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Histidine-Specific Peptide Modification via Visible-Light-Promoted C–H Alkylation

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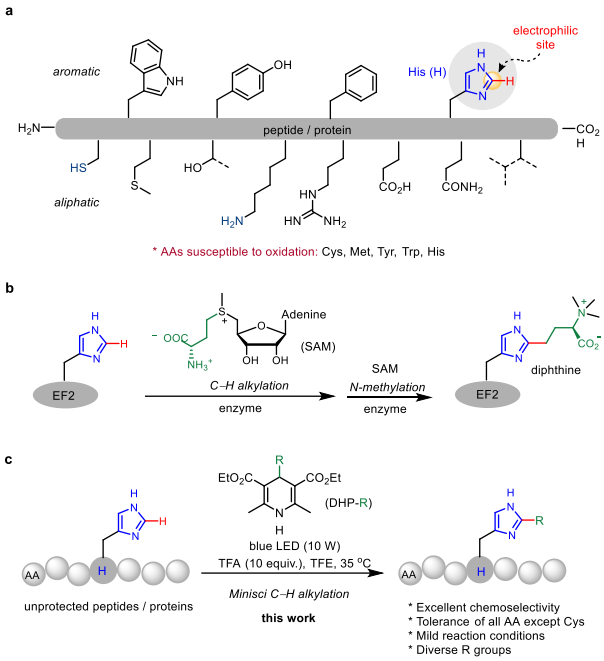
ABSTRCT: Histidine (His) carries a unique heteroaromatic imidazole side chain and plays irreplaceable functional roles in peptides and proteins. Existing strategies for site-selective histidine modification predominantly rely on the N-substitution reactions of the moderately nucleophilic imidazole group, which inherently suffers from the interferences from lysine and cysteine residues. Chemoselective modification of histidine remain one of the most difficult challenges in peptide chemistry. Herein, we report peptide modification via radical-mediated chemoselective C–H alkylation of histidine using C4-alkyl-1,4-dihydropyridine (DHP) reagents under visible light promoted conditions. The method exploits the electrophilic reactivity of the imidazole ring via a Minisci-type reaction pathway. This method exhibits an exceptionally broad scope for both peptides and DHP alkylation reagents. Its utility has been demonstrated in a series of important peptide drugs, complex natural products, and a small protein. Distinct from N-substitution reactions, the unsubstituted nitrogen groups of the modified imidazole ring are conserved in the C–H alkylated products.

INTRODUCTION

Peptides are one of the most important classes of biomolecules, playing a wide range of essential roles in living systems.[1] Naturally occurring or synthetic peptides have long been used as therapeutic agents and continue to serve as invaluable platforms for new drug development.[2] In comparison to *de novo* synthesis, methods for site-selective modification of existing peptides could provide a more straightforward and cost-effective approach to diversify peptides for functional studies.[3] Despite significant development, existing methods for peptide modification are largely limited to reactions of nucleophilic residues such as cysteine (Cys)[4] and lysine (Lys).[5] Moving beyond the functionalization of these nucleophilic residues would dramatically expand the arsenal of peptide functionalization methods.[6] In this context, direct functionalization of ubiquitous C–H bonds could offer a different set of reactivity and selectivity for peptide modification.[7] In contrast to the development of C–H functionalization of small molecules,[8] peptide C–H functionalization is still in its infancy.[9] The presence of various interfering functional groups on peptides poses a significant obstacle to chemoselectivity (Scheme 1a).

Histidine (His) carries an electron-deficient heteroaromatic imidazole side chain, and plays irreplaceable roles in protein function including metal ion coordination, hydrogen bond donor/acceptor, proton shuttle, and nucleophilic catalysis.[10] The important function and rarity of His residues (approximately 2.2% in proteins) make it an enticing target for peptide modification. Most reported strategies for His modification are predominantly based on the N-alkylation or phosphorylation of the imidazole ring.[11] However, interference of more nucleophilic Cys and Lys residues has significantly limited the scope of these methods. In addition, the N-modifications inevitably abolish the function of



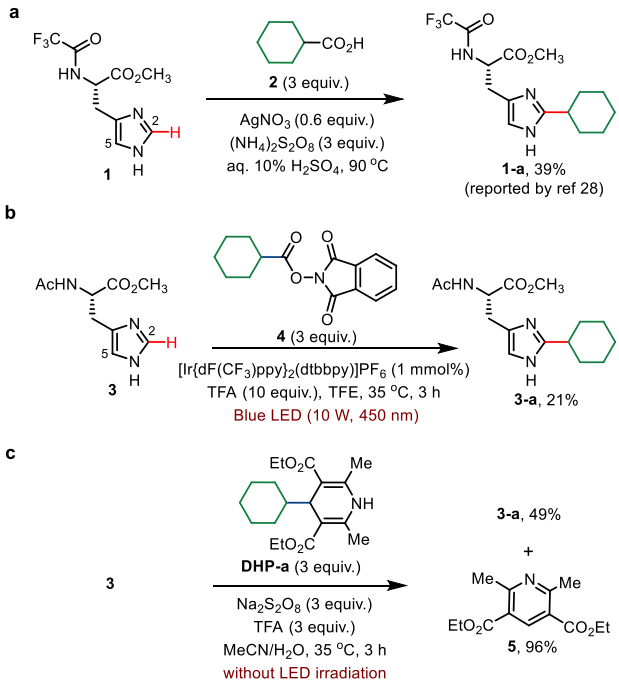
Scheme 1. Histidine-specific peptide modification via radical-mediated chemoselective C–H functionalization. **a**, Representative proteinogenic amino acid residues of peptides and proteins. **b**, Biosynthesis of diphthine via enzyme-catalyzed C_2-selective C–H functionalization of His with S-adenosylmethionine (SAM). **c**, Visible-light-promoted Minisci-type C_2-selective C–H functionalization of His with DHP-R reagents. EF2, translation elongation factor 2. Circles and AAs represent amino acids. DHP = 1,4-dihydropyridine. TFA, trifluoroacetic acid. TFE, trifluoroethanol.

adenosylmethionine (SAM), a key post-translational modification (PTM) for diphthine biosynthesis (Scheme 1b).[12] Although the mechanism of biosynthesis has not been fully elucidated, it appears similar to Minisci-type C–H functionalization of electron-deficient heteroarenes via radical-mediated pathway.[13] Herein, we report a selective, efficient and broadly applicable chemical method for His-specific peptide modification via radical-mediated C_2-selective C–H alkylation of imidazole using various 4-alkyl-1,4-dihydropyridines (DHP) reagents under the irradiation of visible light (Scheme 1c). The critical nitrogen functional groups of histidine are conserved in the alkylated products. This reaction proceeds under mild conditions and exhibits excellent scope for both peptides and DHP reagents. Mechanistic studies indicated that the DHP reagent not only serves as a donor of alkyl radicals, but also as a H atom acceptor thus an oxidant. The avoidance of external oxidant is critical to minimize side reactions of peptides bearing oxidation sensitive residues.

RESULTS AND DISCUSSION

Useful late stage C–H functionalization of peptides demands orthogonal reactivity selective for target C–H bonds and compatibility with various peptide functional groups. Beside interferences from nucleophilic groups, the oxidation of Cys, Met, His, Trp and Tyr need to be avoided.[14] In comparison to electron-neutral Phe and electron-rich Tyr and Trp residues, His carries a unique electron-deficient heteroarene imidazole side chain. Moreover, the nitrogen atom of the imidazole can be protonated under physiological conditions to make the C_2 position of imidazolium a distinct electrophilic site on peptide side chains and backbones. We reasoned that the electrophilicity of the imidazole ring could be exploited by a Minisci-type radical-mediated C–H alkylation reaction for chemoselective modification of His. This proposed Minisci reaction would proceed through attack of nucleophilic alkyl radical on the electrophilic C_2 position of imidazole, followed by single electron transfer oxidation (SET), and deprotonation/re-aromatization to give the alkylated product. Minisci reactions of His have been sporadically studied. Notably, Jain and co-workers reported a C–H alkylation of a simple His derivative with free carboxylic acid as the alkyl radical donor, and $(NH_4)_2S_2O_8$ as the oxidant, under strongly acidic conditions at elevated temperature, giving product **1-a** in moderate yield (Scheme 2a).[15]

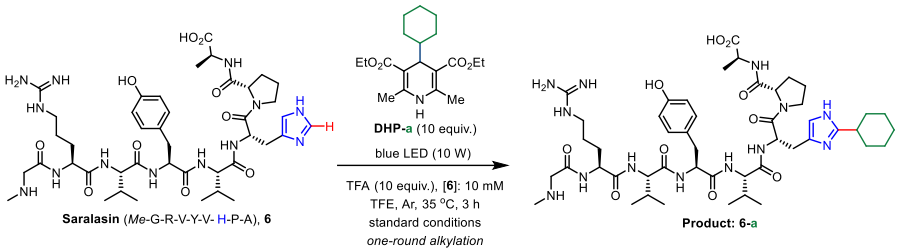
Encouraged by Jain's study and recent advances in photoredox catalysis,[16] we commenced an investigation of Minisci-type C–H alkylation of simple His derivatives under mild operating conditions, with the ultimate aim of application to complex peptide substrates. After extensive experimentations with model His substrate **3**, two protocols stood out. Firstly, reaction of **3** with cyclohexyl *N*-hydroxyphthalimide ester **4** under the irradiation of blue LED light (10 W) using [Ir{dF(CF$_3$)ppy}$_2$(dtbbpy)]PF$_6$ photocatalyst gave the desired C_2-alkylation product **3-a** in 21% isolated yield and with high regio-selectivity (Scheme 2b). However, this reaction exhibited poor compatibility with peptide substrates bearing unprotected Trp, free amine, and C-terminal carboxylate groups.[17] Secondly, reaction of **3** with 3 equivalents of 4-cyclohexyl-1,4-dihydropyridine (**DHP-a**), 3 equivalents of trifluoroacetic acid (TFA) and 3 equivalents of $Na_2S_2O_8$ oxidant at 35 ℃ without irradiation of visible light gave product **3-a** in a 49% isolated yield and with excellent regio-selectivity. Pyridine compound **5** was formed as the major byproduct in > 90% yield (Scheme 2c). A similar non-irradiated protocol for Minisci alkylation of N-heteroarenes using 4-alkyl-1,4-dihydropyridine reagents as alkyl donors was recently reported by Molander and co-workers.[18]



Scheme 2. Minisci-type C–H alkylation of simple histidine derivative. **a**, Alkylation of histidine with carboxylic acid reagent and strong oxidant at elevated temperature. **b**, Preliminary results on C–H alkylation of histidine at ambient temperature under photoredox catalysis. **c**, Preliminary results on oxidant-promoted C–H alkylation of histidine with C$_4$-cyclohexyl 1,4-dihydropyridine (**DHP-a**) without light irradiation.

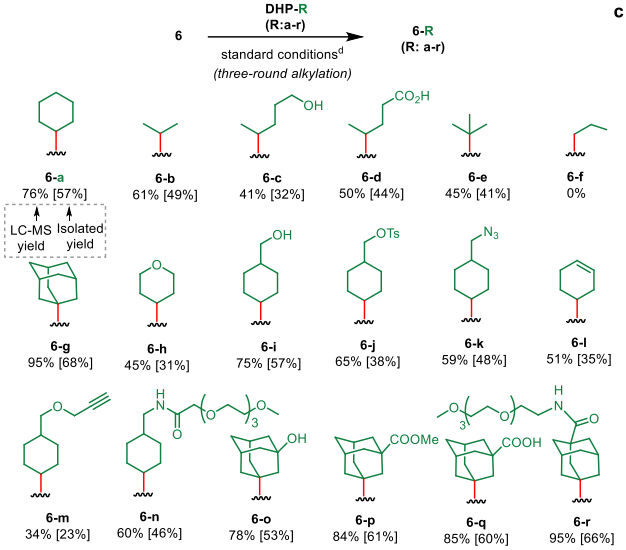
Next, the use of **DHP-a** for C–H alkylation of His was evaluated with more complex substrate saralasin **6**, a potent antihypertensive octapeptide carrying a single mid-chain His (Scheme 3a). As shown in entry 1, the reaction of **6** under Molander's conditions using the combination of **DHP-a** and $Na_2S_2O_8$ without light irradiation gave 21% yield of the desired product **6-a**, a considerable amount of oxidized byproducts (~27%, see Figure S4), and 52% of unconsumed **6** based on liquid chromatography–mass spectrometry (LC-MS) analysis. Undesired oxidation of side chains such as Tyr, Trp and Met is one of the major complicating factors in peptide synthesis and modification, causing serious problem for purification.[14, 19] To our surprise, the formation of undesired oxidation byproducts was significantly suppressed by omitting $Na_2S_2O_8$ oxidant and using excess amount of **DHP-a** and TFA under the visible light irradiation conditions. As shown in entry 2, reaction of **6** with 10 equivalents of **DHP-a** and 10 equivalents of TFA in trifluoroethanol (TFE) solvent under the irradiation of a 10 W blue LED light for 3 hours at 35 °C under argon atmosphere gave 41% of **6-a**, 58% of unconsumed **6**, and little undesired oxidation side products. Besides pyridine **5**, a new byproduct corresponding to a di-hydrogenated form of **DHP-a** was identified (see Scheme 6 for a mechanistic discussion). The conversion of **6** can be improved by a reiterative alkylation procedure. Treating the first alkylation reaction mixture with cold diethyl ether, drying the precipitate and subjecting it to the same alkylation procedure (10 equivalents of **DHP-a** and TFA in TFE), giving **6-a** in 57% LC-MS yield. A third round of precipitation/alkylation operat-

a



Entry	Change from standard conditions, reagents (equiv.)	LC-MS yield of 6-a (%)[a]
1	TFA (10 → 15); TFE → MeCN/H_2O (1/1); + $Na_2S_2O_8$ (12); dark	21
2	Standard conditions	41 57 (two rounds)[b] 76 (three rounds)[c]
3	DHP-a (10 → 5); TFA (10 → 5)	31
4	DHP-a (10 → 20); TFA (10 → 20)	31
5	3 h → 5 h	42
6	Ar → air	6
7	TFE → hexafluoroisopropanol (HFIP)	18
8	TFE → MeCN	25
9	TFE → MeCN/H_2O (3/1)	8
10	no TFA	15
11	TFA → AcOH	25
12	Dark; 25 °C	10
13	Dark; 45 °C	15
14	Dark; 25 °C; no TFA	0

b



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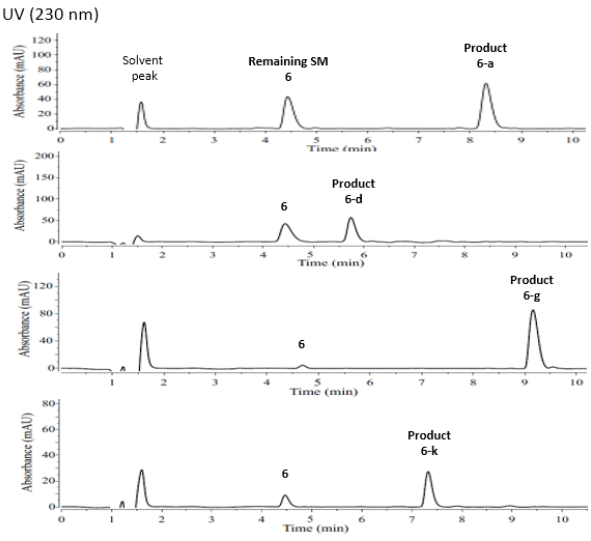
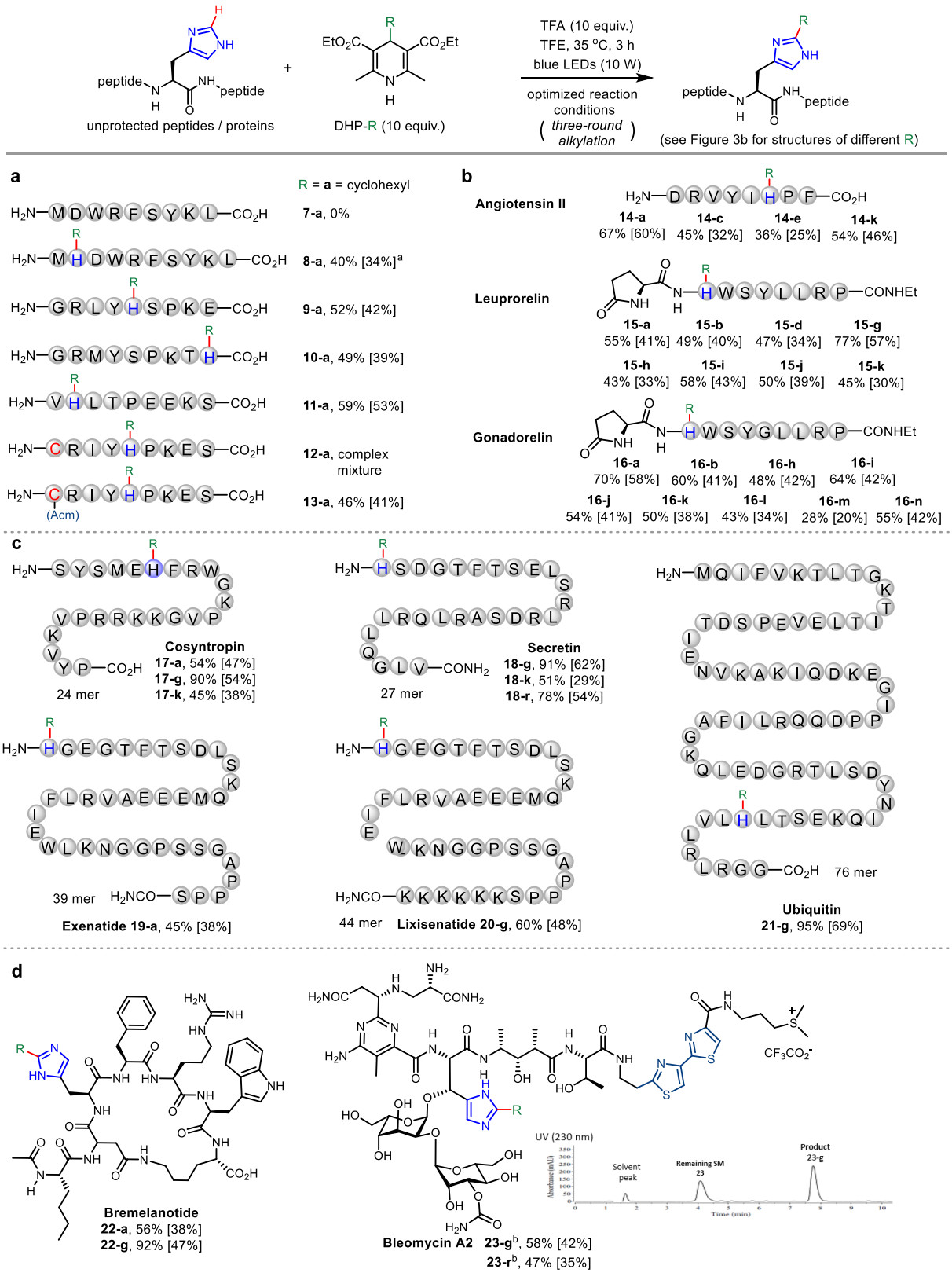


Scheme 3. Development of His-specific alkylation reaction of saralasin **6**. **a**, Optimization of reaction conditions for His-selective functionalization of saralasin **6** with **DHP-a**. **b**, Scope of DHP reagents under optimized conditions using the three-round alkylation procedure. Compounds **6-R** are alkylation products of **6** bearing different R groups (marked in green). **c**, LC trace of solids obtained after diethyl ether precipitation. [a]LC-MS yields were estimated by UV absorption at 230 nm of the peak corresponding to the alkylated peptide product versus the sum of all peptide peaks (also checked by MS) of the crude reaction mixture after precipitation treatment to remove the majority of nonpeptide materials. [b]LC-MS yields after two rounds of alkylation. [c]LC-MS yields after three rounds of alkylation. [d]Standard conditions: peptide (2.0 μmol, 1 equiv.), DHP (20.0 μmol, 10 equiv.), TFA (20.0 μmol, 10 equiv.) in TFE (0.2 mL, 10 mM) under irradiation of blue LED (10 W) under argon (Ar) atmosphere at 35 ℃ for 3 h. After treating the reaction mixture with cold diethyl ether, the precipitate was collected and analyzed by LC-MS (one-round alkylation). The precipitate can be subjected to the same reaction conditions for one or two more times (two or three-round alkylation). The isolated yields by HPLC were provided in square brackets.



Scheme 4. Substrate scope of the visible light-mediated C–H alkylation of His for peptide labeling. **a**, Evaluation of the tolerance of AA side chains with **DHP-a**. **b**, Chemo-selective His C_2-position functionalization of bioactive peptides (8-10 amino acids). **c**, Chemo-selective His C_2-position functionalization of complex bioactive peptides (24-76 amino acids). **d**, Chemo-selective His C_2-position functionalization of cyclic peptide bremelanotide and bleomycin glycopeptide. LC-MS yields were estimated by UV absorption (230 nm) of the peak of alkylated peptide product vs the sum of all peptide peaks (analyzed by MS) of the crude reaction mixture after precipitation treatment to remove the majority of nonpeptide materials. [a]Isolated yields were provided in square brackets. [b]Bleomycin A2 (5.0 μmol, 1 equiv.), **DHP-g**, **-r** (50.0 μmol, 10 equiv.) in TFE (0.5 mL, 10 mM) under irradiation of 10 W blue LED and Ar atmosphere, at 35 ℃, 3 h, two-round alkylation.

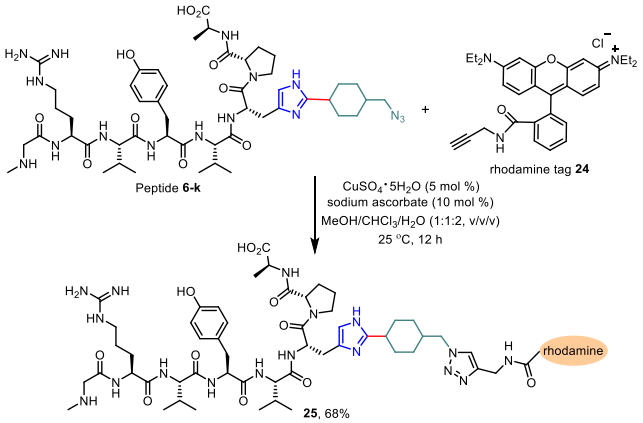
ions gave **6-a** in 76% LC-MS yield (Scheme 3b, 57% isolated yield) with negligible amount of side products (see chromatogram of reaction mixture in Scheme 3c). NMR and tandem MS/MS analyses of **6-a** confirmed the C_2 regioselectivity of the alkylation reaction (see Supporting Information). Several other observations made during reaction optimization are noteworthy: 1) TFE is much better than any other solvents examined (entries 7–9), owing to its special ability to solubilize peptides and facilitate radical-mediated reactions. 2) The addition of TFA significantly improves reaction yield (entries 10 and 11). 3) Small amounts of product are obtained without LED irradiation (entries 12 and 13). In comparison, a reaction performed in the absence of LED irradiation and TFA gave no product (entry 14). This indicates that TFA could promote the activation of DHP reagents to a small extent without light irradiation. The activation is further enhanced by LED irradiation. Similar activations of DHP reagents by heat, Lewis acid, SET oxidant or light irradiation have been reported. 4) Decreases or increases in the loading of DHP reagents or extended reaction time did not improve the reaction efficiency (entries 3–5). 5) Significant amount of oxidized side products (~16%) were formed under air atmosphere (entry 6, see Figure S11).

As shown in Scheme 3b, reactions of saralasin **6** with DHP reagents carrying different C_4-alkyl groups were evaluated using the optimized three-round alkylation protocol. These reagents can be readily prepared in a single step in high yield from a three component reaction of corresponding alkylated aldehyde, ethyl acetoacetate and ethyl 3-aminocrotonate.[18, 20] In general, cyclic or acyclic, and secondary or tertiary alkyl groups work well, forming desired products in moderate to excellent yield based on LC-MS analysis. Primary alkyl groups show significantly lower reactivity (e.g. **6-f**). A wide range of functional groups such as hydroxyl (**6-c**), free carboxylic acid (**6-d** and **6-q**), ether (**6-h**), tosylate (**6-j**), azido (**6-k**), alkene (**6-l**), alkyne (**6-m**), polyethylene glycol (**6-n** and **6-r**) and ester (**6-p**) were tolerated. Notably, >90% yield of **6-g**, **-r** bearing an adamantyl substituent were obtained using **DHP-g**, **-r**. As shown in Scheme 3c, chromatograms of selected reaction mixtures indicated the reactions typically proceeded with high chemo-selectivity, forming trace amounts of side products. All products were analyzed with tandem MS/MS to confirm the site-selectivity of the reaction (see Supporting Information for details).

The scope of this alkylation protocol was next investigated with peptide substrates of varied length and composition (Scheme 4). To our delight, all proteinogenic amino acids except free Cys were compatible with our alkylation protocol using **DHP-a** (Scheme 4a). No alkylation occurred to 9-mer peptide **7**, which has no His but carries three aromatic residues Trp, Phe, and Tyr. Selective His C–H alkylation was achieved in good isolated yields for **8-a** to **11-a**. The reaction of peptide **12** containing a free Cys residue gave complex results (see Figure S122). It is well-known that free thiol group is reactive under radical-mediated conditions.[21] In comparison, alkylation of peptide **13** carrying an acetamidomethyl (Acm)-protected Cys residue worked well to give the desired product **13-a** in 46% LC-MS yield.

This protocol worked well with a series of small-sized His-containing peptides drugs of less than 10 AA residues, such as angiotensin II (a cardiovascular drug), leuprorelin (for treatment of prostate or breast cancer) and gonadorelin (for amenorrhea and hypogonadism). All the peptides reacted with the selected DHPs, affording the desired products **14-a** to **16-n** in moderate to good yields (Scheme 4b). As shown in Scheme 4c, medium-sized peptide drugs (of 24-76 residues) also worked well. Cosyntropin (a synthetic adrenocorticotropic hormone) was alkylated with **DHP-a**, **-g**, and **-k**, giving products **17-a**, **-g** and **-k** in good to excellent yields (45-90% LC-MS yields). Secretin (a diagnostic agent for pancreatic functional diagnoses) reacted with DHPs to give products **18-g**, **-k** and **-r**. Exenatide (a blockbuster drug for treatment of type-2 diabetes) was alkylated with **DHP-a** to provide a 45% LC-MS yield. **20-f**, an analog of lixisenatide (a synthetic type-2 diabetes drug), was obtained in good yield. To our delight, ubiquitin, a small-sized protein of 76 residues, was alkylated with almost full conversion to generate **21-g** (>90% LC-MS yield). The circular dichroism spectrum of **21-g** is identical to that of expressed ubiquitin, indicating intact α-helical structure (see Figure S262).

The versatility of this transformation was also demonstrated in the late stage C–H functionalization of complex peptides bearing various unnatural AA residues (Scheme 4d). Bremelanotide, a cyclic peptide for treatment of female sexual dysfunction, reacted with **DHP-a, -g** to give products **22-a**, **-g** in 56% and 92% LC-MS yield respectively. Bleomycin A2 is a potent glycopeptide antibiotic and anticancer agent.[22] Its structure contains a number of potential interfering groups such as bis-thiazole, pyrimidine, sulfonium ion groups, and an acid-sensitive disaccharide moiety. The histidine residue of bleomycin serves as a ligand for iron, and is essential for biological activity. Modification of the histidine residue via chemical or enzymatic approaches has been previously impossible. We were pleased to find that the alkylation reaction of Bleomycin A2 with admantyl substituted **DHP-g**, **-r** proceeded in 58% and 47% LC-MS yield and with excellent chemoselectivity in the absence of TFA additive using a two-round alkylation procedure.

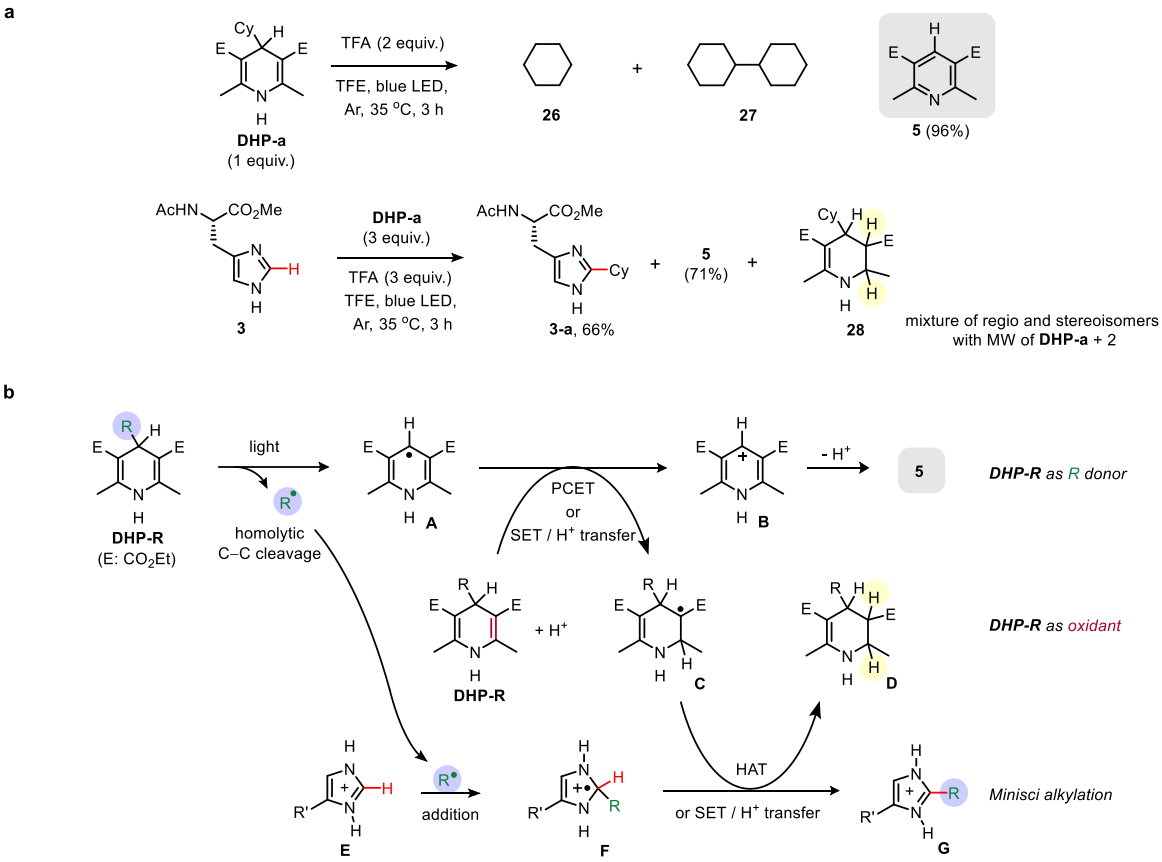


Scheme 5. Secondary labelling of alkylated peptide by azide-alkyne click reaction.

As shown in Scheme 5, alkylated peptide **6-k** bearing an azido handle was subjected to a copper-catalyzed azide-alkyne cycloaddition reaction with a Rhodamine fluorophore to give product **25** in 68% isolated yield.

MECHANISTIC STUDY

Our C_2-selective alkylation of His with DHP reagents likely follows the general mechanism of radical-mediated Minisci-type C–H functionalization of electron-deficient heteroarenes. A critical feature of our protocol is the exclusion of external oxidants, which prevents side reactions of oxidation-prone residues and enables chemoselective His modification in a comple-



Scheme 6. Mechanistic proposal of photo-mediated C–H alkylation of His with DHP reagents. **a**, Control experiments with DHP-a. **b**, Proposed main reaction pathway of C–H alkylation of His featuring a dual role of DHP reagent.

x molecular setting. However, an oxidant is required to balance the net dehydrogenative Minisci reaction. It is well-known that DHP reagents such as Hantzsch esters are excellent reductants. Recent studies by Nishibayashi,[23] Li,[24] Molander,[25] Melchiorre and others[26] have shown that alkyl-substituted DHP reagents (DHP-R) can undergo C-C cleavage to generate an alkyl radical R• under the activation of heat, Lewis acid, SET oxidant or light irradiation.[27] As shown in Scheme 6a, irradiation of **DHP-a** in TFE with TFA gave a mixture of cyclohexane **26**, bis-cyclohexane **27** (trace amount, detected by GC-MS), and pyridine product **5** (>90% yield). This indicates that cyclohexyl radical R• is generated under our reaction conditions. Furthermore, irradiation of simple His substrate **3** with 3 equivalents of **DHP-a** in the presence of 3 equivalents of TFA gave a mixture of C–H alkylation product **3-a** (66% isolated yield), pyridine **5** (71% isolated yield with respect to **DHP-a** used), and a new tetrahydropyridine byproduct **28**. Compound **28** was formed as a mixture of regio- and diastereomeric isomers of reduced **DHP-a**. Formation of **28** was observed in all the C–H alkylation reactions tested. Compound **28** is unstable and decomposes quickly in air. One of its major isomers was isolated and characterized by ^{1}H, ^{13}C-NMR, GC-MS, and high-resolution MS (see Figures S286-S289). These results strongly suggest that, contrary to the conventional role of reductant, DHP reagents serve as the oxidant in our Minisci reaction system.[28]

As outlined in Scheme 6b, we propose the main pathway of our reaction system starts with homolytic C–C cleavage of DHP-R substrate under visible-light irradiation to generate alkyl radical R• and DHP radical intermediate **A**. **A** can be oxidized by SET or proton-coupled electron transfer (PCET) with another molecule of DHP-R to form DHP cation **B** and radical **C**. Deprotonation of **B** gives aromatized pyridine product **5**. The nucleophilic alkyl radical R• reacts with the protonated His substrate **E** to give radical cation intermediate **F**. Oxidation of **F** by **C** via hydrogen atom abstraction (HAT) or SET/H^{+} transfer gives the final alkylated product **G** along with tetrahydropyridine **D**. As an alternative to the homolytic cleavage process, SET between two DHP-Rs under photo irradiation can generate a pair of radical anion and radical cation (see Figure S281). These intermediates can play similar roles as R• donor and electron/hydrogen atom acceptor in the subsequent steps. Overall, DHP-R reagents serve as both R• donor and oxidant (as acceptor of electron or hydrogen atom) in our light-promoted Minisci-type C–H alkylation of His.

CONCLUSION

In summary, we have developed an efficient and broadly applicable method for late-stage modification of peptides via radical-mediated chemoselective C–H alkylation of histidine residues. In contrast to the nucleophilic substitution reactions used in existing histidine modification methods, the new method exploits the electrophilic reactivity of imidazole ring via a Minisci-type reaction pathway under visible light promoted conditions. Mechanistic studies of the reaction indicate that DHP reagents play unprecedented roles of both alkyl radical donor and oxidant. The exclusion of strong external oxidant is critical to suppress oxidation of the peptide substrates. This method exhibits an exceptionally broad scope for both peptides and

DHP alkylation reagents. Importantly, the unsubstituted nitrogen groups on the imidazole ring of modified histidine are conserved. The utility of this methods has been demonstrated in the modification of a series of important peptide drugs, complex natural products, and a small protein. We are optimistic this method will provide a useful tool to label and fine-tune histidine residues of molecules for both biological and medicinal chemistry study.

EXPERIMENTAL SECTION

Typical procedure for chemoselective C–H alkylation of His under LED irradiation: Saralasin **6** (106.5 mg, 93.4 μmol, 1.0 equiv.), **DHP-a** (312.7 mg, 0.93 mmol, 10.0 equiv.), degassed TFA (70 μL, 0.93 mmol, 10.0 equiv.) and TFE (9.35 mL, 10 mM) were added into a glass vial (20 mL). The mixture was gently purged by argon for 2 mins and sealed with a cap. The reaction mixture was stirred under argon atmosphere and irradiated with two blue LEDs (10 W, 3 cm from the vial) for 3 hours. The reaction temperature was kept around 35 ℃ with a cooling fan. The reaction mixture was concentrated with a stream of argon and precipitated with the addition of 10 mL of ice-cold diethyl ether. The precipitate was collected by decantation after centrifugation and dried under reduced pressure. The crude peptide mixture was re-subjected to the same alkylation operation for two more rounds. The final crude product after precipitation treatment was analyzed by LC-MS and purified by preparative reverse phase HPLC to afford a white peptide powder **6-a** as a TFA salt after lyophilization (65.0 mg, 53.2 μmol, 57% yield).

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.

Detailed experimental procedures and spectral data

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Notes

The authors declare no competing financial interest.

Author Contributions

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REFERENCES

(1) Albericio, F.; Kruger, H. G. Therapeutic Peptides. *Future Med. Chem.* **2012,** *4*, 1527-1531.

(2) (a) Craik, D. J.; Fairlie, D. P.; Liras, S.; Price, D. The Future of Peptide-based Drugs. *Chem. Biol. Drug Des.* **2013,** *81*, 136-147; (b) Henninot, A.; Collins, J. C.; Nuss, J. M. The Current State of Peptide Drug Discovery: Back to the Future? *J. Med. Chem.* **2018,** *61*, 1382-1414.

(3) (a) Boutureira, O.; Bernardes, G. J. L. Advances in Chemical Protein Modification. *Chem. Rev.* **2015,** *115*, 2174-2195; (b) deGruyter, J. N.; Malins, L. R.; Baran, P. S. Residue-Specific Peptide Modification: A Chemist's Guide. *Biochemistry* **2017,** *56*, 3863-3873; (c) Taniguchi, A.; Shimizu, Y.; Oisaki, K.; Sohma, Y.; Kanai, M. Switchable Photooxygenation Catalysts that Sense Higher-order Amyloid Structures. *Nat. Chem.* **2016,** *8*, 974-982; (d) Wadzinski, T. J.; Steinauer, A.; Hie, L.; Pelletier, G.; Schepartz, A.; Miller, S. J. Rapid Phenolic O-glycosylation of Small Molecules and Complex Unprotected Peptides in Aqueous Solvent. *Nat. Chem.* **2018,** *10*, 644-652; (e) Antos, J. M.; McFarland, J. M.; Iavarone, A. T.; Francis, M. B. Chemoselective Tryptophan Labeling with Rhodium Carbenoids at Mild pH. *J. Am. Chem. Soc.* **2009,** *131*, 6301-6308.

(4) (a) Spicer, C. D.; Davis, B. G. Selective Chemical Protein Modification. *Nat. Commun.* **2014,** *5*, 4740-4753; (b) Bondalapati, S.; Jbara, M.; Brik, A. Expanding the Chemical Toolbox for the Synthesis of Large and Uniquely Modified Proteins. *Nat. Chem.* **2016,** *8*, 407-418; (c) Zhang, C.; Vinogradova, E. V.; Spokoyny, A. M.; Buchwald, S. L.; Pentelute, B. L. Arylation Chemistry for Bioconjugation. *Angew. Chem. Int. Ed.* **2019,** *58*, 4810-4839.

(5) Amamoto, Y.; Aoi, Y.; Nagashima, N.; Suto, H.; Yoshidome, D.; Arimura, Y.; Osakabe, A.; Kato, D.; Kurumizaka, H.; Kawashima, S. A.; Yamatsugu, K.; Kanai, M. Synthetic Posttranslational Modifications: Chemical Catalyst-Driven Regioselective Histone Acylation of Native Chromatin. *J. Am. Chem. Soc.* **2017,** *139*, 7568-7576.

(6) Taylor, M. T.; Nelson, J. E.; Suero, M. G.; Gaunt, M. J. A Protein Functionalization Platform Based on Selective Reactions at Methionine Residues. *Nature* **2018,** *562*, 563-568.

(7) (a) Noisier, A. F. M.; Brimble, M. A. C–H Functionalization in the Synthesis of Amino Acids and Peptides. *Chem. Rev.* **2014,** *114*, 8775-8806; (b) Wang, W.; Lorion, M. M.; Shah, J.; Kapdi, A. R.; Ackermann, L. Late-Stage Peptide Diversification by Position-Selective C−H Activation. *Angew. Chem. Int. Ed.* **2018,** *57*, 14700-14717.

(8) (a) Cernak, T.; Dykstra, K. D.; Tyagarajan, S.; Vachal, P.; Krska, S. W. The Medicinal Chemist's Toolbox for Late Stage Functionalization of Drug-like Molecules. *Chem. Soc. Rev.* **2016,** *45*, 546-576; (b) Godula, K.; Sames, D. C-H Bond Functionalization in Complex Organic Synthesis. *Science* **2006,** *312*, 67-72; (c) Qin, Y.; Zhu, L.; Luo, S. Organocatalysis in Inert C–H Bond Functionalization. *Chem. Rev.* **2017,** *117*, 9433-9520; (d) Shugrue, C. R.; Miller, S. J. Applications of Nonenzymatic Catalysts to the Alteration of Natural Products. *Chem. Rev.* **2017,** *117*, 11894-11951.

(9) (a) Noisier, A. F. M.; García, J.; Ionuț, I. A.; Albericio, F. Stapled Peptides by Late-Stage C(sp3)−H Activation. *Angew. Chem. Int. Ed.* **2017,** *56*, 314-318; (b) Osberger, T. J.; Rogness, D. C.; Kohrt, J. T.; Stepan, A. F.; White, M. C. Oxidative Diversification of Amino Acids and Peptides by Small-molecule Iron Catalysis. *Nature* **2016,** *537*, 214-219; (c) Ruan, Z.; Sauermann, N.; Manoni, E.; Ackermann, L. Manganese-Catalyzed C−H Alkynylation: Expedient Peptide Synthesis and Modification. *Angew. Chem. Int. Ed.* **2017,** *56*, 3172-3176.

(10) (a) Agostini, F.; Völler, J.-S.; Koksch, B.; Acevedo-Rocha, C. G.; Kubyshkin, V.; Budisa, N. Biocatalysis with Unnatural Amino Acids: Enzymology Meets Xenobiology. *Angew. Chem. Int. Ed.* **2017,** *56*, 9680-9703; (b) Liao, S.-M.; Du, Q.-S.; Meng, J.-Z.; Pang, Z.-W.; Huang, R.-B. The Multiple Roles of Histidine in Protein Interactions. *Chem. Cent. J.* **2013,** *7*, 44-55.

(11) (a) Jia, S.; He, D.; Chang, C. J. Bioinspired Thiophosphorodichloridate Reagents for Chemoselective Histidine Bioconjugation. *J. Am. Chem. Soc.* **2019,** *141*, 7294-7301; (b) Koniev, O.; Wagner, A. Developments and Recent Advancements in the Field of Endogenous Amino Acid Selective Bond Forming Reactions for Bioconjugation. *Chem. Soc. Rev.* **2015,** *44*, 5495-5551.

(12) Zhang, Y.; Zhu, X.; Torelli, A. T.; Lee, M.; Dzikovski, B.; Koralewski, R. M.; Wang, E.; Freed, J.; Krebs, C.; Ealick, S. E.; Lin, H. Diphthamide Biosynthesis Requires an Organic Radical Generated by an Iron–sulphur Enzyme. *Nature* **2010,** *465*, 891-896.

(13) Buratti, W.; Gardini, G. P.; Minisci, F.; Bertini, F.; Galli, R.; Perchinunno, M. Nucleophilic Character of Alkyl Radicals. V. Selective Homolytic α-oxyalkylation of Heteroaromatic Bases. *Tetrahedron* **1971,** *27*, 3655-3668.

(14) Li, S.; Schöneich, C.; Borchardt, R. T. Chemical Instability of Protein Pharmaceuticals: Mechanisms of Oxidation and Strategies for Stabilization. *Biotechnol. Bioeng.* **1995,** *48*, 490-500.

(15) Jain, R.; Cohen, L. A.; El-Kadi, N. A.; King, M. M. Regiospecific Alkylation of Histidine and Histamine at C-2. *Tetrahedron* **1997,** *53*, 2365-2370.

(16) (a) Jin, J.; MacMillan, D. W. Direct Alpha-arylation of Ethers through the Combination of Photoredox-mediated C-H Functionalization and the Minisci Reaction. *Angew. Chem. Int. Ed.* **2015,** *54*, 1565-1569; (b) Proctor, R. S. J.; Davis, H. J.; Phipps, R. J. Catalytic Enantioselective Minisci-type Addition to Heteroarenes. *Science* **2018,** *360*, 419-422; (c) Yi, H.; Zhang, G.; Wang, H.; Huang, Z.; Wang, J.; Singh, A. K.; Lei, A. Recent Advances in Radical C–H Activation/Radical Cross-Coupling. *Chem. Rev.* **2017,** *117*, 9016-9085; (d) Bottecchia, C.; Noël, T. Photocatalytic Modification of Amino Acids, Peptides, and Proteins. *Chem. Eur. J.* **2019,** *25*, 26-42; (e) Fawcett, A.; Pradeilles, J.; Wang, Y.; Mutsuga, T.; Myers, E. L.; Aggarwal, V. K. Photoinduced Decarboxylative Borylation of Carboxylic Acids. *Science* **2017,** *357*, 283-286; (f) Huang, X.; Meggers, E. Asymmetric Photocatalysis with Bis-cyclometalated Rhodium Complexes. *Acc. Chem. Res.* **2019,** *52*, 833-847.

(17) We found that excess of redox-active ester could undergo nucleophilic substitution reaction with free amine to form amide bond even in the presence of TFA. It's also known that C-terminal carboxylic acids of peptides or β-position of trypophan can be activated under these photoredox conditions, see: (a) Bloom, S.; Liu, C.; Kölmel, D. K.; Qiao, J. X.; Zhang, Y.; Poss, M. A.; Ewing, W. R.; MacMillan, D. W. C. Decarboxylative Alkylation for Site-selective Bioconjugation of Native Proteins via Oxidation Potentials. *Nat. Chem.* **2018,** *10*, 205-211; (b) Yu, Y.; Zhang, L.-K.; Buevich, A. V.; Li, G.; Tang, H.; Vachal, P.; Colletti, S. L.; Shi, Z.-C. Chemoselective Peptide Modification via P hotocatalytic Tryptophan β-Position Conjugation. *J. Am. Chem. Soc.* **2018,** *140*, 6797-6800.

(18) Gutiérrez-Bonet, Á.; Remeur, C.; Matsui, J. K.; Molander, G. A. Late-Stage C–H Alkylation of Heterocycles and 1,4-Quinones via Oxidative Homolysis of 1,4-Dihydropyridines. *J. Am. Chem. Soc.* **2017,** *139*, 12251-12258.

(19) Pearlman, R.; Bewley, T. A. Stability and Characterization of Human Growth Hormone. In *Stability and Characterization of Protein and Peptide Drugs: Case Histories;* Wang, J., Pearlman, R., Eds.; Plenum Press: New York, 1993; 5, pp 1-58.

(20) Tewari, N.; Dwivedi, N.; Tripathi, R. P. Tetrabutylammonium Hydrogen Sulfate Catalyzed Eco-friendly and Efficient Synthesis of Glycosyl 1,4-dihydropyridines. *Tetrahedron Lett.* **2004,** *45*, 9011-9014.

(21) Denes, F.; Pichowicz, M.; Povie, G.; Renaud, P. Thiyl Radicals in Organic Synthesis. *Chem. Rev.* **2014,** *114*, 2587-693.

(22) Galm, U.; Hager, M. H.; Van Lanen, S. G.; Ju, J.; Thorson, J. S.; Shen, B. Antitumor Antibiotics: Bleomycin, Enediynes, and Mitomycin. *Chem. Rev.* **2005,** *105*, 739-758.

(23) Nakajima, K.; Nojima, S.; Nishibayashi, Y. Nickel- and Photoredox-Catalyzed Cross-Coupling Reactions of Aryl Halides with 4-Alkyl-1,4-dihydropyridines as Formal Nucleophilic Alkylation Reagents. *Angew. Chem. Int. Ed.* **2016,** *55*, 14106-14110.

(24) Chen, W.; Liu, Z.; Tian, J.; Li, J.; Ma, J.; Cheng, X.; Li, G. Building Congested Ketone: Substituted Hantzsch Ester and Nitrile as Alkylation Reagents in Photoredox Catalysis. *J. Am. Chem. Soc.* **2016,** *138*, 12312-12315.

(25) Gutiérrez-Bonet, Á.; Tellis, J. C.; Matsui, J. K.; Vara, B. A.; Molander, G. A. 1,4-Dihydropyridines as Alkyl Radical Precursors: Introducing the Aldehyde Feedstock to Nickel/Photoredox Dual Catalysis. *ACS Catal.* **2016,** *6*, 8004-8008.

(26) (a) Zhang, H.-H.; Zhao, J.-J.; Yu, S. Enantioselective Allylic Alkylation with 4-Alkyl-1,4-dihydro-pyridines Enabled by Photoredox/Palladium Cocatalysis. *J. Am. Chem. Soc.* **2018,** *140*, 16914-16919; (b) Huang, W.; Cheng, X. Hantzsch Esters as Multifunctional Reagents in Visible-Light Photoredox Catalysis. *Synlett* **2017,** *28*, 148-158.

(27) (a) Buzzetti, L.; Prieto, A.; Roy, S. R.; Melchiorre, P. Radical-Based C−C Bond-Forming Processes Enabled by the Photoexcitation of 4-Alkyl-1,4-dihydropyridines. *Angew. Chem. Int. Ed.* **2017,** *56*, 15039-15043; (b) van Leeuwen, T.; Buzzetti, L.; Perego, L. A.; Melchiorre, P. A Redox-Active Nickel Complex that Acts as an Electron Mediator in Photochemical Giese Reactions. *Angew. Chem. Int. Ed.* **2019,** *58*, 4953-4957.

(28) (a) Eisner, U. Synthesis of 1,2-dihydropyridines. *J. Chem. Soc. D,* **1969,** 1348-1349; (b) Fukuzumi, S.; Hironaka, K.; Tanaka, T. Photoreduction of Alkyl Halides by an NADH Model Compound. An Electron-transfer Chain Mechanism. *J. Am. Chem. Soc.* **1983,** *105*, 4722-4727.