



Science of
Synthesis

Abiotic Reactions in Live Environments

Volume Editors

J. L. Mascareñas

M. Tomás-Gamasa



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Preface

As the pace and breadth of research intensifies, organic synthesis is playing an increasingly central role in the discovery process within all imaginable areas of science: from pharmaceuticals, agrochemicals, and materials science to areas of biology and physics, the most impactful investigations are becoming more and more molecular. As an enabling science, synthetic organic chemistry is uniquely poised to provide access to compounds with exciting and valuable new properties. Organic molecules of extreme complexity can, given expert knowledge, be prepared with exquisite efficiency and selectivity, allowing virtually any phenomenon to be probed at levels never before imagined. With ready access to materials of remarkable structural diversity, critical studies can be conducted that reveal the intimate workings of chemical, biological, or physical processes with stunning detail.

The sheer variety of chemical structural space required for these investigations and the design elements necessary to assemble molecular targets of increasing intricacy place extraordinary demands on the individual synthetic methods used. They must be robust and provide reliably high yields on both small and large scales, have broad applicability, and exhibit high selectivity. Increasingly, synthetic approaches to organic molecules must take into account environmental sustainability. Thus, atom economy and the overall environmental impact of the transformations are taking on increased importance.

The need to provide a dependable source of information on evaluated synthetic methods in organic chemistry embracing these characteristics was first acknowledged over 100 years ago, when the highly regarded reference source **Houben–Weyl Methoden der Organischen Chemie** was first introduced. Recognizing the necessity to provide a modernized, comprehensive, and critical assessment of synthetic organic chemistry, in 2000 Thieme launched **Science of Synthesis, Houben–Weyl Methods of Molecular Transformations**. This effort, assembled by almost 1000 leading experts from both industry and academia, provides a balanced and critical analysis of the entire literature from the early 1800s until the year of publication. The accompanying online version of **Science of Synthesis** provides text, structure, substructure, and reaction searching capabilities by a powerful, yet easy-to-use, intuitive interface.

From 2010 onward, **Science of Synthesis** is being updated quarterly with high-quality content via **Science of Synthesis Knowledge Updates**. The goal of the **Science of Synthesis Knowledge Updates** is to provide a continuous review of the field of synthetic organic chemistry, with an eye toward evaluating and analyzing significant new developments in synthetic methods. A list of stringent criteria for inclusion of each synthetic transformation ensures that only the best and most reliable synthetic methods are incorporated. These efforts guarantee that **Science of Synthesis** will continue to be the most up-to-date electronic database available for the documentation of validated synthetic methods.

Also from 2010, **Science of Synthesis** includes the **Science of Synthesis Reference Library**, comprising volumes covering special topics of organic chemistry in a modular fashion, with six main classifications: (1) Classical, (2) Advances, (3) Transformations, (4) Applications, (5) Structures, and (6) Techniques. Titles will include *Stereoselective Synthesis*, *Water in Organic Synthesis*, and *Asymmetric Organocatalysis*, among others. With expert-evaluated content focusing on subjects of particular current interest, the **Science of Synthesis Reference Library** complements the **Science of Synthesis Knowledge Updates**, to make **Science of Synthesis** the complete information source for the modern synthetic chemist.

The overarching goal of the **Science of Synthesis** Editorial Board is to make the suite of **Science of Synthesis** resources the first and foremost focal point for critically evaluated information on chemical transformations for those individuals involved in the design and construction of organic molecules.

Throughout the years, the chemical community has benefited tremendously from the outstanding contribution of hundreds of highly dedicated expert authors who have devoted their energies and intellectual capital to these projects. We thank all of these individuals for the heroic efforts they have made throughout the entire publication process to make **Science of Synthesis** a reference work of the highest integrity and quality.

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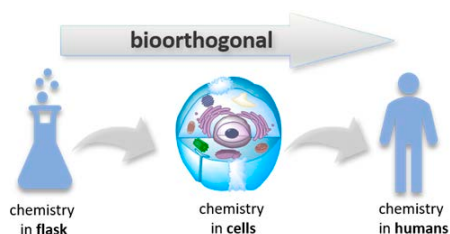
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This contribution aims to trace the origins of bioorthogonal chemistry, from its roots in early alchemy and the Scientific Revolution, to its development as a response to the quest to understand the fundamental principles of life. The development and application of chemical tools to study and manipulate biological processes and biomolecules laid the foundation for modern chemical biology. With the advent of the first chemical reactions that proceed selectively and efficiently without interfering with biological systems, scientists gained a unique set of tools to achieve an unprecedented level of understanding of biological processes at the molecular level. New techniques such as strain-promoted azide–alkyne cycloaddition (SPAAC), tetrazine ligation, and artificial metalloenzymes have further advanced the field. These modern tools of chemistry have significantly extended the potential of bioorthogonal chemistry and hold the promise of revolutionizing healthcare through improved drug delivery and advanced diagnostic tools. By placing these achievements in the context of the history of science, it is clear that this field has a bright future. As our understanding of bioorthogonal reactions deepens and technology evolves, we can anticipate their incorporation into a wide range of practices, potentially ushering in a new era of advanced personalized treatments.

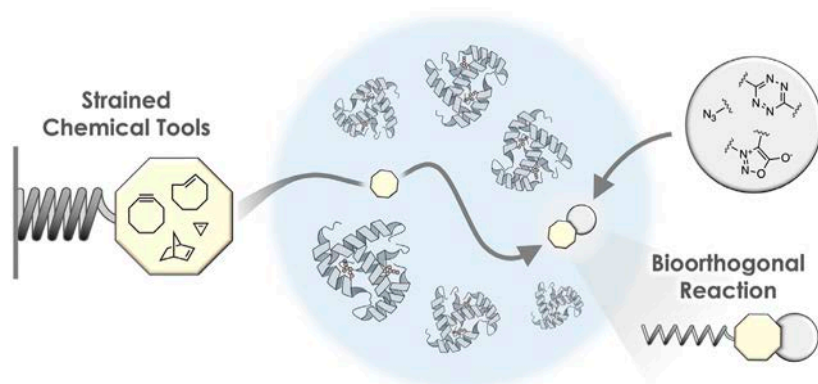


Keywords: bioorthogonal chemistry · chemical biology · scientific revolution · click chemistry · biomolecules · drug delivery · enzymes · personalized medicine

3 Strain-Promoted Bioorthogonal Chemistry

N. Pfeleger^{1b}, A. Schiefer^{1b}, and H. Mikula^{1b}

Strain-promoted bioorthogonal reactions, driven by cycloalkynes and cycloalkenes, have become indispensable in chemical biology. These strained chemical tools enable selective bioorthogonal ligations and bond-cleavage reactions, supporting research and development in various emerging fields such as molecular imaging, targeted drug delivery, and biomolecular engineering. The unique reactivities of strained cycloalkynes and cycloalkenes allow for efficient and precise (bio) molecular transformations, even in complex biological environments. This review provides an overview of strain-promoted bioorthogonal reactions, focusing on their mechanisms, kinetics, and other performance characteristics, while emphasizing their critical role in advancing both fundamental research and translational applications.

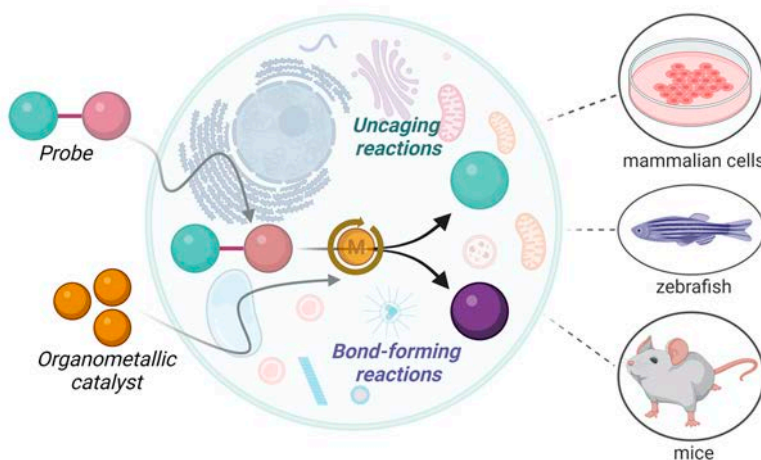


Keywords: click chemistry · bioorthogonal chemistry · cyclooctynes · *trans*-cyclooctenes · cyclopropenes · azides · tetrazines · mesoionics · 1,3-dipolar cycloaddition · inverse-electron-demand Diels–Alder reaction · bioorthogonal bond-cleavage reaction · click-to-release

4 Metal Catalysis in Live Environments

M. Tomás-Gamasa^{ID} and J. L. Mascareñas^{ID}

Bioorthogonal chemistry has revolutionized the field of chemical biology, providing powerful tools for manipulating molecules in biological environments, including living cells and organisms. For many years, these transformations have been mainly limited to the well-known copper-catalyzed azide–alkyne cycloadditions (CuAAC) and metal-free reactions with strained reagents. However, in recent years it has been widely demonstrated that some transition-metal-catalyzed reactions may also be performed in biological settings, enabling, among other applications, the bioimaging of biological targets and structures or prodrug activations. Particularly attractive is the possibility of using organometallic catalysis for performing synthetic, bond-forming reactions, as this can lead to new types of technologies for biological exploration and biomedical discovery.

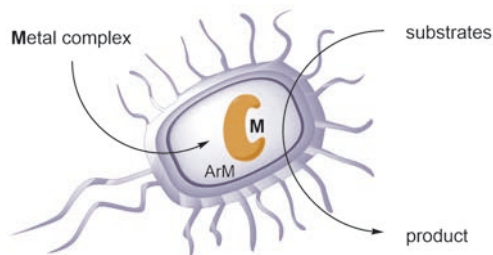


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Keywords: bioorthogonal chemistry · abiotic chemistry · cellular chemistry · metal catalysis · organometallic chemistry · uncaging · bond-forming reactions · prodrugs · fluorescence

5 Artificial Metalloenzymes In Vivo*G. Roelfes* 

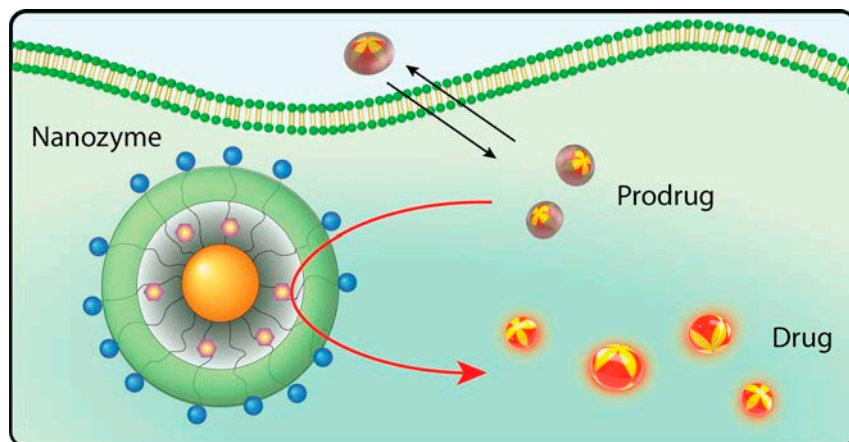
Artificial metalloenzymes (ArMs) are an emerging class of biocatalysts that aim to merge the attractive features of homogeneous catalysis and biocatalysis. While mainly studied *in vitro*, recently there has been a growing interest in the application of artificial metalloenzymes *in vivo*. Reviewed herein are the different approaches to assemble artificial metalloenzymes *in vivo* and the main areas of application: for biocatalytic synthesis and for *in vivo* activation of anti-tumor drugs in or near cancer cells.



Keywords: artificial metalloenzymes · biocatalysis · *in vivo* catalysis · whole-cell biocatalysis · designer enzymes · biocatalytic cascade · proteins · transition-metal catalysis · asymmetric catalysis · biosynthesis · anti-tumor drugs · catalytic uncaging

6 Bioorthogonal Nanozymes for Catalysis in Living Systems*C.-M. Hirschbiegel* , *J. I. García-Peiro* , *Y. A. Cicek* , *J. L. Hueso* ,
and *V. M. Rotello* 

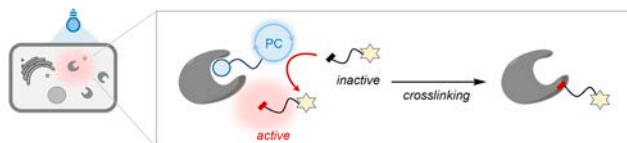
Transition-metal catalysts (TMCs) can be encapsulated into nanomaterials to form bioorthogonal “nanozymes”. This encapsulation extends the lifetime of the catalyst, enhances water solubility, and can improve its catalytic performance. Several nanomaterials can act as host scaffolds. The choice of host nanomaterial impacts the properties of the respective nanozyme and provides modularity for engineering enhanced circulation time and targeting ability. This review examines various bioorthogonal nanozymes and their respective TMCs, discussing their advantages and disadvantages for bioorthogonal catalysis, and providing an outlook on their biomedical applications.



Keywords: bioorthogonal chemistry · nanomaterials · encapsulation · catalyst protection · enzyme-like nanostructures · prodrugs · localized drug release

7 Photocatalysis in Chemical Biology*A. Kohyama^{1b} and N. Winssinger^{1b}*

Controlling the space and time of chemical processes has led to great benefits in biological research. From this perspective, photocatalytic reactions have been developed to be a breakthrough in chemical biology. In combination with a photocatalyst, visible-light irradiation turns on the reaction system by converting an inactive species into an active one. This review showcases the principle of photocatalytic reactions and some applications in chemical biology by focusing on the cooperative use of active species and proximity effects.

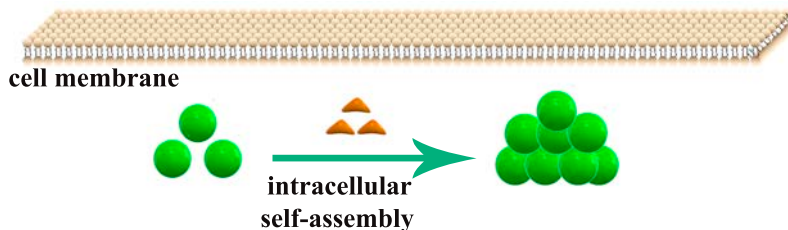


PC = photocatalyst

Keywords: photoredox reactions · single-electron transfer (SET) · singlet oxygen · proximity-inducing labeling · photodynamic therapy (PDT) · uncaging

8 Bioorthogonal Supramolecular Assemblies in Cells*J. Geng^{1b}, N. Sapkota^{1b}, and S. Lin^{1b}*

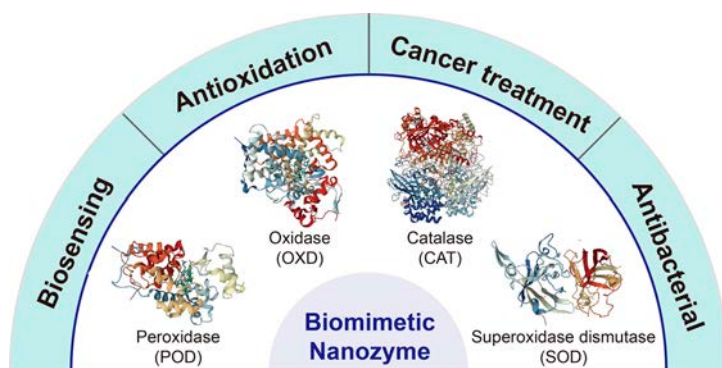
This review addresses the concept and mechanisms of bioorthogonal supramolecular self-assembly in living organisms. It introduces bioorthogonal chemistry, which allows chemical reactions to occur within biological systems without interfering with natural processes. Key mechanisms reviewed include intracellular polymerization, host–guest interactions involving macrocyclic molecules, enzyme-mediated assembly, and peptide self-assembly. These processes mimic the way nature constructs complex structures and are crucial for applications in drug delivery, bioimaging, and diagnostics, paving the way for advancements in personalized medicine and cancer therapy.



Keywords: bioorthogonal chemistry · supramolecular self-assembly · intracellular polymerization · host–guest interactions · enzyme-mediated assembly · peptide self-assembly · drug delivery · bioimaging · diagnostics

9 **Biomimetic Nanozymes**S. Jin^{ID} and Y. Jeong^{ID}

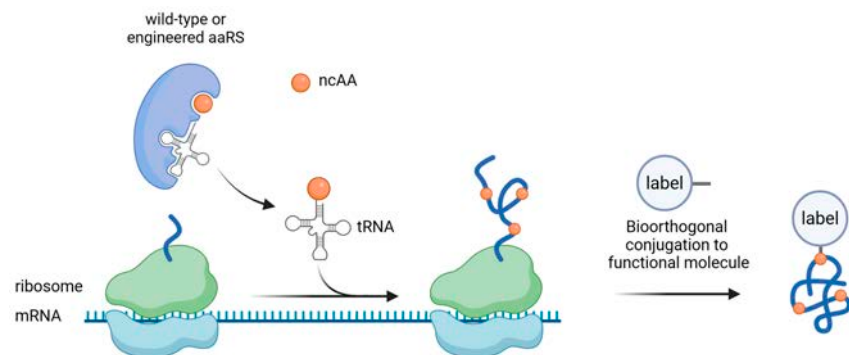
Nanozymes are being studied as suitable alternatives to natural enzymes in live environments because of their biocompatible size with other proteins. Their size can not only provide biocompatibility in biological applications but also enhance catalytic activity and yield benefits for catalyst separation and reuse in industrial applications. Due to these advantages, diverse types of nanozymes have been developed. This review provides an overview of the catalytic mechanisms and classification of biomimetic nanozymes. Additionally, it reviews recent research progress on biomimetic nanozyme based catalysis and its biosensing, bioimaging, and therapeutic applications.



Keywords: nanozyme · artificial enzyme · biomimetic nanozyme · nanomaterials · peroxidase · oxidase · catalase · superoxide dismutase · biosensing · antioxidation · anticancer treatment · antibacterial application

10 **Protein Modification Through Metabolic Incorporation of Noncanonical Amino Acids**B. J. Ignacio^{ID}, L. Herwig, N. van der Zanden^{ID}, K. M. Bongers^{ID}

Metabolic incorporation of noncanonical amino acids (ncAAs) has emerged as a versatile approach to introduce chemical functionalities into proteins without disrupting their native structure or requiring bulky tags. This review focuses on two main strategies for ncAA incorporation: residue-specific proteome-wide labeling and site-specific modification of target proteins. Emphasis is placed on ncAAs with functional side chains that enable further chemical modification, highlighting their applications for studying and manipulating proteins in complex biological environments.



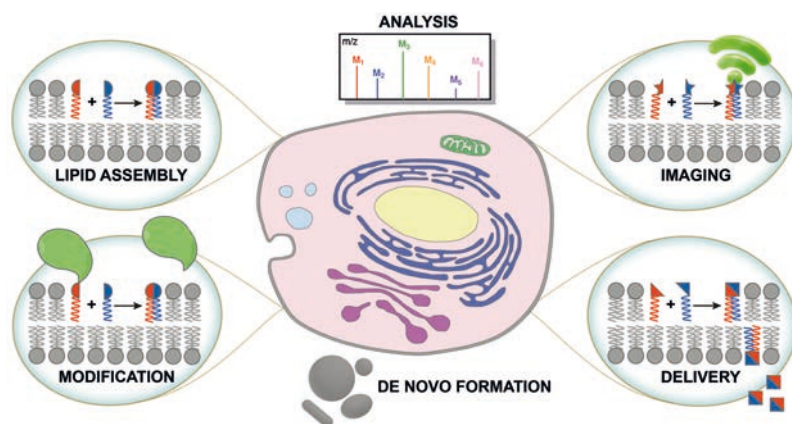
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Keywords: noncanonical amino acids (ncAAs) · metabolic protein labeling · genetic code expansion · residue-specific labeling · site-specific modification · bioorthogonal chemistry · protein engineering

11

Bioorthogonal Tools for the Synthesis, Modification, and Analysis of Lipids*F. A. Souto-Trinei^{1b}, N. K. Devaraj^{1b}, and R. J. Bred^{1b}*

Lipids are essential biomolecules that play crucial roles in many biological processes, including membrane formation, cell signaling, and energy storage. Although the importance of lipids is well-acknowledged, deciphering their biosynthesis, trafficking, and functions has proven challenging due to their structural diversity. This review covers recent advances in the preparation, derivatization, and analysis of lipids in living systems. In particular, we describe several bioorthogonal approaches for controlling and characterizing lipid structure. This powerful toolbox is helpful in numerous applications, including the elucidation of lipid–protein interactions and the development of therapeutics.

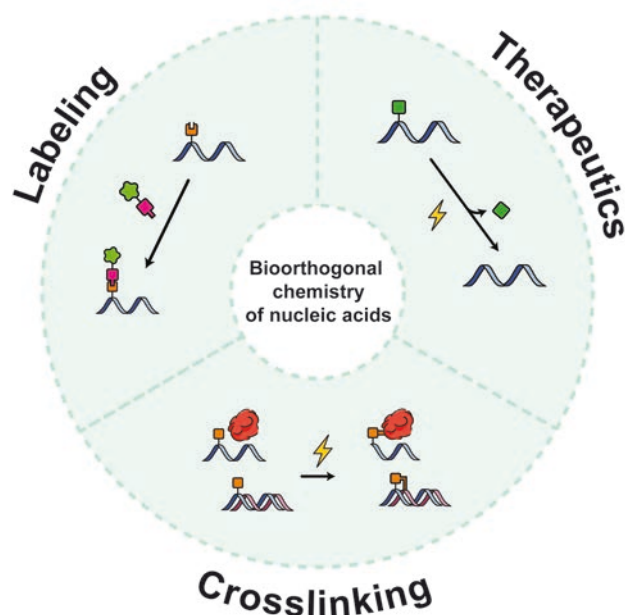


Keywords: lipids · bioorthogonal tools · membranes · supramolecular · self-assembly · phospholipids · sphingosine · ceramide · tetrazines · imaging · protein trafficking · drug delivery

12 **Bioorthogonal Chemistry of Nucleic Acids**

R. M. R. M. Lopes^{1b}, J. Barr, J. H. Meffert^{1b}, A. Colaker, G. Colpaert^{1b}, S. Ghosh^{1b},
E. Cadoni^{1b}, and A. Madder^{1b}

Bioorthogonal chemistry is a powerful tool allowing the modification of DNA and RNA through incorporation of artificial reactive groups that can function in a live environment, without interfering with natural biological processes. This chapter reviews the main strategies for installing bioorthogonal functionalities into nucleic acid derivatives by both synthetic and metabolic means, facilitating subsequent postsynthetic labeling via reactions such as CuAAC, SPAAC, IEDDA, and photoclick. We also focus on those photochemical reactions that allow for the triggered manipulation of the nucleic acid conformation and function using light as a trigger. Furthermore, we review the mechanistic principles, structural considerations, and application of these reactions in living cells and organisms, highlighting their utility in nucleic acid imaging, diagnostics, and therapeutic approaches.

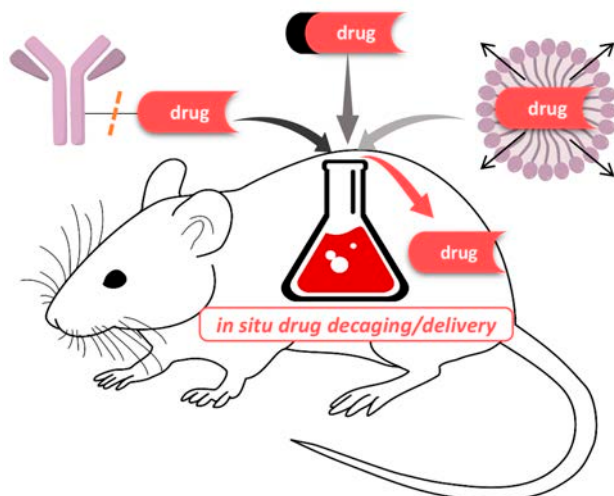


Keywords: bioorthogonal chemistry · nucleic acids · DNA modification · RNA modification · synthetic strategies · metabolic labeling · CuAAC · SPAAC · IEDDA · photoclick · Staudinger reactions · photoswitches · azobenzenes · photochemical activation · imaging · diagnostics · therapeutics · crosslinking

13

Abiotic Chemistry in Living Animals: Therapeutic Applications*K. Porte^{1b}, F. Thoreau^{1b}, S. Papot^{1b}, and F. Taran^{1b}*

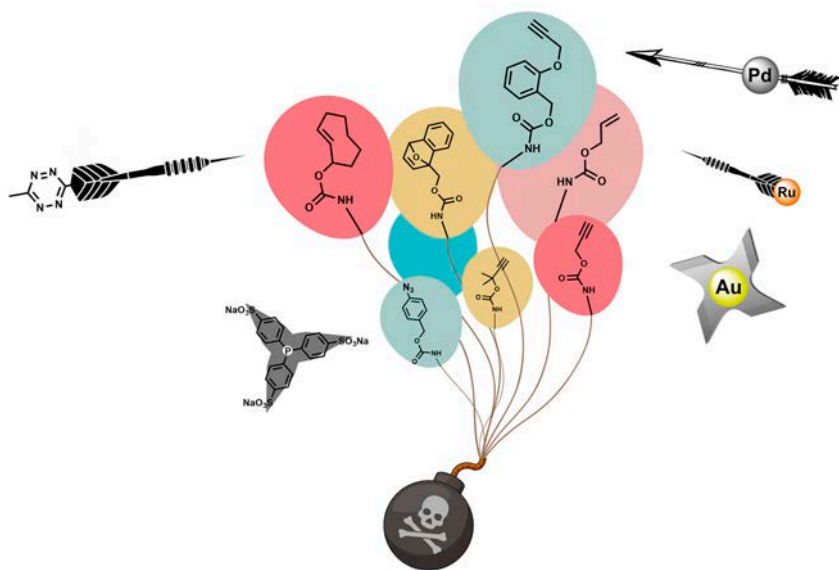
The concept of click chemistry, first reported in 2001, has paved the way for bioorthogonal chemistry, which represents a class of chemical transformations that operate in biological media without interfering with endogenous functional groups. Bioorthogonal reactions including ligation, cleavage, and click-to-release reactions can now be carried out in living organisms, unveiling the potential to modulate drug properties by fostering their selective release or synthesis in targeted tissues as well as their on-demand clearance. Herein, we report innovative approaches that employ bioorthogonal chemistry for therapeutic purposes.



Keywords: bioorthogonal chemistry · click chemistry · ligation reactions · cleavage reactions · click-to-release reactions · in situ chemistry · in vivo chemistry

14 Bioorthogonal Strategies for the Uncaging and Assembly of Drugs*C. Adam*^{ID}, *Á. Lorente-Macías*^{ID}, *S. Croke*^{ID}, and *A. Unciti-Broceta*^{ID}

The bioorthogonal synthesis of drugs offers a unique opportunity for targeting — either molecularly, spatiotemporally, or both — the delivery of active compounds directly to the disease site. Problems such as unfavorable pharmacokinetic (PK) profiles and dose-limiting side effects can be mitigated with the careful deployment of the tools of bioorthogonal chemistry. In order to access medicinal applications, researchers have developed ground-breaking new chemistries for the caging and uncaging, assembly, and molecular targeting of a wide range of clinically approved drugs. This review presents notable examples of bioorthogonal drug synthesis that have emerged from the two main branches of the bioorthogonal field: organic click chemistries and transition-metal catalyzed reactions.



Keywords: bioorthogonal catalysis · click chemistry · click-to-release · transition metals · prodrug · chemotherapy · targeted therapy

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