Conference paper

João M. J. M. Ravasco, Jaime A. S. Coelho, Alexandre F. Trindade*
and Carlos A. M. Afonso*

Synthesis and reactivity/stability study of double-functionalizable strained *trans*-cyclooctenes for tetrazine bioorthogonal reactions

https://doi.org/10.1515/pac-2019-0201

Abstract: The unique ability of the bioorthogonal pairs to withstand and unaffect biological processes while maintaining high selectivity towards each other sparked the interest in better probing and controlling biological functions. In early years, *trans*-cyclooctene (TCO)/tetrazine ligation readily standed out by encompassing most of the bioorthogonal criteria such as its excellent biocompatibility, selectivity and efficiency, and as a result of high HOMO-LUMO gap. Modifications on the TCO scaffold such as cyclopropanation render bicyclononene-based TCOs with high enhancement of its reactivity, whereas other modifications focused on improving the solubility, stability, or enabling the scaffold to act as *click*-to-release drug delivery system. The implementation of facile methods to enhance its versatility is essential for potentiating drug-delivery strategies and expanding the dynamic range of bioorthogonal on/off control. Considering the remarkable properties of bicyclononene-based TCOs we envisioned that the incorporation of an additional vector for functionalization at the cyclopropane moiety could allow access to more complex and double-functionalized TCO probes. Herein we report the synthesis and study of a double-functionalizable strained *trans*-cyclooctenes for tetrazine bioorthogonal reactions.

Keywords: alkenes; catalysis; chemoselectivity; click chemistry; cycloadditions; ICPOC-24; rhodium catalysis; tetrazine; *trans*-cyclooctene.

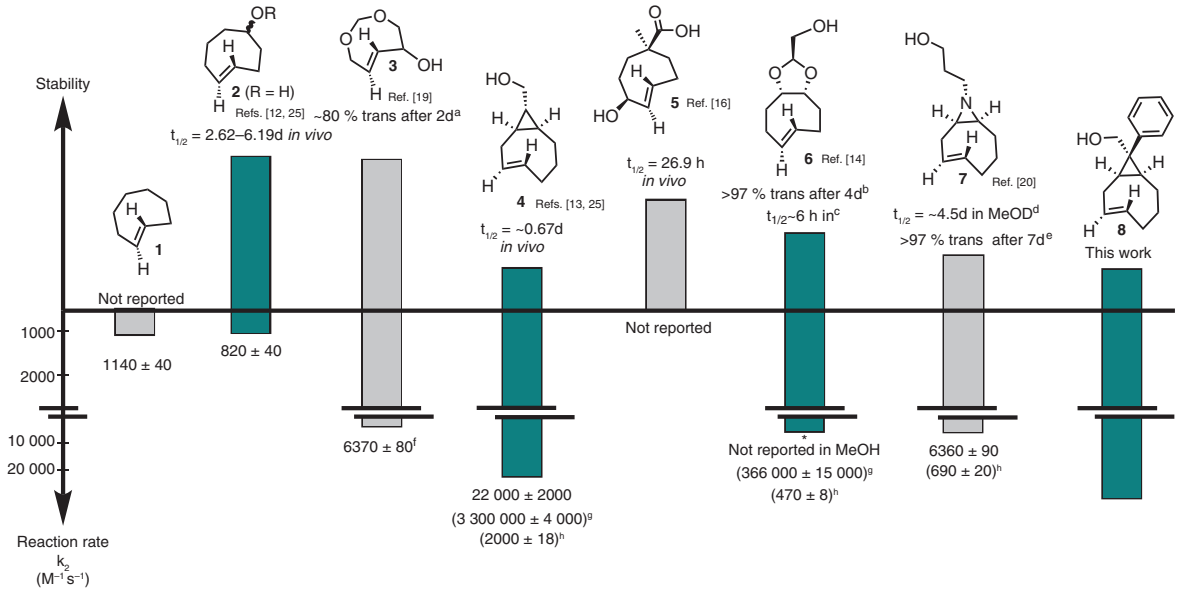
Introduction

Bioorthogonal reactions have become a significant area of research at the interface between chemistry and biology. By allowing the access to chemically modified biomolecules, these transformations are now a stablished way to probe and control biological functions both *in vitro* and *in vivo* [1, 2]. Within the bioorthogonal toolbox, strained molecules play a leading role as powerful handles in various rapid and metal-free click reactions such as strain-promoted azide-alkyne cycloaddition (SPAAC) and inverse electron-demand Diels–Alder (iEDDA) [3–8]. As a result of high HOMO energies alongside with considerable thermodynamic drive due to the release of strain energy, these molecules react with enhanced kinetic rates. Additionally, the efficiency of these transformations is governed by the energy gap between the $HOMO_{dienophile}$ and $LUMO_{diene}$,

Article note: A collection of invited papers based on presentations at the 24th IUPAC International Conference on Physical Organic Chemistry (ICPOC 24) held in Faro, Portugal, 1–6 July 2018.

***Corresponding authors: Alexandre F. Trindade,** Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Av. Prof. Gama Pinto, 1649-003 Lisboa, Portugal; and School of Chemistry, University of Leeds, Leeds LS2 9JT, UK, e-mail: a.fonsecatrindade@leeds.ac.uk; and **Carlos A. M. Afonso,** Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Av. Prof. Gama Pinto, 1649-003 Lisboa, Portugal, e-mail: carlosafonso@ff.ulisboa.pt
João M. J. M. Ravasco and Jaime A. S. Coelho: Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Av. Prof. Gama Pinto, 1649-003 Lisboa, Portugal

which can be tuned by changing the electronic properties of the reagents. In particular, inverse electron-demand Diels–Alder (iEDDA) ligations between symmetrical tetrazines and, electron rich and strained dienophiles have stood out due to its extraordinarily fast kinetics, as well as excellent biorthogonality, criteria which are crucial when working under dilute biological *milieu*. Sauer and co-workers firstly described the role of ring-strain on the acceleration of iEDDA reactions between symmetrical tetrazines and strained dienophiles (*trans*-cyclooctene (TCO) **1** vs. its *cis*-isomer) while excluding relevant differences between the electronic properties of different dienophiles [9, 10]. More recently, *trans*-cyclooctene **2** was paired with tetrazines for bioconjugation purposes as demonstrated by Fox and co-workers. The combination of these reagents allowed for very fast second-order rates ($k_2 > 10^3\ M^{-1}\ s^{-1}$) for iEDDA transformations in aqueous media, while presenting excellent biocompatibility [11, 12]. This discovery opened new avenues in iEDDA bioconjugation research with immediate applications in fundamental biology, imaging and therapeutics (Scheme 1). As described for cyclooctynes, the fusion of a cyclopropane ring into the *trans*-cyclooctene scaffold (*s*-TCO **4**) has led to impressive enhancements on the second-order rate constants resulting in a 19-fold reactivity increase [13]. In the case of these bicyclononene-based TCOs (e.g. *s*-TCO **4**), the additional strained cyclopropane ring forces the bicyclic system to adopt a half-chair like conformation which has been linked to the increase on reactivity. The same effect also resulted in a scaffold with slightly higher aqueous instability (e.g. via a free radical mediated pathway) compared to its unstrained analogs. Although rapid reactivity often comes at the expense of poorer stability in aqueous media, *s*-TCO **4** still presented satisfactory stability with no degradation in water and human serum for 24 h or in the presence of an amine and thiol nucleophiles. Nonetheless, concerns regarding stability alongside with solubility issues led to the development of dioxolane-fused *trans*-cyclooctenes (*d*-TCO **6**). Though less reactive than *s*-TCO **4** at 25 °C, this analog exhibited enhanced stability (43 % isomerization after 5 h in pH 7.4, 30 mM mercaptoethanol) and improved aqueous solubility (log P = 0.94) [14]. Another subtle modifications on the TCO scaffold (Scheme 1) have allowed its application as a *click*-to-release drug delivery system (**5**) [15, 16], in the synthesis of antibody-drug conjugates [17, 18], its incorporation into proteins through genetic expression using non-cannonical amino acids (**3**) [19], and more recently, the intrinsic generation of fluorogenic products upon reaction with simple tetrazine (**7**) [20]. The use of TCOs has been most impactful for its immediate employment in live animal as part of pre-targeting



Scheme 1: Stability/reactivity properties of various TCOs. All k_2 were measured in MeOH, at 25 °C by reaction with 3,6-di-2-pyridyl-1,2,4,5-tetrazine, unless otherwise noted; (a) TCO (6 mM) in D_2O and incubated with 3 equiv cysteamine (18 mM) at 37 °C; (b) 5 mM TCO, human serum, RT; (c) pH 7.4 in D_2O in the presence of glutathione (10 mM); (d) MeOH, covered in aluminium foil; (e) pH 7.4, 3 equiv cysteine; (f) pH 7.4, 20 °C, with Cy5-H-tetrazine; (g) H_2O, 25 °C; (h) in MeOH, using diphenyl-s-tetrazine.

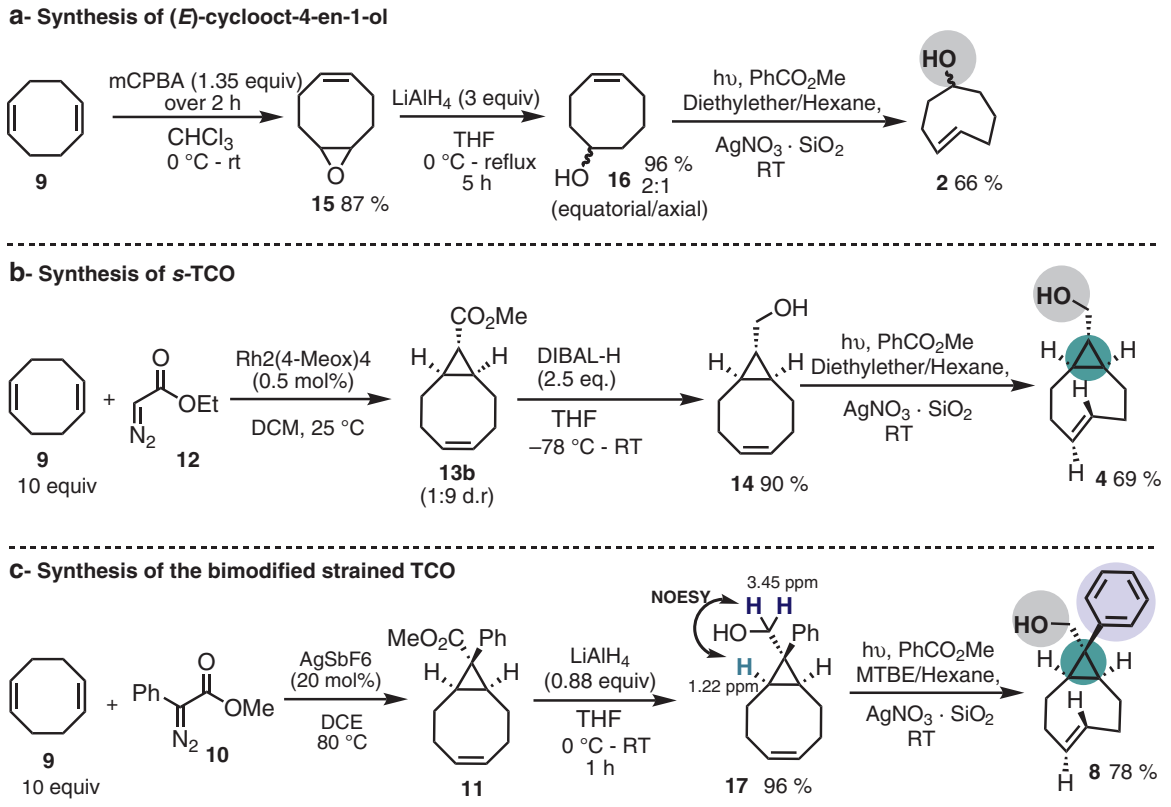
radiolabeling and theranostics strategies [21–28]. Recent applications of a pre-targeting strategy imply the administration of a tumor-binding agent (classically a full antibody) containing a first bioorthogonal handle (e.g. TCO) followed by a subsequent delivery of a second *clickable* handle linked to the imaging or therapeutic radionuclide. Both bioorthogonal reagents should withstand the *in vivo* media for the longest time possible in order to meet each other in sufficient concentration to generate a significant therapeutic effect or imaging signal. For this reason, the *in vivo* application of TCO technology has been limited to lesser-strained TCOs (e.g. **2**), which despite possessing 100-fold slower reactivity than strained TCOs such as **4**, tend to be many-fold less prone to degradation ($t_{1/2}$ = 16 h *in vivo*) [25]. On the other hand, the use of small diabody-bearing TCOs has recently been reported as an attractive approach in nuclear imaging and radioimmunotherapy not only due to their reduced kidney uptake and high tumor targeting, but also to their enhanced clearance from both blood and normal tissues, compared to full mAbs (as rapid as $t_{1/2}$ = 1.92 h) [29]. Bearing such pharmacodynamic and pharmacokinetic (PD/PK) properties, small diabodies present compatible half-lives with more strained TCOs such as **4** ($t_{1/2}$ = 0.67d *in vivo*) [25], and could be an important platform for the employment of *s*-TCOs.

Considering the remarkable properties of bicyclononene-based TCOs we envisioned that the incorporation of an additional vector for functionalization at the cyclopropane moiety could allow access to more complex and double-functionalized TCO probes. Herein, we report the synthesis of a bicyclononene TCO featuring a model quaternary center bearing an aromatic substituent, and the impact of this additional substituent on the kinetics of iEDDA bioconjugations and TCO stability.

Results and discussion

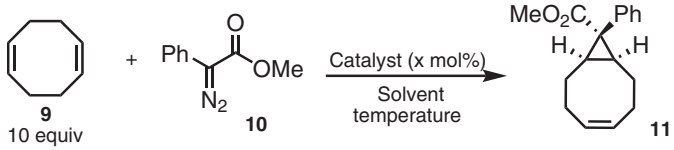
The studies began by synthesizing *trans*-cyclooctenes **4** and **8** via a convergent synthesis involving a cyclopropanation reaction between 1,4-cyclooctadiene (COD) and the corresponding diazo reagent, an ester reduction and a photochemical *cis-trans* isomerization (Scheme 2). We hypothesized that the use of the donor-acceptor methyl 2-phenyl(diazoacetate) **10** as carbene precursor would deliver the desired degree of substitution while introducing two functional groups which open new perspectives for exploring different functionalization strategies. Moreover, the use of a simple phenyl substituted donor-acceptor diazo compounds also allows evaluating the impact of an aromatic chromophore in the photoisomerization process. Davies and co-workers demonstrated that simple cyclic alkenes such as cyclohexene undergo allylic C–H insertion reactions in the presence of rhodium metallocarbenes generated from methyl 2-phenyldiazoacetate, whilst silver salts promoted a cyclopropanation reaction in refluxing dichloromethane [30]. However, no information was available on the efficiency of these methods for cyclic non-conjugated dienes such as our chosen starting material, COD. Initially, we observed that dirhodium(II) tetraacetate remain incapable of delivering the desired cyclopropane **11** from methyl 2-phenyldiazoacetate and COD (Table 1, entry 1). On the other hand, the desired cyclopropane **11** was isolated in 62 % yield by using 20 mol% of $AgSbF_6$ in DCE at 80 °C (Table 1, entry 2). The catalyst loading was reduced to 10 mol% without impacting the reaction outcome (Table 1, entry 2). Further reaction conditions modifications such as neat COD, mixture of toluene/DCE or decreasing temperature to 40 °C led to decrease in the reaction yield or no reaction at all (See Supporting Information). We anticipated that these results were most likely due to limited catalyst solubility. Finally, a screening of other silver salts including those featuring poorly coordinating counter-ions such as tetrafluoroborate led to considerably lower yields as compared to $AgSbF_6$ (Table 1, entry 3).

For the synthesis of **4**, we decided to optimize the synthetic route reported by Fox and co-workers [11] seeking higher selectivity for the *endo* product as this isomer presented superior kinetics in bioconjugation reactions compared to the *exo* diastereomer (both for cyclopropanated cyclooctynes [31, 32] and for *trans*-cyclooctenes [13, 14]). Towards this goal, we screened several typical cyclopropanation catalysts such as, dirhodium(II) catalysts, copper salts and, iron and ruthenium porphyrins (Table 2 and SI). The Rh_2OAc_4-catalyzed cyclopropanation reaction of COD with ethyl diazoacetate delivered cyclopropane **13** as a diastereomeric mixture of 2:1 favoring the *exo* product (Table 2, entry 1). Axial coordination of an *N*-heterocyclic



Scheme 2: Synthetic routes for the synthesis of TCO **2**, **4** and **8**.

Table 1: Reaction conditions optimization for the cyclopropanation reaction of 1,4-cyclooctadiene with methyl 2-phenyldiazoacetate.[a]

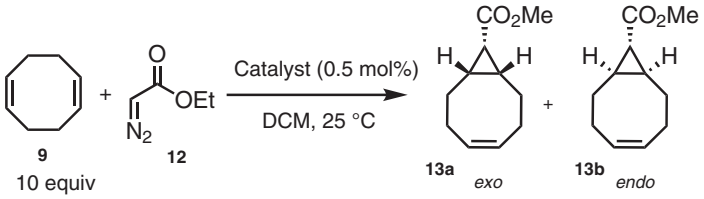


Entry	Catalyst	x (mol%)	Solvent	T (°C)	Isolated yield 11 (%)
1	$Rh_2(OAc)_4$	2	DCM or DCE	–10 to 80	Traces
2[b]	**$AgSbF_6$**	**20 [10]**	**DCE**	**80**	**62 % [60 %]**
3[b]	AgTFA, AgTf, AgBz or $AgBF_4$	10	DCE	80	10 %–33 %

[a]Reactions were carried by slow addition of the diazo (0.3 mmol in 2 mL solvent) over 3 h to a solution of COD (3 mmol in 2 mL solvent and catalyst (x mol%) at a given temperature. Best result is highlighted in bold. [b]Catalyst stirred for 30 min with COD and solvent at 80 °C before the addition of diazo reagent.

carbene (IPr) onto $Rh_2(OAc)_4$ did not result in an active catalyst under the tested reaction conditions (Table 2, entry 2). The screening of a range of other dirhodium(II) catalysts featuring distinct electronic and steric properties did not lead to a direct correlation between the rhodium electrophilic character or ligands steric with the *exo:endo* ratio. Nevertheless, the least electrophilic catalyst, $Rh_2(cap)_4$, led to an enhanced 4:1 *exo:endo* ratio (Table 2, entry 3). In addition, $Rh_2(esp)_2$ and $Rh_2(4\text{-Meox})_4$, which feature bulkier bridging ligands, led to a 1:1 and 1:9 *exo:endo* ratio, respectively, suggesting a role of steric factors in the diastereoselectivity (Table 2, entries 5, 6). Finally, the screening of other catalysts namely, iron and cobalt porphyrins and copper catalysts, did not lead to further improvements (Table 2, entries 7–9 and Supporting Information).

Table 2: Reaction conditions optimization for the cyclopropanation reaction of 1,4-cyclooctadiene with ethyl diazoacetate.[a]



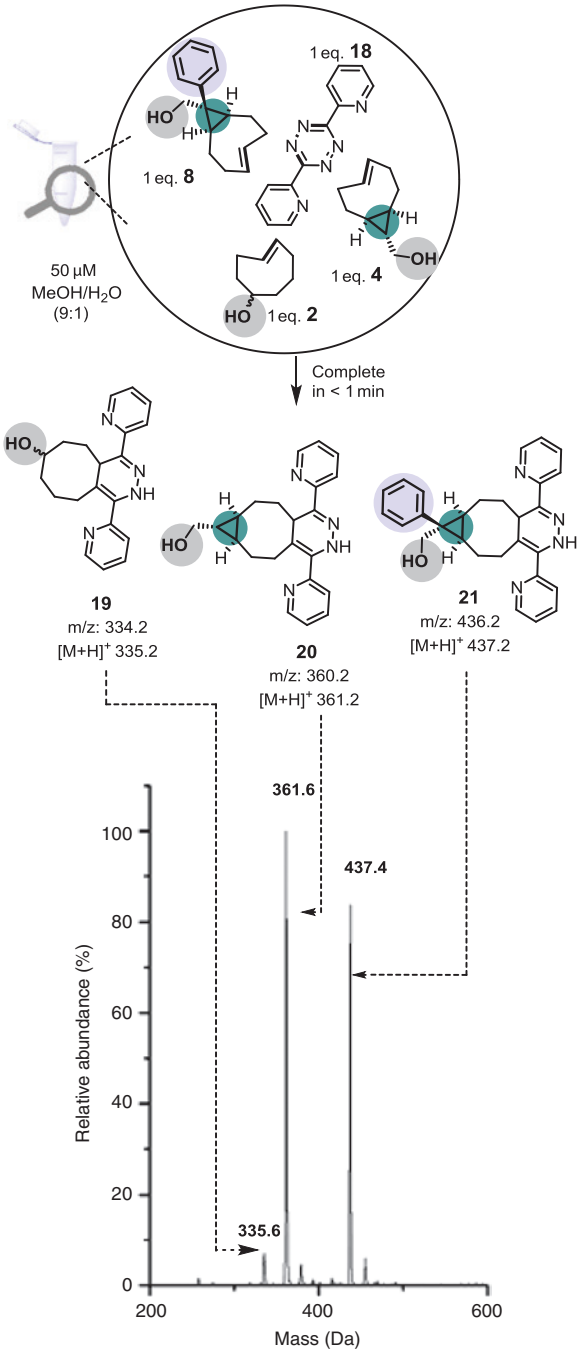
Entry	Catalyst	Yield (%)[b]	*exo:endo*[c]
1	$Rh_2(OAc)_4$	80	2:1
2	$Rh_2(OAc)_4$IPr	N.R.	–
3	$Rh_2(cap)_4$	75	4:1
4	$Rh_2(tfa)_4$	73	7:3
5	$Rh_2(esp)_2$	92	1:1
6	**Rh_2(4-Meox)$_4$**	**85**	**1:9**
7	$Rh_2(oct)_4$	91	2:3
8	$Rh_2(TBSP)_4$	90	2:3
9	Rh_2(MeO-Mandelate)$_4$	95	2:3

[a]Reactions were carried by slow addition of the diazo (0.3 mmol in 2 mL solvent) over 3 h to a solution of COD (3 mmol in 2 mL DCM and catalyst (5 mol%) at 25 °C. Best result is highlighted in bold. [b]Combined isolated yields; [c]crude GC ratio. N.R., no reaction.

In the last step of the synthesis, the cyclooctene ring was photoisomerized using a home-made flow photoreactor with selective capture of the desired *trans*-cyclooctene into an heterogenous $AgNO_3$/silica matrix [11]. TCOs **2**, **4** and **8** were isolated in good yields (66, 69 and 78 %, respectively, Scheme 2). These results show that the presence of the phenyl ring did not interfere with the photochemical isomerization and scavenging processes.

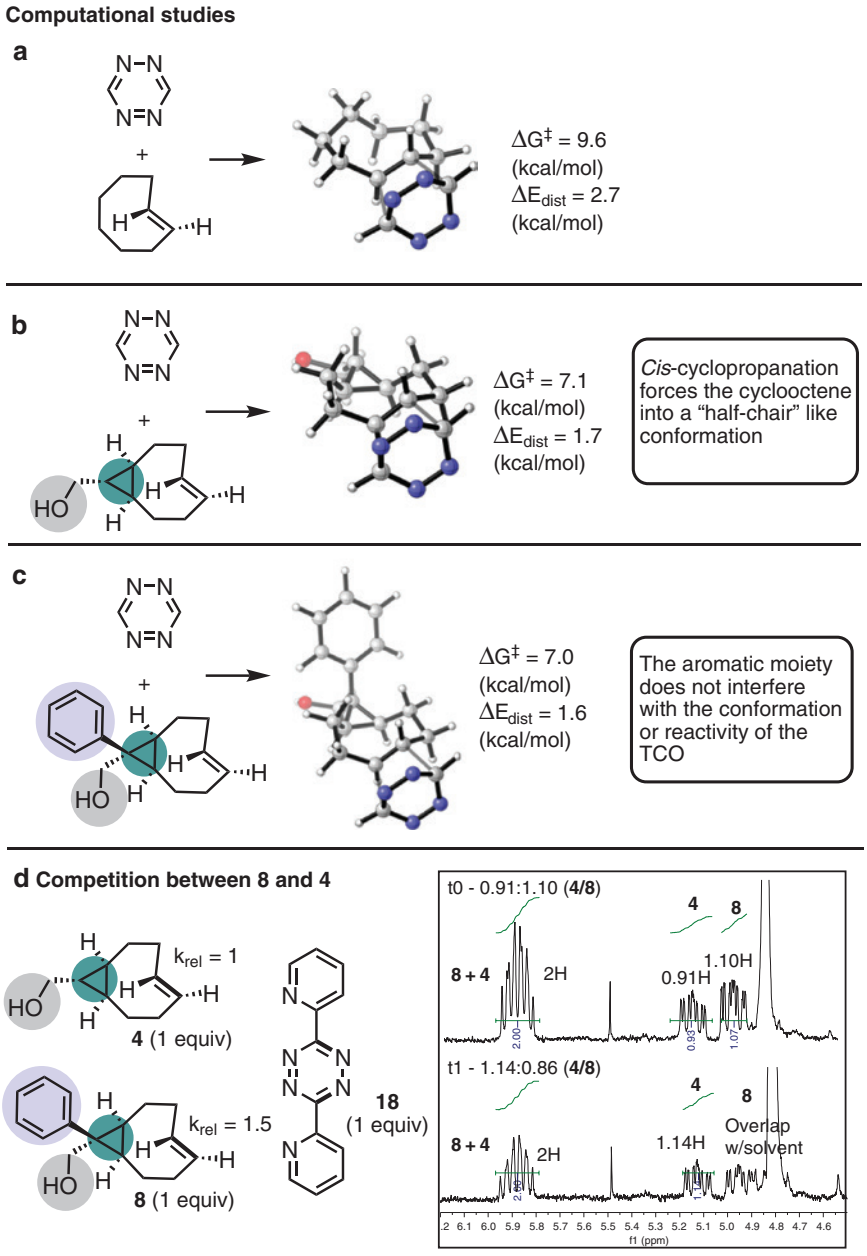
In order to evaluate the impact of the degree of substitution of the cyclopropane ring we studied the reactivity between TCO **2**, **4** and **8** and 3,6-di-2-pyridyl-1,2,4,5-tetrazine (**18**). A competitive experiment performed by incubating an equimolar mixture of TCO **2**, **4** and **8** (50 μM each) with a 50 μM tetrazine **18** solution (9:1 MeOH/H_2O) at 25 °C resulted in the full conversion of the **18** and formation of the corresponding products **19**, **20** and **21**, as observed by ESI-MS analysis (Scheme 3), Products **20** and **21** were found to be predominant relatively to **19**, being product **20** the major. Since initial observations shown that the reaction kinetics were too rapid for reliable rate determination using a UV-Vis spectrometer, we turn our attention to ^{1}H NMR analysis. Thus, the competition experiments between **4** and **8** were performed in pure MeOD allowing us to estimate that TCO **8** ($k_{rel} = 1.54 \pm 0.1$) reacted *ca.* 1.5 times faster with **18** than TCO **4** ($k_{rel} = 1$). This result highlights the vector grow did not introduce a level of steric hindrance that would render the TCO **8** significantly less reactive, such as TCO **2**.

As mentioned previously TCO structure and reactivity also impart important contributions to the reagent stability/degradability [13]. Nonetheless, strained TCOs can still present moderate to good stability, as reported for TCO **4** [13] and for TCO **6** [14], which presented and half-life of ~6 h at pH 7.4 D_2O in 10 mM GSH. Bioorthogonal handles should ideally be stable at biological pH and inert to biological nucleophiles such as thiols and amines, as well as stable under proper storage (preferentially bench-stable) and easy to handle. In order to evaluate the stability of novel TCO **8**, we studied its degradation in various biologically mimetic conditions, as well as in the presence of nucleophiles. TCO **8** showed no decomposition for over 70 h in buffered D_2O (pH 7.4 and 6, 1:1 DMSO/PBS), and similarly to TCO **4**, no decomposition was detected upon exposure to high concentration of an amine nucleophile (30 mM n-pentylamine in CD_3OD) for 24 h. On the other hand, some degradation was observed (12 % after 12 h) in the presence of concentrated ethanethiol (30 mM in CD_3OD), with a more rapid isomerization occurring if exposed to high concentration of reduced GSH in aqueous media (60 % in 12 h in DMSO/PBS 1:1, pH 7.4). Similar observations have been reported for very stable dioxolane-fused *trans*-cyclooctenes such as **6**, which isomerized rapidly under similar conditions (30 mM



Scheme 3: ESI-MS competition assays between TCOs (50 μM each) and 18 (50 μM) in 9:1 MeOH/H_2O.

mercaptoethanol, pH 7.4, 43 % isomerization after 5 h). Furthermore, TCO **8** proved to be stable if bench kept for 12 days as a solid, suffering no degradation. As caution, the TCO was kept under freezer storage and has proven to be stable for at least 6 months. In order to further understand the impact of the added aromatic moiety onto TCO **8** in its reactivity towards tetrazines, we performed DFT calculations at M06L/6-311+G(d,p)//M06L/6-31G(d) level of theory. For this purpose we calculated both activation free energy and distortion energies [5, 8, 33] in the transition state of the reaction between 1,2,4,5-tetrazine (s-tetrazine) with *trans*-cyclooctene, and TCOs **4** and **8**. Transition state calculations in the gas phase for the cycloaddition between s-tetrazine and **4** proceeded with a barrier of $\Delta G^{\ddagger} = 7.1$ kcal mol^{-1} and an $\Delta E_{dist} = 1.7$ kcal mol^{-1} (Scheme



Scheme 4: Optimized transition state structures for the iEDDA reaction of s-tetrazine with (a) *trans*-cyclooctene, (b) **4** and (c) **8.** Calculations were performed at M06L/6-311+G(d,p)// M06L/6-31G(d) level of theory. (d) Competition NMR experiments between **4** and **8** with 3,6-di-2-pyridyl-1,2,4,5-tetrazine in CD_3OD at 22 °C.

4b). These barriers are considerably lower than the activation free energy and distortion energies calculated for *trans*-cyclooctene ($\Delta G^{\ddagger} = 9.6$ kcal mol^{-1}; $\Delta E_{dist} = 2.7$ kcal mol^{-1}; Scheme 4a), which are cconsistent with previous reports where the bicyclononene adopt a half-chair like conformation, increasing its ring strain and accelerating its reactivity towards tetrazines [13]. Finally, introduction of the aromatic ring (TCO **8**) did not significantly change the energy barrier for the iEDDA towards *s*-tetrazine, nor the distortion energy in the transition state ($\Delta G^{\ddagger} = 7.0$ kcal mol^{-1}; $\Delta E_{dist} = 1.6$ kcal mol^{-1}; Scheme 4c) comparing to the highly reactive bicyclononene **4** (Scheme 4b). Such results support our NMR observations where **8** reacts slightly faster than **4**, but still in the same order of magnitude. Furthermore, amongst diastereomers, the isomer **8** has the lowest calculated energies ($\Delta G^{\ddagger} = -0.6$ kcal mol^{-1} than the other *cis*-cyclopropaned TCO isomer), while the *trans*-cyclopropane ring fused diastereomer presented the highest energies as expected (see SI).

Conclusions

Tackling TCO scaffold's sensitivity, responsible for demanding and sometimes serendipitous iterative modifications, we envisioned a cheap silver-catalyzed method to allow double functionalization in TCO scaffolds without compromising reactivity and stability. As the original scaffold only tolerates the appendage of one payload, we successfully developed a method for the synthesis of a new hetero double-functionalized strained TCOs, capable of generating this bioorthogonal handle in moderate to good overall yield. Despite the presence of a quaternary center, the new TCO presented no deleterious reactivity towards tetrazine when compared to its predecessor, the most rapid TCO known (**4**), yet, with a slight decrease in hydrophilicity. Additionally, stability studies in aqueous media and in the presence of mimetics of biological nucleophiles showed that the quaternary center did not cause a detrimental impact on neither *trans-cis* isomerization nor cyclopropane-ring opening comparing to the literature, being completely stable in buffered solutions up to 3 days. We believe the construction of this double modified bioorthogonal handle may pave the way to access more complex and double functionalized homo or heterogenous therapeutic and/or fluorogenic TCO probes in the future.

Acknowledgements: The authors acknowledge Fundação para a Ciência e a Tecnologia (FCT) (Ref. SFRH/BD/120829/2016, PTDC/QEQ-QOR/3644/2014, UID/DTP/04138/2013) for financial support.

References

[1] B. L. Oliveira, Z. Guo, G. J. L. Bernardes. *Chem. Soc. Rev.* **46**, 4895 (2017).
[2] O. S. Walker, S. J. Elsasser, M. Mahesh, M. Bachman, S. Balasubramanian, J. W. Chin. *J. Am. Chem. Soc.* **138**, 718 (2016).
[3] H. Wu, N. K. Devaraj. *Top. Curr. Chem.* **374**, (2016).
[4] B. L. Oliveira, Z. Guo, G. J. L. Bernardes. *Chem. Soc. Rev.* **46**, 4895 (2017).
[5] F. Liu, Y. Liang, K. N. Houk. *Acc. Chem. Res.* **50**, 2297 (2017).
[6] J. C. Jewett, C. R. Bertozzi. *Chem. Soc. Rev.* **39**, 1272 (2010).
[7] E. M. Sletten, C. R. Bertozzi. *Acc. Chem. Res.* **44**, 666 (2011).
[8] R. D. Row, J. A. Prescher. *Acc. Chem. Res.* **51**, 1073 (2018).
[9] F. Thalhammer, U. Wallfahrer, J. Sauer. *Tetrahedron Lett.* **31**, 6851 (1990).
[10] J. Sauer, D. K. Heldmann, J. Hetzenegger, J. Krauthan, H. Sichert, J. Schuster. *Eur. J. Org. Chem.* **12**, 2885 (1998).
[11] M. Royzen, G. P. A. Yap, J. M. A. Fox. *J. Am. Chem. Soc.* **130**, 3760 (2008).
[12] M. L. Blackman, M. Royzen, J. M. Fox. *J. Am. Chem. Soc.* **130**, 13518 (2008).
[13] M. T. Taylor, M. L. Blackman, O. Dmitrenko, J. M. Fox. *J. Am. Chem. Soc.* **133**, 9646 (2011).
[14] A. Darko, S. Wallace, O. Dmitrenko, M. M. Machovina, R. A. Mehl, J. W. Chin, J. M. Fox. *Chem. Sci.* **5**, 3770 (2014).
[15] R. M. Versteegen, R. Rossin, W. Ten Hoeve, H. M. Janssen, M. S. Robillard. *Angew. Chemie – Int. Ed.* **52**, 14112 (2013).
[16] R. Rossin, S. M. J. Van Duijnhoven, W. Ten Hoeve, H. M. Janssen, L. H. J. Kleijn, F. J. M. Hoeben, R. M. Versteegen, M. S. Robillard. *Bioconjug. Chem.* **27**, 1697 (2016).
[17] M. S. Robillard, W. Ten Hoeve, R. M. Versteegen, R. Rossin, F. J. M. Hoeben. Bio-Orthogonal Drug Activation. *WO 2012/156920*, PCT/IB2012/052447, *Filled 16/05/2012*.
[18] S. Hapuarachchige, Y. Kato, D. Artemov. *Sci. Rep.* **6**, 24298 (2016).
[19] E. Kozma, I. Nikić, B. R. Varga, I. V. Aramburu, J. H. Kang, O. T. Fackler, E. A. Lemke, P. Kele. *ChemBioChem.* **17**, 1518 (2016).
[20] S. J. Siegl, A. Vázquez, R. Dzijak, M. Dračínský, J. Galeta, R. Rampmaier, B. Klepetářová, M. Vrabel. *Chem. – A Eur. J.* **24**, 2426 (2018).
[21] T. Stauch, J. F. Scholtes, A. Dreuw. *Chem. Phys. Lett.* **654**, 6 (2016).
[22] O. Keinänen, E. M. Mäkilä, R. Lindgren, H. Virtanen, H. Liljenbäck, V. Oikonen, M. Sarparanta, C. Molthoff, A. D. Windhorst, A. Roivainen, J. J. Salonen, A. J. Airaksinen. *ACS Omega* **2**, 62 (2017).
[23] R. Rossin, T. Lappchen, S. M. van den Bosch, R. Laforest, M. S. Robillard. *J. Nucl. Med.* **54**, 1989 (2013).
[24] A. Rondon, N. Ty, J. B. Bequignat, M. Quintana, A. Briat, T. Witkowski, B. Bouchon, C. Boucheix, E. Miot-Noirault, J. P. Pouget, J. M. Chezal, I. Navarro-Teulon, E. Moreau, F. Degoul. *Sci. Rep.* **7**, 14918 (2017).
[25] R. Rossin, S. M. Van Den Bosch, W. Ten Hoeve, M. Carvelli, R. M. Versteegen, J. Lub, M. S. Robillard. *Bioconjug. Chem.* **24**, 1210 (2013).
[26] P. Adumeau, K. E. Carnazza, C. Brand, S. D. Carlin, T. Reiner, B. J. Agnew, J. S. Lewis, B. M. Zeglis. *Theranostics* **6**, 2267 (2016).

[27] J. P. Meyer, J. L. Houghton, P. Kozlowski, D. Abdel-Atti, T. Reiner, N. V. K. Pillarsetty, W. W. Scholz, B. M. Zeglis, J. S. Lewis. *Bioconjug. Chem.* **27**, 298 (2016).
[28] E. M. F. Billaud, E. Shahbazali, M. Ahamed, F. Cleeren, T. Noël, M. Koole, A. Verbruggen, V. Hessel, G. Bormans. *Chem. Sci.* **8**, 1251 (2017).
[29] S. M. J. van Duijnhoven, R. Rossin, S. M. van den Bosch, M. P. Wheatcroft, P. J. Hudson, M. S. Robillard. *J. Nucl. Med.* **56**, 1422 (2015).
[30] J. L. Thompson, H. M. L. Davies. *J. Am. Chem. Soc.* **129**, 6090 (2007).
[31] J. Dommerholt, S. Schmidt, R. Temming, L. J. A. Hendriks, F. P. J. T. Rutjes, J. C. M. Van Hest, D. J. Lefeber, P. Friedl, F. L. Van Delft. *Angew. Chemie – Int. Ed.* **49**, 9422 (2010).
[32] I. Nikić, J. H. Kang, G. E. Girona, I. V. Aramburu, E. A. Lemke. *Nat. Protoc.* **10**, 780 (2015).
[33] F. M. Bickelhaupt, K. N. Houk. *Angew. Chemie – Int. Ed.* **56**, 10070 (2017).

Supplementary Material: The online version of this article offers supplementary material (https://doi.org/10.1515/pac-2019-0201).