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

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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:

Cyclooctynes - Overview

- Smallest stable, unsubstituted cycloalkyne
- Alkyne bond angle = \( \sim 156^\circ \)
- Strain energy = \( \sim 18 \text{ kcal/mol} \)

- The difference in hydrogenation enthalpies of cyclooctyne versus 4-octyne is \( \sim 10 \text{ kcal/mol} \)

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


Reactions of cyclooctynes:

Trimerization:

Radical Reactions:

Cycloadditions:


Synthesis of Cyclooctynes

- Oxidative and Thermal Decomposition:

\[ \text{NH}_2 \quad \text{HgO} \quad \rightarrow \quad \text{HgO} \quad \rightarrow \quad \text{NH}_2 \]

- Elimination:

\[ \text{LiNR}_2 \quad \rightarrow \quad \text{LiNR}_2 \quad \rightarrow \quad \text{LiNR}_2 \]

- Examples from Bertozzi lab:

\[ \text{KHMDS, TESCI} \quad \rightarrow \quad \text{KHMDS, TESCI} \quad \rightarrow \quad \text{KHMDS, TESCI} \]

1. LiHMDS, TESCI
2. Selectfluor
3. KHMDS, TESCI
4. Selectfluor

Copper-free Click Chemistry with Cyclooctynes

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

\[
\text{PhN}_3 \quad \text{Ph} \quad \text{N} \quad \text{N} \quad \text{N}
\]

"explosionsartig" - like an explosion

- Further Activation - kinetic study:

\[
\text{BnN}_3 \quad \text{Bn} \quad \text{N} \quad \text{N} \quad \text{N}
\]

\[
\text{O} \quad \text{CO}_2\text{H} \quad \text{Bn} \quad \text{O} \quad \text{CO}_2\text{H}
\]

\[
k_{rel} = 1
\]

- Other examples:

\[
\begin{align*}
\text{Bertozi, C.R. et al.; ACS Chemical Biology, 2006, 1(10), 644-648} \\
\text{Jewett, J.C., Bertozi, C.R.; Chem. Soc. Rev. 2010, 39, 1272.}
\end{align*}
\]
Applications - Bioorthogonal Reactions Using Cyclooctynes

- **Protein Labelling:**

  ![Diagram of protein labelling reaction]

  \[ \text{E. coli} \rightarrow \text{PEO-Biotin} \]

  Nessen, M.A., et al.; *J. Proteome Res. 2009*, 8, 3702

- **Lipid Labelling:**

  ![Diagram of lipid labelling reaction]

  Imaging in living cells


- **Glycan imaging:**

  ![Diagram of glycan imaging]

  • Applied in living mice and monitored in real time.

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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%

TMS

KHMDS, Tf\_2O, THF
-78 °C - -45 °C
80%

Unstable

CsF
• No C₃-symmetric product was observed

• To test if DIFBO was indeed formed, in situ trapping with benzyl azide was performed:
Title Paper - Difluorobenzocyclooctyne - Stabilization by $\beta$-Cyclodextrin

1. CsF, CH$_3$CN then Fcc eluting with hexanes
2. Dilution with CH$_3$CN, evaporation of hexanes
3. $\beta$-cyclodextrin, H$_2$O, lyophilization

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

- LCMS trace:
Title Paper - Difluorobenzocyclooctyne - Complexation with \( \gamma \)-Cyclodextrin
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 accommodate two molecules of BIFBO?
3. What is the rate difference between the trimerization event and reaction with O₂?
• 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 β-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.