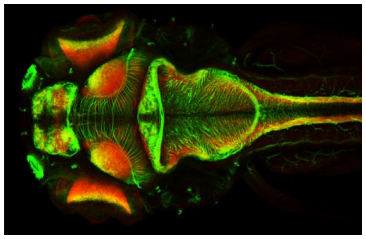
Jewett, J. C.; Bertozzi, C. R. *Chem. Soc . Rev*. **2010**, *39*, 1272-1279.

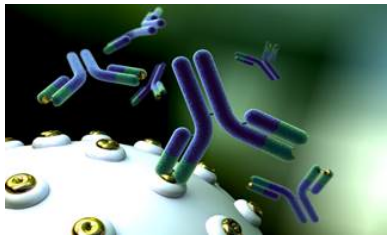
Click Reactions For Post-Translational Protein Modification

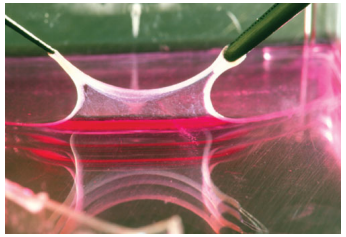
■ Why Do We Want Bioorthoganol Reactions?

Mechanistic Biology

Pharmaceuticals



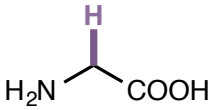




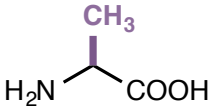
Biomaterials

Click Reactions For Post-Translational Protein Modification

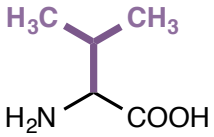
Non-polar



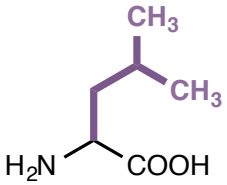
Glycine



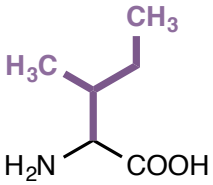
Alanine



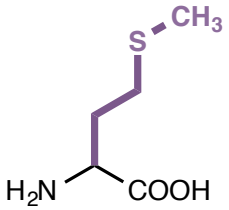
Valine



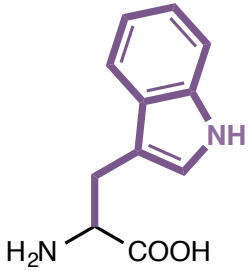
Leucine



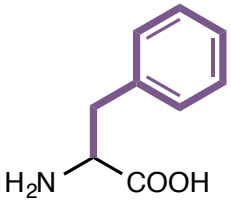
Isoleucine



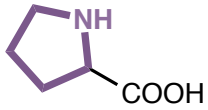
Methionine



Tryptophan

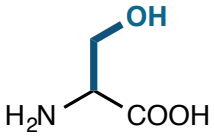


Phenylalanine

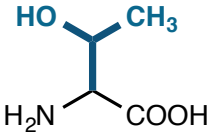


Proline

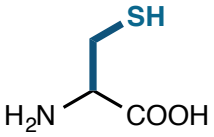
Polar



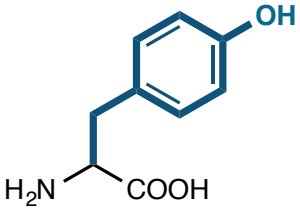
Serine



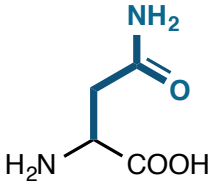
Threonine



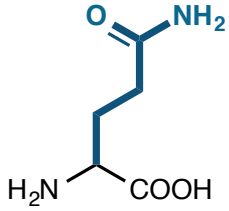
Cysteine



Tyrosine



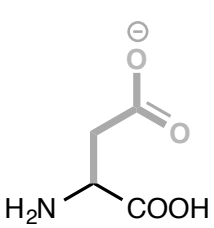
Asparagine



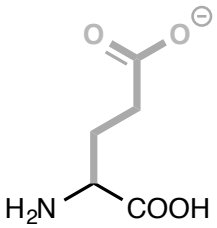
Glutamine

Click Reactions For Post-Translational Protein Modification

Acidic

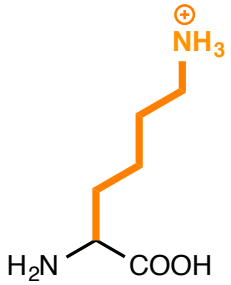


Aspartic acid

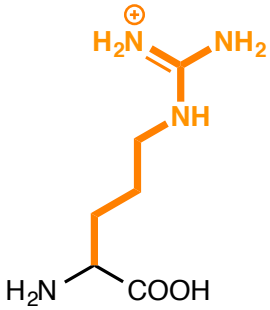


Glutamic acid

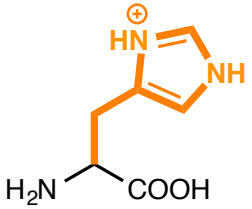
Basic



Lysine



Arginine

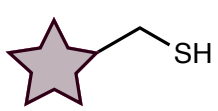


Histidine

Click Reactions For Post-Translational Protein Modification

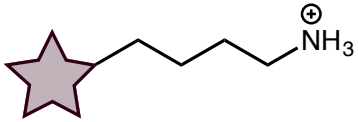
■ Which residues do we target for Click and How?

Cysteine



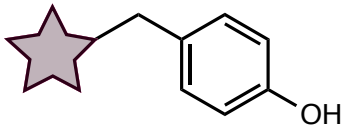
- uncommon residue
- nucleophilic
- good ligand for metals

Lysine



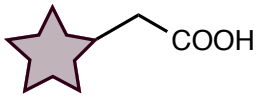
- most abundant residue
- strong nucleophile (as free amine)
-vast literature for reactions of amines

Tyrosine



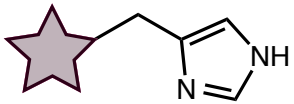
- activated for EAS reactions

Aspartic or Glutamic acid



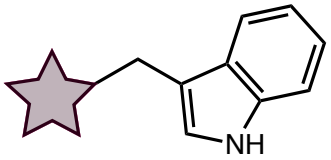
- prevalence
of coupling reactions in biology

Histidine



- good ligand for metals
- prone to acylation

Tryptophan



- electrophilic addition to C3

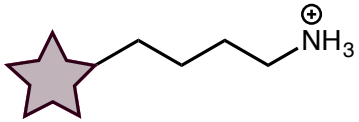
Click Reactions For Post-Translational Protein Modification

■ Which residues do we target for Click and How?

Cysteine

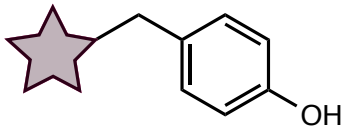
- uncommon residue
- nucleophilic
- good ligand for metals

Lysine



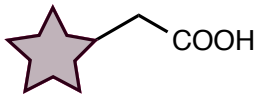
- most abundant residue
- strong nucleophile (as free amine)
-vast literature for reactions of amines

Tyrosine



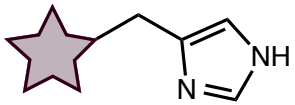
- activated for EAS reactions

Aspartic or Glutamic acid



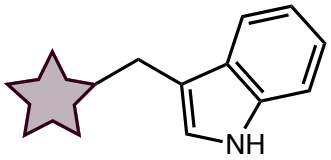
- prevalence
of coupling reactions in biology

Histidine



- good ligand for metals
- prone to acylation

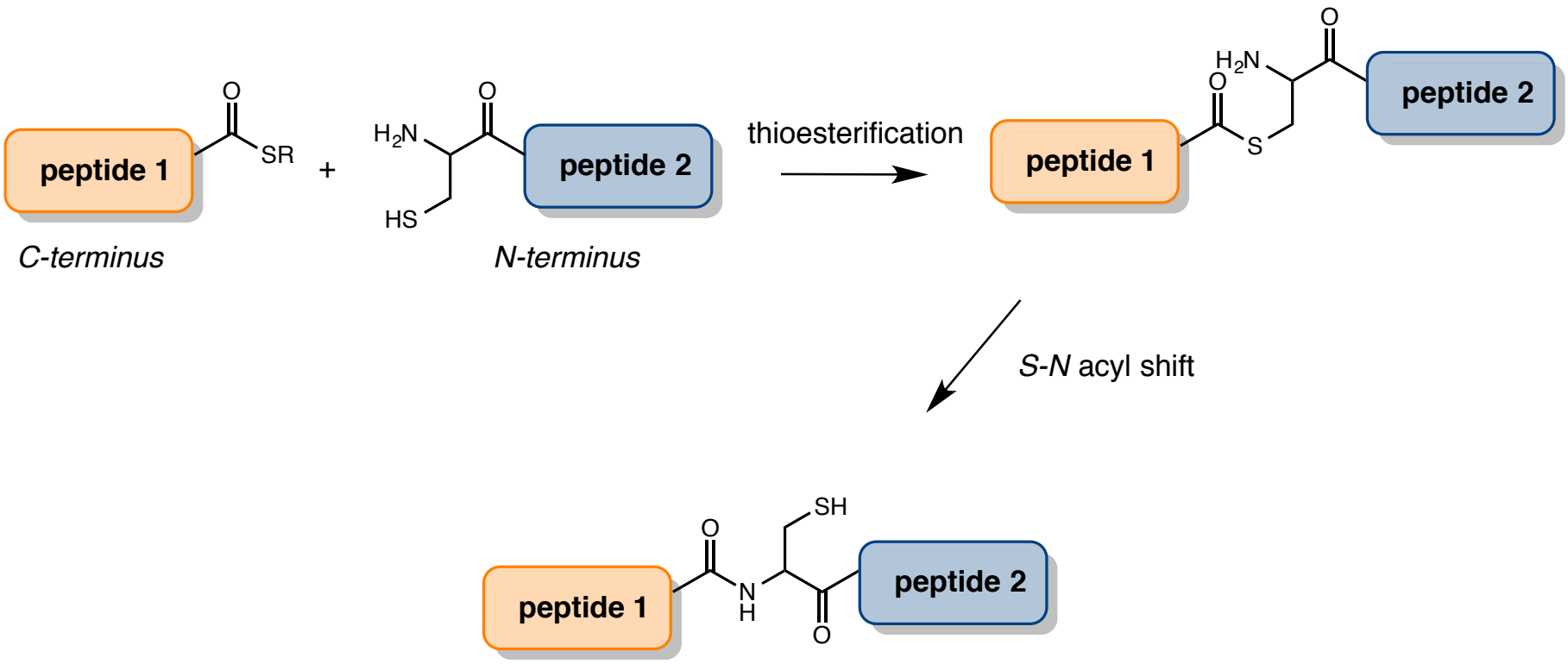
Tryptophan



- electrophilic addition to C3

Click Reactions For Post-Translational Protein Modification

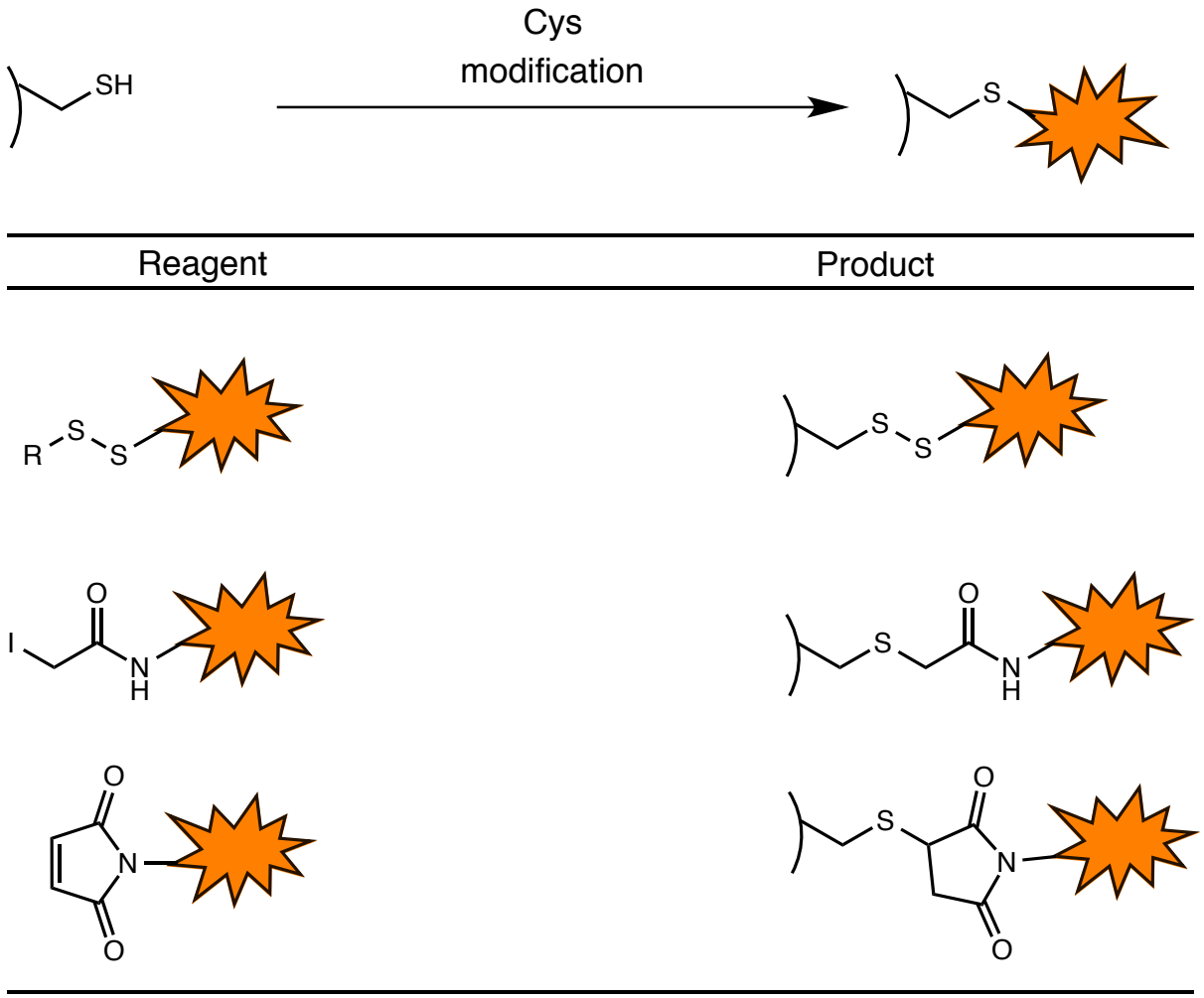
■ Cysteine- Native Chemical Ligation



Hackeng, T. M.; Griffin, J. H.; Dawson, P. E. *P.N.A.S.* **1999**, *96*, 10068-10073.
Malins, L. R.; Payne, R. J. *Curr. Opin. Chem. Biol.* **2014**, *22*, 70-78.

Click Reactions For Post-Translational Protein Modification

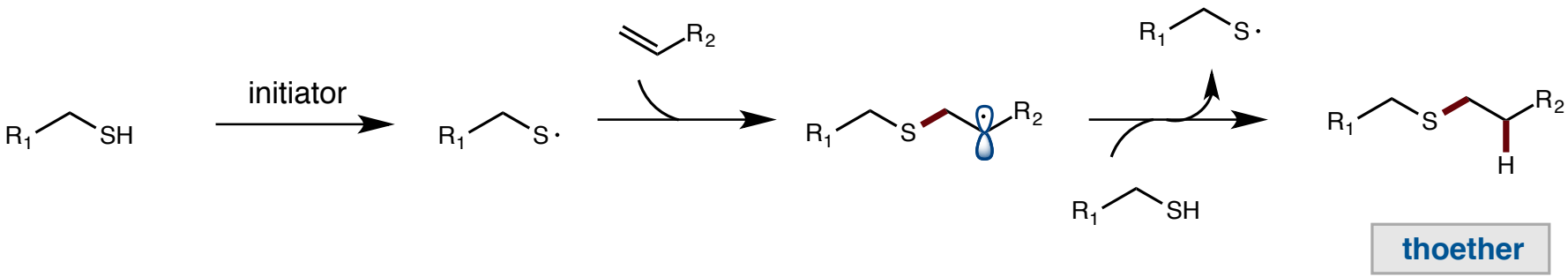
■ Classical Methods for Cysteine Modification



Sletten, E. M.; Bertozzi, C. R. *Agnew. Chem. Int. Ed. Engl.* **2009**, *48*, 6974-6998. and references therein

Click Reactions For Post-Translational Protein Modification

■ Cysteine: Thiol-ene



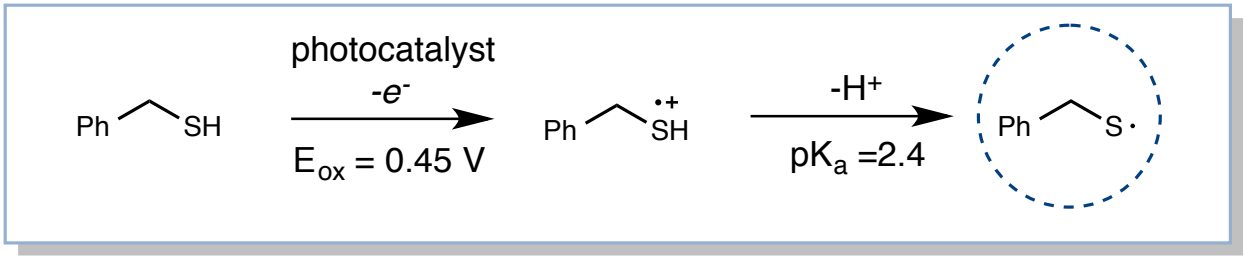
common initiators

AIBN

R_3Sn-H

direct UV irradiation

Yoon et al. ***2013***



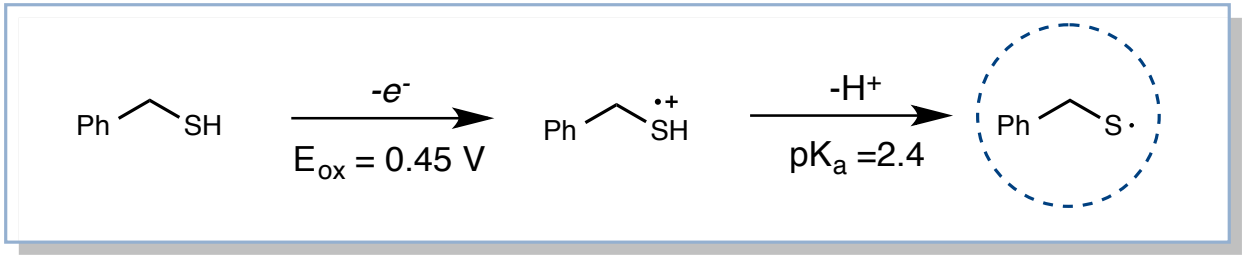
Hoyle, C. E.; Bowman, C. N. *Agnew Chem. Int. Ed.* **2010**, *49*, 1540-1573. and references therein

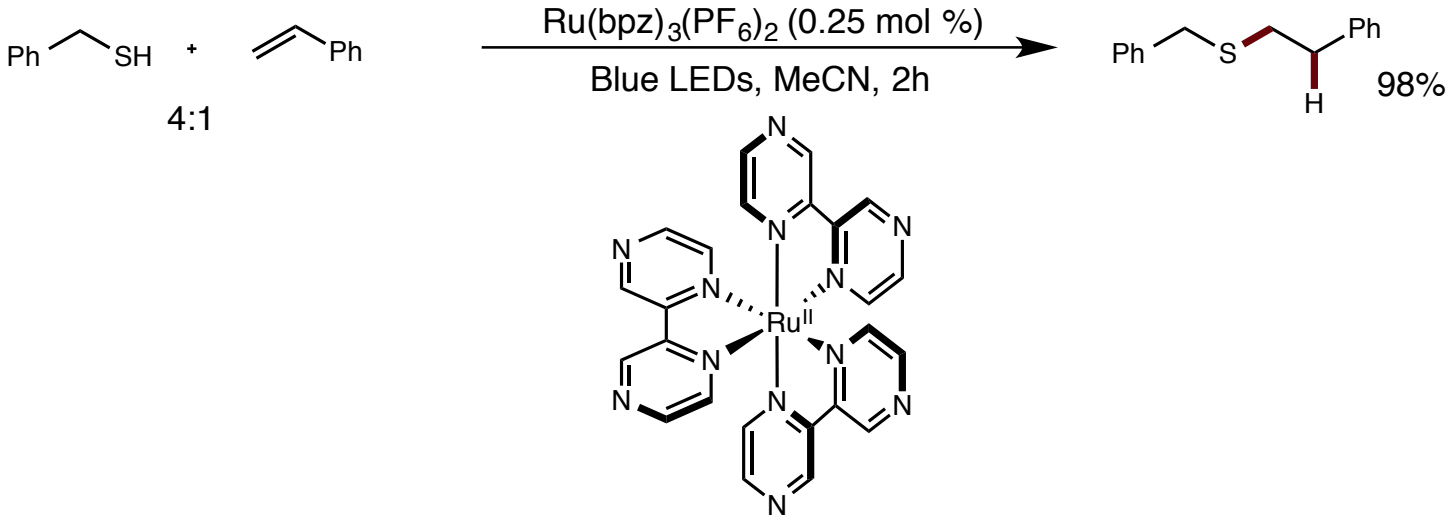
Tyson, E. L.; Ament, M. S. Yoon, T. P. *J. Org. Chem.* **2013**, *78*, 2046-2050.

Click reactions for Post-translational Protein Modification

■ Cysteine: Thiol-ene

Yoon et al. ***2013***





$Ru(bpz)_3(PF_6)_2$

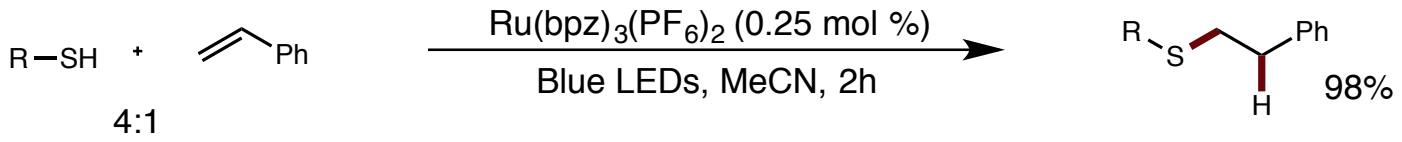
$E_{1/2}^{II*/I}$ = + 1.35 V

Tyson, E. L.; Ament, M. S. Yoon, T. P. *J. Org. Chem.* **2013**, *78*, 2046-2050.

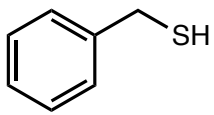
Click Reactions For Post-Translational Protein Modification

■ Cysteine:Thiol-ene

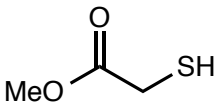
Yoon et al. ***2013***



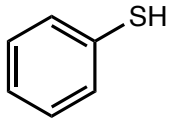
Thiol Scope



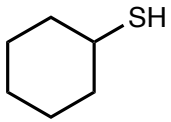
98% (2h)



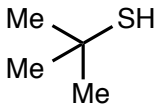
96% (1.5h)



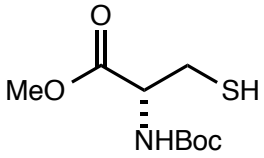
98% (1h)



98% (8h)



86% (20h)



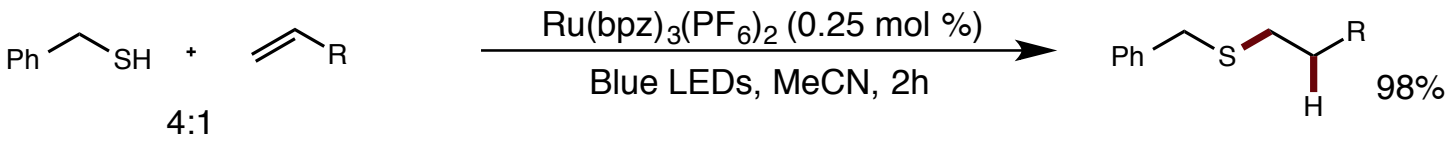
97% (1.5h)

Tyson, E. L.; Ament, M. S. Yoon, T. P. *J. Org. Chem.* **2013**, *78*, 2046-2050.

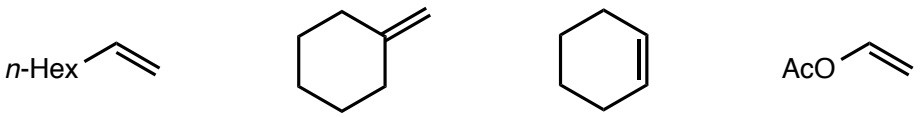
Click Reactions For Post-Translational Protein Modification

■ Cysteine: Thiol-ene

Yoon et al. ***2013***



Alkene Scope



99% (1h) 98% (1h) 98% (6h) 82% (2h)

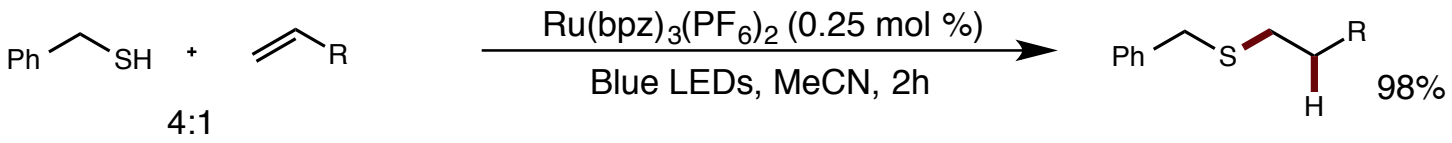
86% (2h) 88% (3h) 80% (3h) 93% (26h)

Tyson, E. L.; Ament, M. S. Yoon, T. P. *J. Org. Chem.* **2013**, *78*, 2046-2050.

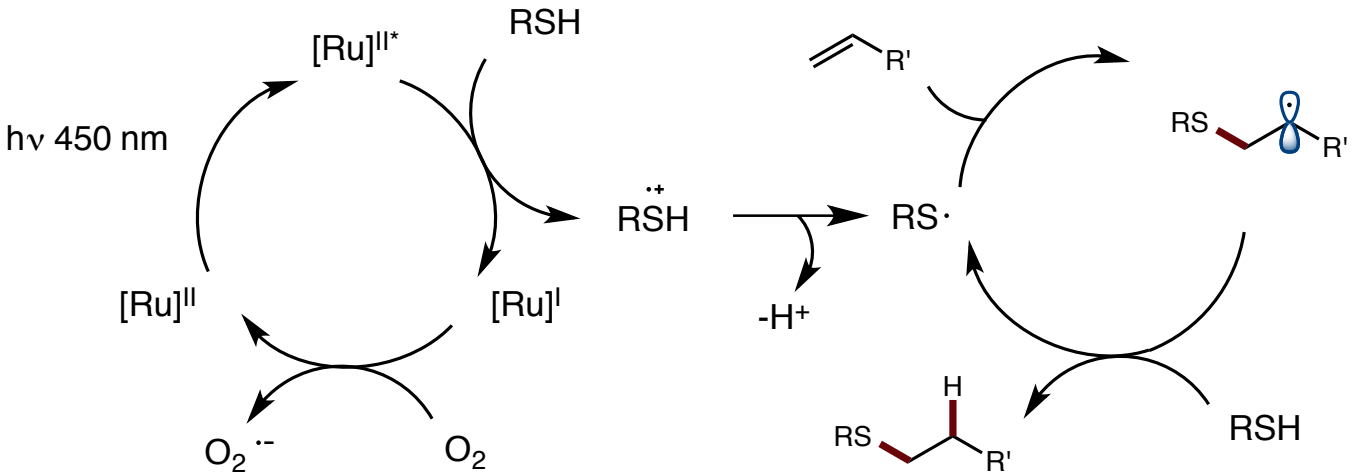
Click Reactions For Post-Translational Protein Modification

■ Cysteine: Thiol-ene

Yoon et al. ***2013***



Proposed Mechanism

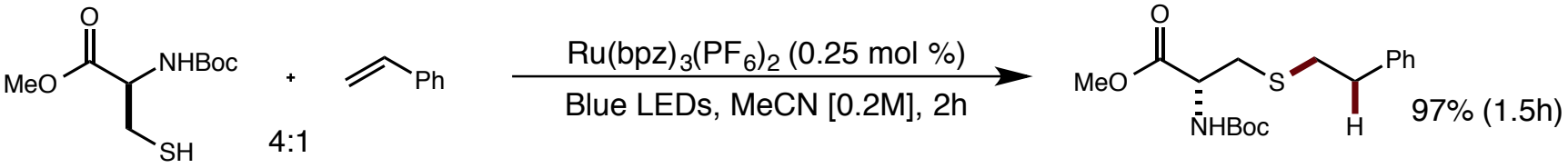


Tyson, E. L.; Ament, M. S. Yoon, T. P. *J. Org. Chem.* **2013**, *78*, 2046-2050.

Click Reactions For Post-Translational Protein Modification

■ Cysteine: Thiol-ene Bio-orthogonal variation

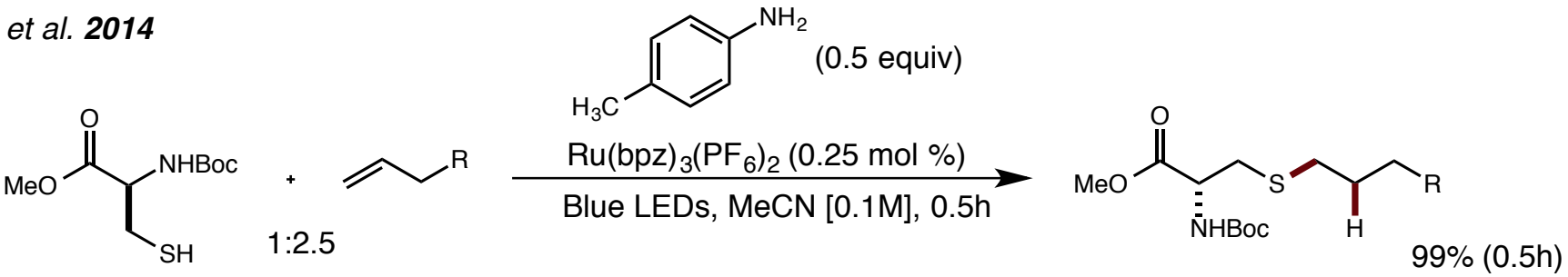
Yoon et al. ***2013***



Bio-orthogonal variation

- amino acid is limiting
- work in dilute, aquesous media
- exhibit high rate of reactivity

Yoon et al. ***2014***



Tyson, E. L.; Niemeyer, Z. L.; Yoon, T. P. *J. Org. Chem.* **2014**, *79*, 1427-1436.
Tyson, E. L.; Ament, M. S. Yoon, T. P. *J. Org. Chem.* **2013**, *78*, 2046-2050.

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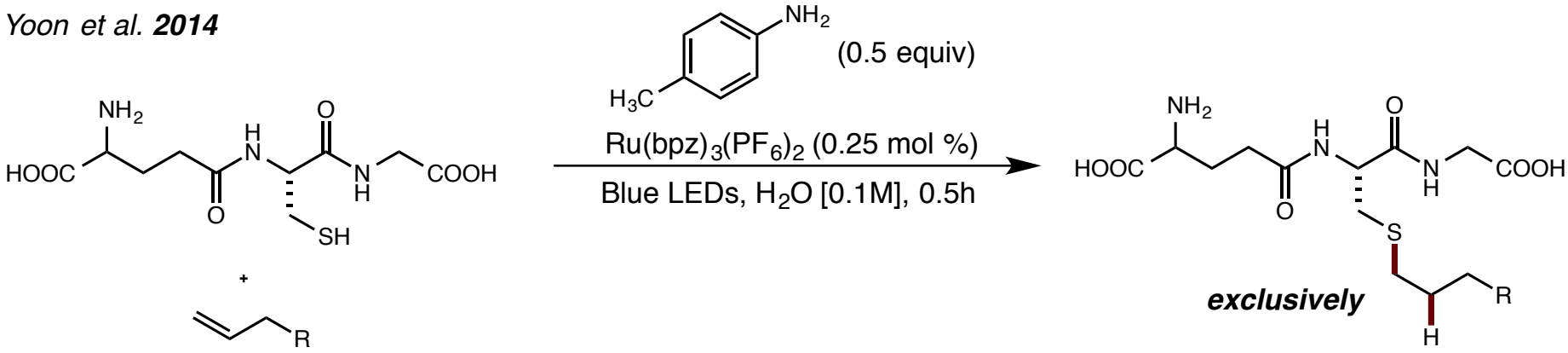
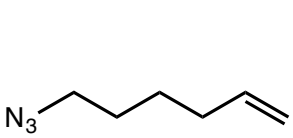
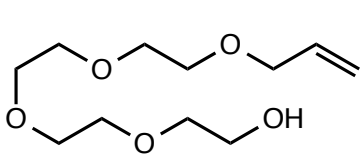
Yoon et al. ***2014***

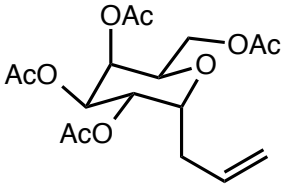




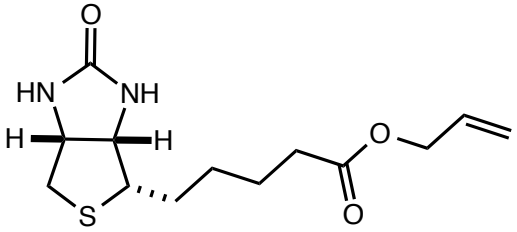
60%



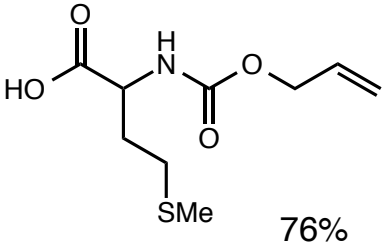
75%



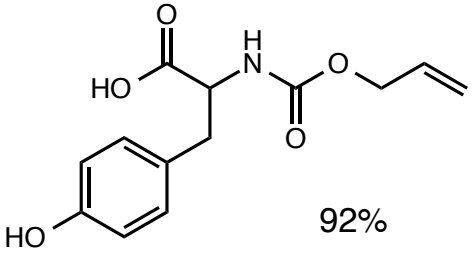
71%



77%



76%



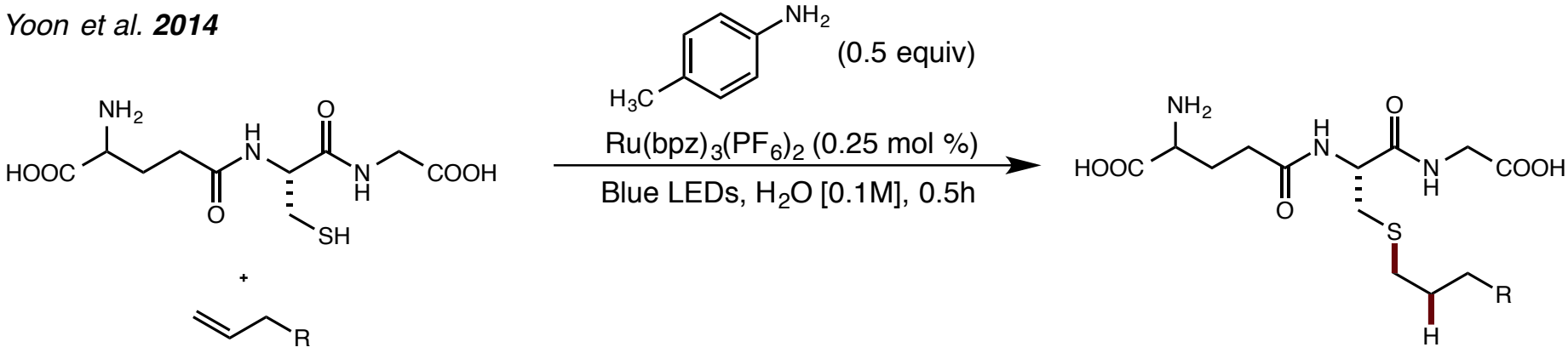
92%

Tyson, E. L.; Ament, M. S. Yoon, T. P. *J. Org. Chem.* **2013**, *78*, 2046-2050.

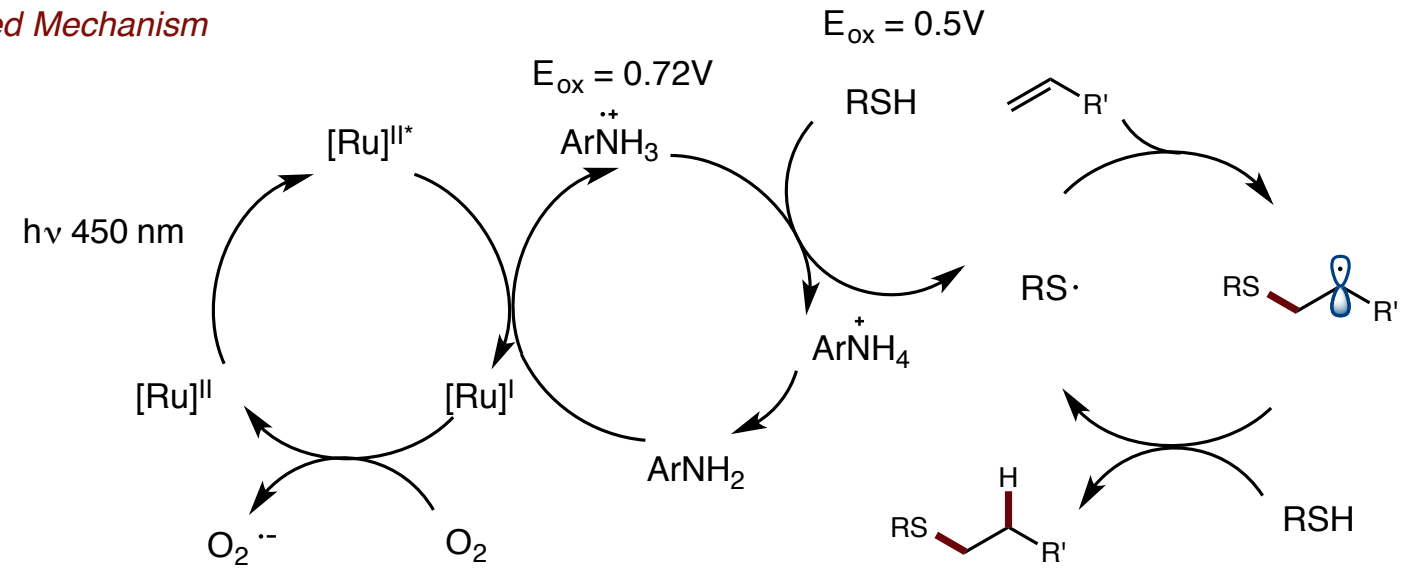
Click Reactions For Post-Translational Protein Modification

■ Cysteine: Thiol-ene Bio-orthogonal variation

Yoon et al. ***2014***



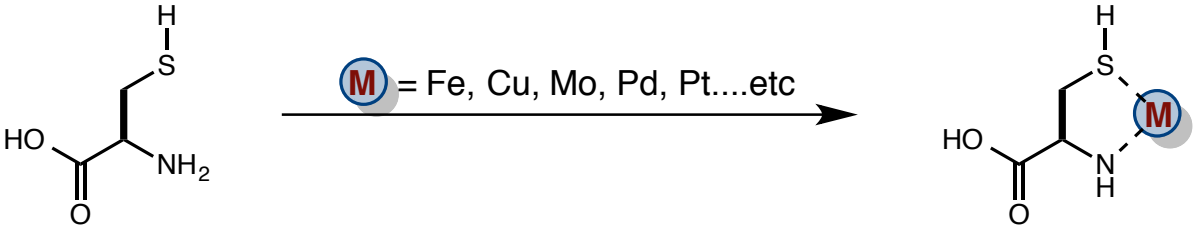
Proposed Mechanism

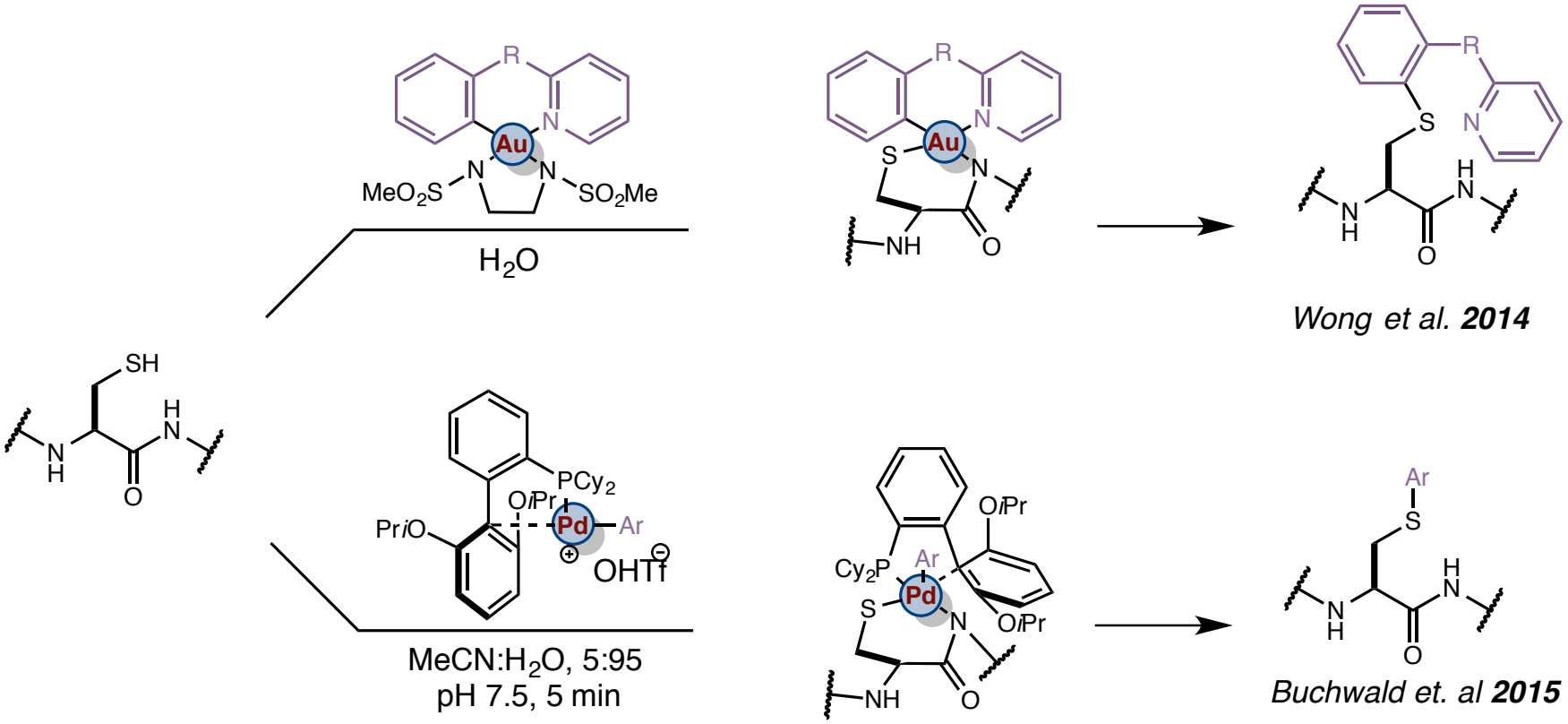


Tyson, E. L.; Ament, M. S. Yoon, T. P. *J. Org. Chem.* **2013**, *78*, 2046-2050.

Click Reactions For Post-Translational Protein Modification

■ Cysteine: Metal-mediated Cross Coupling





Kung. K. K.; Ko, H. M. Cui, J. F.; Chong, H. C.; Leung, Y.C.; Wong, M. K. *Chem. Commun.* **2014**, 50, 11899-11902.
Vinogradova, E. V.; Zhang, C.; Spokoyny, A. M.; Pentelute, B. L. Buchwald, S. L. *Nature* **2015**, *526*, 687-691.

Click Reactions For Post-Translational Protein Modification

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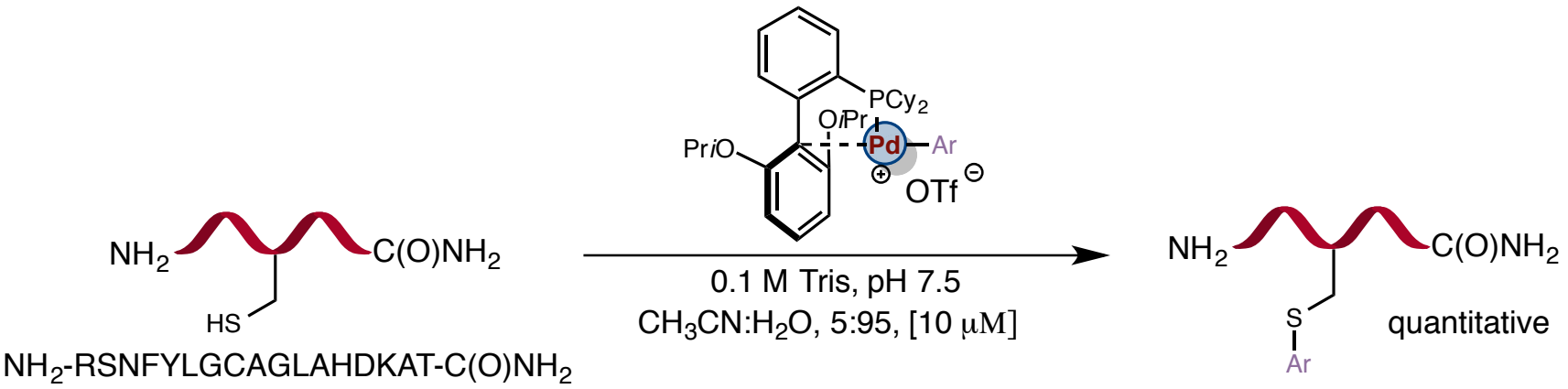
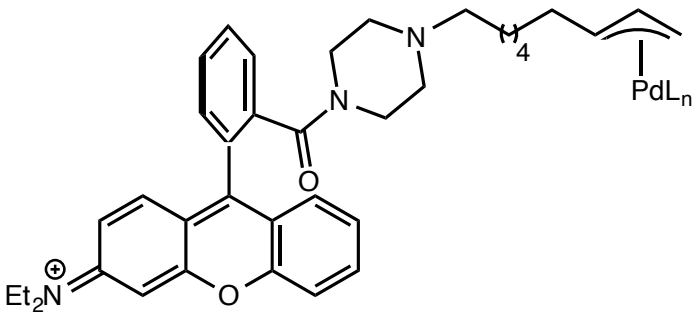
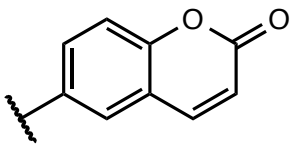


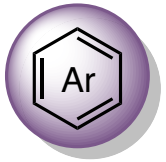
Pd cat. *O*-allylation of tyrosine (*Francis et. al* ***2005***)



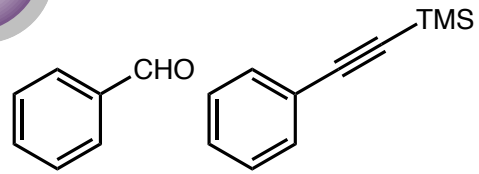
electrophilicity of metal center tunes chemoselectivity

Fluorescent tags

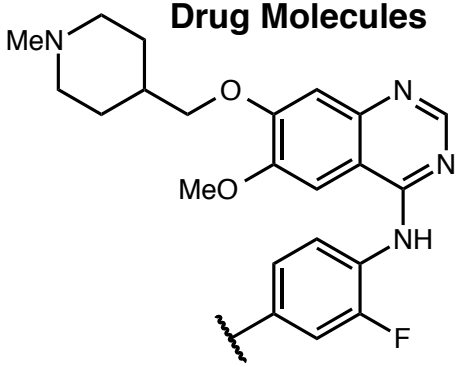




Bioconjugation handles



Drug Molecules

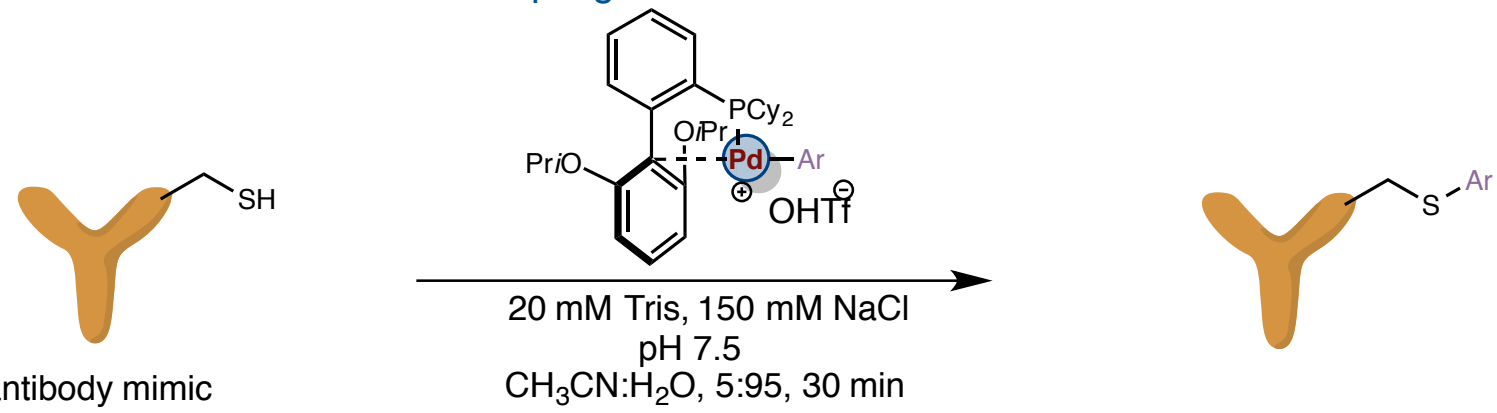


Vandetanib

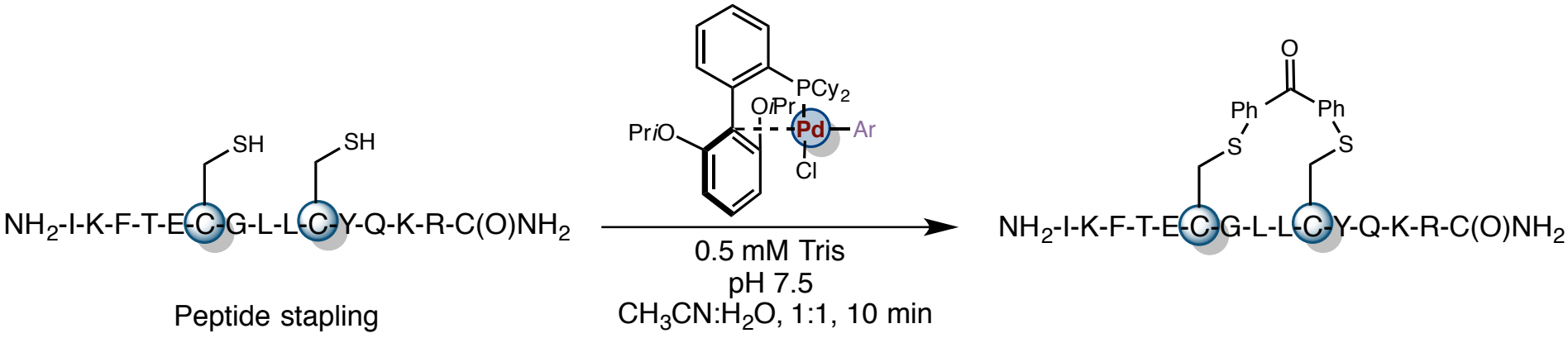
Vinogradova, E. V.; Zhang, C.; Spokoyny, A. M.; Pentelute, B. L. Buchwald, S. L. *Nature* **2015**, *526*, 687-691.

Click Reactions For Post-Translational Protein Modification

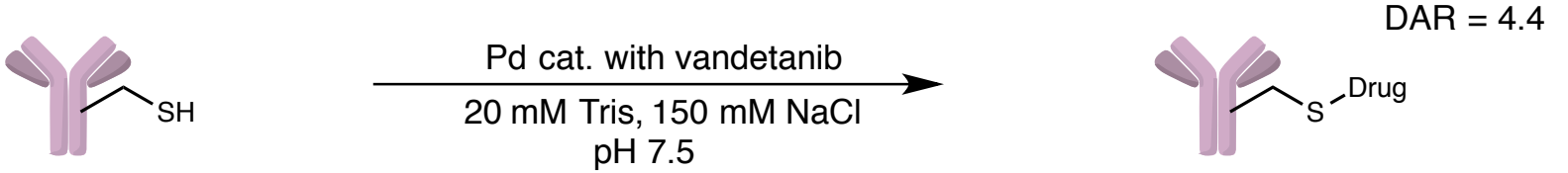
■ Cysteine: Metal-mediated Cross Coupling



antibody mimic



Peptide stapling



Trastuzumab

Trastuzumab-vandetanib conjugate

Vinogradova, E. V.; Zhang, C.; Spokoyny, A. M.; Pentelute, B. L. Buchwald, S. L. *Nature* **2015**, *526*, 687-691.

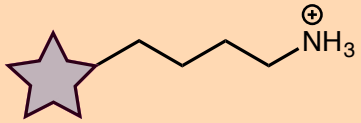
Click Reactions For Post-Translational Protein Modification

■ Which residues do we target for Click and How?

Cysteine

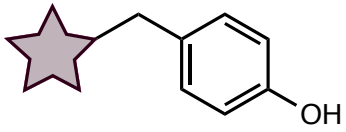
- uncommon residue
- nucleophilic
- good ligand for metals

Lysine



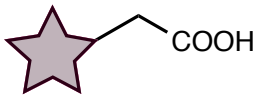
- most abundant residue
- strong nucleophile (as free amine)
-vast literature for reactions of amines

Tyrosine



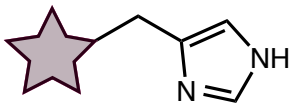
- activated for EAS reactions

Aspartic or Glutamic acid



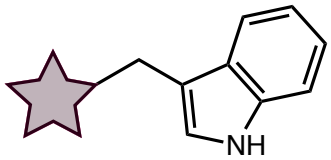
- prevalence
of coupling reactions in biology

Histidine



- good ligand for metals
- prone to acylation

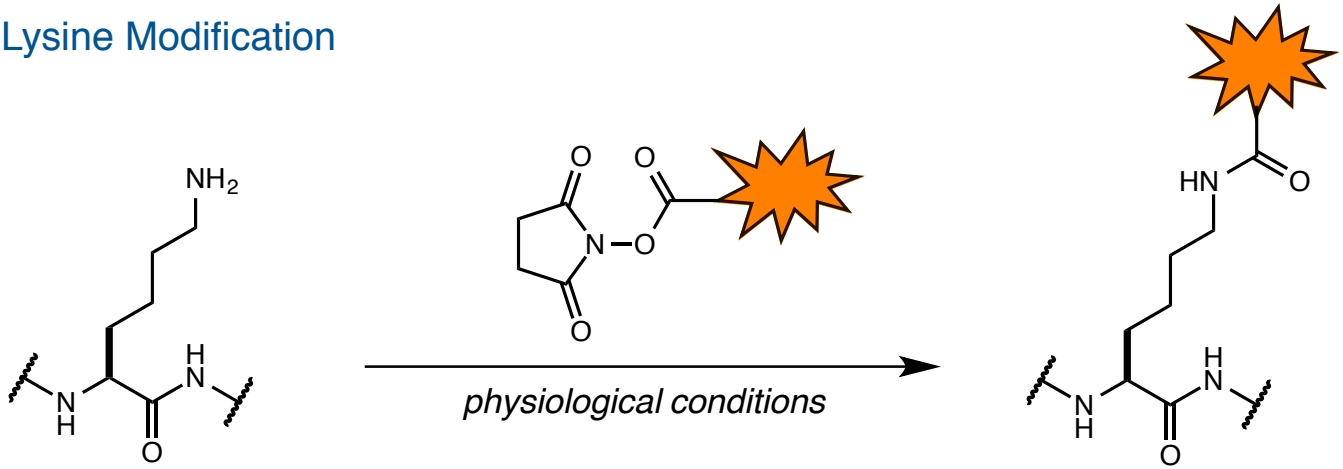
Tryptophan



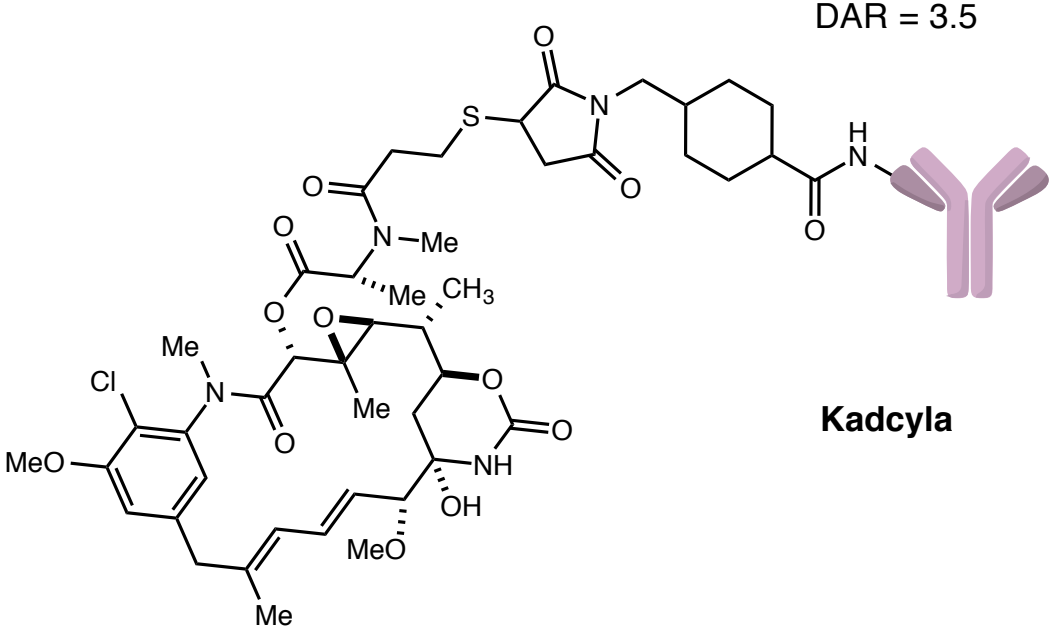
- electrophilic addition to C3

Click Reactions For Post-Translational Protein Modification

■ Classical Lysine Modification



DAR = 3.5

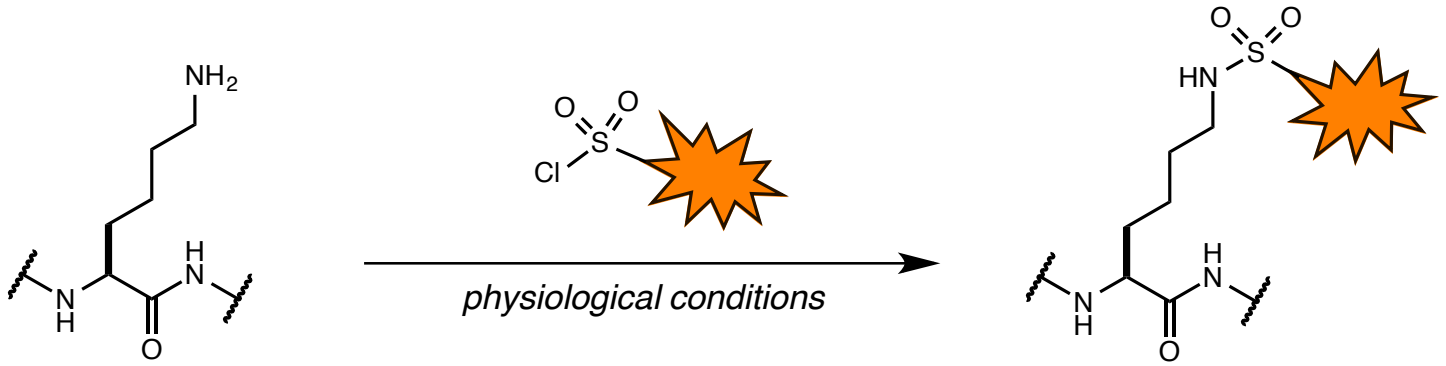


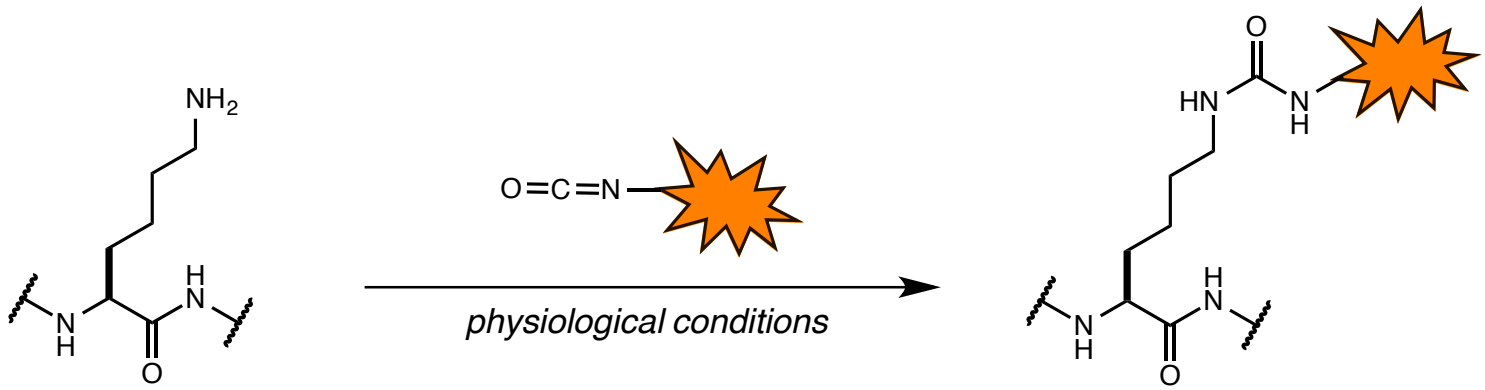
Kadcyla

Jewett, J. C.; Bertozzi, C. R. *Chem. Soc . Rev*. **2010**, *39*, 1272-1279.

Click Reactions For Post-Translational Protein Modification

■ Classical Lysine Modification

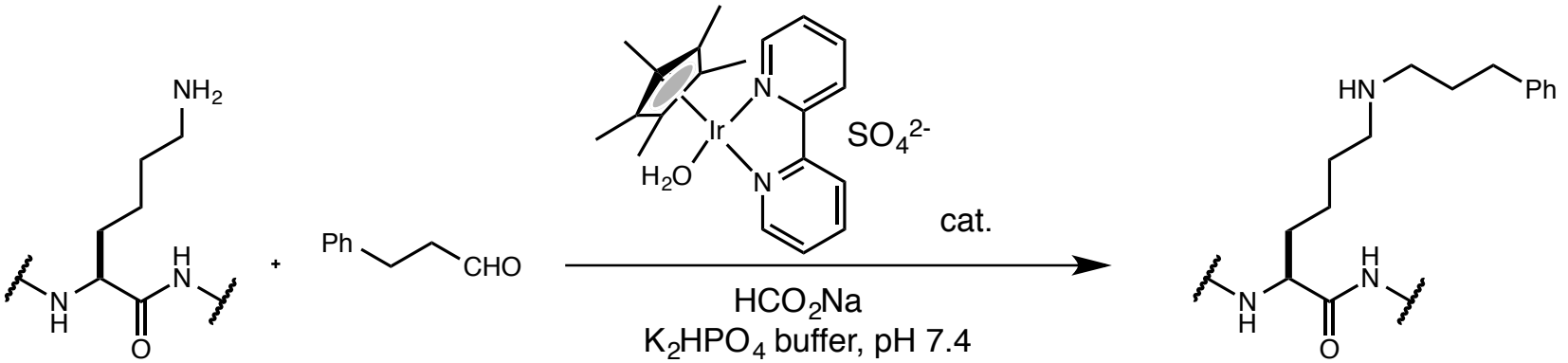


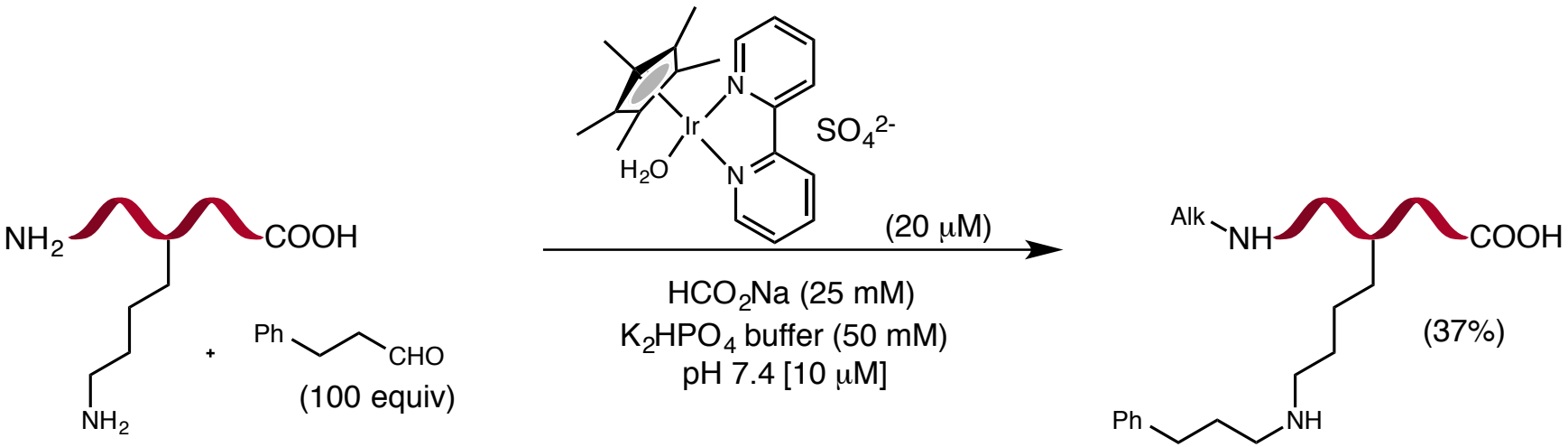


Jewett, J. C.; Bertozzi, C. R. *Chem. Soc . Rev*. **2010**, *39*, 1272-1279.

Click Reactions For Post-Translational Protein Modification

■ Lysine Modification by Transfer Hydrogenation





NH_2-MYKFARLAND-CO_2H NH_2-MYKFARLAND-CO_2H

McFarland, J. M.; Francis, M. B. *J. Am. Chem. Soc.* **2005**, *127*, 13490-13491.

Click Reactions For Post-Translational Protein Modification

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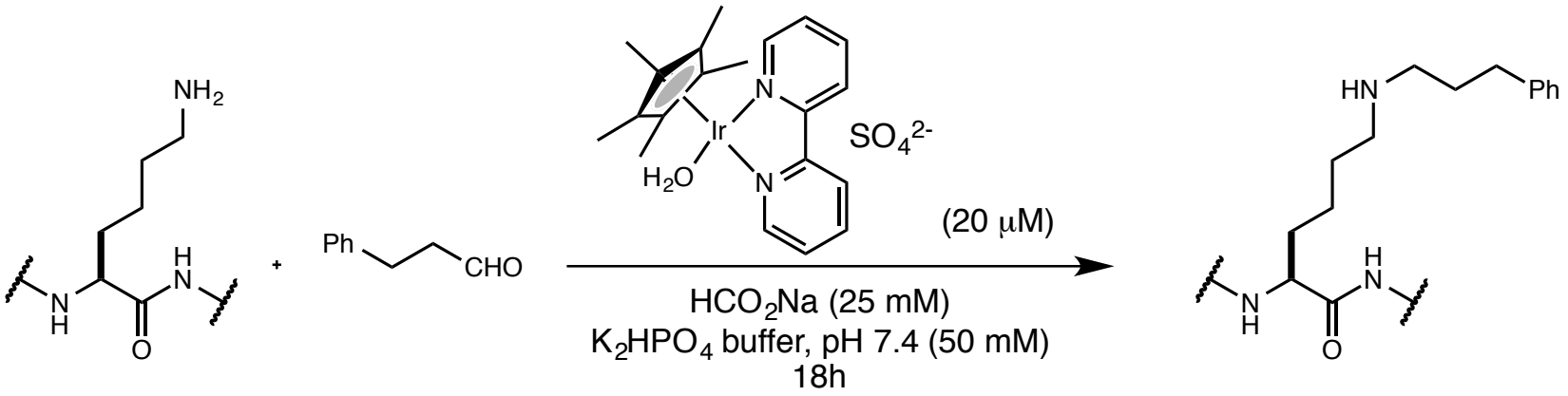
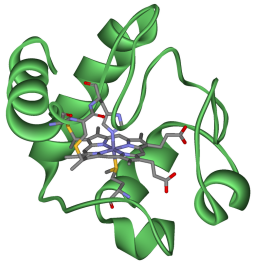
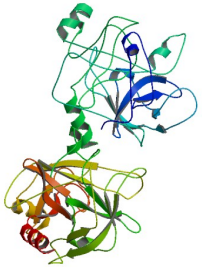




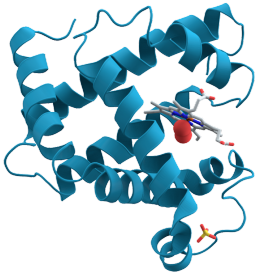
Cytochrome *c*
(19 Lys)

+ 1 Lys 19%
+ 2 Lys 27%
+ 3 Lys 22%
+ 4 Lys 12%



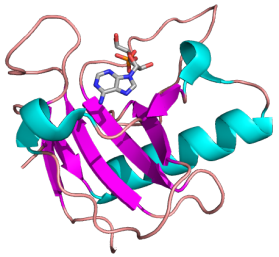
α-Chymotrypsinogen A
(14 Lys)

+ 1 Lys 47%
+ 2 Lys 27%
+ 3 Lys 02%
+ 4 Lys 00%



Myoglobin
(19 Lys)

+ 1 Lys 15%
+ 2 Lys 00%
+ 3 Lys 00%
+ 4 Lys 00%



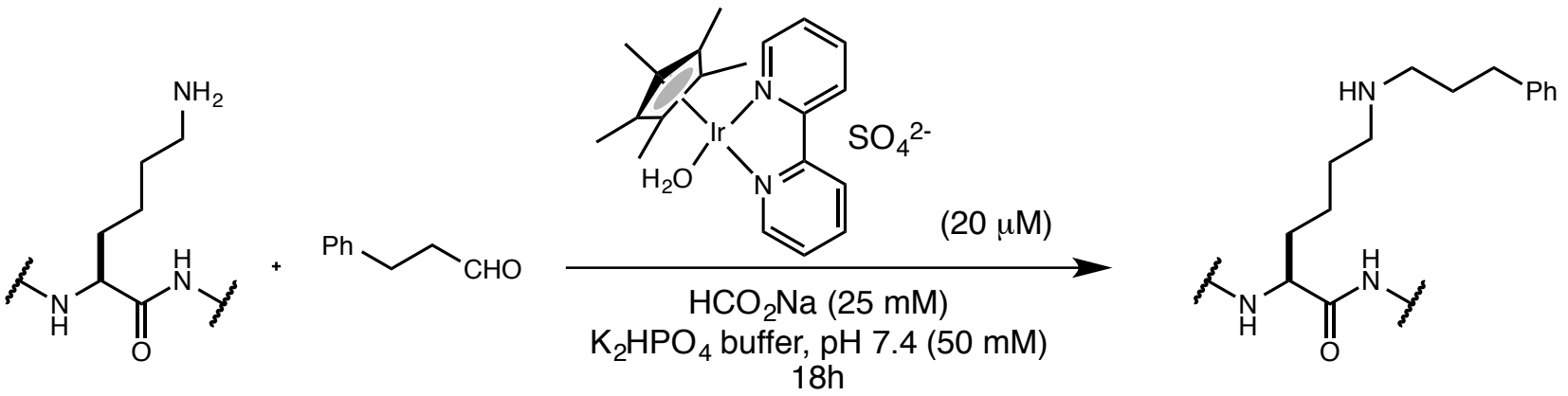
Ribonuclease
(10 Lys)

+ 1 Lys 41%
+ 2 Lys 00%
+ 3 Lys 00%
+ 4 Lys 00%

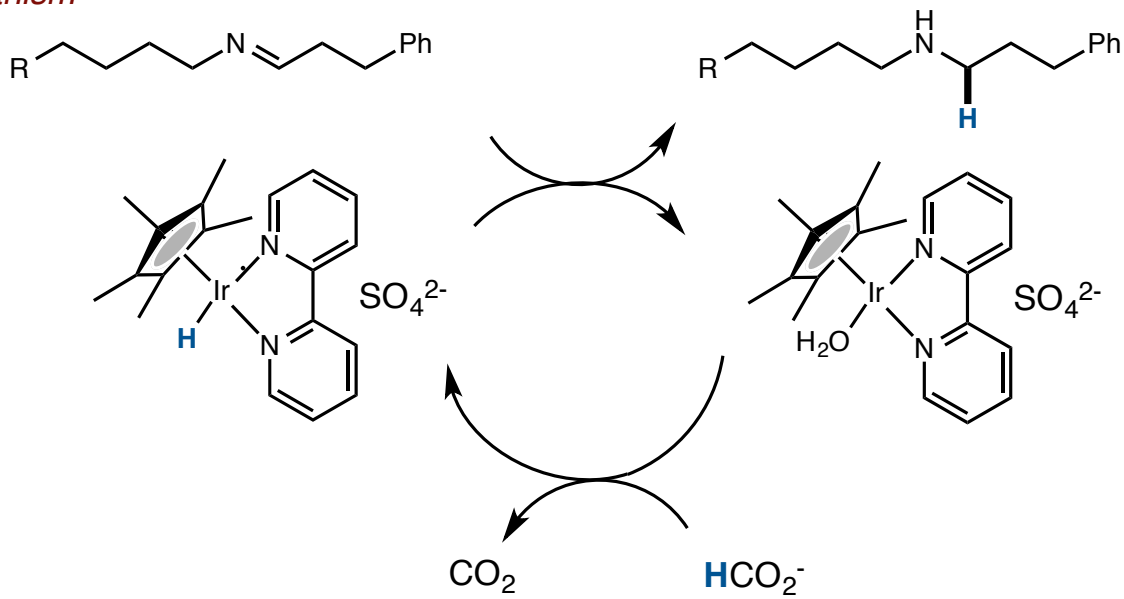
McFarland, J. M.; Francis, M. B. *J. Am. Chem. Soc.* **2005**, *127*, 13490-13491.

Click Reactions For Post-Translational Protein Modification

■ Lysine Modification by Transfer Hydrogenation



Proposed Mechanism



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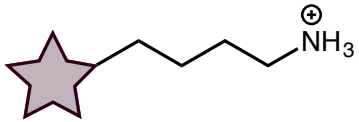
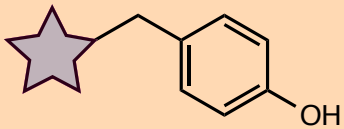


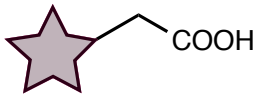
- most abundant residue
- strong nucleophile (as free amine)
-vast literature for reactions of amines

Tyrosine



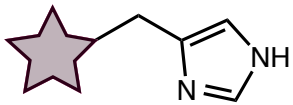
- activated for EAS reactions

Aspartic or Glutamic acid



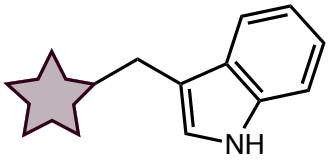
- prevalence
of coupling reactions in biology

Histidine



- good ligand for metals
- prone to acylation

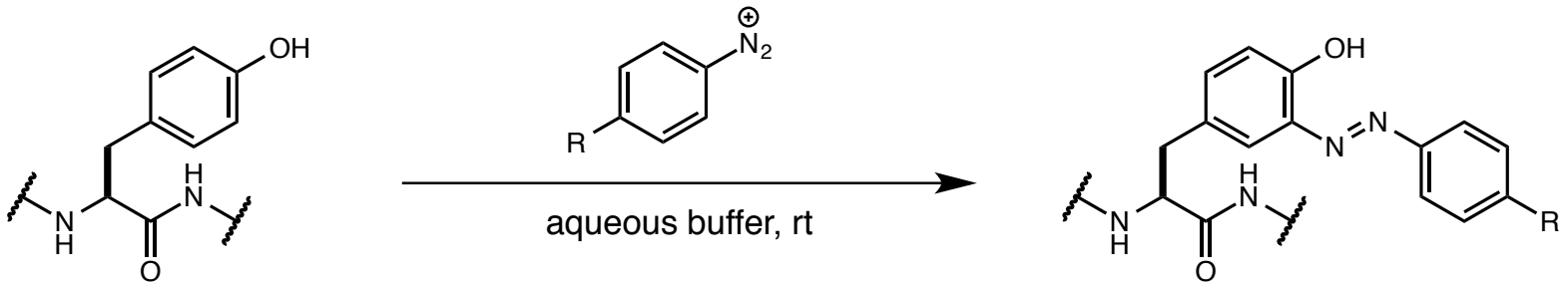
Tryptophan



- electrophilic addition to C3

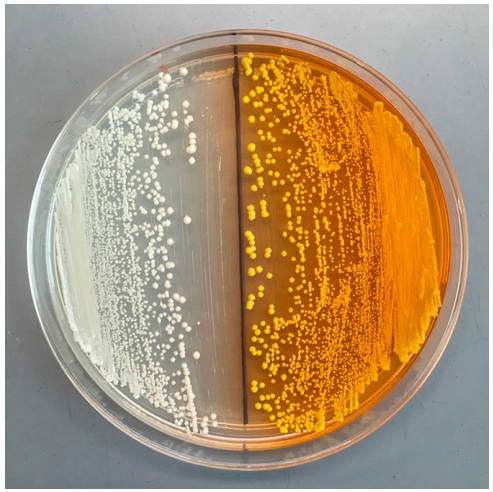
Click Reactions For Post-Translational Protein Modification

■ Tyrosine Classical Modification



bright organge

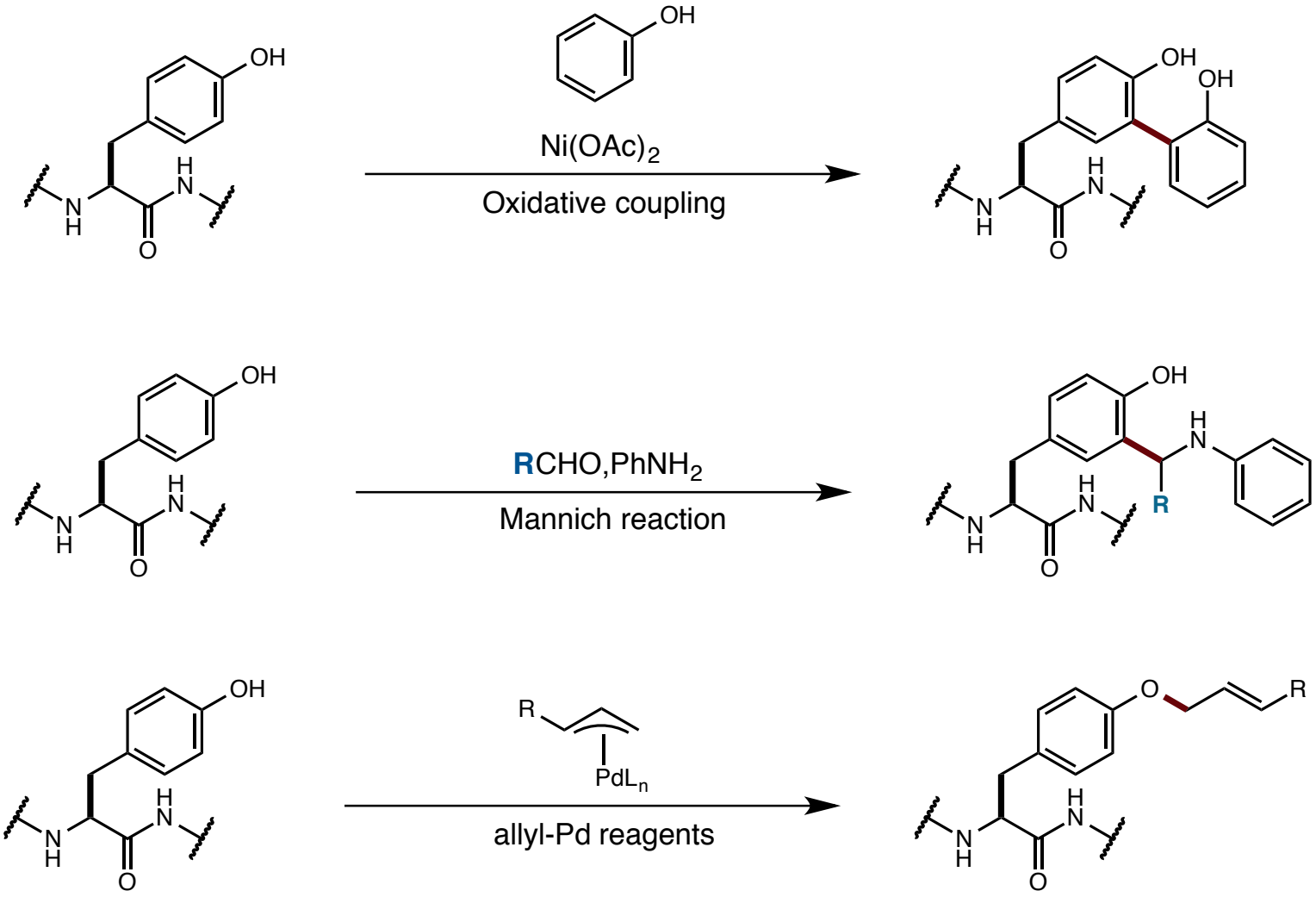
one of the earliest reported Modifications-**1905**



Jewett, J. C.; Bertozzi, C. R. *Chem. Soc . Rev*. **2010**, *39*, 1272-1279.

Click Reactions For Post-Translational Protein Modification

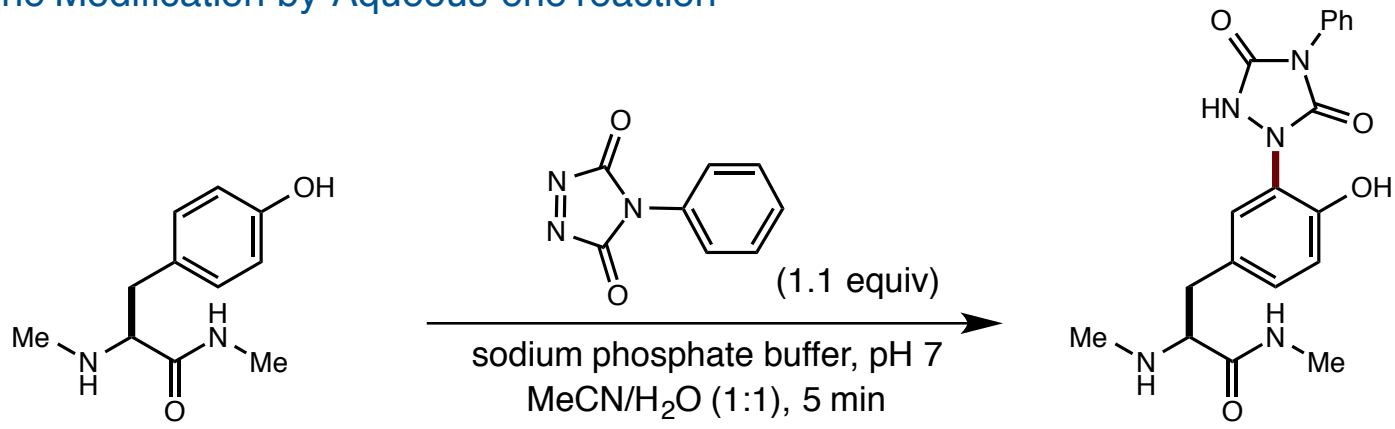
■ Tyrosine Classical Modification



Jewett, J. C.; Bertozzi, C. R. *Chem. Soc . Rev*. **2010**, *39*, 1272-1279.

Click Reactions For Post-Translational Protein Modification

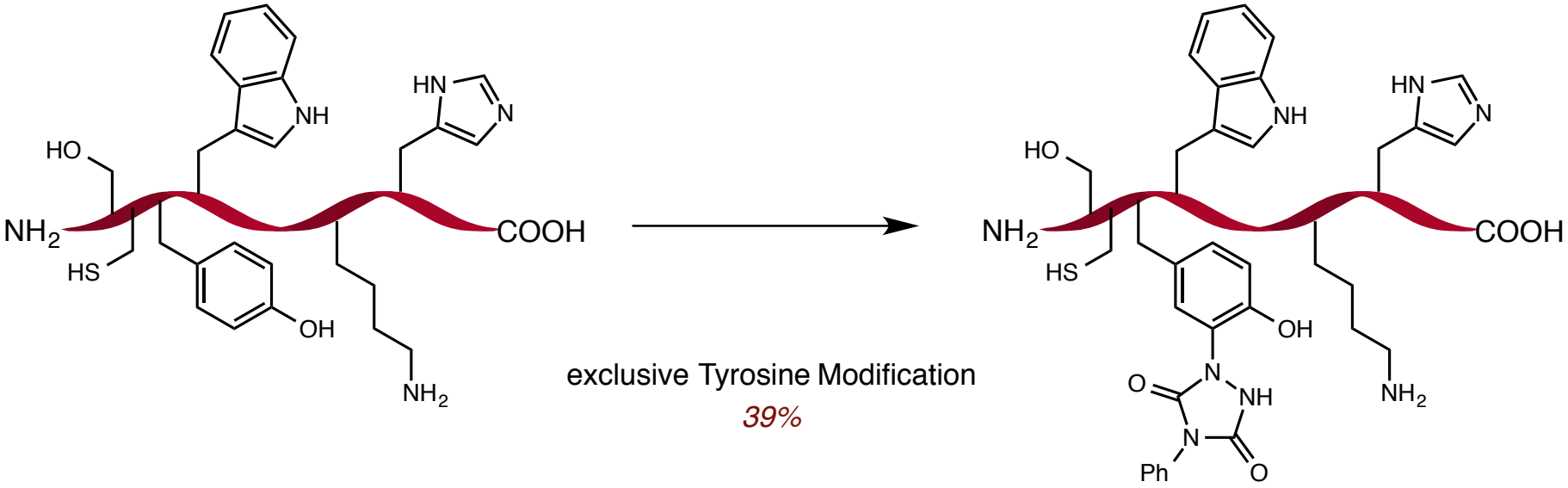
■ Tyrosine Modification by Aqueous-ene reaction



65%

quantitative with 3.3 equiv PTAD

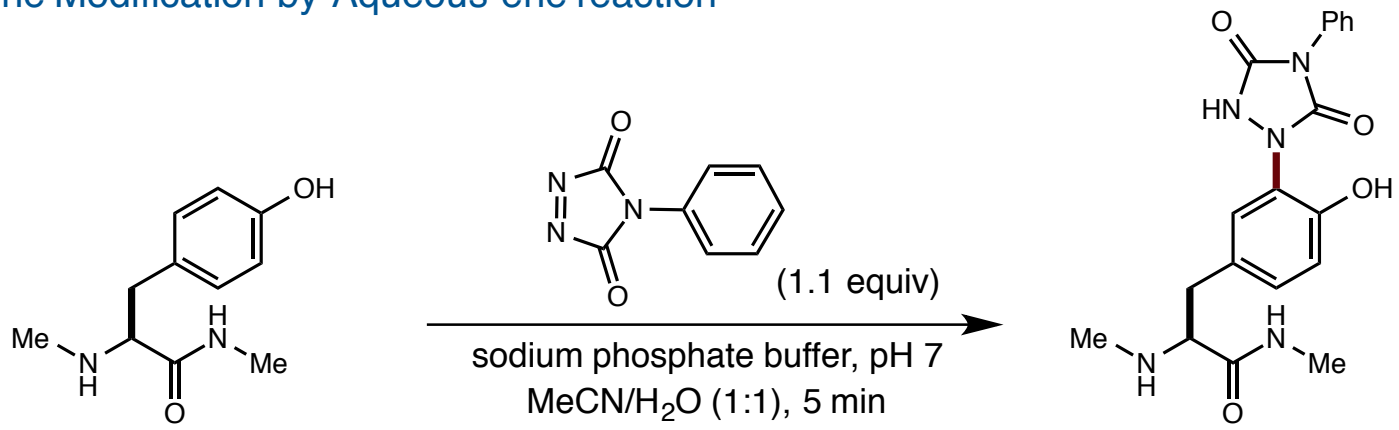
Competition experiments



Ban, H.; Gavrilyuk, J.; Barbas, C. F. III. *J. Am. Chem. Soc.* **2010**, *132*, 1523-1525.

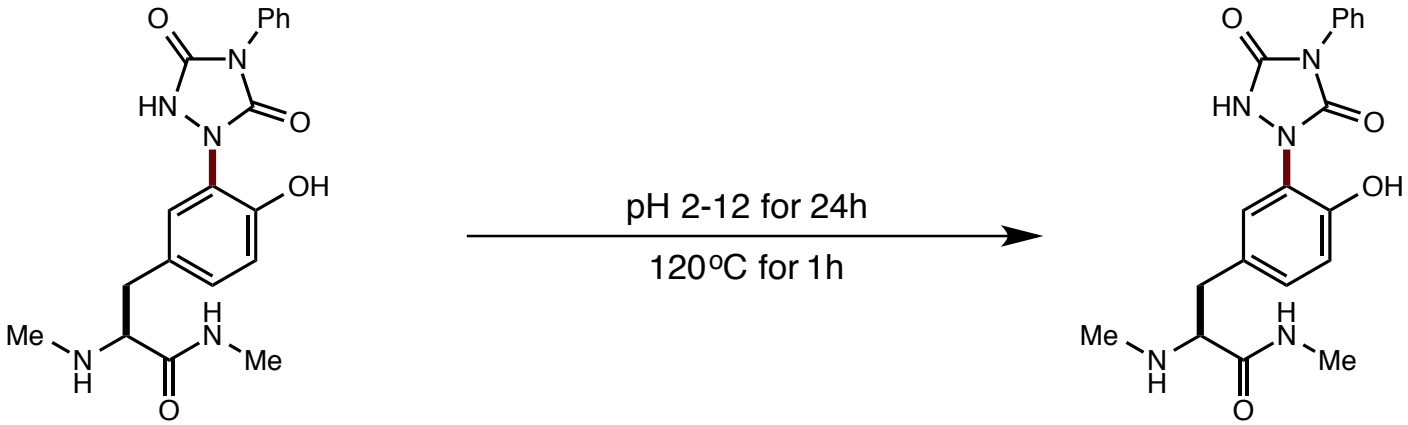
Click Reactions For Post-Translational Protein Modification

■ Tyrosine Modification by Aqueous-ene reaction



65%

quantitative with 3.3 equiv PTAD



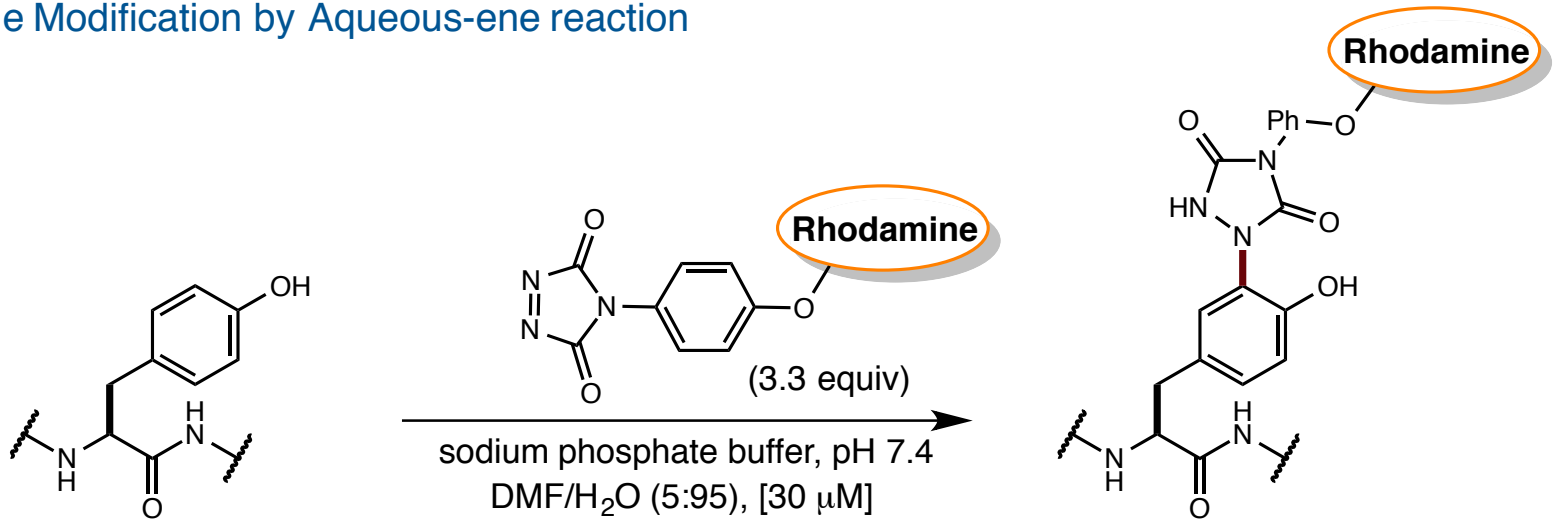
new ***C-N*** bond <u>stable</u> to pH and temperature condtions

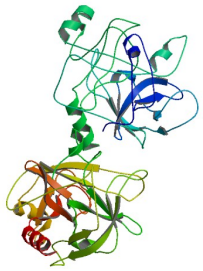
(*89 % recovered*)

Ban, H.; Gavrilyuk, J.; Barbas, C. F. III. *J. Am. Chem. Soc.* **2010**, *132*, 1523-1525.

Click Reactions For Post-Translational Protein Modification

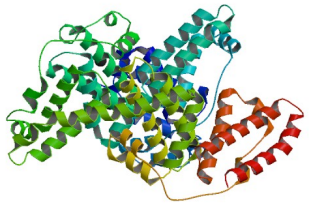
■ Tyrosine Modification by Aqueous-ene reaction





Chymotrypsinogen A
(81%)
(3 Tyr)

Myoglobin
(8 %)
(2 Tyr)



Bovine Serum Albumin
(96 %)
(21 Tyr)

Ban, H.; Gavrilyuk, J.; Barbas, C. F. III. *J. Am. Chem. Soc.* **2010**, *132*, 1523-1525.

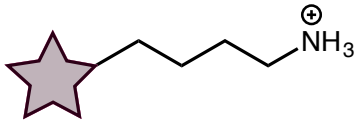
Click Reactions For Post-Translational Protein Modification

■ Which residues do we target for Click and How?

Cysteine

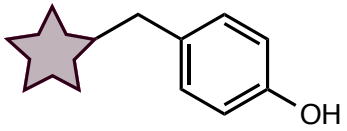
- uncommon residue
- nucleophilic
- good ligand for metals

Lysine



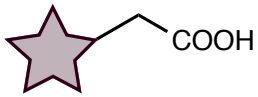
- most abundant residue
- strong nucleophile (as free amine)
-vast literature for reactions of amines

Tyrosine



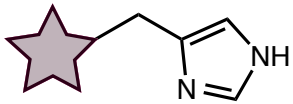
- activated for EAS reactions

Aspartic or Glutamic acid



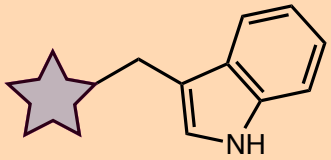
- prevalence
of coupling reactions in biology

Histidine



- good ligand for metals
- prone to acylation

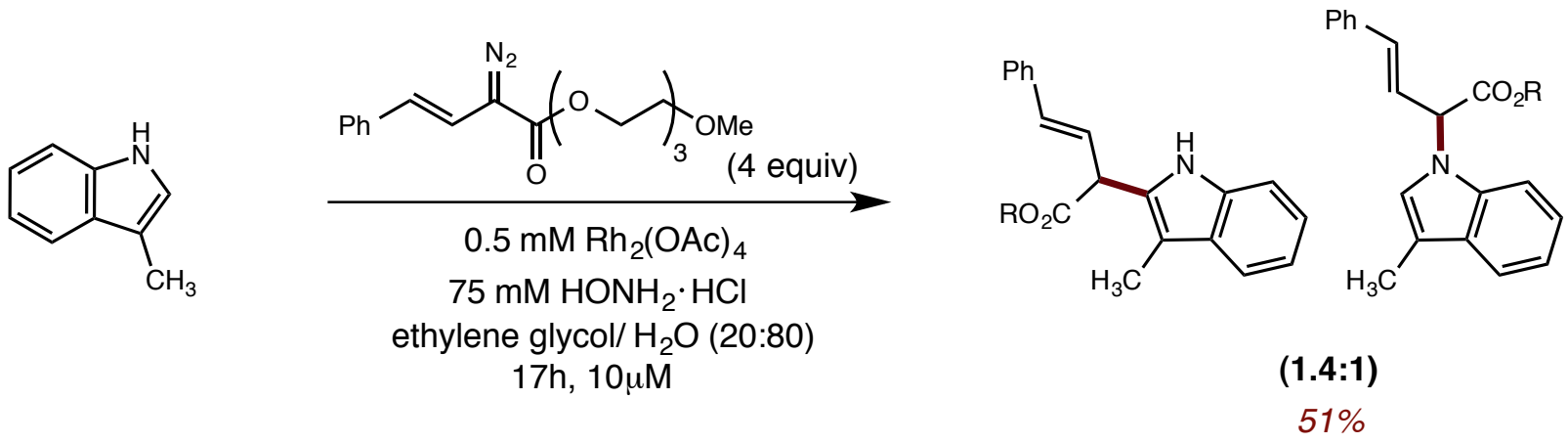
Tryptophan



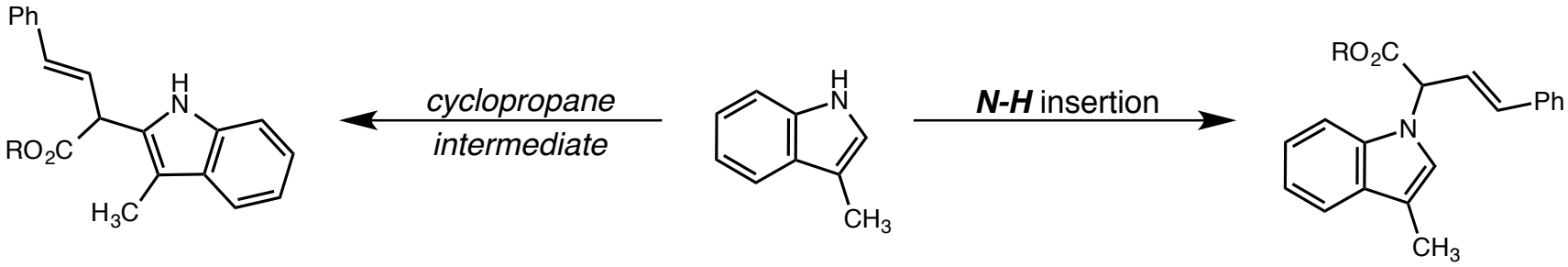
- electrophilic addition to C3

Click Reactions For Post-Translational Protein Modification

■ Tryptophan Modification



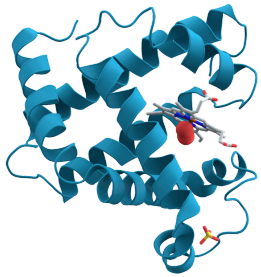
Possible Reaction pathways



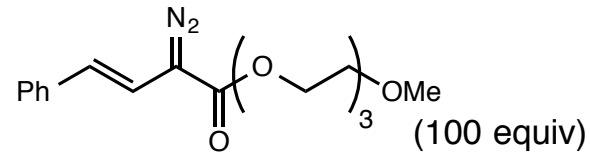
Antos, J. M.; Francis, M. B. *J. Am. Chem. Soc.* **2004**, *126*, 10256-10257.

Click Reactions For Post-Translational Protein Modification

■ Tryptophan Modification

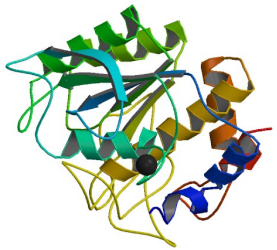


Myoglobin (100 μM)
(2 Trp)

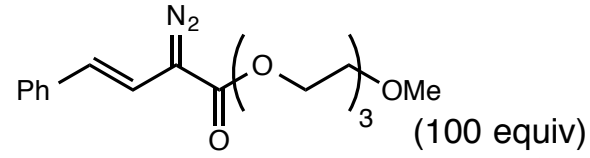


$Rh_2(OAc)_4$ (1.0 equiv)
75 mM $HONH_2 \cdot HCl$
ethylene glycol/ H_2O (20:80)
7h, pH 3.5

Tryptophan Modification products



subtilisin Carlsberg
(100 μM)
(one Trp residue)



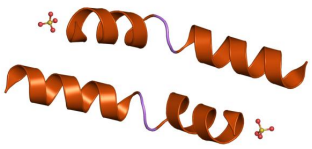
$Rh_2(OAc)_4$ (1.0 equiv)
75 mM $HONH_2 \cdot HCl$
ethylene glycol/ H_2O (20:80)
7h, pH 1.5

Tryptophan Modification product

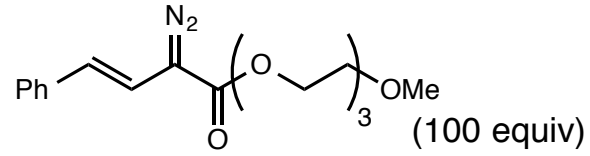
Antos, J. M.; Francis, M. B. *J. Am. Chem. Soc.* **2004**, *126*, 10256-10257.

Click Reactions For Post-Translational Protein Modification

■ Tryptophan Modification

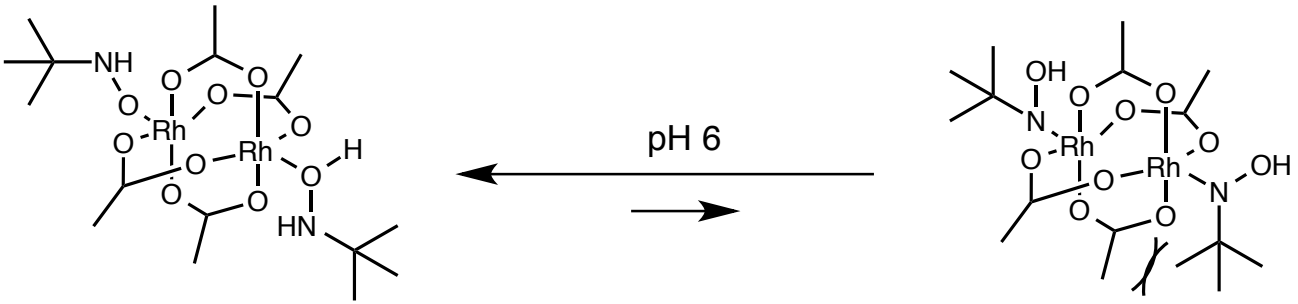


Melittin (100 μM)
(1 Trp)



100 μM $Rh_2(OAc)_4$
75 mM *t*BuNHOH
ethylene glycol/ H_2O (20:80)
7h, pH 6-7

Tryptophan Modification
products



O-bound
Favored

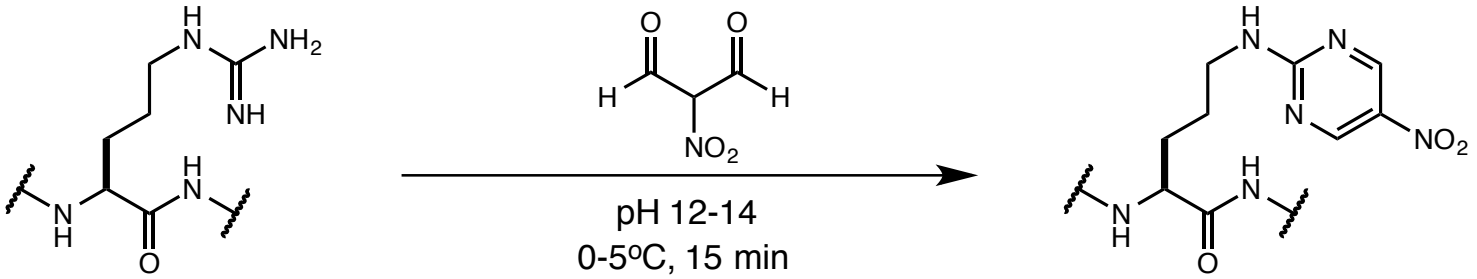
N-bound
Disfavored

*t*BuNHOH promotes carbenoid formation at higher pH 3-9

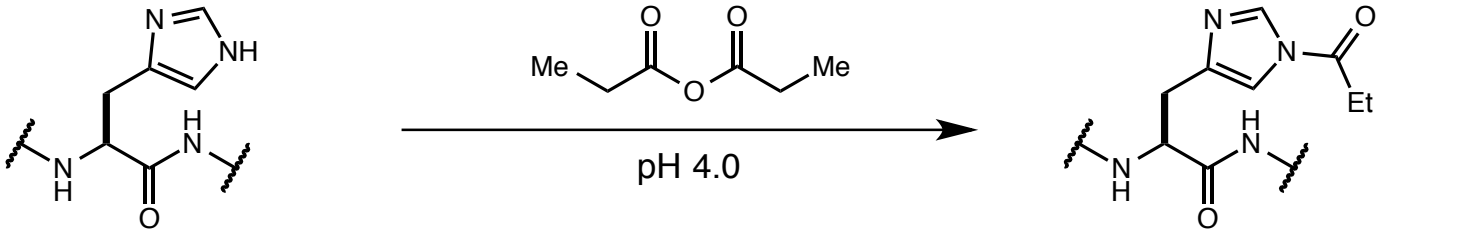
Antos, J. M.; McFarland, J. M.; Lavarone, A. T.; Francis, M. B. *J. Am. Chem. Soc.* **2009**, *131*, 6301-6308.

Click Reactions For Post-Translational Protein Modification

■ Modification of other Residues



Arg sidechain



His sidechain

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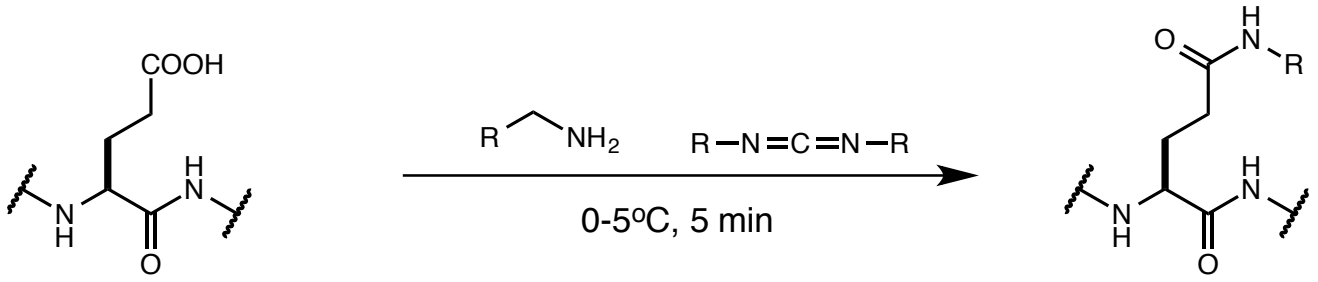
■ Modification of other Residues



Glu sidechain

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Concluding Remarks

- Direct site-selective modification of proteins has evolved over the last 20 years

- Direct modification can be a powerful tool with widespread pharmaceutical and industrial applications

- Research to expand the number of available reactions and surface reidues which can be modified is needed

Questions