PYRROC

an alternative to Copper catalysts in strain promoted azide-alkyne cycloaddition reactions

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Dr. Corinna Gröst

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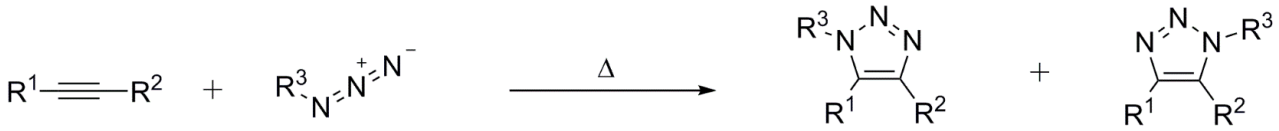
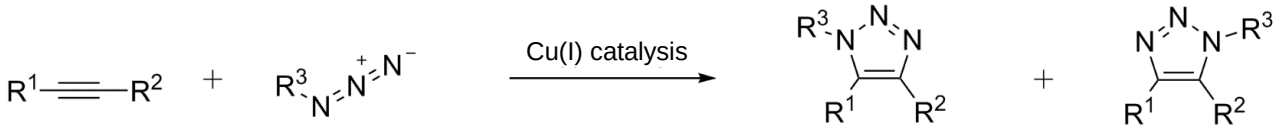
Azide Alkyne Cycloaddition

- Huisgen (1960er): 1,3-dipolar cycloaddition



- Sharpless and Meldal (2002):



➔ **Click Chemistry:**

- Insensitivity against water or oxygen
- Broad applicability
- High yield
- No or easily separable side-products

Angew. Chem., Int. Ed., 1963, ***2***, 565. *Angew. Chem., Int. Ed.,* 2002, **41**, 2596. *J. Org. Chem.,* 2002, ***67***, 3057. *Angew. Chem., Int. Ed.,* 2001, **40**, 2004.

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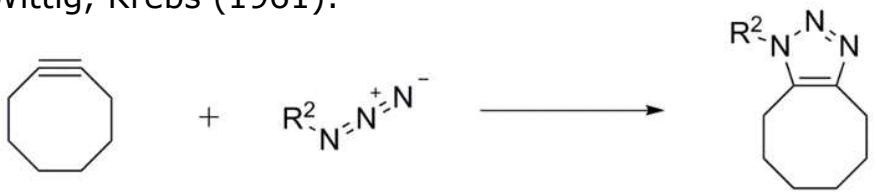
SPAAC

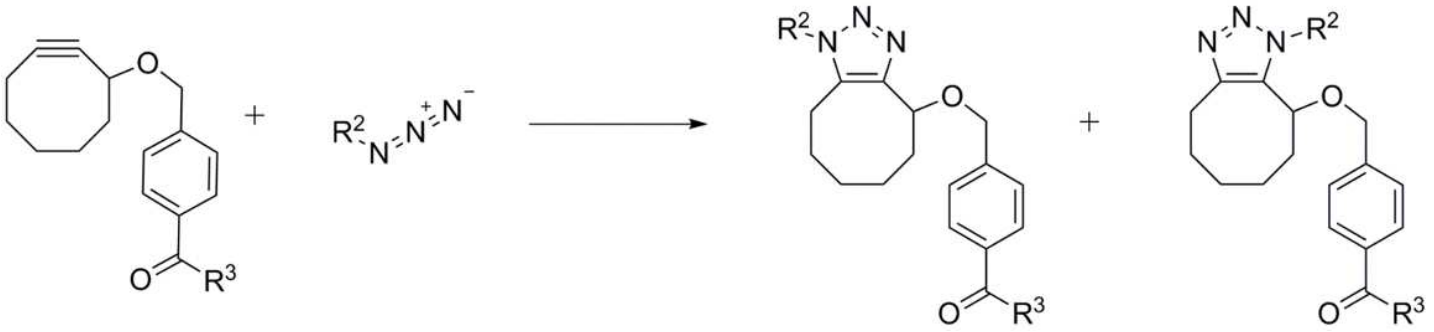
strain-promoted azide-alkyne cycloaddition

- Cu(I) cytotoxic ➔ **click reaction through ring strain**

Wittig, Krebs (1961):



Bertozzi (2004):



Chem. Ber., 1961, ***94***, 3260. *J. Am. Chem. Soc.,* 2004, **126**, 15046.

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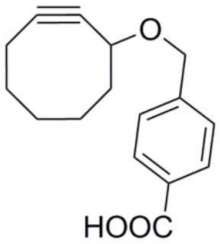
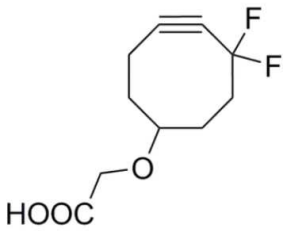
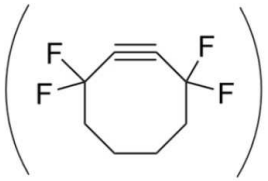
Cyclooctynes



OCT
$k = 2.4 \times 10^{-3}$

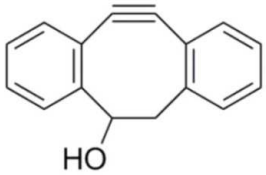


DIFO
7.6×10^{-2}

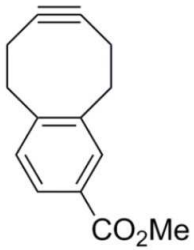


3,3,8,8-Tetrafluorcyclooctin
calc.: 1.8*

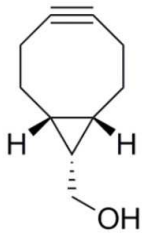
reactivity



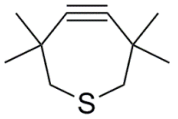
DIBO
5.7×10^{-2}



COMBO
2.4×10^{-1}



***endo*-BCN**
1.4×10^{-1}



TMTH
4.0

k (in $M^{-1}s^{-1}$) determined through ^{1}H-NMR measurement in the reaction with benzyl azide. * calc. for the reaction with MeN_3.

J. Am. Chem. Soc., 2008, **130**, 11486. *J. Am. Chem. Soc.,* 2009, **131**, 8121. *ChemBioChem,* 2011, **12**, 1912. *Chem. - A Europ. J.,* 2012, **18**, 822. *Angew. Chem., Int. Ed.,* 2010, **49**, 9422. *Org. Lett.,* 2014, **16**, 1634. *J. Org. Chem.,* 2012, **77**, 2093.

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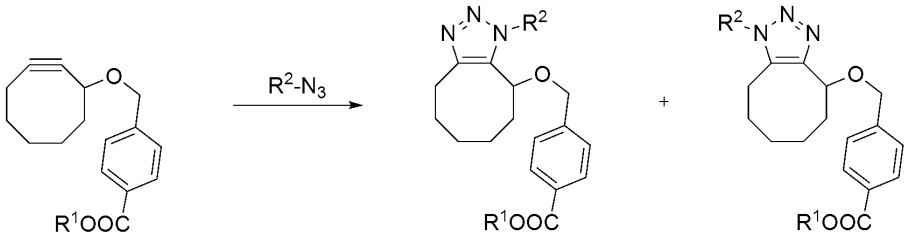
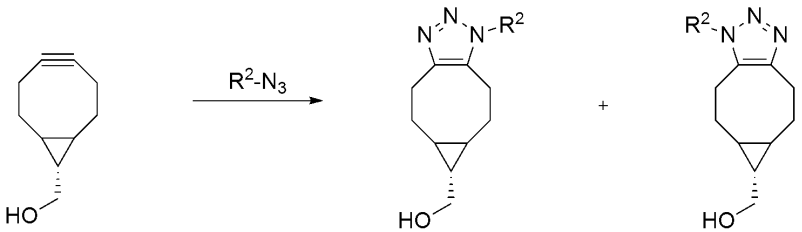
Cyclooctynes

OCT



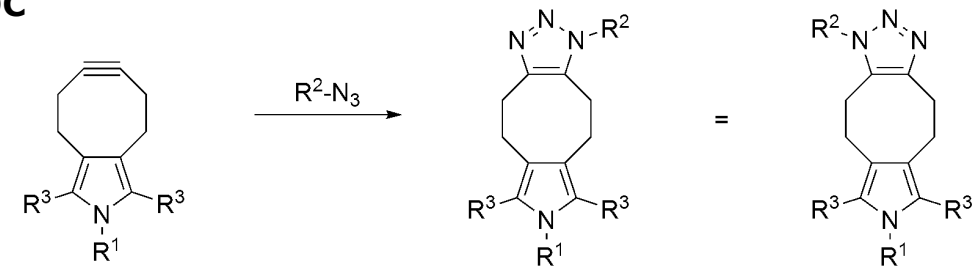
Regioisomers

BCN



Enantiomers or diastereoisomers

PYRROC



Identical substances

Org. Biomol. Chem., 2015, **13**, 3866.

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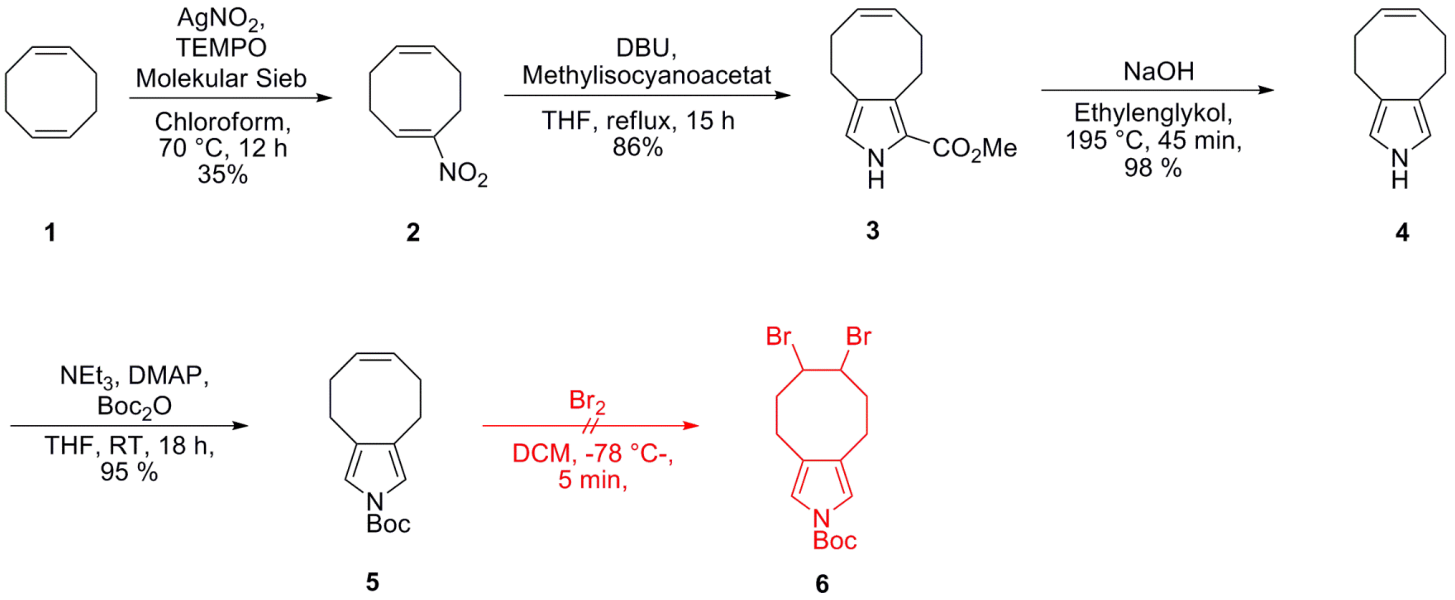

Org. Biomol. Chem., 2015, **13**, 3866.

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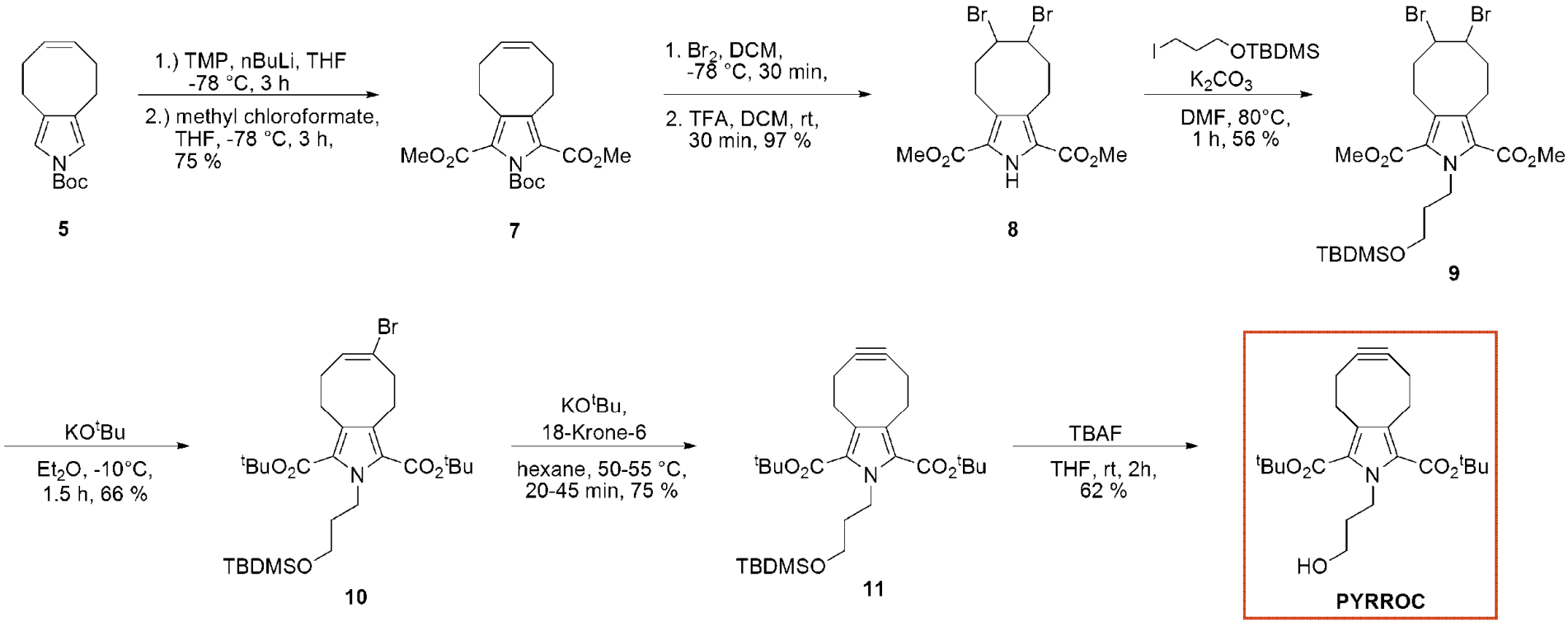

Org. Biomol. Chem., 2015, **13**, 3866.

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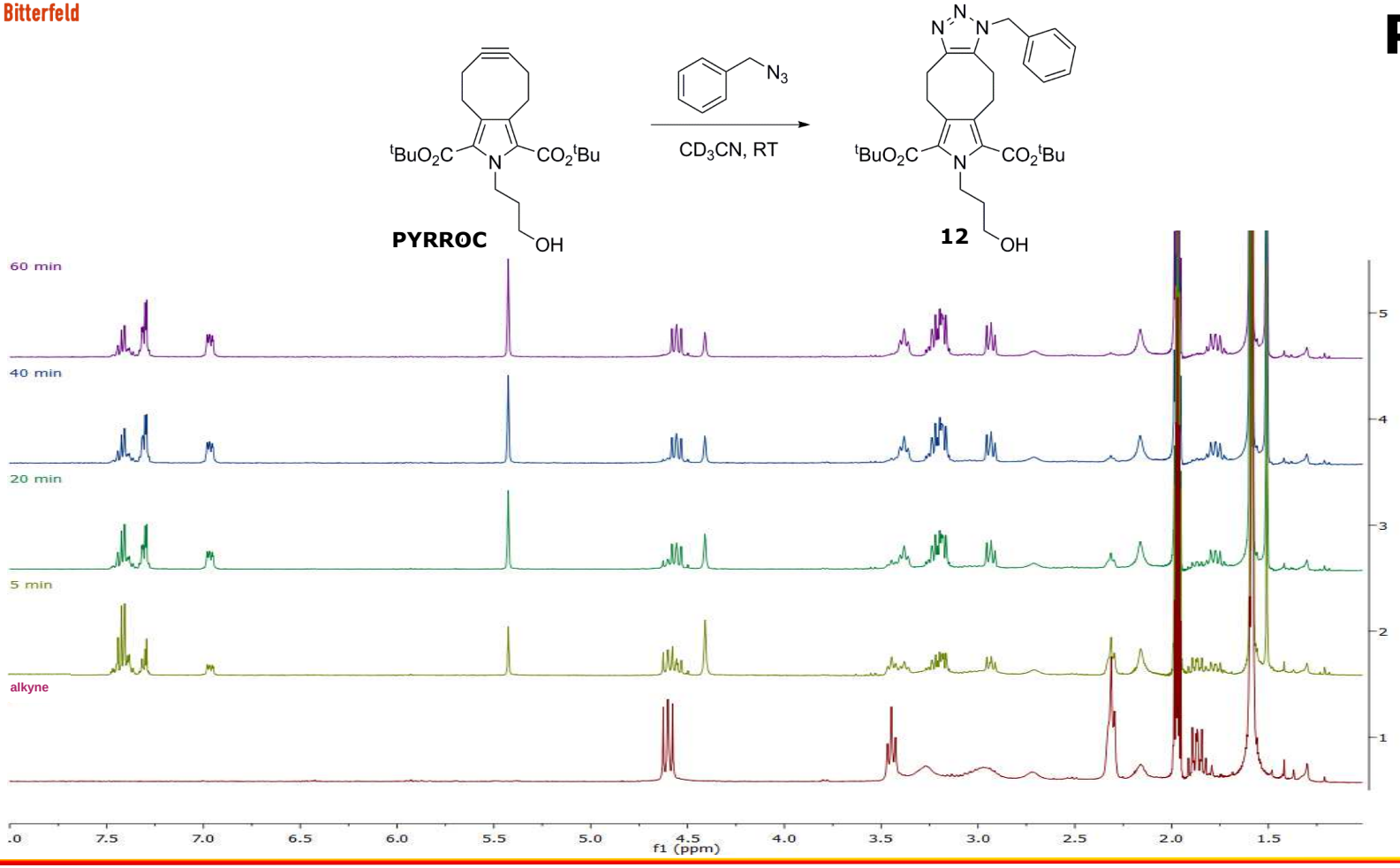
PYRROC



Org. Biomol. Chem., 2015, **13**, 3866.

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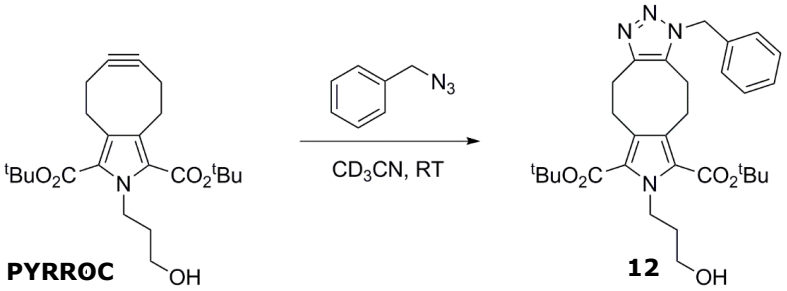
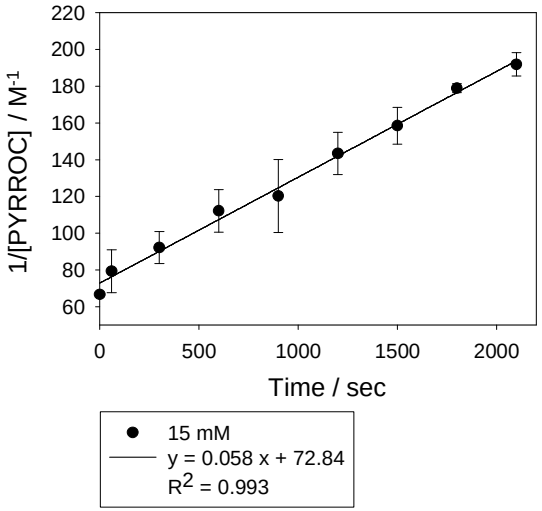
PYRROC





k = 0.058 ± 0.004 M^{-1} s^{-1}

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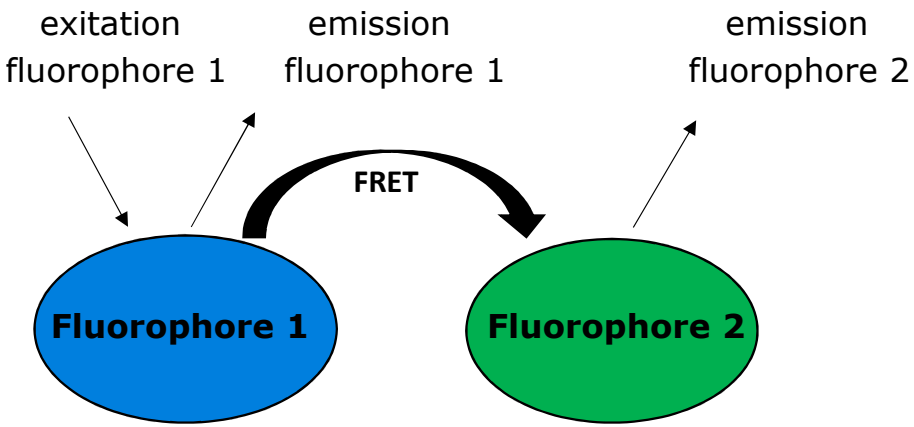
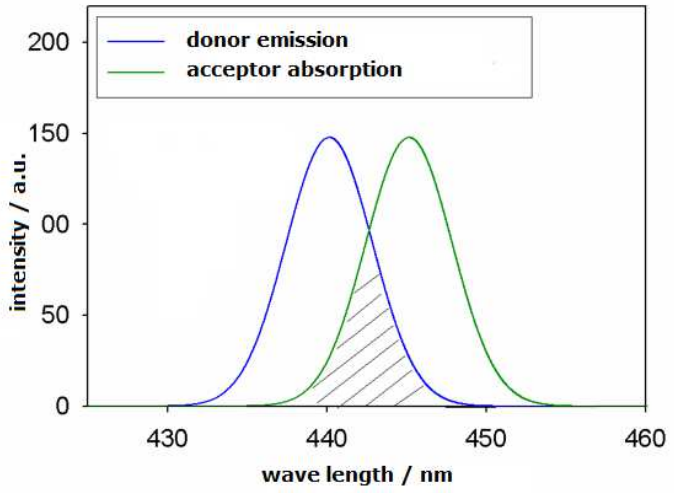
FRET

Förster resonance energy transfer





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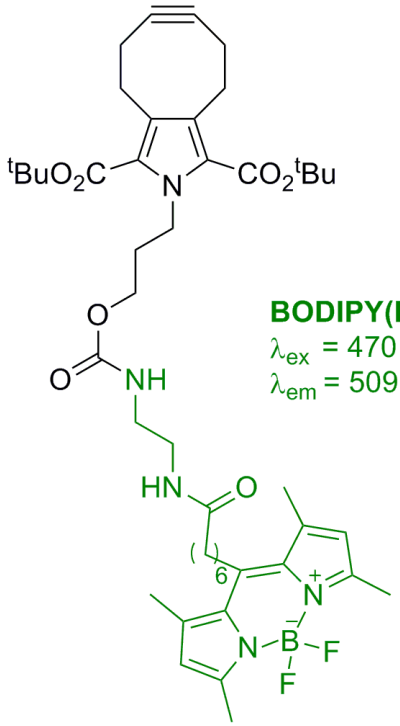
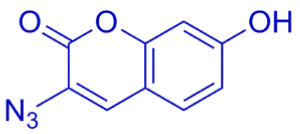


BODIPY(FL)-PYRROC

λ_{ex} = 470 nm

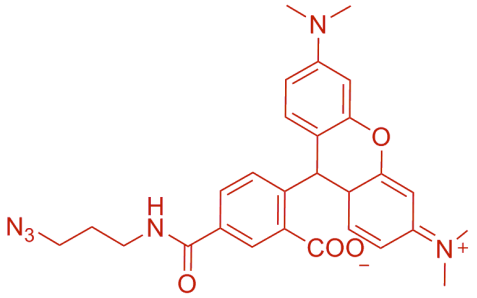
λ_{em} = 509 nm



3-Azido-7-hydroxycoumarine

λ_{ex} = 400 nm

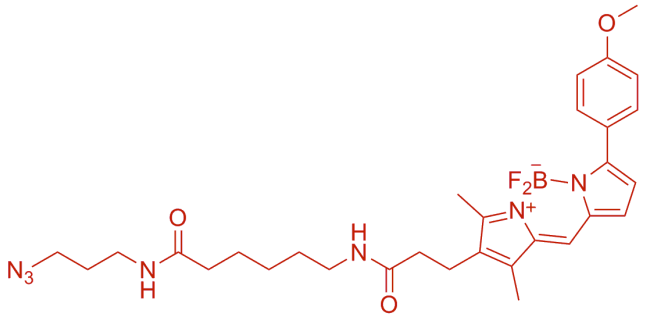
λ_{em} = 470 nm



5-TAMRA-azide

λ_{ex} = 540 nm

λ_{em} = 575 nm



BODIPY(TMR)-azide

λ_{ex} = 545 nm

λ_{em} = 575 nm

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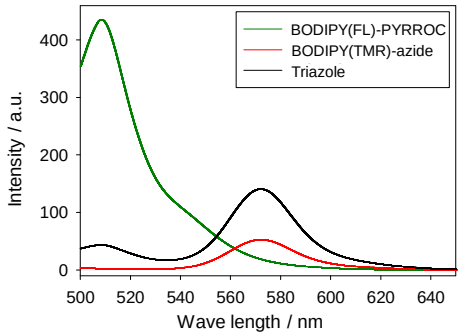
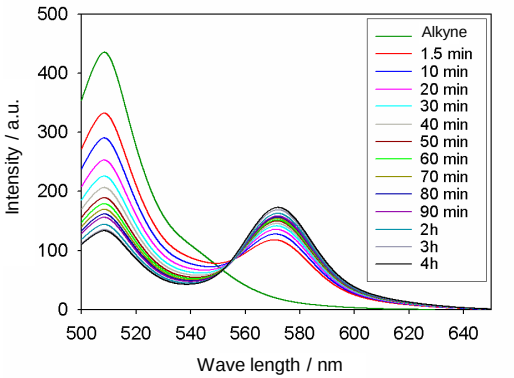
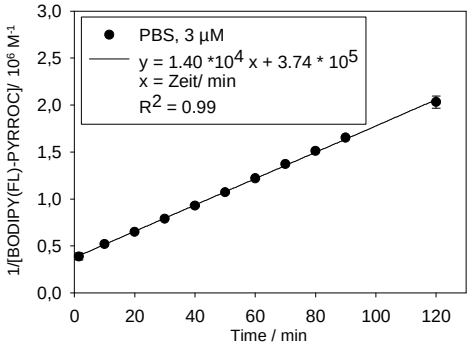
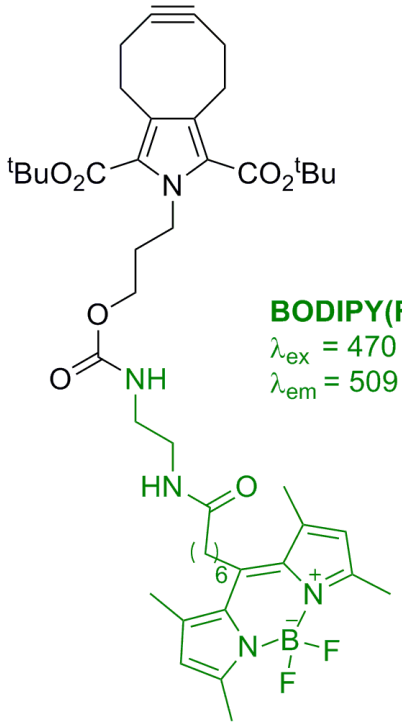
PYRROC



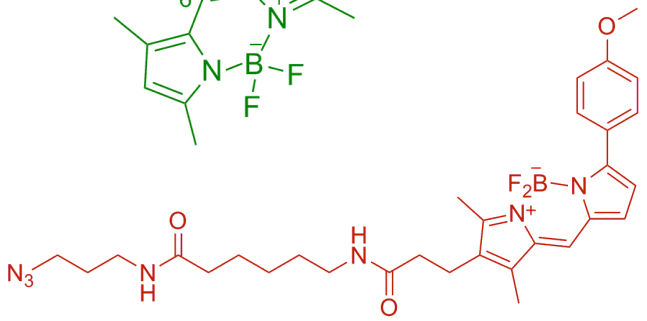




k = 234 ± 2 M^{-1} s^{-1}



BODIPY(FL)-PYRROC
λ_{ex} = 470 nm
λ_{em} = 509 nm



BODIPY(TMR)-azide
λ_{ex} = 545 nm
λ_{em} = 575 nm

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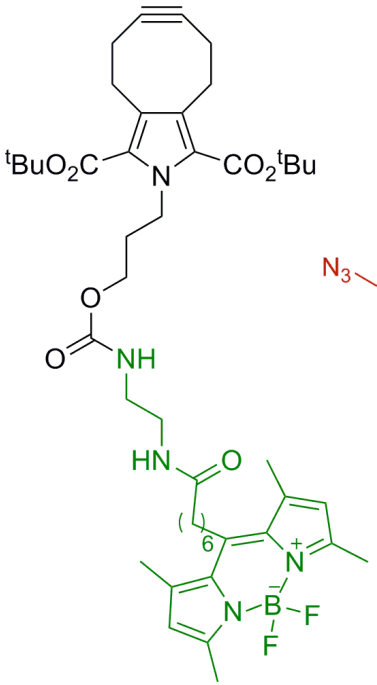
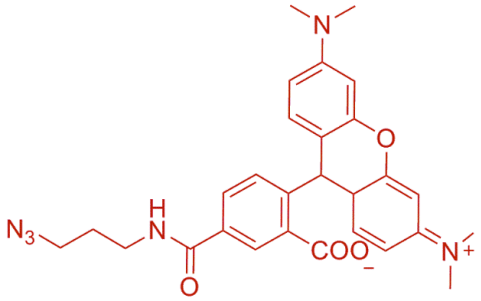
PYRROC

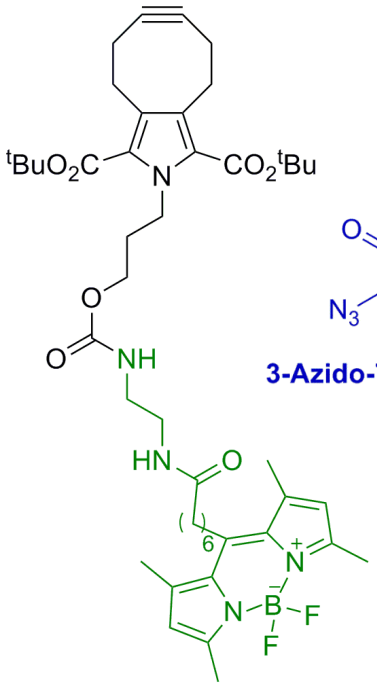


BODIPY(FL)-PYRROC

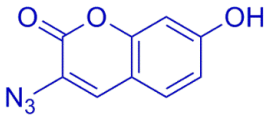


5-TAMRA-azide

k = 13.9 ± 0.3 M^{-1} s^{-1}

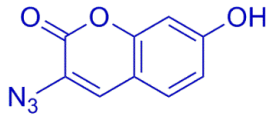


BODIPY(FL)-PYRROC

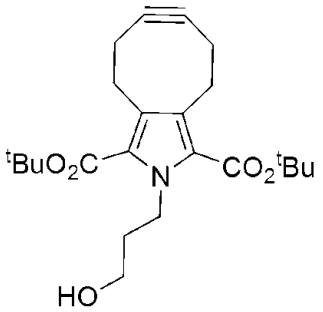


3-Azido-7-hydroxycoumarine

k = 930 ± 26 M^{-1} s^{-1}



3-Azido-7-hydroxycoumarine



PYRROC

k = 492 ± 43 M^{-1} s^{-1}

measured in PBS (phosphate buffered saline)

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Potential applications

Biochemistry

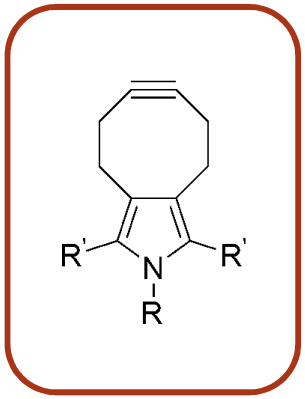
Fluorescence-labelling of biomolecules

in cells

in Organisms

Bioorthogonal reactions

with ≥ 2 functionalities in cells



Material Science

Synthesis of macromolecules and polymers

Drug discovery

Synthesis of compound libraries

Lead structure optimization

Target Guided Synthesis (TGS)

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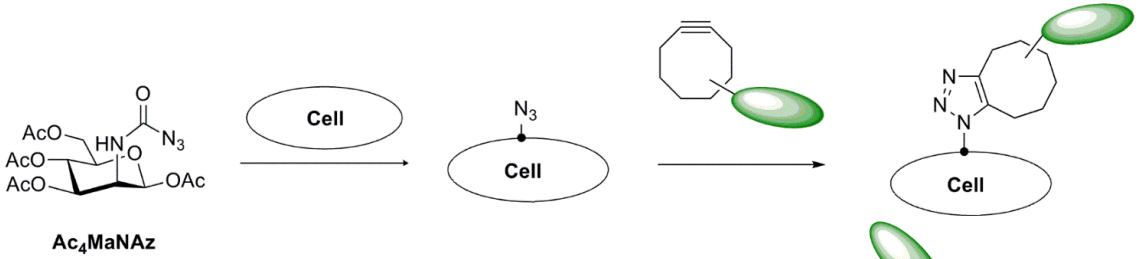
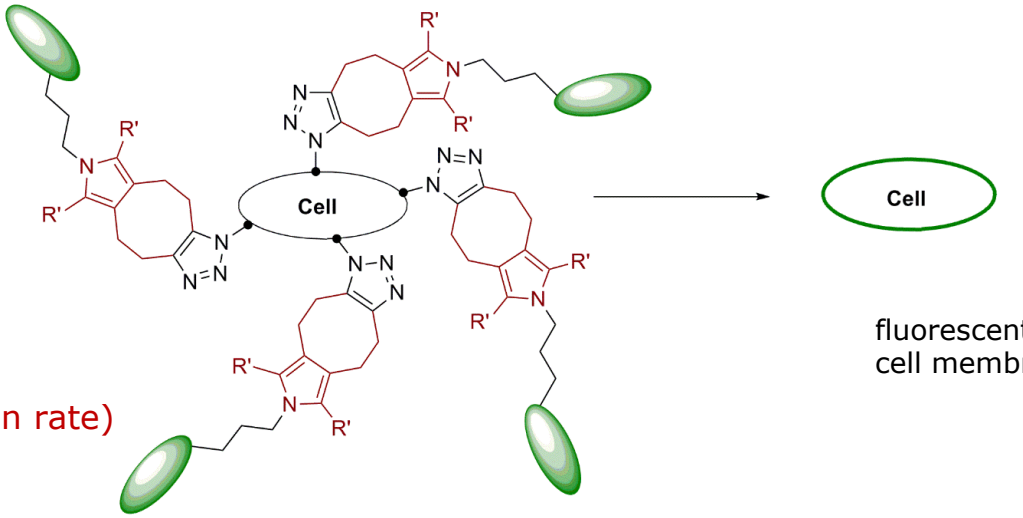
Fluorescence-labelling of biomolecules

Labelling of glycanes, proteins, enzymes

e.g. live cell imaging of cell membrane



with Pyrroc:



fluorescent
cell membrane

+ highly reactive PYRROC in aq. media

+ functunalization to fit special needs (in solubility, size, reaction rate)

J. Am. Chem. Soc., 2008, **130**, 11486. *Chem. Rev.,* 2013, **113**, 4905. *ChemBioChem,* 2015, **16**, 1314. *J. Am. Chem. Soc.,* 2003, **125**, 4686.

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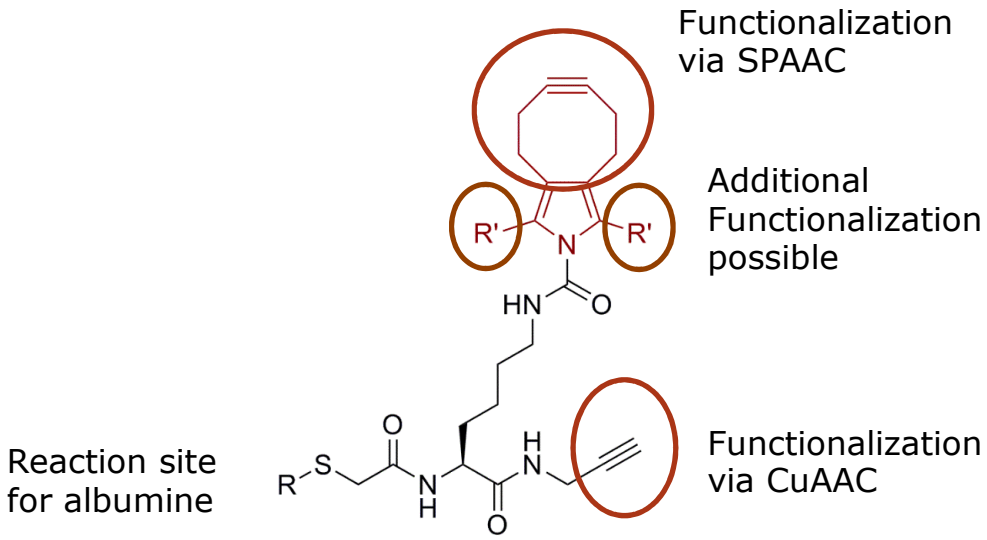
Bioorthogonal reactions

orthogonal synthetic handles

sequential biomolecule conjugations

e.g. development of biotherapeutics, antibody–drug conjugates, synthetic vaccines



Angew. Chem., Int. Ed., 2012, **51**, 6320. *Chem. Soc. Rev.,* 2007, **36**, 1249-62. *Org. Biomol. Chem.*, 2012, **10**, 548.

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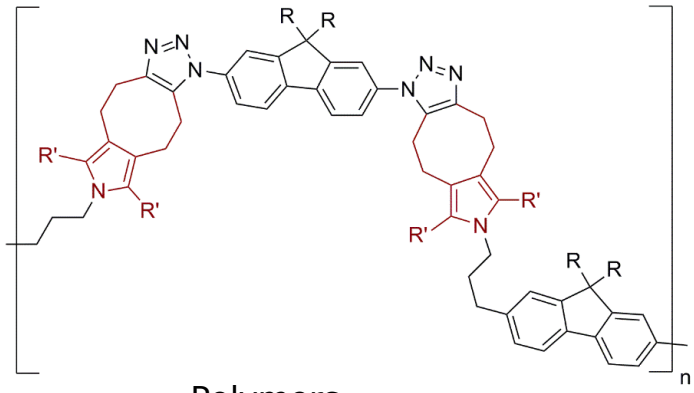
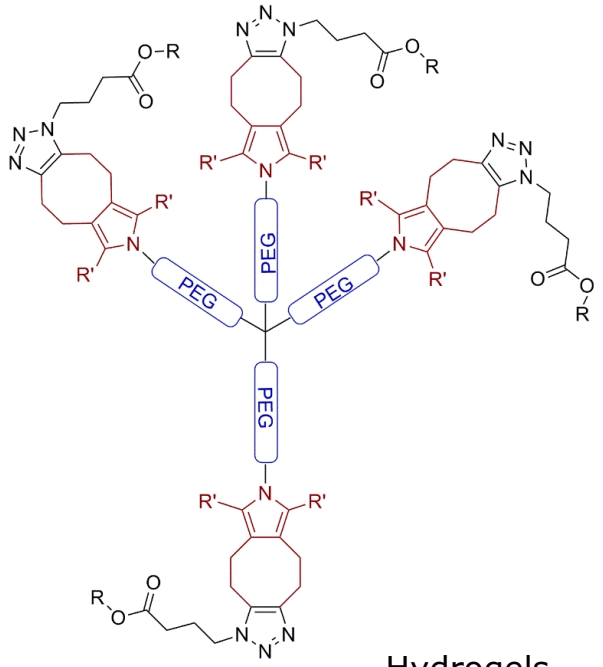
Synthesis of macromolecules



Polymers



Hydrogels

+ more functionalization and branching possible
+ interesting new properties
+ no side products (Cu,…)

Angew. Chem., Int. Ed., 2008, **46**, 1018. *Nat. Chem.*, 2011, **3**, 925. *Chem. Soc. Rev.,* 2007, **36**, 1249-62. *Pharm. Res.* 2012, **29**, 902.

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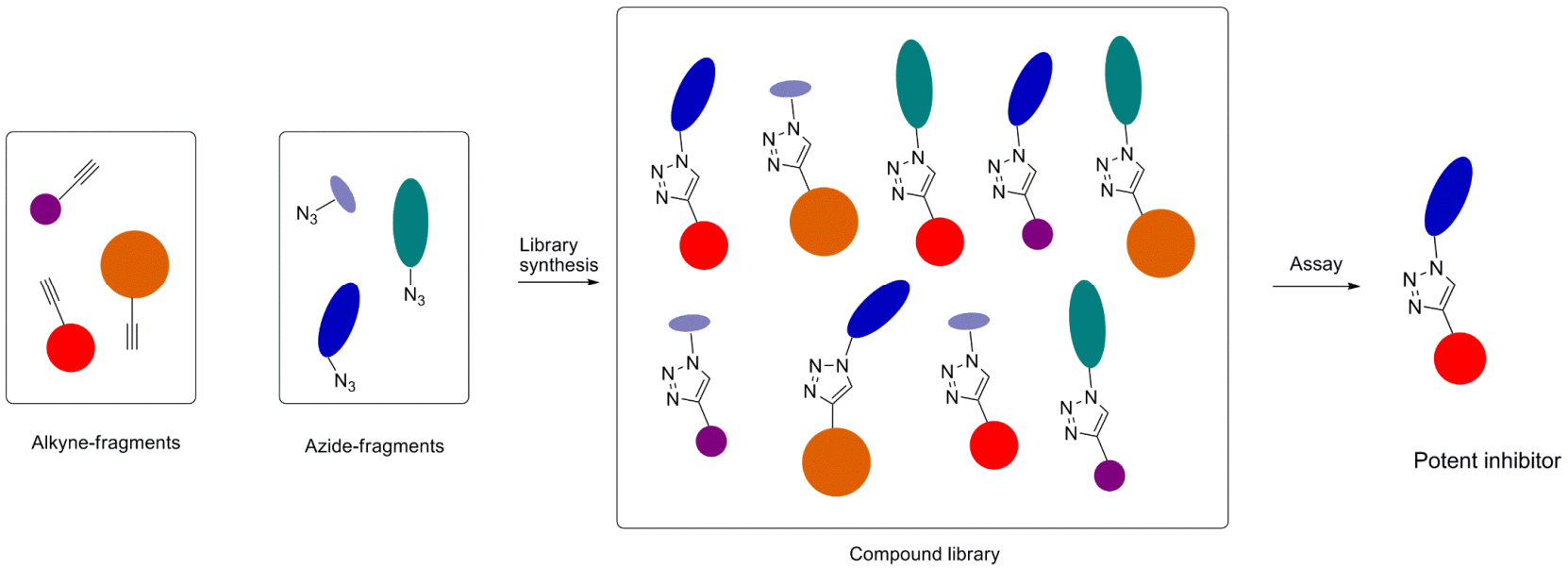
Compound libraries

Synthesis of compound libraries and lead structure optimization through high-throughput screening



+ fast reaction + no side products (Cu,…) + new scaffold

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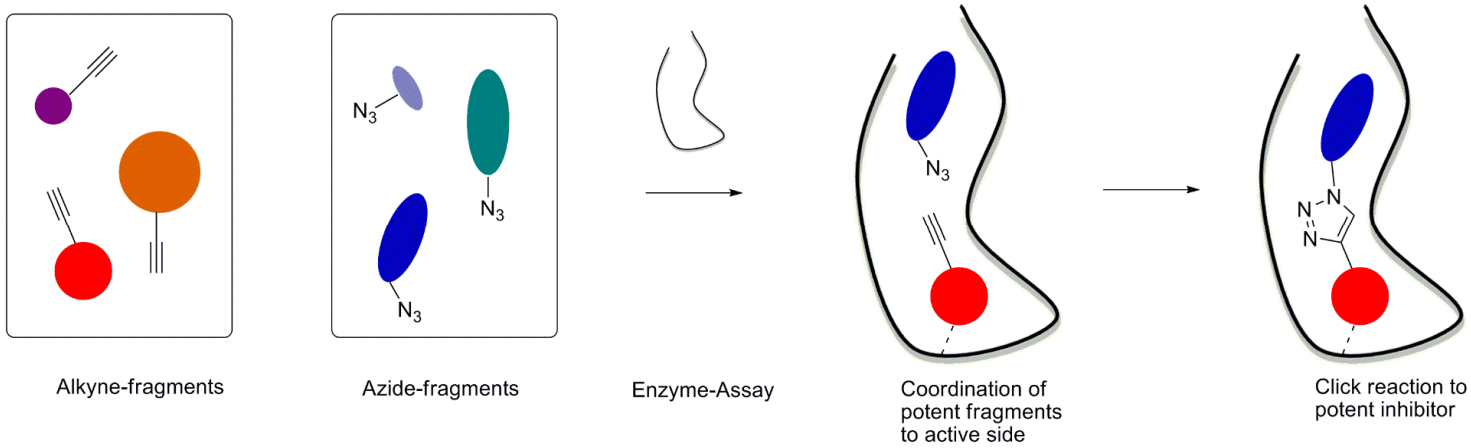
Target Guided Synthesis

Reaction at the active side of the enzyme
e.g. screening for inhibitors of Histonedeacetylase, HIV-1 Protease or Acetylcholinesterase



Angew. Chem., Int. Ed., 2002, **41**, 1053. *Angew. Chem., Int. Ed.*, 2010, **49**, 6817. *Angew. Chem., Int. Ed.*, 2006, **45**, 1435.

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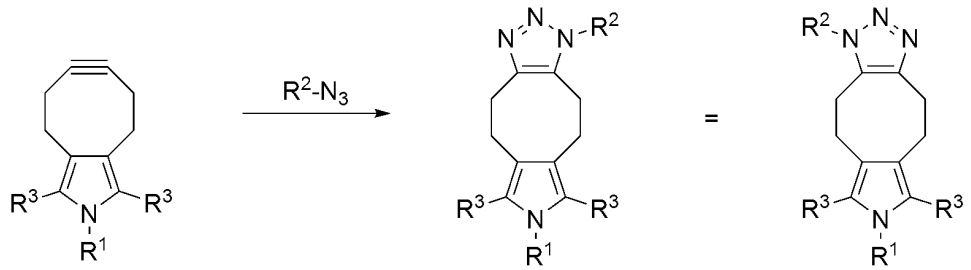
Target Guided Synthesis

Reaction on protein surfaces
inhibition of protein-protein interactions



PYRROC

+ large molecules synthesized inside the cell
+ fast reaction
+ selective
+ Isomer-free preparation of inhibitors favorable

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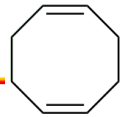
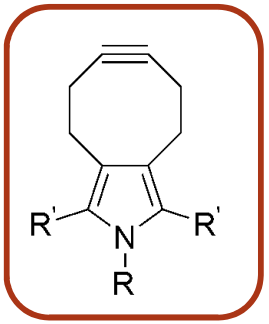
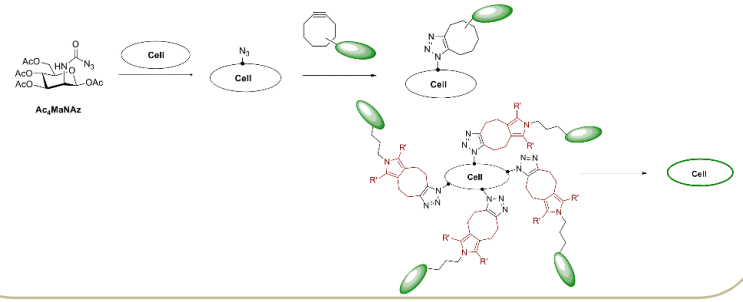
Summary



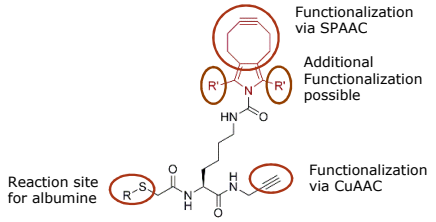


k up to 930 M^{-1} s^{-1}

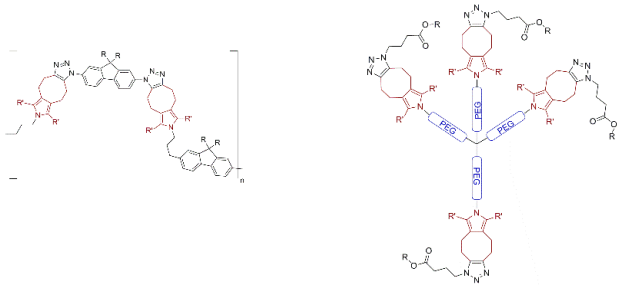
Fluorescence-labelling of biomolecules



Bioorthogonal reactions



Synthesis of macromolecules



Drug discovery