Peptide late-stage C(sp³)–H arylation by native asparagine assistance without exogenous directing groups†

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There is a strong demand for novel native peptide motifs for post-synthetic modifications of peptides without pre-installation and subsequent removal of directing groups. Herein, we report an efficient method for peptide late-stage C(sp³)–H arylations assisted by the unmodified side chain of asparagine (Asn) without any exogenous directing group. Thereby, site-selective arylations of C(sp³)–H bonds at the N-terminus of di-, tri-, and tetrapeptides have been achieved. Likewise, we have constructed a key building block for accessing agouti-related protein (AGRP) active loop analogues in a concise manner.

Introduction

Peptides are increasingly important drug candidates, which are largely employed to treat metabolic disorders, cancer, allergy, and immune and cardiovascular diseases.1 They also represent key tools that modulate biological events mediated by protein–protein interactions (PPIs).2 Native peptides usually suffer from poor pharmacological features due to lack of structural diversity or enzymatic degradation,3 but chemically modified non-natural peptides could feature higher binding affinities to the target, as well as improved pharmacokinetics, stability, and cell permeability.4

The late-stage modification represents an effective strategy to obtain structurally diverse peptides and peptidomimetics. Thus, late-stage modification methods of peptides have been achieved in terms of ariations,5 alkylations,6 and cycloadditions.7 Over the past few years, C–H activation has been recognized as an atom- and step-economical pathway towards molecular syntheses,8 with remarkable applications in materials science,9 the agrochemical industry,10 and drug discovery,11 among others.12 To the best of our knowledge, studies on late-stage functionalizations of peptides via C(sp³)–H activation have been scarcely reported. In this context, Yu13 successfully implemented C(sp³)–H activation of peptides using native N,O- or N,N-bidentate coordination without external auxiliary

† Electronic supplementary information (ESI) available. See DOI: 10.1039/d0sc03830j

Edge Article - Published on 12 August 2020. Downloaded on 6/16/2021 9:41:05 PM. This journal is © The Royal Society of Chemistry 2020
provide a useful strategy employing Asn as an internal directing group for C(sp³)-H functionalization of peptides. The unmodified side chain of Asn combined with the backbone was utilized as the N,N'-bidentate coordination via 5,6-fused bicyclic palladacycles (Fig. 1c) to perform the late-stage peptide C(sp³)-H arylation.

The complex has facilitated the inert C(sp³)-H bond arylation in peptides. Thereby, arylated di-, tri-, and tetrapeptides containing Asn have been assembled. The salient features of our approach comprise (a) C(sp³)-H activation of peptides assisted by a natural amino acid which circumvent the preinstallation and removal of DGs; (b) the first unmodified side chain of the natural amino acid as the endogenous auxiliary assistance applied in C(sp³)-H activation; and (c) discovery of native bidentate assistance through less-strained 5,6-fused bicyclic palladacycles.  

Results and discussion

Optimization of reaction conditions

To validate our hypothesis, we initiated our studies by exploring reaction conditions for the palladium(II)-catalyzed primary C(sp³)-H arylation of N-phthaloyl protected dipeptide 1 with 3-iodotoluene (Tables 1 and S1 in the ESI†). Initial optimization revealed DCE to be the best solvent of choice (Table S1,† entries 1–5), with KF being identified as the optimal additive (entries 1–3). By replacing Pd(OAc)₂ by PdCl₂ as the catalyst the yield of product 2a was excitingly increased to 67% when the amount of AgOAc and KF was increased to 2.5 equivalent (entry 4). Notably, the reaction failed to proceed using AgOTf as the additive (entry 5). While Cu(OAc)₂ gave a dramatically decreased yield (entry 6). Encouraged by the success of the arylation of dipeptides, we next investigated the feasibility of applying this approach to the arylation of tripeptides and tetrapeptides (Scheme 2). Using tripeptide 3a as the substrate, through minor adjustment of the reaction conditions (Table S2 in the ESI†), we were pleased to

Table 1 Optimization of reaction conditions

| Entry | [TM] | Oxidant | Additives | Yield/% |
|-------|------|---------|-----------|---------|
| 1     | Pd(OAc)₂ | AgOAc | NaOAc | 26 |
| 2     | Pd(OAc)₂ | AgOAc | Cs₂CO₃ | Trace |
| 3     | Pd(OAc)₂ | AgOAc | KF | 41 |
| 4     | PdCl₂ | AgOAc | KF | 67b |
| 5     | PdCl₂ | AgOTf | KF | —b |
| 6     | PdCl₂ | Cu(OAc)₂ | KF | 10b |
| 7     | Pd(MeCN)₂Cl₂ | AgOAc | KF | 72b |
| 8     | Pd₃(dbta)₃ | AgOAc | KF | 36b |
| 9     | Pd[PPh₃]₂Cl₂ | AgOAc | KF | 35b |
| 10    | [RuCl₂(p-cymene)]₂ | AgOAc | KF | —b |
| 11    | [Cp*RhCl₂]₂ | AgOAc | KF | —b |
| 12    | Co(OAc)₂ | 4H₂O | AgOAc | KF | —b |

Substrate scope

With the optimal reaction conditions in hand, the substrate scope of a range of aryl iodides was investigated, and the results are summarized in Scheme 1. Both substrates with electron-donating (Me-, MeO-, and t-Bu-) and electron-withdrawing (F-, Cl-, Br-, CF₃-, and CO₂Me-) groups reacted smoothly and afforded the desired products in good yields. Pleasingly, biphenyl and naphthyl moieties were also tolerated, leading to the corresponding products (2m and 2n) in 63% and 64% yields. The reaction performed with good chemoselectivity. Encouraged by the success of the arylation of dipeptides, we next investigated the feasibility of applying this approach to the arylation of tripeptides and tetrapeptides (Scheme 2). Using tripeptide 3a as the substrate, through minor adjustment of the reaction conditions (Table S2 in the ESI†), we were pleased to
find that the arylation of 3a with 1-iodo-4-methoxybenzene 4a could deliver the expected product 5aa in 61% isolated yield.

Then, the scope of substrates was evaluated under the optimized reaction conditions. Satisfyingly, a wide range of aliphatic amino acids, including Leu, Ala, Val, and Lys, at the C-terminus of the tripeptides are compatible with these conditions. In addition, aryl iodides bearing electron-donating as well as electron-withdrawing substituents were tolerated, affording products 5aa–5ej. Given the feasibility of the tripeptide arylation, we expanded the peptide substrates to structurally complex tetrapeptides. The arylation products of tetrapeptides 6fa–6gh could be obtained in moderate yields (50–58%). Phe-containing tetrapeptide 3h could also be arylated albeit with lower yields (6hg–6hk, 25–36%). While considerable progress has been made in C(sp3)–H activation,26 our strategy enabled position-selective arylation of Ala assisted by N,N-bidentate coordination of the Asn in tri- and tetra-peptides.

To further demonstrate that the reaction coordination site is the primary amide of Asn, the control reaction and competition reaction were investigated under the standard conditions (Scheme 3). First, we removed the Asn side chain of dipeptide 1 and replaced it with a methyl group, while retaining the tert-butyl ester of the dipeptide. Therefore, N-phthaloyl protected dipeptide 7 was independently prepared, and subjected to the optimized reaction conditions. It failed to afford arylated products of arylation of C(sp3)–H bonds at the N-terminus (Scheme 3a). Since tripeptides or tetrapeptides both contain Asn bidentate and backbone amide bidentate, it is important to analyze the key role of Asn bidentate in promoting C(sp3)–H functionalization. For the competition experiment between tripeptides 3c and 8a, product ratio of approximately (5cg : 9a = 6 : 1) (Scheme 3b) was obtained.

Mechanistic investigation

Additionally, we probed the catalyst mode of action by means of computational studies at the PW6B95-D4/def2-TZVP+SMD (DCE)//PBE0-D3BJ/def2-SVP level of theory (Fig. 2).27 A detailed analysis between the C–H activation and reductive elimination elementary steps provided support for the C–H activation to be the rate-determining step with an activation energy of 19.6 kcal mol⁻¹, with oxidative addition being energetically more favorable by only 1 kcal mol⁻¹. An alternative pathway
where the NH$_2$ of the terminal amide is deprotonated was also taken into consideration (Fig. S1, see the ESI†). The latter was shown to be overall energetically disfavored, with reductive elimination as the rate-determining step with a high energy barrier of 30.9 kcal mol$^{-1}$. These studies provide strong support for the palladium-catalyzed C(sp$_3$)$\cdots$H arylation to occur through a Pd(II/IV) pathway where the NH of the internal, instead of the terminal amide is deprotonated.

Based on previous reports on palladium-catalyzed amide-directed C–H bond activation and computational studies, we propose a plausible catalytic cycle to be initiated by a facile organometallic C–H activation (Scheme 4). Initially, the palladium catalyst coordinates covalently with the deprotonated NH of the internal amide generating a bidentate coordinated palladium(II) complex A. Subsequently, complex A undergoes slow C(sp$^3$)$\cdots$H bond cleavage to form the 5,6-fused bicyclic palladium complex B. The oxidative addition of the aryl iodide to B affords palladium(IV) intermediate C, which then undergoes reductive elimination followed by protonation leading to the formation of the corresponding arylated product. The silver salt is proposed to accelerate the rate of the oxidative addition or the reductive elimination, while likewise acting as a halide scavenger.$^\text{8,24a,28}$

Agouti-related protein (AGRP) is a potent orexigenic peptide that antagonizes the melanocortin-3 and melanocortin-4 receptors (MC3R and MC4R).$^\text{29}$ This protein has been physiologically implicated in regulating food uptake, body weight control, and energy homeostasis.$^\text{30}$ In attempts to improve the antagonist activity and selectivity of AGRP active loop, previous studies have applied a substitution strategy to prepare AGRP active loop analogues.$^\text{31}$ The results have indicated that some substitutions of amino acid could increase potency of AGRP. However, the synthesis of AGRP loop analogues requires the introduction of modified unnatural amino acids. Some unnatural amino acids are expensive and difficult to synthesize, such as L-4,40-biphenylalanine (Bip) and 3-(2-naphthyl)-L-alanine (Nal(2')). Through C–H activation, the functional group could be installed directly into native peptides, such an approach is
highly efficient, step- and atom-economical. Thus, we attempted to apply our strategy to synthesize new AGRP loop analogues. The arylation products through deprotection of phthaloyl (Phth) gave NH2-free tetrapeptides (details see the ESIF). Tetrapeptides and subsequently were coupled with Cbz–Pro–Pro–Arg(Pbf)–Phe–OH to obtain linear octapeptides, which were cyclized to access AGRP loop analogues. AGRP loop analogues and were obtained through this strategy (see the ESIF synthesis of AGRP loop analogues); the introduction of a bromide atom in potentially enables further late-stage derivatization of this peptide (Scheme 5).

Conclusion

In conclusion, we have developed an efficient strategy for palladium(u/v)-catalyzed late-stage C(sp3)–H arylation of peptides using unprecedented internal Asn. The protocol avoids the additional requirement for installation and removal of exogenous directing groups. Importantly, our approach has provided a novel synthetic route to access the key building block for the synthesis of AGRP loop analogues.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

Generous support by the NSFC (Grant No. 21978273 and 21506190), the CSC (Scholarship to Y. Weng), the DFG (Gottfried-Wilhelm-Leibniz award to L. A.), the University of Goettingen and the Onassis Foundation (fellowship to N. K.) is gratefully acknowledged.

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