

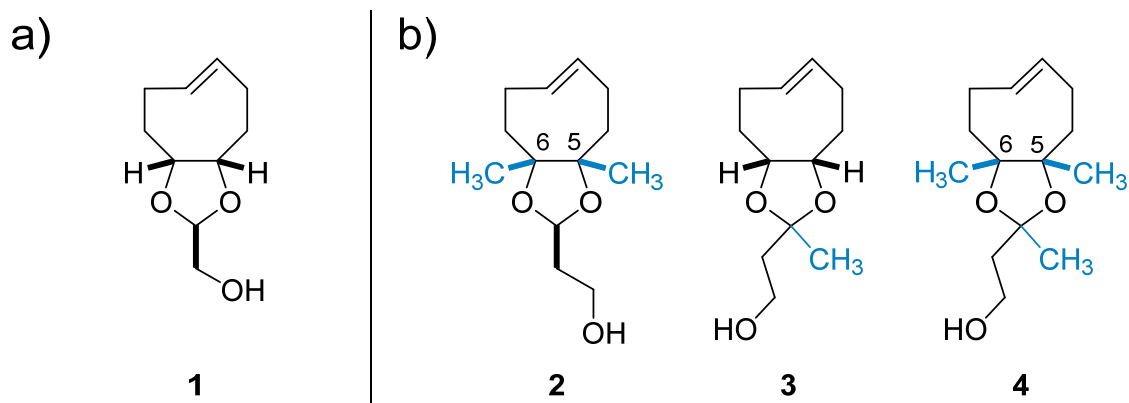
## Highly Reactive Dienophiles for Bioorthogonal Tetrazine Ligations

Martin Wilkovitsch, Dennis Svatunek, and Hannes Mikula

Institute of Applied Synthetic Chemistry, TU Wien (Vienna University of Technology)  
Getreidemarkt 9, 1060 Vienna, Austria  
martin.wilkovitsch@tuwien.ac.at

The reactions between 1,2,4,5-tetrazines and *trans*-cyclooctenes (TCO), known as “tetrazine ligations”, are the fastest bioorthogonal reactions reported so far with second order rate constants of up to  $3.3 \times 10^6 \text{ M}^{-1}\text{s}^{-1}$  [1]. The conformationally strained *trans*-cyclooctene d-TCO (**1**) introduced by Fox and coworkers (Figure 1a) [1] is an additionally strained and thus more reactive TCO that still show moderate stability, in contrast to highly reactive s-TCO [2] with only very limited *in vivo* stability.

The aim of this work is the development of structurally enhanced d-TCO derivatives by introducing methyl groups at key positions in the core structure to increase reactivity while still maintaining sufficient stability (Figure 1b). Bis-methylation at positions 5 and 6 of the TCO core structure (compounds **2** and **4**) and methylation of the acetal carbon (compounds **3** and **4**) have been considered. Based on computational studies compounds **2** and **4** are expected to be significantly more reactive compared to the parent TCO **1**. In addition, the introduced methyl groups are also expected to be beneficial for stability. Results of computational and experimental investigations will be presented.



[1] A. Darko, S. Wallace, O. Dimitrenko, M. M. Machovina, R. A. Mehl, J. W. Chin, J. M. Fox, *Chem. Sci.* **2014**, *5*, 3770-3776;

[2] M. T. Taylor, M. L. Blackman, O. Dimitrenko, J. M. Fox, *J. Am. Chem. Soc.* **2011**, *133*, 9646-9649.