

# Ruthenium Catalysis in Biological Habitats

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Dedicated to Prof. Janine Cossy

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Recent years have witnessed a considerable progress in research aimed at merging transition metal catalysis with chemical and cell biology. Therefore, a crescent number of metal-catalyzed transformations have been shown compatible with biological media and even with living settings. Of the different transition metals used to build these biocompatible catalysts, ruthenium has demonstrated to be particularly powerful, in part because the resulting complexes exhibit a very good balance between reactivity and biological stability. Indeed, ruthenium complexes have demonstrated utility to promote a great variety of reactions in biologically relevant contexts, from deprotection and redox processes to cycloadditions or photocatalytic transformations. Many of these reactions may enable the development of new type of biological tools and pharmacological strategies.

**Keywords:** bioconjugation, bioorthogonal chemistry, fluorescence, organometallic chemistry, transition metals.

## 1. Introduction

Chemical Biology is a scientific discipline aimed at the development of chemical and synthetic tools for the study and manipulation of biological systems. The field includes the area of bioorthogonal chemistry, which encompasses a class of chemical reactions that can take place in biological and living environments in a unharmed way. This term was first coined by Bertozzi in 2003, as part of their work for imaging glycans in live settings.<sup>[1]</sup> After these initial developments, the field has progressed in a quite explosive manner, and has somewhat changed the way in which chemistry interacts with biology.<sup>[2]</sup> Worth to note, Bertozzi, together with Sharpless and Meldal, have received the Nobel Prize in Chemistry in 2022 because of the pioneering work in Bioorthogonal and Click chemistry.

In order for chemical reactions to be considered *bioorthogonal* and therefore amenable for translation to living organisms,<sup>[3]</sup> they must fulfill several characteristics: 1) *chemoselectivity* – the reactants must selectively react with each other in the presence of a

rich milieu with many other biological functional groups; 2) *compatibility with physiological conditions* – namely aqueous media, neutral pH and temperatures below 37 °C; 3) *biocompatibility* of the reactants and reagents; 4) *fast kinetics* – since the reactions are performed usually in the micro- to nano-molar concentration range, a fast rate is needed to overcome the dilution effect and reduce the exposition time to abiotic components; and 5) *stability of reactants and products* – the reaction components should not be degraded or metabolized in the biological media.

Early examples of coupling reactions that are considered bioorthogonal can be traced back to the late 1980s, when Rideout and coworkers developed a bioconjugation technique based on the formation oximes and hydrazones.<sup>[4]</sup> The higher stability of these functional groups towards hydrolysis compared to the parent imines, because of the stabilization effect of the heteroatom adjacent to the imine nitrogen, was critical for their use in aqueous media.<sup>[5]</sup> In this work, the

authors applied the reaction to the synthesis of cell toxins from non-toxic starting materials (*Scheme 1*).<sup>[4]</sup>

In the late 1990s, Bertozzi extended this approach to oxime linkages that covalently modify cell membranes.<sup>[6,7]</sup>

Bertozzi and coworkers have also developed several other bioorthogonal chemical tools. For instance, in a seminal paper in the year 2000, they described a *Staudinger* bioconjugation method.<sup>[8]</sup> In the classical *Staudinger* reaction, an organic azide reacts with a phosphine to form an aza-ylide intermediate, which is spontaneously hydrolyzed by water to deliver the phosphine oxide and the resulting amine. The introduction of a methyl ester in the arylphosphine moiety allows to trap the iminophosphorane species and suppress the hydrolytic pathway, thereby enabling the formation of an amide bond (*Scheme 2*). The simplicity of the reaction and the absence of phosphines and azides in biological environments made this *Staudinger* reaction a promising candidate for bioorthogonal chemistry. However, despite its great selectivity, the poor kinetics of the process, which ranks among the

slowest bioorthogonal transformations, hampered its widespread application.<sup>[9]</sup>

A cutting-edge turn in the field was the development of the strain-promoted 1,3-dipolar cycloaddition between azides and strained alkynes or alkenes,<sup>[10]</sup> and especially the more recent cycloaddition between tetrazines and *trans*-cyclooctenes, which is among the fastest of all bioorthogonal reactions.<sup>[11]</sup>

Whereas these reactions are impressive and have led to many useful applications, the intrinsic high reactivity of the reactants can lead to unwanted, secondary reactions. In this context, the development of bioorthogonal processes promoted by external catalytic reagents is very attractive, as the reactants would remain stable until finding the catalyst.

Particularly attractive is the possibility of using transition metal complexes as catalysts, owing to the enormous breadth and scope of organometallic catalysis. Moreover, the possibility of tuning the physical and catalytic properties of the metal catalysts by modifying their ancillary ligands, represents an additional bonus for this type of strategy. It is however



José Luis Mascareñas completed his PhD in the University of Santiago in 1988 and carried out post-doctoral studies at Stanford University (1989–1990) and Harvard University (1993–1995). He became full professor at the University of Santiago in 2005. He has published over 220 articles and supervised 41 PhD theses. In 2014 he was awarded with an ERC Advanced Grant for his project

METBIOCAT (<http://metbiocat.eu/>) and, more recently, an ERC Proof of Concept (2020). He has been scientific director of CIQUS since 2014. In 2015 he received the Gold medal of the Spanish Society of Chemistry and in 2016, appointed as member of the European Academy of Sciences. His current research splits between a synthetic program aimed at discovering novel methods based on metal catalysis, and a chemical biology program focused on the development of synthetic tools for biological intervention.



Fernando López obtained his PhD in 2003 at the University of Santiago de Compostela (USC). He carried out predoctoral research with Profs. E. M. Carreira and J. F. Hartwig, and postdoctoral studies with Prof. B. L. Feringa. In 2006, he joined the USC as a 'Ramón y Cajal' researcher. In 2008, he was appointed Tenured Scientist at the Spanish National Research Council (CSIC–IQOG) and, in 2018,

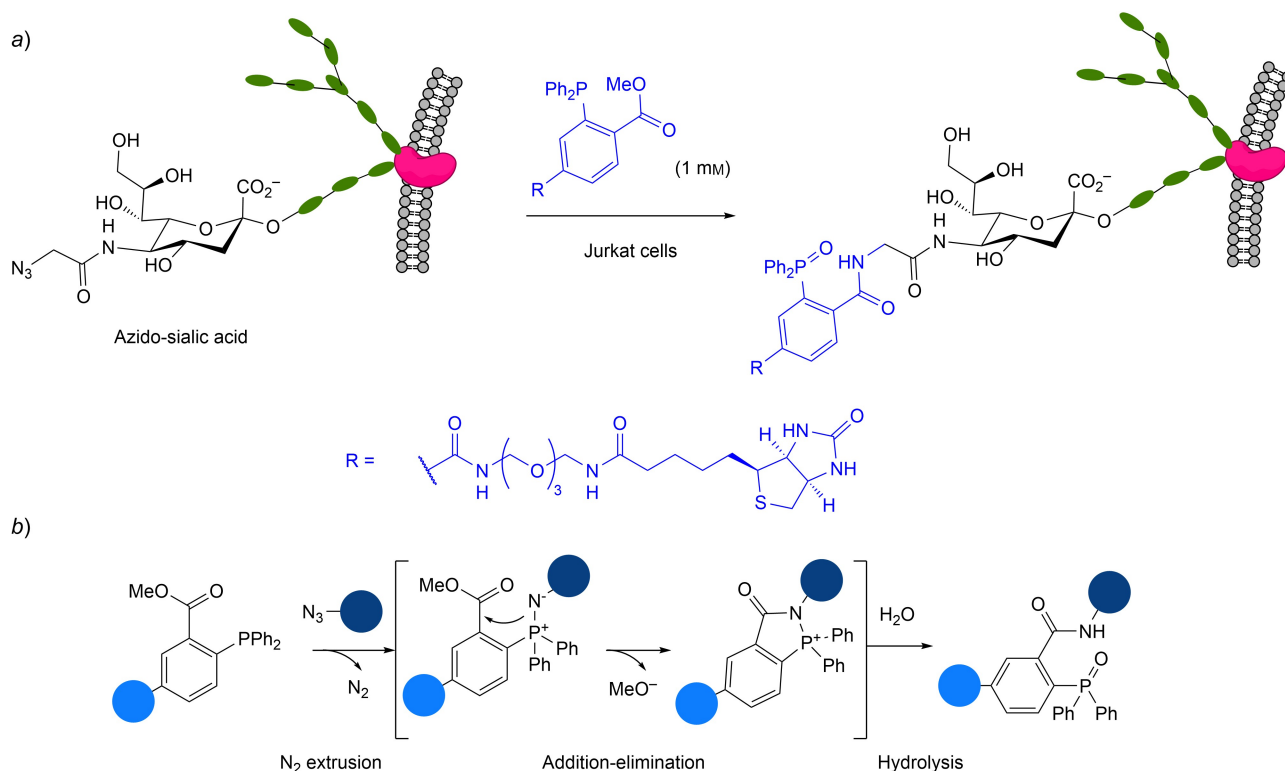
he was promoted to Senior Research Scientist, from 2020 at the MBG-CSIC. Since 2012, he was assigned as PI at the CiQUS (USC). His research is aimed at discovering new, efficient synthetic methods as well as novel biocompatible tools, both based on transition metal catalysis.



Alejandro Gutiérrez-González studied Chemistry at University of La Laguna (Spain). He joined Prof. J. L. Mascareñas and Dr. F. López group, first as a Master student and a predoctoral researcher. In 2021 he joined the group of Prof. A. Fürstner (Germany) in a predoctoral stay. He is finishing his PhD working on new ruthenium-promoted bioorthogonal transformations in biological media.



**Scheme 1.** Formation of a hydrazone in the presence of human red blood cells.<sup>[4]</sup>

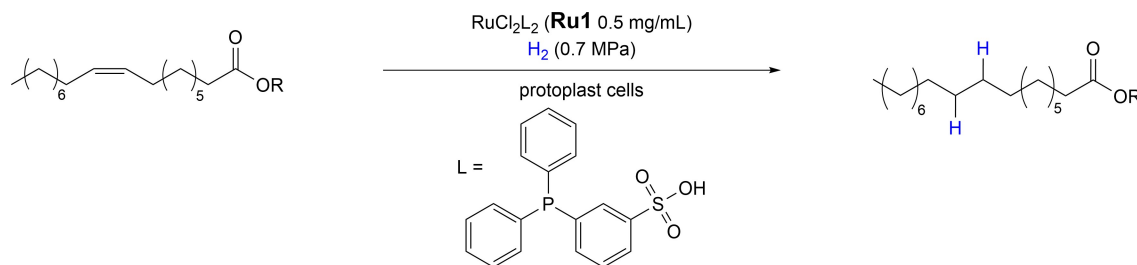


**Scheme 2.** a) Staudinger-Bertozzi bioconjugation: modification of azido sugars in the surface of Jurkat cells. b) Mechanism of the process.<sup>[8]</sup>

true that translating transition metal catalysis to biological environments encompasses numerous challenges, such as the compatibility of the reagents and organometallic intermediates with water and with the biological milieu, or the potential deactivation of the active catalytic species. Nonetheless, it is important to note that the hydrolysis of carbon-metal bonds in many transition metal complexes might not be kinetically easy, and the reaction with reactants containing unsaturated bonds could be preferred.<sup>[12,13]</sup> Indeed, during the last decades a good number of transition metal catalyzed reactions have been shown to be compatible with water,<sup>[12–15]</sup> and, in recent years, the development of transition metal catalyzed bioorthogonal reactions has evolved from mere curiosity to a fruitful field.<sup>[16–18]</sup>

The early examples of transition metal catalyzed transformations in any kind of biological media can be traced back to the early 1980s, when Cséplő and coworkers reported the use of a water-soluble ruthenium catalyst ( $\text{RuCl}_2\text{L}_2$ , **Ru1**,  $\text{L} =$  triphenylphosphine-3-sulfonic acid) to hydrogenate unsaturated fatty acids in the presence of protoplasts (Scheme 3).<sup>[19]</sup> The reduction of the unsaturated fatty acids changes the membrane fluidity as the percentage of unsaturation correlates with the micro viscosity of the cell membranes. Even though the reaction induces the death of most of the cells after two hours, it can be considered as a pioneering example in the field.

A landmark advance was achieved in 2002 by Sharpless and by Meldal, who independently developed the Copper Catalyzed Azide-Alkyne Cycloaddi-



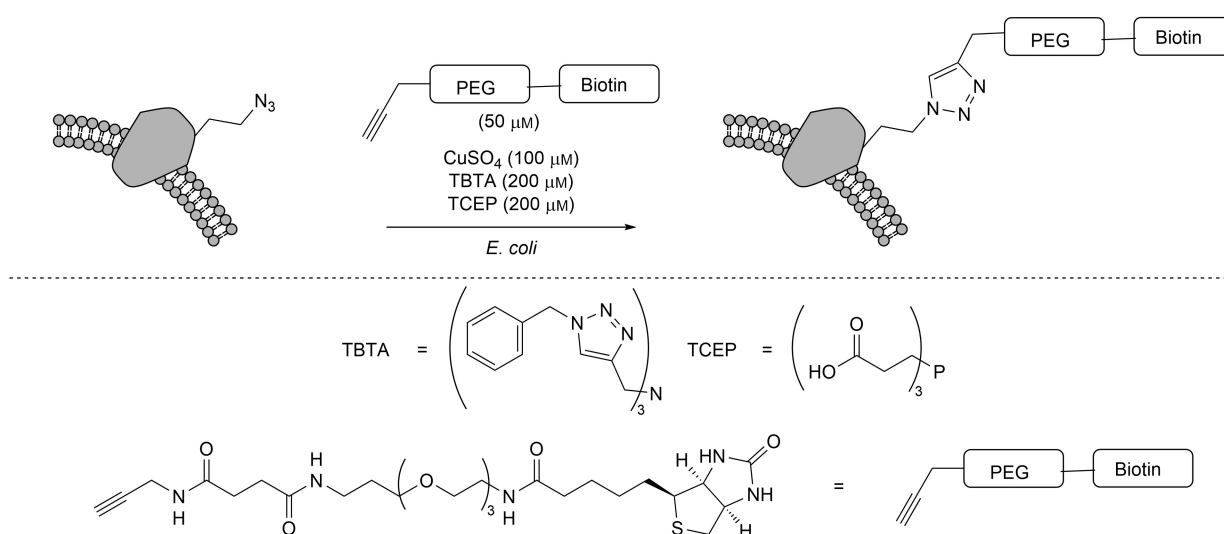
**Scheme 3.** Hydrogenation of phospholipids by a water-soluble ruthenium complex, **Ru1**, in protoplast cells.<sup>[19]</sup>

tion (CuAAC), a reaction in which an organic azide and a terminal alkyne rapidly undergo a formal [3 + 2] cycloaddition mediated by copper(I) catalysts.<sup>[20–22]</sup> The reaction, which involves copper metalacyclic intermediates, can take place in aqueous media, and presents very good orthogonality.<sup>[23]</sup> Moreover, the resulting triazoles, which are obtained with complete regioselectivity, are of intrinsic interest from a biologically point of view, due to their chemical inertness and structural similarity to amides. During these decades, there have been many efforts to translate this ligation to biological and living environments. For instance, *Tirrell* and coworkers overexpressed a genetically engineered protein bearing azidohomoalanine (AHA), a non-natural amino acid, in the surface of *E. coli*, and demonstrated that this azido protein could be conjugated to a biotin-alkyne using the CuAAC (*Scheme 4*).<sup>[24]</sup>

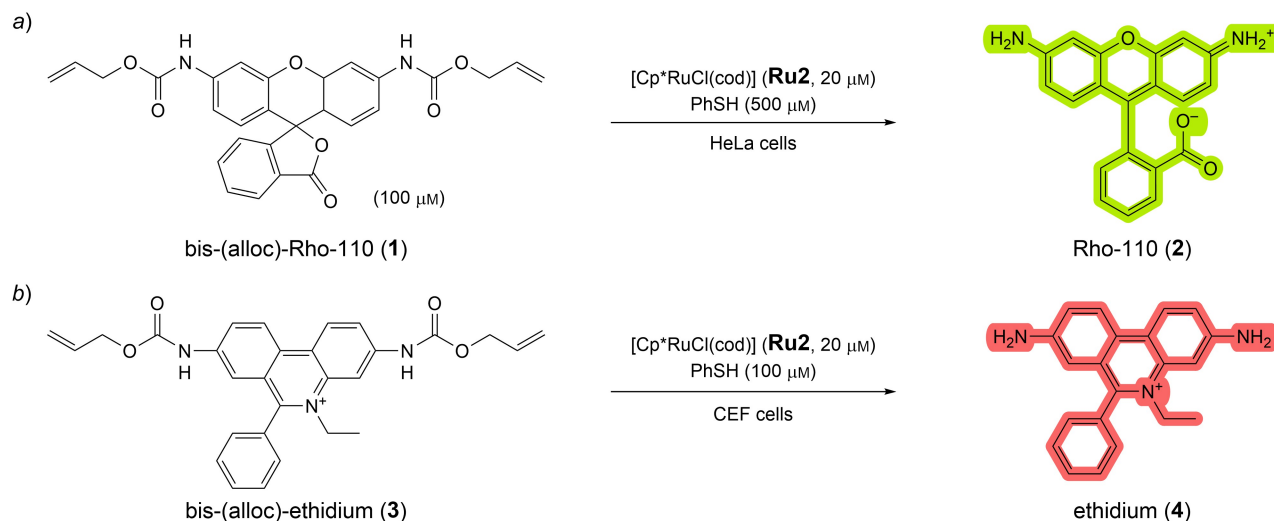
There have been other reports on the use of the CuAAC for promoting bioorthogonal ligations in bio-

logical media, most of them relying on the use copper complexes containing tris(triazole) ligands.<sup>[25,26]</sup> However, the application in living cells has been hampered by the lability and toxicity of the copper(I) salts.<sup>[25,27]</sup>

In 2006, *Meggers* and coworkers showed that the Ru(II) complex [Cp\*<sub>2</sub>Ru(cod)Cl] (**Ru2**) is able to promote the removal of allyl carbamate (*i.e.*, alloc) protecting groups from a Rhodamine-110 caged probe (**1**), not only *in vitro* in aqueous buffers, but also in living HeLa cells (*Scheme 5*).<sup>[28]</sup> Even though the reaction requires the addition of a thiol source like thiophenol to accelerate the process, this report represents a pioneering example on the use of organometallic catalysis in biological media, beyond the CuAAC. Later, in 2014, *Mascareñas* and coworkers further demonstrated the bioorthogonal potential of this ruthenium catalyst, by using it for the uncaging of DNA-binding agents, such as DAPI or ethidium bromide, inside live mammalian cells.<sup>[29]</sup>



**Scheme 4.** CuAAC modification of membrane proteins in *E. coli* expressing azidohomoalanine (AHA).<sup>[24]</sup>



**Scheme 5.** a) Deprotection of bis-(alloc)-Rho-110 in HeLa cells by **Ru2**.<sup>[28]</sup> b) Ruthenium catalyzed deprotection of bis-(alloc)-ethidium.<sup>[29]</sup>

Related uncaging reactions, but for propargyl instead of allyl protecting groups, can also be carried out using metal nanoparticles, such as demonstrated by *Bradley* and *Unciti-Broceta*, using palladium nanospheres.<sup>[30,31]</sup> These authors even used the strategy for the uncaging of cytotoxic drugs like 5-fluoro-1-propargyl-uracil in biological environments.<sup>[30]</sup>

Soon after these seminal discoveries, the development of new metal based bioorthogonal transformations got momentum and several other transformations were sequentially added to the bioorthogonal armory. Most reported examples involve palladium catalysts, both in the form of nanoparticles (NPs),<sup>[30,32,33]</sup> or using discrete Pd complexes,<sup>[34–36]</sup> although these reagents tend to be readily deactivated. Biocompatible transformations promoted by complexes featuring other metals like Ir,<sup>[37,38]</sup> Au,<sup>[39–41]</sup> Cu,<sup>[42–44]</sup> Os,<sup>[45]</sup> or Fe<sup>[46,47]</sup> have also been reported, although in general, the catalytic efficiency is rather low.<sup>[48,49]</sup> In many cases, these metals also present problems of biocompatibility and/or ready deactivation of the catalyst. In this context, ruthenium complexes are the ones that usually lead to the best results. In general, ruthenium complexes present good levels of biocompatibility and can be used for a variety of reactions, not only for deprotection processes.

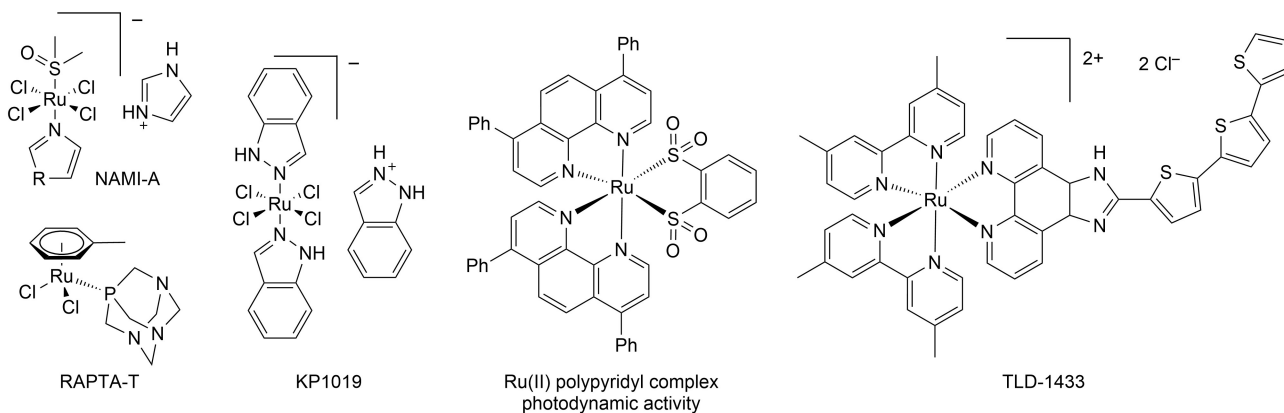
In this review, we will make a brief historical overview on the use of ruthenium catalysts for performing chemical reactions in biologically relevant media and live environments, highlighting their

potential and opportunities for further uses in chemical biology.

## 2. Ru Complexes in Catalysis and Biomedicine

Ruthenium is a polyvalent hard metal, member of the platinum group together with Osmium, Iridium, Rhodium, Palladium and Platinum. It has a quite rich redox chemistry, that ranges from oxidation states  $-2$  (e.g.  $[\text{Ru}(\text{CO})_4]^{2-}$ ), to  $8+$  (e.g.  $\text{RuO}_4$ ). However, ruthenium catalyzed processes usually involve oxidation states from  $2+$  to  $4+$ . Ruthenium complexes usually display octahedral geometries in the oxidation states  $2+$ ,  $3+$  and  $4+$ , although other coordination topologies are also possible.<sup>[50]</sup>

Ruthenium complexes tend to present a good balance between reactivity and stability, and therefore have been widely used as catalysts for a variety of reactions. Most of these reactions entail classical organometallic mechanisms, with ruthenium cycling between different redox states, even though redox neutral ruthenium-promoted reactions have also been described.<sup>[51]</sup> Overall, ruthenium catalysts offer a broad mechanistic diversity, and a wide number of reactions involving different intermediates have been described. These intermediates include Ru  $\pi$ -allyl complexes (in allylation/deallylation reactions), Ru carbenes, vinylidenes and allenylidenes (e.g., in hydration, hydrofunctionalization and cyclopropanation reactions) or ruthenacycles (e.g., in cycloadditions, cycloisomeriza-



**Scheme 6.** Selection of Ruthenium complexes with anticancer or PDT activity.

tions and alkene metathesis). Moreover, several ruthenium polypyridyl and related complexes have been successfully applied as photocatalysts in photochemical transformations entailing energy and electron transfer processes.<sup>[52–54]</sup> Overall, ruthenium catalysts can be used for many types of mechanistically diverse reactions, including deprotections, couplings, isomerizations and cycloaddition processes.

Classically, these reactions have been carried out in organic solvents, and avoiding the presence of water, and/or oxygen; however, it has been increasingly demonstrated that many of these ruthenium catalysts can tolerate aqueous solvents.<sup>[15]</sup> This water compatibility is one of the reasons why ruthenium complexes have also been studied in biomedicine, as potential therapeutic agents.

For instance, a bis-imidazole tetrachlororuthenate(III) complex has demonstrated remarkable activity for the treatment of colorectal cancer (Scheme 6).<sup>[55,56]</sup> Apparently, this and other related ruthenium complexes behave as prodrugs, undergoing a reduction to active Ru(II) derivatives at the target site. Photoactive ruthenium polypyridyl complexes are also being studied for photodynamic therapy due to their capabilities to generate singlet oxygen,<sup>[57,58]</sup> for instance the ruthenium compound TLD-1433 is under clinical trials for the treatment of bladder cancer.<sup>[59]</sup>

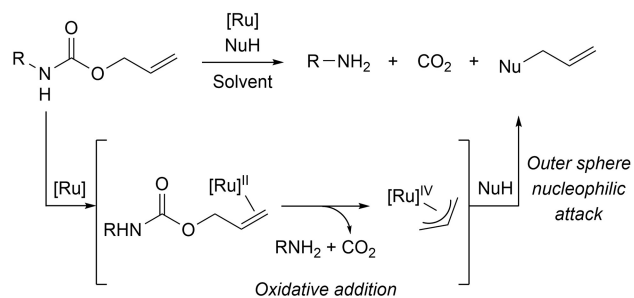
Overall, it seems clear that ruthenium is an extremely versatile metal, that allows the preparation of many types of complexes compatible with aqueous and biological media, thus offering many possibilities for triggering a variety of catalytic processes. We have organized the next discussion considering their participation in bond cleavage and bond forming reactions, oxidation/reduction processes, and, finally, in reactions that are promoted by ruthenium photocatalysts, all of

them taking place in biological and/or living environments.

### 3. Ruthenium-Promoted Bond Cleavage Reactions

#### 3.1. Deallylation Processes

Ruthenium(II) complexes can be effective catalysts for deallylation reactions involving *Tsuji–Trost* like mechanisms.<sup>[60–62]</sup> The reaction requires substrates with good leaving groups and takes place through an initial oxidative addition step to give a  $\pi$ -allyl ruthenium intermediate. In the case of allyl carbamates (alloc), the reaction is especially favored because of the release of CO<sub>2</sub>. To close the catalytic cycle, an outer sphere attack of a sacrificial nucleophile is needed, so that the Ru(II) catalyst can be regenerated (Scheme 7). Importantly, this type of deprotection reactions have been shown to be compatible with alcoholic and aqueous conditions.<sup>[63]</sup>



**Scheme 7.** Mechanism of the ruthenium catalyzed *Tsuji–Trost* deallylation.

Undoubtedly, the most relevant example on the use of this type of reaction in biological environments is the abovementioned pioneering work of Meggers and coworkers on the uncaging of Rhodamine derivatives using  $[\text{Cp}^*\text{RuCl}(\text{cod})]$  (**Ru2**, see Scheme 5,a).<sup>[28]</sup>

The ruthenium-promoted uncaging reactions have also been exploited by other groups for different type of biological applications. For instance, in 2013, Balskus reported the use a ruthenium-promoted deprotection of *N*-alloc-*p*-aminobenzoic acid (alloc-PABA, **5**) to deliver *p*-aminobenzoic acid (PABA, **6**), a nutrient that is key for the growth and survival of PABA-auxotroph organisms. Accordingly, Balskus used three different *E. coli* mutants that lack the ability to produce PABA to demonstrate that, only when the alloc-PABA and the ruthenium catalyst (**Ru2**) were added to a PABA-free culture media, the survival and growth of these *E. coli* auxotroph mutants could be recovered at normal levels (Scheme 8).<sup>[64]</sup>

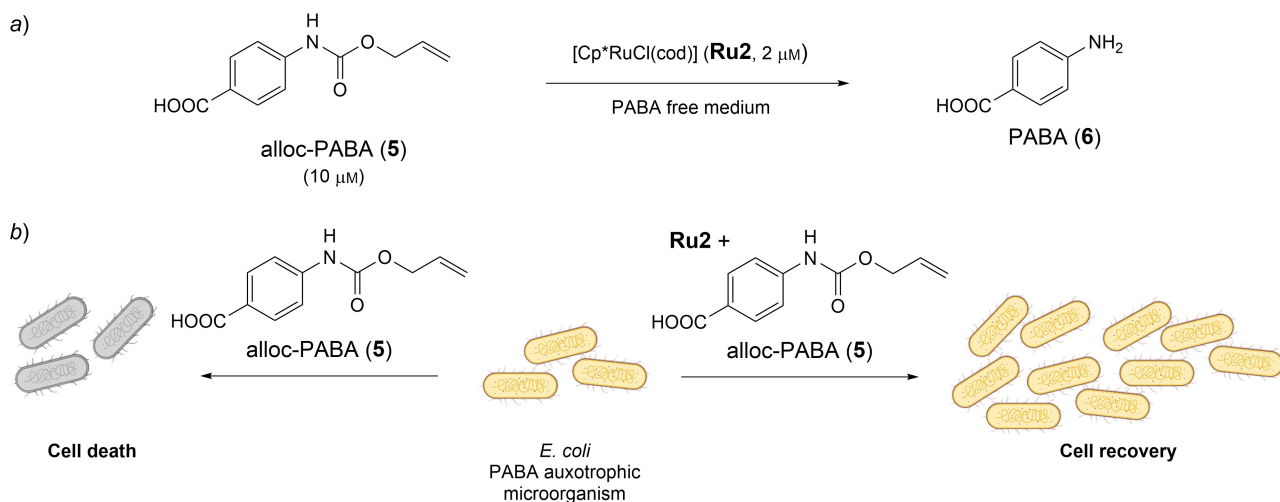
As previously commented, our group reported in 2014 the uncaging of DNA binders in living cells using a Ru(II) catalyst (Scheme 5,b).<sup>[29]</sup> Another interesting example on the applicability of this deallylation process in chemical biology was reported by Rotello and coworkers, demonstrating that the ruthenium catalysts can be encapsulated in gold nanoparticles coated with amphiphilic chains (Scheme 9).<sup>[65]</sup> The incorporation of ammonium groups at the tips of the amphiphilic chains enabled the water solubility of the overall system. The resulting nanozymes can promote the deprotection of bis-(alloc)-Rhodamine 110 in living cells (HeLa cells, Scheme 9,b).<sup>[65]</sup>

Moreover, they found that these nano constructs can be made stimuli-responsive by incorporating cucurbit[7]uril, which selectively coordinates the ammonium groups precluding or shielding the interchange of the ruthenium reagents with the media. The addition of an adamantly ammonium derivate, which exhibits a higher binding affinity for the cucurbit[7]uril moiety, restores the catalytic activity of the ruthenium nanozyme.<sup>[65]</sup>

The Meggers group demonstrated in 2016 that the alloc-removal reactions can be performed with higher efficiency by using a Kitamura type of ruthenium catalysts,<sup>[63]</sup> which features quinaldic acid ligands (Scheme 10).<sup>[66]</sup> Thus, catalyst **Ru3** provided turnover numbers (TON's) of up to 270, while the previous system (**Ru2**) only afforded a TON of 4. Moreover, the catalyst works using glutathione (GSH) as nucleophile, instead of thiophenol, which facilitates its translation to cellular contexts.

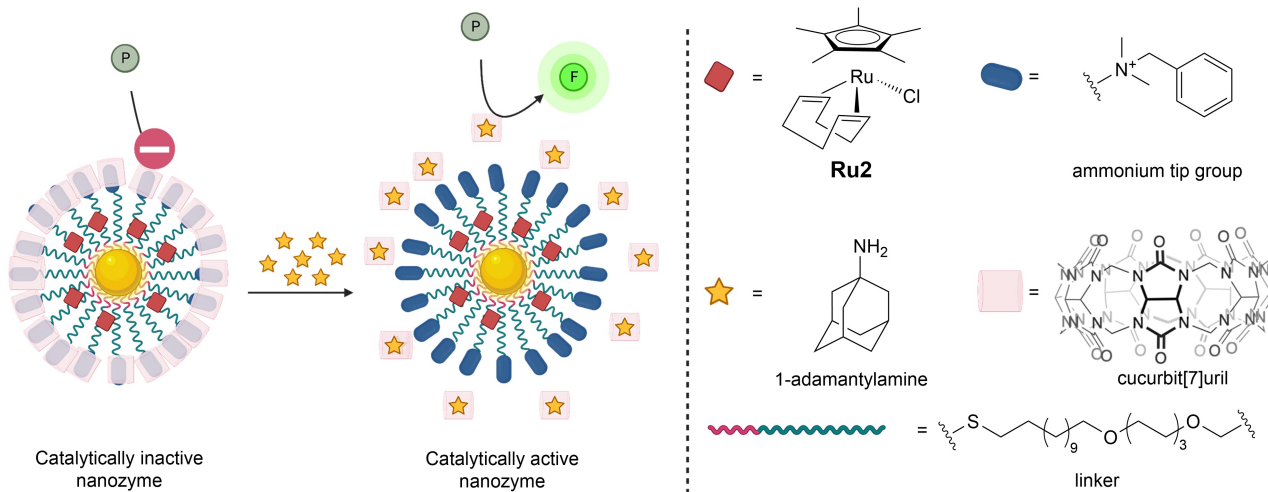
The higher efficiency of this second-generation catalyst allowed for the *in-situ* generation of doxorubicin (**8**) inside HeLa cells. In particular, cells were treated with a high concentration of alloc-doxorubicin (**7**, 100  $\mu\text{M}$ ), which is significantly less toxic than doxorubicin. Upon subsequent treatment with 5  $\mu\text{M}$  of the ruthenium catalyst **Ru3**, the alloc group was removed, and the biological effect of the drug was fully restored, promoting cell death (Scheme 10). This strategy highlights the potential of transition metals for therapeutic applications based on *in situ* activation of pro-drugs.

Soon after, Mascareñas and coworkers took advantage of the structural modularity of this second-

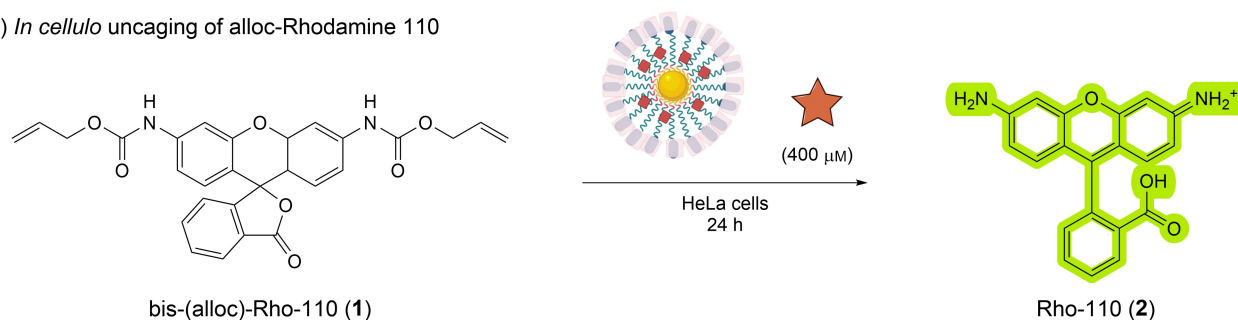


**Scheme 8.** a) **Ru2**-promoted deallylation of alloc-PABA. b) Rescuing of PABA auxotrophic *E. coli* by a ruthenium catalyzed deallylation of alloc-PABA.<sup>[64]</sup>

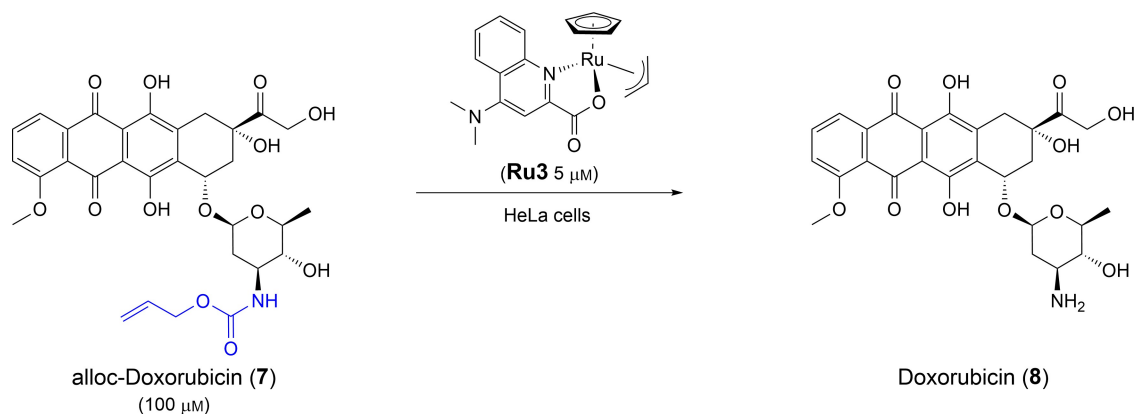
a) Supramolecular regulation of the nanozyme



b) *In cellulo* uncaging of alloc-Rhodamine 110



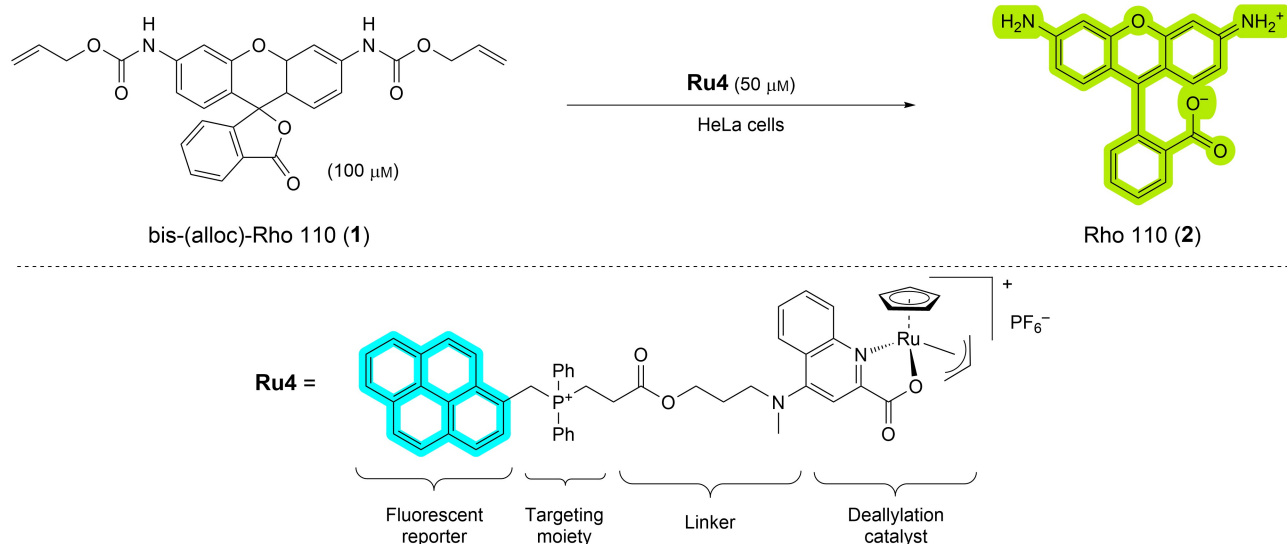
**Scheme 9.** a) Supramolecular regulation of a nanozyme by addition of 1-adamantylamine. b) Nanozyme promoted deprotection of bis-(alloc)-Rho-110.<sup>[65]</sup>



**Scheme 10.** Activation of alloc-Doxorubicin by ruthenium catalyzed deallylation in HeLa cells.<sup>[66]</sup>

generation Ru-catalysts to develop a family of novel ruthenium conjugates which bear a phosphonium moiety attached to the quinaldic acid ligand (**Ru4**, Scheme 11).<sup>[67]</sup> This lipophilic cationic groups exhibit a single positive charge, resonance-stabilized over three phenyl groups and a large hydrophobic surface area.

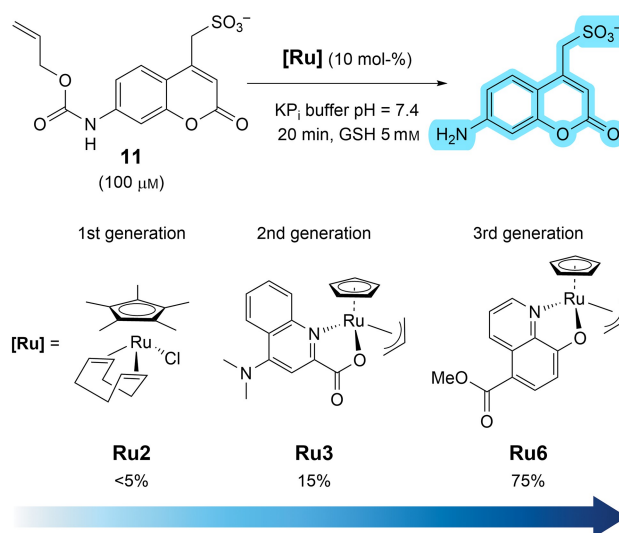
All these features allow the ruthenium complex to be internalized and accumulated preferentially in the mitochondria,<sup>[68]</sup> due to the membrane potential across the inner membrane and, therefore, the reaction is mainly taking place in these specific organelles.



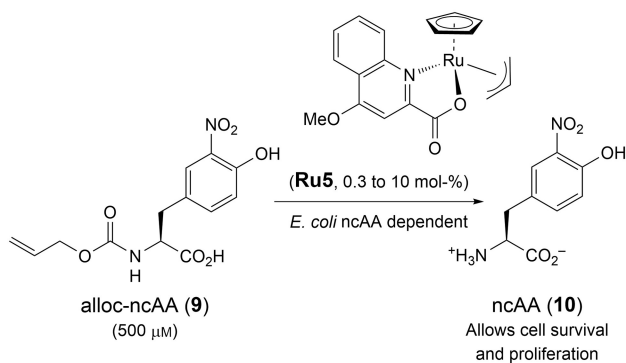
**Scheme 11.** Ruthenium-catalyzed deprotection of bis-(alloc)-Rhodamine 110 in HeLa cell mitochondria.<sup>[67]</sup>

In 2020, Mayer and coworkers used a new variant of this second generation deallylation catalysts (**Ru5**) for the release of an essential non-canonical amino acid (ncAA) within *E. coli*.<sup>[69]</sup> In particular, the authors made the bacterial survival of the *E. coli* mutants dependent of the *in situ* deprotection alloc-ncAA (**9**) by **Ru5** (Scheme 12).

In a new turn to improve catalytic efficiency, Meggers and Völker reported a third-generation catalyst, **Ru6**, which incorporates an 8-hydroxyquinoline moiety instead of the quinaldic acid ligand, enabling the deallylation of the alloc-coumarin **11** to proceed up to 30 times faster compared to the second-generation catalyst **Ru3** (Scheme 13). Moreover, the new catalyst proved to be effective in undiluted blood serum.<sup>[70]</sup>



**Scheme 13.** Deallylation of water soluble alloc-coumarin by different ruthenium catalysts at 100  $\mu\text{M}$ .<sup>[70]</sup>



**Scheme 12.** Ruthenium catalyzed deprotection of alloc-ncAA for the survival of artificial *E. coli* auxotroph.<sup>[69]</sup>

Therefore, discrete ruthenium complexes have demonstrated to be very effective catalysts for deallylation reactions in biological environments, especially in substrates with alloc protecting groups. Palladium salts and complexes can also promote this deallylation reaction, however their efficiency is lower, likely because they are readily deactivated in cellular contexts.

Importantly, ruthenium complexes have also been incorporated into protein scaffolds to form artificial metalloenzymes that can promote the above bond cleavage reactions. In particular, Ward has extensively

worked during the last decade to develop artificial metalloenzymes based on a streptavidin-biotin binding strategy. In this context, this group reported in 2018 the use of a biotin containing ruthenium complex, **Ru7**, which when bound to streptavidin can promote the deallylation reaction of *N*-alloc containing substrates.<sup>[71]</sup> The artificial metalloenzymes were structurally optimized by several rounds of directed evolution cycles. The resulting optimal metalloenzyme was uptaken by HEK-293T cells promoted the deallylation of *N*-alloc-triiodothyronine, **12**. Thus, the released triiodothyronine **13** activates a gene switch that triggers the production of luciferase as readout, enabling the determination of the reaction efficiency (Scheme 14,a).

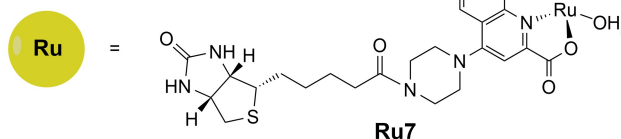
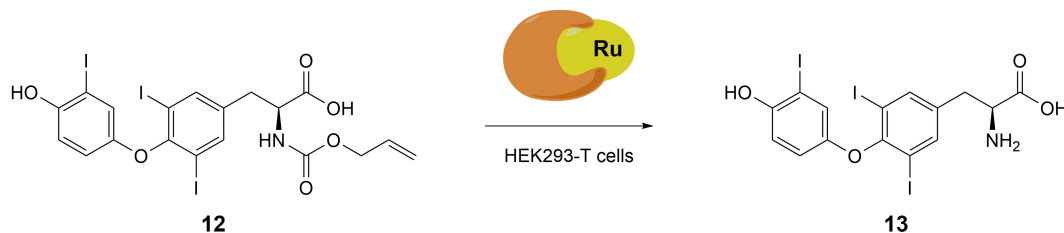
In a new tour the force, *Jessen-Trefzer* reported the preparation of a new type of artificial ruthenium-

containing metalloenzymes using encapsulin proteins. In particular, they use the Halo-tag protein and a third-generation ruthenium deallylation catalyst, **Ru8**, bearing the Halo ligand, to ensemble the artificial metallo-protein. The resulting encapsulin conjugates allowed the formation of catalytic protein compartments, mimicking the compartmentalization of biological processes in cells. This ensemble is capable of promoting the deallylation reaction of alloc-coumarin **14** *in vitro* and in the presence of white blood cells (monocytes J774 A.1, Scheme 14,b).<sup>[72]</sup>

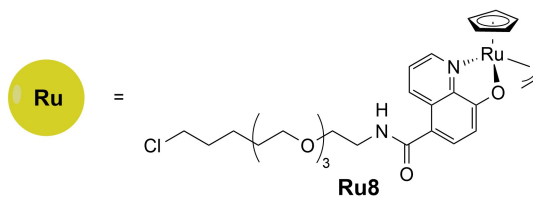
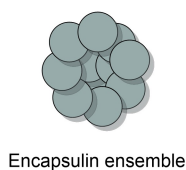
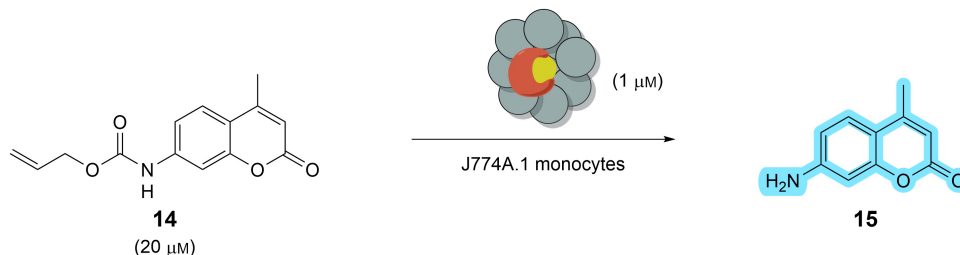
#### 4. Bond Forming Reactions

Ruthenium containing catalysts are particularly powerful as synthetic tools for bond-forming reactions. Their

a) Artificial streptavidin-Ru promoted alloc deprotection of *N*-alloc-triiodothyronine



b) Artificial Halo-Ru-Encapsulin complex, promotes alloc deprotection of allyl (4-methyl-2-oxo-2H-chromen-7-yl)carbamate



**Scheme 14.** a) Streptavidin-Ru artificial metalloenzyme for the alloc deprotection of alloc-triiodothyronine in HEK293-T cells.<sup>[71]</sup> b) Encapsulin Nanoreactor promotes the deprotection of an alloc-coumarin in J774 A.1 cells.<sup>[72]</sup>

applications include coupling processes based on the formation of carbon-carbon and carbon-heteroatom bonds, as well as cycloaddition reactions. Of course, most of these reactions have been optimized in organic solvents, but a number of bond forming processes occurring in biological media and even in living environments have also been developed, as indicated in the next sections.

#### 4.1. Metathesis Reactions

Ruthenium-catalyzed metathesis reactions can be considered among the most versatile transformations in the field of synthetic chemistry. This type of reaction has been extensively applied in industry for polymerization processes, as well as for the synthesis of complex macrocyclic products, including some pharmaceutical drugs currently in the market.<sup>[73]</sup> Remarkably, the reaction has even been carried out in water using soluble complexes such as  $\text{Ru}(\text{H}_2\text{O})_6(\text{tos})_2$ , as originally shown by Grubbs in ring opening polymer-

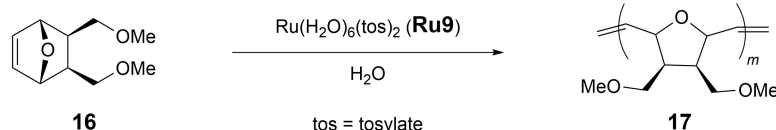
ization processes of cyclic alkenes (**Ru9**, Scheme 15,a).<sup>[74]</sup>

Another water-soluble ruthenium derivative like **Ru10**, which consists of a Hoveyda–Grubbs complex equipped with a polyethylene glycol chain attached to the N-heterocyclic carbene moiety, worked well for ring closing metathesis of diene **18** under aqueous conditions, such as that depicted in Scheme 15,b.<sup>[75]</sup>

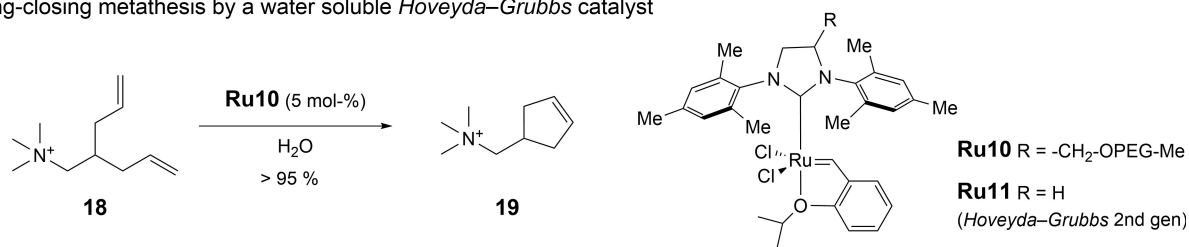
In 2008, Davis and coworkers demonstrated that the 2nd-generation Hoveyda–Grubbs catalyst (**Ru11**) could be used for the modification of proteins bearing an allyl moiety by means of cross-metathesis processes with external alkenes.<sup>[76]</sup> The reactions, which are carried out in aqueous and biological buffers, worked better when allyl sulfides and selenides are used as protein-bearing alkenes, likely because of the assistance of the heteroatom for the stabilization of ruthenium containing intermediates (Scheme 16).<sup>[77,78]</sup>

Along these lines, an important breakthrough in the bioconjugation of proteins using alkene cross-metathesis was achieved by introducing genetic

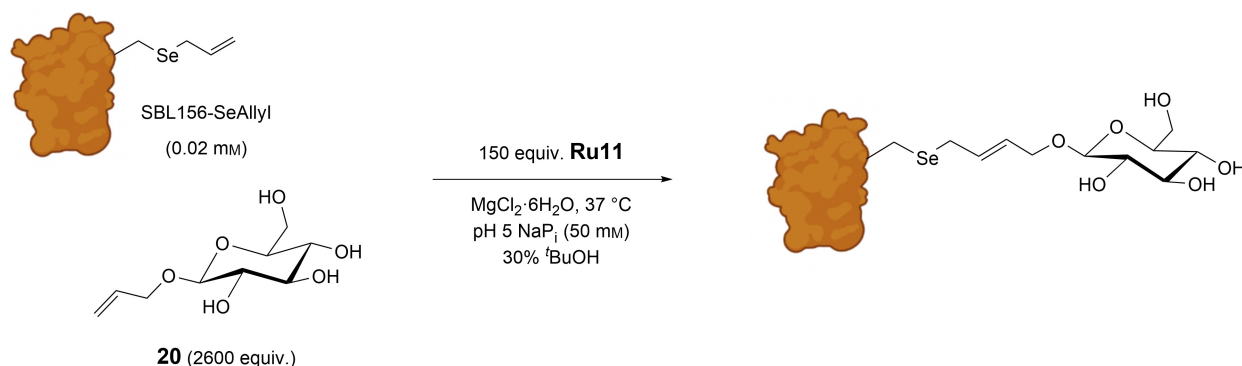
##### a) Ring-opening metathesis polymerization in water



##### b) Ring-closing metathesis by a water soluble Hoveyda–Grubbs catalyst



**Scheme 15.** Ru-catalyzed metathesis of alkenes under aqueous conditions.<sup>[74,75]</sup>



**Scheme 16.** Ruthenium promoted alkene metathesis on allyl-selenide-bearing protein.<sup>[78]</sup>

expansion techniques to easily incorporate non-natural amino acids bearing allyl sulfide moieties, such as allyl-derived methionines.<sup>[79]</sup> Importantly, this strategy enabled the introduction of this reactive motif in specific positions of the protein without performing chemical modifications, prior to the alkene cross-metathesis reaction.

A very elegant application of a ring closing metathesis reactions has been recently described by Ward and coworkers using the Hoveyda–Grubbs 2nd generation catalyst.<sup>[80]</sup> The authors demonstrated that the ring closing metathesis of *o*-allylphenylallyl ethers like **21**, generate 1,4-dihydronaphthalenes (**22**) which, after a spontaneous 1,4-elimination process deliver a naphthalene molecule (**23**) and the oxygen-tethered cargo. Thus, in the case shown in Scheme 17, the release of a coumarin (**24**) allows the monitoring of the process by fluorescence.

Ruthenium metathesis catalysts such as the conjugate **Ru12** were also used by Ward and coworkers to develop artificial metalloenzymes, following the biotin-streptavidin strategy. Through a series of directed evolution rounds these authors found a metallo-streptavidin mutant with increased activity, which was able to catalyze the ring closing metathesis of diene **25** to deliver the corresponding coumarin **26** in the periplasm of living bacteria (*E. coli*, Scheme 18,a).<sup>[81]</sup>

A similar approach but using human serum albumin (HSA) as protein scaffold was developed by Tanaka and coworkers.<sup>[82]</sup> In particular, these authors conjugated a second generation Hoveyda–Grubbs catalyst to a coumarin (as HSA ligand) through a polyethylene glycol tether (Scheme 18,b), and demonstrated that the resulting hybrid can bind to HSA with good affinity. The metalloprotein construct is able to catalyze the ring closing metathesis of the prodrug **27**

to umbelliprenin (**28**), a natural product active against various cancer cell lines.

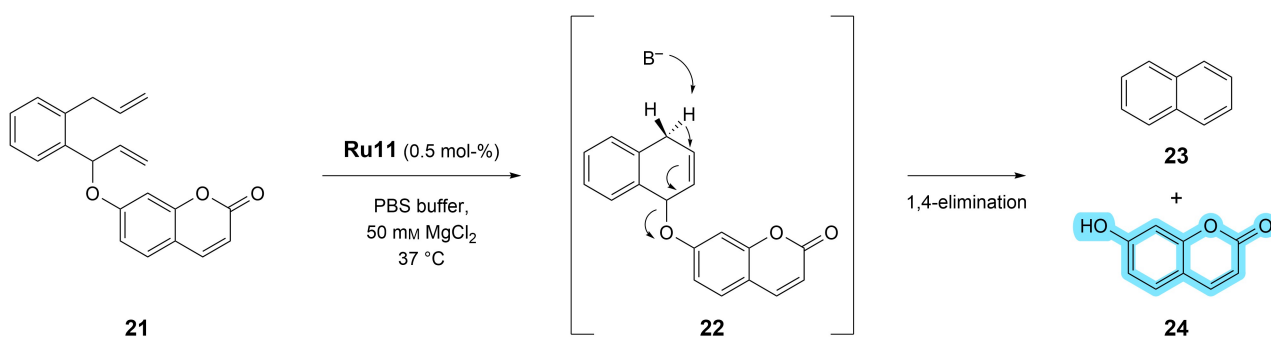
Importantly, the authors showed that the protein scaffold avoids deactivation of the catalyst under physiological conditions, due to the surface charge of the protein that prevents the entrance of biological thiols, like GSH, into the catalytic center. To translate the ring closing metathesis into living mice, they decorated the HSA protein with targeting *N*-glycans, to ensure the cellular recognition and uptake of the metalloenzyme.<sup>[82]</sup>

In 2018, Michel and coworkers used a ruthenium-promoted cross metathesis as a reporter for the presence of bio-ethylene in living cells.<sup>[83]</sup> The approach involved the design and use of a Hoveyda–Grubbs derivative, **Ru14**, which bears a tethered BODIPY moiety. The ruthenium center acts as a quencher for the BODIPY fluorescence, but upon exposure to ethylene gas dissolved in biological media, the reaction promotes an ethylene cross metathesis reaction that releases the fluorescent BODIPY derivative (**29**). The process was carried out in HEK239T cells, using just 2  $\mu$ M of the complex **Ru14**, and allowed to detect the ethylene vapor from ripe fruits (bananas and mangos, Scheme 19).

#### 4.2. Reactions with Carbene Reactants

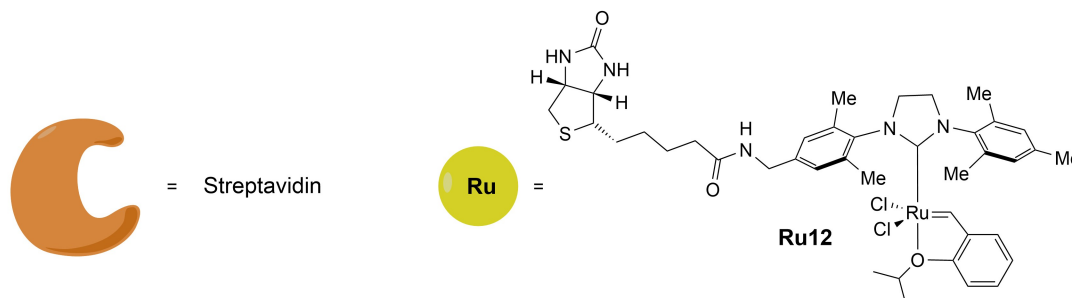
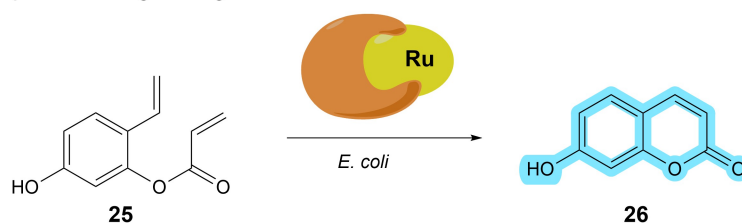
Despite reactions that involve metal carbenes are especially attractive due to the stabilization of divalent carbon by the metal center, examples involving ruthenium carbenes in aqueous or biological media that do not rely on alkene metathesis remain scarce.<sup>[49]</sup>

The first example of a metal catalyzed carbene cyclopropanation in aqueous media was reported using ruthenium ( $[\text{RuCl}_2(p\text{-cymene})]_2$ , **Ru15**) by Nishi-

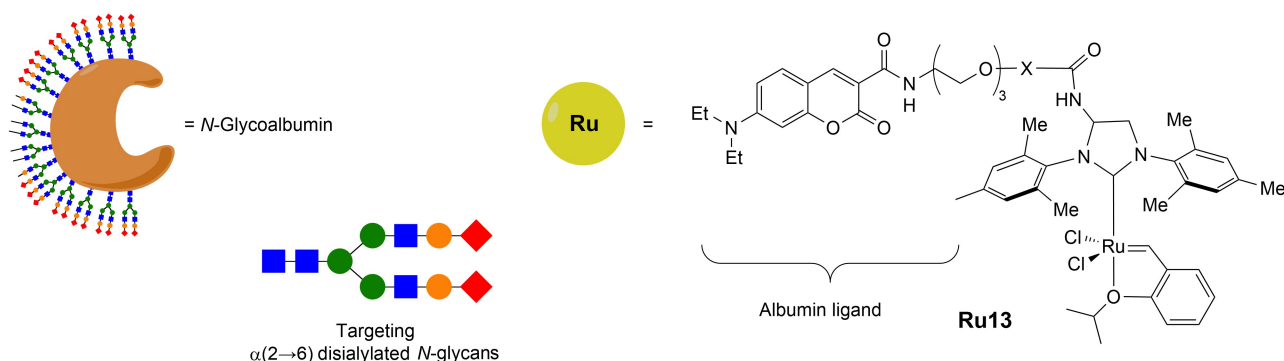
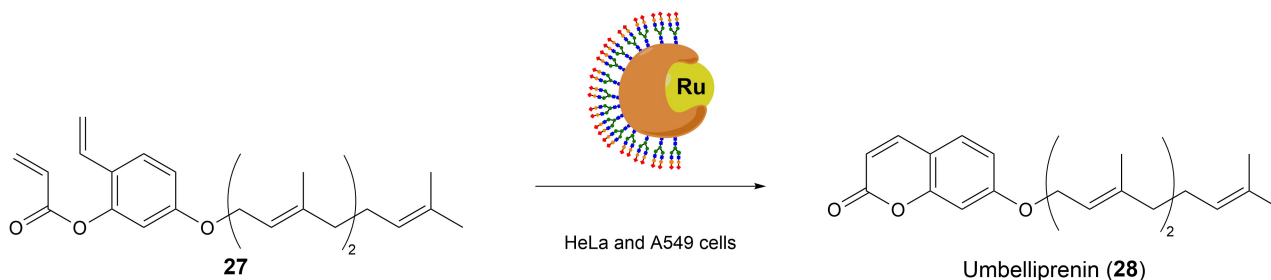


**Scheme 17.** Ruthenium catalyzed ring closing metathesis of *o*-allylphenylallyl ether follow by 1,4-elimination to release a coumarin cargo.<sup>[80]</sup>

a) Artificial streptavidin-Ru promoted ring closing metathesis in *E. coli*



b) Artificial HSA-Ru promoted ring closing metathesis in HeLa and A549 cells



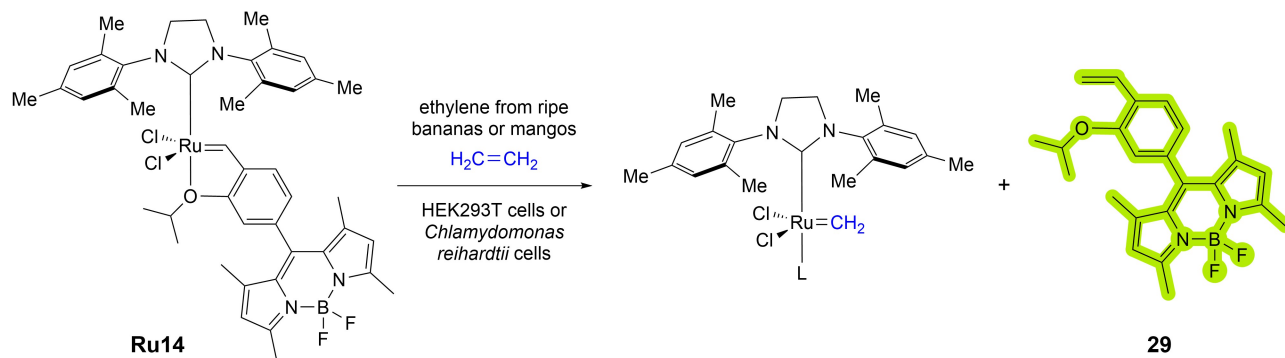
**Scheme 18.** a) Streptavidin-Ru artificial metalloenzyme for ring closing metathesis in *E. coli*.<sup>[81]</sup> b) HSA-Ru artificial metalloenzyme for ring closing metathesis in HeLa and A549.<sup>[82]</sup>

yama in 2001. In this work, Nishiyama and coworkers reported the use of chiral PyBOX ligands and **Ru15**, for the diastereoselective cyclopropanation of styrene, **30**, using (+)-menthyl diazo carboxylate, **31** under aqueous conditions (water/toluene 1:1, Scheme 20,a).<sup>[84]</sup>

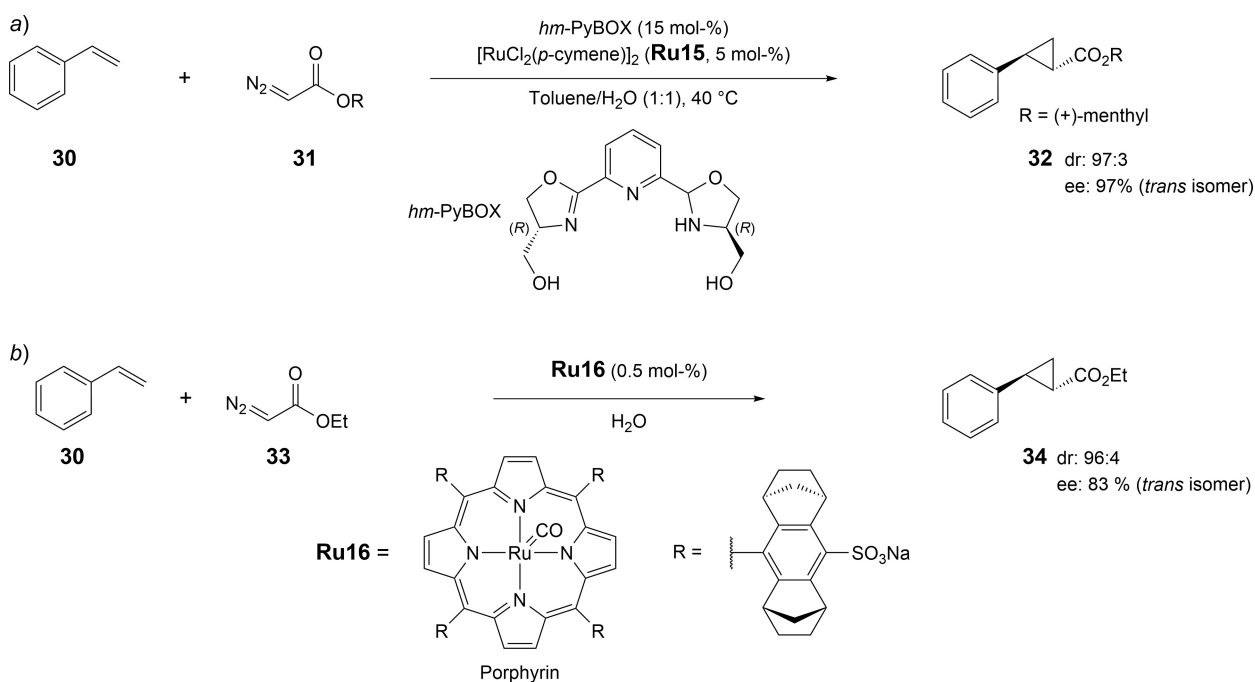
A similar report on a diastereoselective cyclopropanation of styrene was reported by Simonneaux and coworkers, who developed a water-soluble sulfonated porphyrin ruthenium complex, **Ru16**, able to promote

the reaction on water. These conditions are far from been considered bioorthogonal, however they showcase the potential use of ruthenium metal carbenes under aqueous conditions (Scheme 20,b).<sup>[85]</sup>

An example of a carbene transfer for the selective modification of proteins was reported by Che and coworkers using the water-soluble ruthenium glycosylated porphyrin, **Ru17** in PBS. The catalyst **Ru17** was especially efficient for the selective alkylation of the N-



**Scheme 19.** Ethylene detection by a ruthenium-based probe, **Ru14**.<sup>[83]</sup>



**Scheme 20.** a) **Ru15** catalyzed diastereoselective cyclopropanation of styrene in toluene/water.<sup>[84]</sup> b) Water soluble porphyrin **Ru16** catalyzed diastereoselective cyclopropanation of styrene.<sup>[85]</sup>

terminus of peptides and proteins. This selectivity allowed for tagging different proteins like a RNase A or insulin, with a fluorophore tethered to a diazo ester, **35** (Scheme 21).<sup>[86]</sup>

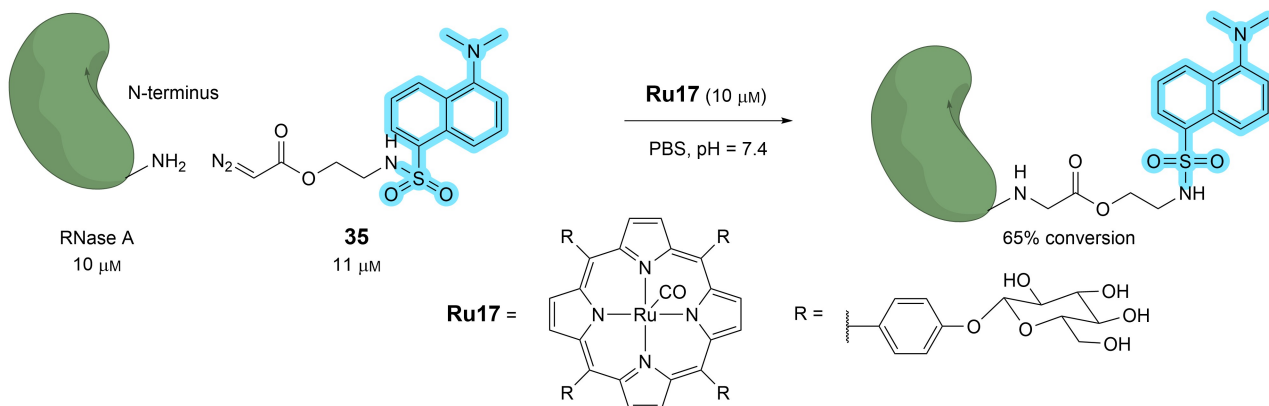
Teply and coworkers reported the use of the  $[\text{Cp}^*\text{RuCl}(\text{cod})]$  (**Ru2**) for a domino process involving the generation of a transient vinyl carbene intermediate from the reaction between enyne **36** and the diazo derivative **37**. The reaction entails an *in situ* generated Ru-carbene **38** and the subsequent trapping of this intermediate in an intramolecular cyclopropanation process (Scheme 22). The reaction works remarkably well in Fetal Bovine serum, *E. coli* lysates, *Rattus*

*norvegicus* urine and in human serum.<sup>[87]</sup> Curiously, despite the good bioorthogonality profile, it was not used for *in cellulo* or *in vivo* catalysis.

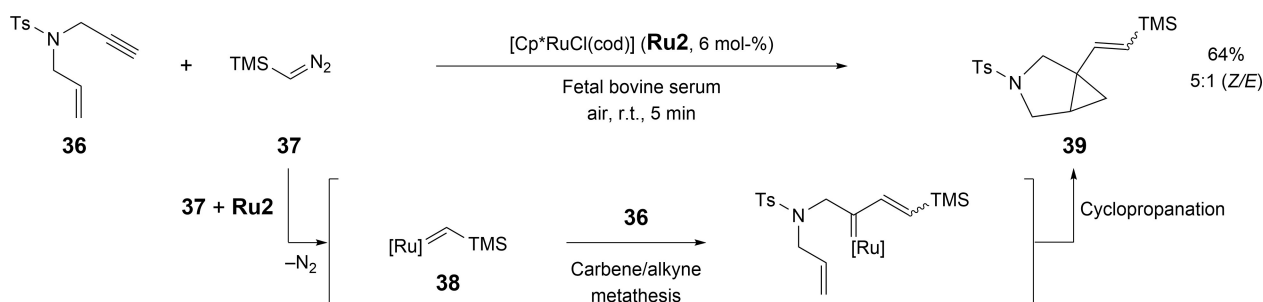
#### 4.3. Hydrosilylation of Alkynes

In addition to inter- or intramolecular reactions involving the formation carbon-carbon bonds, ruthenium catalysts have been sporadically used for other transformations under biologically relevant conditions.

For instance, in 2017, Spring and coworkers described the translation of a ruthenium-catalyzed hydrosilylation of alkynes,<sup>[88,89]</sup> to biologically relevant



**Scheme 21.** Ruthenium glycosylated porphyrin promotes the N–H alkylation of the N-terminus of RNase A.<sup>[86]</sup>



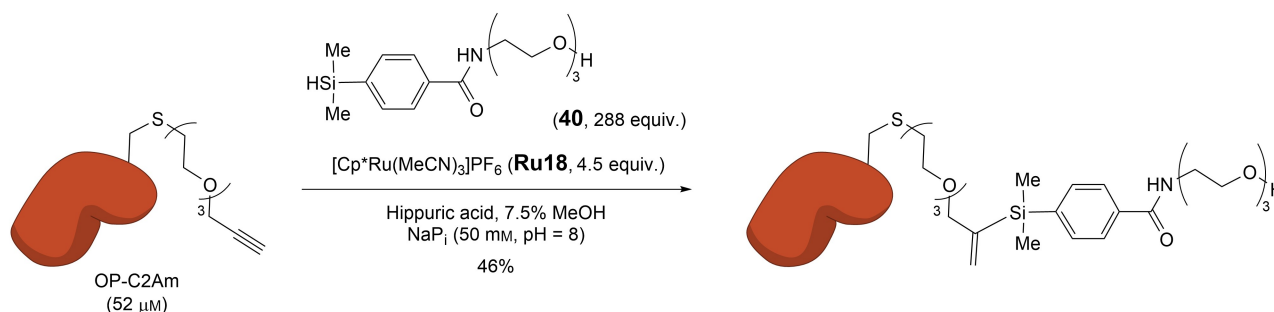
**Scheme 22.** Domino carbene/alkyne-metathesis cyclopropanation in fetal bovine serum catalyzed by **Ru2**.<sup>[87]</sup>

environments.<sup>[90]</sup> The authors showed that the cationic complex [Cp\*Ru(MeCN)<sub>3</sub>]PF<sub>6</sub> (**Ru18**) can catalyze the hydrosilylation of alkyne-containing biomolecules (such as sugars and amino acids), in aqueous buffers.<sup>[90]</sup> The reaction can also be used for the modification of peptides and even proteins (e.g., lysozymes and GFP proteins) equipped with *O*-propargyloxycarbonyl residues. Thus, silanes bearing biotin tags or specific fluorophores can be chemoselectively attached to these proteins under physiological conditions, at a rate comparable to that of other widely

used bioorthogonal reactions. In the case of proteins, the reaction usually requires the use of superstoichiometric quantities of the ruthenium complex (4.5 equiv.) and of the silane (**40**, 288 equiv., Scheme 23).

#### 4.4. Ruthenium-Catalyzed Azide Alkyne Cycloadditions (RuAAC)

As commented before, the CuAAC, which is the reference of Click chemistry, is a fantastic reaction in



**Scheme 23.** Ruthenium promoted hydrosilylation of peptides under aqueous conditions.<sup>[90]</sup>

terms of rate and chemoselectivity, but the copper salts present biocompatibility problems. Therefore, the development of alternatives based on other type of metal catalysts is highly valuable. Of course, ruthenium catalysts could be an excellent choice, owing to the overall good reactivity profiles and the better biocompatibilities. But curiously, despite *Sharpless* and *Fokin* showed that Ru complexes could also be used to promote the AAC in organic solvents, the development of a biocompatible or even water-compatible variants were not described until recently.

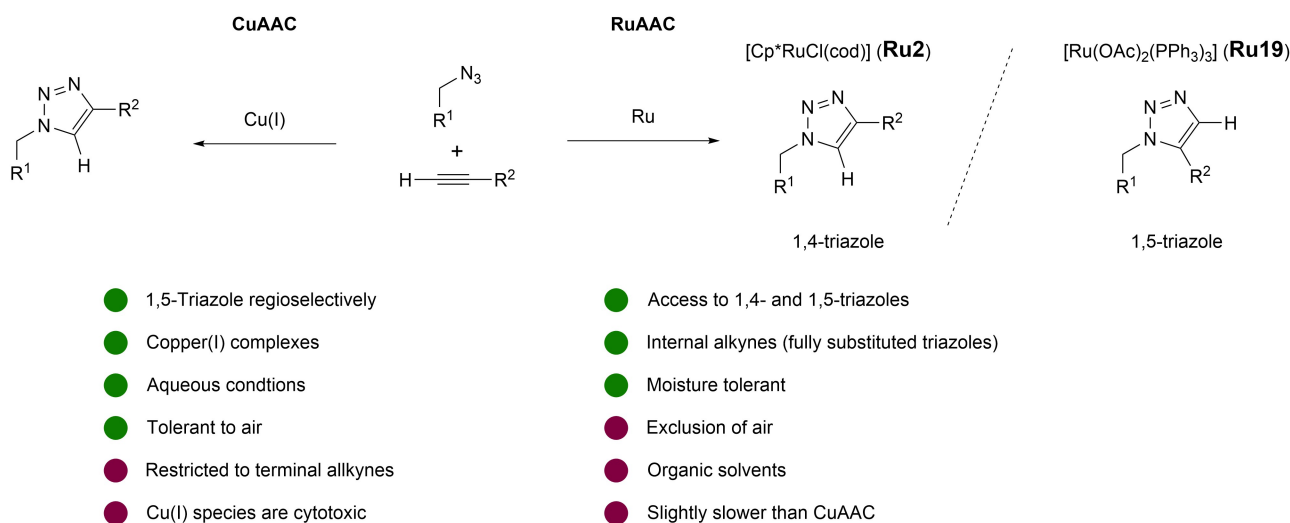
Thus, in the original report by *Sharpless* and *Fokin*, in 2005, these authors found that the ruthenium-catalyzed cycloaddition between organic azides and alkynes (RuAAC) could be achieved in organic solvents (THF or toluene) with complexes like  $[\text{Cp}^*\text{RuCl}(\text{cod})]$  (**Ru2**) or  $[\text{Ru}(\text{OAc})_2(\text{PPh}_3)_3]$  (**Ru19**). Importantly, the reaction proceeded with terminal alkynes as well as with 1,2-disubstituted ones.<sup>[91,92]</sup> Overall, the Ru catalyzed AAC proved to be just slightly slower than the CuAAC, but it could give access to both 1,4- and 1,5-triazole regioisomers. Unfortunately, the reaction usually yields mixtures of both isomers and, moreover, the process was described to require oxygen-free conditions (Scheme 24).<sup>[93,94]</sup>

Using as reference an Ir-catalyzed cycloaddition between thioalkynes and azides which included a single example using water as reaction media,<sup>[95]</sup> our group investigated the behavior of thioalkynes in RuAAC processes. This allowed to discover a highly efficient cycloaddition reaction with a variety of aryl or alkyl azides, using  $[\text{Cp}^*\text{RuCl}(\text{cod})]$  (**Ru2**) as catalyst, in aqueous mixtures and even in water (Scheme 25).<sup>[96]</sup>

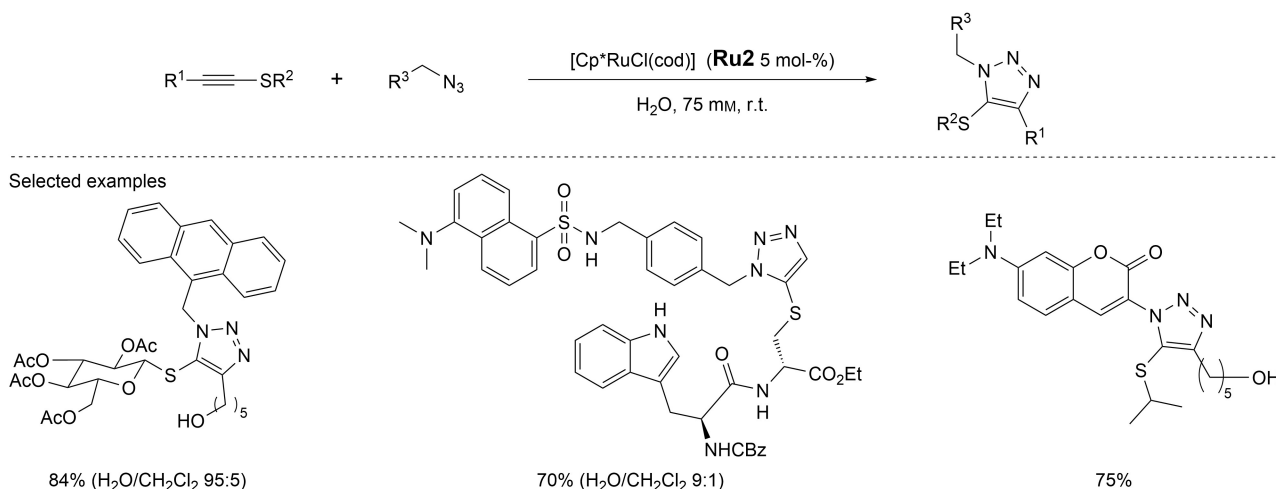
The reaction presented a broad scope with respect of the thioalkyne, and always led to the 1,5-triazole regioisomer, therefore being complementary to the original CuAAC. More importantly, the method turned out to be highly bioorthogonal, as it could be carried out in PBS, cell culture media, cell lysates or even in presence of living bacteria. Interestingly, the reaction can be combined with the CuAAC, so that both cycloadditions could be coupled in a sequential one-pot process.

Despite these promising bioorthogonality features, to obtain good yields of the products this RuAAC had to be performed at the millimolar range (from 10–100 mM, typically at 75 mM), which obviously hampers its applications in biology, wherein highly diluted conditions (typically at the micromolar range) are required.

Curiously, while trying to develop more effective ruthenium catalysts which could allow to overcome these limitations, we found out that cationic Ru complexes, which had previously failed to promote azide–alkyne cycloadditions in organic solvents,<sup>[92]</sup> could be used with thioalkyne substrates, under aqueous conditions. In particular, the complex  $[\text{Cp}^*\text{Ru}(\text{MeCN})_3]\text{PF}_6$  (**Ru18**) allowed for very efficient azide–thioalkyne cycloadditions under aqueous conditions at room temperature.<sup>[97]</sup> More importantly, this catalyst proved to be quite efficient under high dilution conditions, so that good yields and excellent regioselectivities of the desired triazoles could be obtained at low micromolar concentrations. Compared to **Ru2**, cationic complex **Ru18** also exhibits improved chemoselectivity between thioalkynes and alkynes and,



**Scheme 24.** Overall comparison of the CuAAC and the RuAAC.



**Scheme 25.** Ruthenium catalyzed azide thioalkyne cycloaddition under aqueous conditions.<sup>[96]</sup>

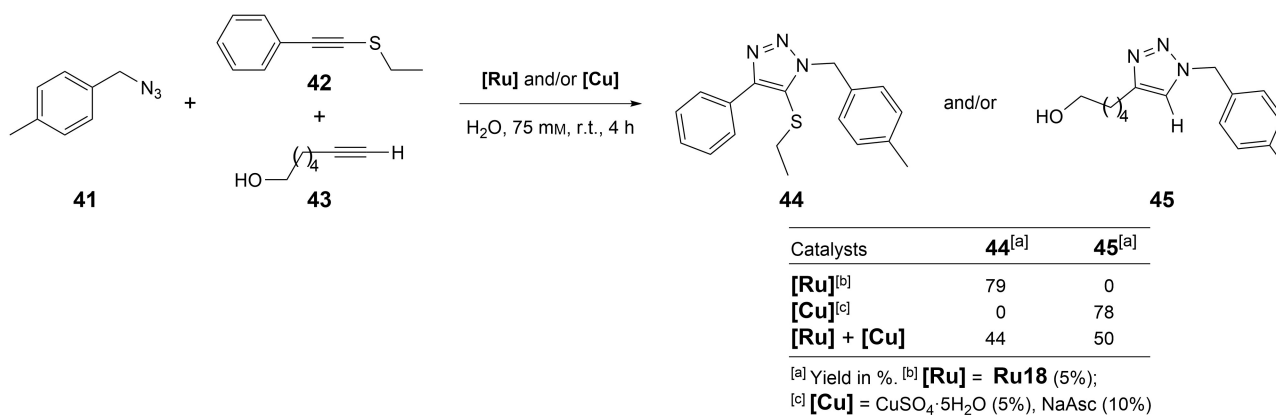
therefore, the CuAAC and RuAtAC reactions can now work in a perfect, mutually orthogonal fashion (Scheme 26).

Considering that the [Cp\*Ru(MeCN)<sub>3</sub>]PF<sub>6</sub> (**Ru18**) can be synthesized by arene displacement of closely related [Cp\*Ru(arene)]PF<sub>6</sub> sandwich complexes, we investigated the viability of using these complexes as precatalysts. We found that irradiating the otherwise catalytically inert complex, [Cp\*Ru(pyrene)]PF<sub>6</sub> (**Ru20**) with light (365 nm), leads to a catalytically active complex, which is able to promote the RuAtAC between azide **46** and thioalkyne **42** with very high efficiency, even at high dilution (100 μm of **46**, 200 μm of **42**). Moreover, the reaction is not only efficient in water, as it can also be carried out in highly complex biological media such as cell culture media or cell lysates (Scheme 27).

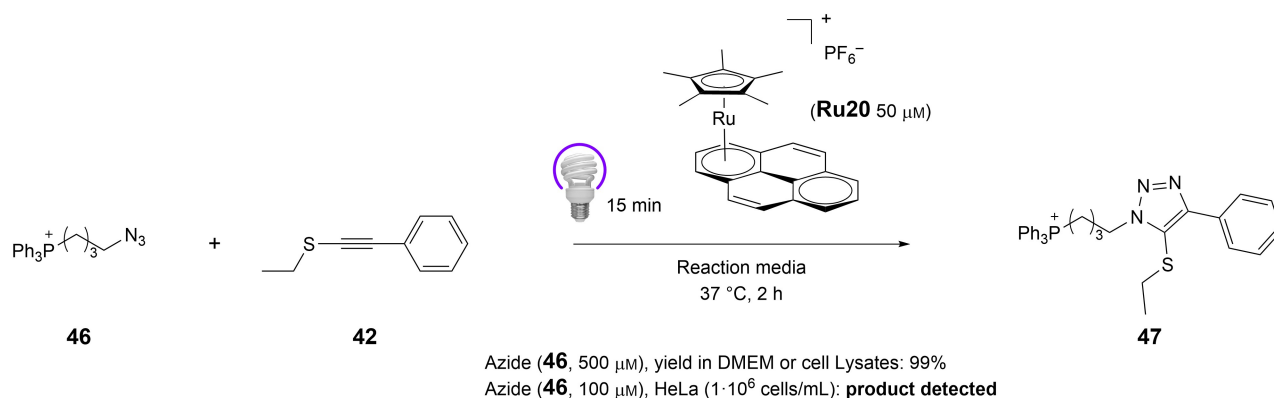
With these new cationic complexes, we were able to modify peptides and oligonucleotides using the RuAtAC, and even carry out the reaction in the presence of HeLa cells (in suspension), although the attempts to translate it to the interior of living cells failed.

#### 4.5. [2 + 2 + 2] Cycloadditions

A key element in the bioorthogonal success of the Cu or Ru-catalyzed azide-alkyne cycloadditions is the ready coordination of metal complex to the azide nitrogen and to the alkyne. Performing other type of metal-catalyzed bioorthogonal cycloadditions doesn't seem easy unless one could somewhat favor a pre-coordination of the metal to a desired partner in the presence of other biological components. This might



**Scheme 26.** Orthogonality for the RuAtAC and the CuAAC.

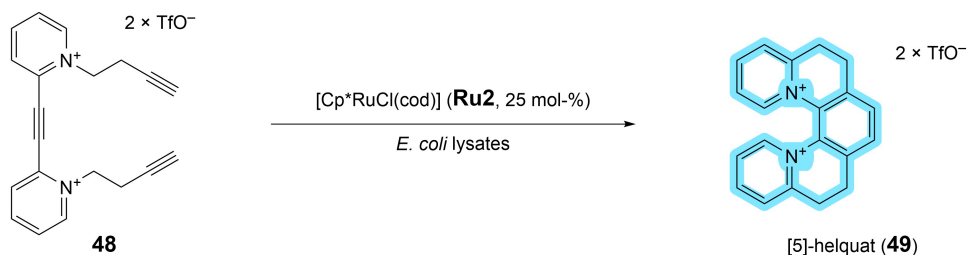


**Scheme 27.** Photoactivatable ruthenium complexes for azide thioalkyne cycloadditions in water, biorelevant media, under high dilution conditions.<sup>[97]</sup>

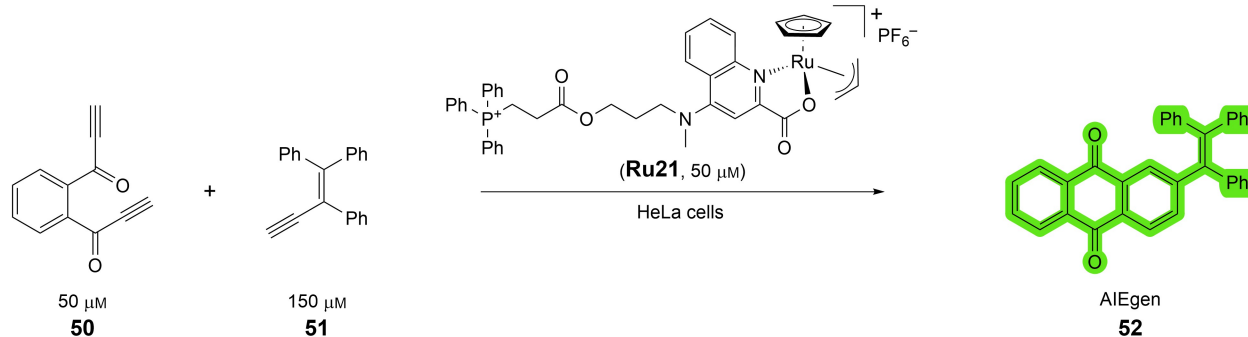
be achieved by using bidentate type of coordination units, that could facilitate the trapping of the metal. This strategy was preliminary explored by *Teplý* and coworkers in the development of a formal ruthenium catalyzed [2+2+2] cycloaddition of the triyne **48** to afford the aromatic polycycle **49**. The reaction, promoted by the complex **Ru2**, was found to proceed under aqueous conditions and even in presence of bacteria lysates (*E. coli*) as reaction media (Scheme 28,a).<sup>[87]</sup>

Our group has recently demonstrated that it is possible to perform this type of annulations in a more challenge intermolecular scenario. In particular, ruthenium complexes such as **Ru21** can promote the formal [2+2+2] cycloaddition of a diyne **50** and an alkyne **51**, in aqueous mixtures and also in cell lysates. More importantly, the annulation can even be performed in live mammalian cells, as demonstrated by the detection of the fluorescent anthraquinone product **51** by microscopy. This product is an aggregation induced

a) Ruthenium catalyzed [2+2+2] cycloaddition to form [5]-helquat in *E. coli* lysates



b) Ruthenium catalyzed [2+2+2] cycloaddition to form AiEgen anthraquinones in HeLa cells



**Scheme 28.** a) Ruthenium catalyzed intramolecular [2+2+2] cycloaddition to form [5]-helquat in *E. coli* lysates. b) Ruthenium catalyzed [2+2+2] intermolecular cycloaddition in HeLa cells to form an AiEgen anthraquinone.<sup>[87,98]</sup>

emission dye (AiEgen), that, by itself, cannot be internalized into cells because of its intrinsic tendency to aggregate; however, it can be efficiently produced inside cells using the abovementioned ruthenium catalyzed cycloaddition (Scheme 28,b).<sup>[98]</sup>

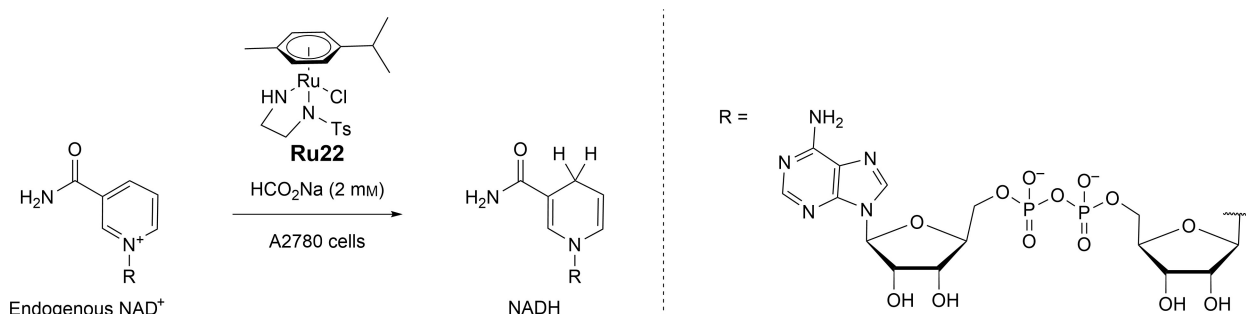
## 5. Reactions Involving Oxidation or Reduction Processes

Ruthenium catalysts have been extensively used in organic chemistry for functional group transformations involving oxidation or reduction processes.<sup>[54,99,100]</sup> Exploring whether some of these transformations could be translated to biological habitats is an important challenge that has been approached by several groups, especially by Sadler and coworkers.

For instance, these authors reported in 2012 the reduction of NAD<sup>+</sup> in living cancer cells using sodium

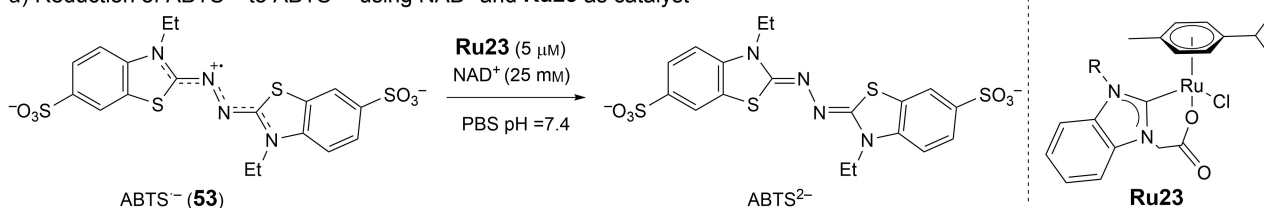
formate as hydride source and a ruthenium(II) sulfonamide ethylenediamine complex **Ru22** as catalyst. The method allowed to control the level of NAD<sup>+</sup> in living cancer cells (A2780) and, therefore, the reductive stress of the cell (Scheme 29).<sup>[101,102]</sup> In another case, the same group reported the use of a chiral catalyst that allows to reduce pyruvate with sodium formate as hydride source to D- or L-lactate, depending on the chirality of the metal center. However, in this case an osmium analog was the one chosen for cellular experiments, due to its better performance in the reduction process.<sup>[45]</sup>

A piano stool arene ruthenium complex, **Ru23**, has been applied by Tennyson and coworkers to reduce a radical monoanion 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonate) (ABTS, **53**) using oxidized nicotinamide adenine dinucleotide (NAD<sup>+</sup>) as reducing agent (Scheme 30). Although NAD<sup>+</sup> is usually employed as oxidant, in this particular case the authors showed

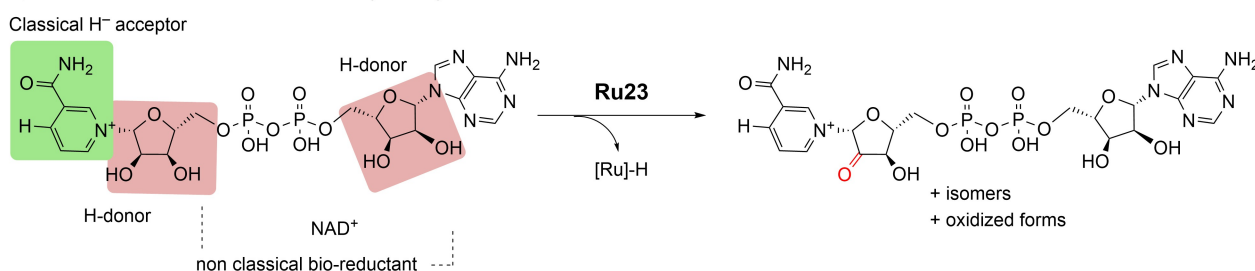


**Scheme 29.** Ru22-promoted reduction of endogenous NAD<sup>+</sup> by formate oxidation.<sup>[101]</sup>

a) Reduction of ABTS<sup>•+</sup> to ABTS<sup>2-</sup> using NAD<sup>+</sup> and **Ru23** as catalyst



b) Structure of NAD<sup>+</sup> and **Ru23** catalyzed hydride abstraction



**Scheme 30.** Ruthenium promoted non-conventional oxidation of NAD<sup>+</sup> and ABTS reduction under aqueous conditions.<sup>[103]</sup>

that the complex **Ru23** was able to promote the oxidation of the hydroxy groups of the ribose backbone of NAD<sup>+</sup> by hydride abstraction, generating a Ru hydride species capable of promoting the reductions processes under aqueous conditions.<sup>[103]</sup>

Other metal-mediated intracellular reductions mediated by iridium or rhodium catalysts have been developed,<sup>[104]</sup> but in general ruthenium complexes tend to be superior in terms of versatility and biocompatibility.

In 2019, our group reported a redox-neutral ruthenium catalyzed isomerization of allylic alcohols to ethyl ketones, a reaction that involves the generation of ruthenium hydride intermediates. The process is efficiently promoted by the ruthenium(IV) bis-allyl complex **Ru24**, in aqueous buffers,<sup>[105]</sup> it is highly bioorthogonal, compatible with many biological milieus and, importantly, can also be performed in live mammalian cells (HeLa, Vero and A549 cells, *Scheme 31,a*). Moreover, ESI-MS experiments not only demonstrated the presence of the product inside the cells, but also allowed to calculate the turnover number (TON) for the intracellular isomerization (TON = 23 for **Ru24** at 10  $\mu$ M), confirming the catalytic nature of the process. Also, we showed that the *in situ* isomerization of bis-allylic alcohols allows to generate  $\alpha,\beta$ -unsaturated ketones that can act as *Michael*

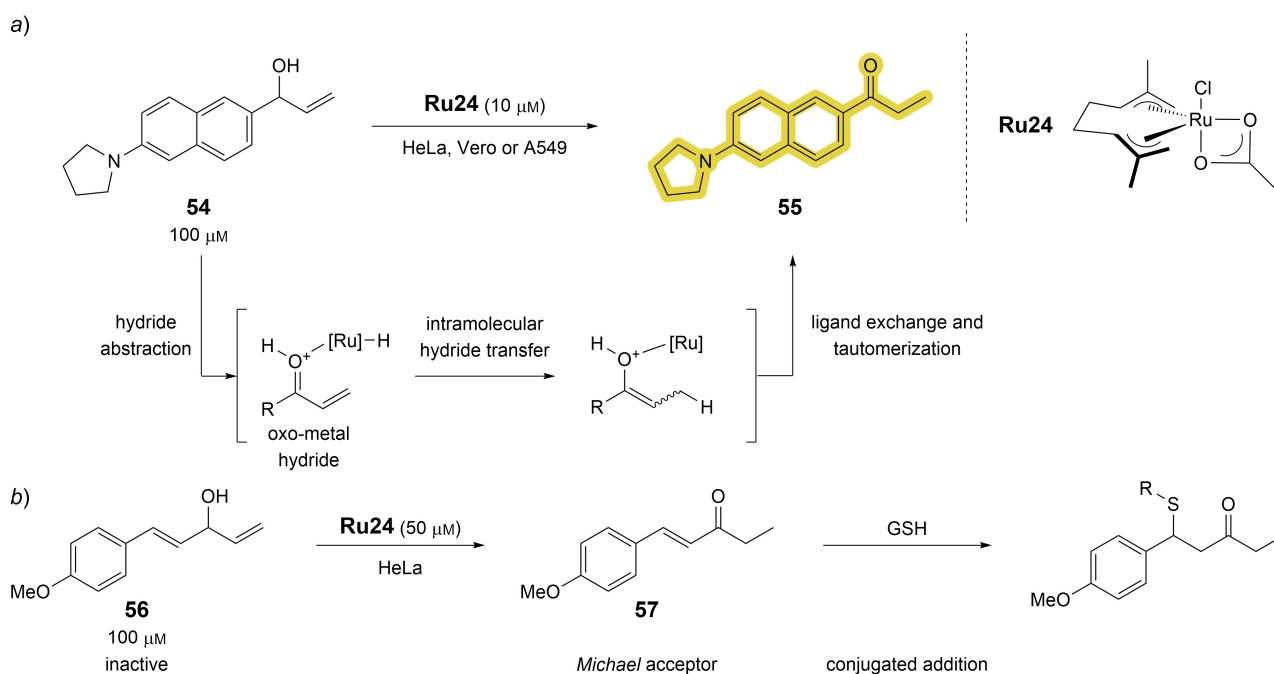
acceptors depleting the GSH reserves of the cell (*Scheme 31,b*).

## 6. Bioorthogonal Photocatalysis

Over the last decade, photoredox catalysis has emerged as a very powerful tool in organic synthesis. Photocatalysis, apart from being environmentally friendly and sustainable, allows to form challenging bonds and enable novel transformations under soft conditions. Its rapid and broad adoption in industry for the synthesis of fine chemicals and drug candidates clearly illustrates its synthetic power and potential.<sup>[106]</sup>

From a chemical biology perspective, the use of light is an attractive feature, as many photosensitizers absorb light at longer wavelength than many biological polymers (proteins, DNA, polysaccharides...). This might allow for both a spatial and temporal control of the reactivity, due to the discretional nature of the irradiation.<sup>[107]</sup>

Ruthenium complexes like [Ru(bpy)<sub>3</sub>]Cl<sub>2</sub> (**Ru25**) are amongst the most widely used photocatalysts for synthetic applications. They are usually irradiated with visible light, which promotes MLCT processes, so that the resulting excited species can trigger SET or energy transfer processes with appropriate substrates. The



**Scheme 31.** a) Ruthenium catalyzed isomerization of allylic alcohols in living HeLa, Vero or A549 cells. b) Ruthenium catalyzed synthesis of GSH depleting agent **57** inside HeLa cells.<sup>[105]</sup>

biological applications of these photocatalysts have been mainly focused on the field of photodynamic therapy (PDT) in which reactive oxygen species are generated;<sup>[57,108,109]</sup> however applications in the field of bioorthogonal chemistry remain in its infancy.

Nonetheless, some recent biorelevant applications have been reported. Gaunt and coworkers, for instance, developed a hypervalent iodine compound (**58**), capable of selectively targeting methionine residues in native proteins, to yield a highly functionalized  $\alpha$ -sulphonium diazo-ester.<sup>[110]</sup> These proteins, equipped with the diazo compounds, were elegantly modified by photocatalytic reactions promoted by **Ru25**, using alkyl derivatives of the Hantzsch ester (e.g., **60**) and irradiation with a blue light (40 W Kessil lamp). The  $\alpha$ -keto carbenes generated upon irradiation (**59**), in a mixture of water and acetonitrile, react with the alkylating agent **60** to provide the protein derivative **61** (88 % conversion, Scheme 32).

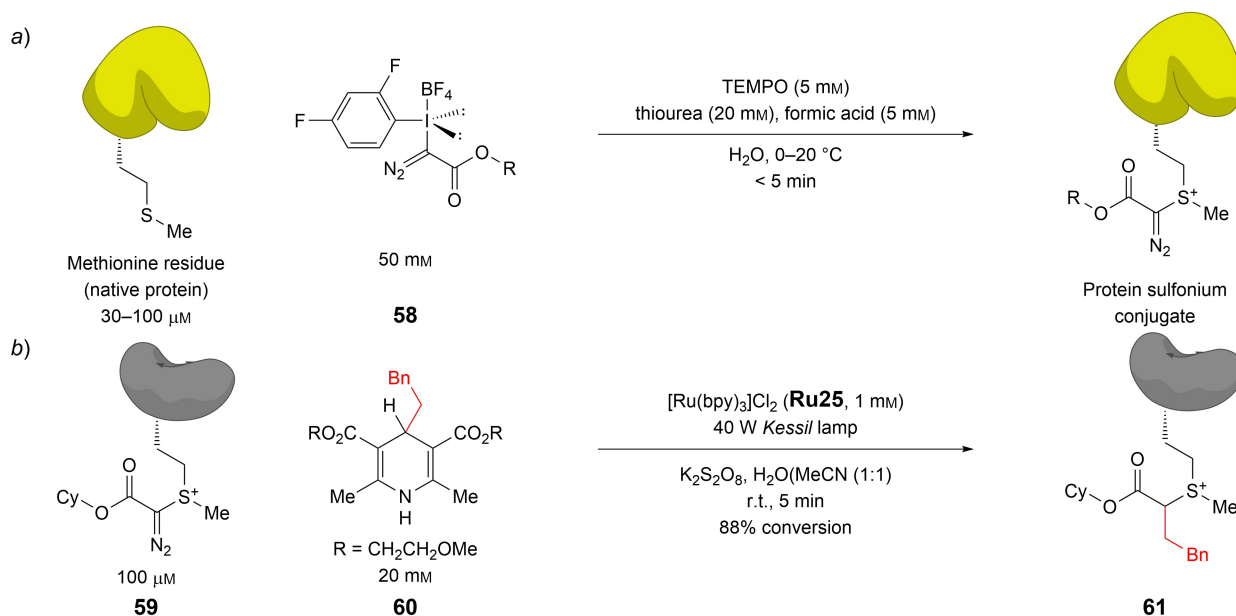
The main area in which the ruthenium photocatalysis has played a key role in bioorthogonal ligations involves the modification of tyrosine-containing proteins (aqueous buffer).<sup>[111]</sup> The first applications can be traced back to the late 90's when Kodadek reported the use of **Ru25** for the photocatalytic cross-linking of proteins through tyrosine moieties.<sup>[112]</sup> The photochemically generated C-centered-tyrosine radical reacts with a radical acceptor in close proximity, usually another tyrosine or a cysteine, to form a bond

which crosslinks these proteins (Scheme 33). This process allows to elucidate the structure of multi-system proteins due to the crosslinking between their units.

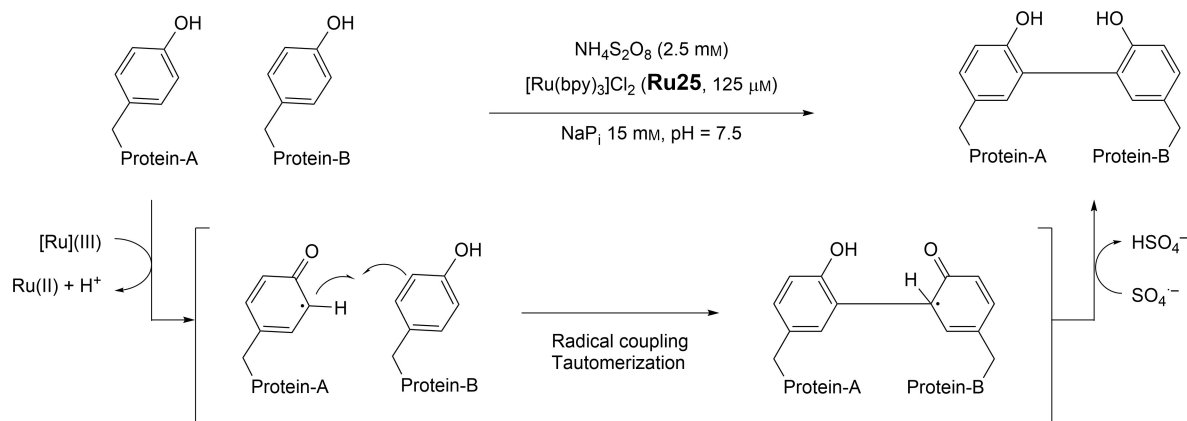
A similar approach has been used for proximity labeling reactions. Nakamura reported several [Ru(bpy)<sub>3</sub>]<sup>+</sup> derivatives bearing protein-ligands for photo-labeling of a host protein (Scheme 34). In this case the tyrosine radical traps a 4-(dimethylamino) benzamide derivative, which bears a dye or a biotin molecule to enable the determination of the reaction efficiency by fluorescence or by streptavidin affinity assays.<sup>[113–116]</sup>

Winssinger and coworkers reported the use of streptavidin protein dimer as template for the photo-reduction of aryl azide caged fluorophores.<sup>[117]</sup> The streptavidin allows for the binding of a biotin containing polypyridyl ruthenium photocatalyst and a unit of the aryl azide caged probe. Upon binding of the fragments and irradiation at 455 nm, energy transfer between the photocatalyst and the aryl azide promotes N<sub>2</sub> extrusion, followed by the self-immolative cleavage of the fluorophore (Rh = Rhodamine, Scheme 35). The protein dimer serves as a template to bring closer the photocatalyst and the substrate, allowing the reaction to proceed 30 times faster than under free conditions.

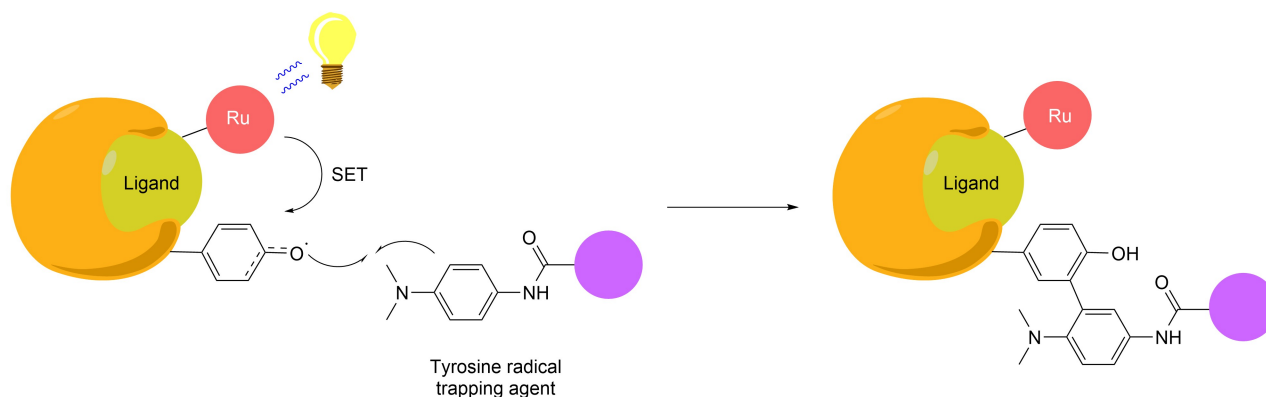
A similar strategy but using microRNAs templates has also been used by Winssinger and coworkers working even in live vertebrate (zebrafish



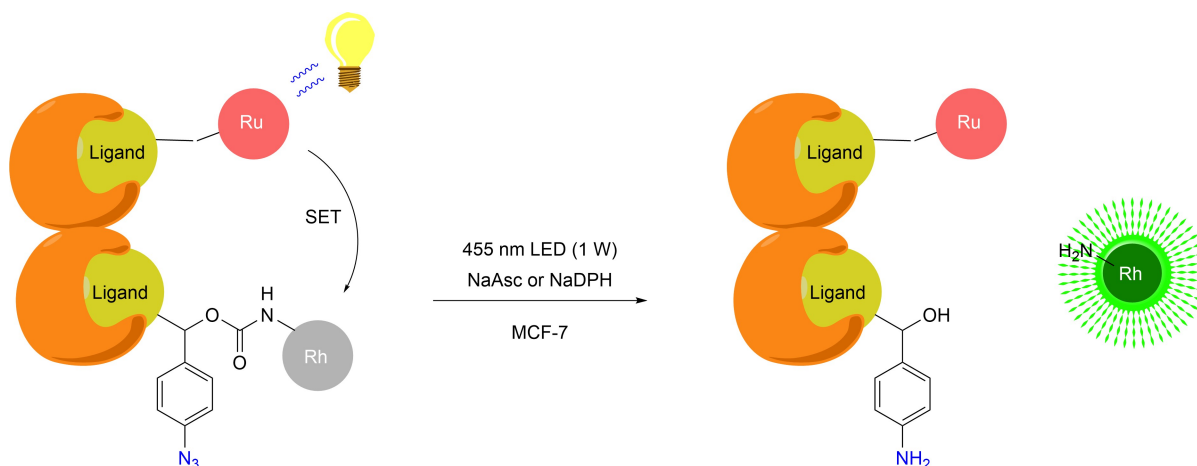
**Scheme 32.** a) Modification of methionine residues in native proteins by the hypervalent iodine compound **58**. b) Ruthenium promoted photofunctionalization of protein sulfonium conjugates.



**Scheme 33.** Ruthenium promoted tyrosine dimerization and crosslinking of proteins.<sup>[112]</sup>



**Scheme 34.** Ruthenium promoted tyrosine labeling of proteins.<sup>[113]</sup>



**Scheme 35.** Protein templated photoreduction of aryl azides.

embryos).<sup>[118]</sup> The authors also reported similar photo-reductions using pyridinium linkers.<sup>[119–121]</sup>

One of the latest examples in the use a ruthenium photocatalyst for a biological application was also reported by *Winssinger* and consisted of a *trans* to *cis*



**Scheme 36.** Ruthenium catalyzed *trans*-to-*cis* isomerization of *trans*-Res-3 M.

isomerization of double bonds.<sup>[122]</sup> Upon excitation of the ruthenium photocatalyst and intersystem crossing to the triplet state, there is an energy transfer to the acceptor. The excited acceptor now can isomerize to the less thermodynamically favor *cis*-compound. They studied the isomerization of different pharmacological agents also in cellular settings. This strategy allows for the *in-situ* activation of pharmaceutical agents like combretastatin A-4 and resveratrol analogs (Scheme 36).

## 7. Final Remarks

Ruthenium-based compounds have found widespread applications in chemical biology and biomedicine, likely because of the good balance between reactivity and stability offered by this type of metal complexes. The applications range from biomedical uses as stoichiometric agents to catalytic reactions in biological environments and even in live settings. After the cutting-edge contribution of Meggers in 2006, a great variety of bioorthogonal uncaging and bond-forming reactions promoted by ruthenium catalysts have been developed.

The robust performance of ruthenium catalysts in deallylation reactions warrants applications for the 'in vivo' activation of pro-drugs, even in a biomarker or cell-specific manner. Synthetic-like reactions that could allow the *in-situ* assembly of biologically active products are also highly attractive and will be further developed. In this context, there is still much room for translating new ruthenium catalyzed reactions to the bioorthogonal arena. This is for instance the case for photocatalytic processes, owing to the well-known use of ruthenium photocatalysts in biomedicine.

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## Data Availability Statement

The data that support the findings of this study are openly available in minerva at <https://minerva.usc.es/xmlui/?locale-attribute=gl>, reference number 0.

## Author Contributions

A. G.-G., F. L. and J. L. M. all contributed to writing and editing of the manuscript.

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