[*n*+1]CPPs and their Potential as Bioorthogonal Imaging Reagents

Phil and Penny Knight Campus for Accelerating Scientific Impact

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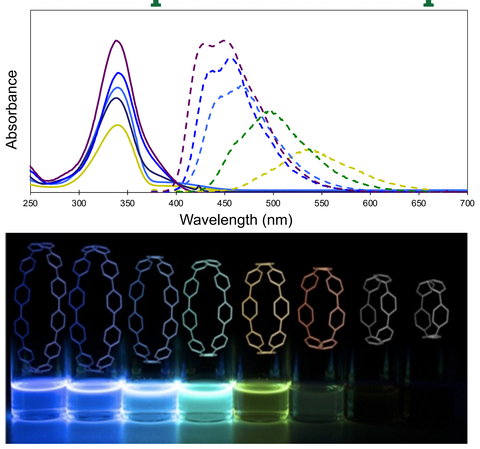
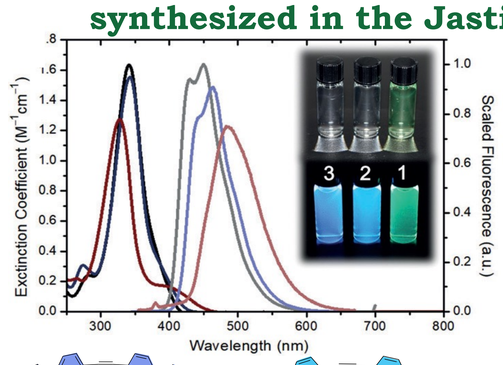
Cycloparaphenylenes

CPPs have unique fluorescent properties

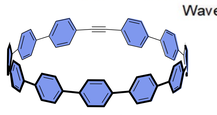


[*n*]CPPs have unique photophysical properties including common absorption and size-dependent fluorescence with red-shifted emission as the size of the hoop decreases Darzi, E. R.; Jasti, R. *Chem. Soc. Rev.* **2015,** *44*, 6401-6410.

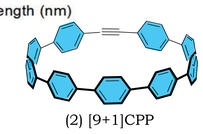
Alkyne containing CPPs were first synthesized in the Jasti Lab



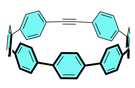
Alkyne containing CPPs ([n+1]CPPs) increase reactivity and can undergo a ligation reaction called the Strain-Promoted Azide-Alkyne cycloaddition (SPAAC)



(3) [11+1]CPP

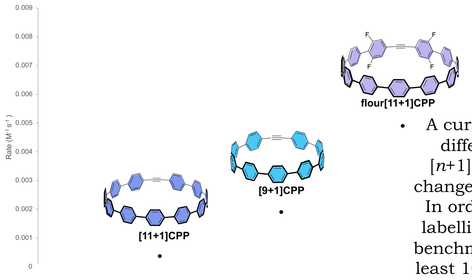


(2) [9+1]CPP



(1) [7+1]CPP

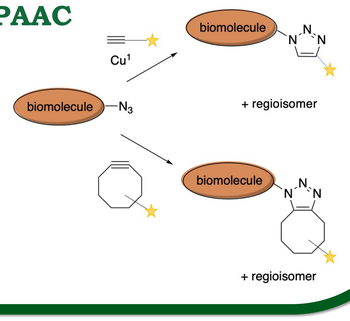
Schaub, T. A.; Margraf, J. T.; Zakharov, L.; Reuter, K.; Jasti, R. *Angew. Chem. Int. Ed.* **2018**, *57*, 16348-16353.



- A current study shows how different modulations of [*n*+1]CPPs can translate to changes in kinetics/reactivity. In order for a bioorthogonal labelling reaction to occur, a benchmark rate constant of at least 10^{-3} has been discovered

Labelling biomolecules with [*n*+1]CPPs via the SPAAC reaction

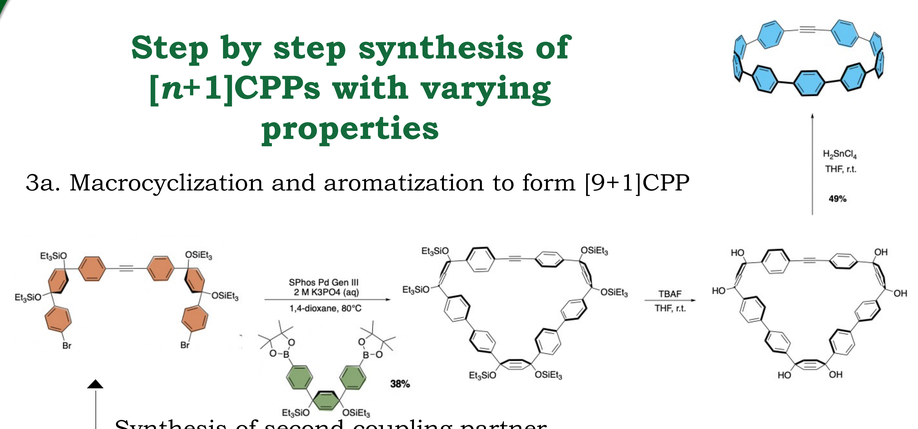
The reactive and fluorescent properties of [*n*+1]CPPs suggest their potential use as bioorthogonal labelling reagents via the Strain-promoted azide-alkyne cycloaddition (SPAAC reaction)



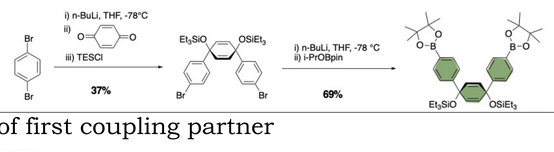
Synthesis of [9+1]CPP and *m*[9+1]

Step by step synthesis of [*n*+1]CPPs with varying properties

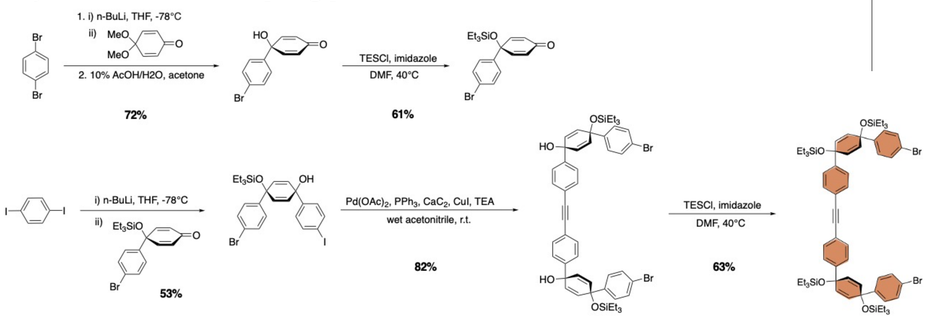
3a. Macrocyclization and aromatization to form [9+1]CPP



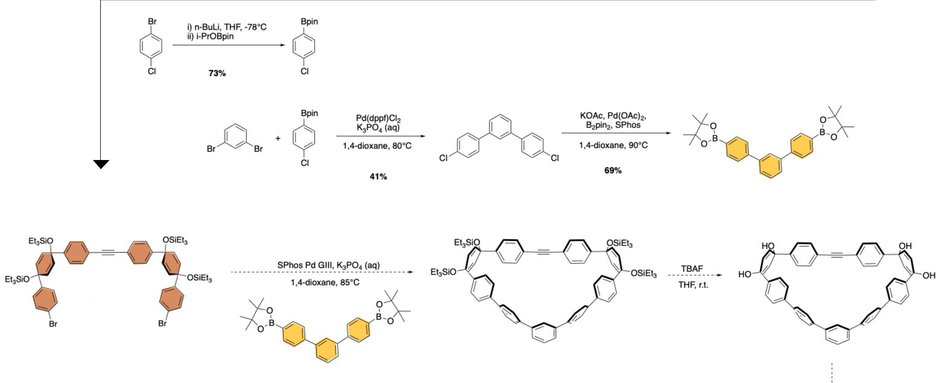
Synthesis of second coupling partner



Synthesis of first coupling partner



Synthesis of second coupling partner

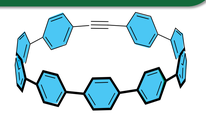


Macrocyclization and aromatization to form *m*[9+1]CPP

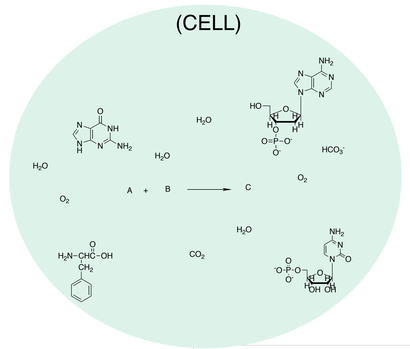
Synthesis of both [9+1] and *m*[9+1] will allow for comparison between two CPPs with varying reactivity, stability, and fluorescence. These CPPs will be used for proof-of-concept biology labelling reactions

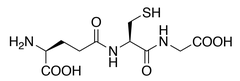
Proof of Concept Biology reactions

Assessing stability of [*n*+1]CPP in solution of biological nucleophiles



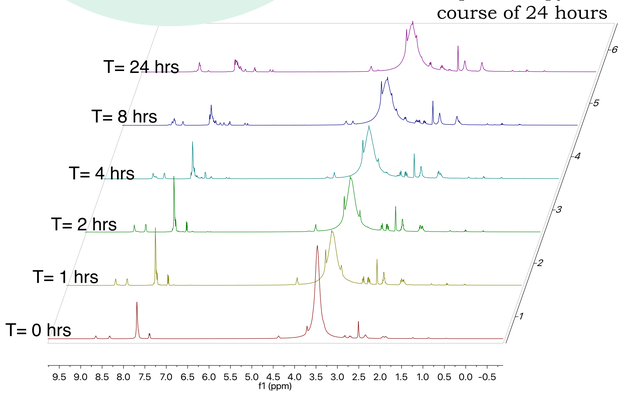
[9+1]CPP



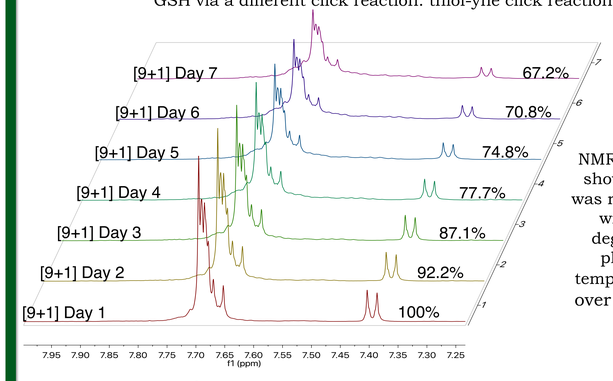


GSH

To assess the stability of [9+1]CPP in a solution of biological nucleophiles, [9+1]CPP was incubated in L-glutathione (GSH) in d-DMSO at physiological temperature (37˚C) and monitored by 1H NMR spectroscopy over the course of 24 hours



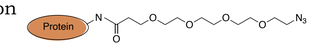
NMR spectroscopy showed the [9+1]CPP was reactive with the GSH over the course of 24 hours. After further analysis it was discovered the alkyne reacted with the thiol group in GSH via a different click reaction: thiol-yne click reaction.



NMR spectroscopy showed [9+1]CPP was relatively stable with minimal degradation at physiological temperature (37˚C) over the course of 7 days.

Key results

- The [9+1]CPP is relatively stable at physiological temperature (37 ˚C) with minimal degradation
- The [9+1]CPP can react with thiol groups via the thiol-yne click reaction. This suggests its potential use for various click reactions in addition to the SPAAC reaction



Future directions

To label a model protein with various [*n*+1]CPPs via the SPAAC reaction