Bioorthogonal Chemistry

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February 13, 2013

Wednesday Literature Talk

Outline

- What is It and Why Do We Care?
- Historical Background
- Staudinger Ligation
- Copper-free Click Chemistry
- Tetrazine Cycloadditions
- Other Examples
- Future Directions

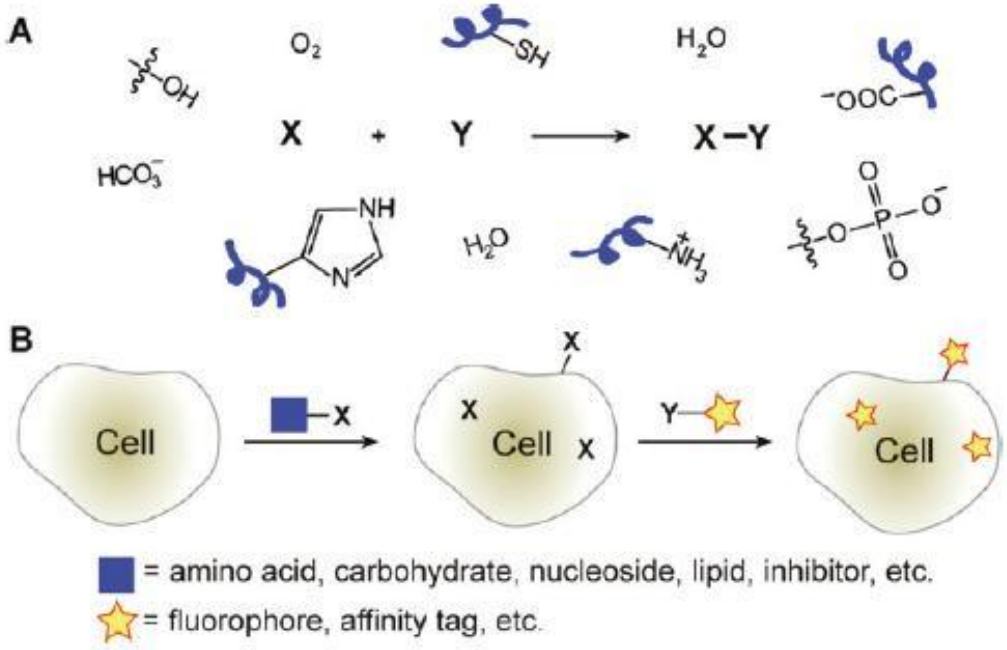
What Are We Talking About Here?

- “But what if the challenge [of synthesis] were inverted, wherein the target structure was relatively simple but the environment in which the necessary reactions must proceed was so chemically complex and uncontrollable that no two functional groups could combine reliably and selectively under such cond[illegible]lyn Bertozzi, UC Berkley

Acc. Chem Res., **2011**, *44*, 651.

What Is It?

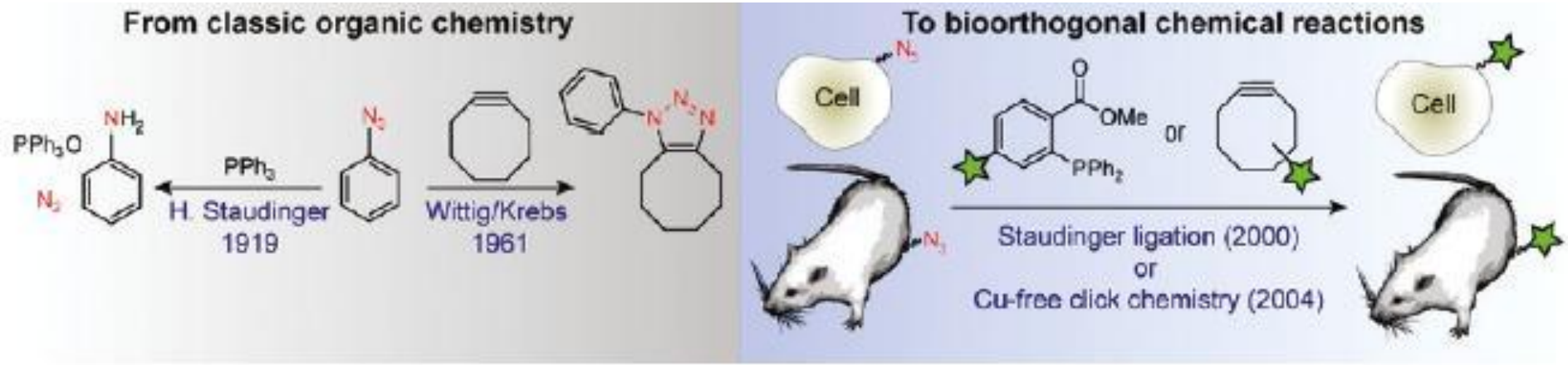
- **Bioorthogonal chemistry**– chemical reactions that neither interact with nor interfere with a biological system.



Acc. Chem. Res., **2011**, *44*, 666.

So Why Do We Care?

- Takes classic organic reactions and redesigns them with biological systems in mind
- Allows for more efficient/ non-toxic drug delivery, biological imaging, and material science



Requirements of Bioorthogonality

1. Functional groups used must be inert to biological moieties
2. FG’s must be selective for one another and nontoxic to organisms
3. Reaction must work in biological media
4. Must have very fast kinetics, particularly at low concentrations and in physiological condtions ($k_2 > 10^{-4}$ $M^{-1}s^{-1}$)
5. Helpful, s small

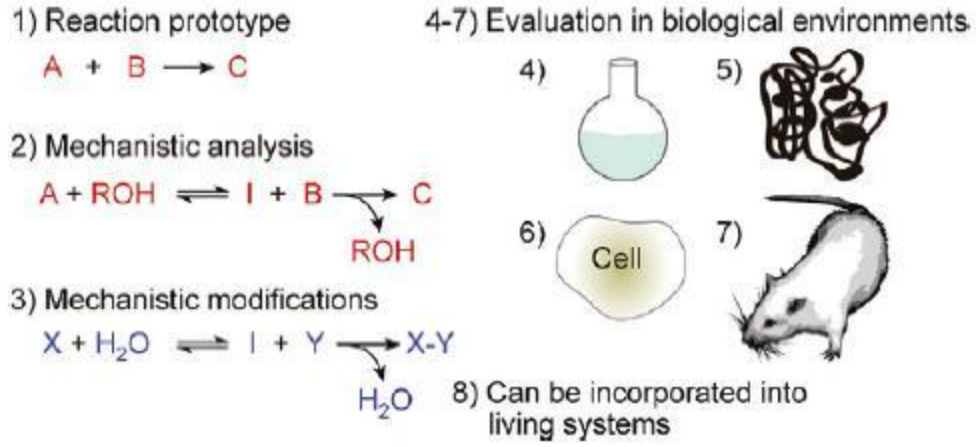


FIGURE 3. A step-by-step guide to developing a bioorthogonal reaction.

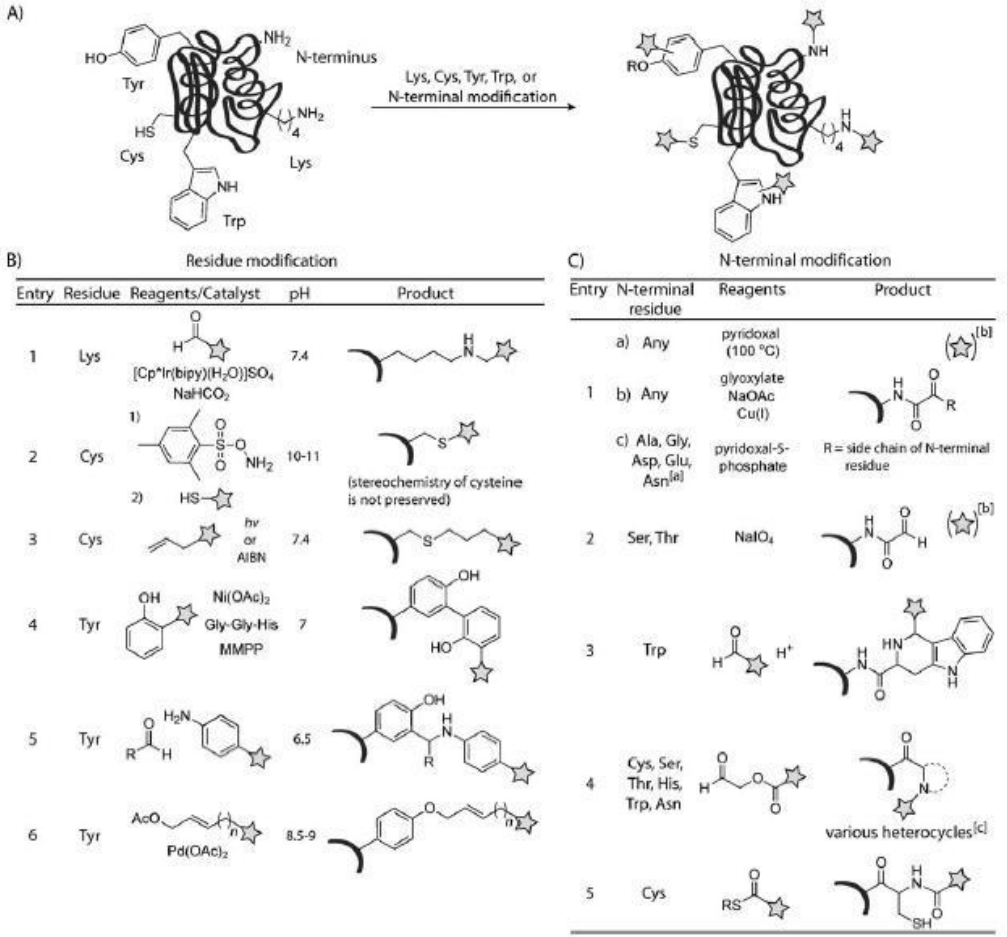
Types of Bioorthogonal Transformations*

1. Nucleophilic Additions
2. 1,3-Dipolar Cycloadditions
3. Diels-Alder Reactions
4. Metal-Catalyzed Couplings
5. [2+2+2] Cycloadditions

*Published to date

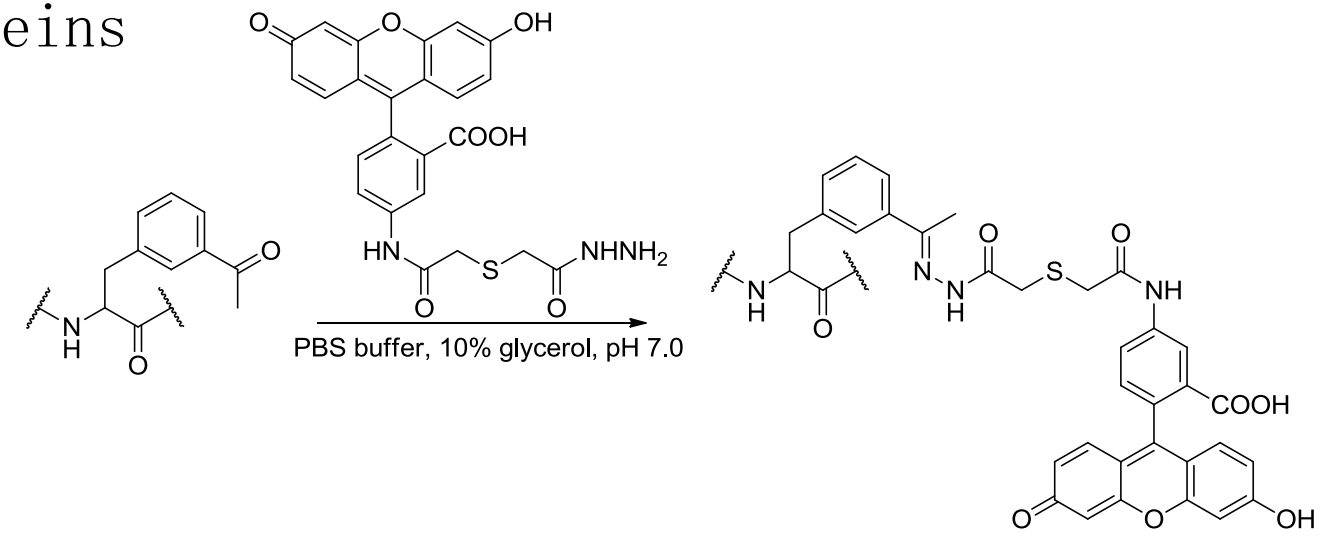
Historical Background

- Bioconjugation generally involves modification of amino acid residues to modify a protein in some wa
- Cysteine and lysine are mos commonly modified due to terminal thiol or amine group, respectively
- Used mostly to attach fluorophores or to immobilize proteins on a surface
- Most reactions not possible for *in vivo*

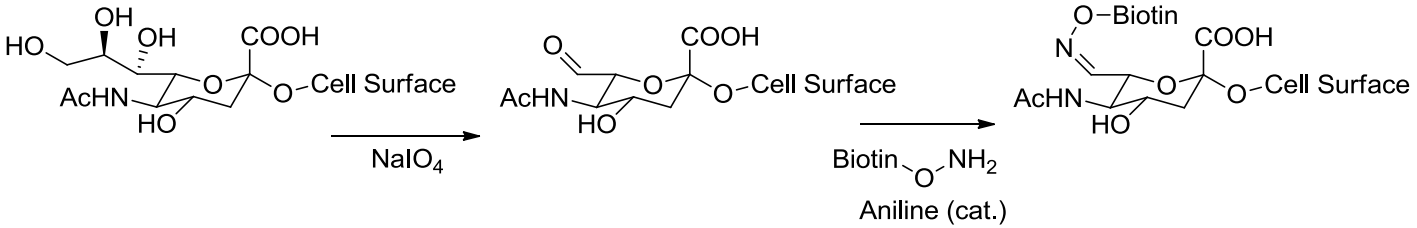


Condensation of Carbonyls with Amines

- Schultz showed succesful intercellular labeling of proteins



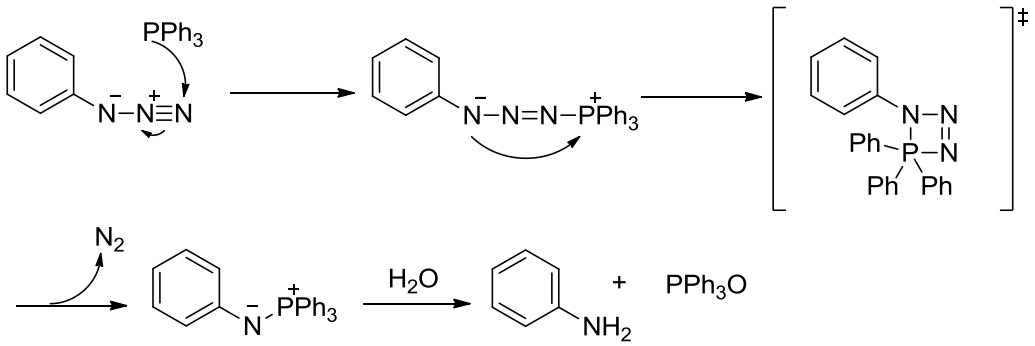
- Paulson has made improvements recently to compensate for prior shortcomings



Biochemistry, **2003**, *42*, 6735.

Staudinger Reduction

- In 1919 Staudinger showed that azides could be reduced with PPh_3 and water

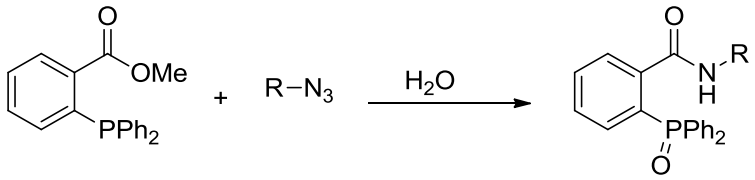


- Very mild method to reduce many azides
- Trapping of product prior to hydrolysis could lead to coupled product

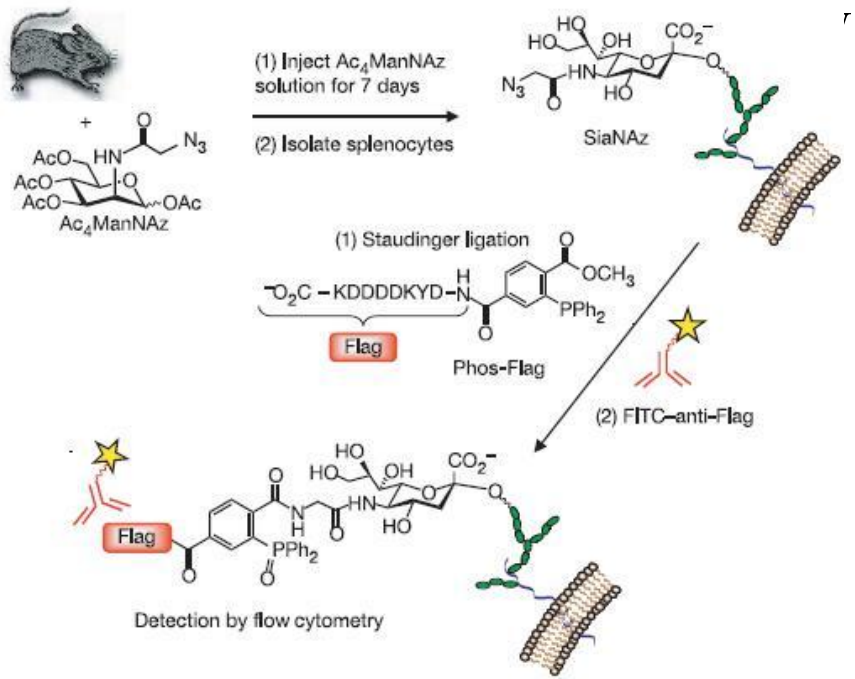
Helv. Chim. Acta., **1919**, *2*, 635.

Staudinger Ligation

- Bertozzi modified classic reaction to appease biological demands



- First example of [illegible] taking place *in vivo*



Science, **2000**, *287*, 2007.
Nature, **2004**, *430*, 873.

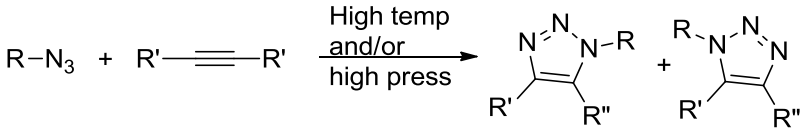
Staudinger Ligation

- Azide as bioorthogonal coupling partner was ground breaking
- Used for labeling proteins or sugars with fluorescent linkers for labeling disease or therapeutic targeting
- Advantages: Small azide, very selective
- Disadvantages: Slow kinetics (k_2 = 0.002 $M^{-1}s^{-1}$) limits reactivity at low concentrations, phosphine reagents slowly oxidized in air

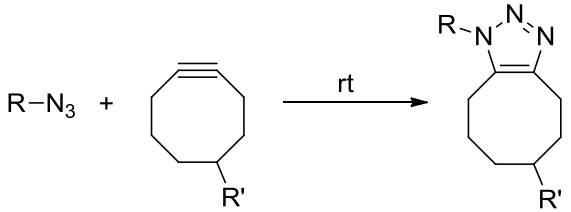
Acc. Chem. Res., **2011**, *44*, 666.

[3+2] Cycloadditions With Azides/Alkynes

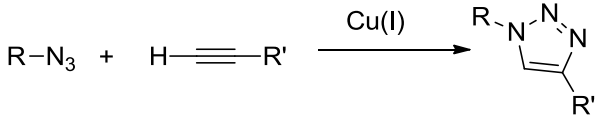
- Michael first reported cycloaddition in 1893, but Huisgen studied mechanisms and reaction rates in the 60s



- Wittig and Krebs reported strained cyclooctyne reacted like ‘an explosion’ with azide in 1961



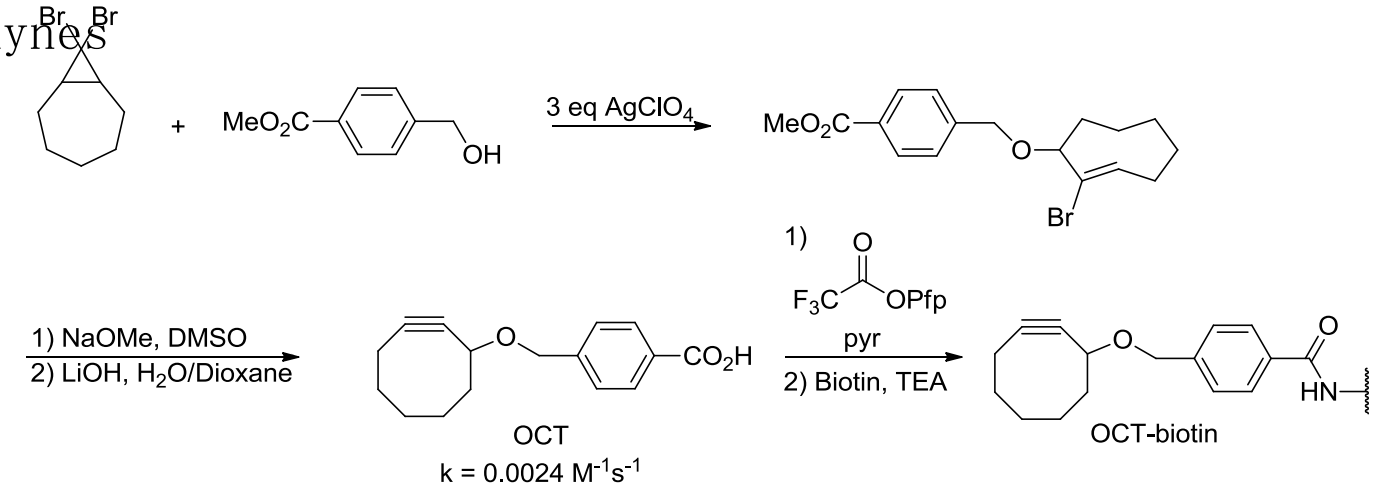
- Modern Cu catalyzed ‘click’ chemistry introduced by Sharpless and Meldal in 2002



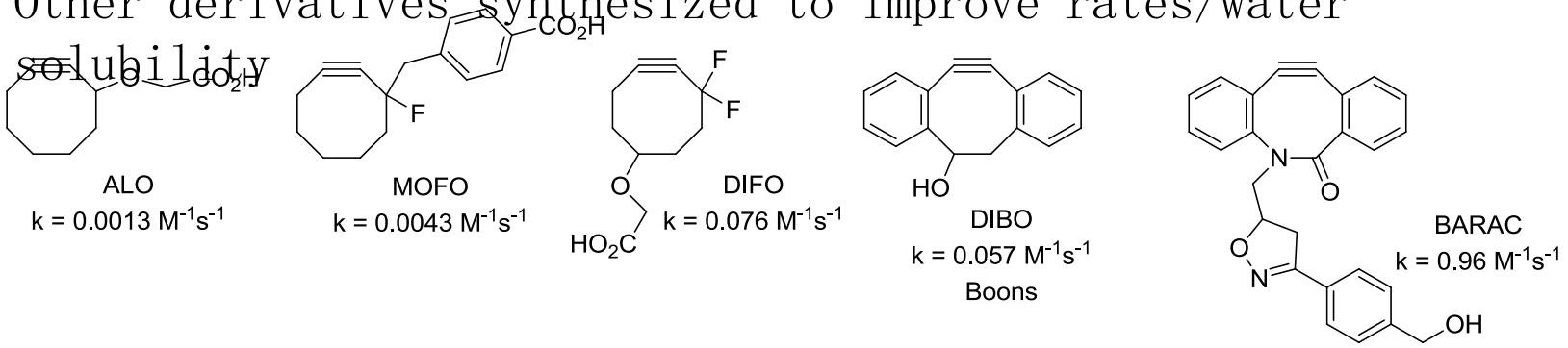
Chem. Ber., **1961**, *94*, 3260.
Angew. Chem. Int. Ed., **2002**, *41*, 2596.
J. Prakt. Chem., **1893**, *48*, 94.
Angew. Chem. Int. Ed. Engl., **1963**, *2*,

Copper-Free Click Chemistry

- Bertozzi developed Wittig-inspired strained (~18 kcal/mol) alkynes



- Other derivatives synthesized to improve rates/water solubility



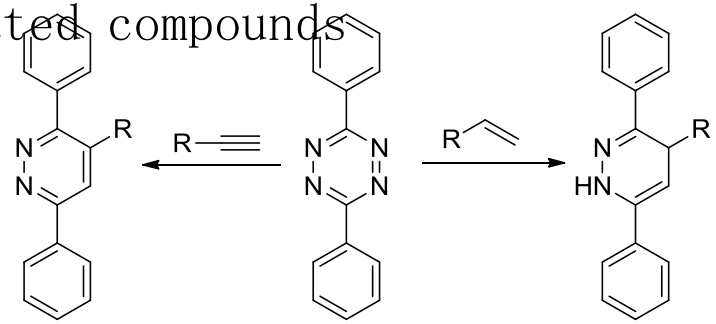
JACS, **2004**, *126*, 15046.
Acc. Chem. Res., **2011**, *44*,

Copper-Free Click Chemistry

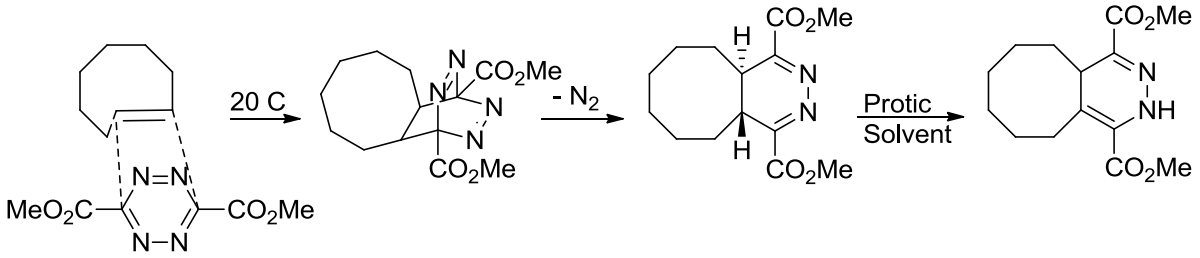
- Still most common bioorthogonal reaction used, due to wide variety of derivatives and many groups working on perfecting it
- Advantages: Small azide, much faster rates with modified alkynes
- Disadvantages: Starting materials can be difficult/costly to synthesize, rates still relatively low, esp for expensive coupling partners

Inverse-Demand Diels-Alder With Tetrazines

- Carboni and Lindsey first reported pyradazine/dihydropyrdazine synthesis from tetrazines and various unsaturated compounds



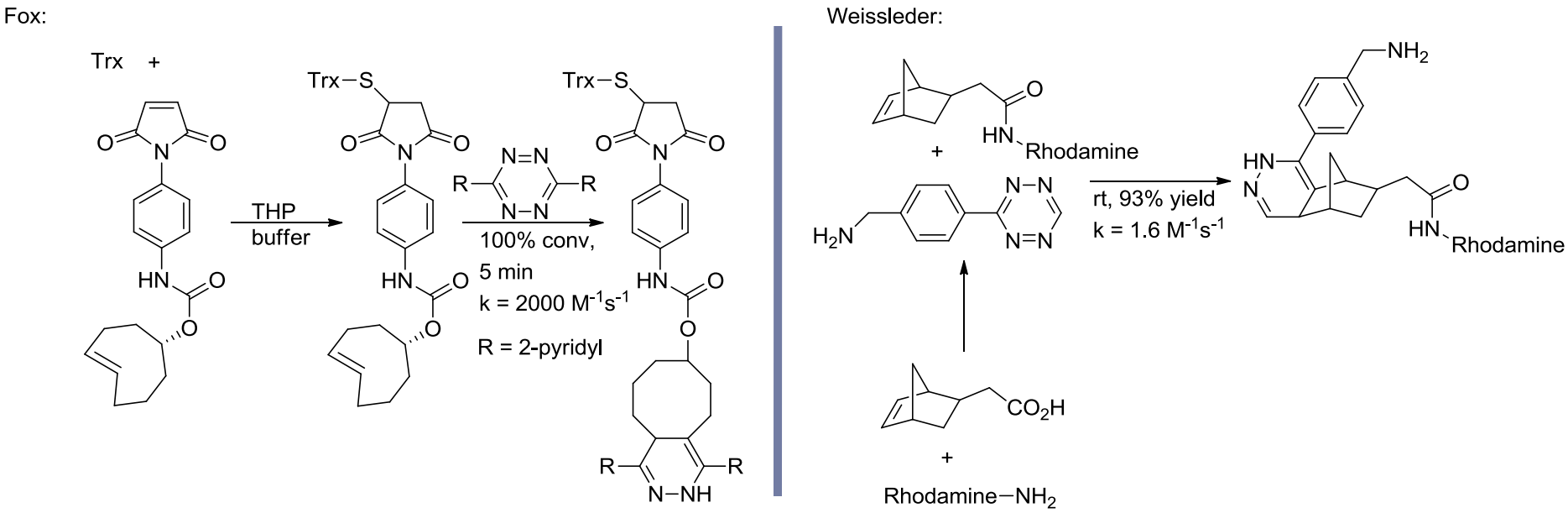
- Sauer described extremely fast reaction of electron deficient tetrazines with strained dienophiles



JACS, **1959**, *81*, 4342.
Tetrahedron Lett., **1990**, *31*,

Initial Biological Tetrazine Cycloadditions

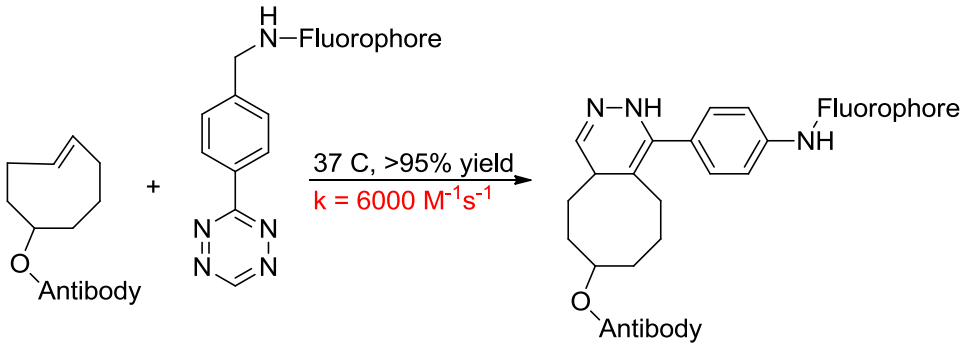
- Fox and Weissleder independently began study of this promising new reaction



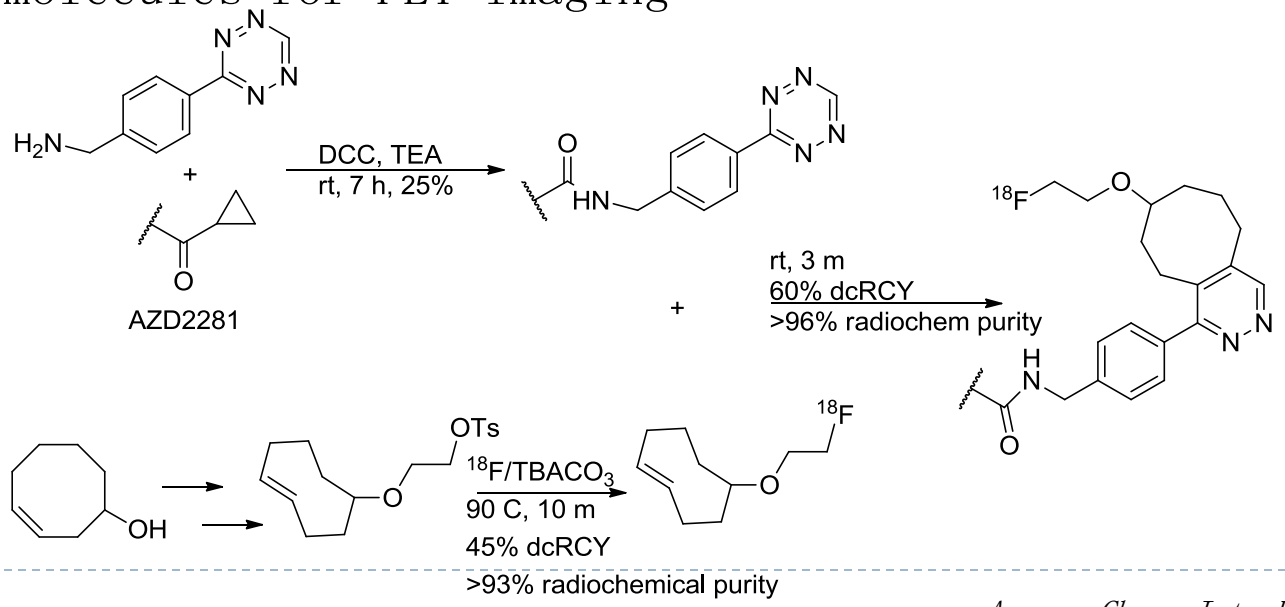
JACS, **2008**, *130*, 13518.
Bioconjugate Chem., **2008**, *19*,

Current Tetrazine Cycloadditions

- Weissleder combined the two strategies with amazing results



- Due to fast kinetics, this method is useful for ^{18}F labeling of biomolecules for PET imaging



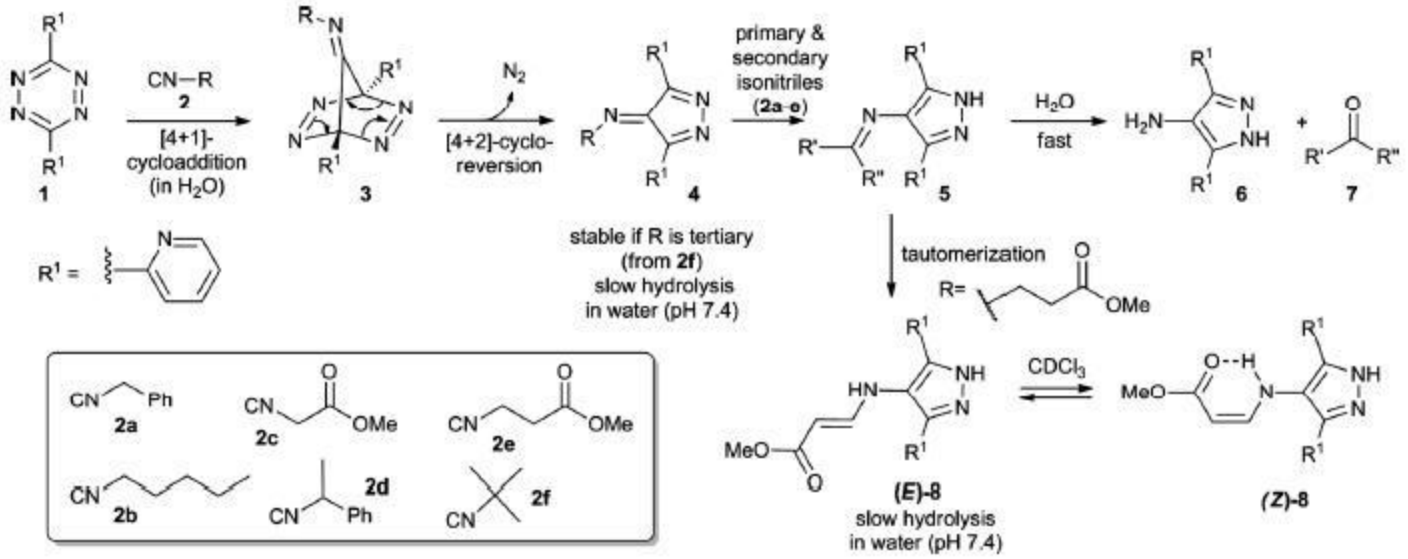
Angew. Chem. Int. Ed., **2009**, *48*, 7013.

Tetrazine Cycloadditions

- Many *in vitro* protein, glycan, and fatty acid labeling, PET imaging (due to fast kinetics), not many *in vivo* studies to date
- Advantages: Easy to synthesize starting materials, no catalyst, extremely fast reaction rates
- Disadvantages: Tetrazine derivatives are much larger than azides, not regio/stereoselective

[4+1] Cycloadditions With Isonitriles

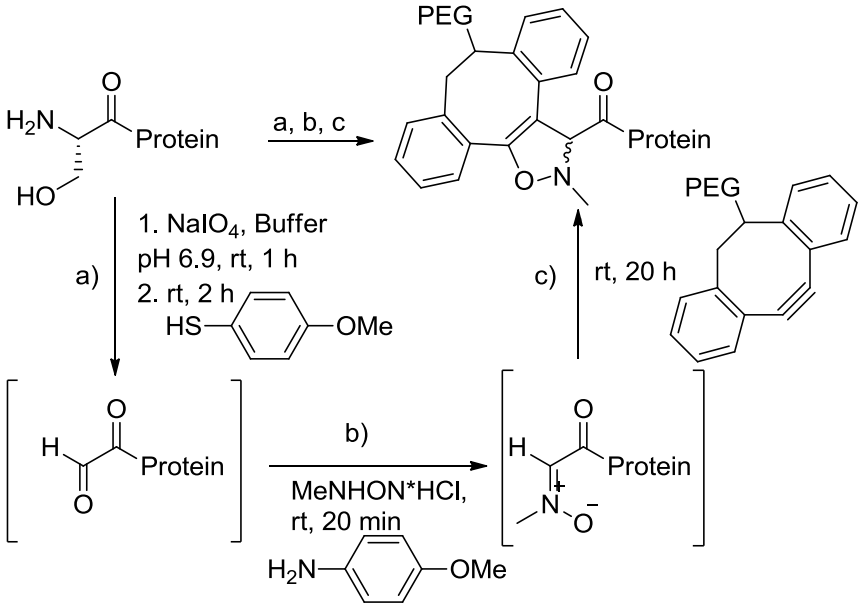
- Leeper developed ‘click’ analogue with isonitriles and tetrazines based on [4+1] reported by Seitz in 1982
- Fluorophore labeling of proteins
- Advantages: New type of bioorthogonal reaction
- Disadvantages: Only 3° or propanoate isonitriles tolerated, and probably not orthogonal to other reactions



Org. Biomol. Chem., **2011**, *9*, 7303.

Alkyne-Nitrone Cycloaddition (SPANC)

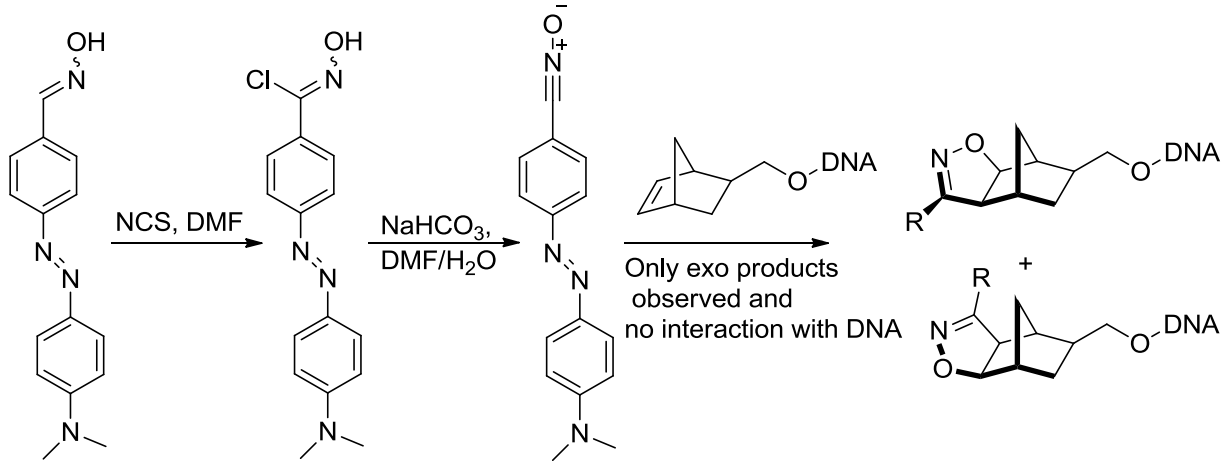
- Boons and van Delft augmented ‘Cu-free click’ to utilize nitrones, increasing rates of reaction
- Used for site specific protein labeling
- Advantages: Faster kinetics than azides, use of functionalized nitrones allows for diverse products
- Disadvantages: Necessary addition of stabilizing additives not ideal for *in vivo* applications, few examples of use to date



Angew. Chem. Int. Ed., **2010**, *49*, 3065.

Norbornene 1,3-Dipolar Cycloaddition With Nitrile Oxides

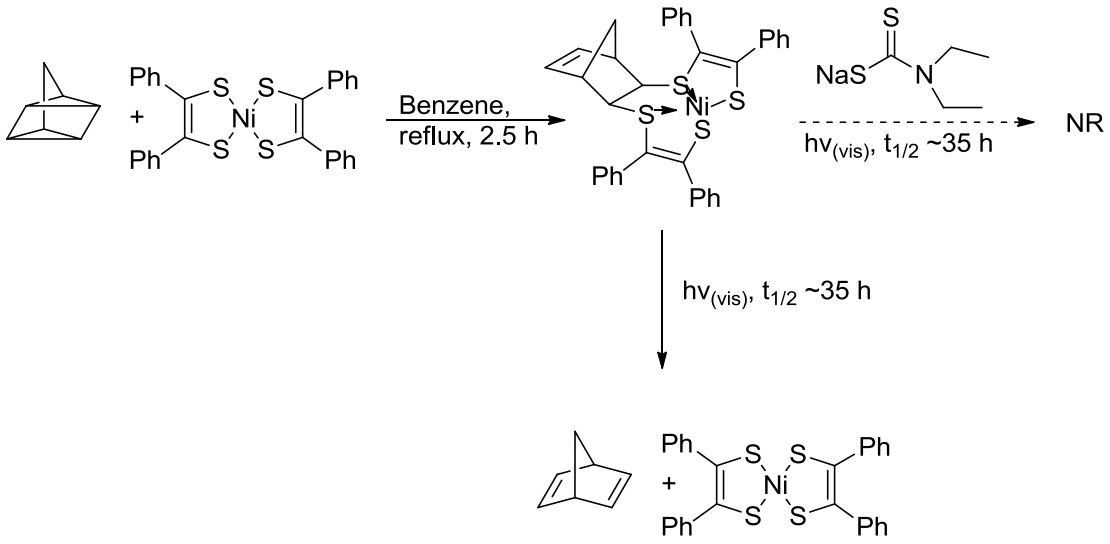
- Carell developed ‘click’ modifcation based on Huisgen’s work with nitrile oxides.
- Only shown to be used for labeling DNA with biomarkers
- Advantages: No interaction with DNA, no need for large excess of marker, orthogonal to Cu-free ‘click’
- Disadvantages: Few applications, attaching norbornene to DNA is difficult



Org. Lett., **2009**, *11*, 2405.

[2+2+2] Cycloadditions

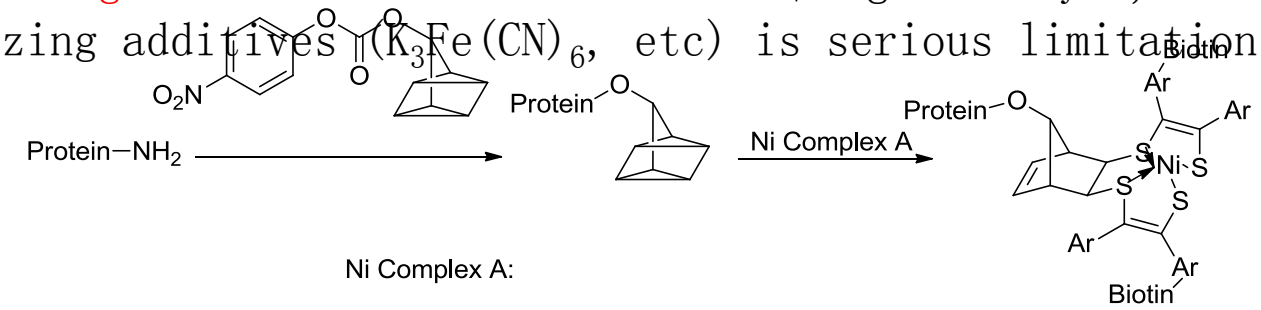
- Quadricyclane has ~80 kcal/mol of ring strain, which allows for cycloaddition with electron deficient π systems fairly easily
- 1986- Sugimori reported a Ni bis(dithiolene) complex that reacted rapidly



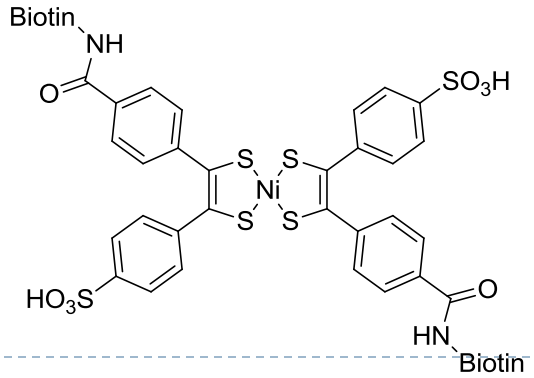
Chem. Lett., 1986, 2109.

Quadricyclane Ligation

- Bertozzi developed usable version of Sugimori's reaction for bio conditions
- Used for *in vitro* protein labeling only so far
- Advantages: New reaction orthogonal to known ones with comparable kinetics (k_2 = .25 $M^{-1}s^{-1}$), small and nontoxic coupling partners
- Disadvantages: Not useful in live cells/organisms yet, need for stablizing additives ($K_3Fe(CN)_6$, etc) is serious limitation



Ni Complex A:



JACS, **2011**, *133*, 17570.

Future Directions

- The perfect bioorthogonal reaction has not yet been developed
- Need for more reactions that can be orthogonal to one another
- Group 15 has been huge, so perhaps Sb or Bi could work as well
- Perhaps phosphorus, sulfur chemistry could be beneficial
- Use of ultrasound and other biocompatible energy forms to increase reaction rates

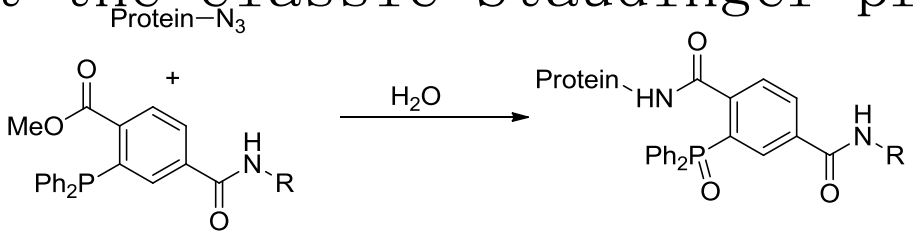
Angew. Chem. Int. Ed., **2009**, *48*, 6974.

Questions?

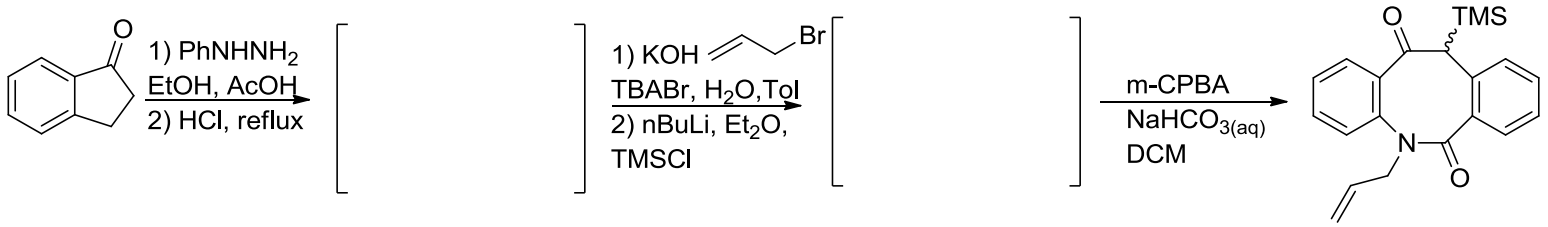
- Thank your for your attention!

Volunteers?!?!?!?!

- Question 1: Show mechanism and explain why you don't get the classic Staudinger product.



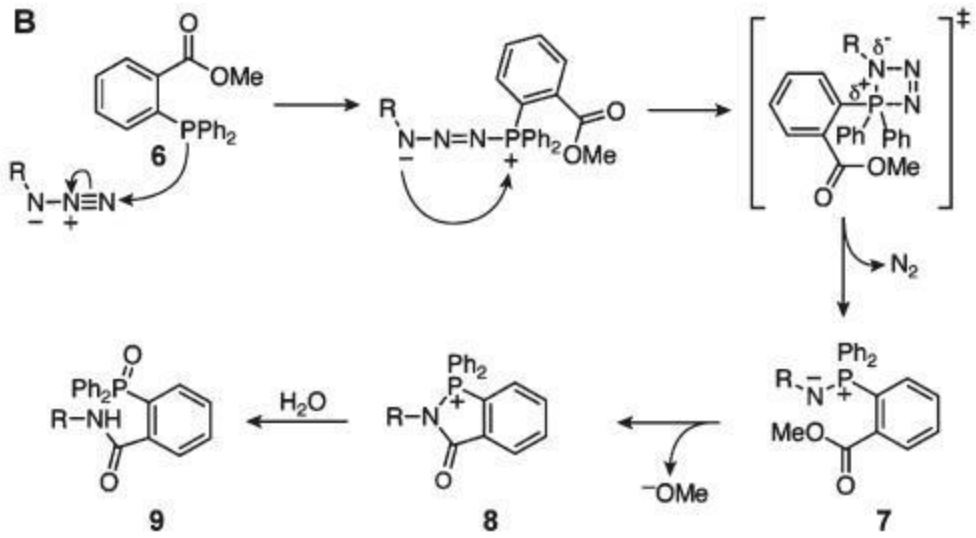
- Question 2: Predict products:



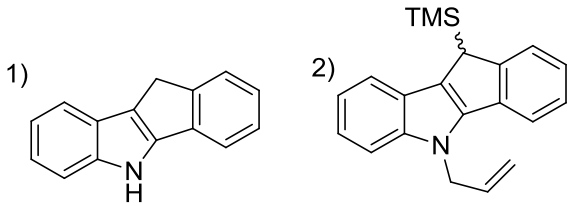
- Question 3:

Answers!!!

Question 1:



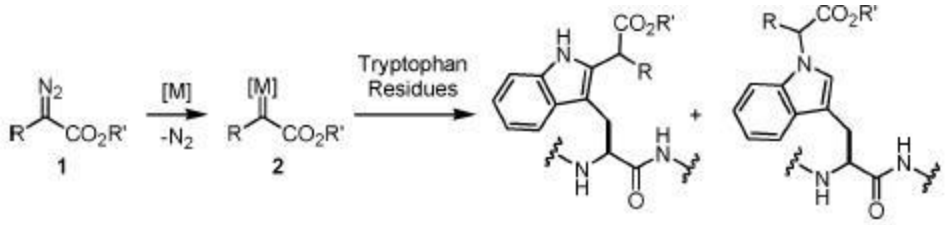
Question 2:



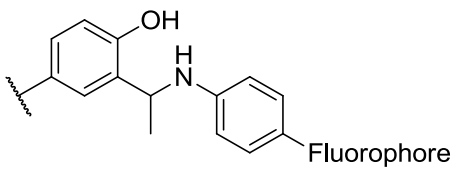
Answers!!!

Question 3:

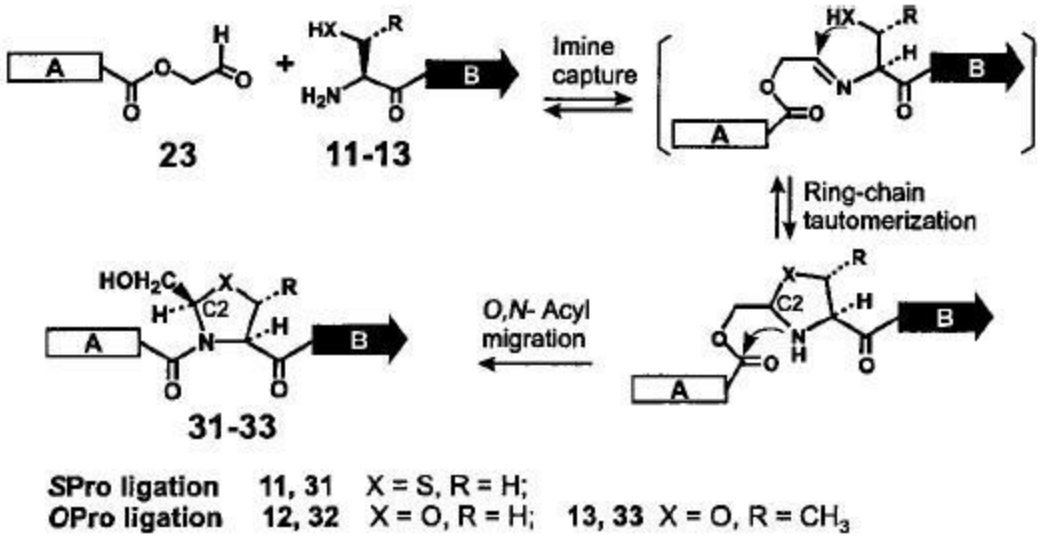
a)



b)



c)



SPro ligation **11, 31** X = S, R = H;
OPro ligation **12, 32** X = O, R = H; **13, 33** X = O, R = CH_3