The Bioorthogonal Click Reaction of Cyclobutenes and Tetrazines

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Purpose

The relatively new field of bioorthogonal or copper-free “click” chemistry has flourished in recent years because of the potential to conduct rapid and selective ligations under conditions compatible with living cells (Bertozzi, 2003). This research seeks to expand on the narrow set of cycloalkene and tetraneous pairings of bioorthogonal chemistry that are suitable for inverse electron-demand Diels-Alder 4+2 cycloaddition (the “click” mechanism) with the introduction and characterization of 3-substituted cyclobutene as a viable alternative.

Background

Why choose click chemistry?
- Wide in scope
- Rapid ligation rate
- Selective: No undesired byproducts
- Stable and irreversible
- Effective means of immobilizing or linking molecules

A recent innovation
- Azide-alkyne Huisgen 1,3-dipolar cycloaddition (2001)
- Sharpless-Fokin Copper(I)-catalyzed Azide-alkyne cycloaddition or CuAAC (2002)
- Bertozzi Bioorthogonal cycloaddition (2003)

The popular copper catalyzed click reaction

Bioorthogonal Chemistry

Bioorthogonal strain-promoted cycloadditions encompass a wider variety of molecules and reaction conditions than azide-alkyne cycloadditions. Strained cycloalkenes are paired with a tetraneous in this spontaneous (uncatalyzed) reaction. Three types are typically employed:

- Cyclobutene
- Cyclopropene
- Nonlanones
- 1,2,4,5-Tetrazine

The tetraneous and these cycloalkenes are also discrete as functional groups in a biological system and do not perturb regular functions. It is for this reason the bioorthogonal click reaction has been useful in biochemical imaging (fluorescent and PET).

Materials & Methods

- Amphiphilic cyclobutene
  (hydrophobic + hydrophilic ends) for coordinated formation of self-assembled monolayer (SAM)
- Thiol (—SH) functionalized tail to take advantage of strong gold-sulfur affinity
- Formation of SAM
- 1,2,4,5-tetrazine partner
- Incorporated redox indicator for quantitative measurement
- The bioorthogonal click reaction on monolayer surface

Work on the synthesis of amphiphilic cyclobutenes developed by Sittiwong, et. al.*

Four step pathway established for conversion of carboxylic acid functionalized tail (omwego) to thiol

1,2,4,5-tetrazine with incorporated redox indicator synthesized in two part pathway followed by a coupling step

Synthesis

Tetrazine Synthetic Pathway

Redox Indicator Synthesis

Conclusions

Through a nine step synthetic pathway of our cyclobutene (initially established by Wantanee Sittiwong), as well as a unique tetraneous synthetic method, we have developed a model system with which to explore the use of cyclobutene in inverse electron-demand Diels-Alder 4+2 reactions. As a result of this research, we hope that cyclobutene garners acceptance as a new and effective bioorthogonal molecule.

Future Work

- SAM formation
- Click reaction kinetics in collaboration with Prof. Rebecca Lai:
  Stability, Selectivity, Ligation Rates
- Improving synthetic yields and/or shortening pathways
- Other cyclobutene functionalization or constituents
- Possible biochemical applications
  I. Chemical sensors
  II. Imaging properties
  III. Bioconjugations

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