Difluorobenzocyclooctyne: Synthesis, Reactivity, and Stabilization by β-Cyclodextrin

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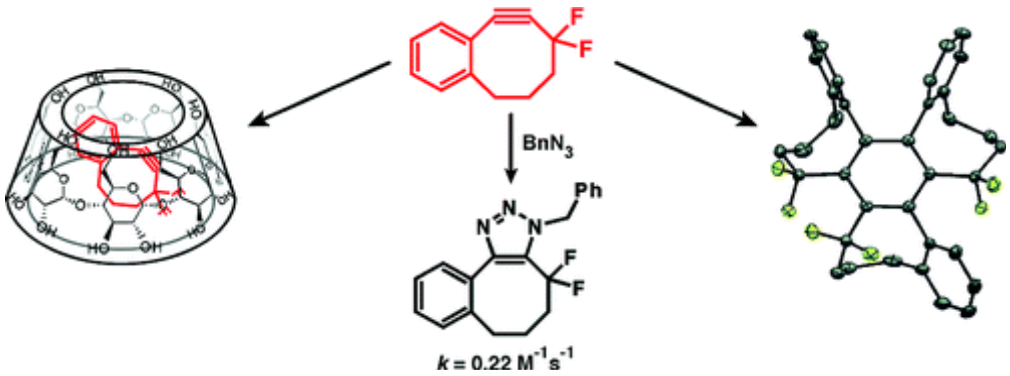
Departments of Chemistry and Molecular and Cell Biology and Howard Hughes Medical Institute, University of California, Berkeley, California 94720, and The Molecular Foundry, Lawrence Berkeley National Laboratory, Berkeley, California 94720



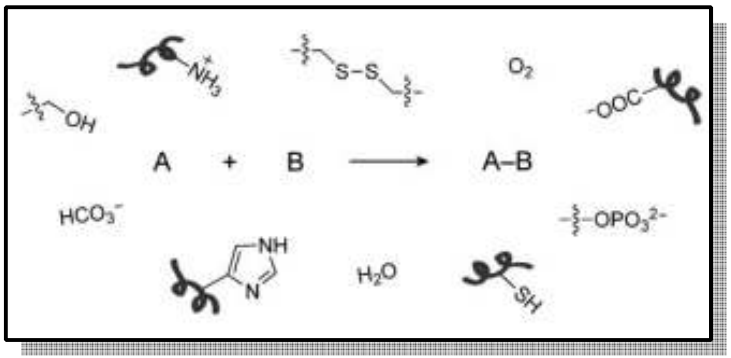
Current Literature Presentation

September 25th, 2010

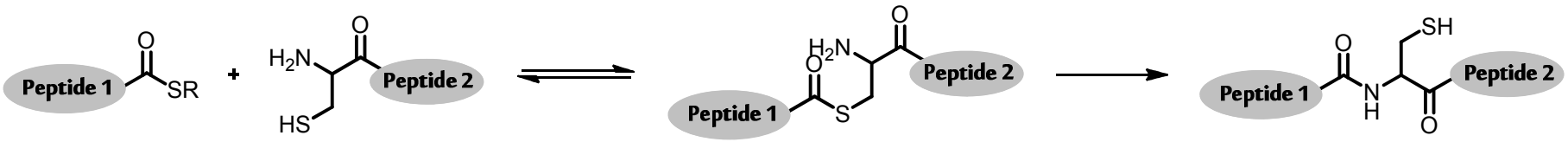
Nolan Griggs, Ph.D.

Bioorthogonal Reactions - Overview

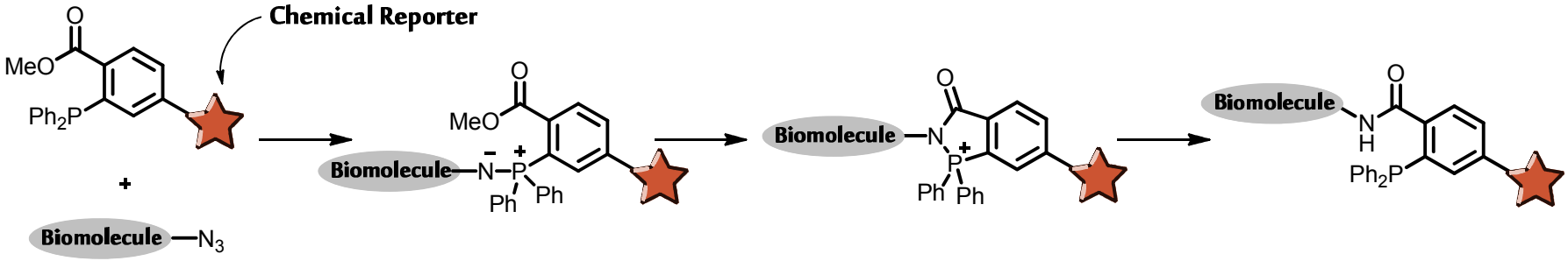
Definition: "Chemical reactions that do not interfere with biological processes."



Native Chemical Ligation (NCL) - Kent and co-workers, 1994:

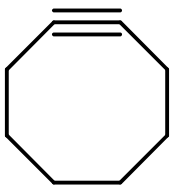


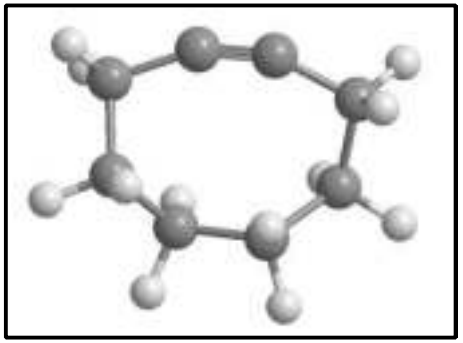
Staudinger Ligation - Bertozzi and co-workers, 2000:



Reveiw: Bertozzi, C.R., Sletten, E.M.; *Angew. Chem. Int. Ed.* **2009**, 6974.

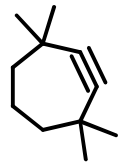
Cyclooctynes - Overview





- Smallest stable, unsubstituted cycloalkyne
- Alkyne bond angle = ~156 °
- Strain energy = ~18 kcal/mol

- The difference in hydrogenation enthalpies of cyclooctyne versus 4-octyne is ~10 kcal/mol[1]



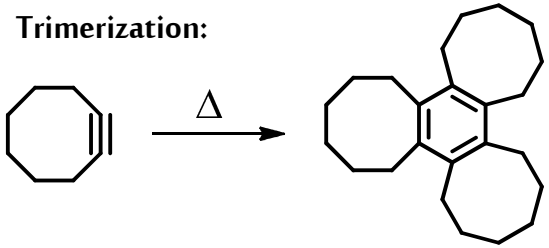
Note: 3,3,7,7-tetramethylcycloheptyne has been prepared and isolated:

Kimling, H., Krebs, A.; *Angew. Chem. Int. Ed.* **1971**, 509.

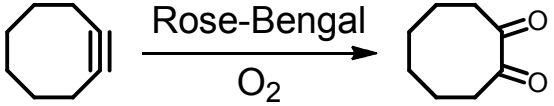
1. Turner, R.B., Mallon, B.J. et al.; *J. Am. Chem. Soc.* **1973**, 790.

Reactions of cyclooctynes:

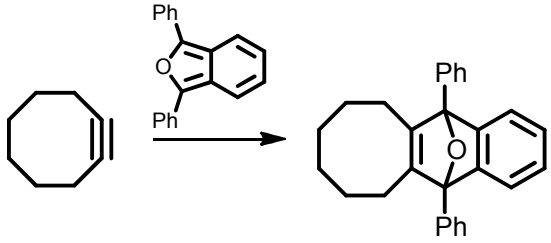
Trimerization:



Radical Reactions:



Cycloadditions:

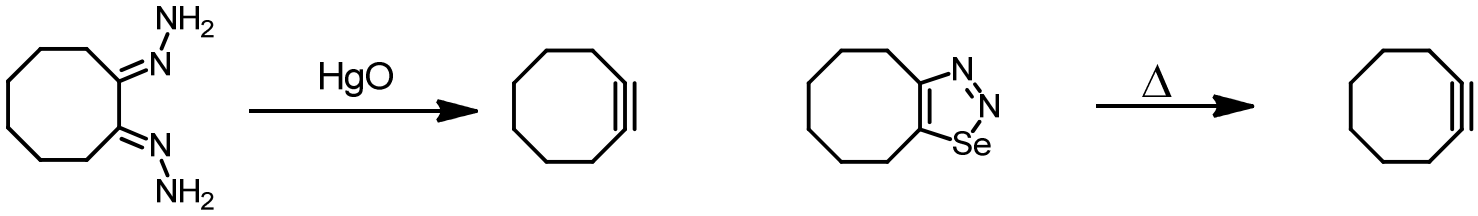


Meier, H. *Synthesis*, **1972**, 5, 235.

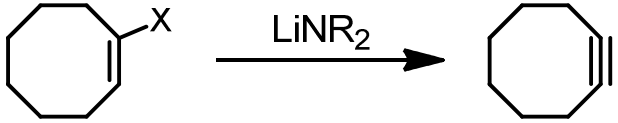
Krebs, A., Wilke, J.; *Topics in Current Chemistry,* **1983**, 109, 189-233

Synthesis of Cyclooctynes

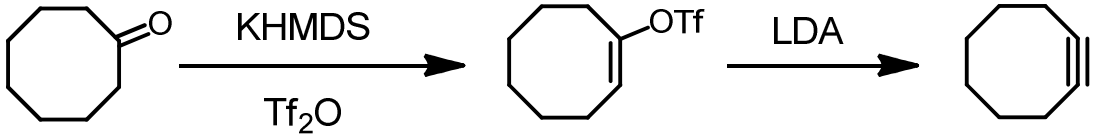
- **Oxidative and Thermal Decomposition:**

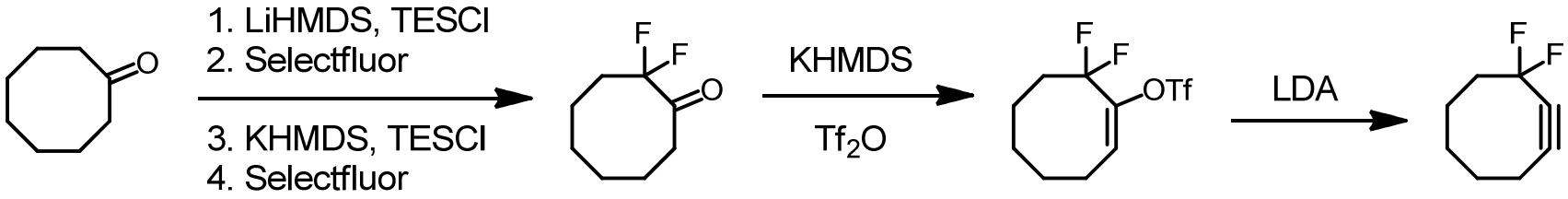


- **Elimination:**



- **Examples from Bertozzi lab:**

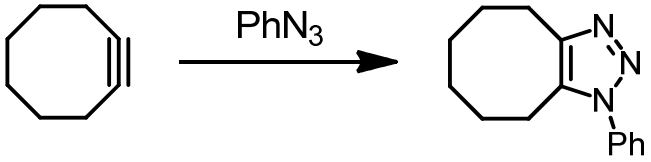




Jewett, J.C., Bertozzi, C.R.; *Chem. Soc. Rev.* **2010**, 39, 1272.

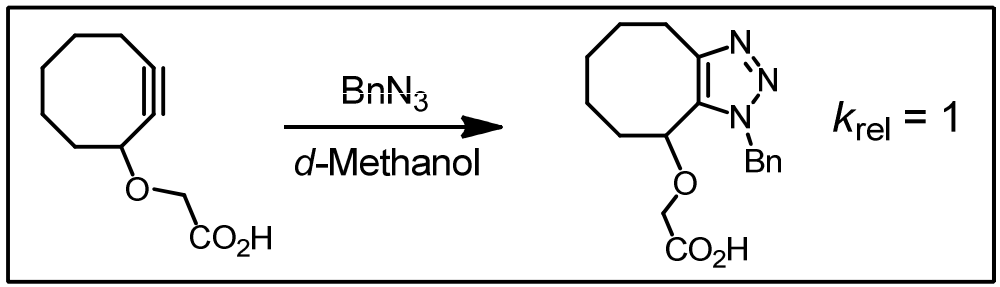
Copper-free Click Chemistry with Cyclooctynes

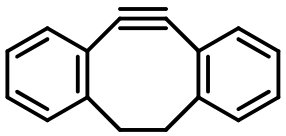
- **Cyclooctynes react with azides to give triazole products without the use of Cu catalysis:**



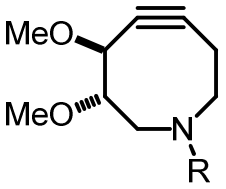
"explosionsartig" - like an explosion
Krebs, A., Wittig, G.; *Chem Ber.* **1961**, 94, 3260.

- **Further Activation - kinetic study:**

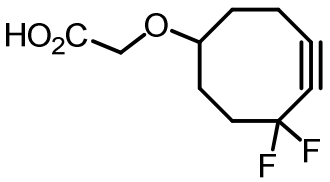




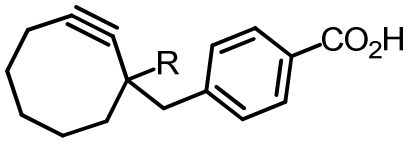
k_{rel} = ~40



k_{rel} = 1.5



k_{rel} = 60



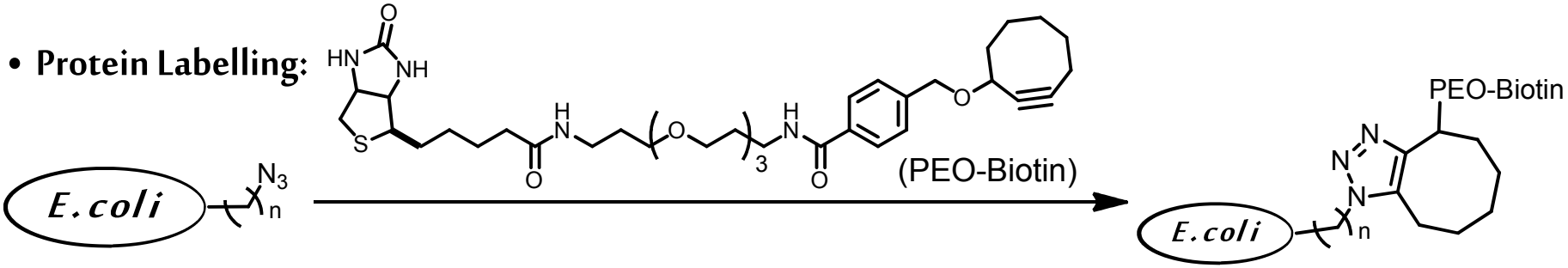
R = H - k_{rel} = 1
R = F - k_{rel} = 3

Bertozzi, C.R. et al.; *ACS Chemical Biology,* **2006**, 1(10), 644-648

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

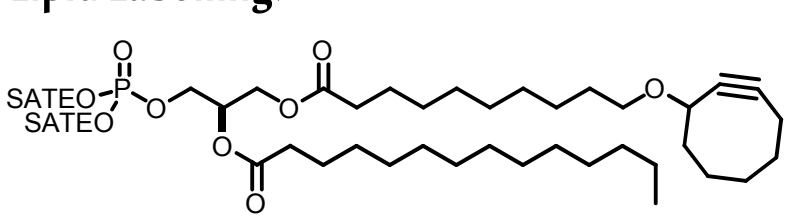
Applications - Bioorthogonal Reactions Using Cyclooctynes

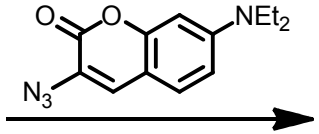
- **Protein Labelling:**



Link, A.J., et al. *PNAS* **2006**, 103, 10180
See also: Fernandez-Suarez, M., et al.; *Nat. Biotech.* **2007**, 25, 1483
Nessen, M.A., et al.; *J. Proteome Res.* **2009**, 8, 3702

- **Lipid Labelling:**

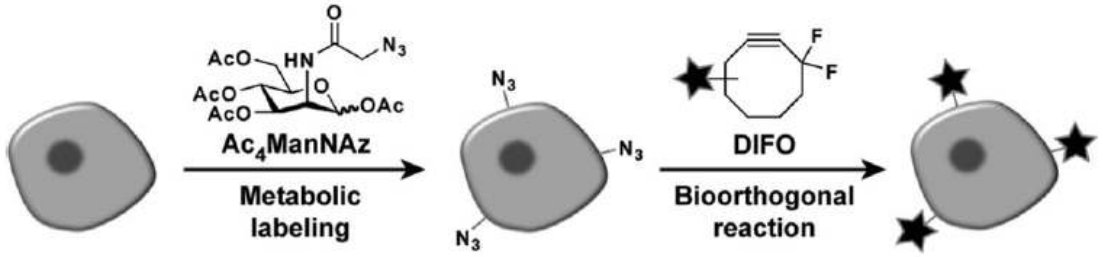




Imaging in living cells

Neef, A.B., Schultz, C.; *Angew. Chem. Int. Ed.* **2009**, 48, 1498.

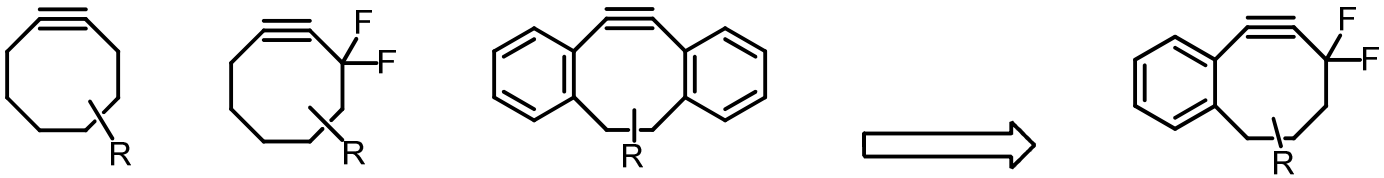
Glycan imaging:



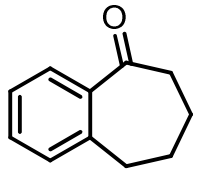
- Applied in living mice and monitored in real time.

Baskin, J.M et al. *PNAS* **2007**, 104, 16793

Title Paper - Difluorobenzocyclooctyne - Synthesis

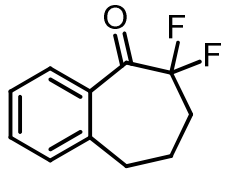


OCT $k_{rel} = 1$ | **DIFO** $k_{rel} = 40$ | **DIBO** $k_{rel} = 30$ | **DIFBO** $k_{rel} = 110$



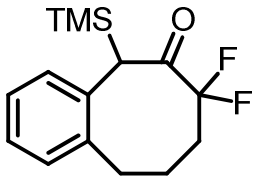
1-benzosuberone

1. hexylamine, TFA (cat)
   Dean Stark, cyclohexane
2. Selectfluor then HCl (3M)
   70 % - 2 Steps



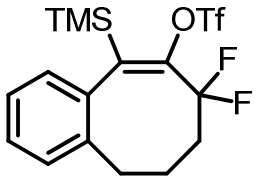
$AlMe_3$, $TMSCHN_2$

CH_2Cl_2, -78 °C
97%

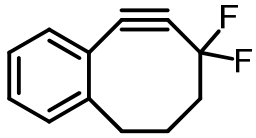


KHMDS, Tf_2O, THF

-78 °C - -45 °C
80%

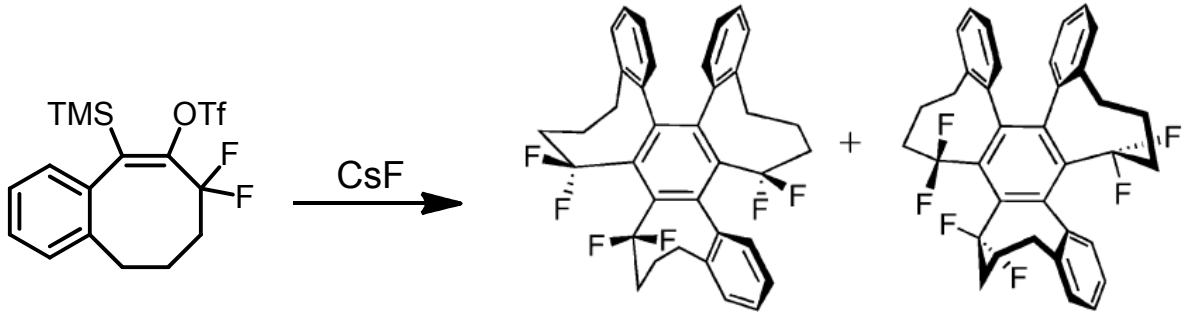


CsF

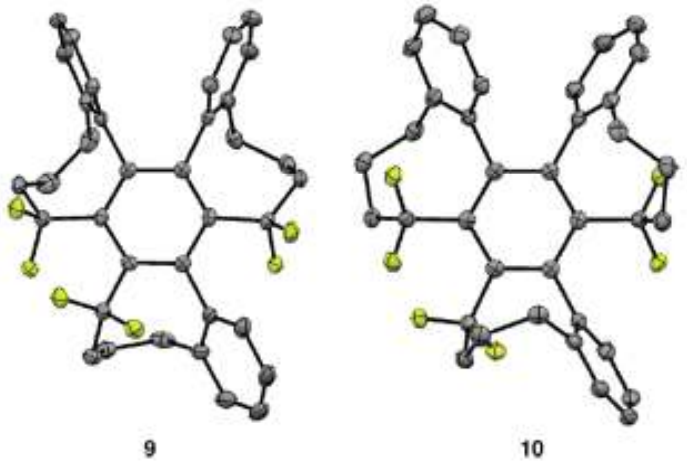


Unstable

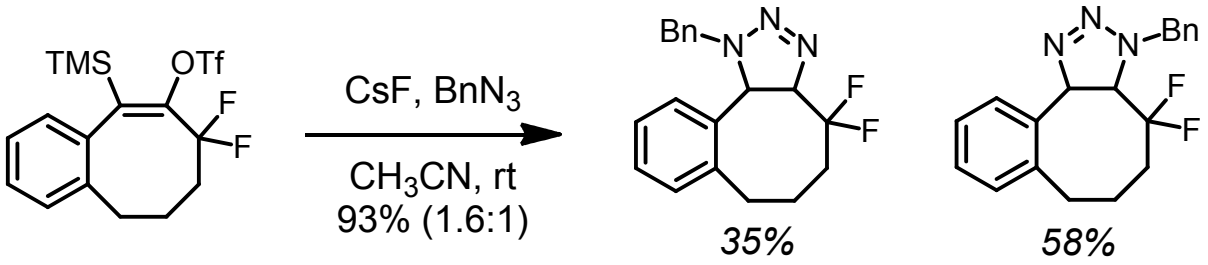
Title Paper - Difluorobenzocyclooctyne - Reactivity



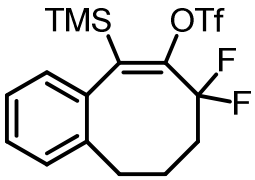
- No C_3-symmetric product was observed



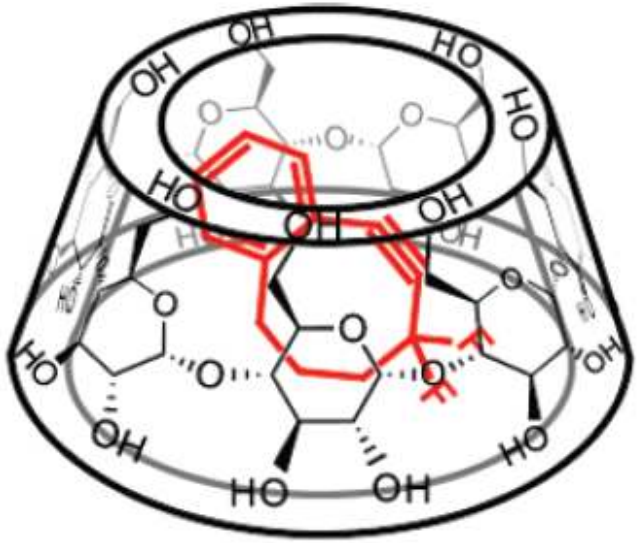
- To test if DIFBO was indeed formed, in situ trapping with benzyl azide was performed:



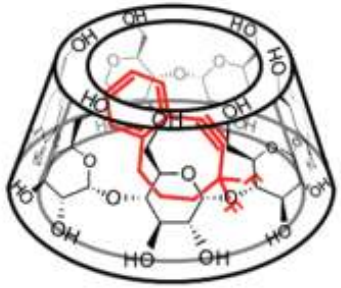
Title Paper - Difluorobenzocyclooctyne - Stabilization by β-Cyclodextrin



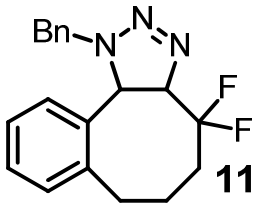
1. CsF, CH_3CN
   then Fcc eluting with hexanes
2. Dilution with CH_3CN, evaporation of hexanes
3. β-cyclodextrin, H_2O, lyophilization



- **Isolated as a stable, white powder**
- **Characterized as the inclusion complex using extensive solution and solid-state NMR techniques**



BnN_3, H_2O, DMSO



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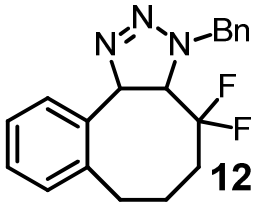
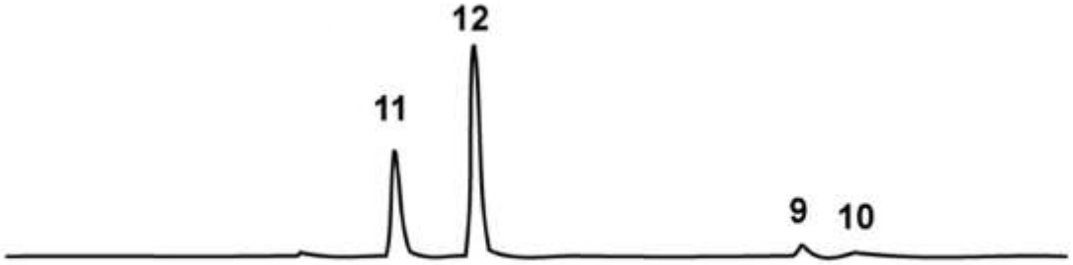
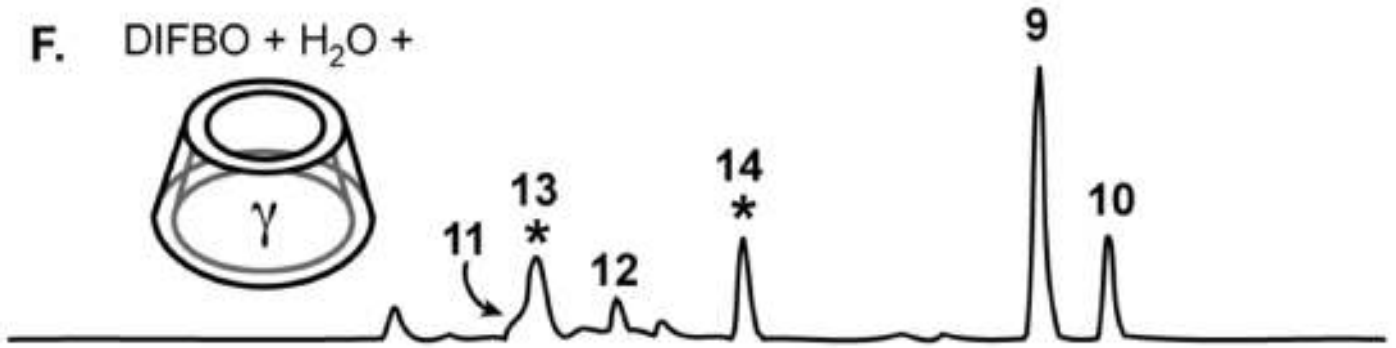


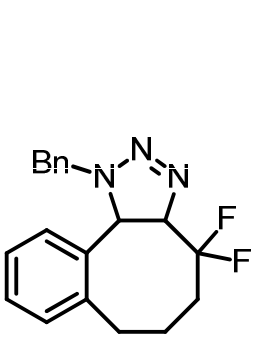
12

- **LCMS trace:**

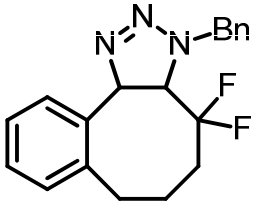


Title Paper - Difluorobenzocyclooctyne - Complexation with γ-Cyclodextrin

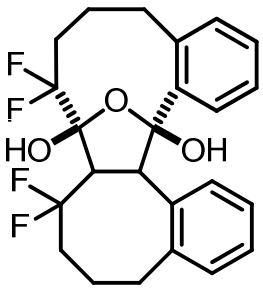




11



12



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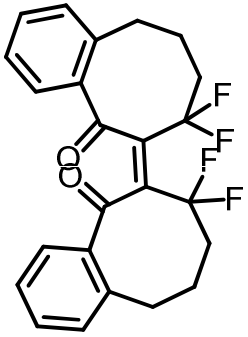

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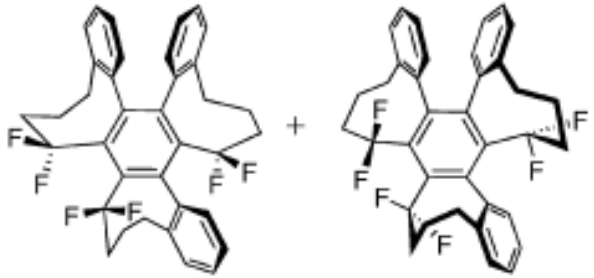
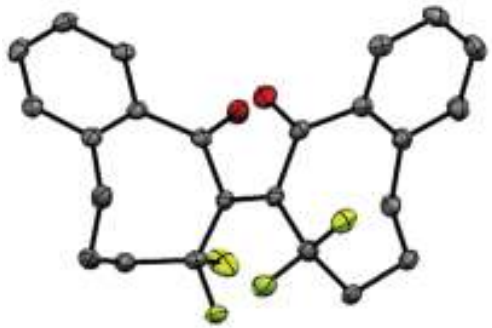


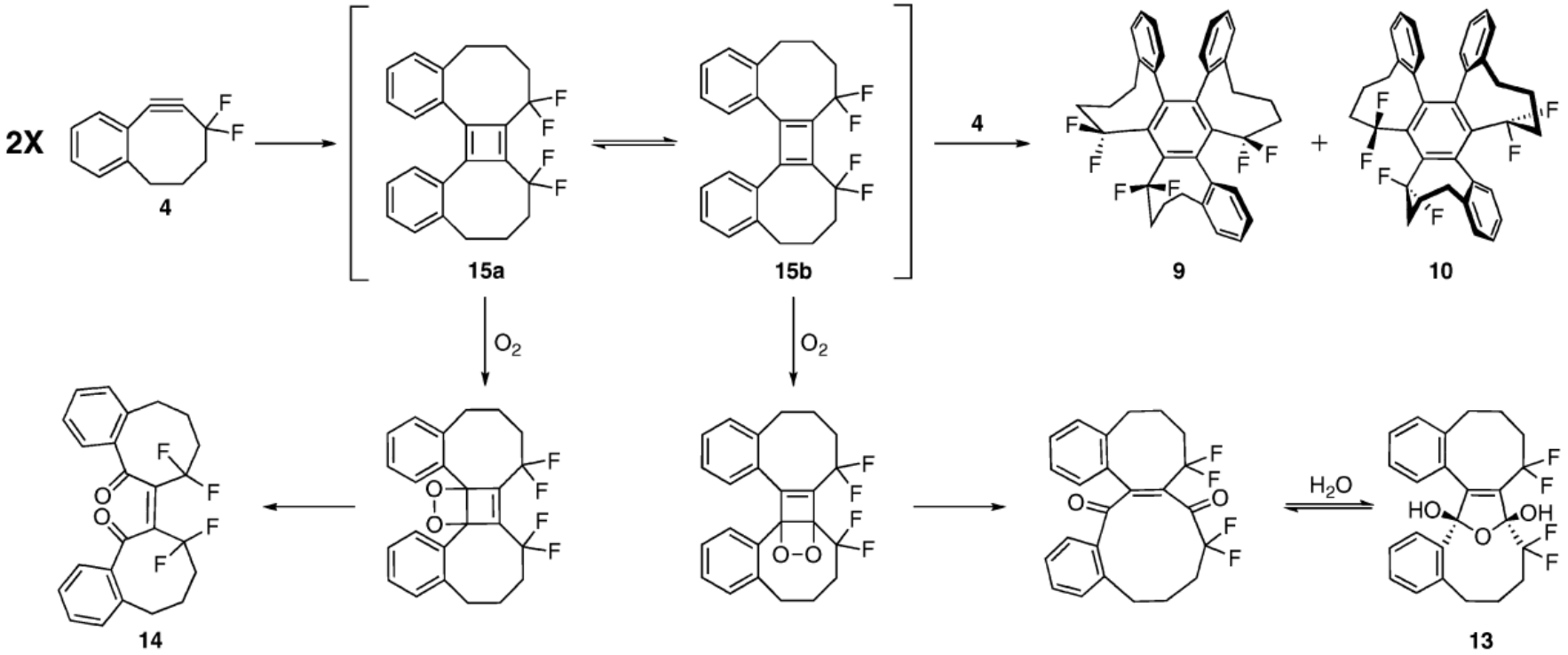
9 10

13



14

Title Paper - Proposed Mechanism for the Formation of Dimers 13 and 14



- **The formation of both dimers raises interesting questions:**
  1. **Does the dimerization event occur selectively inside the γ-cyclodextrin cavity?**
  2. **Can the γ-cyclodextrin cavity accomidate two molecules of BIFBO?**
  3. **What is the rate difference between the trimerization event and reaction with O_2?**

Title Paper - Conclusions and Significance

- A new substituted cyclooctyne (DIFBO) has been discovered with drastically superior kinetics in 2+3 cycloadditions with benzyl azide.

- Due to the enhanced reactivity of DIFBO, complexation with b-cyclodextrin was found to stabilize DIFBO allowing for easy storage and manipulation.

- The inclusion complex of DIFBO and γ-cyclodextrin produced two compounds presumed to arise from a single antiaromatic intermediate, thus providing a . possible means to further study antiaromaticity.