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Tryptophan-specific peptide modification through metal-free photoinduced N-H alkylation employing *N*-aryl glycines

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ABSTRACT

Tryptophan (Trp) carries a unique heteroaromatic indole side chain and plays a critical role in peptide or protein modification. Herein, we have reported a metal-free photoinduced N-H alkylation strategy using *N*-aryl glycines for specific modification of tryptophan-containing peptides. The robustness of our approach is demonstrated by its wide substrate scope, excellent isolated yields, as well as almost unobservable side effects. Using this highly efficiently metal-free condition, alkylated Trp-containing peptides can be smoothly assembled. This study provides a reliable and practical tool for the chemo-selective modification of various tryptophan containing oligopeptides.

Chemo-selective modifications of peptides and proteins have gained increased interest in drug development, which enhances peptide druggability or creates additional reactivity [1–5]. To date, a number of elegant strategies have been employed to increase drug efficiency, target affinity and cell penetration [6–9], such as transition-metal catalysis [10], multicomponent reaction [11,12] and enzyme catalysis [13,14]. However, most of traditional methods are often hampered by challenges including poor solubility, expensive catalysts, stringent reaction conditions, and difficult maneuverability [15]. Recently, photoredox catalysis has emerged as an ideal platform for post-synthetic modifications of peptides and proteins, owing to its mild and highly selective nature, as well as its compatibility with biological conditions [16]. And it has also been successfully utilized in the construction of cyclic peptides [17], label natural proteins and cells [18], as demonstrated in various studies. Tryptophan represents the rarest proteinogenic amino acid with a frequency of only 1.4%, compared to the 5% average frequency of other amino acids [19]. Yet it has gained widespread attention as a promising target for post-synthetic modifications due to its active chemical properties and crucial roles in protein stabilization and recognition [20–24]. Currently, most of photocatalytic strategies focus on adding electron-deficient units to the C2 site of tryptophan, including trifluoromethyl [25], difluoromethyl [26] and various α-carbonyl alkyl groups (Fig. 1A) [27]. Meanwhile, a photocatalytic tryptophan β-position modification has been reported [28], which exhibits remarkable chemo-selectivity for both simple and endogenous peptides, such as glucagon and GLP-1 amide (Fig. 1B). In addition, it is noteworthy that the NH of the indole ring is also an attractive target for photocatalytic modification. For instance, Studer's group successfully accomplished the insertion of photochemically generated siloxycarbenes into the *N*-H bond of tryptophan (Fig. 1C) [29].

In peptide drug optimization, alkylation modification is an important approach for expanding the structural diversity of peptide candidate drugs in drug optimization [30]. Recently, photoinduced decarboxylative alkylation has led to wide-spread attention amongst chemists, pharmacologists and biologists [31–37]. Glycine holds significant value as it is one of the most crucial and abundantly accessible natural amino acids [38–40]. Numerous studies have demonstrated that *N*-aryl glycines can efficiently generate α-amino radicals under visible light irradiation. These radicals can then undergo oxidation, leading to the transformation of iminium ions, which can ultimately be trapped with a diverse range of nucleophiles (Fig. 2A). In 2019, Le's group developed a novel photocatalyst-free decarboxylative aminoalkylation method using visible light to activate *N*-aryl glycines, allowing for efficient synthesis of disparate imidazo[1,2-α]pyridines *via* iminium ion captured by various nucleophilic reagents [41]. In 2020, Yu and co-workers efficiently assembled dihydroquinoxalin-2(1*H*)-ones and tetrahydroimidazo[1,5-*a*]quinoxalin-4(5*H*)-ones *via* visible light-induced heterogeneous g-C_3N_4-catalyzed decarboxylative reaction of quinoxalin-2(1*H*)-ones with *N*-aryl glycines [42]. More recently, Chen and co-workers also developed a new method

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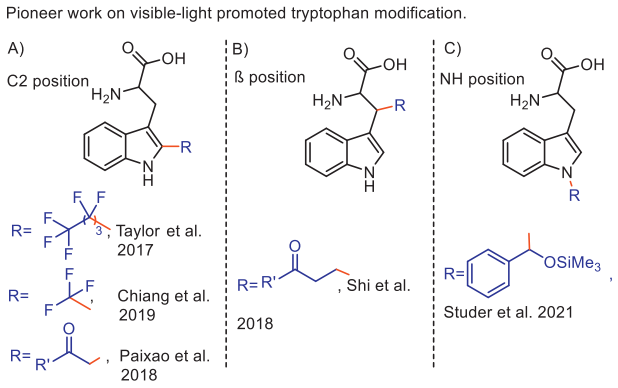


Fig. 1. Pioneer work on visible-light promoted tryptophan modification.

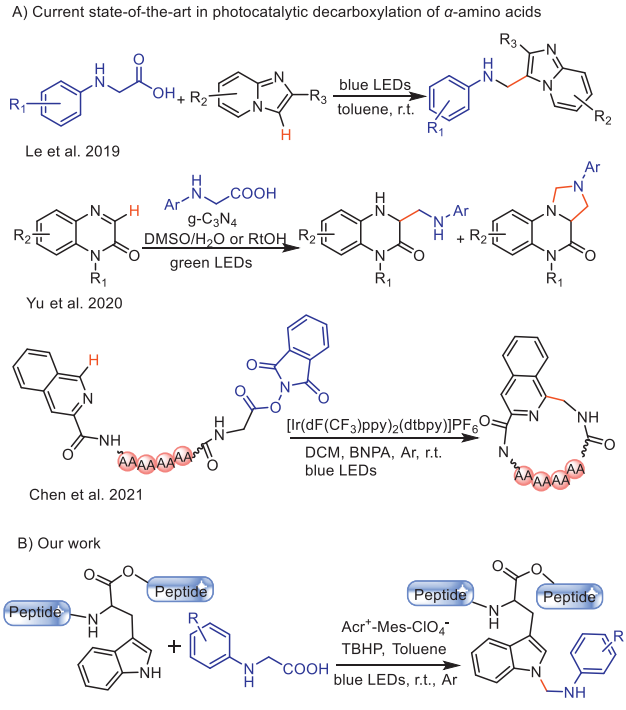
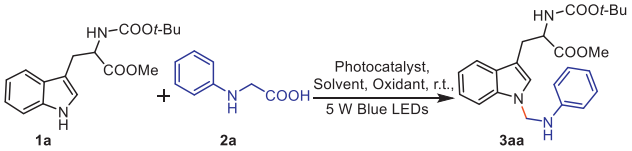


Fig. 2. (A) Current state-of-the-art in photocatalytic decarboxylation of α-amino acids. (B) Chemo-selective Trp-containing peptide alkylation modification through indole (NH) photocatalytic conjugation.

to construct peptide macrocycles *via* radical-mediated intramolecular C–H alkylation reactions under photocatalytic decarboxylation of glycine [43]. Inspired by those publications, we herein reported a metal-free photoinduced post-synthetic alkylation of Trp-containing peptides with *N*-aryl glycines (Fig. 2B), which exhibited commendable chemo-selectivity, good substrate scope and outstanding isolated yields. We anticipated that this approach may be effectively employed in the discovery of new therapeutic agents.

At the outset of our studies, Boc-Trp-OMe (**1a**) and *N*-4-phenyl glycine (**2a**) were chosen as the potential model materials for the optimization investigation (Table 1). We were delighted to obtain the desired product **3aa** in 30% yield without the use of a photosensitizer. And this transformation was conducted by employing TBPB as the oxidant and conducting the reaction at room temperature under 5 W blue LED lights in CH_2Cl_2 (entry 1). Subsequently, different oxidants, including air, DTPB and TBHP, were examined, a better yield of 50% was obtained when using TBHP (entries 2–4). For improving the reaction efficiency, diverse photocatalysts were introduced. After a comprehensive screening process of

Table 1
Optimization of the reaction conditions.[a]



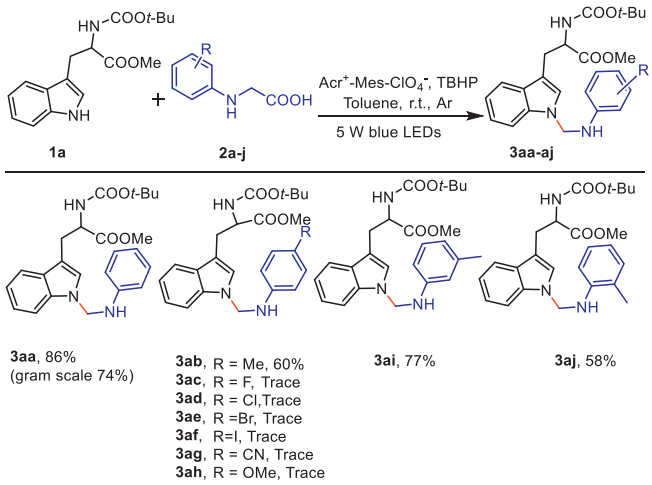
Entry	Catalyst	Oxidant	Solvent	Yield (%)[b]
1	-	TBPB	CH_2Cl_2	30
2	-	Air	CH_2Cl_2	Trace
3	-	DTBP	CH_2Cl_2	Trace
4	-	TBHP	CH_2Cl_2	50
5	*fac*-Ir(ppy)$_3$	TBHP	CH_2Cl_2	28
6	Ru(bpy)$_2$Cl$_2$	TBHP	CH_2Cl_2	Trace
7	Solvent Red 43	TBHP	CH_2Cl_2	Trace
8	Ir{dFCF$_3$ppy}$_2$(bpy)PF$_6$	TBHP	CH_2Cl_2	Trace
9	Eosin Y	TBHP	CH_2Cl_2	34
10	Ir(ppy)$_2$(dtbbpy)PF$_6$	TBHP	CH_2Cl_2	Trace
11	Ir(dFppy)(dtbpy)PF$_6$	TBHP	CH_2Cl_2	Trace
12	Acr$^+$-Mes-BF$_4^-$	TBHP	CH_2Cl_2	60
13	Acr$^+$-Mes-ClO$_4^-$	THBP	CH_2Cl_2	68
14	Acr$^+$-Mes-ClO$_4^-$	TBHP	Toluene	86
15	Acr$^+$-Mes-ClO$_4^-$	TBHP	DMF	Trace
16	Acr$^+$-Mes-ClO$_4^-$	TBHP	DMSO	Trace
17	Acr$^+$-Mes-ClO$_4^-$	TBHP	THF	Trace
18	Acr$^+$-Mes-ClO$_4^-$	TBHP	MeOH	Trace
19	Acr$^+$-Mes-ClO$_4^-$	TBHP	EtOH	Trace
20	Acr$^+$-Mes-ClO$_4^-$	TBHP	CH_3CN	60
21	-	TBHP	Toluene	54
22[c]	Acr$^+$-Mes-ClO$_4^-$	TBHP	Toluene	0

[a] Reaction conditions: the mixture of **1a** (1.0 equiv., 0.1 mmol), **2a** (2.0, equiv., 0.2 mmol), photocatalyst (3 mol%), oxidant (2.0 equiv., 0.2 mmol), and solvent (anhydrous, 1 mL) was irradiated by 5 W blue LED light under Ar at room temperature for 72 h.
[b] Isolated yield based on **1a**.
[c] The reaction was performed in darkness.

various photocatalysts, we were pleased to observe that Acr$^+$-Mes-ClO$_4^-$ emerged as the optimal choice for this transformation (entries 5–13). Next, further examination on various solvents revealed that toluene resulted in a satisfactory reaction yield of 86% for this reaction (entries 14–20). When there is a lack of photocatalyst, the reaction yield decreased from 86% to 54% (entry 14 as to entry 21), which verifies the role of Acr$^+$-Mes-ClO$_4^-$ in promoting the reaction. In addition, the control experiment confirmed that the lack of visible light resulted in the absence of the desired product **3aa**, providing compelling evidence for the crucial role of visible light in facilitating this reaction (entry 22).

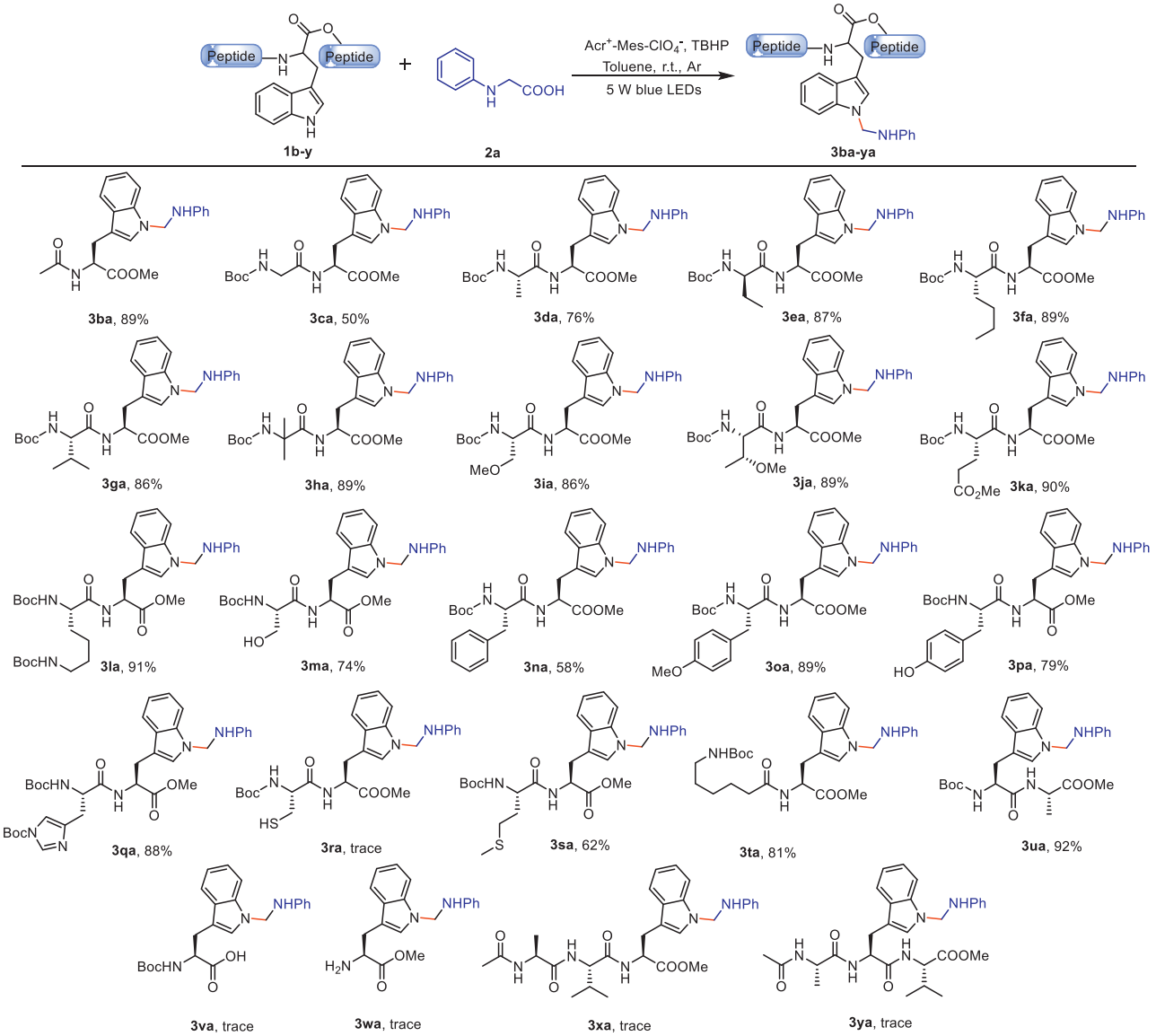
With the optimized reaction conditions in hand, we proceeded to investigate the substrate scope of *N*-aryl glycines **2** (Scheme 1). As described in Scheme 1, the reaction between Boc-Trp-OMe (**1a**) and various *N*-aryl glycines **2a-j**, containing either electron-rich or electron-deficient substituents on the aryl ring, was examined. As expected, substrate **2b** with a *para*-positioned -Me group on the phenyl moiety was smoothly converted to **3ab** in 60% yield. Meanwhile, steric hindrance did not affect the efficiency of the reaction, as evidenced by the good yields of the desired compounds **3ai** and **3aj** (77% and 58%, respectively), which were obtained from *ortho*- or *meta*-substituted *N*-aryl glycines as the starting compounds. To our surprise, it was found that *N*-aryl glycines containing various electron-withdrawing groups (-F, -Cl, -Br, -I, and -CN) or even a strong electron-donating group (-OMe) were not suitable for this transformation. And only trace amounts of the desired products (**3ac-3ah**) were detected.

After establishing a reliable method for the selective N-H alkylation of tryptophan, we proceeded to the versatility of our strategy to various Trp-containing peptides **1b-1y**. As shown in Scheme 2, an array of peptide structures allowed tryptophan-

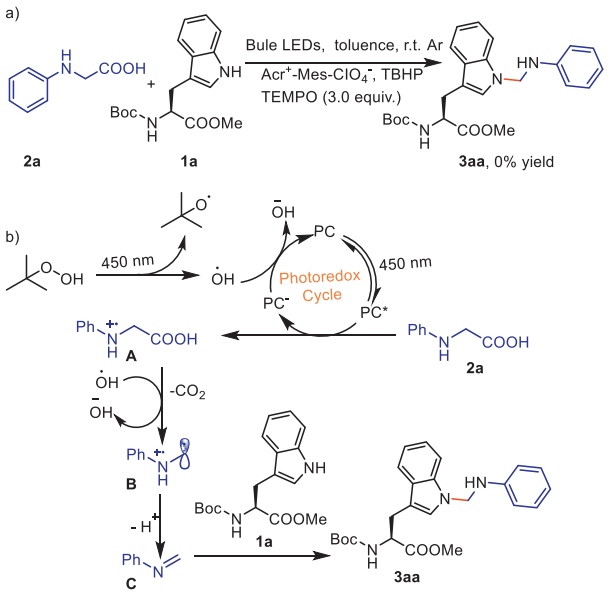


Scheme 1. Scope of *N*-aryl glycines **2**. Reaction conditions: **1a** (1.0 equiv., 0.1 mmol), **2a–j** (2.0 equiv., 0.2 mmol), Acr^{+}-Mes-ClO_4^{-} (3 mol%), TBHP (2.0 equiv., 0.2 mmol), anhydrous toluene (1 mL), 5 W blue LEDs, Ar, 72 h, isolated yields.

functionalization at the N-H position of the indole ring. In the case of the Ac-Trp-OMe (**1b**), the desired product **3ba** was isolated in 89% yield under the optimized reaction conditions. Then, we evaluated the reaction efficiency of various dipeptides with aliphatic side chains including glycine (**1c**), alanine (**1d**), 2-aminobutyric acid (**1e**), norleucine (**1f**), valine (**1g**), 2-amino-2-methylpropionic acid (**1h**), affording the corresponding products in moderate to excellent yields (**3ca-3ha**). This finding suggested that the spatial effect of the side-chain has a negligible influence on the efficiency of the reaction. Subsequently, Trp-derived dipeptides **1i-1l** containing polar uncharged side chains also delivered the desired products in outstanding yields. Serine and threonine, each with methoxyl side chains, together with glutamine's esterified side chains, efficiently participated in this transformation. It was worth noted that serine (**1m**) characterized by the hydroxyl side chain, also successfully underwent this transformation, delivering the product **3ma** in 74% yield. Gratifyingly, dipeptides (**1n-1q**) featuring aromatic side chain were smoothly converted to the products, especially substrate **1p** with tyrosine reacted cleanly to give **3pa** in 79% isolated yield. While the dipeptide Boc-Cys-Trp-OMe (**1r**) with thiol-



Scheme 2. Photocatalyzed N–H alkylation of a variety of tryptophan derivates and tryptophan-containing peptides. Reaction conditions: **1b-y** (1.0 equiv., 0.1 mmol), **2a** (2.0 equiv., 0.2 mmol), Acr^{+}-Mes-ClO_4^{-} (3 mol%), TBHP (2.0 equiv., 0.2 mmol.), anhydrous toluene (1 mL), 5 W blue LEDs, Ar, 72 h, isolated yields.



Scheme 3. Proposed mechanism.

containing side chains did not success in the transformation, substrate **1s**, featuring a methylthio group, yielded the corresponding product **3sa** with a 62% efficiency. Additionally, our method could be successfully applied to the substrate **1t** with the long chain fatty acid, rendering the alkylated compound **3ta** in 81% yield. And we also studied the effect of tryptophan residues' position in the peptide sequence, whether it was located at the C-terminus or N-terminus. It was found that this did not impact with a 92% yield of **3ua** achieved. Unfortunately, this reaction was incompatible with exposed amino or carboxyl groups (**3va-3wa**). And extending the peptide chain to three monomers yielded only trace amounts of the desired products (**3xa**-**3ya**). This could be attributed to the low solubility of the tripeptide in toluene, which led to low reaction efficiency.

To gain a better understanding of the reaction mechanism, the control experiment was conducted. As shown in Scheme 3a, additional of the radical scavenger TEMPO (3.0 equiv.) showed obvious negative influence on the model reaction, effectively proving a radical mechanism for this reaction. Based on this control experiment and previous literatures [44,45], a tentative mechanism for this decarboxylative coupling process was proposed, as depicted in Scheme 3b. After initial excitation of the photocatalyst Acr^+-Mes-ClO_4^- (PC), *N*-aryl glycine **2a** was oxidized by the excited state photocatalyst (PC*) to generate the aminyl cation **A**. The intermediate **A** underwent a proton transfer and decarboxylation process to yield α-amino cation radical **B**, which subsequently lost a hydrogen ion to form the imine intermediate **C**. Finally, the electron-rich tryptophan derivative **1a** captured the mine intermediate **C** to produce the desired alkylated product **3aa**. Furthermore, the reduced photocatalyst PC^- was expected to undergo reoxidation by $HO^{\bullet}$, effectively regenerating the photoactive catalyst PC for the next run.

In summary, a metal-free photoinduced N-H alkylation strategy for the specific modification of tryptophan-containing peptides is reported. This reaction employed *N*-aryl glycines to efficiently generate α-amino radicals under visible light irradiation, exhibiting broad substrate scope and functional group tolerance. It provides a mild and efficient access to the N-H activation of tryptophan indole ring. Given the growing importance of Trp-containing bioactive peptides, this protocol will become increasingly attractive in medicinal chemistry research.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.cclet.2023.109244.

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