BOOK OF ABSTRACTS

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RADIOLABELED 1,2,4,5-TETRAZINES AS BIOORTHOGONAL IMAGING TOOLS

Christoph Denk,[a],* Martin Wilkovitsch,[a] Thomas Wanek,[b]
Claudia Kuntner-Hannes,[b] Dennis Svatunek[a] and Hannes Mikula[a]

[a] Vienna University of Technology, Institute of Applied Synthetic Chemistry, Vienna, Austria
[b] Austrian Institute of Technology, Biomedical Systems, Austria
* christoph.denk@tuwien.ac.at

Labeled antibodies are highly specific imaging probes that are incompatible with short-lived radionuclides due to their slow accumulation. This dilemma can be circumvented by pretargeting using bioorthogonal reactions (Figure).[1,2] These fast and biocompatible *"click"* type ligations are capable of forming a covalent linkage between a pre-administered marker compound and a labeled pull down reagent (PDR) *in vivo*.[2] Due to particularly high ligation rates the inverse electron demand Diels-Alder reaction (IEDDA) of strained dienophiles with 1,2,4,5-tetrazines (Tz) is especially suited for *in vivo* application.[1-3]

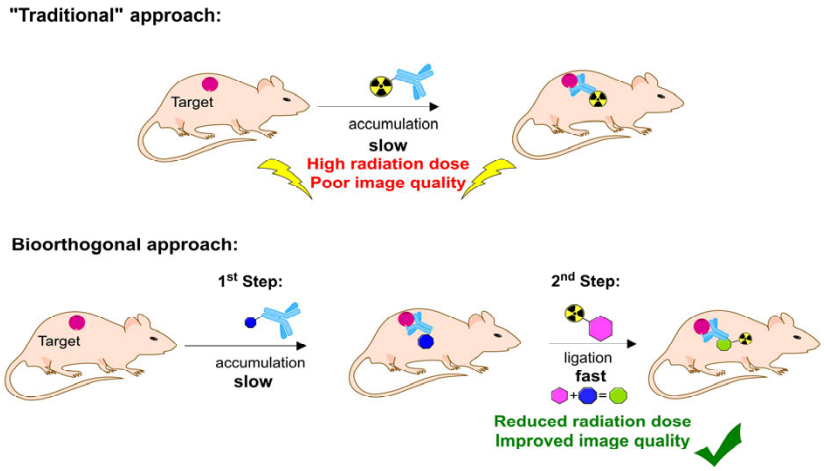

Figure: Comparison of "traditional" immuno-imaging vs a bioorthogonal approach.

In addition to fast ligation rates PDRs must exhibit homogenous biodistribution, good *in vivo* stability and fast excretion. Within this contribution the development and application of several radiolabeled tetrazines, serving as small molecule imaging agents, will be presented. 1,2,4,5-Tetrazines labeled with the positron emitter fluorine-18 were thought to be inaccessible due to reported degradation during radiolabeling.[4] In 2014 our group was able to prepare the first ^{18}F-tetrazine by trading increased stability for reduced IEDDA reactivity.[5] This initial development our ^{18}F-tetrazines were constantly improved. The newest developments include ^{18}F-Tz derivatives with ~200-fold elevated ligation rate that still possess excellent *in vivo* stability, thus showing great promise as secondary imaging agent. Tetrazines for therapeutic application bearing radionuclides of iodine or the α-emitter astatine-211 were furthermore synthesized and evaluated.
We are convinced that radiolabeled tetrazines can overcome problems associated with slow-accumulating vectors (such as antibodies and other nanomedicines), and should allow for many applications in the field of medicine, medicinal chemistry and drug development.

[1] R. Rossin et. al., *J. Nucl. Med.,* **2013**, *54*, 1989.
[2] B.M.Zeglis et. al., *J. Nucl. Med.,* **2013**, *54*, 1389.
[3] J.P. Meyer et. al., *Bioconjugate Chem.,* **2016**, *27*, 2791.
[4] Z. Li et. al., *Chem. Comm.,* **2010**, *46*, 8043.
[5] C. Denk et. al., *Angw. Chem. Int. Ed.,* **2014**, *53*, 9655.

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DEVELOPMENT OF BIOORTHOGONAL 2-NITROIMIDAZOLE BASED HYPOXIA SENSITIVE PROBES

Barbara Sohr,[a,#] Christoph Denk,[a] Thomas Wanek[b] and Hannes Mikula[a,*]

[a] Institute of Applied Synthetic Chemistry, Vienna University of Technology (TU Wien), Vienna, Austria
[b] Health and Environment Department, Biomedical Systems, Austrian Institute of Technology, Seibersdorf, Austria
Recipient of a DOC Fellowship of the Austrian Academy of Sciences
* hannes.mikula@tuwien.ac.at

Hypoxia is a low oxygen condition often observed in a variety of solid tumors and associated with high therapy resistance, invasiveness and metastasis. To properly plan and adjust cancer treatment, it is of vital importance to identify, measure and localize hypoxic regions. Current methods, e.g. PET imaging using ^{18}F-labeled hypoxia sensitive probes such as ^{18}F-FAZA, lack satisfactory tumor-to-background ratios attributed to slow tumoral retention, unspecific accumulation in normal (normoxic) tissue and washout of the probe.[1]

In this work, we focused on the development of novel hypoxia sensitive probes that enable two-step pretargeting strategies based on bioorthogonal chemistry. The inverse electron demand Diels-Alder (IEDDA)-initiated conjugation between 1,2,4,5-tetrazines and strained dienophiles (e.g. *trans*-cyclooctenes, TCOs) has been proven to be a highly efficient and fast bioorthogonal ligation[2] and was therefore used within this study.

The above-mentioned two-step process will consist of (1) administration of a TCO-tagged hypoxia sensitive probe that is specifically trapped in hypoxic cells and (2) administration of a tetrazine-modified compound, e.g. as pull down reagent (PDR) that selectively binds to the probe through bioorthogonal ligation. Depending on the nature of the tetrazine-modified agent the pretargeted hypoxia probe can be used for a wide range of applications (Figure 1) making it a remarkably versatile chemical tool for biomedical research.

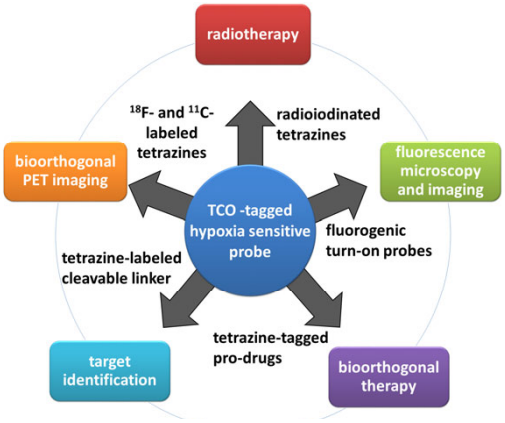

Figure 1: Possible applications for hypoxia probes.

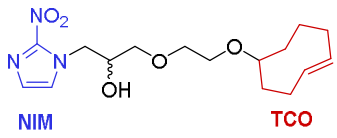

Figure 2: TCO-tagged hypoxia probe.

To achieve hypoxia sensitivity a 2-nitroimidazole (NIM) moiety was incorporated in our target compounds (as shown exemplarily in Figure 2). NIM is known to specfically accumulate in hypoxic cells after intracellular reduction of the NO_2 moiety.[3]

IEDDA ligation of the prepared probes was investigated using already available PDRs to acquire kinetic data and facilitate characterization of conjugation products. Furthermore, *in vitro* stability, cell permeability and accumulation in hypoxic cells were studied.

Results on the synthesis and *in vitro* evaluation of TCO-tagged NIM derivatives as bioorthogonal hypoxia probes will be presented.

[1] S. Carlin, J.L. Humm, *J. Nucl. Med.,* **2012**, 53, 1171.
[2] M.L. Blackman, M. Royzen, J.M. Fox, *J. Am. Chem. Soc.,* **2008**, 130,13518.
[3] J.D. Chapman, A.J. Franko, J. Sharplin, *Br. J. Cancer,* **1981**, 43, 546.

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HIGHLY REACTIVE DIENOPHILES FOR BIOORTHOGONAL TETRAZINE LIGATIONS

Dennis Svatunek, Maximilian Haider and Hannes Mikula*

Institute of Applied Synthetic Chemistry, Vienna University of Technology (TU Wien), Vienna, Austria
* hannes.mikula@tuwien.ac.at

The tetrazine-TCO ligation, the reaction between 1,2,4,5-tetrazines and *trans*-cyclooctenes (TCO), is the fastest bioorthogonal reaction reported so far with second order rate constants of up to 3 300 000 $M^{-1}s^{-1}$.[1] High reactivity is of significance for reactions intended to be used in biological environments, which are commonly performed at very low concentration.
The two-step reaction of an electron deficient tetrazine and TCO is initiated by an inverse electron demand Diels-Alder cycloaddition followed by a retro-Diels-Alder reaction under the loss of nitrogen. Highly reactivity TCOs are the conformationally strained *trans*-cyclooctenes s-TCO (**1**) and d-TCO (**2**) introduced by Fox and coworkers (Figure 1a).[1,2] They show incredibly high reaction rates, however, *in vivo* stability is limited.
Within this work we aimed to improve the reactivity and stability of known *trans*-cyclooctene derivatives and to develop new TCOs with improved properties.
Reactivity of proposed structures are first evaluated *in silico* using established DFT methods (M06-2X/6-311+G(d,p)).[3,4] Most promising structures are selected and further investigated experimentally regarding their stability and reactivity. Explored structures include derivatives of known TCOs, for example bis-methylated s-TCO (**3**) and d-TCO (**4**) (Figure 1b), and *trans*-cyclooctenes with new structural features such as the bridged TCO **5** (Figure 1c).

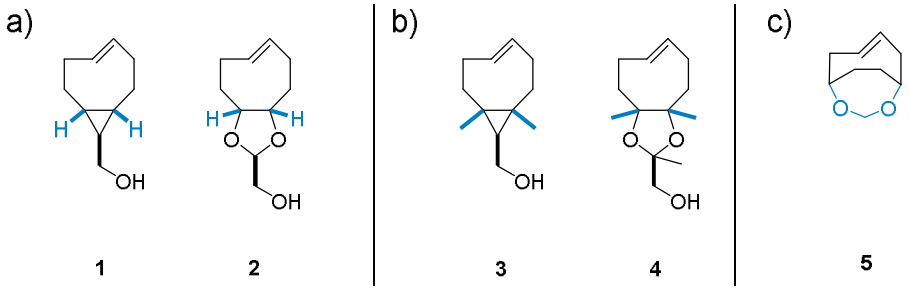

Figure 1: a) Chemical structure of s-TCO (**1**) and d-TCO (**2**); b) Bis-methylated TCOs; c) Bridged TCO

DFT calculations show lowered free energies of activation for bis-methylated derivatives **3** and **4** in comparison to their parent compounds **1** and **2**. The additional methyl groups are furthermore expected to be beneficial for stability. Computational investigation of the novel TCO **5** and similar structures suggests reactivities comparable to s-TCO (**1**).
Results on quantum chemical calculations, the synthesis and evaluation of novel TCOs will be presented.

[1] A. Darko, S. Wallace, O. Dmitrenko, M. M. Machovina, R. a. Mehl, J. W. Chin, J. M. Fox, *Chem. Sci.,* **2014**, *5*, 3770.
[2] M. T. Taylor, M. L. Blackman, O. Dmitrenko, J. M. Fox, *J. Am. Chem. Soc.,* **2011**, *133*, 9646.
[3] C. Denk, D. Svatunek, S. Mairinger, J. Stanek, T. Filip, D. Matscheko, C. Kuntner, T. Wanek, H. Mikula, *Bioconjug. Chem.,* **2016**, *27*, 1707.
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