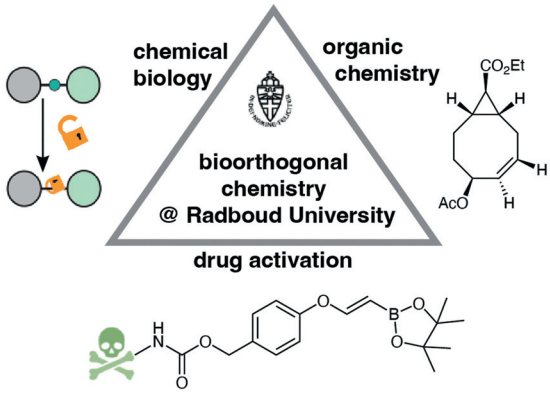
Synlett F. P. Rutjes et al. THIEME OPEN ACCESS Account

Bioorthogonal Chemistry at Radboud University: Past, Present and Future

Floris P. J. T. Rutjes*
Kimberly M. Bonger*
Kevin Neumann*

Institute for Molecules and Materials, Radboud University, Nijmegen, the Netherlands
floris.rutjes@ru.nl
k.bonger@science.ru.nl
kevin.neumann@ru.nl

Published as part of the Cluster
Japan/Netherlands Gratama Workshop



Received: 21.01.2024
Accepted after revision: 14.02.2024
Published online: 14.03.2024 (Version of Record)
DOI: 10.1055/s-0042-1751569; Art ID: ST-2024-01-0015-A

Crowd-Reviewed

Abstract Over the past two decades, bioorthogonal chemistry has profoundly impacted various chemistry-related fields, including chemical biology and drug delivery. This transformative progress stems from collaborative efforts involving chemists and biologists, underscoring the importance of interdisciplinary research. In this Account, we present the developments in bioorthogonal chemistry within our Institute for Molecules and Materials at Radboud University. The chemistry disclosed here spans from strained alkynes and alkenes to drug release and bioconjugation strategies, mirroring the extensive scope provided by bioorthogonal chemistry. By reflecting on the chemistry originating at Radboud University, this Account emphasizes that teamwork is essential for driving significant progress in bioorthogonal chemistry.

Keywords bioorthogonal chemistry, chemical biology, strained alkynes, tetrazines, drug activation

1 Introduction

Historically, chemical transformations have been carried out under strictly defined conditions to ensure optimal reaction outcomes. These conditions often involve the use of anhydrous solvents, high concentrations of reagents, prolonged reaction times, and elevated temperatures. The growing significance of biotherapeutics and advancements in chemical biology have urged chemists to develop chemical reactions that not only exhibit a high level of chemoselectivity but also demonstrate biocompatibility. Carolyn Bertozzi was the first to coin the term 'bioorthogonal chemistry', referring to reaction systems that typically proceed at low micromolar concentrations with a high degree of chemoselectivity while maintaining overall biocompatibility.[1] Ultimately, this allowed chemists to conduct synthetic chemistry within a biological environment.

After years of intense research, an impressive repertoire of reactions has been reported that can be considered as truly bioorthogonal, pushing the boundaries of synthetic organic chemistry.[2] Our 'Chemistry Institute' at Radboud University – known as the Institute for Molecules and Materials – has made significant contributions to these advancements. For example, pioneering work on strained alkynes for strain-promoted azide–alkyne click chemistry was developed at Radboud University in Nijmegen during

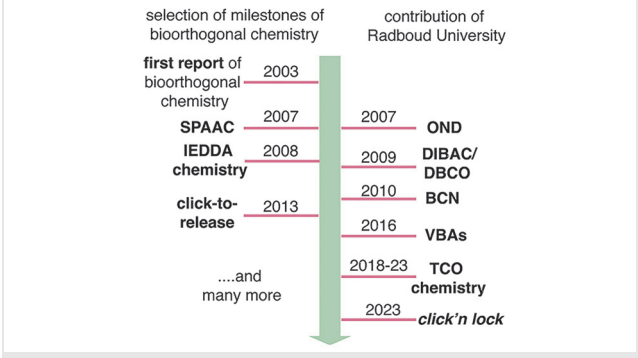


Figure 1 Timeline of developments in the field of bioorthogonal chemistry including contributions from Radboud University.

the early stages.[3] This Account reflects on the developments that originated from Radboud University and highlights their impact on the emerging field of bioorthogonal chemistry (Figure 1).

One can debate the exact origin of our institute's focus on bioorthogonal chemistry, but our strong emphasis on interdisciplinary research and education at Radboud University certainly has played an essential role. This emphasis extends beyond traditional subdisciplines of chemistry and includes research collaborations with biologists, clinicians, and physicists, but also broader educational programmes such as the Bachelor's programme of Molecular Life Sciences. It was this interdisciplinary nature that paved the way for early work in bioorthogonal chemistry, a discipline that emerged at the interface of chemistry and biology with applications in biomedical research and medicine. Professors Floris Rutjes and Floris van Delft conducted pioneering work on metal-free click chemistry tools at our institute, laying the foundation for the modern field of bioorthogonal chemistry.[4]

Since then, our institute has attracted numerous early career academics who have developed into established group leaders with international recognition in the field of bioorthogonal chemistry. The group led by Dr. Kim Bonger has not only developed vinyl boronic acids as new chemical tools for bioorthogonal chemistry but has also reported metabolically active amino acid derivatives with bioorthogonal functionalities, enabling the study of protein synthesis at a molecular level.[5,6] Other faculty members expanded the use of bioorthogonal chemistry into the fields of carbohydrate and RNA chemistry, namely Dr. Thomas Boltje and Dr. Willem Velema, respectively.[7,8] Finally, new faculty member Dr. Kevin Neumann and his group exploit bioorthogonal chemistry for applications in nanomedicine and biotherapeutics.[9] In this Account, we provide specific examples

Biographical Sketches

Floris Rutjes received his PhD from the University of Amsterdam in 1993 with Prof. W. N. Speckamp and Prof. H. Hiemstra and conducted postdoctoral research with Prof. K. C. Nicolaou at The Scripps Research Institute, La Jolla, USA. In 1999 he became full professor in organic synthesis at Radboud University, Nijmegen. He was awarded amongst others the Gold Medal of the Royal Netherlands Chemical Society (KNCV, 2002), the AstraZeneca Award for Research in Organic Chemistry (2003), Most Entrepreneurial Scientist of the Netherlands (2008), and Chemistry Europe Fellow (2022). He is elected member of the Netherlands Academy of Engineering and the Academia Europaea. Currently, he is director of the Institute for Molecules and Materials at Radboud University and vice-president of the European Chemical Society (EuChemS).

Kim Bonger obtained her PhD in (bio)organic chemistry from Leiden University in 2008 working under the supervision of Prof. Dr. Gijs van der Marel and Prof. Dr. Hermen Overkleeft. She then switched fields to molecular biology and cell biology and spent four years as a postdoc with Prof. Dr. Thomas Wandless at Stanford University. In 2013 she started her independent research career in chemical biology at the Radboud University in Nijmegen, the Netherlands, where she was promoted to associate professor in 2021. Her research focuses on the development of novel bioorthogonal chemistry and chemoenzymatic methods for imaging, target discovery, and precision medicine. She further develops molecular probes and explores new chemical biology strategies to understand and modulate the cellular mechanisms involved in (auto)immune diseases.

Kevin Neumann is an assistant professor at Radboud University in Nijmegen, the Netherlands. After obtaining his MSc in Stuttgart, Germany, he joined the group of Prof. Mark Bradley for his PhD studies at the University of Edinburgh. From 2018 to 2021, Kevin worked in the group of Prof. Jeffrey Bode as a postdoctoral fellow at ETH Zurich, Switzerland. Since 2021, Kevin is assistant professor in Nijmegen, the Netherlands and is leading an interdisciplinary group that works in the fields of chemical biology, organic chemistry, and nanomedicine. Among other things, his group focuses on the development of new click strategies for precision synthesis of therapeutical active biomolecules including proteins and cyclic peptides.

alongside a historical overview of achievements associated with bioorthogonal chemistry at Radboud University, showcasing the power of interdisciplinarity in modern chemistry for creating impact.

2 Providing BCN as a Robust Bioorthogonal Tool for Chemical Biology and Beyond (by Floris Rutjes)

Since the initial introduction of bioorthogonal chemistry by Bertozzi and her colleagues, based on copper click chemistry, researchers have sought to identify more biocompatible, stable, and efficient reagents. Bertozzi's group reported strain-promoted alkyne–azide cycloaddition (SPAAC).[10] In brief, the ring strain alters the bond angles of the sp-hybridized carbons to ca. 160°, thus changing the geometry towards the transition state of the cycloaddition. It's noteworthy that cyclooctynes and their rapid reactions with phenylazides were reported in the 1950s by the teams around Blomquist and Prelog at Cornell University and ETH Zurich, respectively.[11,12] At Radboud University, a team led by Floris van Delft introduced cyclooctynes as suitable reagents for rapid strain-promoted cycloaddition with nitrones, referred to as SPANC, in 2010.[13] At that time, available strained alkynes suffered from lengthy synthesis routes. For instance, dibenzo-azacyclooctyne **1** (DIBAC, nowadays commonly termed DBCO) required nine steps, while difluorooctyne **2** (DIFO) needed eight synthetic steps (Figure 2).[14,15] Additionally, these strained alkynes are asymmetrical, eventually resulting in several isomers as products, complicating product isolation and characterization.

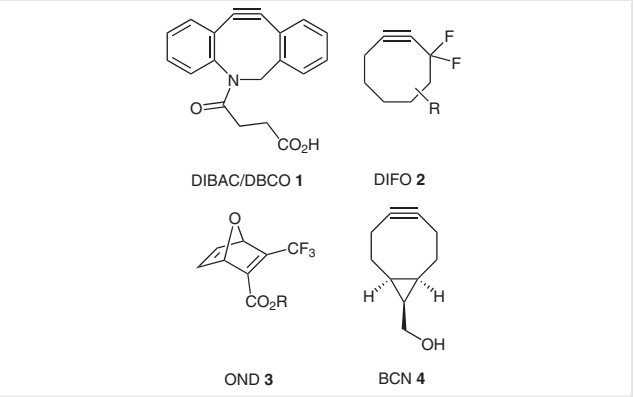
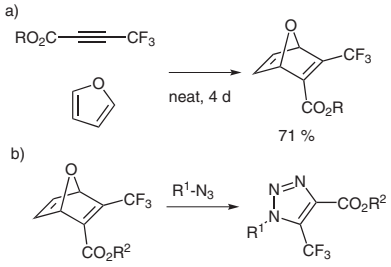


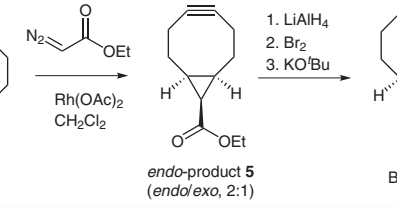
Figure 2 Chemical structures of strained alkenes and alkynes highlighted in chapter 2.

Prior to studying strained cyclooctynes, we developed the strained and electron-deficient oxanorbornadiene **3** (OND), synthesized in one step from commercially available starting materials, as a versatile molecule for (bio)conjugation.[16] OND readily reacts with azides in a cycloaddition reaction, forming the corresponding triazole upon retro-Diels–Alder reaction and release of furan (Scheme 1). Although the reaction rate is modest compared to cyclooctynes, its good water solubility and ease of synthesis, have led to various applications.[17] This early work on bioorthogonal chemistry made us realize that an often-overlooked key aspect is the synthetic availability of bioorthogonal reagents.



Scheme 1 a) Synthesis of OND in a one-step procedure from commercially available alkynes and furan; b) reaction of OND with azides. Subsequent retro-Diels–Alder reaction provides the triazole.

It was in the second half of 2010 that Dr. Floris van Delft and myself reported bicyclo[6.1.0]nonyne **4** (BCN) as a readily available and remarkably stable strained alkyne (Scheme 2).[4a] Inspired by the fact that benzoannulation increases the reactivity of cyclooctynes towards strain-promoted 1,3-dipolar cycloadditions as demonstrated for DIBO and DIBAC, we envisioned that fusion with a cyclopropane should have a similar effect while limiting lipophilicity. The synthesis of BCN was accomplished in four steps, yielding approximately 60% overall and started from commercially available 1,5-cyclooctadiene which was converted into **5** by addition of ethyl diazoacetate in the presence of rhodium acetate. The resulting diastereomeric mixture was readily separated by column chromatography.



Scheme 2 Synthesis of BCN **4** in four steps. The last three steps including reduction, bromination, and elimination are performed sequentially and require only a final purification step.

Stability was assessed in the presence of glutathione without signs of degradation. Initial experiments with benzyl azides revealed second-order rate constants of 0.29 and 0.19 $M^{-1}s^{-1}$ for *endo*-BCN and *exo*-BCN, respectively, compared to second-order rate constants of $7.6 \cdot 10^{-2}$ M^{-1} s^{-1} for DIFO.[10] Conveniently, BCN displays a C_s symmetry, overcoming challenges typically associated with its asymmetri-

cal counterparts, such as DIBAC/DBCO and DIFO. In the following years, BCN was not only applied in the fields of chemical biology but also in the fields of materials science and supramolecular chemistry. For example, in collaboration with the group of Prof. Jan van Hest, we demonstrated that BCN-based crosslinkers enable the chemical triggering of shape transformations in polymersomes.[18] Besides many applications in research, BCN is used in several antibody–drug conjugates (ADCs) that are currently undergoing evaluation in phase 1 clinical trials, driven by the company Synaffix (currently Lonza, Oss, Netherlands).

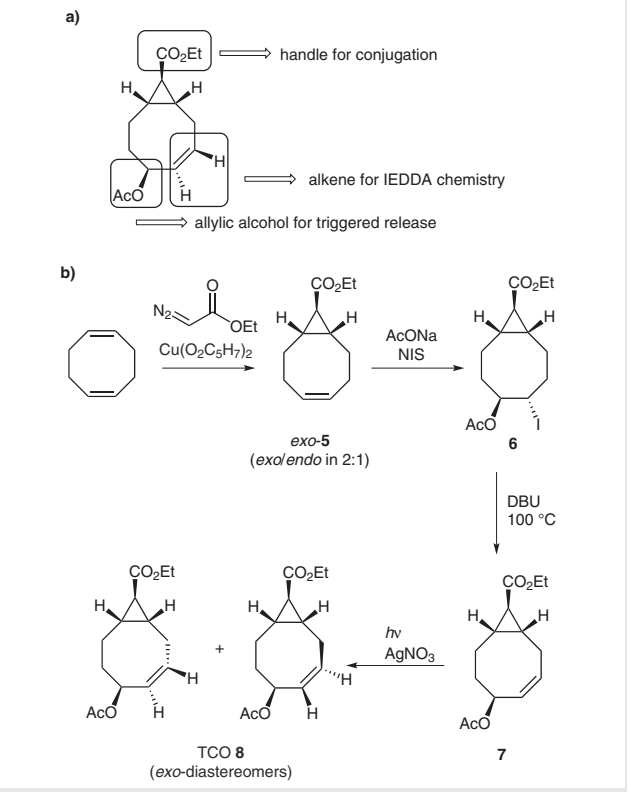
Thus, BCN has served us and many chemical biologists as a robust tool for bioorthogonal chemistry. In particular, its feasible synthesis and stability are unmatched. One disadvantage though that applies to most reaction partners for 1,3-dipolar cycloadditions is the relatively low second-order rate constants, with k_2 ranging from 0.1 to 5 M^{-1} s^{-1}. This has led us and others to turn towards alternative bioorthogonal tools.

3 Towards Readily Available Click-to-Release *trans*-Cyclooctenes (by Floris Rutjes)

Around the same time when we reported BCN as a readily available and stable reagent for 1,3-dipolar additions, the group of Joseph Fox revisited the inverse electron-demand Diels–Alder (IEDDA) reactions of tetrazines and reported their use for bioorthogonal chemistry.[19] Reactions between tetrazines and dienophiles not only display high levels of chemoselectivity but also high second-order rate constants in comparison to SPAAC. In particular, reactions between *trans*-cyclooctene (TCO) and electron-deficient tetrazines exhibit unmatched reaction kinetics with k_2 up to 3,300,000 M^{-1} s^{-1}.[20] These unique properties have made the IEDDA cycloaddition the reaction of choice when working under dilute biological conditions or even *in vivo*.[21]

Once again, one bottleneck of this powerful bioorthogonal tool was the synthesis of the strained 8-membered ring, in this case, *trans*-cyclooctene. In particular, (di)functionalized *trans*-cyclooctenes possess synthetic challenges and often require multistep synthetic routes, significantly impacting the field of bioorthogonal chemistry.

Many applications of TCO rely on its ability to be either pretargeted or used for 'click-to-release' chemistry, describing the release of cargos in the allylic position from TCOs upon IEDDA and subsequent isomerization.[22] Notable, the so-called 'click-to-release mechanism' was developed by the team of Marc Robillard associated to Tagworks Pharmaceuticals which are nowadays also based in Nijmegen. Inspired by our earlier success employing a fused cyclopropane, we sought to explore the possibility of providing readily available strained TCOs susceptible to further modifications and 'click-to-release' chemistries. Eventually, we and others reported a five-step synthesis that provides TCOs displaying competitive rate constants in IEDDA.[23] Our synthesis started from commercially available *cis*,*cis*-1,5-cyclooctadiene which was transformed into compound **5** (Scheme 3). During this synthesis, a key intermediate was the acetylated iodohydrin **6** and its subsequent elimination toward cyclooctene **7**. Over the years, our group has gained significant expertise in flow chemistry and reported, already in 2018, a continuous-flow protocol that enabled efficient photoisomerization toward *trans*-cyclooctenes **8**.[24,25] In brief, substituting the traditionally employed silica gel column with a liquid–liquid extraction module allowed the production of up to 2.2 g/h of specific TCOs. A similar setup was employed for the isomerization of our difunctionalized TCO. Altogether, we have been able to provide a robust synthetic route that allowed access to difunctional TCOs in only four steps, including the widely employed releasable one. The future will show if this TCO as a bioorthogonal tool holds similar success compared to our previously reported BCN.



Scheme 3 a) Structural features displayed by difunctionalized *trans*-cyclooctenes; b) key step in the synthetic route toward TCO **8** is acetylated iodohydrin **6**.

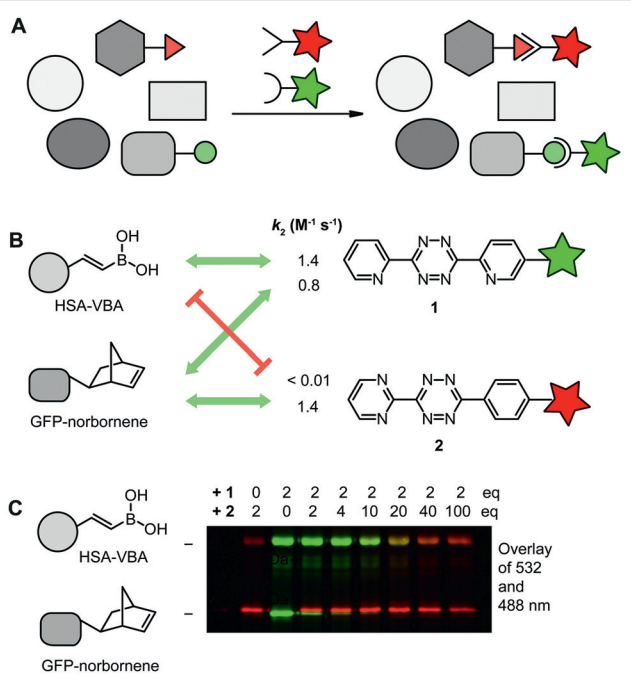


Figure 3 The coordination of VBA with the pyridyl substituent induces proximity of the reagents and improved reaction rates. This unique feature allowed the orthogonal bioorthogonal reaction of two proteins labeled. Figure adapted from ref. 27.

4 Giving Molecules Guidance (by Kimberly Bonger)

The development of the IEDDA reaction with tetrazines and dienophiles have revolutionized the field of bioorthogonal chemistry. The superior reaction rates allowed researchers to perform reactions in very dilute conditions needed for in vivo applications. Especially the cycloaddition with strained alkenes, such as TCO, proved suitable and several groups have applied these reagents for imaging and targeted drug delivery. Yet, the stability of these dienophiles in vivo remains challenging.[26] In our group, we were interested to see if we could obtain more stable, yet reactive dienophiles. As the IEDDA proceeds faster with electron-rich dienophiles, we envisioned that the reactivity of the otherwise unreactive linear alkenes may be improved by introducing electron-donating substituents. While alkoxy substituents resulted only in slight improved reactivity, we were very excited to see that the boronic acid substituents showed unexpectedly high reaction rates that were order of magnitude faster than the unsubstituted alkenes.[5] We envisioned that this unique reactivity was partly attributed to the formation of a charged boronate in aqueous environment. While this may explain part of the reactivity, we additionally found that the vinyl boronic acids were especially reactive to tetrazines with a pyridyl substituent, while they render inactive to tetrazines containing a phenyl or a pyrimidyl substituent. We hypothesized that the boronic acid coordinates to the pyridyl substituent, thereby inducing proximity of the reagents to facilitate the cycloaddition reaction. The more acidic pyrimidyl substituent or the noncoordinating phenyl substituent are less preferred coordinating substituents and therefor are not that reactive with VBAs.

The coordination-induced cycloaddition of tetrazines with VBAs and the large difference in reaction rates with tetrazines containing coordinating- or noncoordinating tetrazines, allowed us to introduce orthogonality within the iEDDA with other strained alkenes for dual orthogonal protein modification. In this example, we modified two proteins containing either a VBA or norbornene dienophile. We were able to react the norbornene selectively and fully with a pyrimidyl tetrazine after which the VBA was reacted with a pyridyl tetrazine in a sequential reaction step (Figure 3).[27]

It is generally accepted that tetrazines containing electron-withdrawing substituents react fastest in IEDDA reactions, but they are also rather unstable in aqueous conditions. The unique coordinating reactivity of VBAs prompted us to also explore other potential coordinating tetrazines.

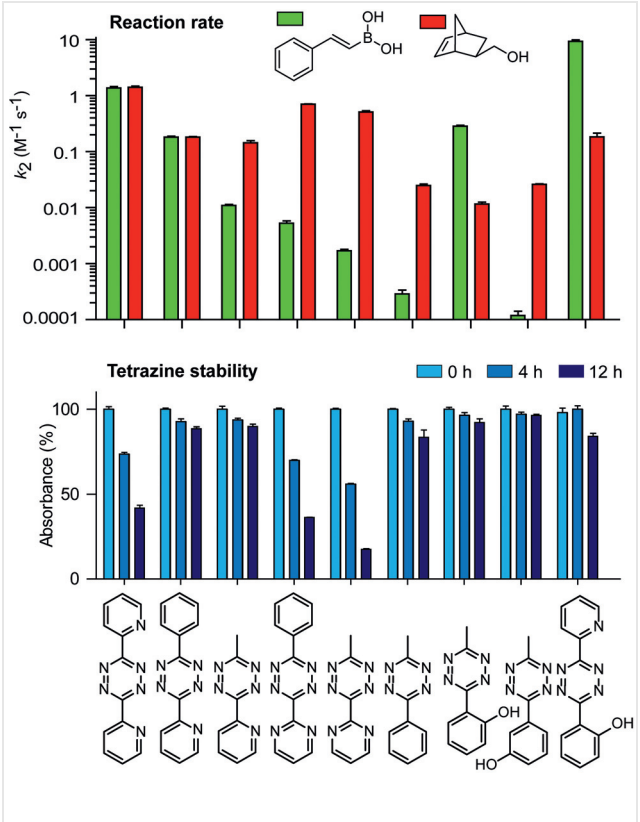


Figure 4 a) Second-order reaction rates of a panel of tetrazines with VBA and norbornene. Rates are measured in 50% methanol/PBS at 20 °C. b) Measured absorption of the tetrazines in 5% DMSO/PBS at 20 °C over time. Figure adapted from ref 28.

We envisioned that the more electron-rich phenolic substituents would provide more stable tetrazines, while still allowing fast reaction with VBAs. Indeed, a striking difference in reaction rate was observed with *o*-phenol-substituted tetrazines that showed more than 5 orders of magnitude improved reaction rates compared to *m*-phenol-substituted tetrazines in the reaction with VBA (Figure 4).[28] Additionally, the tetrazines proved fully stable in aqueous conditions for at least 12 h. Expectedly, the more electron-rich phenol-substituted tetrazines react poorly with norbornene, providing the additional possibility to perform orthogonal bioorthogonal IEDDA reactions with noncoordinating- and more electron-withdrawing tetrazines.

To further explore the scope of the VBA–tetrazine IEDDA reaction we additionally explored VBAs for click-to-release chemistry. We envisioned that VBAs are especially useful for this application as they are water-soluble and stable under aqueous conditions. We designed a VBA containing a self-immolative linker to cage a doxorubicin toxin (Figure 5).[29] We were excited to observe cell death only when applying the construct and an uncaging phenol-containing coordinating tetrazine, while no cell death was observed when subjecting the cells to the caged doxorubicin alone. The more electron-rich phenol tetrazine allowed selective reaction with the VBA cage, while no reaction was observed with vinyl ethers, a caging modality that was reported before by the groups of Devaraj and Bernardes.[30] While this reaction provides an orthogonal decaging strategy, we observed that the reaction rates were rather low to be used in living systems, likely due to the increased electron density present on the boronic acid due to the alkoxy substituent.

In our experience, we have observed great benefits of using VBAs in bioorthogonal reactions. The presence of high amounts of free thiols present in cells challenges many bioorthogonal reactions as they perform nucleophilic addition with strained alkynes or alkenes. VBAs are inert and stable under aqueous conditions. We have used activity-based probes containing VBA handles for labeling and imaging the proteasome.[31] Indeed, using this probe we could image the catalytic activity of proteasome subunits inside living cells. Additionally, VBAs are also hydrophilic and very soluble which is a great benefit when the use of more lipophilic reactants, such as DBCO, proved challenging due to unfavorable physicochemical properties.

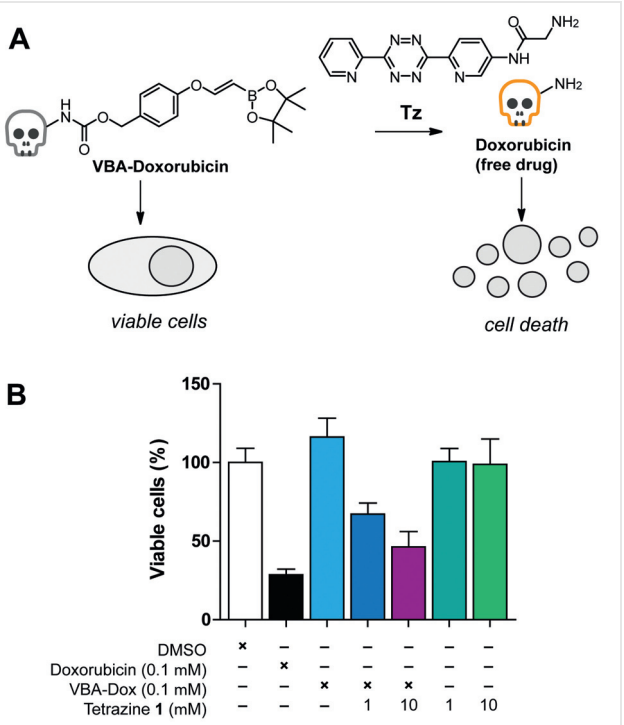
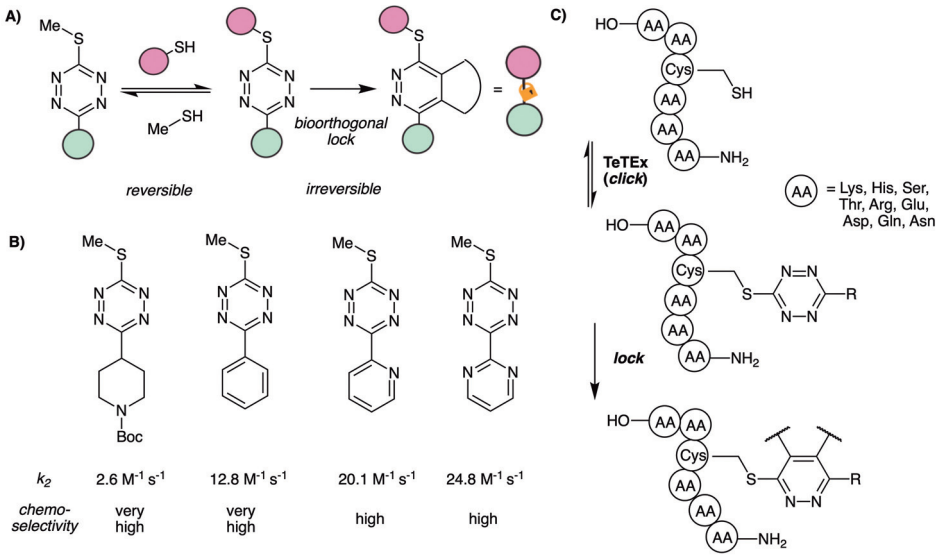


Figure 5 A) VBA-based click-to-release strategy. Doxorubicin was caged with a VBA connected to a self-immolative linker. B) Cell death was only observed upon addition of decaging tetrazine. Figure adapted from ref 29.

5 Next Generation of Bioconjugation Strategies: Dynamic Click Chemistry (by Kevin Neumann)

A longstanding challenge in synthetic chemistry is the modification of complex (bio)molecules with temporal control. At the very beginning of my independent career, we envisioned that the use of a reversible click reaction with an on-demand switch towards irreversibility offers numerous applications in synthetic chemistry and chemical biology. For example, one can imagine that this chemistry may find applications in proteome-wide screening of cysteines. In principle, such a system requires i) a rapid reversible click transformation, ii) a (bio)orthogonal reaction that induces irreversibility at any given point, and iii) the possibility to use the reactivity handles to incorporate chemical functionality. Thiol–maleimide chemistry, in principle, fulfils many of these aspects, namely reversibility and the possibility to incorporate complexity.[32] Yet, while the switch to irreversibility is possible via hydrolysis, this process is typically difficult to control precisely. Instead, K. Gavriel, a talented PhD candidate in my group, sought to employ 1,2,4,5-tetrazines and to take advantage of their ability to undergo two forms of click transformations, namely aromatic nucleophilic substitutions and IEDDA chemistries.[33] This work was inspired by early efforts related to the aromatic nucleophilic substitution of 1,2,4,5-tetrazines, allowing access to stapled peptides and crosslinked polymeric networks.[34] The reversibility of the reported bis-sulfide-functionalized tetrazines under biological relevant conditions, however, remained elusive, which we attributed to the high electron density displayed by the typically employed bis-sulfide tetrazine. We hypothesized that an asymmetric sulfide



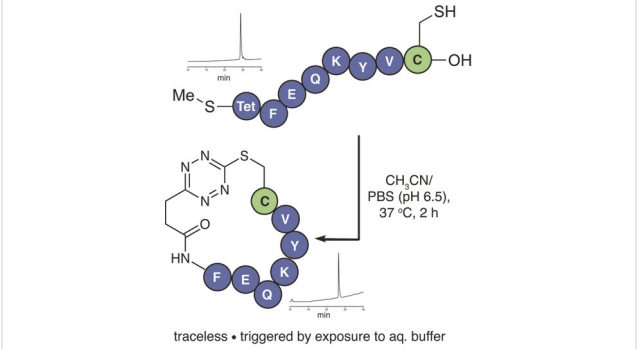
Scheme 4 A) The exchange between thiols and sulfide-bearing tetrazines is reversible. Upon exposure to a dienophile and subsequent IEDDA reaction, the reaction is locked and irreversible. B) TeTEx kinetics depend strongly on electronic properties of the tetrazine. C) TeTEx is chemoselective and allows modification of peptides and proteins.

tetrazine would not only enable conjugation of complex cargos such as biotin or drug molecules but, importantly, also tailor the reactivity towards aromatic nucleophilic substitution.

To confirm our hypothesis, we accessed a range of tetrazines that displayed varying electronic properties (Scheme 4).[33] Initial experiments not only confirmed our hypothesis that the tetrazine–thiol exchange (TeTEx) on asymmetric tetrazines rapidly occurs in aqueous environment but also displays a reversible nature. Conveniently, by employing methylthiol-substituted tetrazines,[35] the reaction proceeds to full conversion, while other substitutes provided an equilibrium.

This is because methanethiol can be removed from the reaction mixture by simply saturating the solution with inert gas such as nitrogen. As hypothesized, second-order rate constants depend on the electronic properties, with electron-deficient tetrazines such as pyrimidine-substituted tetrazines displaying rate constants higher than 20 M^{-1} s^{-1}. In stark contrast to existing cysteine modifications, TeTEx allows switching from reversible to irreversible modifications of complex biomolecules enabled by the reaction of tetrazines with dienophiles towards pyridazines via IEDDA chemistry. We demonstrated this on-demand switch on representative peptide scaffolds by employing different dienophiles, with BCN being the most efficient lock. Our results revealed that those dienophiles afford locked products that initially provide pyridazines, while dienophiles that afford dihydropyridazines are prone to hydrolysis. Independently of us, the group around Joseph Fox elegantly employed the rapid exchange of tetrazine sulfides and thiols as a click reaction for applications in proteome-wide screening of cysteines.[36] In this case, the capability of locking the reaction partners was employed for pull-down assays.

Our group envisioned similar usage but, in addition, was keen on further applying dynamic chemistry for synthetic applications in the field of peptide and protein chemistry. In particular, the cyclization of peptides often suffers from undesired side reactions, such as the formation of dimers and trimers. By utilizing the exchange between tetrazine sulfides and thiols as a dynamic click reaction, we anticipated that higher concentrations could be tolerated during peptide cyclization. Initial concerns related to the compatibility of the tetrazine sulfide scaffold during solid-phase peptide synthesis (SPPS) have proven unjustified, and a small library of tetrazine-terminated peptides was accessed



Scheme 5 The N-terminal installation of methylsulfide tetrazine enabled traceless cyclization with internal and C-terminal cysteines in the absence of additional coupling reagents or extensive protecting group reshuffling. Instead, the cyclization is triggered by buffered aqueous solutions. Figure adapted from ref 37.

(Scheme 5).[37] Interestingly, we observed that silanes, as scavengers, provide better results than pure water. Cyclization is triggered by exposure to buffered aqueous environments, which might prove powerful in the future when applied in high-throughput screenings. Ultimately, TeTEx was employed for the cyclization of peptide scaffolds with a selection of representative amino acids, with the hope of sparking interest in other groups to use this cyclization strategy that avoids extensive protecting group reshuffling or exotic activation reagents.

6 Conclusions

Since the first report by Bertozzi and co-workers over 20 years ago, the field of bioorthogonal chemistry has witnessed many exciting advances, profoundly impacting other fields such as chemical biology and drug delivery. Naturally, this progress is driven by a collaborative effort from both chemists and biologists, making interdisciplinary research indispensable. In this Account, we aim to illustrate this essential aspect by reflecting on the developments related to bioorthogonal chemistry within our own chemistry institute at Radboud University. A closer look at the research described herein exemplifies that chemists can contribute in many ways to a diverse field such as bioorthogonal chemistry. For example, the expertise in synthetic organic chemistry provided by Rutjes and co-workers has resulted in the design of new click tools and conceptually new synthetic routes, allowing scalable synthesis. The group of Kimberly Bonger is enhancing the field of bioorthogonal chemistry by employing a chemical biology perspective. In another example, the groups of Neumann and Bonger demonstrate that bioorthogonal chemistry is a unique chemical tool for developing new advances in medicine. Both, our research lines and that of others highlight the importance of interdisciplinary and collaborative efforts. While individual contributions are valuable, ultimately, one can conclude that teamwork and collaborative efforts are essential.

Conflict of Interest

The authors declare no conflict of interest.

Funding Information

We are grateful for continuing financial support including from the Nederlandse Organisatie voor Wetenschappelijk Onderzoek (NWO), the European Research Council (ERC), and the Dutch Society for Immunology (NVVI). K.B. received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation program (grant agreement No 802940) and the NWO gravitation program 'Institute for Chemical Immunology' (NWO-024.002.009).

Acknowledgment

This Account covers several years of research related to bioorthogonal chemistry at Radboud University and we would like to thank everyone who contributed to this work. While there are too many names to mention individually, we would like to emphasize the contribution of Dr. Floris L. van Delft, Prof. Jan C. M. Hest, and Prof. Roeland J. M. Nolte.

References

(1) (a) Hang, H. C.; Yu, C.; Kato, D. L.; Bertozzi, C. R. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 14846. (b) Bertozzi, C. R. *Acc. Chem. Res.* **2011**, *44*, 651. (c) Sletten, E. M.; Bertozzi, C. R. *Angew. Chem. Int. Ed.* **2009**, *38*, 6974.
(2) (a) Patterson, D. M.; Prescher, J. A. *Curr. Opin. Chem. Biol.* **2015**, *28*, 141. (b) Devaraj, N. K. *ACS Cent. Sci.* **2018**, *8*, 952. (c) Thirumurugan, P.; Matosiuk, D.; Jozwiak, K. *Chem. Rev.* **2013**, *113*, 4905.
(3) Debets, M. F.; van Berkel, S. S.; Dommerholt, J.; Dirks, A. J.; Rutjes, F. P. J. T.; van Delft, F. L. *Acc. Chem. Res.* **2011**, *9*, 805.
(4) (a) Dommerholt, J.; Schmidt, S.; Rinske, T.; Hendriks, L. J. A.; Rutjes, F. P. J. T.; van Hest, J. C. M.; Lefeber, D. J.; Friedl, P.; van Delft, F. L. *Angew. Chem. Int. Ed.* **2010**, *49*, 9422. (b) Debets, M. F.; van der Doelen, C. W J.; Rutjes, F. P. J. T.; van Delft, F. L. *ChemBioChem* **2010**, *9*, 1168.
(5) Eising, S.; Lelivelt, F.; Bonger, K. M. *Angew. Chem. Int. Ed.* **2016**, *55*, 12243.
(6) Ignacio, B. J.; Dijkstra, J.; Mora, N.; Slot, E. F. J.; van Weijsten, M. J.; Storkebaum, E.; Vermeulen, M.; Bonger, K. M. *Nat. Commun.* **2022**, *14*, 3367.
(7) (a) Büll, C.; Heise, T.; van Hilten, N.; Pijnenborg, J. F. A.; Bloemendal, V. R. L. J.; Gerrits, L.; Kers-Rebel, E. D.; Ritschel, T.; den Brok, M. H.; Adema, G. J.; Boltje, T. J. *Angew. Chem. Int. Ed.* **2017**, *12*, 3309. (b) Büll, C.; Heise, T.; Beurskens, D. M. H.; Riemersma, M.; Ashikov, A.; Rutjes, F. P. J. T.; van Kuppevelt, T. H.; Lefeber, D. J.; den Brok, M. H.; Adema, G. J.; Boltje, T. J. *Chem. Biol.* **2015**, *10*, 2353.
(8) Velema, W. A. *Chem. Commun.* **2023**, *59*, 6148.
(9) (a) Neumann, K.; Gambardella, A.; Bradley, M. *ChemBioChem* **2019**, *7*, 872. (b) Neumann, K.; Gambardella, A.; Lilienkampf, A.; Bradley, M. *Chem. Sci.* **2018**, *9*, 7198. (c) Remmers, R. C. P. A.; Neumann, K. *Biomater. Sci.* **2023**, *11*, 1607.
(10) Baskin, J. M.; Prescher, J. A.; Laughlin, S. T.; Bertozzi, C. R. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 16793.
(11) Blomquist, A. T.; Liu, L. H. *J. Am. Chem. Soc.* **1953**, *9*, 2153.
(12) Prelog, V.; Schenker, K.; Künig, W. *Helv. Chim. Acta* **1953**, *59*, 471.
(13) Ning, X.; Temming, R. P.; Dommerholt, J.; Guo, J.; Ania, D. B.; Debets, M. F.; Wolfert, M. A.; Boons, G.-J.; van Delft, F. L. *Angew. Chem. Int. Ed.* **2010**, *17*, 3065.
(14) Codelli, J. A.; Baskin, J. M.; Agard, N. J.; Bertozzi, C. R. *J. Am. Chem. Soc.* **2008**, *34*, 11486.
(15) Debets, M. F.; van Berkel, S. S.; Schoffelen, S.; Rutjes, F. P. J. T.; van Hest, J. C. M.; van Delft, F. L. *Chem. Commun.* **2010**, *46*, 97.
(16) van Berkel, S. S.; Dirks, A. J.; Debets, M. F.; van Delft, F. L.; Cornelissen, J. J. L. M.; Nolte, R. J. M.; Rutjes, F. P. J. T. *ChemBioChem* **2007**, *8*, 1504.
(17) (a) van Dongen, S. F.; Verdurmen, W. P.; Peters, R. J.; Nolte, R. J. M.; Brock, R.; van Hest, J. C. M. *Angew. Chem. Int. Ed.* **2010**, *49*, 7213. (b) Krause, A.; Kirschning, A.; Dräger, G. *Org. Biomol.*

Chem. **2012**, *10*, 5547. (c) Jirawutthiwongchai, J.; Krause, A.; Dräger, G.; Chirachanchai, S. *ACS Macro Lett.* **2013**, *2*, 177.
(18) van Oers, M. C. M.; Rutjes, F. P. J. T.; van Hest, J. C. M. *J. Am. Chem. Soc.* **2013**, *44*, 16308.
(19) Blackman, M. L.; Royzen, M.; Fox, J. M. *J. Am. Chem. Soc.* **2008**, *41*, 13518.
(20) Darko, A.; Wallace, S.; Dmitrenko, O.; Machovina, M. M.; Mehl, M.; Ryan, A.; Chin, J. W.; Fox, J. M. *Chem. Sci.* **2014**, *5*, 3770.
(21) Mitry, M. M. A.; Greco, F.; Osborn, H. M. I. *Chem. Eur. J.* **2023**, *20*, e202203942.
(22) (a) Versteegen, R. M.; Rossin, R.; ten Hoeve, W.; Janssen, H. M.; Robillard, M. S. *Angew. Chem. Int. Ed.* **2013**, *52*, 14112. (b) Versteegen, R. M.; ten Hoeve, W.; Rossin, R.; de Geus, M. A. R.; Janssen, H. M.; Robillard, M. S. *Angew. Chem. Int. Ed.* **2018**, *33*, 10494.
(23) (a) Sondag, D.; Maartense, L.; de Jong, H.; de Kleijne, F. F. J.; Bonger, K. M.; Löwik, D. W. P. M.; Boltje, T. J.; Dommerholt, J.; White, P. B.; Blanco-Ania, D.; Rutjes, F. P. J. T. *Chem. Eur. J.* **2023**, *6*, e202203375. (b) Kuba, W.; Sohr, B.; Keppel, P.; Svatunek, D.; Humhal, V.; Stöger, B.; Goldeck, M.; Carlson, J. C. T.; Mikula, H. *Chem. Eur. J.* **2022**, *28*, e202203069. (c) Liu, B.; ten Hoeve, W.; Versteegen, R. M.; Rossin, R.; Kleijn, L. H. J.; Robillard, M. S. *Chem. Eur. J.* **2023**, *29*, e202300755.
(24) Blanco-Ania, D.; Maartense, L.; Rutjes, F. P. J. T. *ChemPhotoChem* **2018**, *10*, 898.
(25) Royzen, M.; Yap, G. P. A.; Fox, J. M. *J. Am. Chem. Soc.* **2008**, *12*, 3760.
(26) Fang, Y.; Judkins, J. C.; Boyd, S. J.; am Ende, C. W.; Rohlfing, K.; Huang, Z.; Xie, Y.; Johnson, D. S.; Fox, J. M. *Tetrahedron* **2019**, *75*, 4307.
(27) Eising, S.; Xin, B.-T.; Kleinpenning, F.; Heming, J. J. A.; Florea, B. I.; Overkleeft, H. S.; Bonger, K. M. *ChemBioChem* **2018**, *15*, 1648.
(28) Eising, S.; Engwerda, A. H. J.; Riedijk, X.; Bickelhaupt, M. F.; Bonger, K. M. *Bioconjugate Chem.* **2018**, *29*, 3054.
(29) Lelieveldt, L.; Eising, S.; Weijen, A.; Bonger, K. M. *Org. Biomol. Chem.* **2019**, *17*, 8816.
(30) (a) Wu, H.; Alexander, S. C.; Jin, S.; Devaraj, N. K. *J. Am.Chem. Soc.* **2016**, *138*, 11429. (b) Jiménez-Moreno, E.; Guo, Z.; Oliveira, B. L.; Albuquerque, I. S.; Kitowski, A.; Guerreiro, A.; Boutureira, O.; Rodrigues, T.; Jiménez-Osés, O.; Bernardes, G. J. L. *Angew. Chem. Int. Ed.* **2017**, *56*, 243.
(31) Eising, S.; van der Linden, N. G. A.; Kleinpenning, F.; Bonger, K. M. *Bioconjugate Chem.* **2018**, *29*, 982.
(32) Ravasco, J. M. J. M.; Faustino, H.; Trindade, A.; Gois, P. M. P. *Chem. Eur. J.* **2019**, 43.
(33) Gavriel, K.; van Doeselaar, D. C. A.; Geers, D. W. T.; Neumann, K. *RSC Chem. Biol.* **2023**, *4*, 685.
(34) For a selection of publications, see: (a) Santos, T.; Rivero, D. S.; Pérez-Pérez, Y.; Martín-Encinas, E.; Pasán, J.; Daranas, A. H.; Carrillo, R. *Angew. Chem. Int. Ed.* **2021**, *60*, 18783. (b) Rivero, D. S.; Paiva-Feener, R. A.; Santos, T.; Martín-Encinas, E.; Carrillo, R. *Macromolecules* **2021**, *54*, 10428. (c) Brown, S. P.; Smith, A. B. III. *J. Am. Chem. Soc.* **2015**, *137*, 4034.
(35) Bickem, L.; Gavriel, K.; Neumann, K. *Eur. J. Org. Chem.* **2024**, *27*, e202301117.
(36) Tallon, A. M.; Xu, Y.; West, G. M.; am Ende, C. W.; Fox, J. M. *J. Am. Chem. Soc.* **2023**, *29*, 16069.
(37) Geers, D. W. T.; Gavriel, K.; Neumann, K. *J. Pept. Sci.* **2024**, *30*, e3548.