Photochemical Chemoselective Alkylation of Tryptophan-containing Peptides

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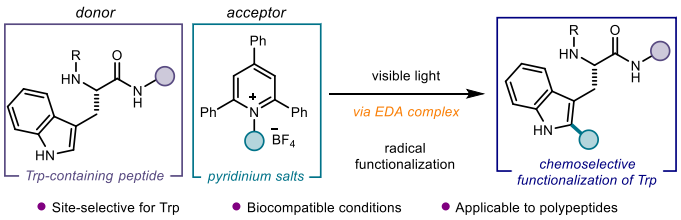
Supporting Information Placeholder



ABSTRACT: We report a photochemical method for the chemoselective radical functionalization of tryptophan (Trp)-containing peptides. The method exploits the photoactivity of an electron donor-acceptor complex generated between the tryptophan unit and pyridinium salts. Irradiation with weak light (390 nm) generates radical intermediates right next to the targeted Trp amino acid, facilitating a proximity-driven radical functionalization. This protocol exhibits high chemoselectivity for Trp residues over other amino acids and tolerates biocompatible conditions.

Photocatalysis is attracting an increasing interest from the chemistry community, mainly because of its operational simplicity and the potential to promote radical processes under mild conditions.[1] Recent studies demonstrated that visible light is also useful for promoting chemistry in complex biological systems.[2] Needed for these applications are reactions that proceed under mild conditions while exhibiting high functional group tolerance and compatibility in aqueous environments – all requirements that photochemistry and radical reactivity excellently fulfil. For example, photochemical methods were recently used for the selective peptide and protein functionalization of natural amino acids by means of radical processes.[3] These strategies for protein bioconjugation[4] can play a central role in the development of novel biologically active protein conjugates for applications in biology and medicine. The reported photoredox systems generally target specific amino acids via redox activation based on single-electron transfer (SET) manifolds.[3] However, discriminating amino acids on the basis of their redox properties can be difficult, thus leading to undesired off-target modifications at other residues.

To achieve complete selectivity for specific amino acids, we sought to design a strategy that uses visible light to generate radicals only in close proximity of the targeted amino acid. Specifically, we surmised that the photochemical activity of transiently generated electron donor-acceptor (EDA) complexes[5] could serve to achieve this goal (Figure 1a). In this strategy, electron-rich molecules (the *donor*) and electron-poor compounds (the *acceptor*) can form colored EDA complexes through diffusion-controlled association.

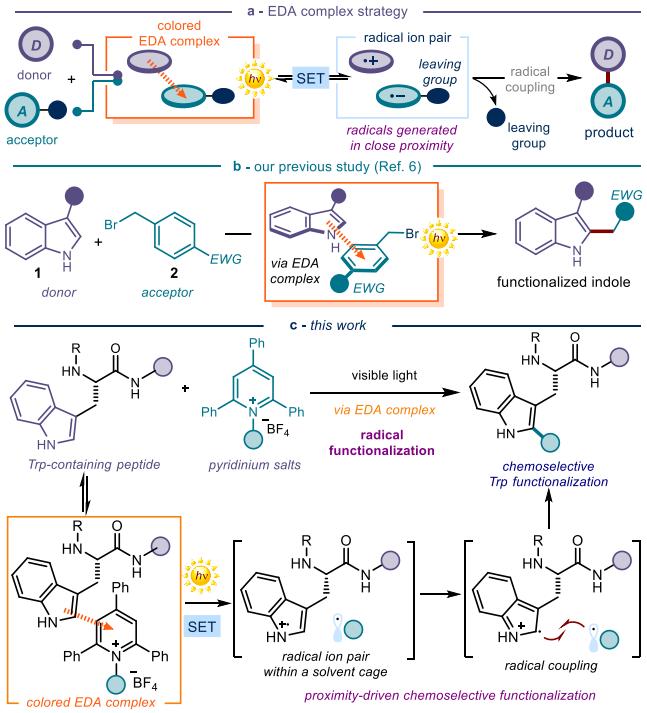


Figure 1. (a) Photochemical EDA complex strategy to generate radicals. **(b)** Our previous work on EDA-complex-mediated C-2 alkylation of indoles. **(c)** The proposed photochemical strategy to generate radicals in close proximity of tryptophan and the resulting chemoselective functionalization process; SET: single-electron transfer; EWG: electron-withdrawing group.

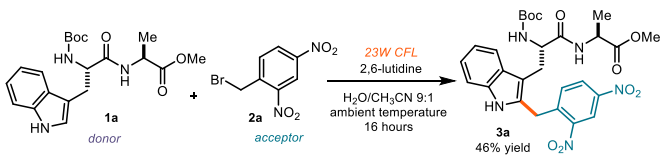
These new chemical entities can then be electronically excited upon light irradiation. A photoinduced SET then affords a radical ion pair within the solvent cage. Finally, radical recombination delivers the product via a proximity-driven radical trap.

We recently used the EDA complex strategy to promote the C-H benzylation of 3-substituted indoles **1** (Figure 1b),[6] which act as donors in EDA complex formation[7] with electron-deficient benzyl bromides **2**. Notably, the indole moiety is the characteristic structure of the canonical amino acid *tryptophan* (Trp). Tryptophan is the rarest of all amino acids (only 1.3% in occurrence), but it is present in about 90% of all proteins. A photochemical method targeting this underexplored amino acid would therefore considerably enrich the protein functionalization toolbox. Previous strategies for Trp functionalization relied on transition metal catalysts.[8] Metal-free approaches were also developed that mainly capitalized on radical reactivity manifolds.[9] Iridium-based photoredox catalysts have also been used to promote radical functionalization of Trp.[10] Recently, there have been a few reports of photochemical protocols that do not require the use of an exogenous photoredox catalyst.[11] In particular, a recent study[11c] exploited the direct excitation of the indole nucleus within Trp residues to elicit radical formation and selective functionalization. However, this approach required irradiation with high-energy light (λ = 302 nm).

Here, we report the successful implementation of a visible-light-driven EDA-complex-mediated radical functionalization protocol for the selective modification of tryptophan in short peptides (Figure 1c). The chemistry can be performed under biocompatible conditions (aqueous solvent) and only requires weak light (λ = 390 nm) as the activating factor. The protocol exhibits high chemoselectivity for Trp residues over other amino acids: this is because the underlying EDA complex mechanism secures the generation of radicals right next to the targeted Trp amino acid, thus facilitating a proximity-driven radical functionalization. Overall, the process relies on a biomimetic mechanism for it exploits the intrinsic tendency of Trp to form photoactive charge transfer complexes in biological systems.[12]

We started our investigations using the simplified dipeptide Boc-Trp-Ala-OMe (**1a**) as the model donor substrate (Scheme 1). The choice of the electron-poor 2,4-dinitrobenzylbromide **2a** was informed by its ability to form EDA complexes with indole frameworks.[6] Since our aim was to develop a method for the chemoselective functionalization of Trp under biocompatible conditions, the experiments were conducted in a water/CH_3CN mixture (9:1) at ambient temperature. A simple compact fluorescent light (CFL) bulb was used for irradiation. These conditions secured the formation of the C-2 alkylated dipeptide **3a** in 46% yield. A control experiment established that the absence of light completely suppressed the reactivity.

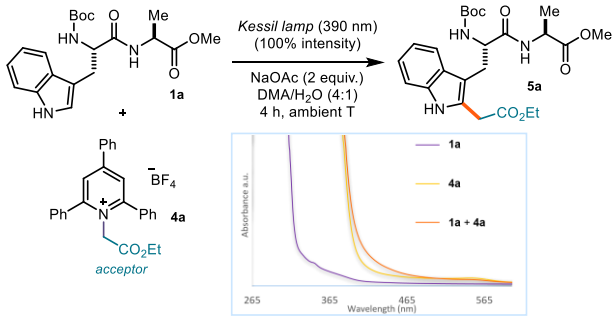
Scheme 1. Preliminary studies: reaction performed using equimolar amounts of **1a** and **2a**.



These initial studies indicated that our photochemical strategy could secure the chemoselective functionalization of the Trp unit under mild conditions and in an aqueous environment. However, despite extensive efforts (detailed in section B of the Supporting Information), we could not increase the overall efficiency of the process using **2a** as the radical precursor.

We therefore evaluated other electron-poor compounds with a known tendency to serve as acceptors for EDA complex formation and as radical precursors (see section C5 in the Supporting Information for details). In particular, using UV/Vis spectroscopic studies, we established that the glycine-derived Katritzky pyridinium salt[7b,13] **4a** could form a visible-light-absorbing EDA complex upon aggregation with dipeptide **1a** (see inset in the figure of Table 1, measurement conducted in DMA). We therefore evaluated if the photoactivity of this EDA aggregate could drive the selective radical alkylation of Trp within **1a**.

Table 1. Optimization studies.[a]



entry	deviation from standard conditions	% yield[b]
1	none	75 (68)
2	in DMF/H_2O (4:1)	70
3	in DMA	35
4	no NaOAc	60
5	no light	0
6	under air	<5
7	1 mmol scale	62 (55)

[a] Reactions performed under an argon atmosphere on a 0.1 mmol scale for 4 h using 3 equiv. of **4a**. [b] Yield of **5a** determined by ^{1}H NMR analysis of the crude mixture using 1,3,5-trimethoxybenzene as the internal standard; yields of isolated **5a** are reported in brackets.

The experiments were conducted in a DMA/water system (4:1 ratio, 0.2 M) using 3 equiv. of the Katritzky salt **4a**, 2 equiv. of NaOAc as the base, and under illumination by a Kessil lamp emitting at 390 nm, since this set-up secured a more reliable irradiation of the mixture. The reaction was complete after 4 hours to afford the C2 functionalized indole product **5a** in 68% yield (entry 1). The process could be performed in a different aqueous system maintaining the same efficiency (DMF/water, entry 2). Interestingly, promoting the reaction in pure DMA afforded lower yield (entry 3), highlighting the beneficial effect of water. The reaction also proceeded in the absence of base, although with a slightly lower efficiency (entry 4). Control experiments demonstrated that the reaction requires light[14] and an argon atmosphere to perform well (entries 5 and 6). Conducting the model reaction on a 1 mmol scale only slightly affected the process' efficiency (**5a** formed in 55% yield, entry 7), demonstrating the practical utility of the method.

We then evaluated the synthetic potential of this strategy under the optimized conditions described in Table 1, entry 1. We

examined the reactivity of different Trp-containing dipeptides **1**. We used the pyridinium salt **4b** bearing a benzyl substituent on the ester moiety, since this radical precursor offered a good reactivity and a more straightforward analysis of the products (Figure 2).

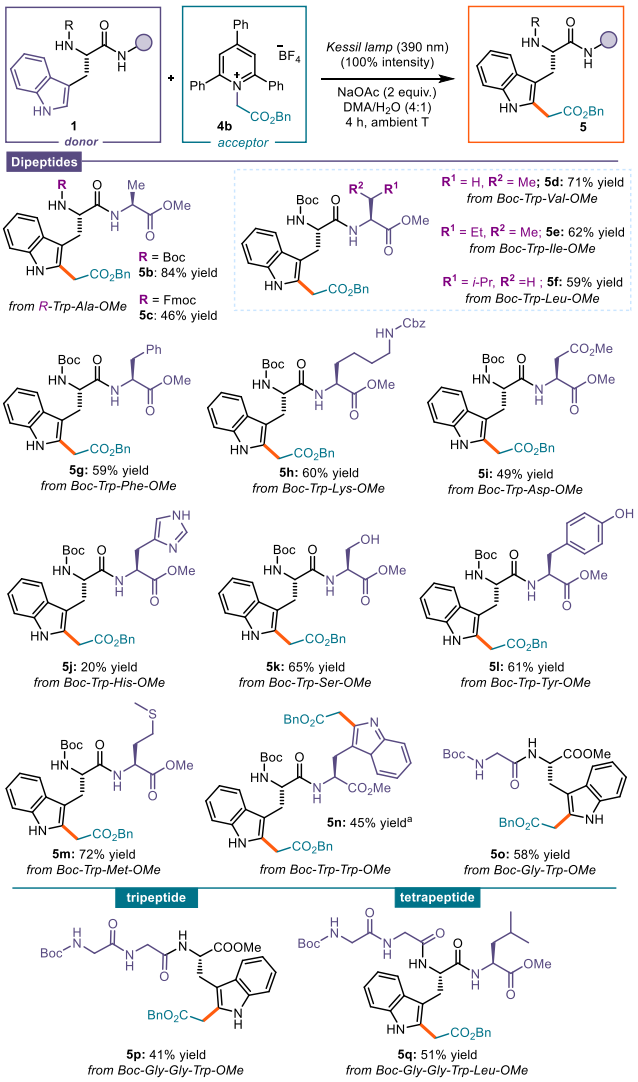


Figure 2. Photochemical chemoselective alkylation: scope of the Trp-containing peptides. Reactions performed on a 0.1 mmol scale using 3 equiv. of **4b**. Yields of the isolated products **5** are reported below each entry (average of two runs per substrate). [a]Using 6 equiv. of **4b** and 4 equiv. of NaOAc.

The *N*-protecting group in **1** could be swapped from Boc to Fmoc maintaining a useful reactivity (adducts **5b** and **5c**). *N*-Boc protected dipeptides bearing a large variety of aminoacidic residues were selectively alkylated at the C2 carbon of the Trp unit (products **5d**-**5n**). Substrates containing a secondary amine and an ester moiety reacted smoothly to afford the corresponding products **5h** and **5i** in good yields. A dipeptide containing a histidine residue could be selectively functionalized at the indole moiety, albeit with a low yield (adduct **5j** isolated in 20% yield). The protocol also tolerated oxidizable functionalities, including the hydroxyl groups within Ser- and Thr-containing dipeptides (products **5k** and **5l**, respectively) and the thioether unit of methionine (adduct **5m**). The dipeptide Boc-Trp-Trp-OMe, bearing two indole units, could be efficiently difunctionalized using an excess (6 equiv.) of radical precursor **4b** (product **5n**). In all experiments, the method's mild conditions ensured the stereochemical integrity of the aminoacidic residues (a single diastereomer was consistently formed). In addition, glycine residues remained untouched in all cases (e.g. product **5o**), which stands in contrast to a recently reported EDA-complex-based photochemical functionalization method with pyridinium salts.[15] As a limitation of the system, unprotected aminoacidic residues, such as a fully unprotected tryptophan and the dipeptide Boc-Trp-Asp-OH, did not react under the optimized conditions. Also a cysteine-containing dipeptide was not amenable to this functionalization protocol (see Figure S4, section H in the Supporting Information, which includes a list of moderately successful and unsuccessful substrates).

In addition to dipeptides, this method was expanded to include the chemoselective functionalization of more complex Trp-containing oligopeptides. A tripeptide (Boc-Gly-Gly-Trp-OMe) and a tetrapeptide (Boc-Gly-Gly-Trp-Leu-OMe) both offered good reactivity, affording the corresponding products **5p** and **5q** in 41% and 51% yield, respectively. Overall, the method secured the selective functionalization of Trp independently of its position along the peptide sequence e.g. Trp located at both the *N*- and *C*-terminal positions (products **5o** and **5p**) or within the peptide sequence (adduct **5q**).

We then evaluated the radical precursors **4** suitable for this photochemical method, using dipeptide Boc-Trp-Ala-OMe **1a** as the model substrate (Figure 3). The pyridinium salts **4** were readily prepared from amino acid precursors.[13]

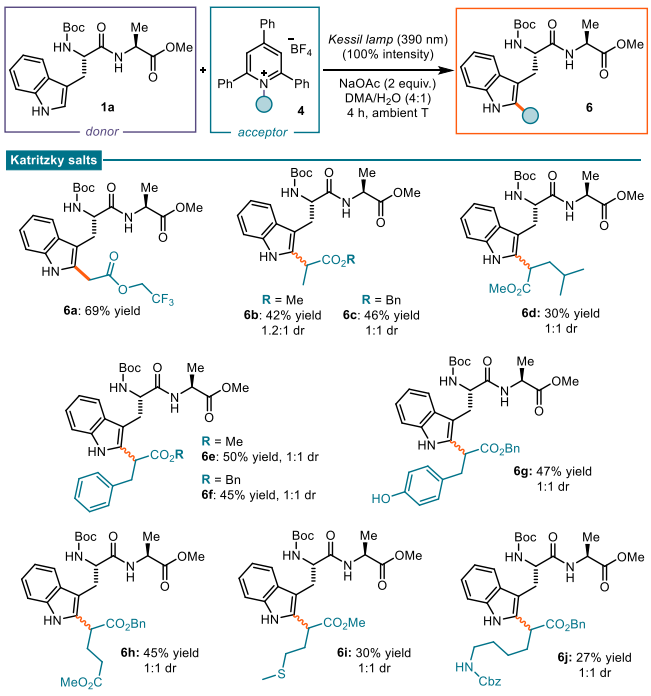


Figure 3. Photochemical chemoselective alkylation of Trp-containing dipeptide **1a**: scope of the radical precursors **4**. Reactions performed on a 0.1 mmol scale using 3 equiv. of **4**. Yields of the isolated products **6** are reported below each entry (average of two runs per substrate).

A primary radical precursor derived from CF_3-containing glycinate afforded good yield (product **6a**). Secondary radical precursors could also be used to alkylate the Trp unit (adducts **6b**-**6j**), although the newly generated stereogenic center could

not be controlled (diastereomeric ratio of about 1:1). A variety of functional groups were used to adorn the Trp unit within the final products. Specifically, Katritzky salts derived from phenylalanine and tyrosine showed good reactivity, affording the corresponding products **6e**-**6g**. The use of methionine and Cbz-lysine-derived Katritzky salts allowed thio and amino moiety to be installed in adducts **6i-6j**, respectively, albeit with moderate reactivity. A pyridinium salt bearing an *N*-cyclohexyl moiety, which would generate a nucleophilic alkyl radical, remained completely unreacted.[16]

In summary, we have developed a photochemical method for the alkylation of tryptophan-containing peptides. The protocol exhibits high chemoselectivity for Trp residues over other amino acids, tolerates biocompatible conditions, and only requires weak light as the activating factor. The availability of starting materials (all substrates are derived from amino acids), the operational simplicity, and the mild reaction conditions make this protocol potentially useful for the functionalization of more complex biomolecules. Investigations along these lines are ongoing in our laboratory.

ASSOCIATED CONTENT

Supporting Information

Experimental procedures and spectral data. The Supporting Information is available free of charge on the ACS Publications website.

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Author Contributions

‡B.L. and X.T. contributed equally.

Notes

The authors declare no competing financial interest.

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(14) Performing the model reaction in Table 1 under 460 nm irradiation (a wavelength that the individual substrates **1a** and **4a** cannot absorb, while their mixture can) afforded the alkylation product **5a** in 17% yield, see section C2 of the Supporting Information for details. This experiment is consonant with the photoactivity of the EDA complex being responsible for the generation of radicals.

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(16) While we propose a mechanism based on radical combination within the solvent cage (Figure 1c), a radical chain propagation proceeding via the addition of a radical into the indole nucleus cannot be excluded. This alternative chain propagation manifold is shown in Scheme S1, section I of the Supporting Information. We could not collect evidence to clearly discriminate among these reaction mechanisms since our attempts to determine the quantum yield of the model reaction were frustrated by the heterogeneity of the reaction mixture (NaOAc is partially insoluble in the reaction mixture), which precluded a homogeneous illumination, a crucial requirement for a reliable quantum yield determination.