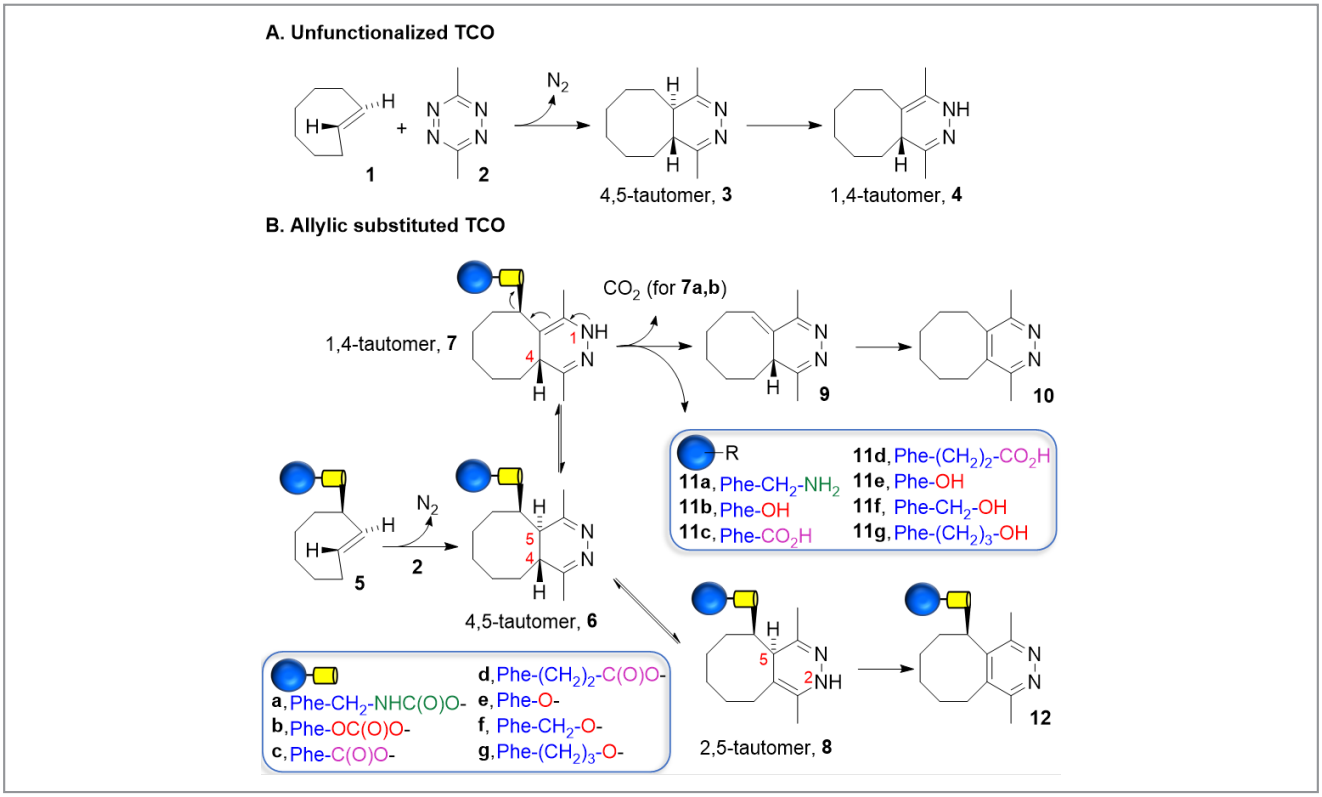
Chemically Cleavable Antibody–Drug Conjugates: Drug Release in One Click

Since the introduction of the Staudinger ligation, the field of bioorthogonal chemistry has grown rapidly, and today's bioorthogonal toolbox includes a plethora of reactions that are highly selective and function in aqueous and complex media at physiological pH. Among these, the inverse-electron-demand Diels–Alder (IEDDA) cycloaddition between strained alkenes and tetrazines (TZs) has proven to be an extraordinary tool due to exceptional speed allowing very low reagent concentrations, potentially enabling in vivo click chemistry for medical applications. This reaction has already been applied in a range of fields, such as materials chemistry, chemical biology (protein modification in vivo, study of dynamic processes in living cells, and high-resolution imaging, among others) and nuclear medicine (radiolabeling of sensitive molecules, and pretargeted radioimmunoimaging and therapy). At first, the IEDDA reaction was viewed only as a powerful ligation tool. Until a few years ago.

In 2013 Dr. Marc Robillard from Tagworks Pharmaceuticals (The Netherlands) and co-workers developed a bioorthogonal bond cleavage reaction, the IEDDA pyridazine elimination, to enable selective elimination chemistry in living systems (*Angew. Chem. Int. Ed.* **2013**, *52*, 14112–14116). "In this modification of the IEDDA cycloaddition, the strained alkene is a *trans*-cyclooctene (TCO) modified at the allylic position with a suitable leaving group, which in our first design was an amine linked to the TCO as a carbamate (Scheme 1)," said Dr. Robillard. He continued: "We hypothesised that, upon TCO reaction with a TZ and release of N_2, the 4,5-dihydropyridazine



Scheme 1 A) IEDDA conjugation B) IEDDA pyridazine elimination of the axial isomers of TCO carbamate, carbonate, esters, and ethers. R. M. Versteegen et al. Click-to-Release from *trans*-Cyclooctenes: Mechanistic Insights and Expansion of Scope from Established Carbamate to Remarkable Ether Cleavage *Angew. Chem. Int. Ed.* **2018**, 57, 10494–10499 © Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission.

(**6**) tautomerises to the 1,4 analogue (**7**) which then undergoes an electron cascade resulting in elimination of CO_2 and free amine. We set out to apply this new elimination reaction in the chemically triggered release of drugs from tumour-bound antibody–drug conjugates (ADCs) to expand the scope of amenable ADC targets from internalizing cancer receptors to those that do not internalise, and to targets in the tumour stroma. Current ADCs are designed to release their drug inside the cancer cell by means of proteases, pH-induced linker hydrolysis, or disulfide-to-thiol reduction promoted by reductases, and therefore can only be used with internalizing cancer receptors. The IEDDA pyridazine elimination has been used in other applications as well, including local prodrug activation for cancer therapy (*ACS Cent. Sci.* **2016**, *2*, 476-482), T-cell activation (*ACS Chem. Biol.* **2018**, *13*, 1569–1576), and protein profiling and uncaging in living cells (*ACS Cent. Sci.* **2016**, *2*, 325–331; *Chem. Commun.* **2017**, *53*, 8443–8446), amongst others."

In a recent article (*Angew. Chem. Int. Ed.* **2018**, *57*, 10494–10499) the group further expanded the scope of the IEDDA pyridazine elimination by demonstrating that, besides carbamate-derived amines, other chemical functionalities can also be liberated following the reaction between an allylic-substituted TCO and a TZ. "In this work, we synthesised TCOs comprising a range of allylic substituents (Scheme 1): aromatic carbonate (**5b**), aromatic and aliphatic esters (**5c**,**d**), and aromatic, benzylic and aliphatic ethers (**5e–g**)," explained Dr. Robillard. With these TCO derivatives the group carried out a thorough evaluation on the formation and disappearance of the dihydropyridazine tautomers and elimination products formed after IEDDA reaction with TZs. The relatively slow tautomerisation in $CDCl_3$ allowed them to study closely the release reaction using ^{1}H NMR and GCMS before moving to more relevant buffered aqueous solutions. "We were able to further support our original hypothesis, namely that the 1,4-dihydropyridazine tautomer is the species producing fast release," remarked Dr. Robillard. He continued: "We also found that the non-releasing 2,5-tautomer can convert slowly into the 1,4-tautomer, thereby contributing to the release and leading to a biphasic release profile. Furthermore, we were particularly pleased to find that ethers, even aliphatic ethers, could also be cleaved in a high yield with this strategy, given the stability

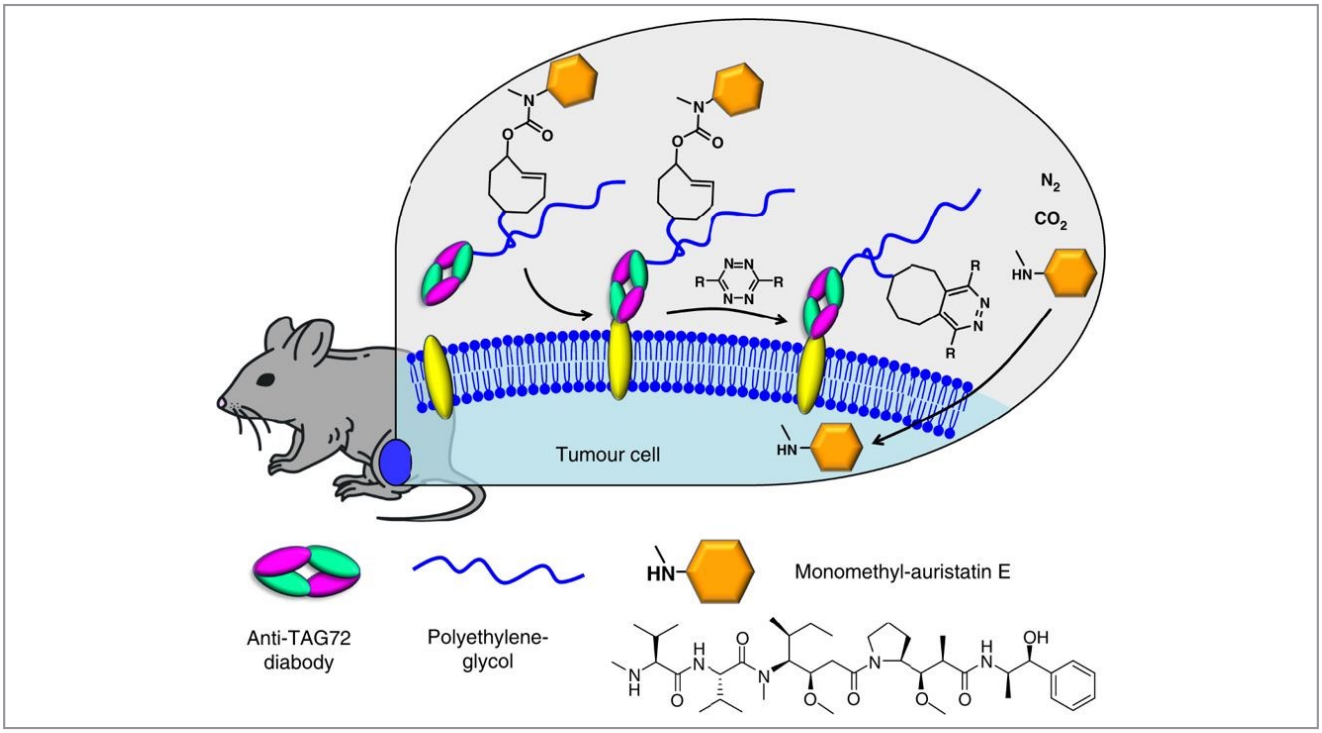


Figure 1 Triggered drug release using "click-to-release" chemistry in vivo: on-tumour liberation of a cell-permeable drug (monomethylauristatin E; MMAE) from a TCO-linked ADC following systemic administration of a TZ activator. Reprinted from R. Rossin et al. *Nat. Commun.* **2018**, *9*, 1484 (Creative Commons Attribution 4.0 International license http://creativecommons.org/licenses/by/4.0/).

of the ether bond and the poor leaving group nature of alkoxides." This indicated that the elimination is mainly governed by the formation of the rapidly eliminating 1,4-dihydropyridazine tautomer, and less by the nature of the leaving group. According to Dr. Robillard, expanding the scope of this cleavage reaction will allow the use of drugs lacking amenable amines, such as duocarmycins. Furthermore, it expands the scope of chemical functionalities that can be unmasked in the context of chemical biology and synthetic chemistry.

"In parallel with the abovementioned mechanistic studies, we conducted the first therapeutic evaluation of chemically cleavable ADCs in mouse models of human cancer, which recently appeared in *Nature Communications* (*Nat. Commun.* **2018**, *9*, 1484)," said Dr. Robillard. This was a collaborative project between Tagworks and several other companies (SyMO-Chem, Avipep, Levena, Syncom) and was carried out at the laboratories of the Radboud University Medical Center and Radboud University, in The Netherlands. "The ADC used in this study is based on a diabody targeting TAG72, a non-internalizing pan-carcinoma target widely expressed in a range of epithelial-derived human adenocarcinomas such as ovarian, colorectal and breast cancers. The ADC carries four bifunctional TCOs linked via a carbamate to monomethylauristatin E (MMAE), a potent and cell-permeable antimitotic agent (Figure 1)," explained Dr. Robillard.

This click-cleavable ADC (tc-ADC) was tested in two mouse models of colon and ovarian carcinomas, in a side-by-side comparison with an analogous ADC containing the protease-sensitive valine–citrulline linker (vc-ADC), designed for intracellular release and used in the marketed ADC Adcetris. "At first, however, we dedicated considerable effort to developing a suitable TZ-based activator, capable of effective on-tumour reaction with the TCO and efficient MMAE release from the ADC," said Dr. Robillard. He continued: "After our first proof-of-concept study on chemically triggered drug release in vivo (*Bioconjugate Chem.* **2016**, *27*, 1697–1706), we realised that sustained circulation of the activator is the key for success. The 3,6-bisalkyl TZs that give high release have a relatively low reactivity and, as small molecules, they clear from circulation too quickly, precluding quantitative on-tumour reaction. Therefore, in our recent study we designed a 3,6-bisalkyl TZ activator containing a PEG_{11}-DOTA (DOTA = 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid) moiety that showed a 12 minute half-life in blood and low retention in non-target tissues. Sequential administration of the tc-ADC and activator pair was shown to lead to high ADC uptake in tumours and complete on-tumour IEDDA reaction, producing high tumour levels of free MMAE one and three days post-ADC activation. The presence of a DOTA chelator in the activator structure presented the opportunity to add a gamma-emitting radiometal (indium-111) and to demonstrate by SPECT-CT imaging that ADC activation took place throughout the tumour." The group's subsequent efficacy studies demonstrated a potent therapeutic effect for the chemically cleavable tc-ADC, with a markedly delayed tumour growth in the human colorectal cancer model (LS174T) and pronounced and durable tumour regression for at least four months (the duration of the study) in the ovarian cancer tumour model (OVCAR-3; Figure 2). On the contrary, the gold standard (enzymatically cleavable) vc-ADC failed to control tumour growth in both models. The limited therapeutic effect observed in the ovarian model for vc-ADC was most likely due to extracellular protease-based MMAE release.

"Overall, the IEDDA pyridazine elimination has already proven to be a very versatile reaction with diverse applications in medicine, chemical biology and synthetic chemistry

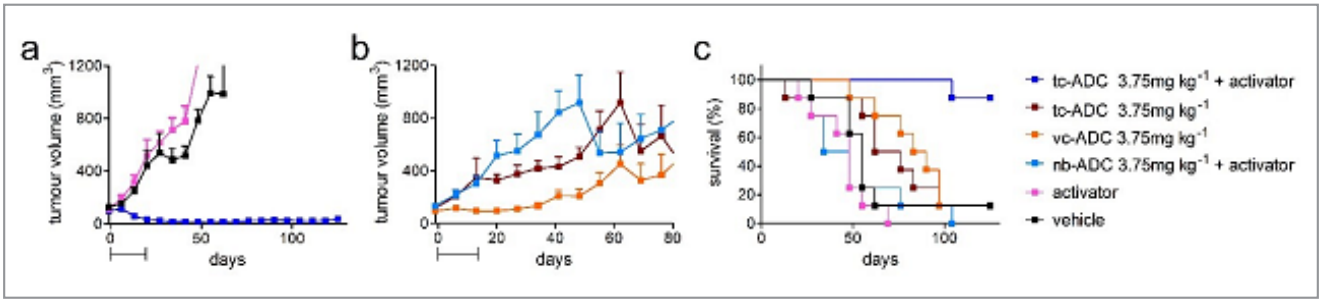


Figure 2 Mean OVCAR-3 tumour volumes (with SEM) in mice that within two weeks received i.v. (a) four cycles of the combination of tc-ADC with activator, activator alone, or vehicle, in comparison to mice that received i.v. four cycles of (b) tc-ADC alone, vc-ADC alone, or the combination of non-binding nb-ADC with activator. (c) Survival curves. The bars below the x axis indicate the treatment periods. Adapted from R. Rossin et al. *Nat. Commun.* **2018**, *9*, 1484 (Creative Commons Attribution 4.0 International license http://creativecommons.org/licenses/by/4.0/).

and we expect the number of applications to continue to grow," said Dr. Robillard. He concluded: "We believe that extracellular click-to-release is one of the key applications for this powerful technology, as it expands the scope of current ADC therapy and it allows other therapeutic targets to be addressed. The therapeutic proof of concept shown in *Nature Communications* is an important step towards the use of such click-to-release approaches in the clinic."

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