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Photochemistry

Photocatalytic Modification of Amino Acids, Peptides, and Proteins

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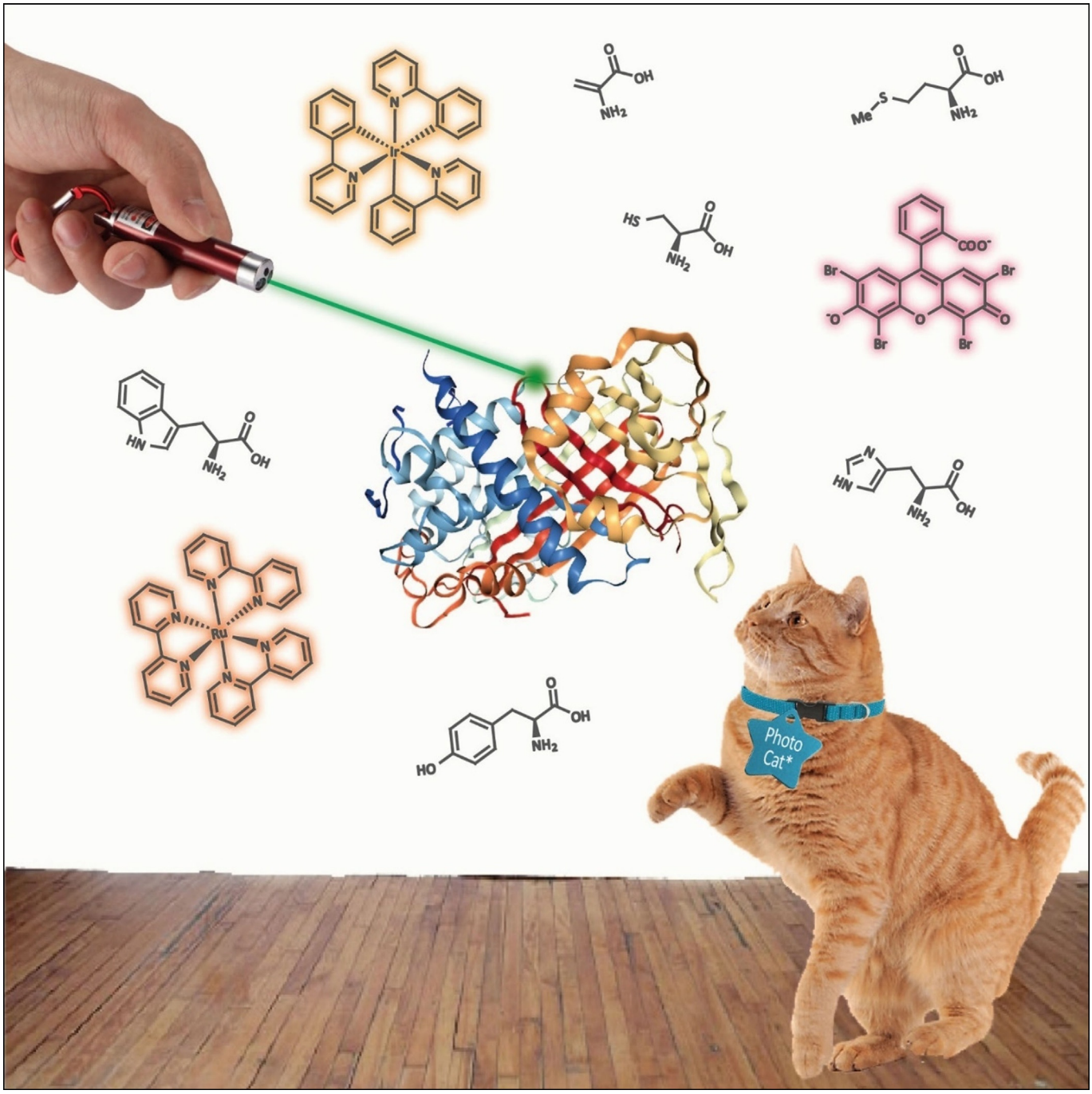

Abstract: In the last decade, visible-light photoredox catalysis has emerged as a powerful strategy to enable novel transformations in organic synthesis. Owing to mild reaction conditions (i.e., room temperature, use of visible light) and high functional-group tolerance, photoredox catalysis could represent an ideal strategy for chemoselective biomolecule modification. Indeed, a recent trend in photoredox catalysis is its application to the development of novel methodologies for amino acid modification. Herein, an up-to-date overview of photocatalytic methodologies for the modification of single amino acids, peptides, and proteins is provided. The advantages offered by photoredox catalysis and its suitability in the development of novel biocompatible methodologies are described. In addition, a brief consideration of the current limitations of photocatalytic approaches, as well as future challenges to be addressed, are discussed.

1. Introduction

Visible-light photoredox catalysis has received increasing attention in the last decade as a powerful strategy to enable unprecedented transformations in organic synthesis.[1] In photoredox catalysis, the ability of transition-metal-based or organic photocatalysts to harvest visible light and convert it into an electrochemical potential is exploited to activate organic substrates.[1a] Specifically, upon absorption, photocatalysts reach an excited state in which they are prone to engage in single electron transfers (SETs) with organic substrates acting as either electron donors or acceptors; thus de facto activating them and resulting in the formation of radical intermediates.[1d] Compared with other catalytic approaches, photoredox catalysis offers the advantage of enabling the activation of organic substrates under mild reaction conditions, while making use of visible-light irradiation as a sustainable source of energy. Moreover, because of the inability of the majority of organic substrates to absorb light in the visible spectrum, together with the fact that most organic molecules possess an activation barrier that cannot be overcome at room temperature, photoredox-based reactions typically exhibit high selectivities, with little or no side reactions observed.[2]

As a consequence of growing interest in peptides as drug candidates, and due to the undeniable importance of antibody–drug conjugates in current state-of-the-art therapeutics, the need for novel bioconjugation strategies is constantly on the rise.[3] In other words, selective chemical transformations aimed at the modification of native or non-native amino acids, as well as robust techniques that allow the incorporation of exogenous entities (e.g., drugs, tracers, or tools for immobilization) in peptides and/or proteins, are of fundamental importance in chemical biology.[4] However, traditional organic chemistry approaches are often inadequate solutions for bioconjugation because their biocompatibility is usually limited. Ideally, bioconjugation strategies should provide selective transformations that result in the formation of stable conjugates, while providing mild and biocompatible reaction conditions (i.e., room temperature, atmospheric pressure, physiological pH, aqueous buffered solutions as solvent).[5]

Despite many advances in the field of bioconjugation, innovative strategies to answer the remaining open challenges (e.g., modification of elusive amino acids, general strategies for regioselective modification of exposed residues in proteins) would greatly contribute to enlarging the toolbox of available methods for post-translational modification methods.[6]

Upon looking at the intrinsic advantages offered by visible-light photoredox catalysis, the reasons in favor of its application to the development of novel methodologies for bioconjugation become apparent. First, the use of visible light to drive chemical transformations is advantageous both in terms of sustainability (i.e., light is a green, traceless reagent) and in preserving the delicate nature of bioactive molecules (as opposed to UV irradiation, which is often disruptive towards the conformational integrity of proteins).[1c,7] Second, photoredox-based reactions can be conducted at room temperature and generally proceed smoothly in buffers or in aqueous mixtures; thus offering biocompatible reaction conditions.[8] Moreover, the reaction kinetics of photocatalytic transformations can be easily controlled, owing to their strong dependence on photon flux.[9] Consequently, the vast majority of photoredox reactions can be easily quenched by simply switching off the light. Such a straightforward on/off approach to control the reaction progression is an attractive feature for bioconjugation methods because it allows the need for quenchers to be circumvented and can simplify subsequent purification methods. Keeping all of these inherent advantages in mind, the recent trend of applying photoredox catalysis to biomolecule modification comes as no surprise.

2. Photocatalytic Modification of a Residue at the Single Amino Acid Level in Peptides and Proteins

Herein, prominent examples of photocatalytic methodologies applied to the modification of single amino acids, peptides, or proteins are presented. For clarity, the selected examples are

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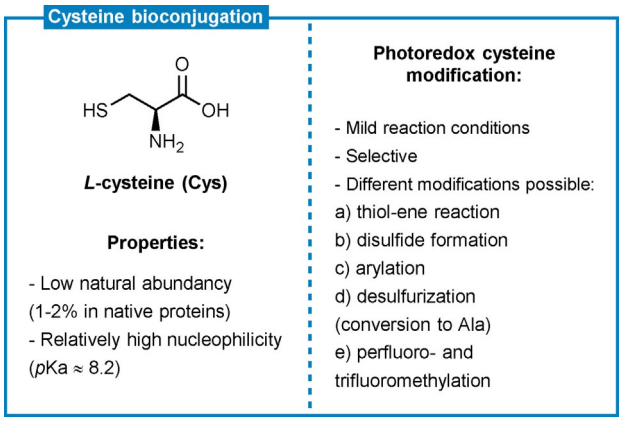
based on whether the presence of a photocatalyst (or a photosensitizer) and irradiation with near-UV or visible light is required for the reaction to proceed. As such, examples based on the use of photoaffinity labeling for protein modification or an in-depth discussion on photodynamic therapy (PDT) for therapeutic purposes fall beyond the scope of this review. For extensive coverage of these topics, we refer to numerous reviews already published.[10]

Among the 20 proteinogenic amino acids, only a few residues represent viable targets for the development of successful bioconjugation methods. Typically, bioconjugation methodologies rely on the intrinsic reactivity of different amino acids to achieve chemoselectivity (e.g., nucleo-/electrophilicity or acid–base behavior). Alternatively, the accessibility of one specific residue in proteins or peptides (i.e., position in the sequence or spatial orientation in the overall structure) can be exploited to achieve site selectivity in the presence of other reactive amino acids. A straightforward approach to classify methods for amino acid modification is to organize them based on the target residue. Thus, the following discussion illustrates pertinent reports on the photocatalytic modification of amino acids, starting from the most targeted residues (i.e., Cys and Tyr) and then moving on to less explored approaches.

It should be noted that the scope of this review is not only to provide an overview of prominent examples, in which photocatalytic methodologies have been successfully applied to the modification of single amino acids, peptides, or proteins, but also to illustrate the potential of such methodologies as powerful, yet compatible, alternatives to commonly used post-translational modifications.[11]



Scheme 1. Overview of the photoredox modification of cysteine.

2.1. Cysteine

Among endogenous amino acids, cysteine represents one of the most targeted residues for the post-translational modification of peptides and proteins.[12] The reason behind the popularity of biomodification strategies targeting cysteine can be found in its low natural abundance (≈1–2% in proteins), together with its relatively high nucleophilicity. The combination of these characteristics offer the possibility of enabling the site-selective introduction of relevant moieties (Scheme 1). Furthermore, cysteine displays high nucleophilicity, particularly in its deprotonated thiolate form ($pK_a \approx 8.2$, depending on the conformation, neighboring residues, and reaction mixture), which makes it an ideal target for rapid and efficient modification. Depending on the pH, excellent site selectivity for cysteine residues can be obtained, even in the presence of other nucleophilic sites, such as lysine and histidine. Despite these apparent advantages, cysteine modification has rarely been used in vivo due to the high concentration of free thiols within the cell (e.g., glutathione).

The thiol–ene click approach, that is, the reaction between a thiol and an alkene, is a very efficient transformation to establish a C–S linkage through reaction.[13] This reaction is typically initiated by a radical initiator or UV light. However, due to the incompatibility of peptides and proteins with high-energy UV light,[7c] several reports have appeared in recent years on the development of visible-light thiol–ene transformations by using photocatalysts, including $[Ru(bpy)_3]^{2+}$,[14] $[Ru(bpz)_3]^{2+}$,[15] and 9-mesityl-10-methylacridium$^+$ (bpy = 2,2′-bipyridine, bpz = 2,2′-bipyrazine).[16] These catalysts are able to absorb visible light to reach an excited state that is reductively quenched to generate a thiyl radical cation (Scheme 2). Upon deprotonation, a thiyl radical is formed, which can subsequently add to an olefin in an anti-Markovnikov fashion. The resulting alkyl radical can abstract a hydrogen atom from the remaining thiol substrate to generate the desired thiol–ene adduct and an equivalent of thiyl radical. The reduced photocatalyst is most likely reoxidized by molecular oxygen, which closes the photocatalytic cycle.[15]

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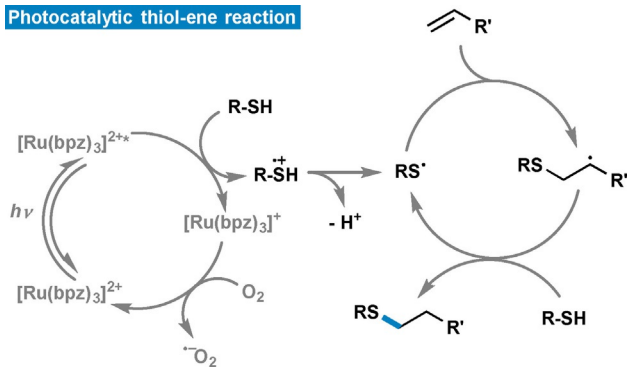
Timothy Noël currently holds a position as Associate Professor at the Eindhoven University of Technology. His research interests range from organic chemistry to chemical engineering and encompass more specifically flow chemistry, organic synthesis, and synthetic catalytic methodology development. His work has received several awards, including most recently the VIDI award (2015), the Thieme Chemistry Journal Award (2016), and the DECHEMA prize (2017).

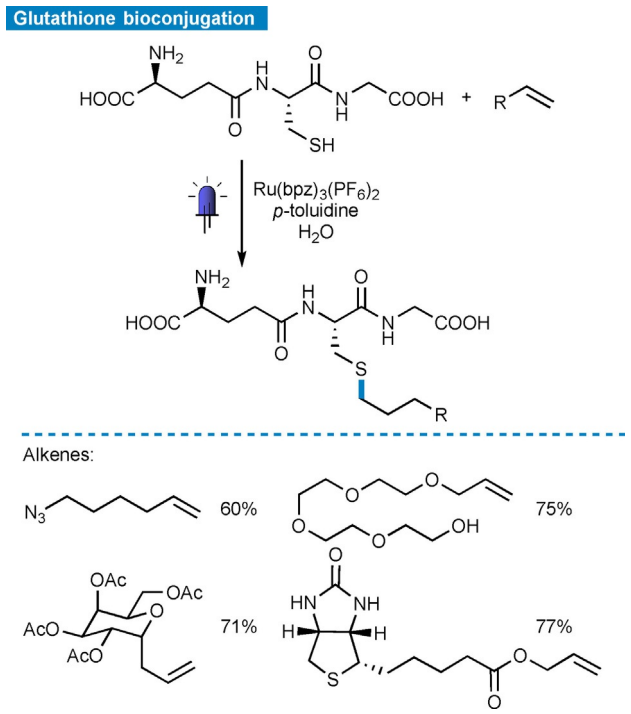


Scheme 2. Mechanism of the photocatalytic thiol–ene reaction.

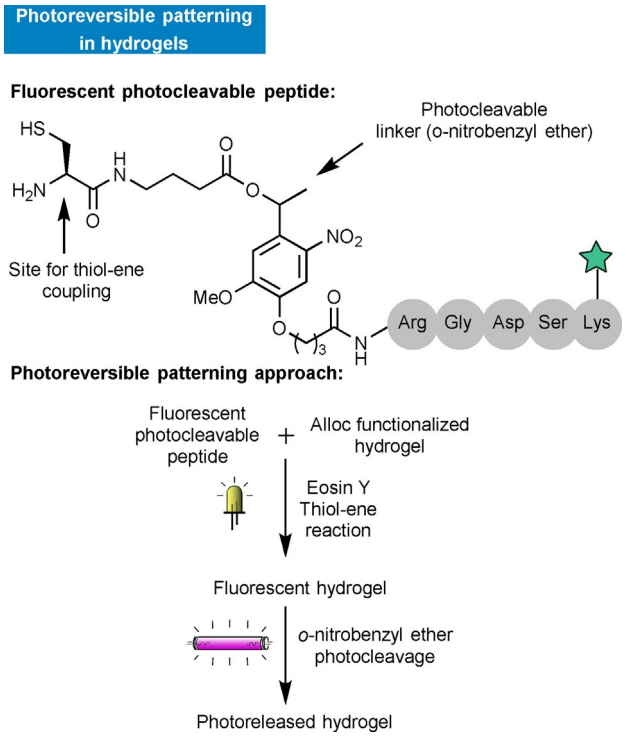
The biocompatibility of this transformation was demonstrated by Yoon et al.[17] They developed a visible-light photocatalytic protocol that allowed glutathione to be coupled with various olefins in aqueous media. $[Ru(bpz)_3]^{2+}$ was used as a photocatalyst and *p*-toluidine served as a redox mediator. The addition of *p*-toluidine allows the photo-oxidation of thiols to the corresponding thiyl radical to be catalyzed more efficiently. This proved crucial to enable the use of the thiol component as the limiting reagent. A variety of biologically relevant olefinic coupling partners, containing azides, polyethylene glycol (PEG) oligomers, protected sugars, and biotin, were efficiently hydrothiolated under these conditions (Scheme 3).

An intriguing example of a biocompatible thiol–ene photocatalytic transformation was developed by Anseth and DeForest.[18] They developed a hydrogel that could be reversibly patterned with a short peptide fragment by using biorthogonal, light-driven reactions (Scheme 4). The hydrogel was functionalized with allyloxycarbonyl (Alloc) moieties, which served as coupling partners for the thiol–ene coupling. The photoreversible patterning agent, Ac-C-(PL)RGDSK-(AF_{488})-NH_2 (AF_{488} = Alexa Fluor 488), based on the cell-adhesion ligand RGD (Arg-Gly-Asp), was synthesized to contain a photoreactive cysteine moiety and a photocleavable *o*-nitrobenzyl ether linker (PL). This peptide was subsequently coupled to the Alloc olefin through an Eosin Y mediated thiol–ene click reaction. Notably, the peptide loading (0–1 mM) on the hydrogel could be controlled by varying either the irradiation time (0–120 s) or the concentration of the organic dye Eosin Y (2.5–10 μM, λ_{max} = 515 nm). The peptide could be subsequently released by carrying out cleavage of the *o*-nitrobenzyl ether group through exposure of the patterned hydrogel to UV light (λ = 325–415 nm). Hence, the hydrogel can be reversibly patterned simply by changing the light source. In addition, spatial control is possible by selectively exposing certain regions of the hydrogel to light. The authors claimed that the wavelengths were cytocompatible; thus allowing this method to be used in the presence of living cells.

The formation of disulfide bonds between two cysteine residues is another modification that is mainstream in various proteins. Disulfides play an essential role in protein folding and in the structural integrity of proteins. Air oxidation is one of the most straightforward methods to establish cysteine oxidation for the construction of the corresponding S–S bond, but suffers from slow reaction kinetics and unwanted dimerization re-

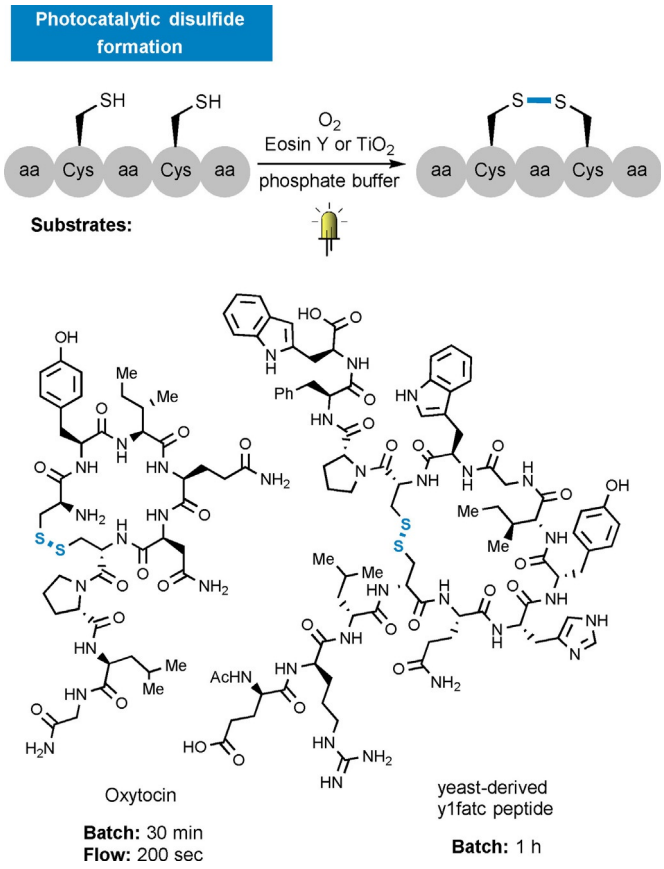


Scheme 3. Visible-light-induced photocatalytic coupling of glutathione with various olefins in aqueous media.



Scheme 4. Photoreversible patterning in hydrogels through Eosin Y catalyzed thiol–ene coupling and UV-induced photocleavage of a *o*-nitrobenzyl ether protecting group. Alloc = allyloxycarbonyl.

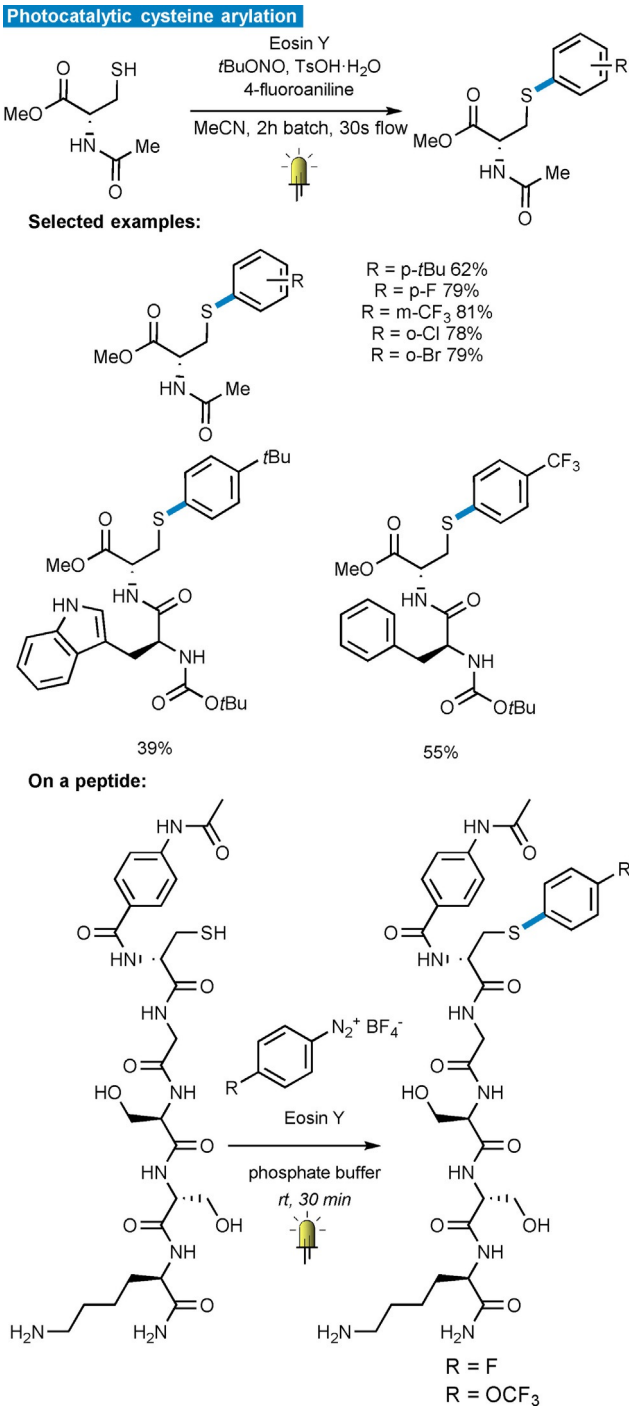
actions. To overcome these limitations, Noël et al. developed a photocatalytic variant that allowed the oxidation process to be significantly accelerated.[19] The reaction uses either Eosin Y or TiO_2 as the photocatalyst in the presence of a base (Scheme 5).



Scheme 5. Photocatalytic disulfide formation in peptides. The reaction can be catalyzed by Eosin Y or by a heterogeneous TiO_2 photocatalyst.

The reaction can be carried out in aqueous phosphate buffer (pH 7.4) to forge an intramolecular disulfide bond to yield cyclic peptides, such as oxytocin and the native form of a yeast-derived y1fatc peptide, within a reaction time of 0.5–1 h. Interestingly, the method can be carried out on fully deprotected peptides and is tolerant towards various sensitive amino acid residues, including histidine, tryptophan, tyrosine, arginine, and glutamine. Given its dependency on molecular oxygen, the reaction is sensitive to gas-to-liquid mass-transfer limitations, and thus, to reaction scale.[20] To overcome these limitations, the authors developed a continuous-flow protocol that enabled the Eosin Y catalyzed photocatalytic synthesis of oxytocin within a residence time of 200 s.

Noël and co-workers developed a photocatalytic arylation protocol based on the classical Stadler–Ziegler reaction.[21] The protocol involves the use of aryldiazonium salts (E_{red} as high as 0.5 V versus a standard calomel electrode (SCE)), which generate aryl radicals in the presence of Eosin Y as the photocatalyst (Scheme 6). These electrophilic aryl radicals can be easily trapped by cysteine, resulting in the formation of a covalent

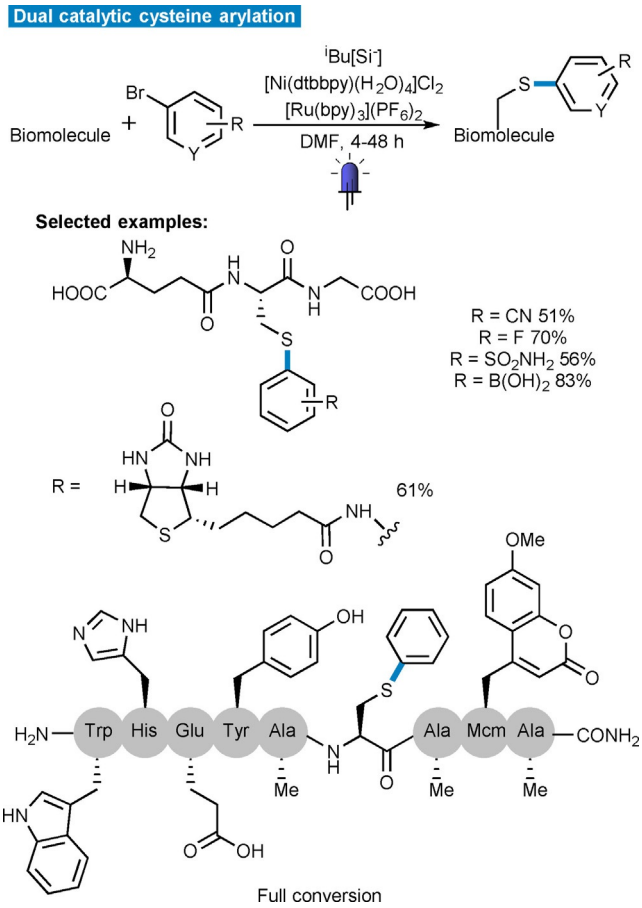


Scheme 6. Visible-light-induced cysteine arylation with benzene diazonium salts as aryl radical precursors. The reaction was demonstrated on a single amino acid, on dipeptides, and on a large peptide. TsOH = toluenesulfonic acid.

C–S bond. Various aryl amines with a wide variety of electron-deficient and -donating groups can be engaged in this transformation to generate the required diazonium salts in situ with *tert*-butyl nitrite and *p*TsOH. The reaction can be carried out in 2 h in batch, whereas the reaction is completed within 30 s in flow. More complex and fully deprotected peptide substrates could be arylated in a phosphate buffer solution within an irra-

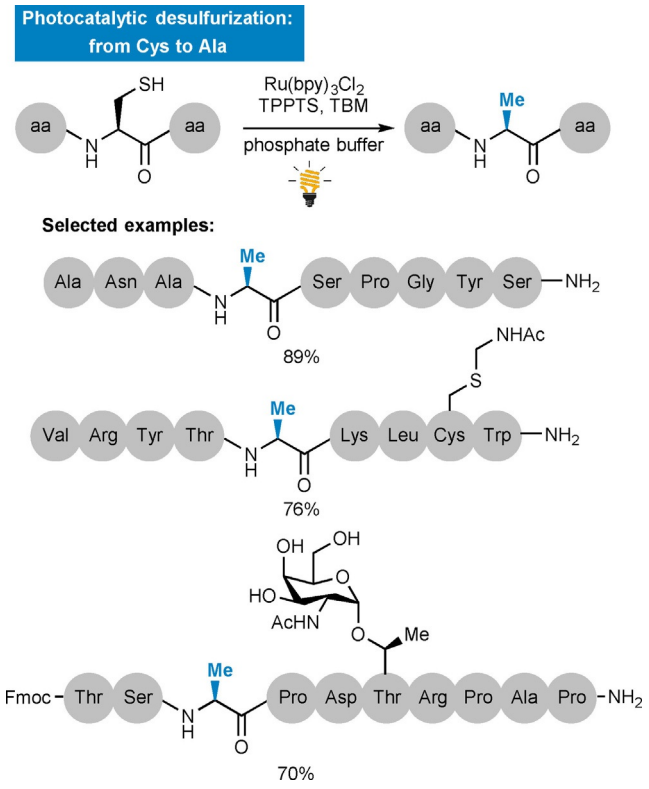
diation time of 30 min in batch by using preformed diazonium salts.

Another approach to cysteine arylation was developed by Molander et al. by using a dual nickel/photocatalytic cycle.[22] The reaction utilizes aryl bromides as coupling partners and proceeds in the presence of ammonium bis(catechol) silicate as a hydrogen atom transfer reagent, $[Ni(dtbbpy)(H_2O)_4]Cl_2$ (dtbbpy = 4,4′-di-*tert*-butyl-2,2′-dipyridyl) as the cross-coupling catalyst, and $[Ru(bpy)_3](PF_6)_2$ as the photocatalyst in DMF (Scheme 7). Glutathione was used as a benchmark example that showed excellent reactivity towards a diverse set of aryl bromides with various functional groups. Interestingly, the method was also applicable to a fully deprotected cysteine-containing 9-mer peptide containing other reactive residues, such as Trp, His, Glu, and Tyr, with aryl bromide (20 equiv).



Scheme 7. Dual catalytic approach towards cysteine arylation with a ruthenium photocatalyst and a nickel catalyst.

Guo et al. developed an efficient visible-light photocatalytic protocol to desulfurize cysteine moieties into alanine within peptides (Scheme 8).[23a] Notably, the reaction can be carried out in aqueous phosphate buffer solution. $[Ru(bpy)_3]^{2+}$ was used as a photocatalyst to generate cysteine radicals, which can react subsequently with a water-soluble phosphine (i.e., TPPTS). The corresponding phosphoranyl radical is converted through β scission into an alkyl radical, which abstracts a



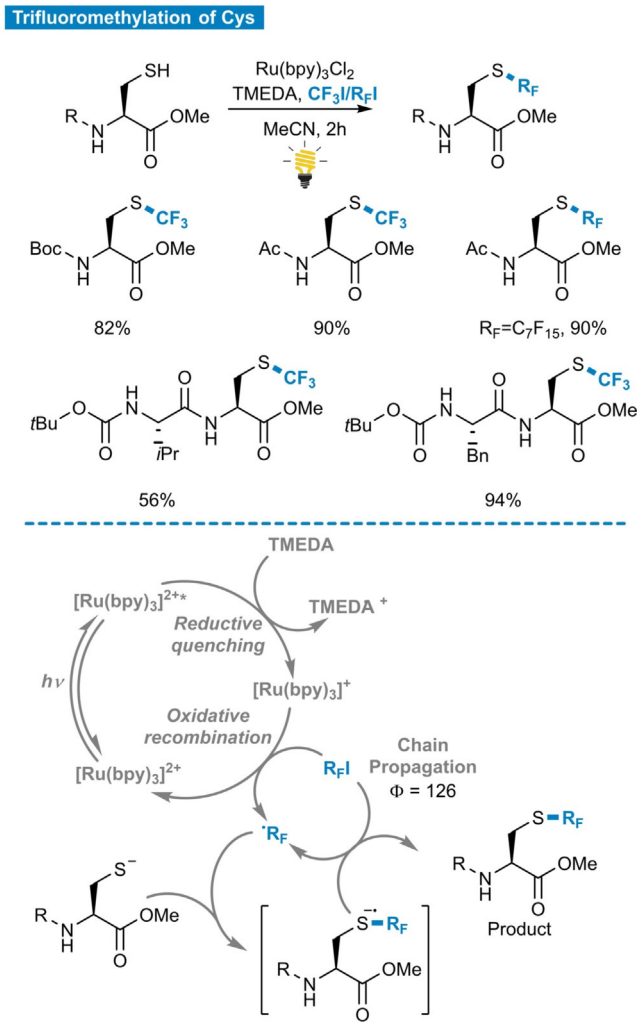
Scheme 8. Visible-light photocatalytic desulfurization of cysteine to alanine. TPPTS = 3,3′,3″-phosphinidynetris(benzenesulfonic acid)trisodium salt, TBM = *tert*-butyl mercaptan, Fmoc = fluorenylmethyloxycarbonyl.

proton from cysteine to generate the alanine residue. Notably, a broad variety of different, deprotected peptide sequences could be subjected to the reaction protocol to provide the target alanine-containing peptide in good to excellent yield.

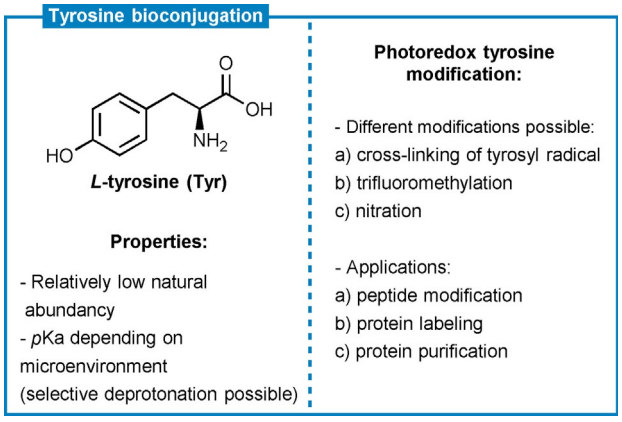
The incorporation of fluorinated moieties is of great interest to alter the metabolic stability, lipophilicity, and bioavailability of peptides.[24] Furthermore, complex biological interactions can be investigated by means of ^{19}F NMR spectroscopy, if fluorinated peptides are used.[25] Noël et al. developed a perfluoroalkylation strategy that could be applied to cysteine and some short peptide fragments (Scheme 9).[26] After excitation with blue light-emitting diodes (LEDs), $[Ru(bpy)_3^{2+}]^*$ can be reductively quenched by TMEDA to generate $[Ru(bpy)]_3^+$, which is subsequently oxidized to generate an electrophilic R_F radical. This R_F radical can react with cysteine to forge the S–R_F bond. Quantum yield experiments showed that this reaction followed a radical chain pathway. By using this method, various R_F groups can be introduced in good to excellent yields.

2.2. Tyrosine

Tyrosine is another interesting residue that enables site-selective modification because it is relatively rare on protein surfaces. Furthermore, the reactivity of individual tyrosyl residues can be tuned by selective deprotonation because the pK_a depends on the microenvironment around the residue (Scheme 10).



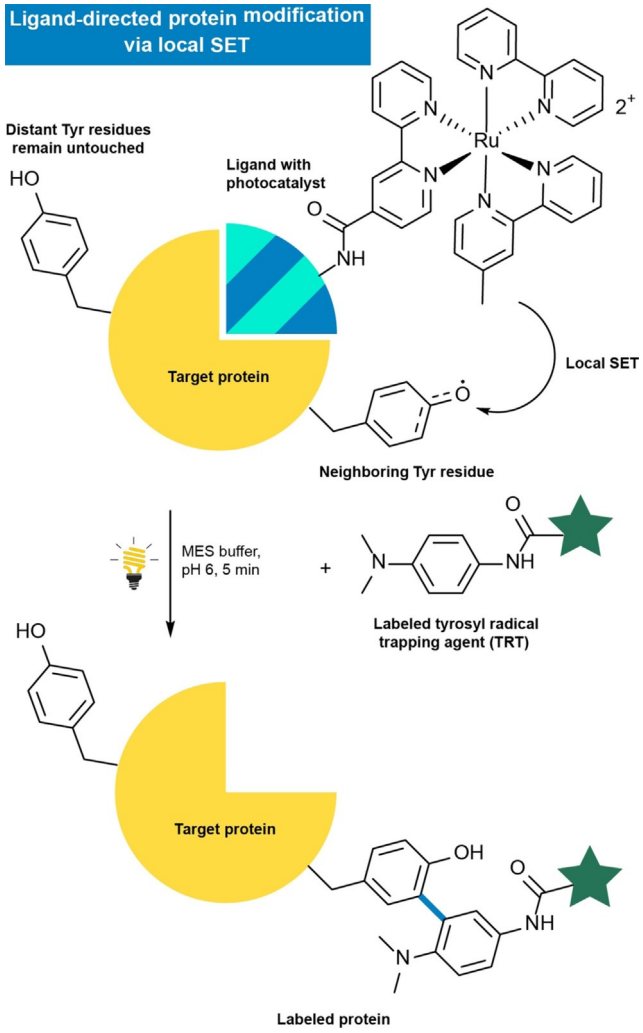
Scheme 9. Photocatalytic trifluoromethylation and perfluoroalkylation of cysteine and its proposed mechanism. TMEDA = tetramethylethylenediamine, Boc = *tert*-butyloxycarbonyl, Bn = benzyl.



Scheme 10. Overview of the photoredox modification of tyrosine.

One of the earliest reports on the photocatalytic modification of proteins was disclosed by Kodadek and co-workers.[27] They reported a powerful cross-linking strategy that targeted surface tyrosyl residues by using visible-light photocatalysis. The cross-linking reaction is initiated by the formation of a tyrosyl radical, which can subsequently couple with other nucleophilic sites, for example, another Tyr residue. Interestingly, the reaction is extremely fast and results in 60% yield after a light pulse of only 0.5 s.[28] It was observed that efficient cross-linking was obtained only for those proteins that possessed electron-rich aromatic residues in the proximity of the protein–protein interface.[29] Furthermore, the reaction was inhibited in the presence of a large excess of other free amino acids, such as tryptophan, tyrosine, and cysteine. Another potential limitation is that the reaction requires ammonium persulfate as an oxidative quencher, which is cell-impermeable and can oxidize biomolecules directly.

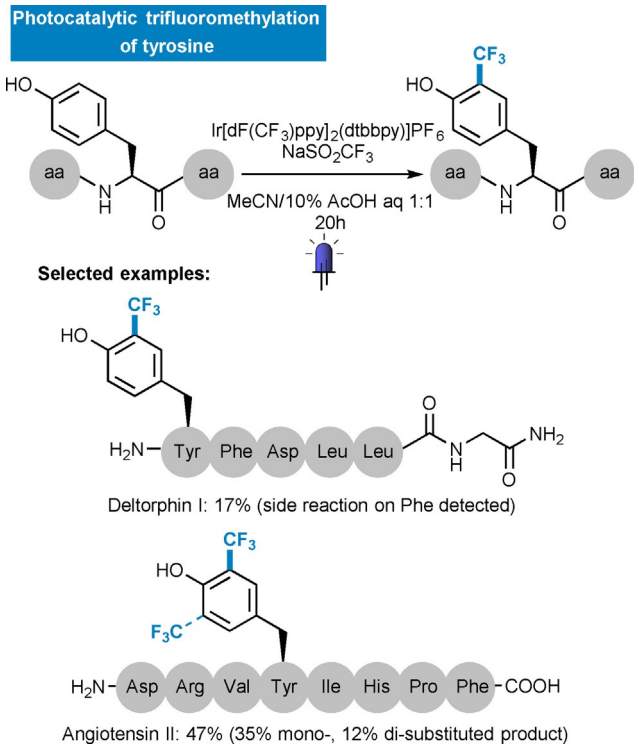
Nakamura et al. developed a broadly applicable photocatalytic method to enable protein modification by using $[Ru(bpy)_3]^{2+}$.[30] The photocatalyst generates tyrosyl radicals upon light irradiation, which subsequently react with various radical trapping reagents containing an *N*′-acyl-*N*,*N*-dimethyl-1,4-phenylenediamine (Scheme 11). The method was success-



Scheme 11. Ligand-directed tyrosine modification through a locally induced SET. MES = 2-(*N*-morpholino)ethanesulfonic acid.

fully applied to the modification of peptides (e.g., angiotensin II) and proteins (e.g., bovine serum albumin).[31] Notably, to demonstrate the potential of this method to enable selective protein modification through a local SET, the authors synthesized a benzenesulfonamide-conjugate ruthenium-based photocatalyst. This benzenesulfonamide moiety functions as a ligand for the protein carbonic anhydrase and, upon protein–ligand interaction, a significantly higher modification efficiency of nearby tyrosine residues was observed. This method was also applicable to selectively target native carbonic anhydrases in the cell lysate. This interesting finding was next used to allow for the selective purification and chemical labeling of target proteins from protein mixtures on the surface of affinity beads.[32] The authors also applied this strategy to achieve simultaneous protein labeling and knockdown through local singlet oxygen generation.[33] The residues that were most prone to singlet oxygen oxidation were histidine, methionine, and tryptophan, but cysteine and tyrosine were left untouched.

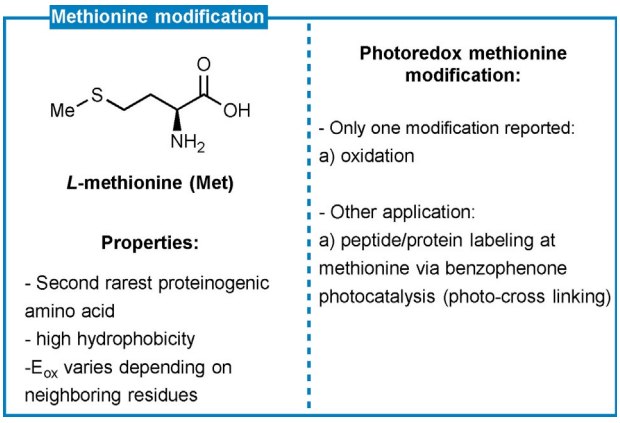
Researchers at Merck have developed a photocatalytic approach to the installation of trifluoromethyl moieties on tyrosine side chains.[34] The photocatalytic method utilizes $NaSO_2CF_3$ (20 equiv) and $[Ir\{dF(CF_3)ppy\}_2(dtbbpy)]PF_6$ (15 mol%; $dF(CF_3)ppy$ = 3,5-difluoro-2-[5-(trifluoromethyl)-2-pyridinyl-N]-phenyl-C) in a mixture of CH_3CN/10% aqueous AcOH, under irradiation for 20 h (Scheme 12). The method displayed good selectivity towards tyrosine in the presence of histidine and phenylalanine side chains. However, in the presence of tryptophan residues, a slightly higher selectivity was observed for tryptophan over tyrosine. The photocatalytic method displayed greater yields for the trifluoromethylated products than those of analogous non-photocatalytic radical trifluoromethylation reactions. A diverse set of biologically relevant peptides were modified by using this strategy, including deltorphin I, angiotensin I, angiotensin II, β-casomorphin, dermorphin, and even insulin.



Scheme 12. Photocatalytic trifluoromethylation of tyrosine.

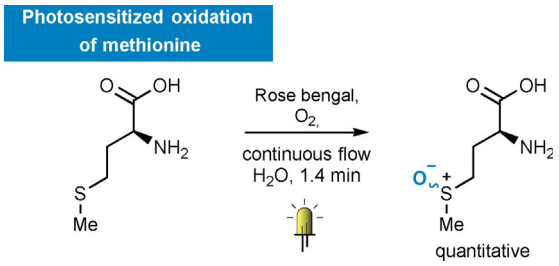
2.3. Methionine

Few examples of conjugation methodologies involving methionine can be found in the literature.[35] This is largely because, compared with the other sulfur-containing proteinogenic amino acid (i.e., Cys), methionine exhibits an intrinsically lower nucleophilicity and high hydrophobicity (Scheme 13).[3a,4a]



Scheme 13. Overview of the photoredox modification of tyrosine.

Thus, one of the biggest challenges in the development of bioconjugation methods for methionine is to achieve selectivity under pH-neutral conditions in the presence of other competing and more nucleophilic residues (i.e., Lys, Cys, Tyr, Ser). However, because all competing nucleophilic residues exist in a protonated form at low pH, selectivity towards methionine could be observed in methods employing acidic conditions.[3a,36] Nevertheless, no photocatalytic methodologies for methionine bioconjugation have been currently reported. On the other hand, photocatalytic methods for the chemical modification of the individual amino acid do exist, often exploiting the fact that this amino acid is prone to oxidation. In biological systems, the oxidation of methionine was found to be a relevant post-translational modification that controlled several signaling pathways at a cellular level.[37] Moreover, methionine sulfoxide, that is, the oxidized form of methionine, has been investigated for its role in the pathogenesis of neurodegenerative diseases and oxidative stress.[38] The photocatalytic oxidation of methionine was described by Monbaliu and co-workers (Scheme 14).[39] In this scalable continuous-flow protocol, the oxidation of methionine through the formation of singlet oxygen was obtained with Rose bengal as a photosensitizer. The biphasic reaction (i.e., liquid reagent and molecular oxygen) was performed in a glass, mesoscale, commercially available photoreactor and afforded quantitative yields within



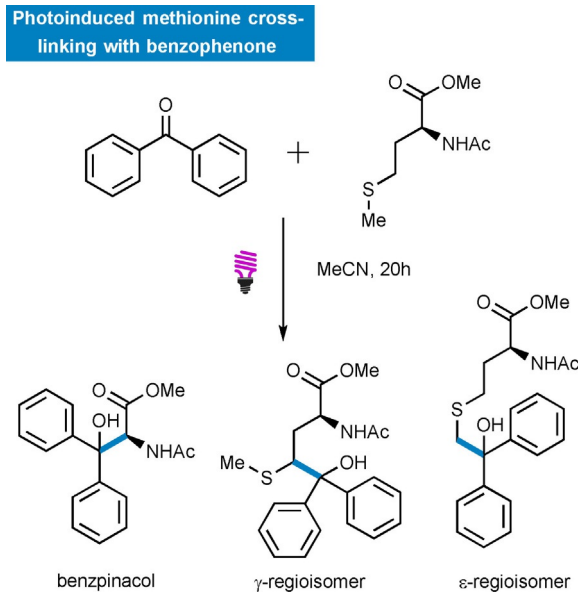
Scheme 14. Photosensitized oxidation of methionine in a continuous-flow reactor.

a reaction time of 1.4 min and a productivity of up to 132 g day^{-1}.

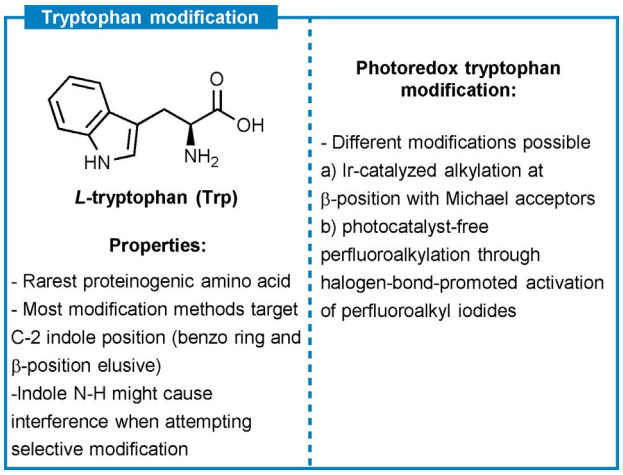
Interestingly, reports from the literature suggest that the oxidation potential of methionine is largely influenced by its neighboring residues (with an oxidation potential ranging from 1.2 to 1.8 V).[40] Thus, by selecting a photocatalyst with suitable redox potentials, it is conceivable to imagine the development of a photocatalytic strategy in which methionine oxidation could selectively occur in the presence of other nucleophilic residues.

Other light-induced selective modifications of methionine have been described in the field of benzophenone photochemistry.[41] Specifically, benzophenone-mediated photolabeling at methionine in peptides and proteins plays an important role in chemical biology.[42] Despite the fact that, in principle, all amino acids can be targeted for photolabeling with benzophenone, several studies in the literature confirm that methionine is often the preferred labeling site in proteins.[43] Ourisson and co-workers studied the chemo-, regio-, and stereoselectivities of different *N*-acetylamino acid esters towards the photochemical reaction with benzophenone.[44] As depicted in Scheme 15, cross-linking in the presence of Ac-Met-OMe yielded benzpinacol and the γ- and ε-regioisomers. Moreover, the "methionine preference" observed in proteins in the reaction with benzophenone was exploited for the development of the methionine proximity assay; a method used for the study of the interaction between peptide ligands and their receptors.[45]

Because such methods cannot be considered photocatalytic, but rather photochemical (i.e., benzophenone is not only the absorbing species, but is also a coupling partner in the reaction), they are not discussed in depth herein. For extensive coverage of the topic, we refer to recent reviews.[41,46]



Scheme 15. Different products observed in the photoinduced cross-linking of *N*-Ac-methionine with benzophenone.

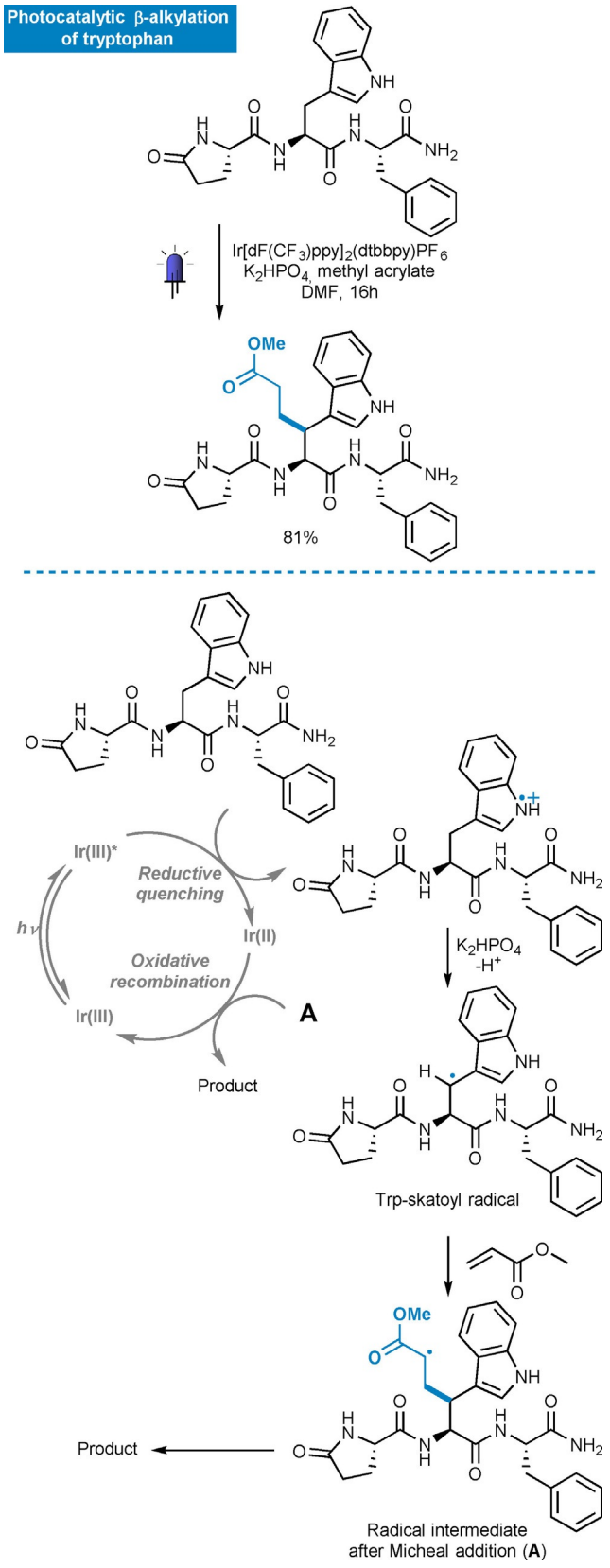


Scheme 16. Overview of the photoredox modification of tryptophan.

2.4. Tryptophan

With a calculated abundance of about 1.3 %, tryptophan is the rarest of all proteinogenic amino acids.[47] However, it was estimated that approximately 90 % of all proteins contained at least one Trp residue.[48] In other words, many proteins contain only one Trp in their sequence. Thus, methodologies affording chemoselective modification of Trp would result in site-selective bioconjugation on a vast number of endogenous substrates (Scheme 16). To date, the majority of methods developed for Trp conjugation showed that the C-2 position of indole was the most reactive, whereas other positions on the aromatic ring or β to the stereocenter proved harder to modify.[4a,49] Moreover, the free NH on the indole moiety of Trp is incompatible with some transition-metal-based methodologies; hence rendering the use of protecting groups necessary.[50] Thus, in this context, the development of novel photocatalytic methodologies selectively targeting Trp would represent a positive advancement in the field of photoredox catalysis.

In a recent example, Shi and co-workers reported a photocatalytic and chemoselective β-modification of tryptophan (Scheme 17).[51] Notably, the selective transformation of the β position was essentially unprecedented in the literature, with only one report illustrating a similar modification, albeit through an enzymatic approach. The authors postulated that the observed selectivity at the β position might derive from the formation of a stabilized Trp-skatolyl radical. Specifically, a

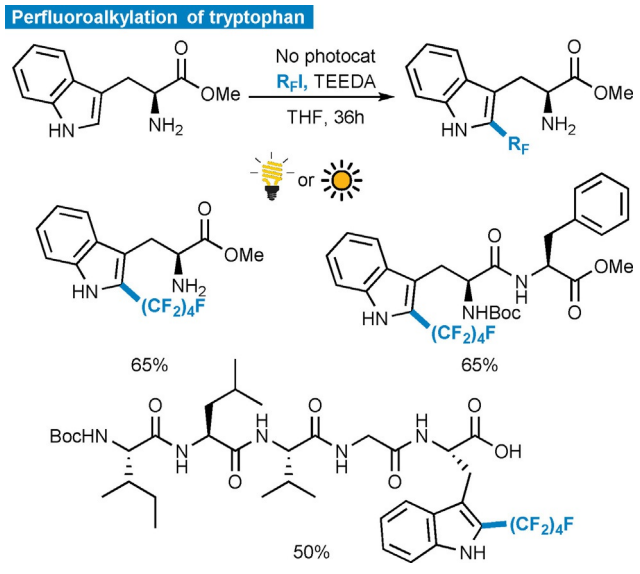


Scheme 17. Photocatalytic β-alkylation of tryptophan and its proposed mechanism.

first SET from the excited state of the photocatalyst (i.e., $[Ir\{dF(CF_3)ppy\}_2(dtbbpy)]PF_6$) to N in the indole moiety of Trp would result in the formation of a radical cation, in which the benzylic proton (i.e., the β proton) exhibited higher acidity. Thus, in the presence of K_2HPO_4, extraction of the β proton affords the stabilized Trp-skatolyl radical. Finally, this radical can undergo conjugation with any Michael acceptor (Scheme 17, intermediate **A**), ultimately affording the desired bioconjugation product after back electron transfer to the photocatalyst.

A broad range of Michael acceptors proved compatible with the transformation, and the selectivity and tolerance of the methodology towards other amino acids was demonstrated with a small array of synthetic and natural peptides (e.g., glucagon and GLP-1). Depending on the conformation and accessibility of other amino acids, competitive reactions with Lys and His residues were observed. Interestingly, if the reaction was performed on human insulin, which does not contain any Trp residues, decarboxylation of the C-terminal residue on the β chain was observed. This result differed from that reported by MacMillan and co-workers, who observed selective decarboxylation of the C terminus in the α chain of insulin under similar photoredox conditions (see Section 3).[52]

Another visible-light-induced modification of tryptophan was reported by Chen and co-workers.[53] In this case, the authors observed that perfluoroalkyl iodides could be activated in the presence of tertiary amines (i.e., *N*,*N*,*N*′,*N*′-tetraethylethylenediamine (TEEDA)) or with THF as a solvent to form complexes that absorbed in the near-UV range. Under these photocatalyst-free conditions, and under the irradiation of a compact fluorescence light or sunlight, the perfluoroalkylation of the C-2 indole position of tryptophan was demonstrated on the amino acid alone and on short peptides (Scheme 18).

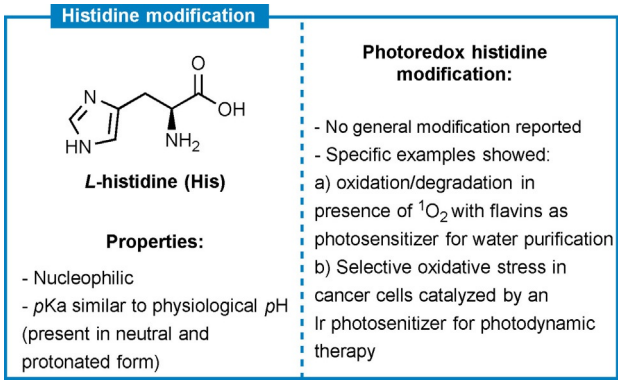


Scheme 18. Photoinduced perfluoroalkylation of tryptophan.

2.5. Histidine

Among proteinogenic amino acids, histidine is the only residue with a pK_a in the physiological range.[3a] Both its protonated

and neutral forms are present at physiological pH; thus rendering this amino acid a major player in all enzymatic and protein interactions requiring the abstraction or donation of protons (Scheme 19).[54] Moreover, due to the high affinity of His for metal ions, this residue is often involved in the coordination of metal cofactors in proteins.[55]



Scheme 19. Overview of the photoredox modification of tyrosine.

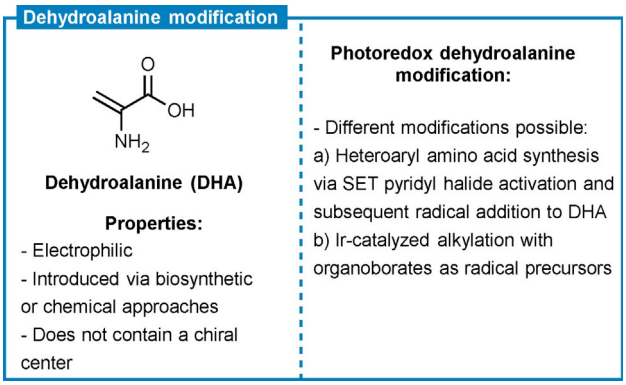
Although few studies on histidine bioconjugation can be found in the literature, the photocatalytic modification of this amino acid mainly remains an unmet challenge.[56] However, few reports describe the tendency and mechanism concerning the oxidation of His with singlet oxygen.[57] In one example, different photosensitizers were tested for their ability to generate singlet oxygen in sunlight-based systems for water disinfection. Interestingly, it was found that, relative to Met, Tyr, and Trp, His showed the highest degradation rate in the presence of singlet oxygen; thus proving the high reactivity of this residue in a photodegradation environment.[58] Although these findings are not aimed at the development of methodologies for protein modification, they do show the preferential reactivity of His in the presence of other amino acids; thus suggesting that attempts to investigate this reactivity might lead to chemoselective modifications of His.

Another field in which evidence of His oxidation in the presence of singlet oxygen has been encountered is PDT. PDT is a noninvasive treatment approved as an alternative to standard oncological therapies or for the treatment of psoriasis, age-related macular degradation, and other skin-related pathologies.[59]

In PDT, major efforts have been devoted, over the years, to the development of new photosensitizers, with the purpose of increasing light penetration in tissues and to enhance the spatial precision of treatments.[10a,60] An example towards such photosensitizers is the novel organoiridium complex reported by Sadler and co-workers.[61] The photosensitizer exhibited a long phosphorescence lifetime and allowed the selective induction of cancer cell death in cell cultures by causing oxidative damage on key His residues, which were overexpressed in tumor cells.

2.6. Dehydroalanine (DHA)

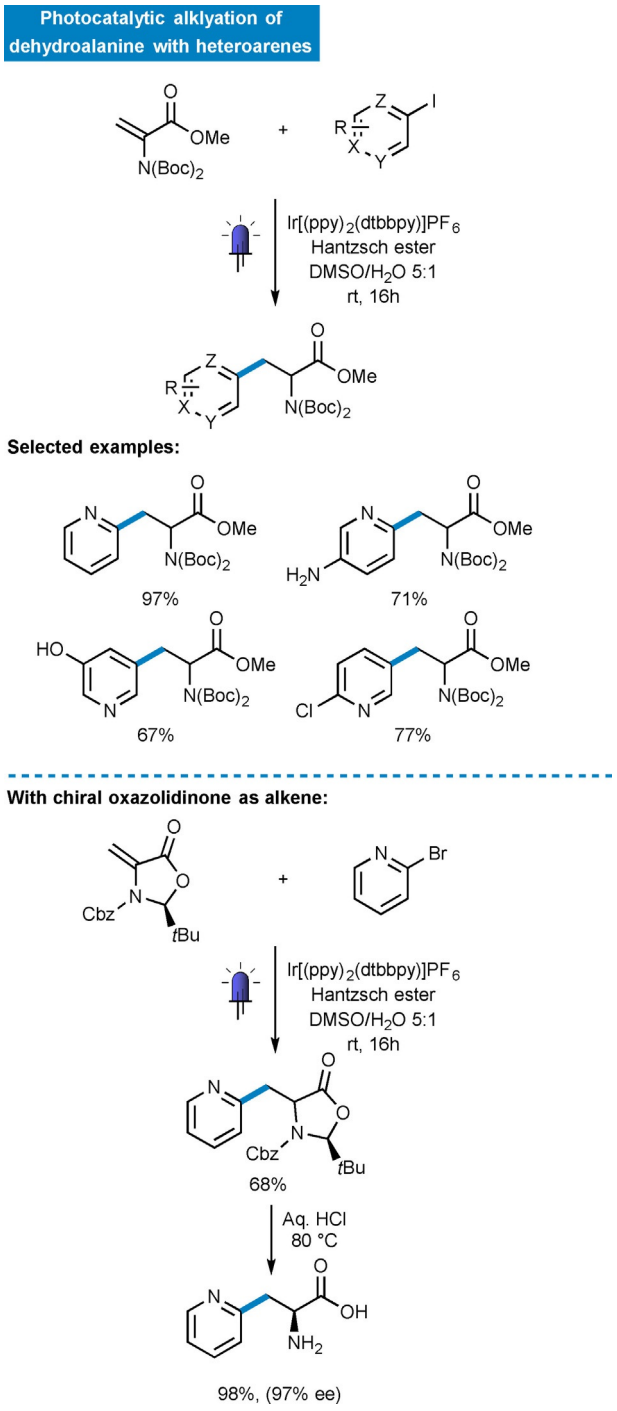
The incorporation of noncanonical amino acids into peptides and proteins offers several advantages.[3b] For instance, these residues can be exploited to increase the metabolic stability, affinity, and physical properties of peptide drug candidates.[62] Moreover, including noncanonical amino acids in peptides or proteins can provide strategies to achieve effective and selective post-translational modification methods. One prominent example of a noncanonical amino acid that enables selective transformations is DHA (Scheme 20). In nature, DHA is found in



Scheme 20. Overview of the photoredox modification of DHA.

peptides of microbial origin and has been observed in proteins as the result of post-transcriptional modifications.[63] The incorporation of DHA residues into peptides and proteins has been demonstrated both biosynthetically[64] and through chemical approaches (e.g., activation/elimination of the thiol moiety in Cys or starting from selenocysteine).[6b,65] Unlike most proteinogenic amino acids, DHA is a good electrophile, and is therefore interesting from a synthetic perspective because it exhibits good reactivity towards nucleophiles. Therefore, several post-translational modifications of DHA based on transition-metal catalysis or Michael additions have been reported.[66]

In 2017, Jiu and co-workers reported the possibility of activating pyridyl halides through a photoredox-induced SET and demonstrated the subsequent addition of these radical species to DHA derivatives.[67] Optimized reaction conditions comprised the use of $[Ir(ppy)_2(dtbbpy)]PF_6$ as a photocatalyst, an excess of the Hantzsch ester as a reductant, and a solvent mixture of DMSO/H_2O. The developed protocol proved efficient for the preparation of a wide range of β-heteroaryl α-amino acid derivatives (Scheme 21). Notably, to obviate the fact that the use of DHA as electrophilic alkenes inevitably results in the formation of products as racemic mixtures, the authors adapted the procedure to use a chiral oxazolidinone as the alkene partner.[68] The product resulting from the transformation exhibited good diastereocontrol, and, following concurrent carbamate cleavage and hemiaminal hydrolysis, gave access to the desired β-heteroaryl α-amino acid derivatives in high stereochemical purity (i.e., 97% *ee*).



Scheme 21. Photocatalytic alkylation of DHA with pyridyl halides as radical precursors. If a chiral oxazolidinone was employed in lieu of DHA, complete diastereocontrol of the obtained product was achieved. After concurrent carbamate cleavage and hemiaminal hydrolysis, a β-heteroaryl α-amino acid derivative was obtained in high stereochemical purity. Cbz = carboxybenzyl, *ee* = enantiomeric excess.

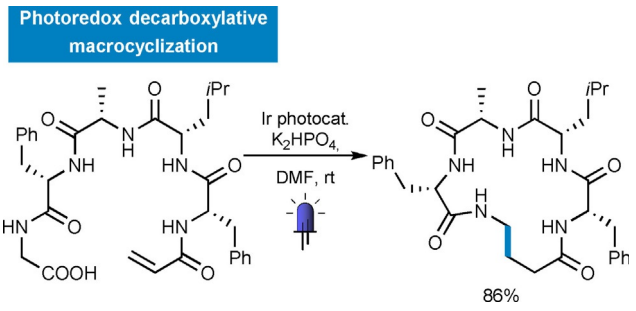
More recently, de Bruijn and Roelfes reported the late-stage functionalization of DHA-containing antimicrobial peptides (e.g., thiostrepton and nisin) by means of photoredox catalysis.[69] Specifically, under irradiation with blue LEDs and by employing $[Ir\{dF(CF_3)ppy\}_2(dtbbpy)]PF_6$ as a photoredox catalyst, the formation of carbon-centered radicals starting from trifluoroborate salts was achieved. These carbon-centered radicals were then trapped by the dehydrated amino acids present in thiostrepton and nisin. Interestingly, in the case of thiostrepton, chemoselective modification of the DHA-16 residue was obtained, whereas in the case of nisin a triple-modified product (corresponding to the modification of both DHA present in the sequence and of one dehydrobutyrine residue) was observed.

3. Other Methods

In this section, relevant photocatalytic methods that are not aimed at the selective modification of a single canonical amino acids are described. For instance, examples of photoredox decarboxylation reactions applied to the C terminus of peptides and proteins, resulting in either alkylated or cyclized derivatives, are described. Moreover, strategies demonstrating decarboxylation/alkylation at the single amino acid level at the C terminus on a small array of residues are discussed. The following examples cannot be categorized according to the targeted amino acid, but instead they represent valuable approaches that could be applied to any sequence of interest. Finally, we opted to include a relevant example on the photocatalytic deprotection of carboxylic acids, which could be of significance in the context of peptide synthesis.

3.1. Photocatalytic decarboxylation strategies: Side-chain or C-terminal modifications

Among the transformations enabled by photoredox catalysis, the decarboxylation of carboxylic acids has been thoroughly investigated.[70] In this context, MacMillan and co-workers described the photoredox decarboxylation of native amino acids to achieve site- and chemoselective bioconjugation.[71] In their first report, the Ir-catalyzed decarboxylation of C-terminal carboxylates was exploited to achieve intramolecular cyclization; thus affording a novel and mild strategy for the synthesis of cyclic peptides. Specifically, the excited state of $[Ir\{dF(CF_3)ppy\}_2(dtbbpy)]PF_6$ can be reductively quenched by the C-terminal carboxylate; thus generating, after CO_2 extrusion, a nucleophilic $C(sp^3)$ radical. The intramolecular addition of the nucleophilic α-amino radical to a pendant Michael acceptor placed at the N terminus of the sequence results in the formation of the desired macrocyclic peptide (Scheme 22). No-

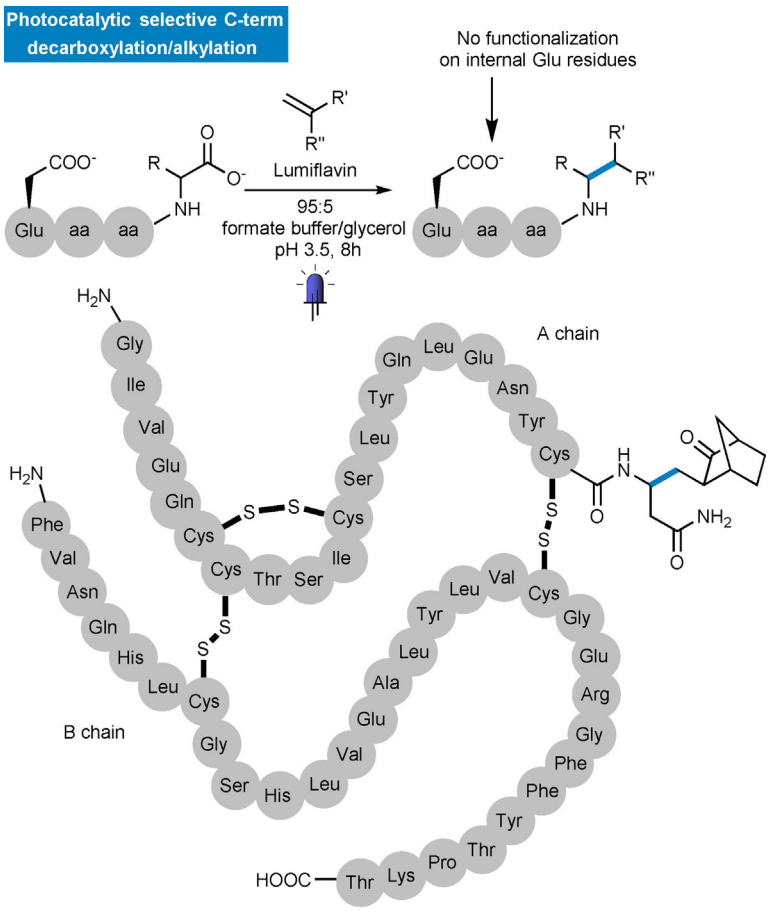


Scheme 22. Photocatalytic decarboxylative macrocyclization of peptides.

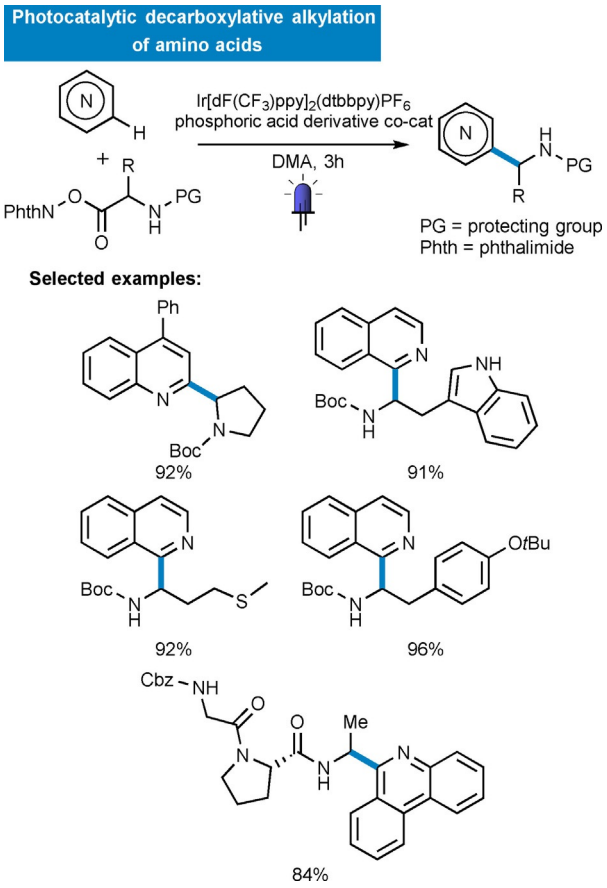
tably, the selective oxidation of the C-terminal carboxylate group over other acid-containing side chains (i.e., aspartate or glutamate) was achieved, owing to its lower pK_a and oxidation potential. A range of macrocyclic peptides, including challenging medium-sized peptidic rings, were prepared following the methodology, and the synthesis of a cyclic somatostatin receptor agonist was showcased.

In a later report, a similar methodology based on the visible-light induced decarboxylation of the C-terminal position was applied to the alkylation of a set of biologically active peptides and proteins (Scheme 23).[52] To render the methodology broadly applicable and compatible with biologically relevant reaction conditions, the authors identified riboflavin tetrabutyrate as an efficient water-soluble photocatalyst. Selectivity and compatibility towards all canonical amino acids was demonstrated, with the sole exception of tyrosine, which underwent competitive oxidation even at lower pH. However, the use of a less oxidizing flavin photocatalyst (i.e., lumiflavin) partially solved the issue. Sequences incorporating other side-chain carboxylates underwent selective decarboxylation at the C terminus. As mentioned earlier, this was rationalized by taking into account that the C-terminal carboxylate was more readily oxidized due to the stabilizing effect of the adjacent nitrogen atom on the α-amino radical generated upon decarboxylation. Finally, the broad applicability of the methodology was demonstrated for a series of tetramers and longer biologically active peptides (e.g., angiotensin II, bradykinin, bivalirudin). Notably, human insulin was also successfully alkylated, while maintaining its structural integrity; decarboxylation of the C terminus occurred selectively at chain A of the dimer.

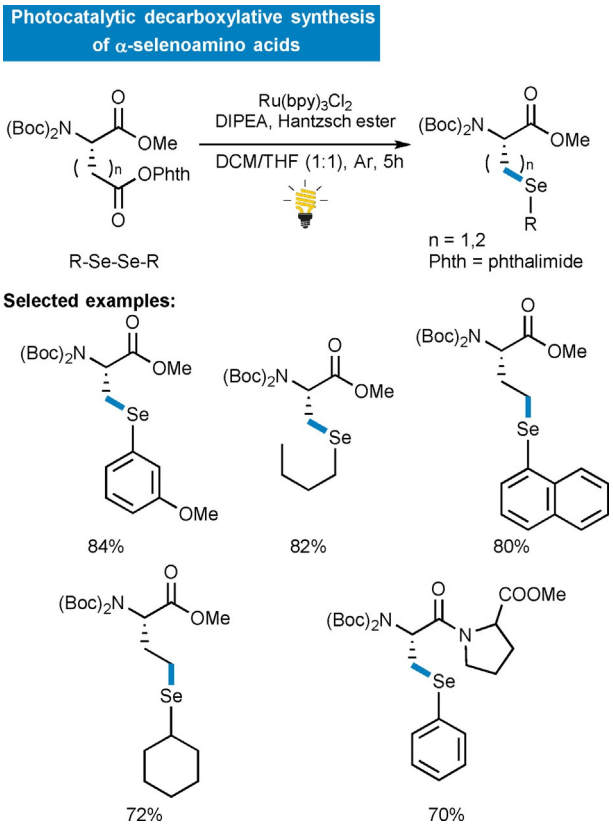
Another C-terminal decarboxylative alkylation of amino acids was reported by Fu and co-workers.[72] In this case, prefunctionalization of the C-terminal amino acid as a redox-active *N*-(acyloxy)phthalimide ester was necessary to enable its decarboxylation. Upon decarboxylation, the generated α-amino radical was then employed to attack an N-heteroarene; thus resulting in the formation of the desired alkylated product. Notably, the same reactivity would be impossible to achieve with amino acids under standard Minisci-like conditions, due to the tendency of the α-aminoalkyl radical to overoxidize to the iminium cation. For the reaction to occur in optimal yields, the use of a [Ir{dF(CF$_3$)ppy}$_2$(dtbbpy)]PF$_6$ photocatalyst (exhibiting a redox potential within the reach of the redox-active ester) and an acid cocatalyst (used to enhance the electrophilicity of the N-heteroarene) were deemed to be essential. The reaction scope was first demonstrated on a series of N-protected amino



Scheme 23. Photocatalytic C-terminal decarboxylation followed by alkylation with Michael acceptors and its application to the A-chain alkylation of human insulin.



Scheme 24. Photocatalytic decarboxylative alkylation of amino acids via photoredox-active phthalimide esters.



Scheme 25. Photocatalytic decarboxylative synthesis of α-amino acids. DIPEA = *N*,*N*-diisopropylethylamine, DCM = dichloromethane.

acid esters and then on a small array of di- and tripeptides, affording the desired products in good to excellent yields (Scheme 24).

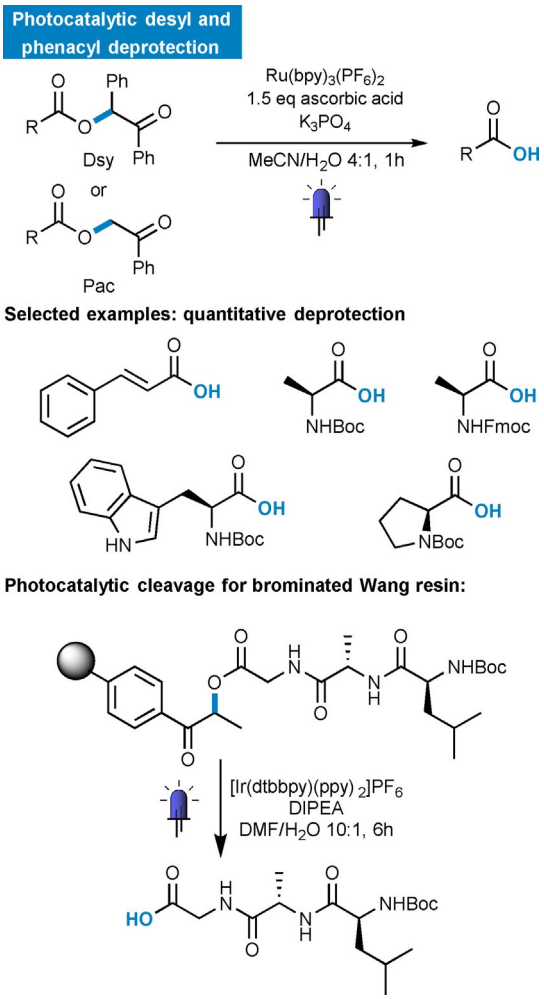
In a follow-up paper, the same group employed *N*-(acyloxy)-phthalimide ester derivatives of Asp and Glu to enable the synthesis of chiral α-selenoamino acids.[73] Specifically, in the presence of [Ru(bpy)$_3$Cl$_2$], the α-amino radical generated upon decarboxylation of the phthalimide ester was reacted with diorganyl diselenides; thus resulting in the formation of a C–Se bond. Notably, the chirality of the amino acid precursor was maintained throughout the reaction and both aliphatic and aromatic diselenides underwent the reaction in good yields (Scheme 25).

3.2. Photocatalytic removal of protecting groups: Application to peptide synthesis

In the context of peptide synthesis, a prominent role is occupied by the use of appropriate and orthogonal protecting groups, which are fundamental in preventing the formation of undesired bonds and side reactions.[74] As mentioned in the Introduction, one of the advantages of photoredox approaches is the possibility to easily switch on and off ongoing transformations by simply initiating or stopping irradiation of the reaction mixture. This straightforward control over the reaction kinetics is one of the most attractive features that renders the implementation of photoredox catalysis advantageous in bioconjugation methods.

Accordingly, the implementation of photoredox-based methods for the protection and deprotection of amino acids would greatly contribute to expanding the plethora of existing orthogonal protection methods. Ideally, with the development of protection/deprotection methods based on photoredox catalysis and compatible with solid-phase peptide synthesis; a novel visible-light-induced approach for both the synthesis and chemical modification of amino acids could be conceived.

Putting this concept into practice, Zeitler and Speckmeier reported a mild and selective photocatalytic deprotection protocol for carboxylic acids based on either phenacyl (Pac) or desyl (Dsy) protecting groups.[75] The deprotection proceeded quantitatively in the presence of catalytic amounts of [Ru(bpy)$_3$](PF$_6$)$_2$ under blue-light irradiation. Furthermore, the use of a mixture of MeCN/H$_2$O (4:1), as well as the presence of K$_3$PO$_4$ and a slight excess of ascorbic acid as a reductant, proved essential in affording the desired deprotected products. The authors speculated that K$_3$PO$_4$ played a key role in buffering the reaction pH, deprotonating ascorbic acid, and in keeping the reaction mixture biphasic. The efficiency of the deprotonation reaction was demonstrated on a series of aromatic and aliphatic carboxylic acids both for the Dsy and Pac groups (Scheme 26). Moreover, under slightly modified conditions (i.e., an Ir photocatalyst, DIPEA, and a solvent mixture of DMF/H$_2$O),



Scheme 26. Photocatalytic deprotection of Dsy and Pac protecting groups.

the applicability of the methodology to the cleavage of a tripeptide from a brominated Wang resin was demonstrated.

4. Summary and Outlook

This review focused on the implementation of photoredox catalysis for the modification of amino acids, peptides, and proteins. As shown by the different examples described herein, photocatalytic approaches offer the possibility of carrying out diverse chemical modifications of amino acids under mild and, in some cases, biocompatible reaction conditions. Nevertheless, more advances are needed to achieve the broad applicability of photoredox methodologies for protein modifications. To date, one of the major hurdles that has prevented the widespread use of photoredox methods is the poor solubility of transition-metal photocatalysts in aqueous reaction media. With this in mind, more efforts to replace these metal complexes with suitable, and equally powerful, organic photocatalysts or efforts towards the use of heterogeneous photocatalysts could yield beneficial results, while simplifying purification methods. Moreover, the majority of reported photoredox methodologies for amino acid modification require the use of stoichiometric amounts of organic bases (often used as sacrificial electron donors in the catalytic cycle) or oxidants. The use of such reagents is often incompatible with the delicate nature of proteins; thus attempts towards the development of additive-free methodologies will be required in the long run to redefine optimized reaction conditions. In this context, a recently reported oxidant-free C–H activation of glycine through a cross-coupling hydrogen evolution reaction might be a nice source of inspiration for alternative reaction pathways that allow direct amino acid modification.[76] Another limitation hampering the extensive applicability of photoredox methods is that current methodologies are directed only to a limited set of different amino acid residues. We anticipate that various strategies will be developed in the near future that allow for the multisite labeling of proteins. For example, novel strategies that afford improved regioselective modification of proteins might be achieved by matching the redox potential of a specific residue with an appropriate photocatalyst. Moreover, owing to recent advances in the field of C–H activation through the combination of photoredox/transition-metal catalysis (i.e., dual catalysis), we foresee the development of novel methodologies suitable for the activation of aliphatic amino acid residues.

Finally, the use of enabling technologies, such as microreactor technology and automation protocols, might significantly facilitate the development of novel photoredox methodologies for protein modification. For instance, they could contribute to the reduction of the amount of materials needed for optimization, while allowing the facile control of multiple reaction conditions.

Overall, we hope that this collection of photocatalytic methods for biomolecule modification will appeal to a chemical biology audience, and will encourage increasing interest on the topic.

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Conflict of interest

The authors declare no conflict of interest.

Keywords: amino acids · bioconjugation · peptides · photochemistry · redox chemistry

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