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A new ligation system for protein conjugation

Alicia J. Curti
University of Nebraska-Lincoln, acurti@huskers.unl.edu

Patrick H. Dussault
University of Nebraska-Lincoln, pdussault1@unl.edu

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Alicia Curti and Pat Dussault*, Department of Chemistry, University of Nebraska-Lincoln

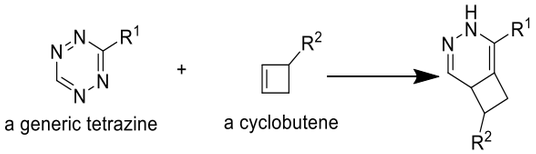
Cyclobutene "Click" Chemistry

Click chemistry describes crosslinking reactions in which pairs of functional groups selectively "click" together to form linkages. The idea of our project is to explore the use of cyclobutene-containing fatty acids as a new class of "click" reagents as a tool for protein modification.

There is great interest in performing click reactions under biologically relevant conditions, in the presence of cells, and even within cells. The best known click reaction, the cycloaddition of alkynes and azides, is highly specific, can be conducted in the presence of buffers and proteins, and forms stable cycloadducts. However, use in biological systems is limited by the need to employ a toxic copper catalyst to achieve useful reaction rates. A number of approaches have been investigated to achieve fast click reactions that do not require a catalyst (Bertozzi 2015).

The cycloaddition of 1,2,4,5-tetrazines with strained alkenes has emerged as an effective alternative to copper-catalyzed azide/alkyne reactions; the strain in the starting alkene lowers the cost of accessing the reaction transition state. To date, cyclobutenes have been looked at very little as substrates for click chemistry.

Example of a tetrazine/cyclobutene "click" reaction



Applications

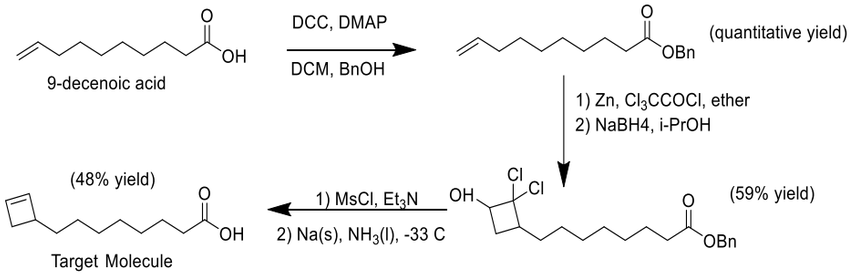
If we are able to successfully react cyclobutene containing amphiphiles with certain analytes in biological systems, this could be used to create or change proteins in cells or to track movement in cells by conjugating fluorescently marked molecules to proteins. If the reactions occur at low enough concentrations, they will be able to be used within cells.

Overview

This study examines a new class of amphiphilic reagents designed to functionalize proteins for "click" crosslinking in biological systems. Amphiphiles are molecules which have polar and nonpolar regions. Amphiphilic cyclobutenes are being used for this project because they have a carboxyl "head" group which allows them to be selectively attached to a protein; and a cyclobutene "tail" allowing click reactions with a dye, nanoparticle, or another protein. Questions that I am seeking an answer to in this study include: Can I functionalize a protein with an amphiphilic cyclobutene? Can this protein/cyclobutene conjugate be employed for "click" reactions, in reactions under conditions relevant to use on or within cells? Can we use this approach to "click" together two proteins?

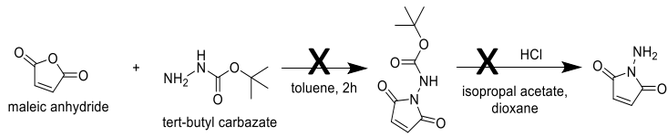
Synthesis of Cyclobutene Amphiphile

The cyclobutene amphiphile was prepared in five steps from commercially available 9-decenoic acid using a method previously developed by the Dussault group (Sittiwong 2014). The key transformations are a cycloaddition and reduction to introduce a dichlorocyclobutanol, and the introduction of the strained double bond through a dissolving metal reduction.

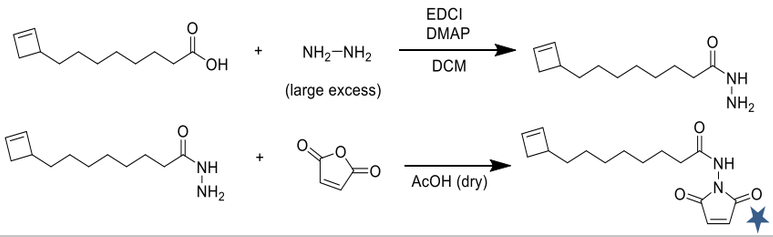


Preparation of the Conjugate

My initial plan to create a maleimide functionalized cyclobutene fatty acid (starred structure below) was unsuccessful (right).

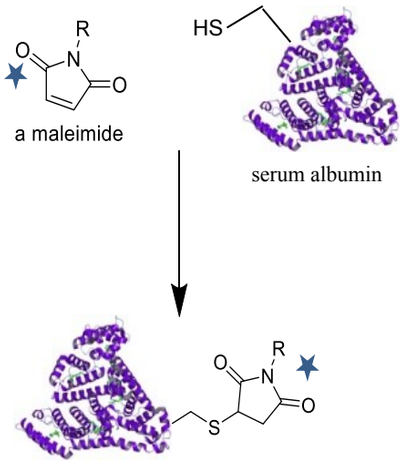


I am currently attempting to prepare the same reagent based upon an altered route (below).



Using BSA/HSA as a Model

I plan to link the maleimide-functionalized cyclobutene to serum albumin (either bovine or human), an inexpensive and well understood model protein which has one free cysteine suitable for reaction with the maleimide. The cyclobutene-functionalized albumin will then be investigated for click reagents with commercially available tetrazines.



References

Bertozzi, C. R.; Organic Letters (2011), 13: 5937-5939

Sittiwong, W.; et. al.; ChemMedChem (2014), 9(8): 1838-1849

Acknowledgements

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