Visible-light-mediated catalyst-free synthesis of unnatural α-amino acids and peptide macrocycles

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The visible light induced, photocatalysts or photoabsorbing EDA complexes mediated cleavage of pyridinium C-N bond were reported in the past years. Here, we report an ionic compound promote homolytic cleavage of pyridinium C-N bond by exploiting the photonic energy from visible light. This finding is successfully applied in deaminative hydroalkylation of a series of alkenes including naturally occurring dehydroalanine, which provides an efficient way to prepare β-alkyl substituted unnatural amino acids under mild and photocatalyst-free conditions. Importantly, by using this protocol, the deaminative cyclization of peptide backbone N-terminals is realized. Furthermore, the use of Et₃N or PPh₃ as reductants and H₂O as hydrogen atom source is a practical advantage. We anticipate that our protocol will be useful in peptide synthesis and modern peptide drug discovery.
Peptides are indispensable bioactive components for exerting biological function normally in various cells. Thus, therapeutic peptide is deemed to be an exquisite substitution of an endogenous molecule which possesses high affinity and selectivity against a pharmacologically diverse set of biological targets. Although remarkable achievements have been made in peptide research, poor membrane permeability, low metabolic resistance, and bioavailability still are insurmountable barriers to endogenous bioactive peptide therapeutic. In this context, several methods have been developed and applied to solve these problems, such as incorporation of unnatural amino acids (UAAs) or macrocyclizations of linear peptides.

In recent years, with the rapid development of photochemistry, visible light promoted radical coupling reactions have become important pathway for building chemical bonds. Owing to mild reaction conditions and excellent functional group tolerance, photoinduced chemical transformations provided an excellent strategy for chemoselective biomolecule modification, which are widely applied in the modification of amino acids, peptides, and proteins. The modification of amino acids is an important strategy for preparing UAAs, such as the modification of glycine, cysteine, tyrosine, tryptophan, histidine, etc. Dehydroalanine (Dha) is a naturally occurring amino acid, and also can be easily prepared from Ser, Cys, and selenocystein, which is preinserted at the position of interest in peptides and proteins. In recent years, Dha has been used as a versatile backbone for synthesis of UAAs. Although these methods provided efficient ways for preparation of various UAAs, the accessing of β-alkyl substituted UAAs were still limited, and the reactions required either transition metal catalysts or stoichiometric amount of metal reagents. Furthermore, the compatible methods for modification of Dha unit in peptides are still rare, and the related peptide cyclization protocol based on functionalization of Dha is still underdeveloped.

The traditional peptide cyclization methods are based on lactamization and disulfide bond formation. In the past decades, with the advent of new generations of peptide pharmaceuticals, varying the nature of ring-forming linkage in peptide macrocycles became a necessity. In this context, transition metal-catalyzed peptide macrocyclization strategies are gaining increasing popularity, and include C–H activation, oxidative cross-couplings, heteroatom ligation, and radical reactions, etc. MacMillan and co-workers recently reported a visible-light-promoted decarboxylative Giese reaction, which provided an efficient way for macrocyclization of peptide backbone C-terminal in an alternative manner besides lactam bond (Fig. 1).

Primary amines are naturally occurring and chemically diverse starting materials, and deaminative reaction of primary amines has emerged as an important strategy for generating alkyl radicals. Katritzky salts derived from ω-1° and ω-2° amines, as well as imines derived from sterically encumbered ω-3° primary amines are important alkyl radical precursors. In line with our interests in photocatalysis and peptide synthesis, we here report a visible light induced, ionic compounds promoted C–N cleavage of Katritzky salts, which is successfully used in the preparation of β-alkyl substituted UAAs and macrocyclization of peptide. Notably, Et3N and PPh3 are effective to act as single-electron reductants for the reactions.

**Results**

**Design plan.** The Katritzky-type alkyl substituted pyridinium (1) can generate an alkyl radical through a photoinduced dissociative electron transfer. However, the light absorption of alkyl

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**Fig. 1 Design plan.** a Photoredox decarboxylative macrocyclization. b UV-Vis absorption spectrum of 1 with ionic compounds. c Visible light induced ionic compound promoted homolytic fragmentation of pyridinium C–N bond.
Hantzsch esters, or aryl amines) to form a light-absorbing highly dependent on a suitable electron donor (e.g., indoles, EDA complexes triggered SET to alkyl pyridinium, which were promote the release of alkyl radical. Compared with reported lytic C–N bond, irradiation, C–N homolysis would unveil more rich photochemistry. To validate our proposal in Fig.1c, a suitable single-electron reductant (SER) is required. Tertiary amines are previously widely used as SERs. Besides tertiary amines, we speculated that electron-rich tertiary phosphines could serve as SER for the reaction. We assume phosphine could provide a radical cation, which would then react with H2O to give the intermediate 3. The latter could act as an SER again by supplying a second electron and yield phosphate oxide. Overall, the proposed photochemical mechanism to generate C(sp^3)-centered radicals by using phosphate and H2O as SER was not realized before.

### Investigation of reaction conditions.

For our initial explorations (Table 1), we selected N-Ac-Dha methyl ester (4a) (0.1 mmol) as Michael acceptor and N-Boc-protected cyclic pyridinium salt 1 as alkyl radical precursor. Most of the tested tertiary alkyl amines and trivalent tertiary phosphines could serve as SER for the reaction, while Et3N (entry 1) and PPh3 (entry 2) gave the best results, respectively. Notably, in the case when PPh3 was used, stoichiometric amount of triphenylphosphine oxide (Ph3PO) was formed as a byproduct, indicating that PPh3 and H2O were all involved in SET process. 5a was obtained in 50% yield in the absence of K2CO3 under condition A, which might be attributed to the formation of the EDA complex between Katritzky salt 1 and Et3N (entry 3). It is worth noting that the yield of 5a dramatically increased to 97% in the presence of K2CO3 (entry 1). The yield of 5a decreased to 13% in the absence of KOCH3 under condition B (entry 4). In addition to K2CO3 and KOCH3, fluoride additives could also increase the outcome (entries 5 and 6, respectively). Without Et3N or PPh3, only a trace amount of desired product formed (entry 7). The reaction gave diminished yield in the absence of H2O (entry 8). We assumed that H2O might play two roles in the reaction. One is increasing the solubility of an ionic compound; the other is involving in the SET process when PPh3 is used as an SER reagent (Fig. 1c).

**Table 1 Control experiments.**

| Entry | Changes from condition A or B | Yield of 5a (%)^a |
|-------|-------------------------------|------------------|
| 1     | no change from condition A    | 97% (94%)        |
| 2     | no change from condition B    | 88% (84%)        |
| 3     | no K2CO3 under condition A    | 50%              |
| 4     | no KOCH3 under condition B    | 13%              |
| 5     | KF instead of K2CO3 under condition A | 63% |
| 6     | CsF instead of KOCH3 under condition B | 41% |
| 7     | without Et3N or PPh3          | trace            |
| 8     | without H2O                   | <54%             |
| 9     | no light                      | N.D.             |
| 10    | no light, 60 °C               | N.D.             |

^aYield was determined by 1H NMR using 4-bromobenzaldehyde as an internal standard. The value within parentheses refers to isolated yield.

### Substrate scopes.

Having established the optimal reaction conditions (Table 1, entries 1 and 2), we examined a series of alkenes (4b–4l) as shown in Fig. 2a. Similar to Dha, another naturally occurring dehydroamino acid derived alkene, namely dehydrobutyrine, also reacted smoothly to give the corresponding product 5b in 80% yield (4:1 d.r.) under condition A and 76% yield (4:1 d.r.) under condition B. Other alkenes, such as diethyl fumarate, vinyl sulfone, or dimethyl maleonate derived alkene were all compatible in the reaction with good yields (5c–5e, 78–95%). The Michael acceptors bearing amide groups also worked well under optimal conditions to give the desired products in uniformly good yields (5f–5j). Furthermore, we
expanded the reaction scope to β-thiolated amino acid derivative (4k) which was readily prepared from L-cysteine and dehydroalanine derived Karady-Beckwith alkene (4l). The corresponding hydroalkylation products 5k and 5l were obtained in high yields and good r.r. (rotamers mixture ratio)\(^{35}\) or d.r.

Subsequently, we shifted our attention to the scope of Katritzky salts for the synthesis of unnatural α-amino acids using a series of secondary alkyl substituted pyridinium (Fig. 2b). Uniformly good yields were obtained (7a–7b, up to 92%). Importantly, this reaction could be extended to glycine derived Katritzky salt (7b, 92 and 85%), which provided the possibility for deaminative N-terminal macrocyclization of the linear peptide backbone. Furthermore, more complex pyridinium radical precursors bearing various functional groups and structural motifs were tested under condition A and B, which provided corresponding products 7f–7r in moderate to excellent yields. Unfortunately, unactivated primary alkyl substrates failed to give the desired hydroalkylation products (When the unactivated primary alkyl substrate was used in the reaction, significant amounts of byproduct from radical coupling of primary alkyl \(^{14}\) dihydropyridine radical with unactivated primary alkyl \(^{14}\) radical was obtained. See supplementary information for details.).

Next, a variety of Dha containing peptides was prepared to test the functional group tolerance of this reaction with various amino acid residues incorporated. To our delight, peptides bearing glycine, proline, leucine, tryptophan, phenylalanine, or tyrosine units were able to participate in the deaminative conjugation to furnish the desired adducts in yields between 76 and 95% with
other amino acid residues untouched (Fig. 3a–9i). Based on excellent performance on modification of peptides, we turned our attention to deaminative N-terminals macrocyclization of linear peptides. As shown in Fig. 3b, a series of peptides that incorporate a structurally diverse set of amino acids can be successfully cyclized using this deaminative method (11a–11e, 28–40% isolated yields after HPLC purifications). Regrettably, the attempt to macrocyclize Dha contained peptide “on resin” during solid-phase peptide synthesis procedure has failed.

Mechanistic studies. To gain some detailed information for the reaction, a series of mechanistic studies were carried out (see Supplementary information for details). As shown in Fig. 4a, radical trapping experiments indicated that the combination of ionic compounds (such as K₂CO₃, KOCH₃, and CsF) and pyridinium were able to harness photonic energy from visible light and promoted C–N homolysis of Katritzky salt to generate an alkyl radical. In isotope tracking experiments (Fig. 4b, c), when D₂O was used instead of H₂O under Condition A and B, deuterated product 5a–D and 7a–D were obtained in 86 and 34% yield, respectively, revealing that H₂O acted as hydrogen atom source to provide an H⁺ for the hydroalkylation process. Furthermore, stoichiometric amount of triphenylphosphine oxide (Ph₃PO) was formed as the byproduct, which suggested that PPh₃ could possibly provide two electrons in a stepwise way during the SET process (Fig. 4b). The UV-Vis absorption of individual reagents or mixtures were shown in Fig. 4d. None of Et₃N, PPh₃, pyridinium (1), or the mixture of [PPh₃ + 1] showed significant

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**Fig. 3 Modification and macrocyclization of peptides.** a Diastereomers were measured by 1H NMR. b Diastereomers were measured by HPLC. a Isolated yield on 0.2 mmol scale (condition A and condition B). b For 11a and 11c–11e, isolated yields on 0.02 mmol under condition A; for 11b, isolated yield on 0.05 mmol under condition A.
Fig. 4 Mechanistic studies. a Radical trapping experiments. b Isotope tracking experiments of Condition B. c Isotope tracking experiments of Condition A. d Analysis of UV-Vis absorption spectra.
absorption over 400 nm. However, basic ionic compounds, namely K₂CO₃ and KOCH₃, could dramatically increase the absorption of pyridinium (I) in the visible light region, respectively. Interestingly, the combination [Et₃N + 1] showed a slight bathochromic shift, which might be due to the formation of EDA complex between I and Et₃N⁵². Although this EDA complex could possibly mediate the cyclation process (Table 1, entry 3), the yield was significantly lower than for K₂CO₃ promoted reaction (50% vs 97%). Some ionic compounds, such as Cs₂CO₃, K₂PO₄, and NaOCH₃, provide higher levels of visible light absorption; however, only moderate yields were obtained (Table S3 and Fig. S8). The use of KBF₄ and NaBF₄ as ionic additives did not give any desired product, which indicated that BF₄⁻ counter anion in Katritzky salt was not crucial for the transformation (Table S3, Table S13, and Fig. S8).

On the basis of these preliminary results, a possible mechanism is proposed (Fig. 5) (Although the mechanistic investigations suggested that a visible light promoted C-N homolysis of Katritzky salts in the presence of ionic compounds is highly possible in our reaction, the EDA complex pathway cannot be completely ruled out at this stage. See supplementary information for details.). Initially, C-N homolysis of Katritzky salt occurred in the presence of basic ionic compounds under visible light irradiation, followed by the formation of an alkyl radical (R¹) and pyridinium radical cation. Et₃N (PPh₃) was oxidized to nitrogen radical cation (phosphine radical cation) with the formation of 2,4,6-triphenyl-phosphine oxide. The formation of triphenylphosphine oxide. The N bond by exploiting the photonic energy from visible light. This finding was successfully applied in deaminative hydroalkylation of a series of alkenes including naturally occurring Dha, which provided an efficient way to prepare β-alkyl substituted UAs under mild and catalyst-free conditions. Importantly, by using this protocol, the deaminative cyclization of peptide backbone N-terminals was realized. Furthermore, the use of Et₃N or PPh₃ as reductants and H₂O as hydrogen atom source is a significantly practical advantage. Further investigating the mechanism of ionic compounds improved visible light absorption of pyridinium and other synthetic applications are currently underway and will be reported in due course.

Methods

General procedure of condition A. To an oven-dried 10 mL quartz test tube with a stirring bar was added alkene (0.2 mmol), alkyl pyridinium salt (0.5 mmol, 2.5 equiv), Et₃N (0.4 mmol, 2 equiv), and K₂CO₃ (0.2 mmol, 1 equiv). Then, the air was withdrawn and backfilled with Ar (three times). CH₃CN (3 mL) and H₂O (6 mmol, 30 equiv) were added. The mixture was transferred to a violet LED photoreactor (24-W, 420–430 nm), where it was irradiated for 12 h. Then, the reaction was quenched with water (5 mL), extracted with ethyl acetate, washed with brine, dried over anhydrous sodium sulfate, concentrated in vacuo, and purified by column chromatography (hexane/acetonitrile) to afford the product.

General procedure of condition B. To an oven-dried 10 mL quartz test tube with a stirring bar was added alkene (0.2 mmol), alkyl pyridinium salt (0.4 mmol, 2.0 equiv), PPh₃ (0.48 mmol, 2.4 equiv), and KOCH₃ (0.6 mmol, 3.0 equiv). Then, the air was withdrawn and backfilled with Ar (three times). Acetone (3 mL) and H₂O (5 mmol, 25 equiv) were added. The mixture was transferred to a violet LED photoreactor (24-W, 410–420 nm), where it was irradiated for 12 h. Then, the reaction was quenched with water (5 mL), extracted with ethyl acetate, washed with brine, dried over anhydrous sodium sulfate, concentrated in vacuo, and purified by column chromatography (hexane/acetonitrile) to afford the product.

Data availability

The authors declare that the data supporting the findings of this study, including experimental details and compound characterization, are available within the article and its Supplementary information file. All data are available from the corresponding author upon request.

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