A NEW BIOORTHOGONAL HANDLE FOR IEDDA-TRIGGERED BOND CLEAVAGE REACTION IN CELLS

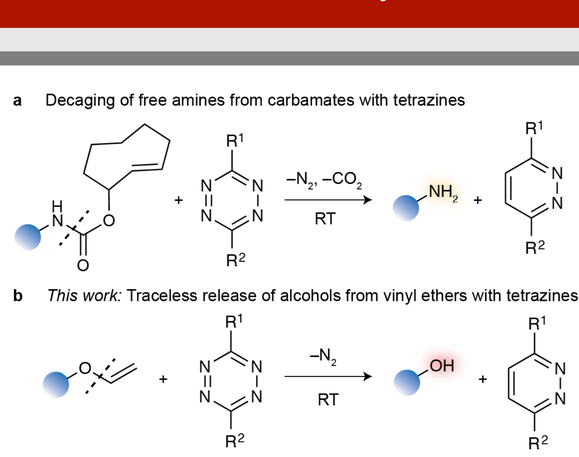
Ester Jiménez-Moreno,[1] Zijian Guo,[1] Bruno Oliveira,[1] Ana Guerreiro,[2] Inês S. Albuquerque,[2] Annabel Kitowski,[1] Omar Boutureira,[1] Tiago Rodrigues,[2] Gonzalo Jiménez-Osés,[3] Gonçalo J.L. Bernardes[1,2]

1) Department of Chemistry, University of Cambridge, Lensfield Road, CB2 1EW Cambridge, United Kingdom
2) Instituto de Medicina Molecular, Faculdade de Medicina da Universidade de Lisboa, Av. Prof. Egas Moniz, 1649-028 Lisboa, Portugal
3) Departamento de Química, Universidad de La Rioja, UA-CSIC. 26006 Logroño, Spain

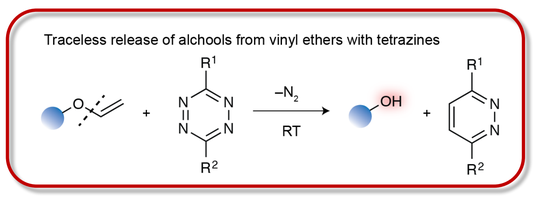
e-mail: ej306@cam.ac.uk

INTRODUCTION

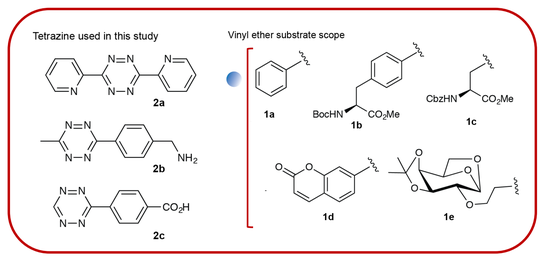
The cleavage of a protecting group from a protein or drug under bioorthogonal conditions enables accurate spatiotemporal control over protein or drug activity. Despite recent advances in the decaging of proteins and small molecules, the ability to release alcohol-containing molecules has been elusive. Strategies based on IEDDA elimination reactions with tetrazines are particularly attractive for decaging relevant molecules in cells and interrogating biology, due to the favourable kinetics and the abiotic nature of tetrazines. Typically, IEDDA elimination reactions have been used with strained alkene protecting groups connected through a carbamate, resulting in a cascade release of a primary amine.[1] However, the reduced metabolic stability of strained alkenes constitutes a major caveat for its utility. Here, we report the development of a vinyl ether–tetrazine system as IEDDA reaction partners for the traceless decaging of alcohol-containing molecules.



REACTION DEVELOPMENT

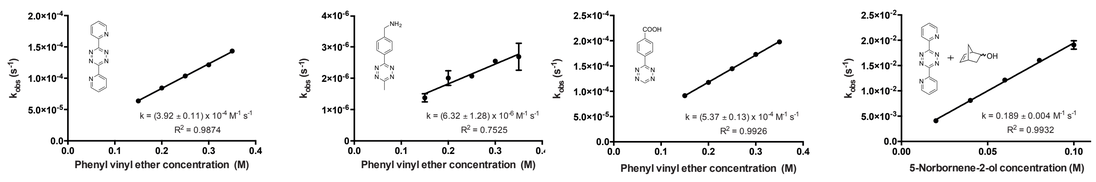


- **Several chemotypes:** protected amino acids serine and tyrosine, an 1,6-anhydro sugar, a fluorophore and a drug.
- Decaging in **good yields**.
- Stable under **biocompatible conditions** (PBS pH 7.4 at 37 ºC)



Compound	Alcohol	Pyridazine	Conversion	Stability
1a	61%	49%	100%	100%
1b	68%	65%	73%	100%
1c	57%	72%	100%	77%
1d	50%	47%	56%	100%
1e	65%	50%	100%	n.d.

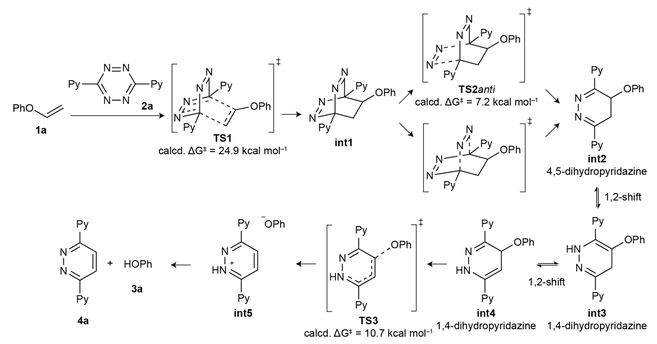
KINETIC STUDIES

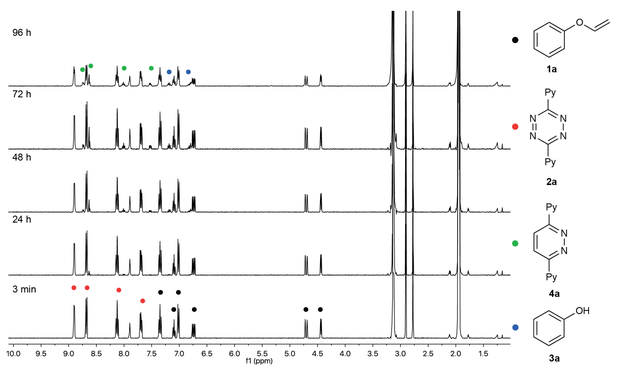


MECHANISTIC STUDIES

QUANTUM MECHANICS

- IEDDA cycloaddition is the **rate-limiting step.**
- Very fast retro-Diels Alder, tautomerization and decaging through an elimination reaction.

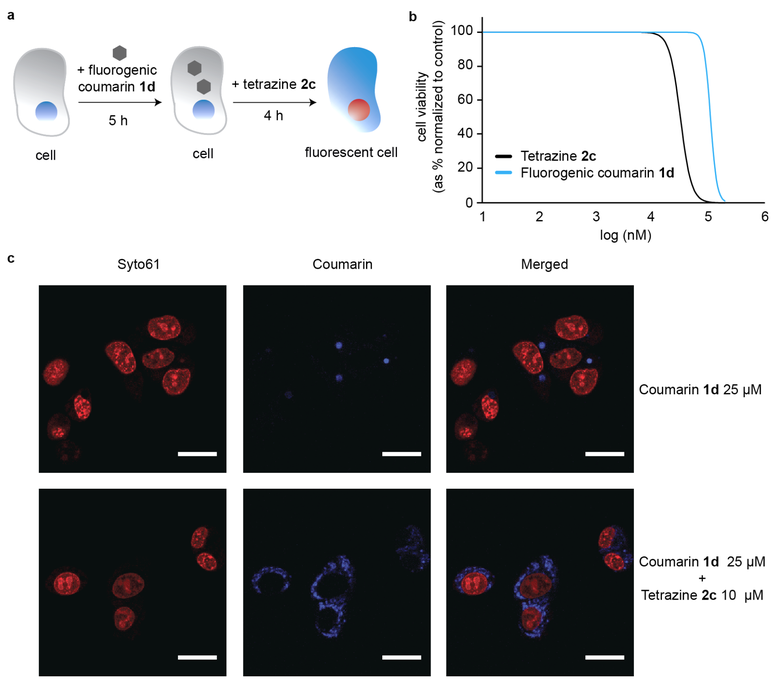




^{1}H NMR

No peaks corresponding to any intermediate of the reaction (δ 2.5–6 ppm) were observed, supporting that the Diels-Alder cycloaddition is the rate-limiting step.

CELL STUDIES

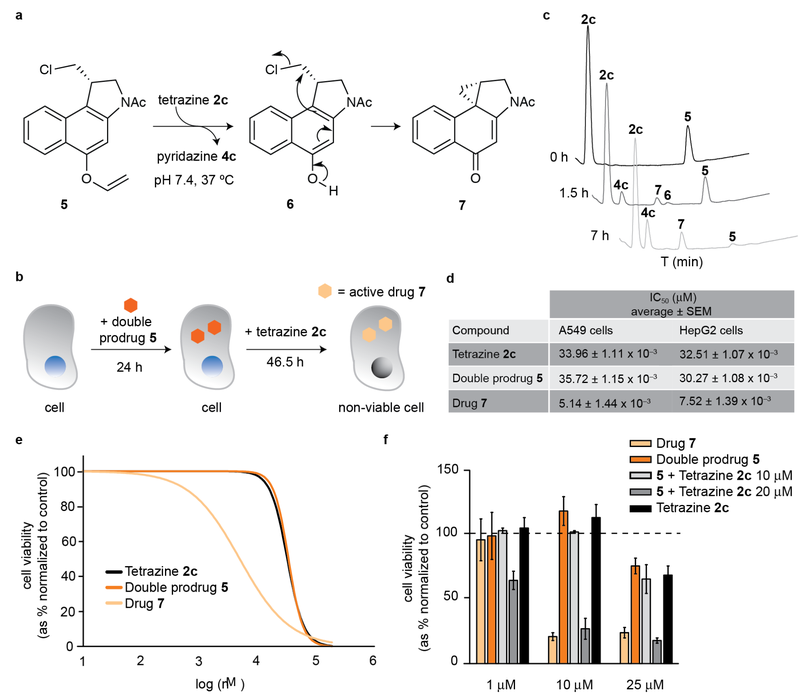


DECAGING OF A VINYL ETHER FLUOROGENIC PROBE

- Non-fluorescent coumarin derivative **1d** and tetrazine **2c**.
- Confocal microscopy showed **turn-on fluorescence** of the released coumarin

SPATIOTEMPORAL DELIVERY OF A DUOCARMYCIN-LIKE DRUG

- Compound **5** can be sucessfully decaged to obtain product **7**
- Incubation of A549 cells with double prodrug **5** and tetrazine **2c:** at 10 μM the product formed upon tetrazine-decaging of **5** is as toxic as the drug **7** alone
- Complete **drug activation** in cells



Conclusions

Here we describe a vinyl ether–tetrazine pair as IEDDA reaction partners for the efficient traceless decaging of alcohol-containing molecules in live cells. This spatiotemporal delivery method may find broad applicability in chemical biology and molecular medicine allowing the development of new traceless drug delivery strategies and precise control of protein function in vivo.

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1) J. Li, S. Jia, P. R. Chen, *Nat. Chem. Biol.* **2014**, *10*, 1003–1005
2) R. M. Versteegen, R. Rossin, W. ten Hoeve, H. M. Janssen, M. S. Robillard, *Angew. Chem.* **2013**, *125*, 14362–14366; *Angew. Chem. Int. Ed.* **2013**, *52*, 14112–14116