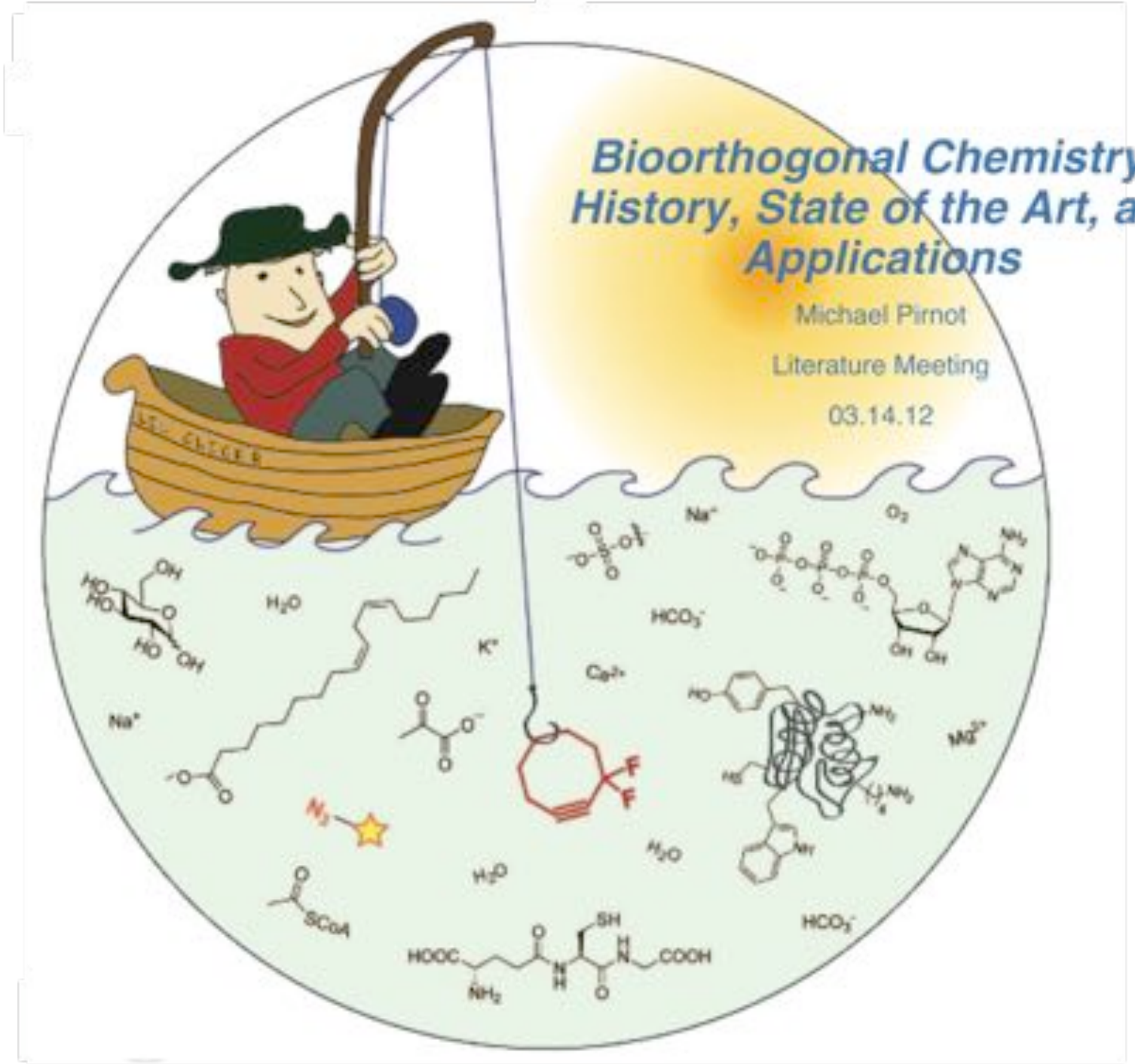

Bioorthogonal Chemistry - History, State of the Art, and Applications
Michael Pirnot
Literature Meeting
03.14.12

The Living Cell

Living cells are a complex network of interacting biopolymers, ions, and metabolites.

Complex cellular processes cannot be observed when the components are in their purified, isolated forms.

Thus, we are forced outside of the artificial confines of a test tube and into the dynamic living cell.

There is a burgeoning interest in developing methods where a reporter tag can be attached to a cellular component for aid in visualization and/or isolation.

Prescher, J. A.; Bertozzi, C. R. *Nat. Chem. Biol.* **2005**, *1*, 13-21.

David Goodshell, artist and biologist

Bioconjugation Chemistry

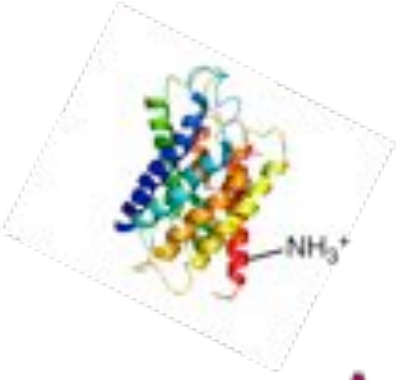
Bioconjugation chemistry - a selective coupling of two biomolecules together in a covalent linkage

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

Sletten, E. M.; Bertozzi, C. R. *Angew. Chem. Int. Ed.* **2009**, *48*, 6974-6998.

Bioorthogonal Chemistry

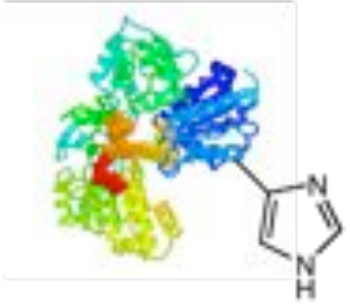
Bioorthogonal chemistry - a chemical reaction that can occur inside living systems without interfering with native biochemical processes



H_2O

A **B** ⟶ **A-B** O_2

HCO_3^-



Sletten, E. M.; Bertozzi, C. R. *Angew. Chem. Int. Ed.* **2009**, *48*, 6974-6998.

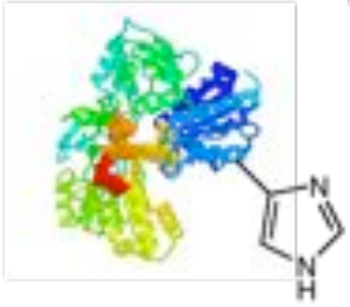
Bioorthogonal Chemistry

Bioorthogonal chemistry - a chemical reaction that can occur inside living systems without interfering with native biochemical processes

B

H_2O

HCO_3^-



O_2

Sletten, E. M.; Bertozzi, C. R. *Angew. Chem. Int. Ed.* **2009**, *48*, 6974-6998.

Bioorthogonal Chemistry - Requirements

water as the solvent

ambient temperature

non-toxic reagents

physiological pH

reaction product must be stable

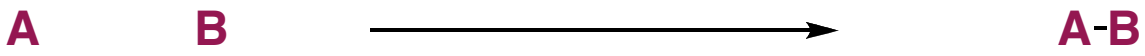
no cross-reactivity

cell-permeable reagents

fast reaction kinetics

Sletten, E. M.; Bertozzi, C. R. *Angew. Chem. Int. Ed.* **2009**, *48*, 6974-6998.

A Brief Consideration of Reaction Kinetics



most bioorthogonal reactions are bimolecular in nature with second-order rate constants

[product] is approximately $k_2[A]_0[B]_0t$ k_2 is typically anywhere from 10^{-3} to 10^3 $M^{-1}s^{-1}$

$k_2 = 2.3\ M^{-1}s^{-1}$, [A] = [B] = 1 μM, time = 1 hr

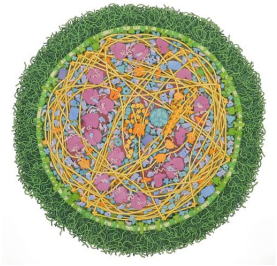
~ 0.8 % labeling of product

Sletten, E. M.; Bertozzi, C. R. *Angew. Chem. Int. Ed.* **2009**, *48*, 6974-6998.

Lim, R. K. V.;Lin, Q. *Chem. Commun.* **2010**, *46*, 1589-1600.

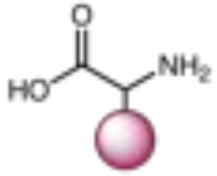
Incorporation of Unnatural Amino Acids

unnatural amino acid natural amino acid X

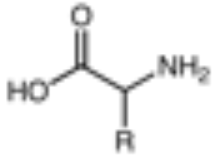


+

+



-



cell is auxotrophic for amino acid X

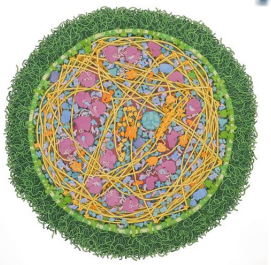
gene for aaRS (aminoacyl tRNA synthetase)

global replacement of X with unnatural amino acid

Link, A. J.; Mock, D. A.; Tirrell, D. A. *Curr. Opin. Biotechnol.* **2003**, *14*, 603.

Cowie, D. B.; Cohen, G. N. *Biochim. Biophys. Acta* **1957**, *26*, 252.

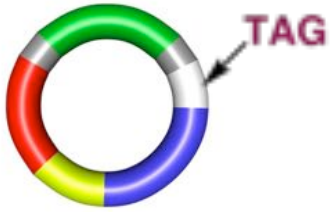
Incorporation of Unnatural Amino Acids



cell

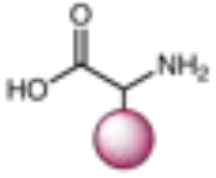
gene for mutant aaRS

gene for tRNA



gene for protein

site-specific incorporation
of unnatural amino acid



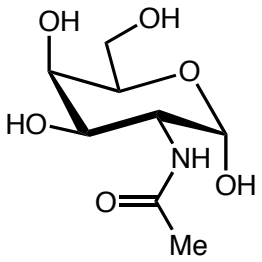
unnatural amino acid

Wang, L.; Schultz, P. G. *Angew. Chem. Int. Ed.* **2005**, *44*, 43.

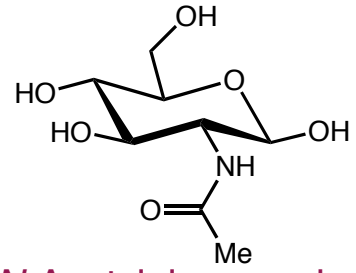
Noren, C. J.; Anthony-Cahill, S. J.; Griffith, M. C.; Schultz, P. G. *Science* **1989**, *244*, 182.

Bain, J. D.; Glabe, C. G.; Dix, T. A.; Chamberlin, A. R.; Diala, E. S. *J. Am. Chem. Soc.* **1989**, *111*, 8013.

Biosynthesis of mucin-type O-linked glycoproteins



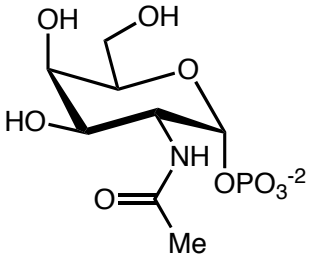
N-Acetylegalactosamine
GalNAc



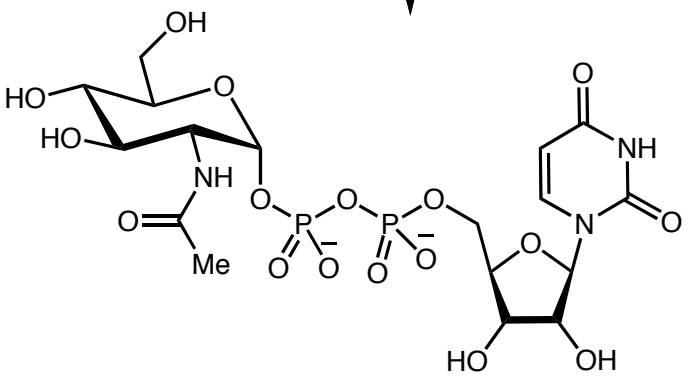
N-Acetylglucosamine
GlcNAc

GalNAc
1-kinase

GlcNAc
salvage
pathway



GalNAc-1-P



UDP-GlcNAc

Hang, H. C.; Yu, C.; Kato, D.; Bertozzi, C. R. *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 14846-14851.

Dube, D. H.; Prescher, J. A.; Quang, C. N.; Bertozzi, C. R. *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 4819-4824.

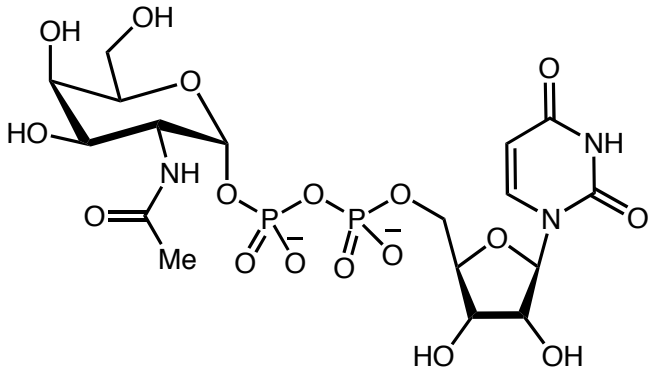
Biosynthesis of mucin-type O-linked glycoproteins

GalNAc-1-P

UDP-GlcNAc

UDP-GalNAc
pyrophosphorylase

UDP-Glc/GlcNAc
C_4-epimerase



UDP-GalNAc

Hang, H. C.; Yu, C.; Kato, D.; Bertozzi, C. R. *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 14846-14851.

Dube, D. H.; Prescher, J. A.; Quang, C. N.; Bertozzi, C. R. *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 4819-4824.

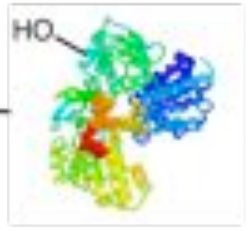
Biosynthesis of mucin-type O-linked glycoproteins

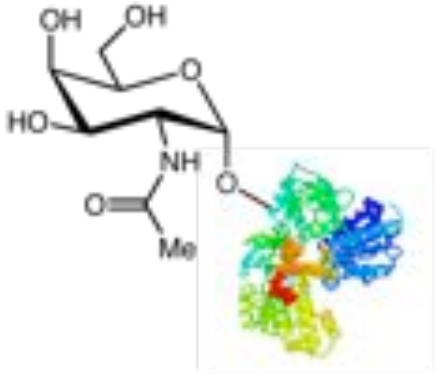
UDP-GalNAc

UDP-GalNAc transporter

UDP-GalNAc

N-acetylgalactosaminyltransferases (ppGalNAcTs)





UDP +

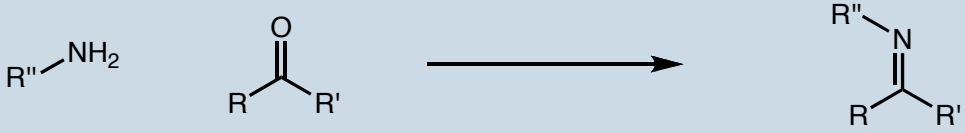
Golgi Lumen

Hang, H. C.; Yu, C.; Kato, D.; Bertozzi, C. R. *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 14846-14851.

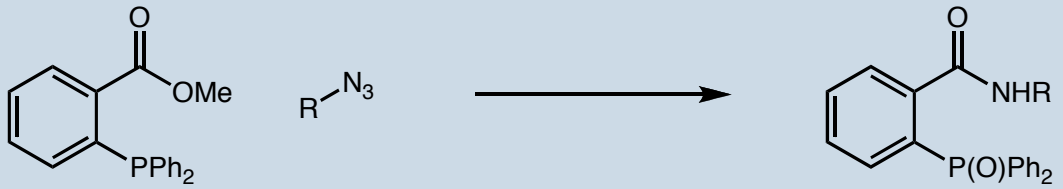
Dube, D. H.; Prescher, J. A.; Quang, C. N.; Bertozzi, C. R. *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 4819-4824.

Bioorthogonal Chemistry

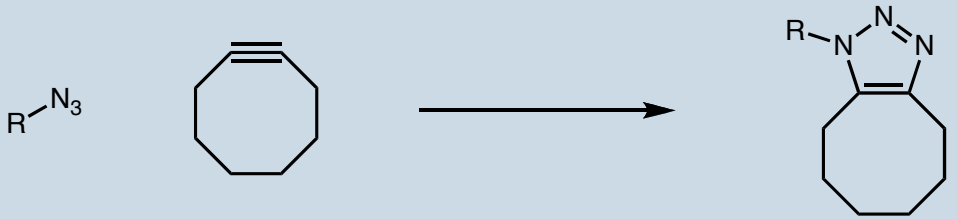
condensation chemistry



Staudinger ligation



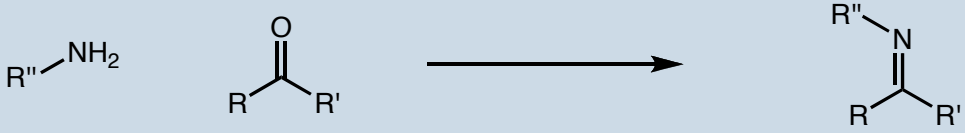
[3+2] cycloadditions



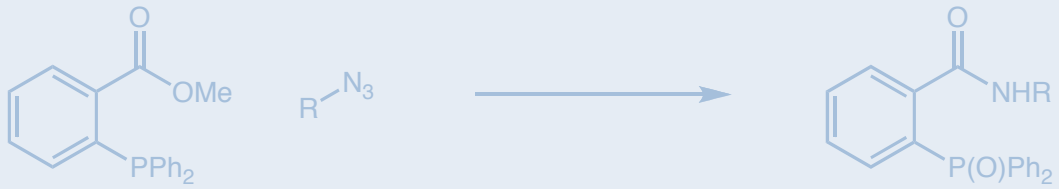
olefin chemistry

Bioorthogonal Chemistry

condensation chemistry

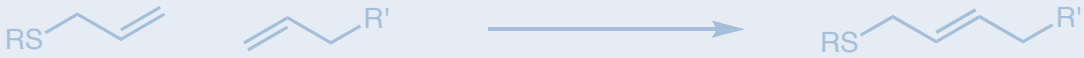


Staudinger ligation

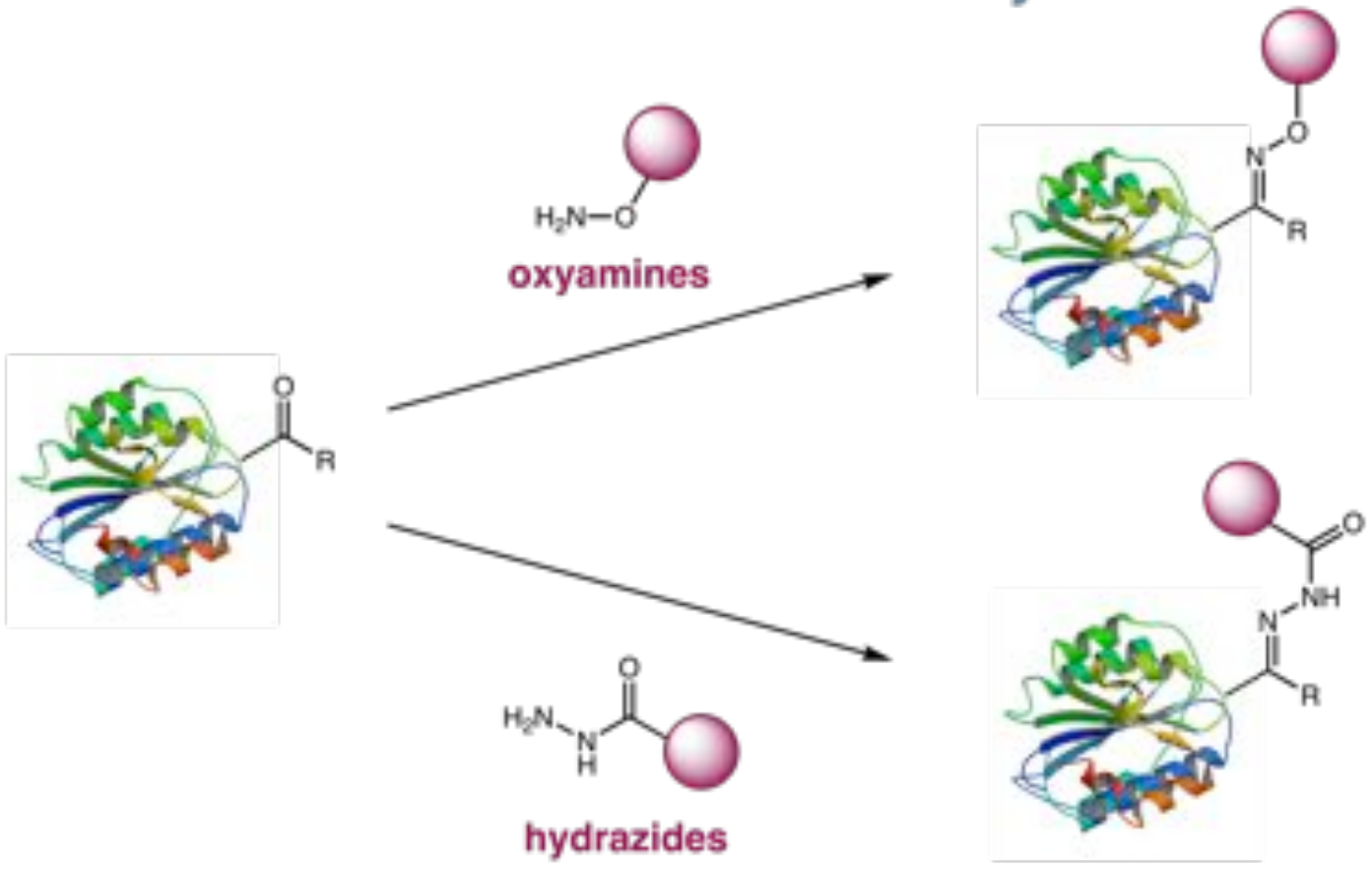


[3+2] cycloadditions

olefin chemistry



Condensation Chemistry

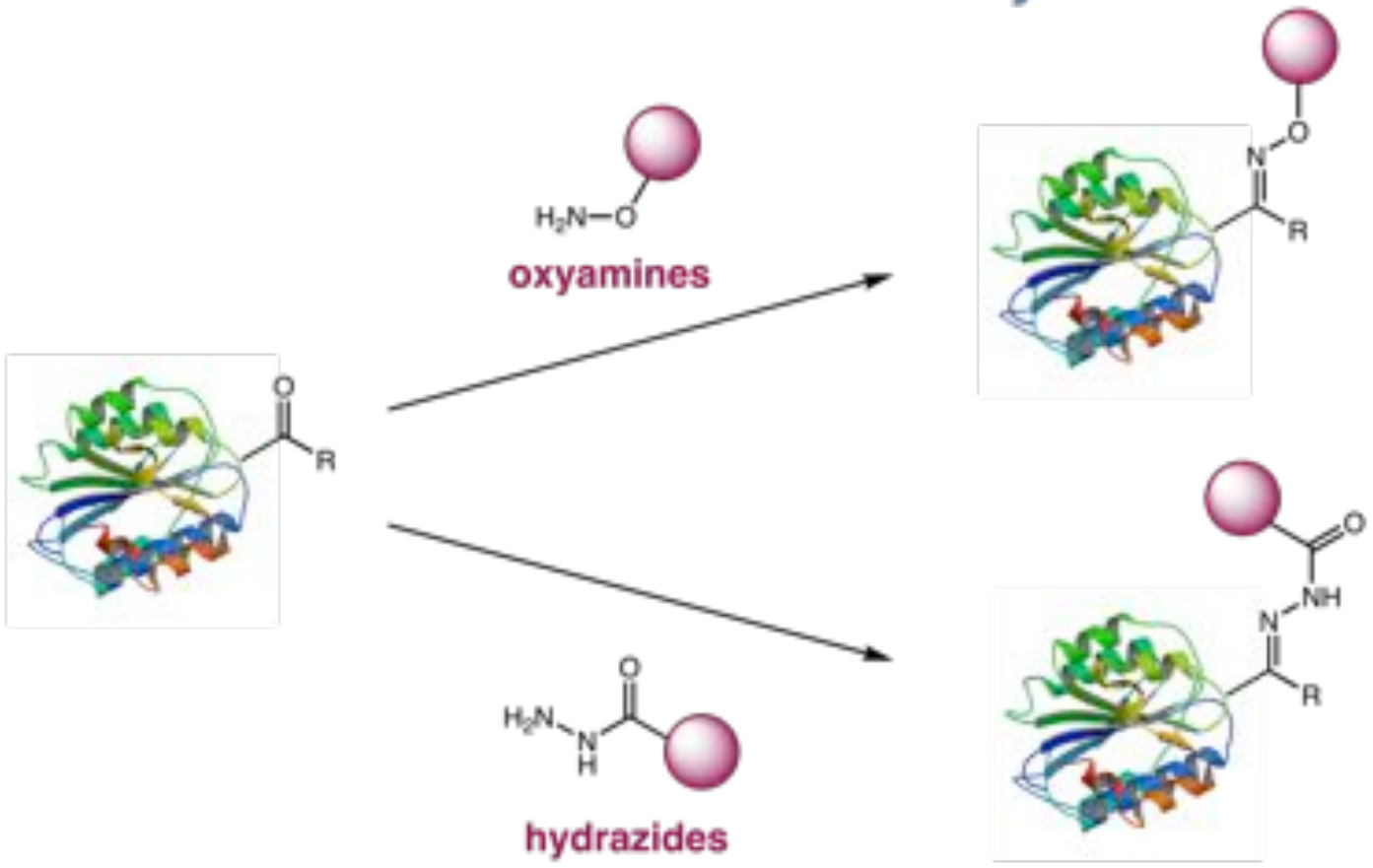


hydrazides and oxyamines are commonly used for this condensation chemistry

biological nucleophiles can condense but the equilibrium favors the starting material

Jencks, W. P. *J. Am. Chem. Soc.* **1959**, *81*, 475.

Condensation Chemistry



this chemistry is not widely employed inside cells or within live organisms

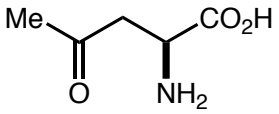
is applicable to cell surfaces for aldehydes and ketones are not present there

Jencks, W. P. *J. Am. Chem. Soc.* **1959**, *81*, 475.

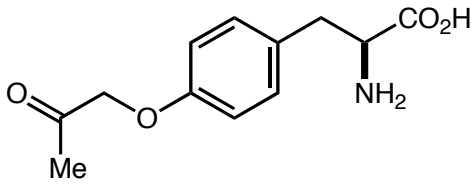
Condensation Chemistry

- Unnatural amino acid mutagenesis was performed on T4 lysozyme

incorporated at two solvent accessible sites - Ser^{44} and Ala^{82}



5% efficiency



30% efficiency

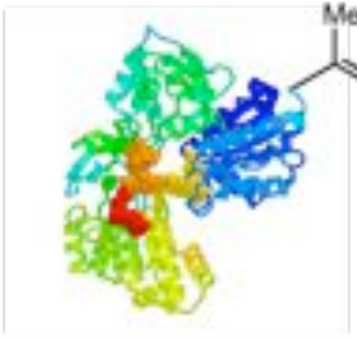
determined by catalytic activity, SDS-PAGE, and autoradiography

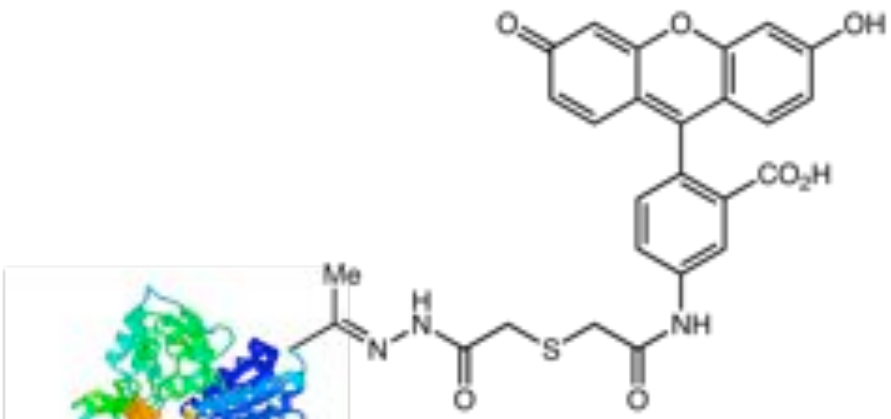
Cornish, V. W.; Hahn, K. M.; Schultz, P. G. *J. Am. Chem. Soc.* **1996**, *118*, 8150-8151.

Zhang, Z.; Smith, B. A. C.; Wang, L.; Brock, A.; Cho, C.; Schultz, P. G. *Biochemistry* **2003**, *42*, 6735-6746.

Condensation Chemistry

- Hydrazone Formation





condensation proceeds in roughly 50% efficiency

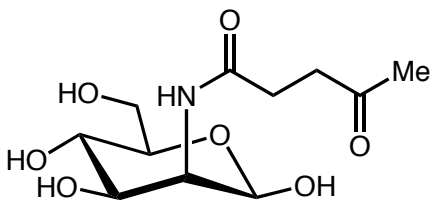
wild-type displays no fluorescence while the mutant does

Cornish, V. W.; Hahn, K. M.; Schultz, P. G. *J. Am. Chem. Soc.* **1996**, *118*, 8150-8151.

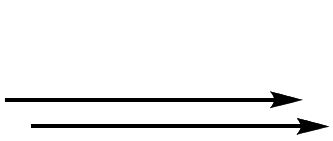
Zhang, Z.; Smith, B. A. C.; Wang, L.; Brock, A.; Cho, C.; Schultz, P. G. *Biochemistry* **2003**, *42*, 6735-6746.

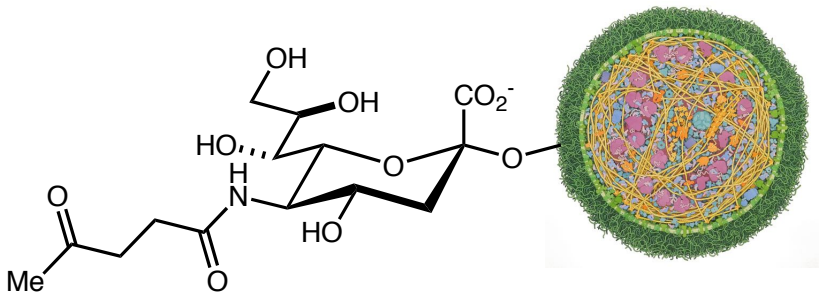
Condensation Chemistry

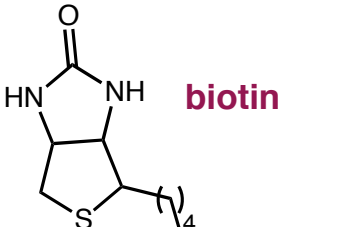
- Cell surface remodeling



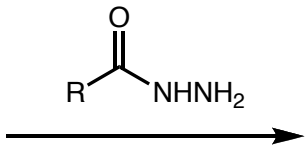
ManLev
a sialic acid analogue



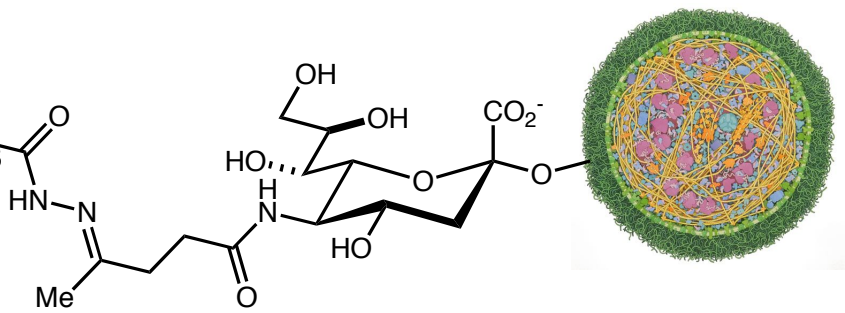




biotin



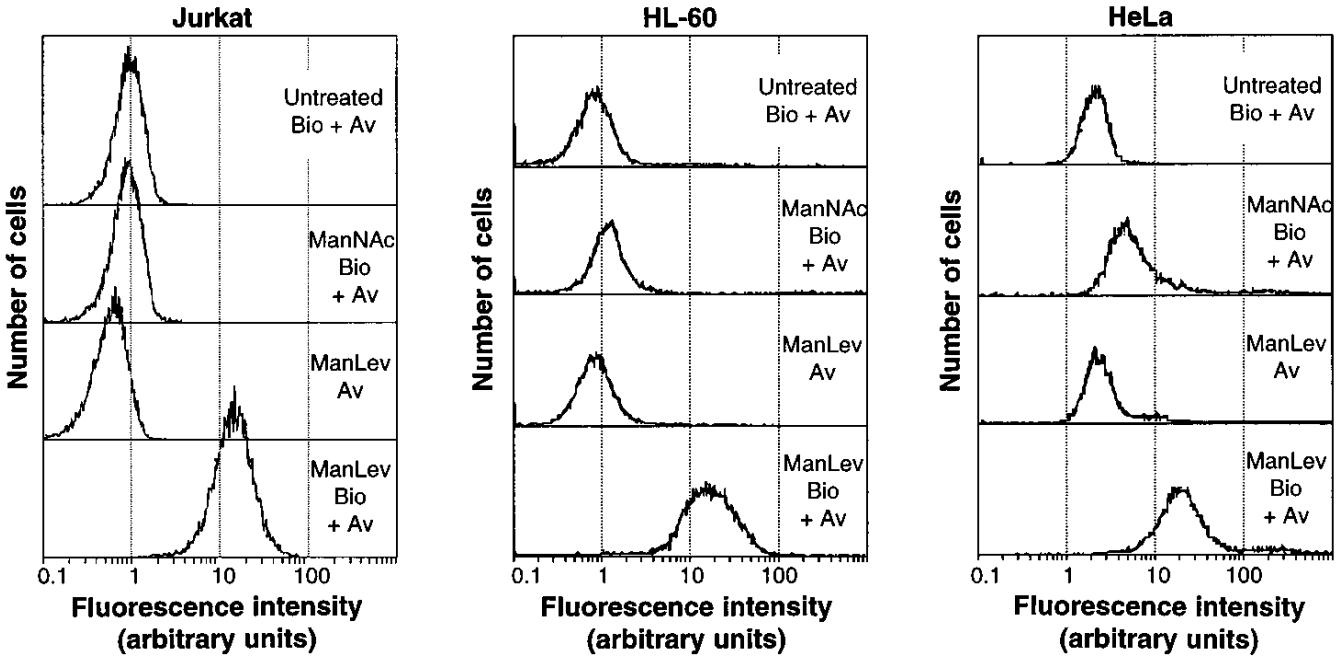
Hydrazide



Keppler, O. T.; Horstkorte, R.; Pawlita, M.*; Schmidts, C.; Reutter, W. Glycobiology* **2001**, *11*, 11R- 18R.
Mahal, L. K.; Yarema, K. J.; Bertozzi, C. R. *Science* **1997**, *276*, 1125-1128.

Condensation Chemistry

- Cell surface remodeling

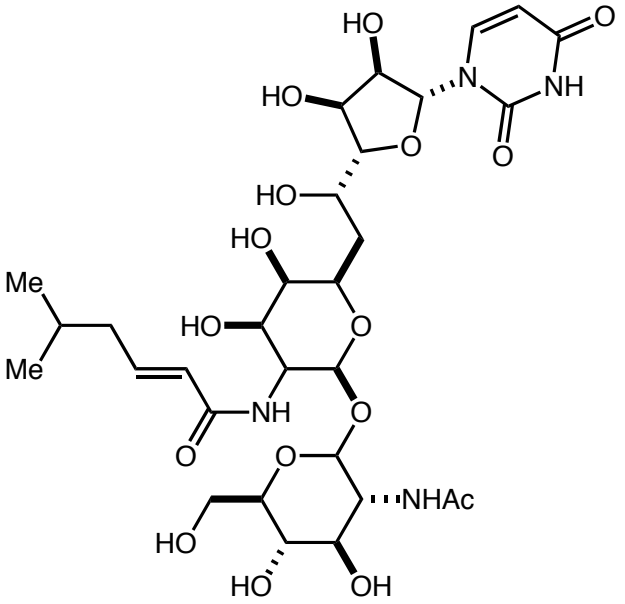


cells were analyzed by flow cytometry after staining with FITC (fluorescein isothiocyanate) avidin

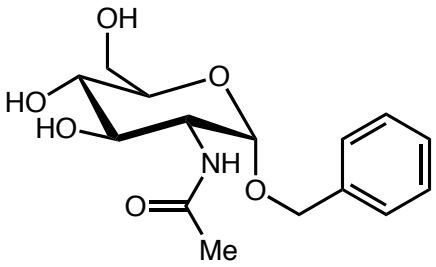
Mahal, L. K.; Yarema, K. J.; Bertozzi, C. R. *Science* **1997**, *276*, 1125-1128.

Condensation Chemistry

tunicamycin



alpha-benzyl *N*-acetylglucosamine



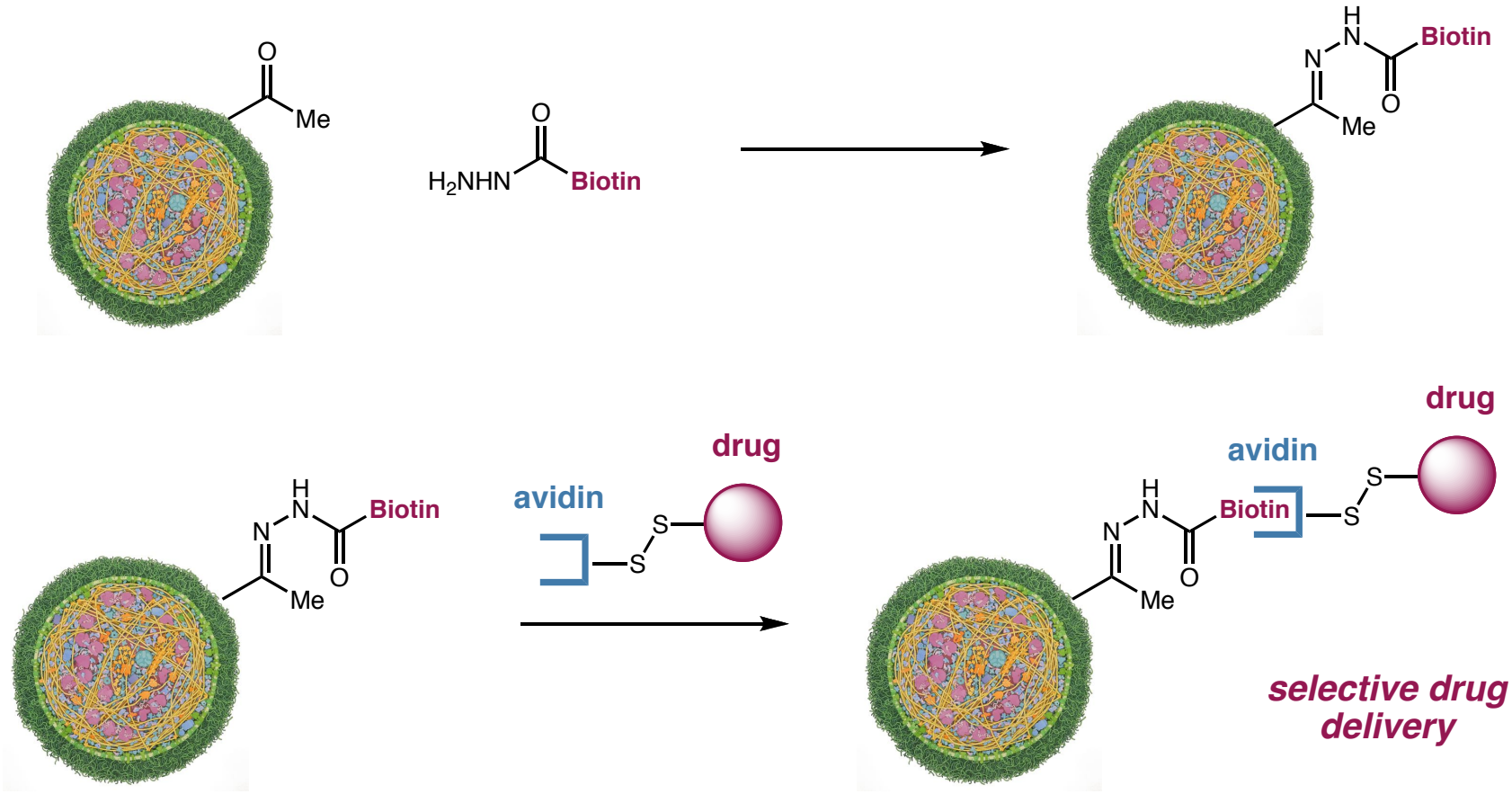
Addition of tunicamycin, a known inhibitor of N-linked protein glycosylation, inhibits ketone expression with ManLev treatment in Jurkat cells.

Ketone expression was blocked on HL-60 and HeLa cells via alpha-benzyl *N*-acetylgalactosamine, an inhibitor of O-linked glycosylation.

Mahal, L. K.; Yarema, K. J.; Bertozzi, C. R. *Science* **1997**, *276*, 1125-1128.

Condensation Chemistry

- Selective drug delivery?



Mahal, L. K.; Yarema, K. J.; Bertozzi, C. R. *Science* **1997**, *276*, 1125-1128.

Condensation Chemistry

- Selective drug delivery?

ricin
inhibits protein synthesis

toxicity of the conjugate was dependent on the expression of ketones

cells with high ketone expression (~700,000 ketones per cell) were sensitive to lethal doses of ricin

LD_{50} between 1 to 10 nM

cells with low ketone expression (~50,000 ketones per cell) showed no toxicity

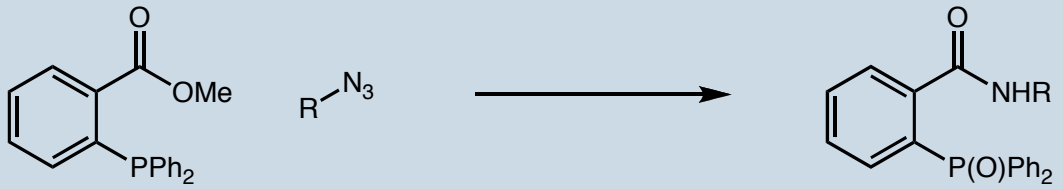
indicates the potential for cell surface engineering to support selective drug delivery

Mahal, L. K.; Yarema, K. J.; Bertozzi, C. R. *Science* **1997**, *276*, 1125-1128.

Bioorthogonal Chemistry

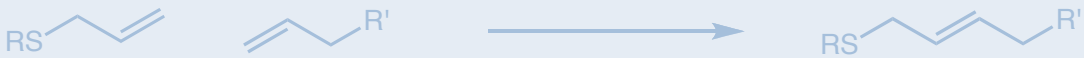
condensation chemistry

Staudinger ligation

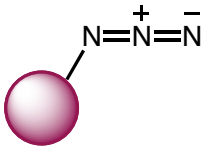


[3+2] cycloadditions

olefin chemistry



Azide - A Powerful Chemical Reporter



absent from biological systems

possesses orthogonal reactivity to most biological functional groups

the azide is small, so biological perturbation is minimal

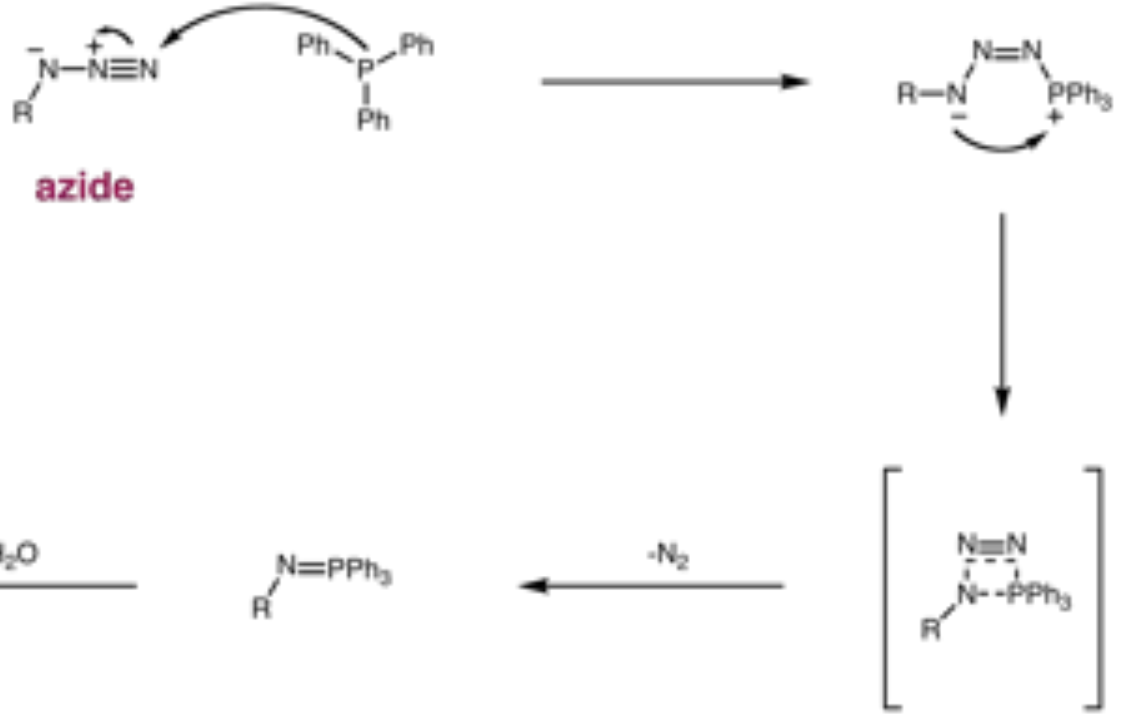
first used as a chemical reporter in 2000 in the Staudinger Ligation

Griffin, R. J. *Prog. Med. Chem.* **1994**, *31*, 121.
Hendricks, S. B.; Pauling, L. *J. Am. Chem. Soc.* **1925**, *47*, 2904.
Saxon, E.; Bertozzi, C. R. *Science* **2000**, *287*, 2007.

The Staudinger Reduction

- inspiration for the Staudinger ligation came from the Staudinger reduction

Hermann Staudinger

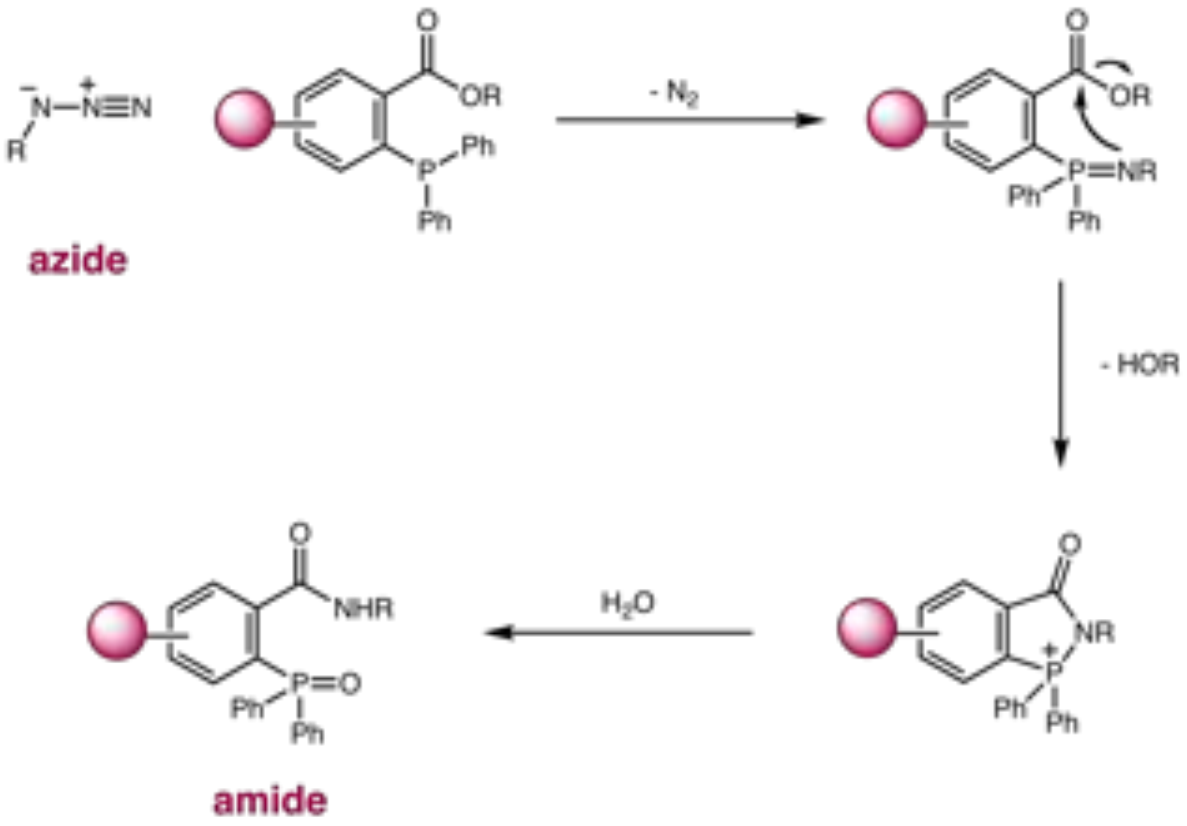


Staudinger, H.; Meyer, J. *Helv. Chim. Acta.* **1919**, *2*, 635.

The Staudinger Ligation

- inspiration for the Staudinger ligation came from the Staudinger reduction

Carolyn Bertozzi

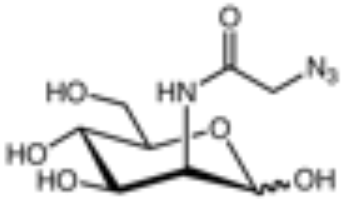


Saxon, E.; Bertozzi, C. R. *Science* **2000**, *287*, 2007.

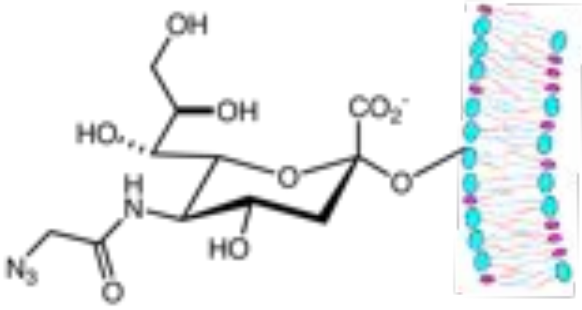
Staudinger, H.; Meyer, J. *Helv. Chim. Acta.* **1919**, *2*, 635.

The Staudinger Ligation

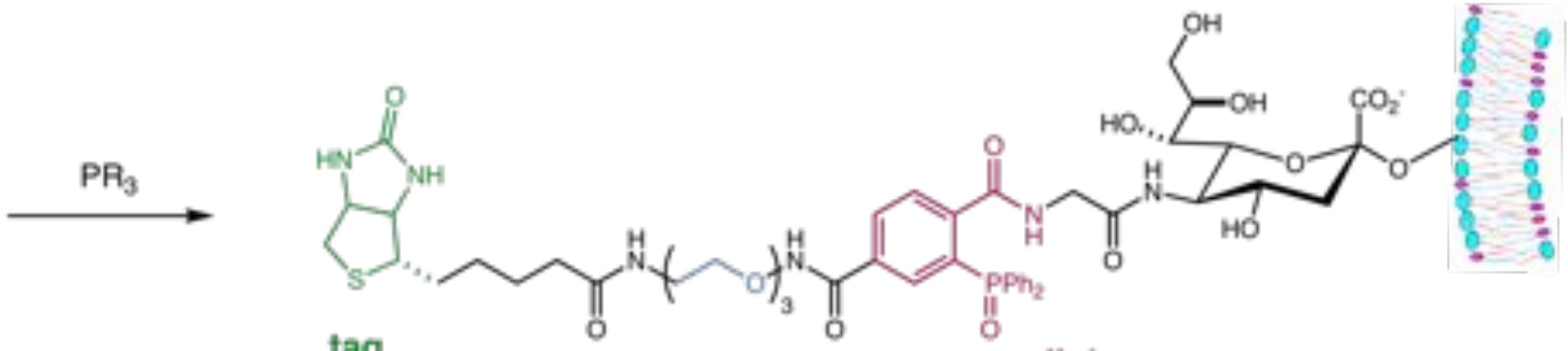
- Cell surface engineering



azidosugar



sialic acid analogue



Saxon, E.; Bertozzi, C. R. *Science* **2000**, *287*, 2007.

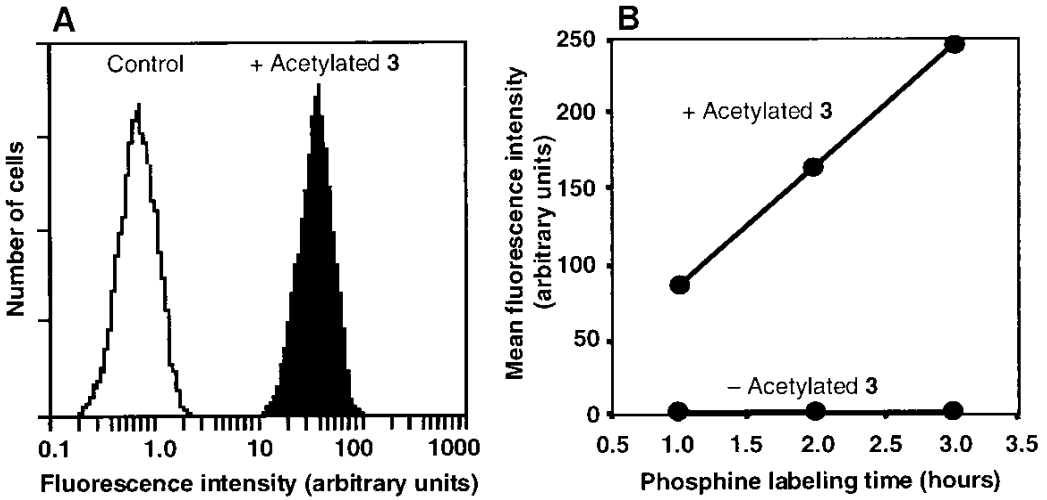
The Staudinger Ligation

Jurkat cells were incubated with azidoacetylmannosamine at a concentration 20 mM for three days.

Cell viability was tested with incubation for up to six days.

Cells were washed and reacted with phosphine for 1 hour at a concentration of 1 mM.

Stained with FITC-avidin and analyzed by flow cytometry.



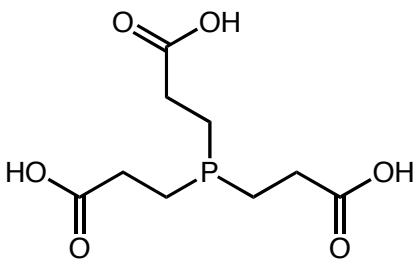
Saxon, E.; Bertozzi, C. R. *Science* **2000**, *287*, 2007.

The Staudinger Ligation

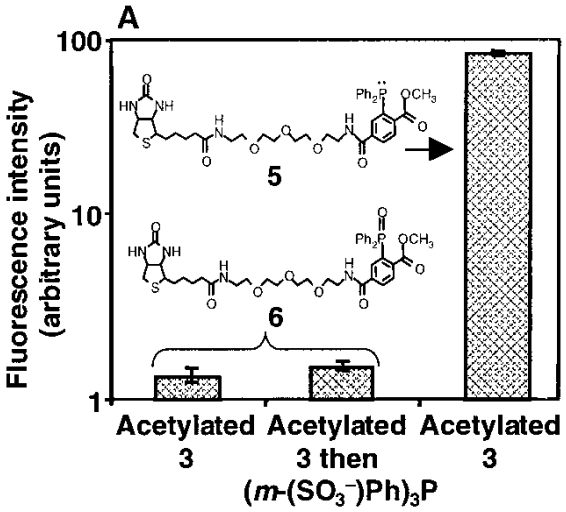
Biological Controls -

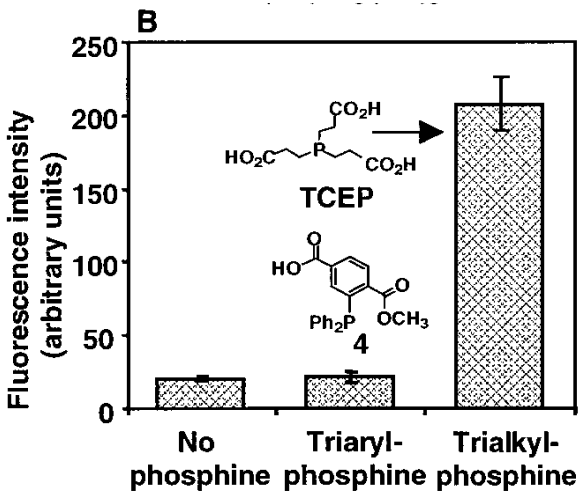
Could a Staudinger reduction be taking place and the phosphine oxide localizes in or outside the cell?

Could the phosphine be reducing disulfide bonds and not be completely bioorthogonal?



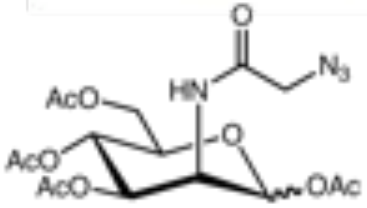
TCEP





Saxon, E.; Bertozzi, C. R. *Science* **2000**, *287*, 2007.

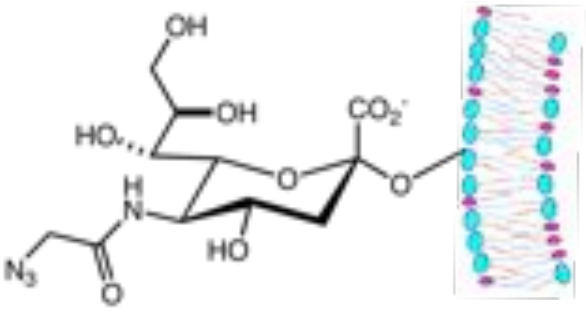
Staudinger Ligation in Living Animals



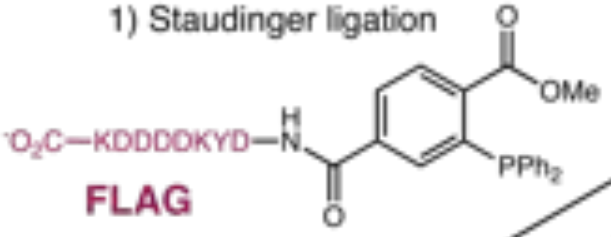
$Ac_4ManNAz$

1) Inject $Ac_4ManNAz$ solution for seven days

2) Isolate splenocytes

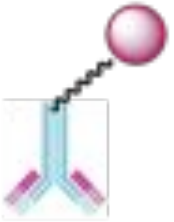


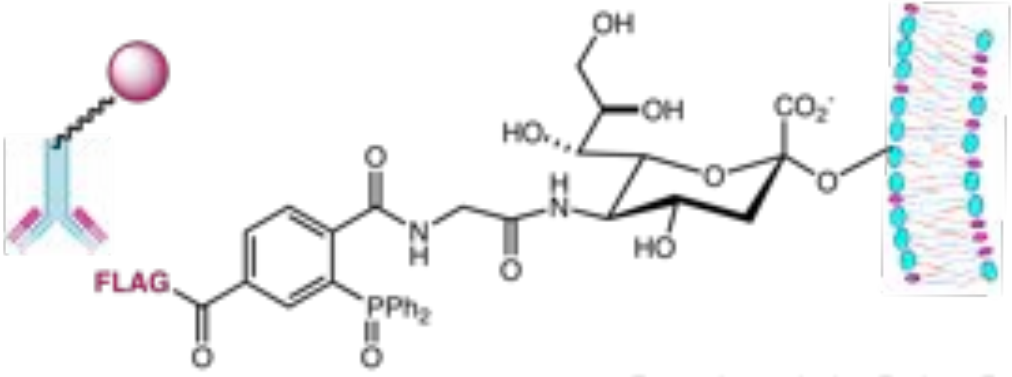
1) Staudinger ligation



FLAG

2) FITC-anti-flag





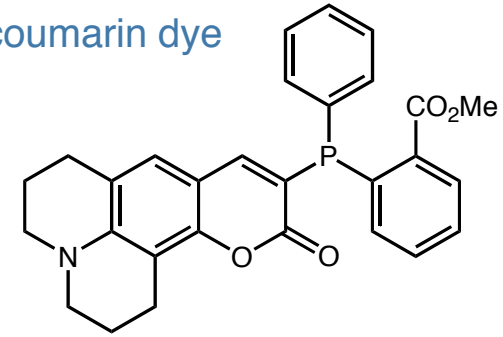
Detection by flow cytometry

Prescher, J. A.; Dube, D. H.; Bertozzi, C. R. *Nature* **2004**, *430*, 873-877.

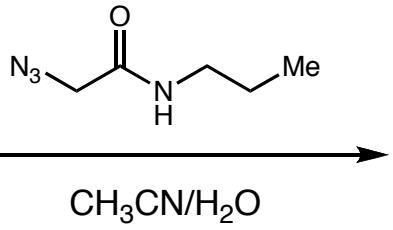
Activation of a Fluorgenic Dye via the Staudinger Ligation

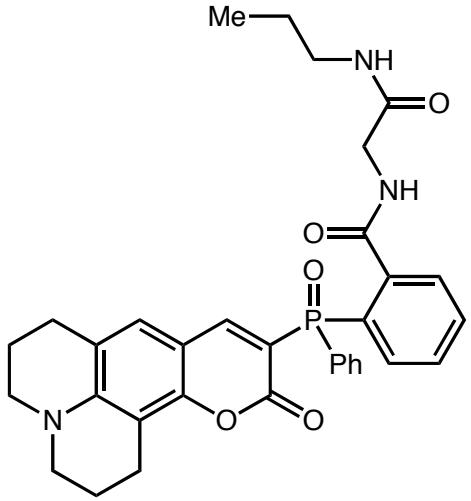
The lone pair of electrons on phosphorus quench the excited fluorophore

coumarin dye

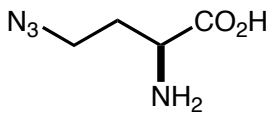


not fluorescent





strongly fluorescent



L - azidohomoalanine

Phosphine dye was reacted with recombinant murine dihydrofolate reductase bearing azidohomoalanine residues.

Unnatural amino acids incorporated during overexpression in a methionine auxotrophic *E. coli* strain.

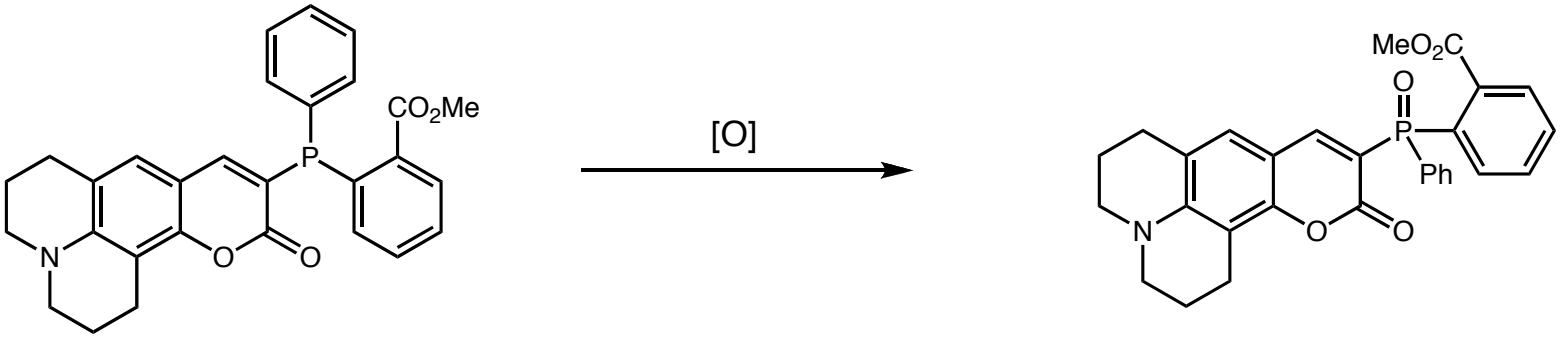
Fluorescence was observed over background.

No washing or Western blotting necessary.

Lemieux, G. A.; de Graffenried, C. L.; Bertozzi, C. R. *J. Am. Chem. Soc.* **2003**, *125*, 4708-4709.

Activation of a Fluorgenic Dye via the Staudinger Ligation

A small problem



not fluorescent

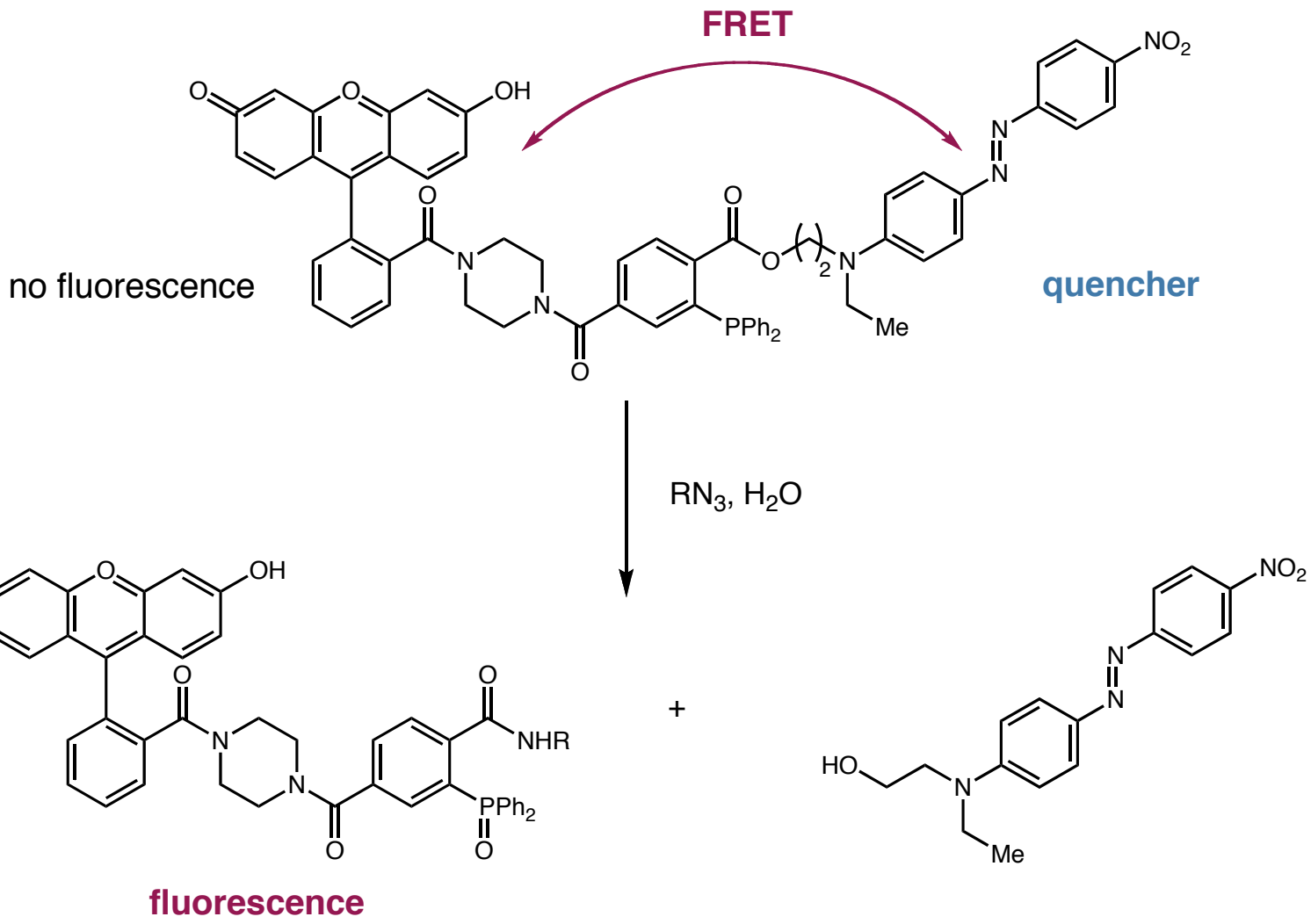
strongly fluorescent

Oxidation of the probe by air would provide background fluorescence.

Hangauer, M. J.; Bertozzi, C. R. *Angew. Chem. Int. Ed.* **2008**, *47*, 2394.

Activation of a Fluorgenic Dye via the Staudinger Ligation

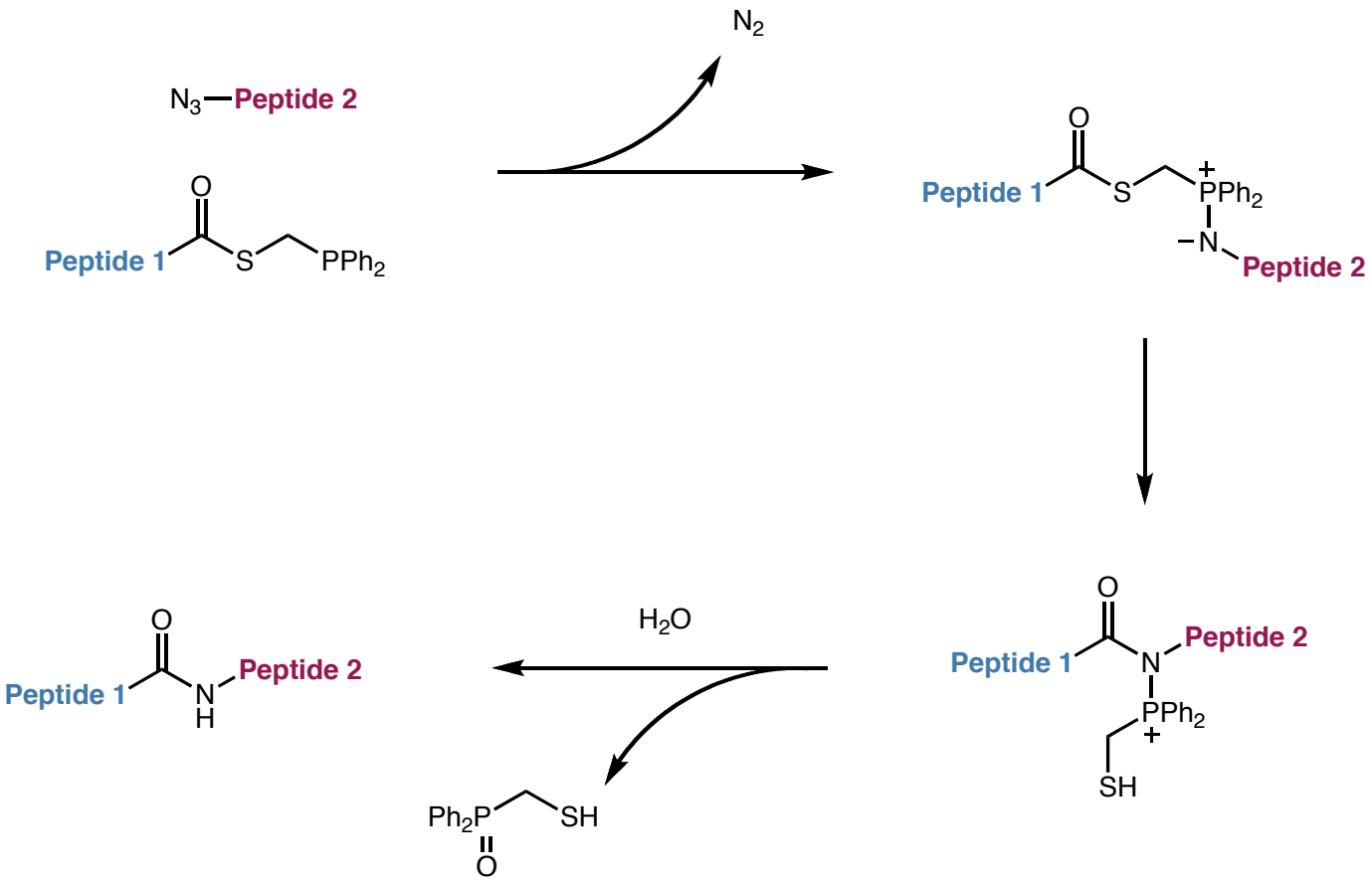
Fluorescence resonance energy transfer (FRET) based probe



Hangauer, M. J.; Bertozzi, C. R. *Angew. Chem. Int. Ed.* **2008**, *47*, 2394.

"Traceless" Staudinger Ligation

Peptide coupling by the traceless Staudinger ligation

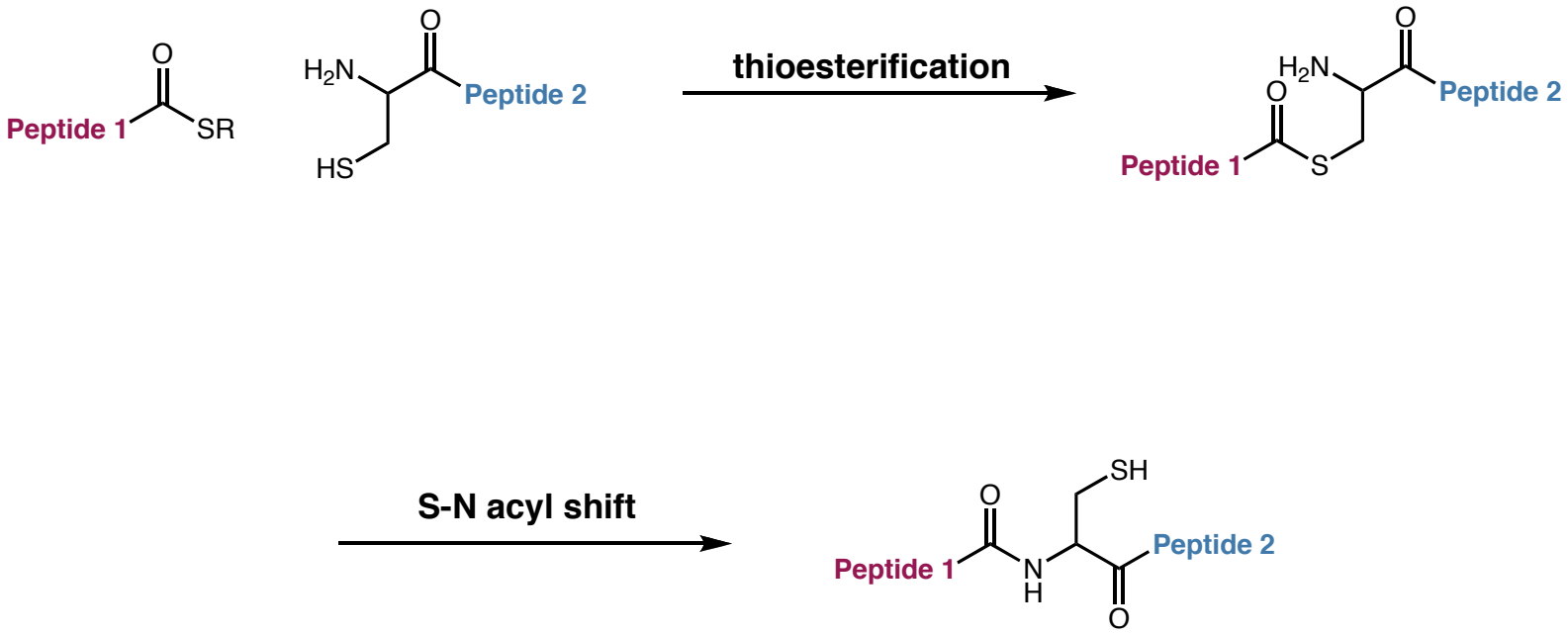


Nilsson, B. L.; Kiessling, L. L.; Raines, R. T. *Org. Lett.* **2000**, *2*, 1939.

Soellner, M. B.; Nilsson, B. L.; Raines, R. T. *J. Am. Chem. Soc.* **2006**, *128*, 8820.

"Traceless" Staudinger Ligation

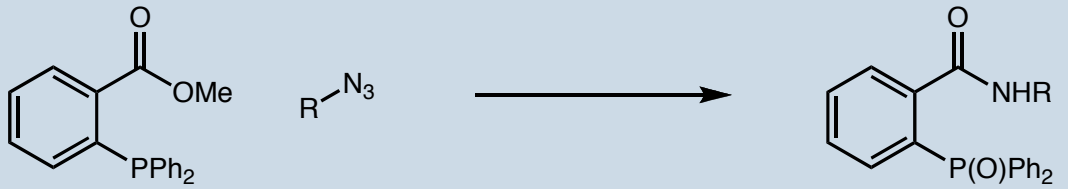
Traceless Staudinger ligation is reminiscent of native chemical ligation



Dawson, P. E.; Muir, T. W.; Clarklewis, I.; Kent, S. B. H. *Science* **1994**, *266*, 776.

Staudinger Ligation

Staudinger ligation



The Staudinger ligation has been used to probe biomolecules within living animals.

Second-order kinetics where the rate-determining step is purported to be attack of the phosphine on the azide.

$k = 10^{-3}\ M^{-1}s^{-1}$ (very slow)

High concentrations of the phosphine are often necessitated (> 250 uM)

Attempts to increase phosphine nucleophilicity has increased the suspectibility of phosphine oxidation.

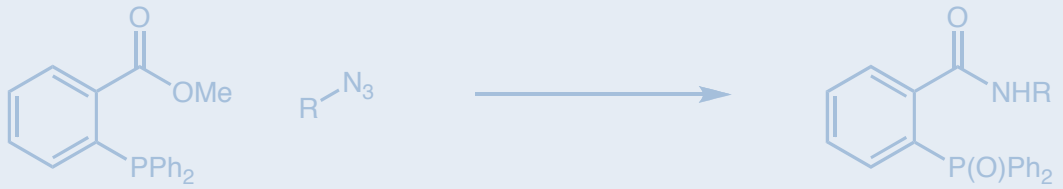
Lin, F. L.; Hoyt, H. M.; Van Halbeek, H.; Bergman, R. G.; Bertozzi, C. R. *J. Am. Chem. Soc.* **2005**, *127*, 2686.

Prescher, J. A.; Dube, D. H.; Bertozzi, C. R. *Nature* **2004**, *430*, 873.

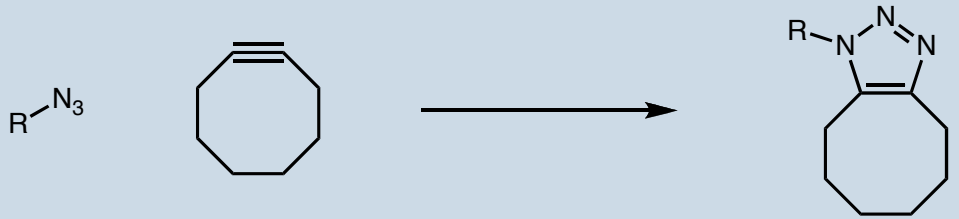
Bioorthogonal Chemistry

condensation chemistry

Staudinger ligation

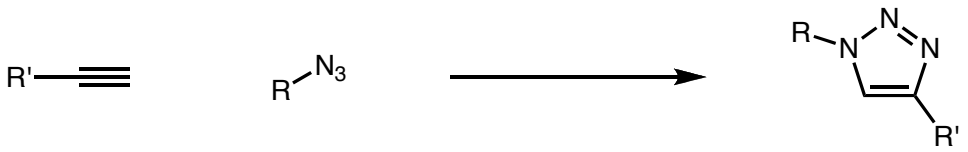


[3+2] cycloadditions



olefin chemistry

[3 + 2] Cycloadditions

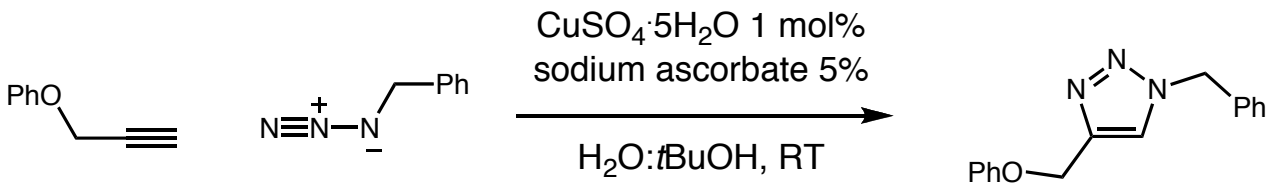


First discovered by Arthur Michael in 1893.

In the 1950s, Rolf Huisgen proposed that the reaction proceeds through a 1,3-dipolar cycloaddition.

High temperatures and pressure required made this reaction largely impractical.

In separate efforts, Meldal and Sharpless reported the copper-catalyzed azide-alkyne 1,3-dipolar cycloaddition (CuAAC).

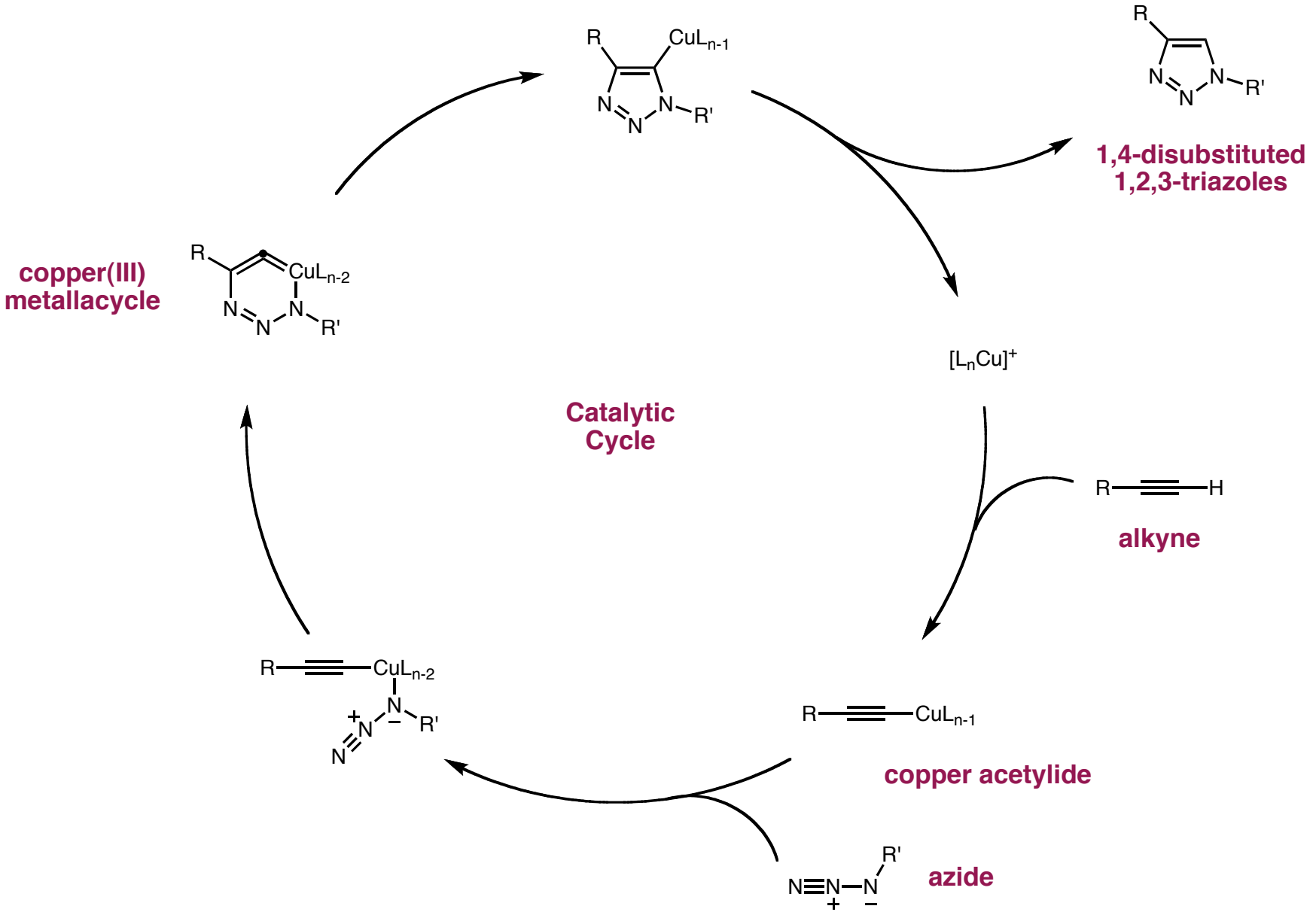


91%

Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem. Int. Ed.* **2002**, *41*, 2596.

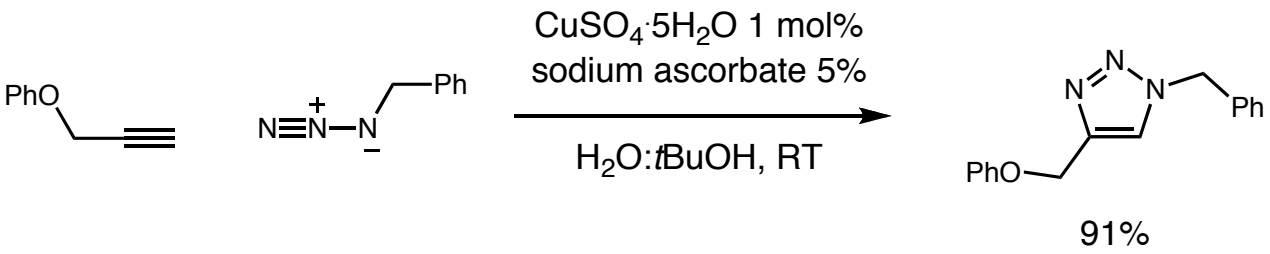
Tornoe, C. W.; Christensen, C.; Meldal, M. *J. Org. Chem.* **2002**, *67*, 3057.

[3 + 2] Cycloadditions



Himo, F.; Lovell, T.; Hilgraf, R.; Rostovtsev, V. V.; Noodleman, L.; Sharpless, K. B.; Fokin, V. V. *J. Am. Chem. Soc.* **2005**, *127*, 210-216.

Copper(I) Toxicity



Finn, Sharpless, and coworkers reported the first biomolecule coupling application of [3+2] cycloaddition through the attachment of dyes to the cowpea mosaic virus.

CuAAC is not widely employed, due to copper(I)'s toxicity.

E. Coli stops dividing after exposure to 100 uM CuBr for 16 hours.

Mammalian cells and zebrafish embryos can survive low concentrations of copper (I) (< 500 uM) but considerable cell death is observed above 1 mM.

Wang, Q.; Chan, T. R.; Hilgraf, R.; Fokin, V. V.; Sharpless, K. B.; Finn, M. G. *J. Am. Chem. Soc.* **2003**, *125*, 3192-3193.

Chan, T. R.; Hilgraf, R.; Sharpless, K. B.; Fokin, V. V. *Org. Lett.* **2004**, *6*, 2853.

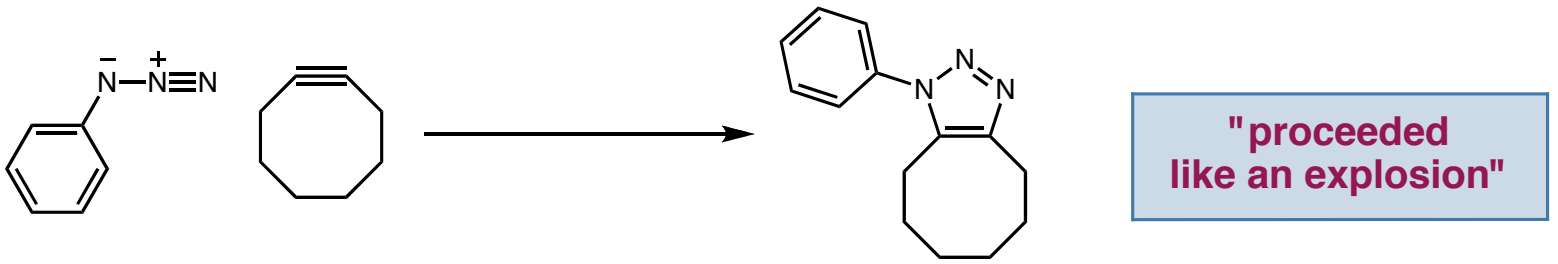
Link, A. J.; Tirrell, D. A. *J. Am. Chem. Soc.* **2003**, *125*, 11164.

Speers, A. E.; Adam, B. F.; Cravatt, B. F. *J. Am. Chem. Soc.* **2003**, *125*, 4686.

[3+2] Cycloaddition - Ring Strain

Strain-promoted azide cycloadditions were first investigated by Alder and Stein in the 1930s.

In the 1960s, Krebs and Wittig commented that phenylazide and cyclooctyne



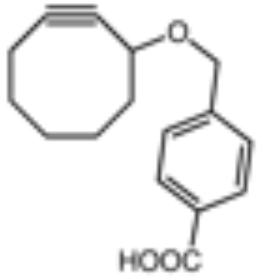
"proceeded like an explosion"

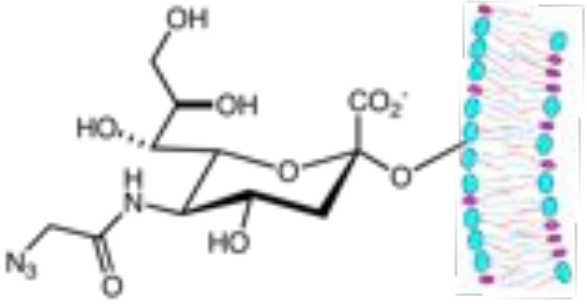
massive bond angle deformation of the acetylene to 163°
~ 18 kcal/mol of ring strain

Wittig, G.; Krebs, A. *Chem. Ber.* **1961**, *94*, 3260.
Alder, K.; Stein, G. *Justus Liebigs Ann. Chem.* **1931**, *485*, 211.

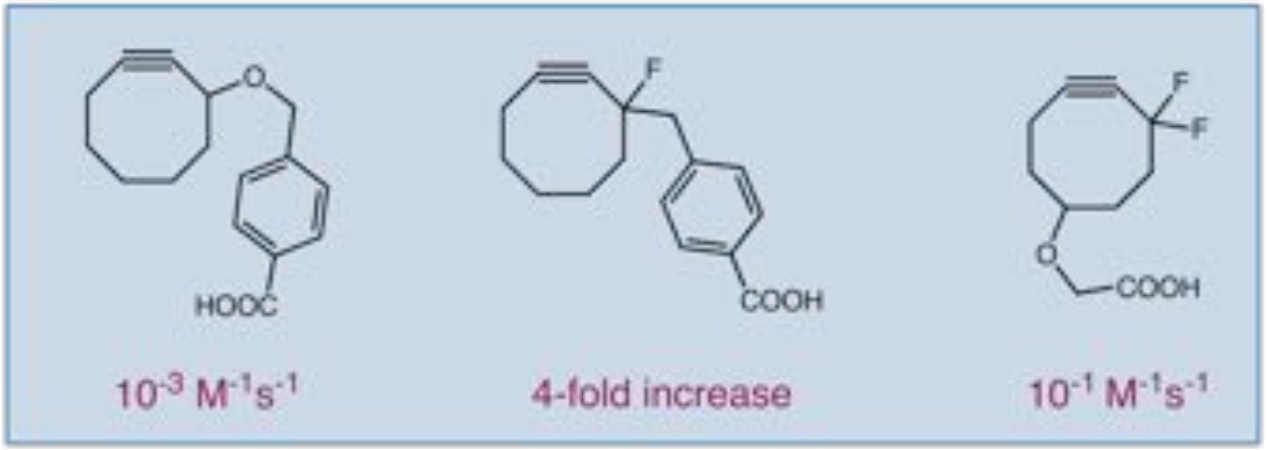
[3+2] Cycloaddition - Ring Strain

Cyclooctynes react smoothly on cell-surface glycans with no apparent cytotoxic effects.





Reactions kinetics is still an issue.

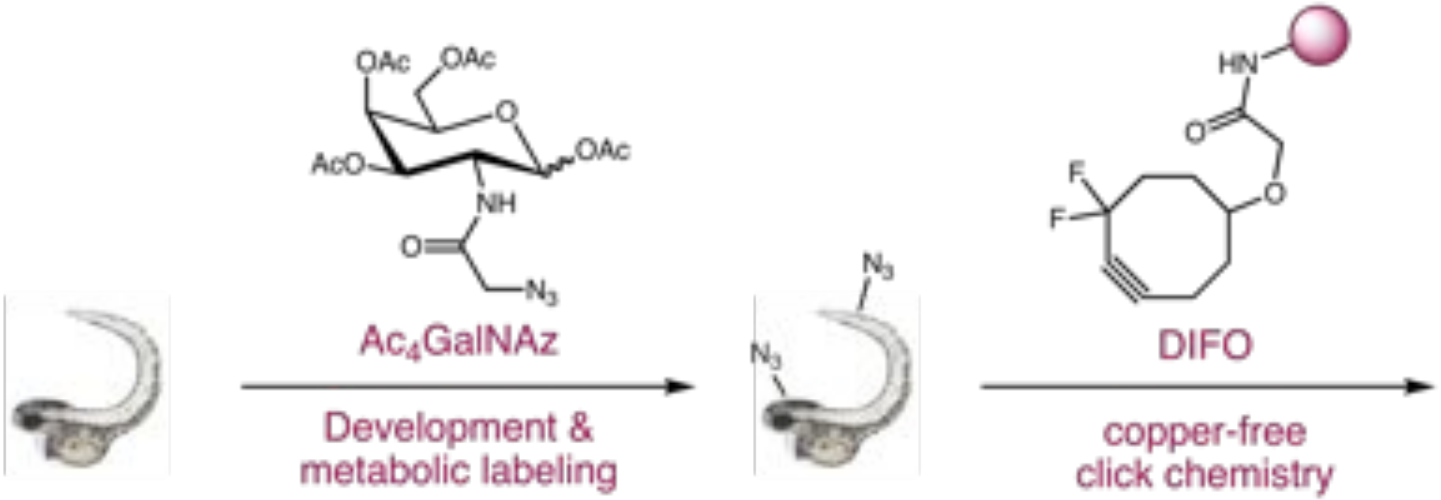


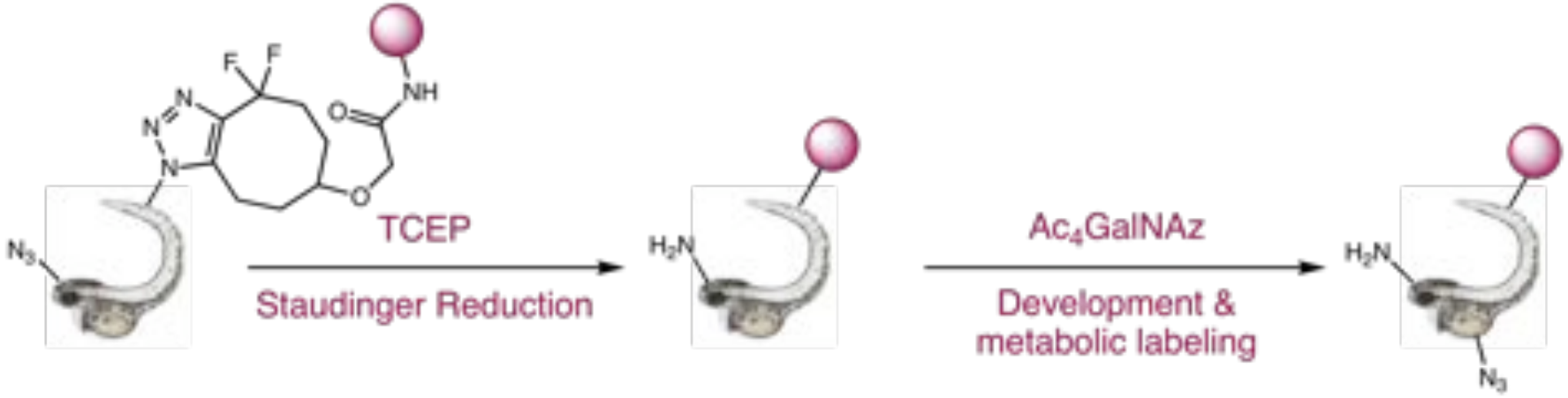
Agard, N. J.; Prescher, J. A.; Bertozzi, C. R. *J. Am. Chem. Soc.* **2004**, *126*, 15046.

Agard, N. J; Baskin, J. M.; Prescher, J. A.; Lo, A.; Bertozzi, C. R. *ACS Chem. Biol.* **2006**, *1*, 644.

Baskin, J. M.; Prescher, J. A.; Laughlin, S. T.; Agard, N. J.; Chang, P. V.; Bertozzi, C. R. *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 16793.

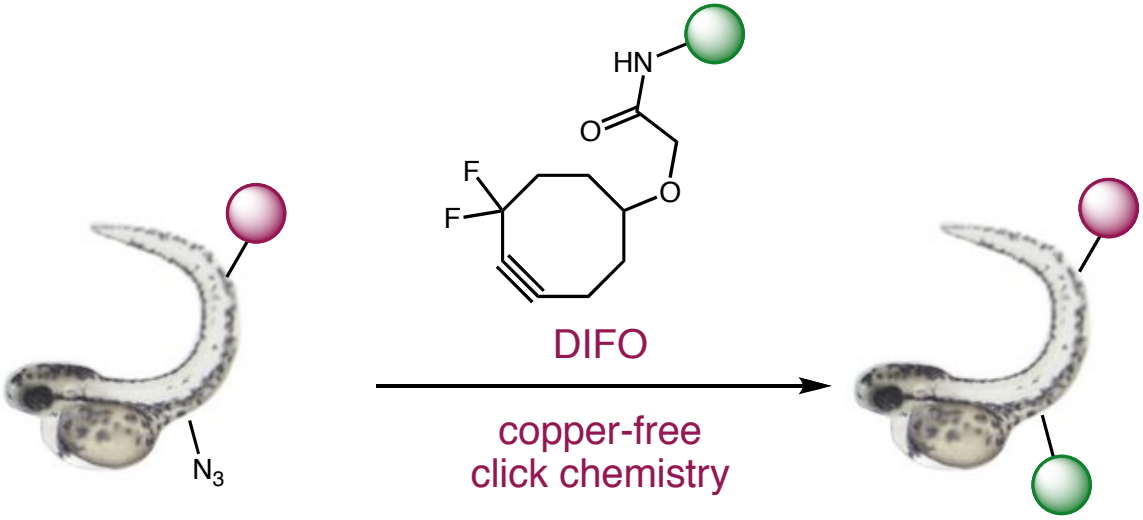
In Vivo Imaging of Zebrafish Embryos





Laughlin, S. T.; Baskin, J. M.; Amacher, S. L.; Bertozzi, C. R. *Science* **2008**, *320*, 664.

In Vivo Imaging of Zebrafish Embryos



Laughlin, S. T.; Baskin, J. M.; Amacher, S. L.; Bertozzi, C. R. *Science* **2008**, *320*, 664.

In Vivo Imaging of Zebrafish Embryos

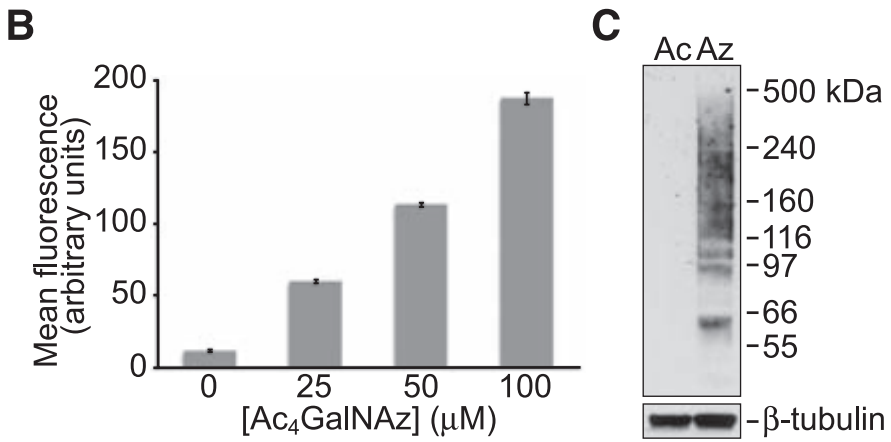
First confirmed that zebrafish glycan biosynthetic enzymes are tolerant of the unnatural sugar.

Zebrafish cell line ZF4 was incubated with various doses of Ac_4GalNAzm reacted with DIFO-488 and analyzed by flow cytometry.

Azide-labeled cell lysates were further characterized by treatment with a DIFO-Flag peptide conjugate.

Observed high-molecular weight species were consistent with labeled glycoproteins.

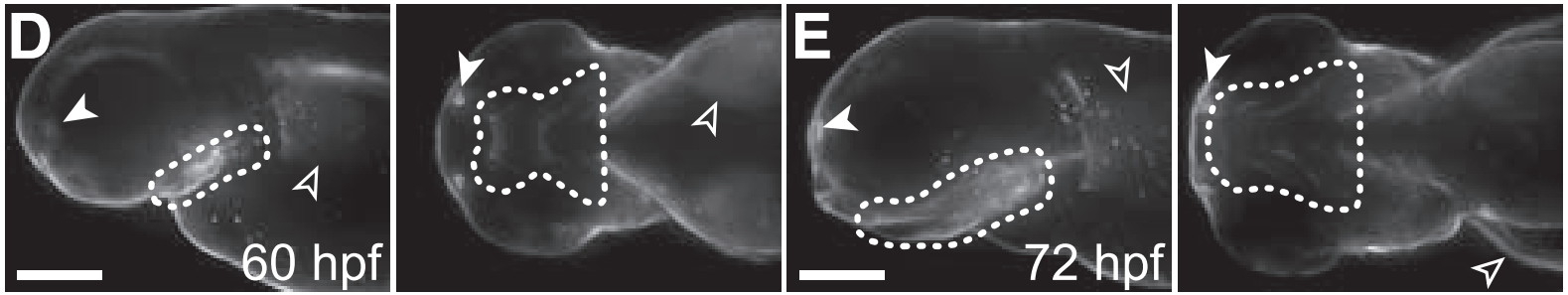
Flag-containing species (glycoproteins like b-hexosaminidase, b-integrin, nicastrin) were known or predicted sites of mucin-type O-linked glycosylation.



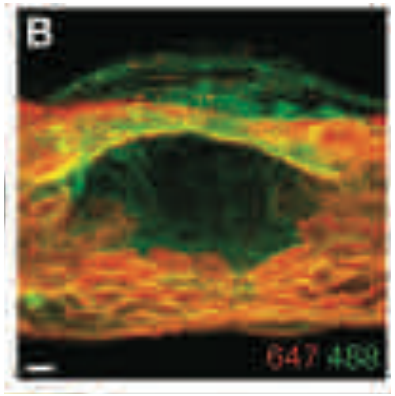
Laughlin, S. T.; Baskin, J. M.; Amacher, S. L.; Bertozzi, C. R. *Science* **2008**, *320*, 664.

In Vivo Imaging of Zebrafish Embryos

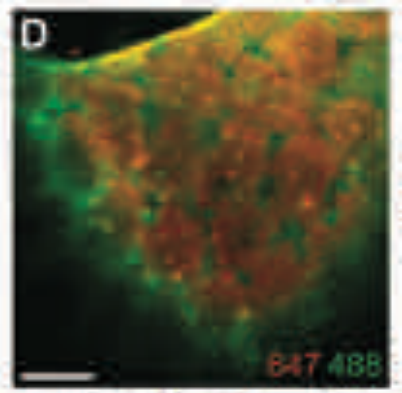
From 60 hours post-fertilization (hpf) to 72 hpf, a burst in fluorescence intensity in the jaw region, pectoral fins, and olfactory organs.



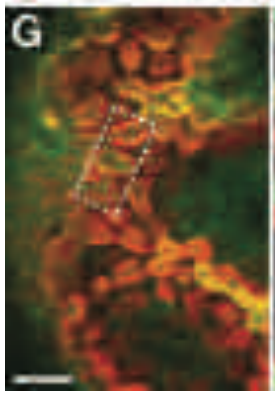
These three areas are reexamined and reacted with **DIFO-647** between 60 and 61 hpf and **DIFO-488** between 61 and 62 hpf.



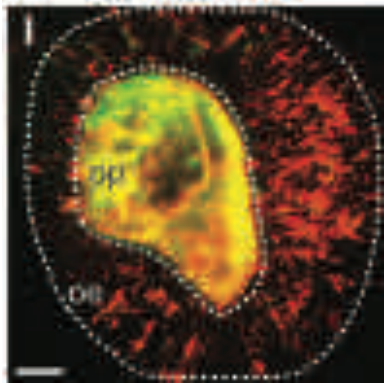
mouth region



pectoral fin



jaw region

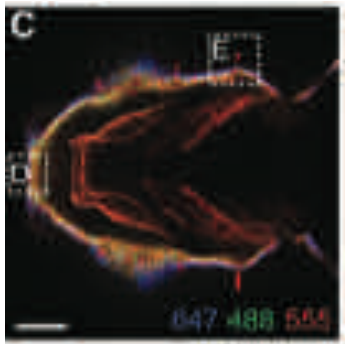


olfactory region

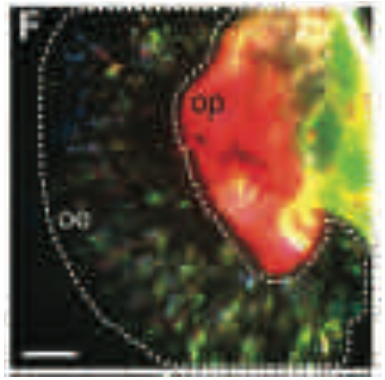
Laughlin, S. T.; Baskin, J. M.; Amacher, S. L.; Bertozzi, C. R. *Science* **2008**, *320*, 664.

In Vivo Imaging of Zebrafish Embryos

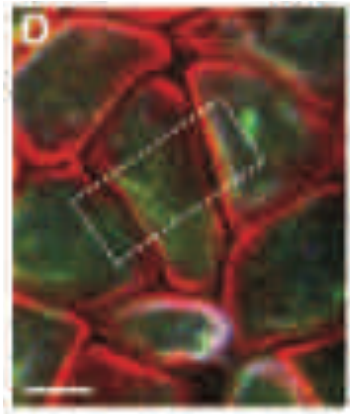
Three-dye bioimaging - **DIFO-647** between 60 and 61 hpf, **DIFO-488** between 62 and 62 hpf, and then **DIFO-555** between 72 and 73 hpf.



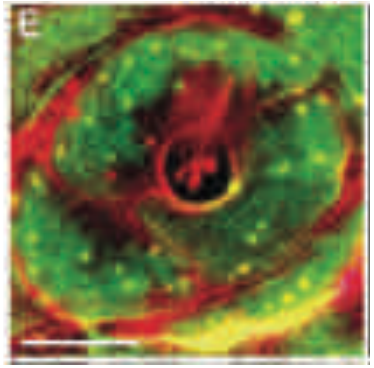
jaw region



olfactory region



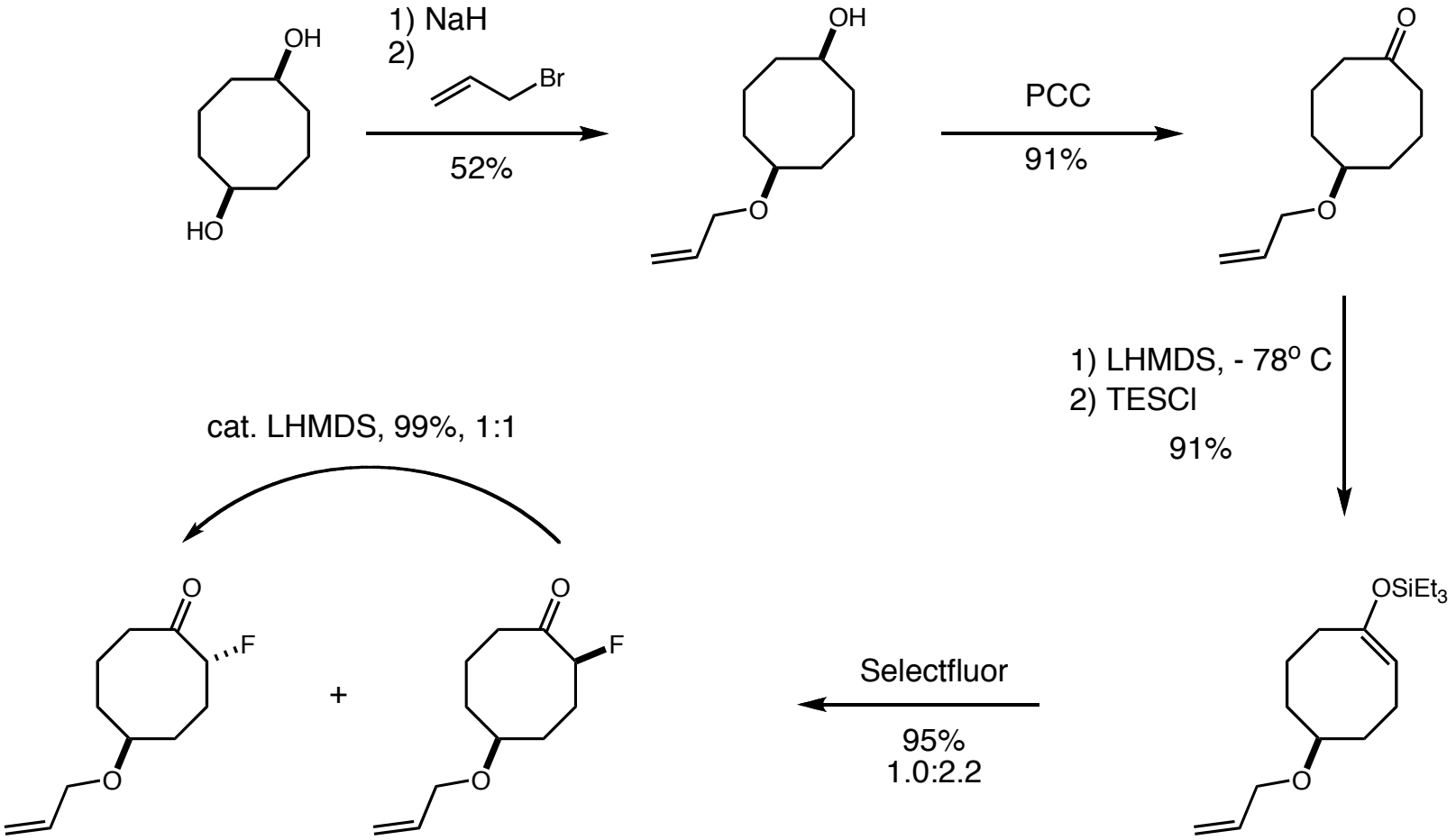
cells



kinocilia

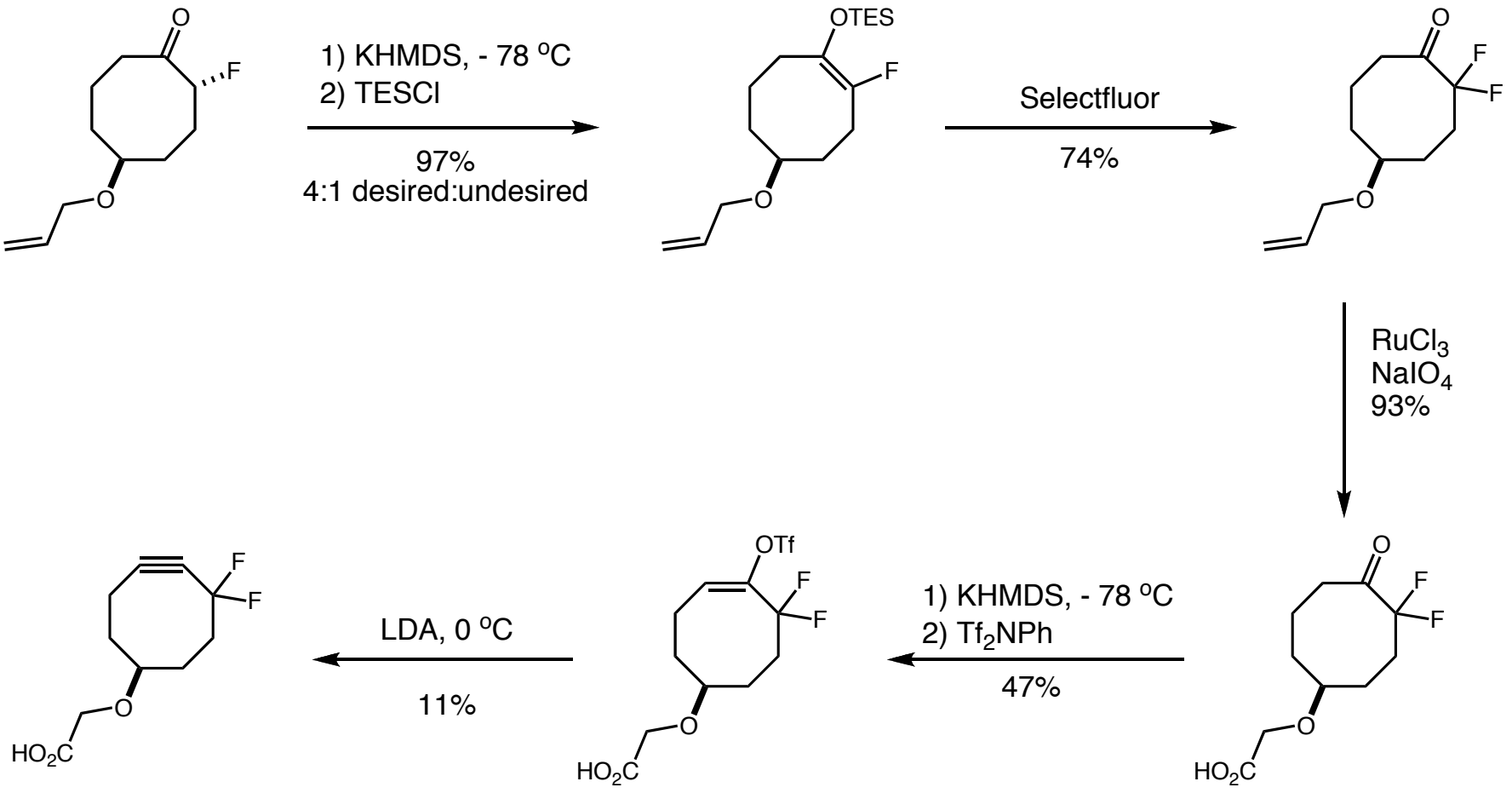
Laughlin, S. T.; Baskin, J. M.; Amacher, S. L.; Bertozzi, C. R. *Science* **2008**, *320*, 664.

DIFO Synthesis



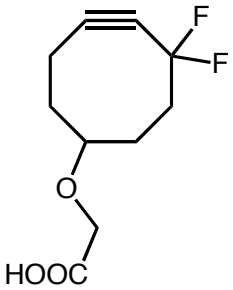
Baskin, J. M.; Prescher, J. A.; Laughlin, J. T.; Agard, N. J.; Chang, P. V.; Miller, I. A.; Lo, A.; Codelli, J. A.; Bertozzi, C. R. *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 16793.

DIFO Synthesis

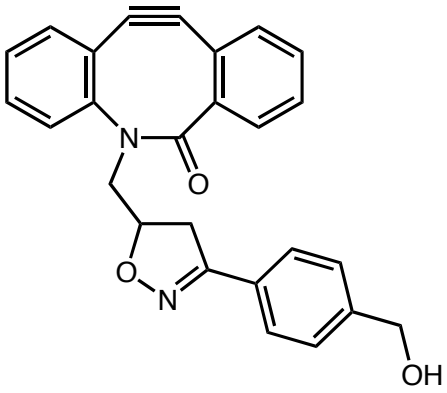


Baskin, J. M.; Prescher, J. A.; Laughlin, J. T.; Agard, N. J.; Chang, P. V.; Miller, I. A.; Lo, A.; Codelli, J. A.; Bertozzi, C. R. *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 16793.

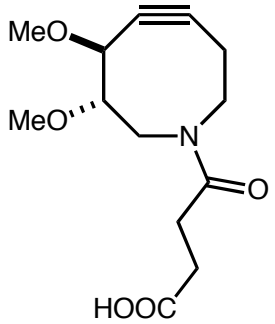
Cyclooctyne Analogues



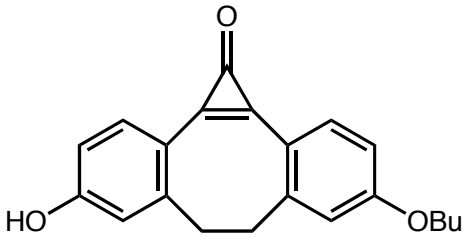
DIFO, Bertozzi Lab
$k = 0.076\ M^{-1}s^{-1}$



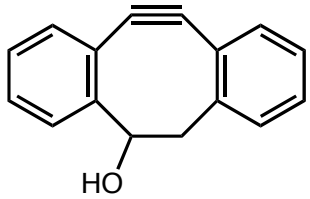
BARAC, Bertozzi Lab
$k = 0.96\ M^{-1}s^{-1}$



DIMAC, Bertozzi Lab
more water soluble
$k = 0.003\ M^{-1}s^{-1}$



Boons and Popik Labs
photocaged
$k = 0.076\ M^{-1}s^{-1}$



DIBO, Boons Lab
5 steps
nontoxic, $k = 0.057\ M^{-1}s^{-1}$

Sletten, E. M.; Bertozzi, C. R. *Org. Lett.* **2008**, *10*, 3097.

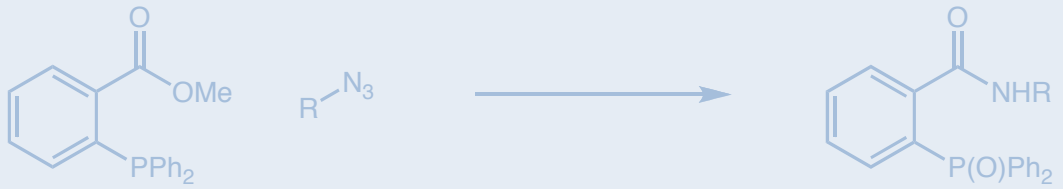
Ning, X. H.; Guo, J.; Wolfert, M. A.; Boons, G. J. *Angew. Chem. Int. Ed.* **2008**, *47*, 2253.

Sletten, E. M.; Bertozzi, C. R. *Acc. Chem. Res.* **2009**, *44*, 666-676.

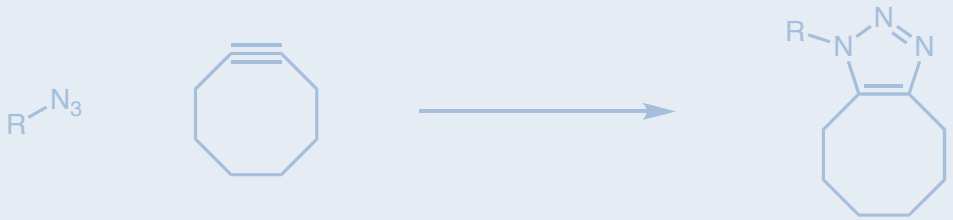
Bioorthogonal Chemistry

condensation chemistry

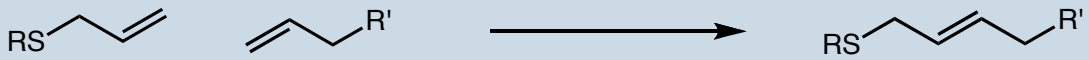
Staudinger ligation



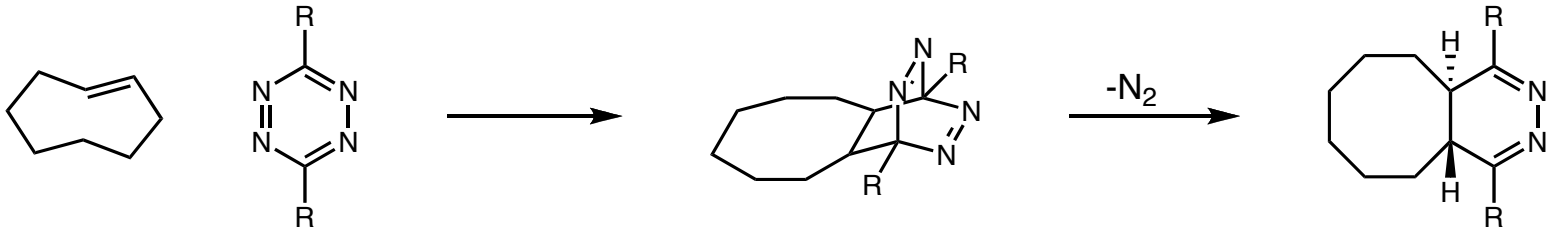
[3+2] cycloadditions



olefin chemistry



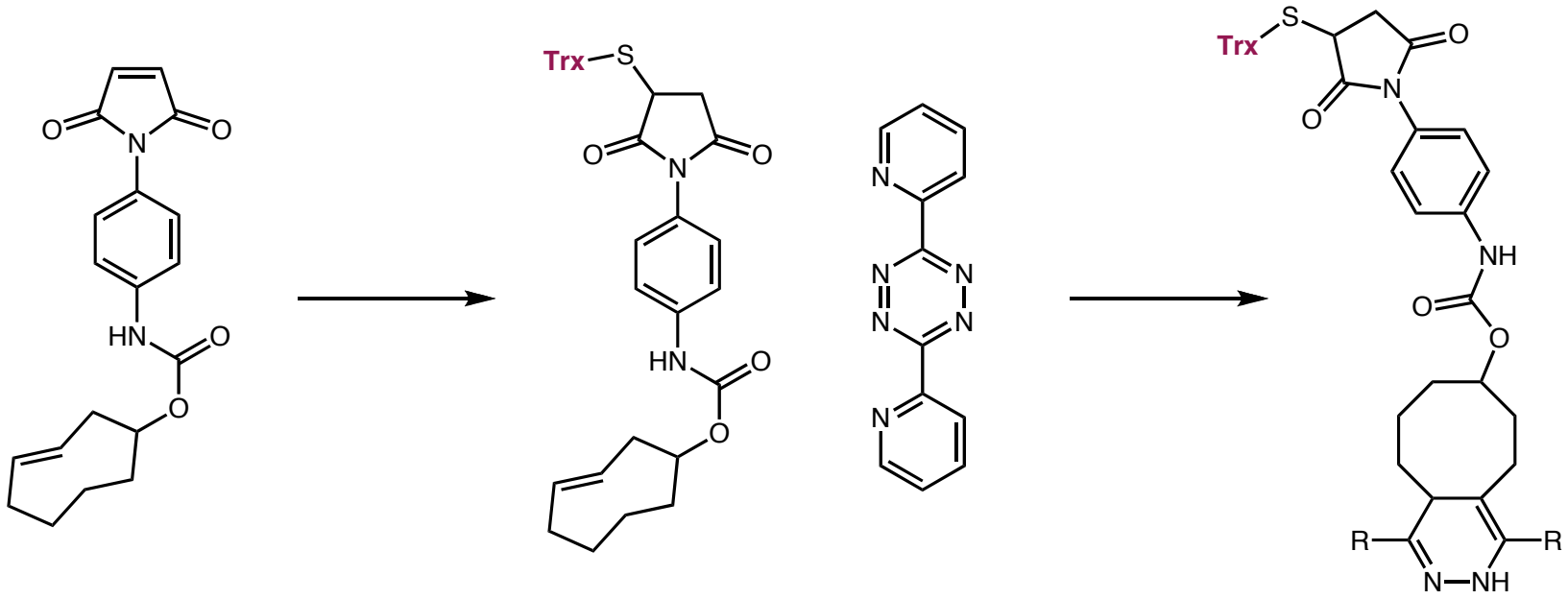
Inverse-Electron Demand Diels-Alder



$k_2 = 2000\ M^{-1}s^{-1}$

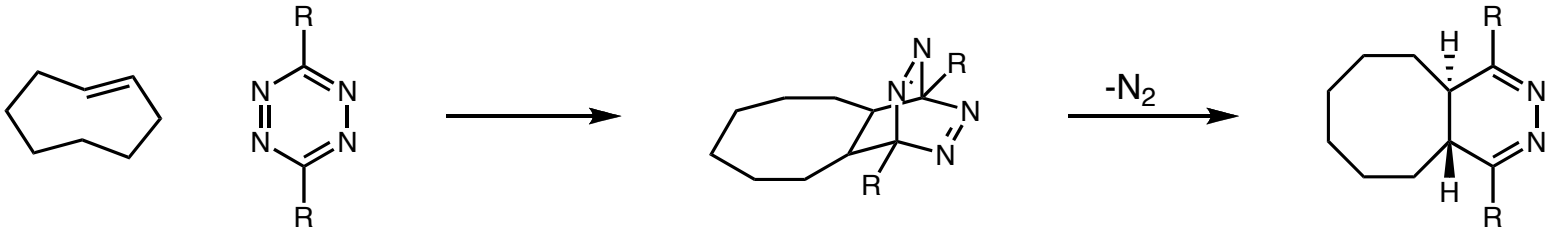
thioredoxin

Trx—SH

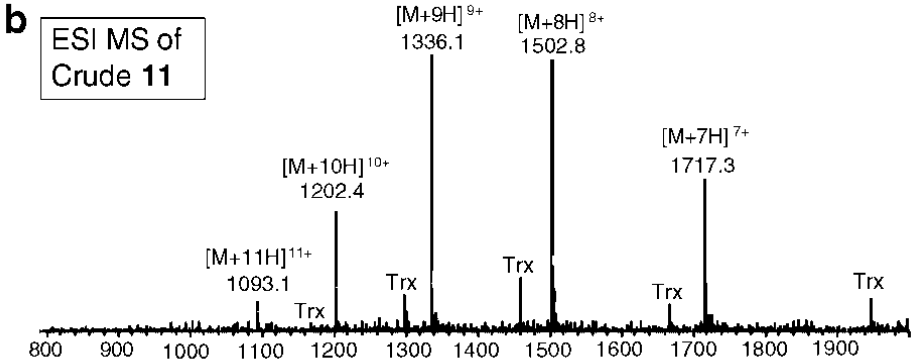


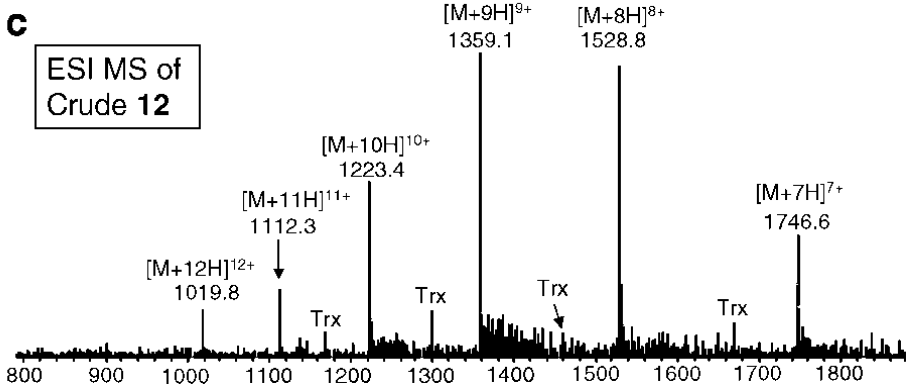
Blackman, M. L.; Royzen, M.; Fox, J. M. *J. Am. Chem. Soc.* **2008**, *130*, 13518-13519.

Inverse-Electron Demand Diels-Alder



$k_2 = 2000\ M^{-1}s^{-1}$





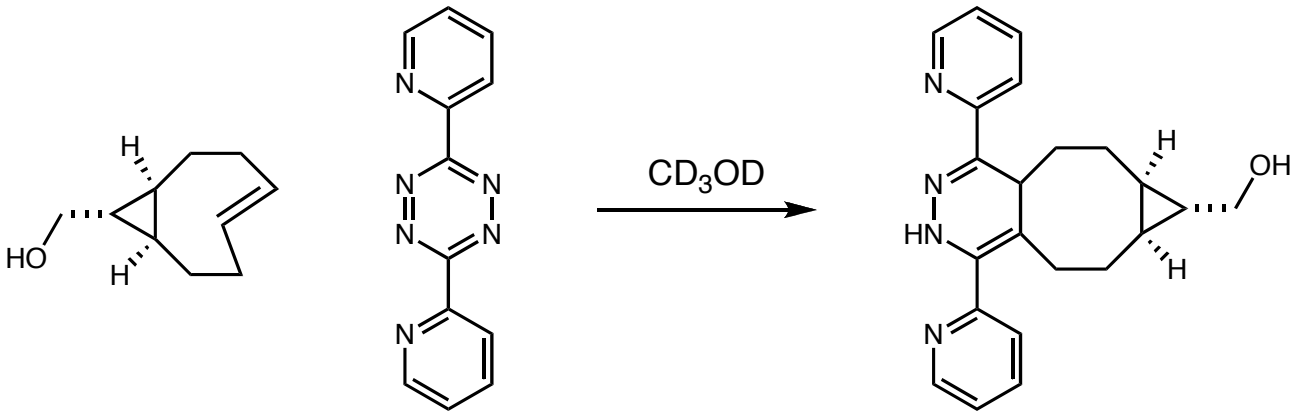
Trx = 11700 Da

Michael product = 12014 Da

Diels-Alder product = 12222 Da

Blackman, M. L.; Royzen, M.; Fox, J. M. *J. Am. Chem. Soc.* **2008**, *130*, 13518-13519.

Inverse-Electron Demand Diels-Alder

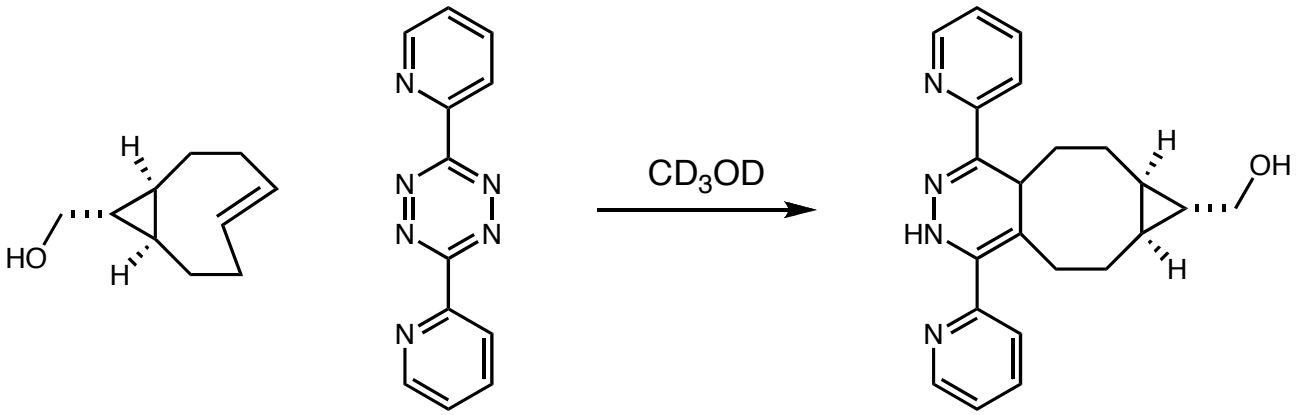


$k_2 = 22{,}000\ M^{-1}s^{-1}$

trans-cyclooctene ring is designed via computation (M06L/6-311+G(d,p)-optimized transition structure)

Taylor, M. T.; Blackman, M. L.; Dmitrenko, O.; Fox, J. M. *J. Am. Chem. Soc.* **2011**, *133*, 9646-9649.

Inverse-Electron Demand Diels-Alder



$k_2 = 22{,}000\ M^{-1}s^{-1}$

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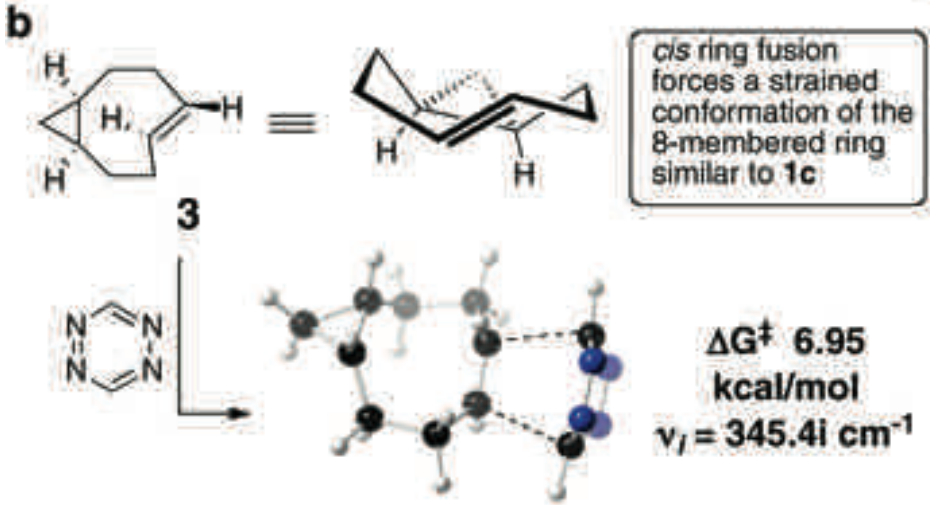
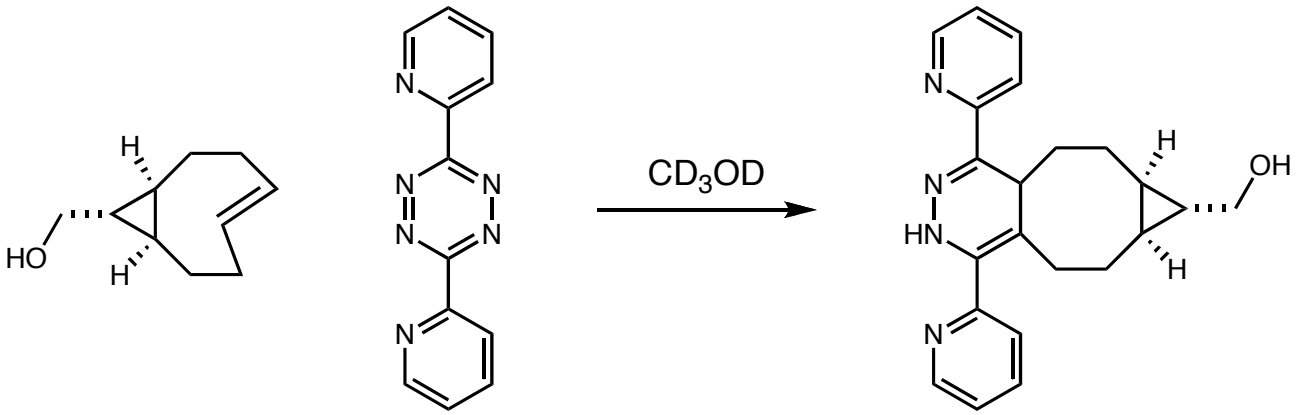


Taylor, M. T.; Blackman, M. L.; Dmitrenko, O.; Fox, J. M. *J. Am. Chem. Soc.* **2011**, *133*, 9646-9649.

Inverse-Electron Demand Diels-Alder



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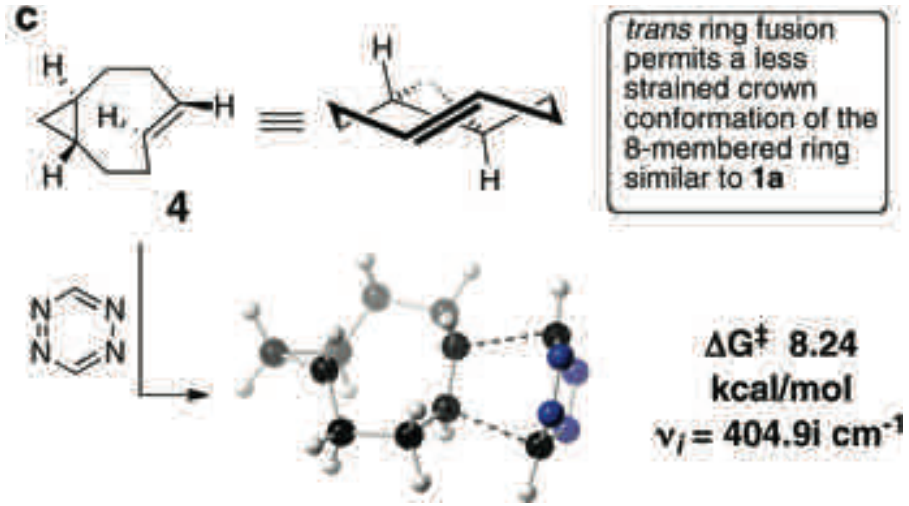
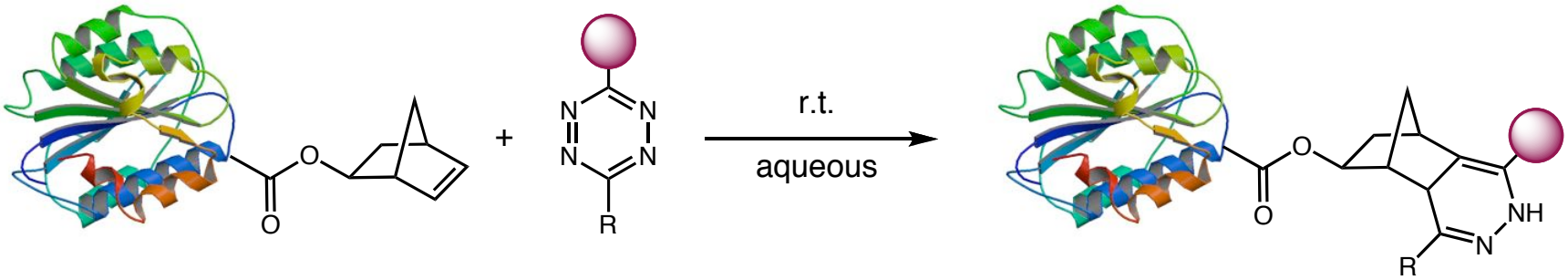


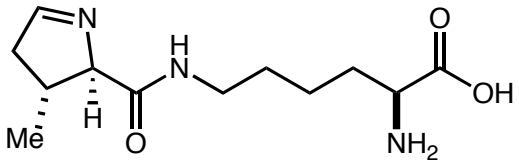
Taylor, M. T.; Blackman, M. L.; Dmitrenko, O.; Fox, J. M. *J. Am. Chem. Soc.* **2011**, *133*, 9646-9649.

Inverse-Electron Demand Diels-Alder

Genetically encoded norbornene directs site-specific cellular protein labelling



Orthogonal synthetase/tRNA pair used to install a norbornene-containing amino acid



Pyrrolysine
"The 22nd Amino Acid"

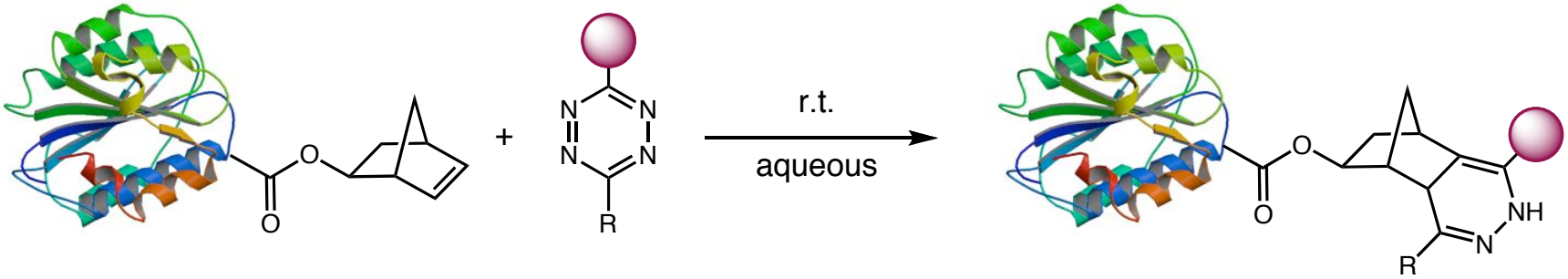
Genetically coded amino acid used by some methanogenic archaea.

Pyrrolysyl-tRNA synthetase/$tRNA_{CUA}$ is orthogonal to endogenous tRNAs and aminoacyl-tRNA synthetases in *E. coli* and eukaryotic cells.

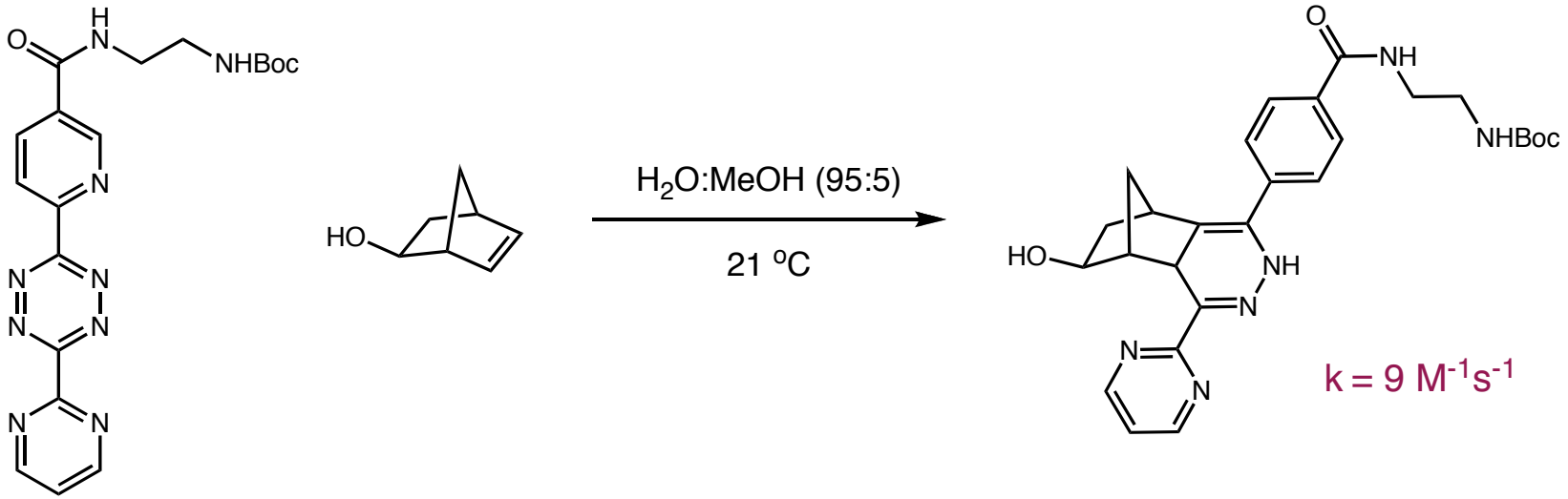
Lang, K.; Davis, L.; Torres-Kolbus, J.; Chou, C.; Deiters, A.; Chin, J. W. *Nature Chem.* **2012**, *Advanced Online Publication*.

Inverse-Electron Demand Diels-Alder

Genetically encoded norbornene directs site-specific cellular protein labelling



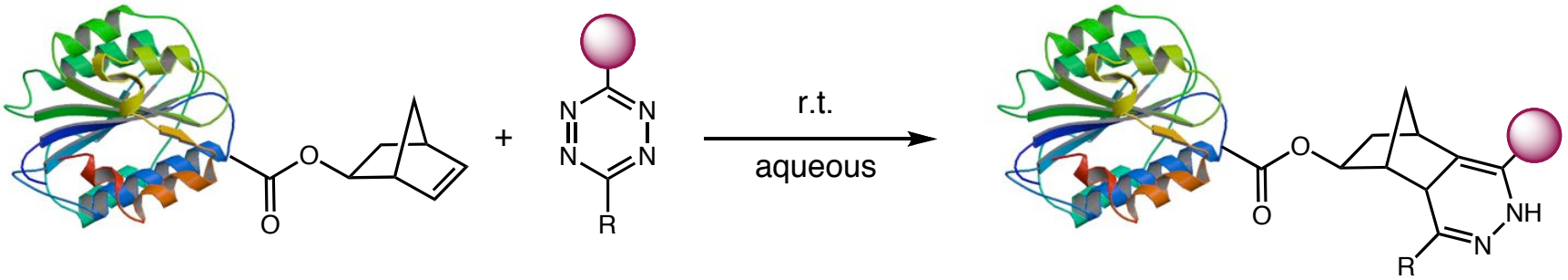
Orthogonal synthetase/tRNA pair used to install a norbornene-containing amino acid



Lang, K.; Davis, L.; Torres-Kolbus, J.; Chou, C.; Deiters, A.; Chin, J. W. *Nature Chem.* **2012**, *Advanced Online Publication*.

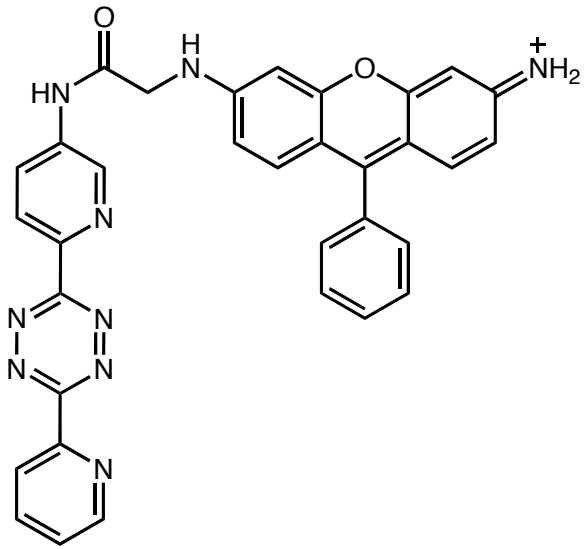
Inverse-Electron Demand Diels-Alder

Genetically encoded norbornene directs site-specific cellular protein labelling



The fluorescence of certain probes increased by a 5-10 fold increase after the cycloaddition

"turn-on" fluorophorogenic probes - tetrazine can quench fluorophore via energy transfer



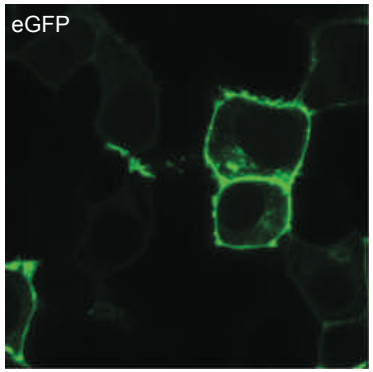
tetramethylrhomdamine (**TAMRA**)

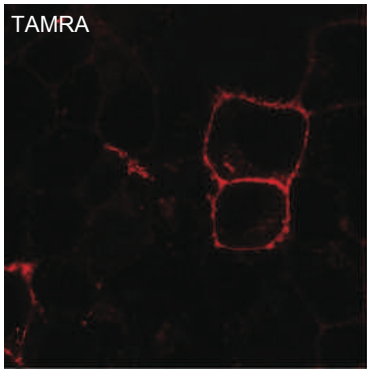
Lang, K.; Davis, L.; Torres-Kolbus, J.; Chou, C.; Deiters, A.; Chin, J. W. *Nature Chem.* **2012**, *Advanced Online Publication.*

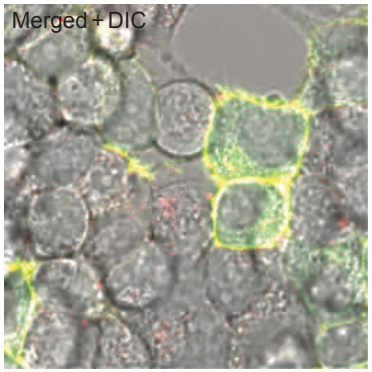
Inverse-Electron Demand Diels-Alder

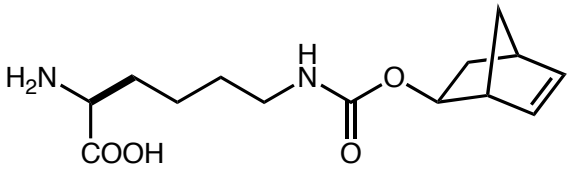
Cycloaddition tested with mutated epidermal growth factor receptor (EGFR-GFP)

2 (1 mM)

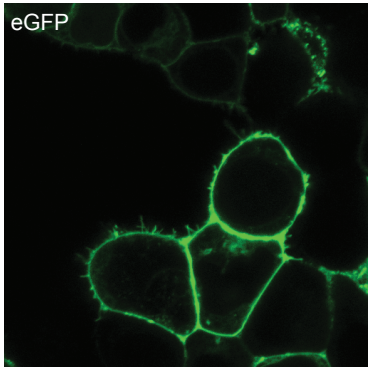


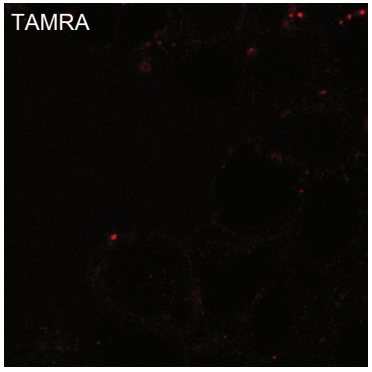


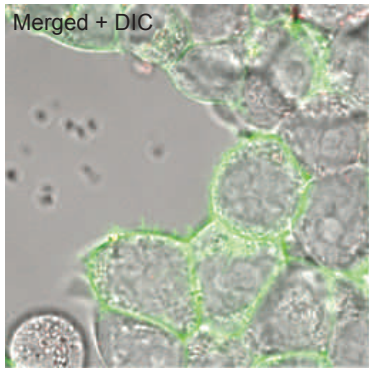


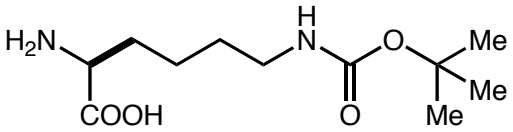


3 (1 mM)



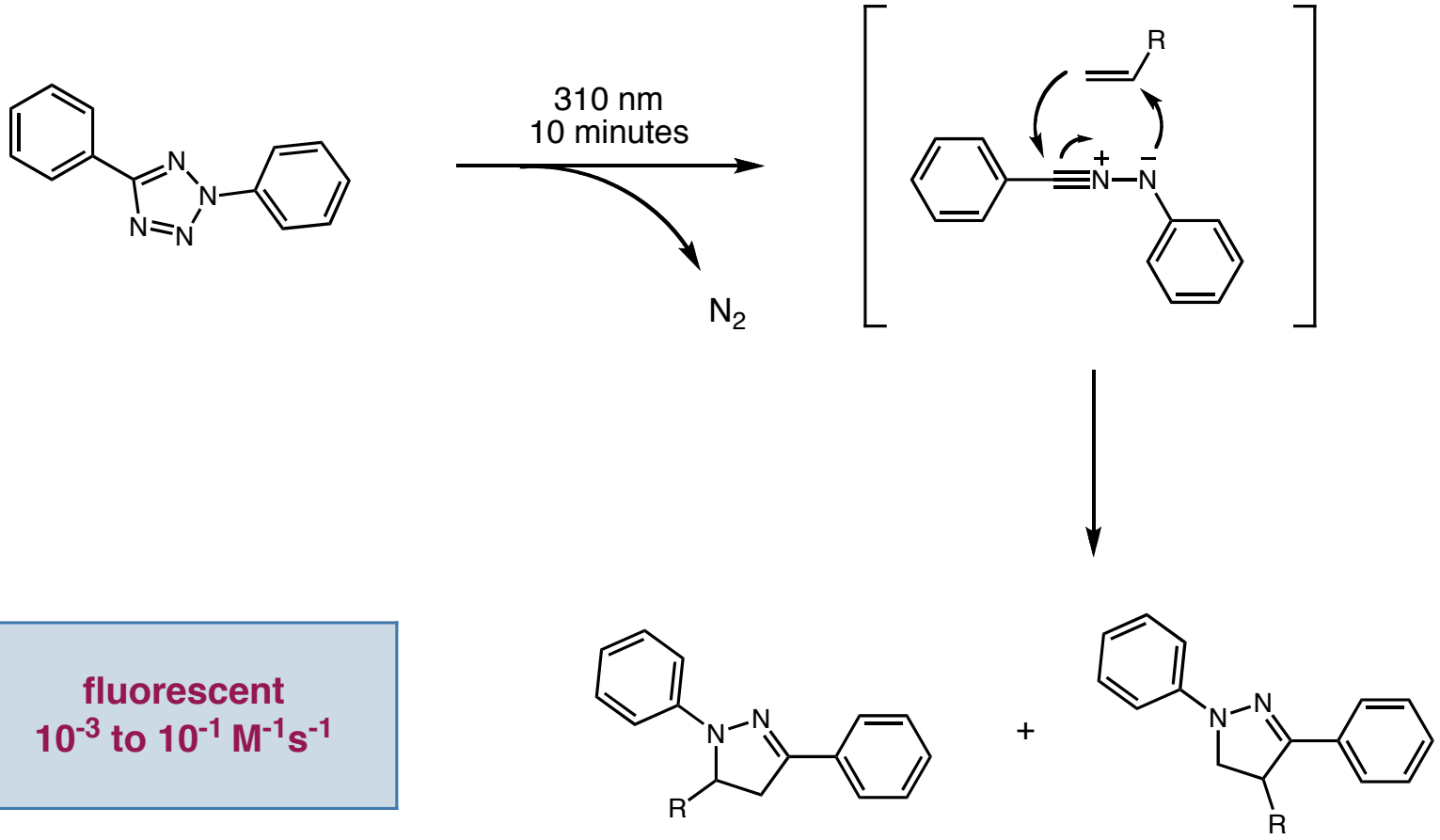






Lang, K.; Davis, L.; Torres-Kolbus, J.; Chou, C.; Deiters, A.; Chin, J. W. *Nature Chem.* **2012**, *Advanced Online Publication*.

Photochemical 1,3-Dipolar Cycloaddition



fluorescent
10^{-3} to 10^{-1} $M^{-1}s^{-1}$

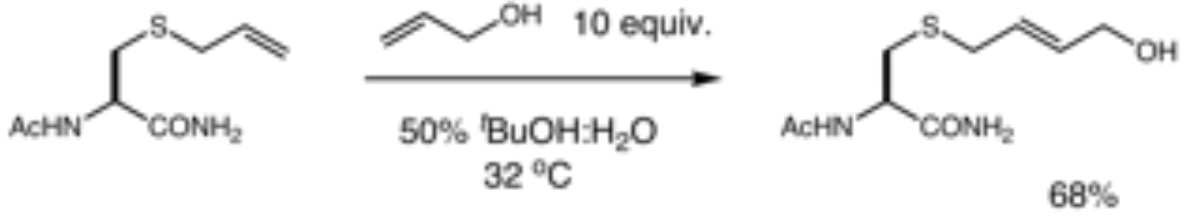
Song, W.; Wang, Y.; Qu, J.; Madden, M. M.; Lin, Q. *Angew. Chem. Int. Ed.* **2008**, *47*, 2832.

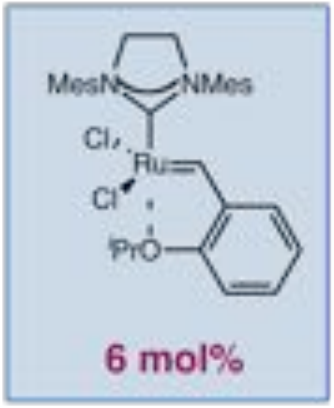
Wang, Y.; Hu, W. J.; Song, W.; Lim, R. K. V.; Lin, Q. *Org. Lett.* **2008**, *10*, 3752.

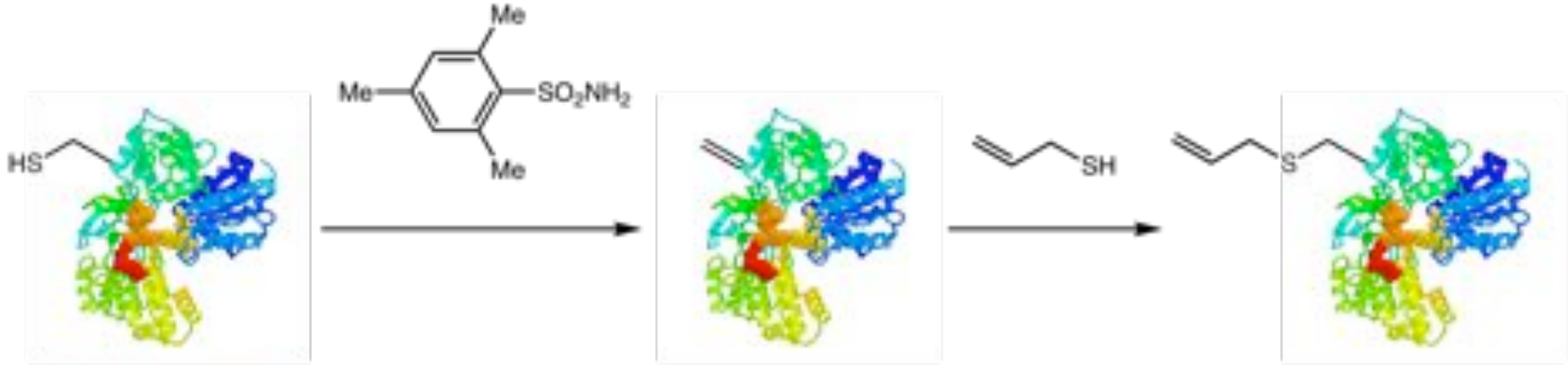
Song, W.; Wang, Y.; Lin, Q. *J. Am. Chem. Soc.* **2008**, *130*, 9654.

Bioorthogonal Cross-Metathesis

Interestingly, allyl sulfides are required due to purported sulfur coordination to the ruthenium center.







serine protease
subtilisin *Bacillus lentus* (SBL)

Bernardes, G. J. L.; Chalker, J. M.; Errey, J. C.; Davis, B. G. *J. Am. Chem. Soc.* **2008**, *130*, 5052-5053.

Lin, Y. A.; Chalker, J. M.; Floyd, N.; Bernardes, G. J. L.; Davis, B. G. *J. Am. Chem. Soc.* **2008**, *130*, 9642-9643.

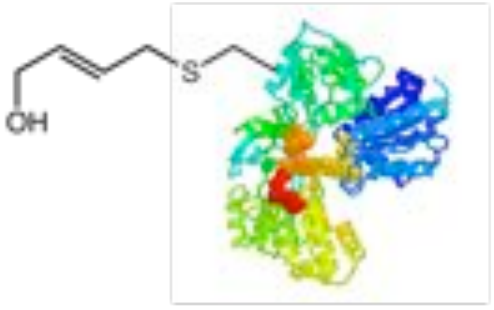
Bioorthogonal Cross-Metathesis

0.01 mM

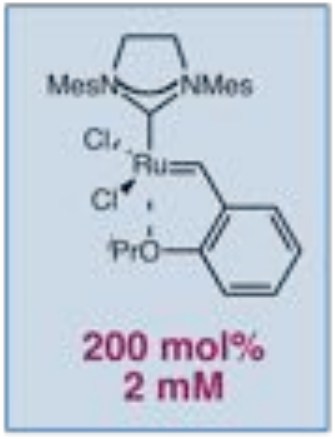
$MgCl_2$ or NaCl

100 mM

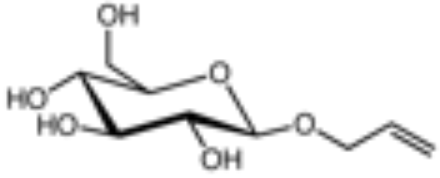
30% ^{t}BuOH:H_2O
37 °C



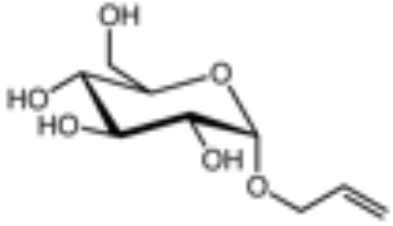
90% yield



200 mol%
2 mM



50%



60%

Salts, such as $MgCl_2$ or NaCl, were added to disrupt any nonproductive chelation to ruthenium

Bernardes, G. J. L.; Chalker, J. M.; Errey, J. C.; Davis, B. G. *J. Am. Chem. Soc.* **2008**, *130*, 5052-5053.

Lin, Y. A.; Chalker, J. M.; Floyd, N.; Bernardes, G. J. L.; Davis, B. G. *J. Am. Chem. Soc.* **2008**, *130*, 9642-9643.

Future Outlook

Groups 15 elements have been particularly lucrative.

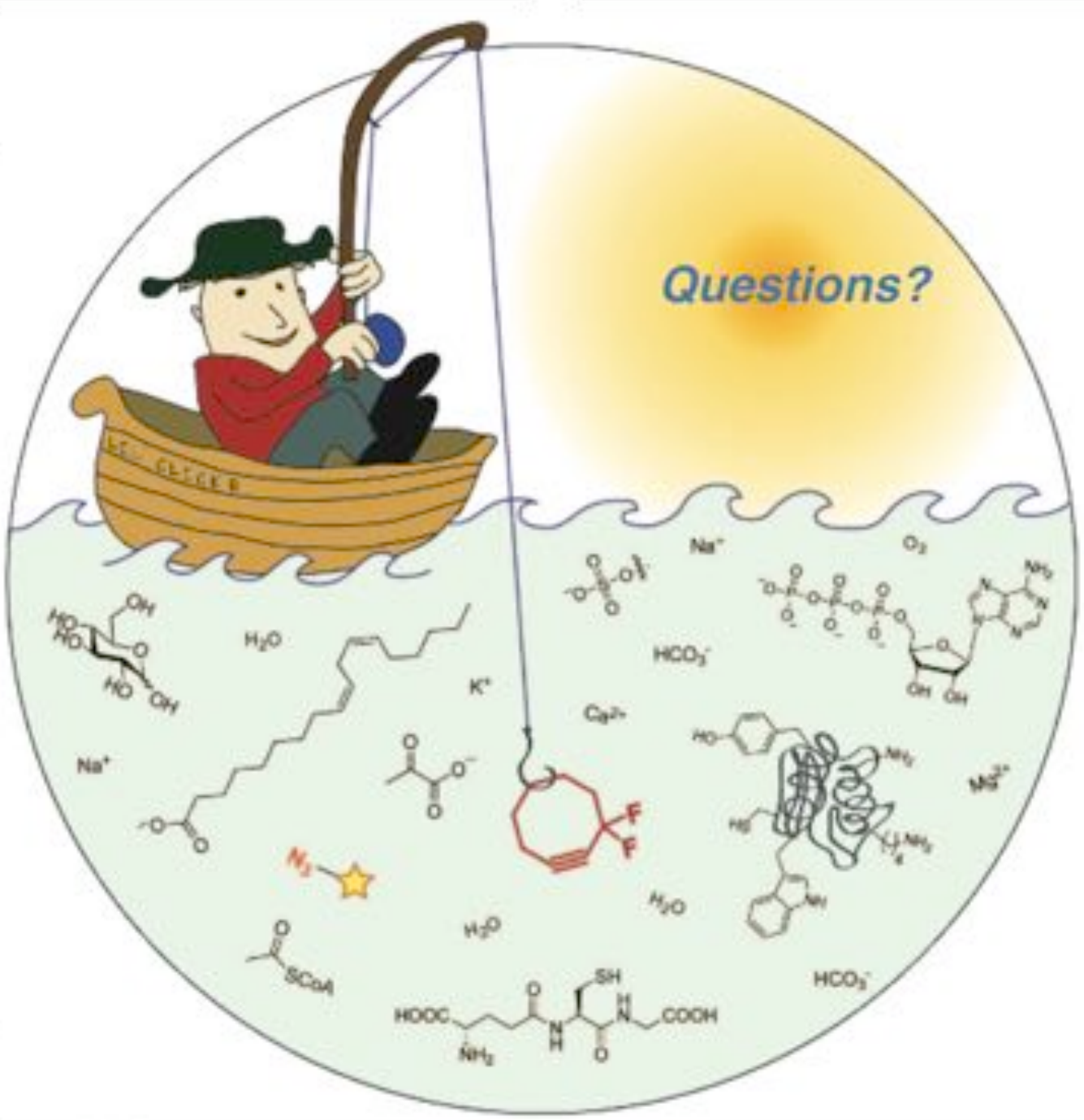
Perhaps larger elements in this group, like bismuth or antimony, may be of use.

Pericyclic reactions have been very promising as well, due to concerted mechanisms that leave little room for interruption from other components.

Applications with other sources of energy, such as light or ultrasound, may occur.

We may see the extension of chemical reporters to other small-molecule metabolites.

Sletten, E. M.; Bertozzi, C. R. *Angew. Chem. Int. Ed.* **2009**, *48*, 6974-6998.


Questions?
Na+
O2
HCO3-
H2O
K+
Ca2+
Na+
Mg2+
N3
F
F
H2O
H2O
SCoA
HOOC
NH2
SH
COOH
HCO3-