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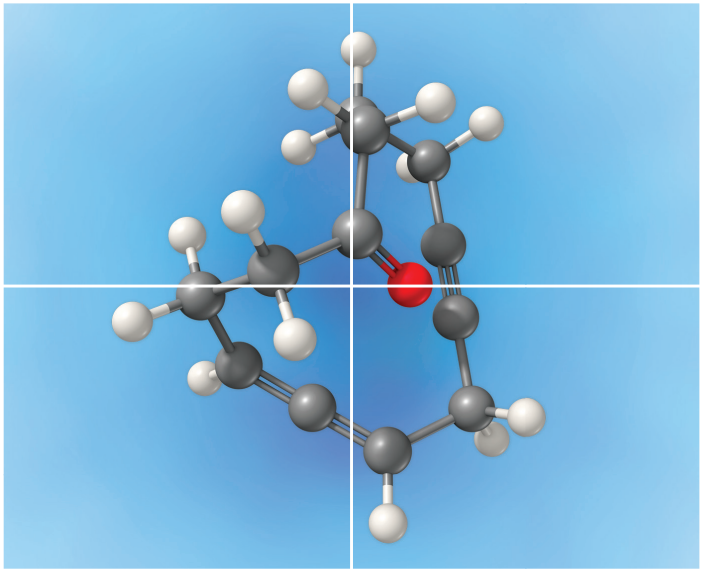

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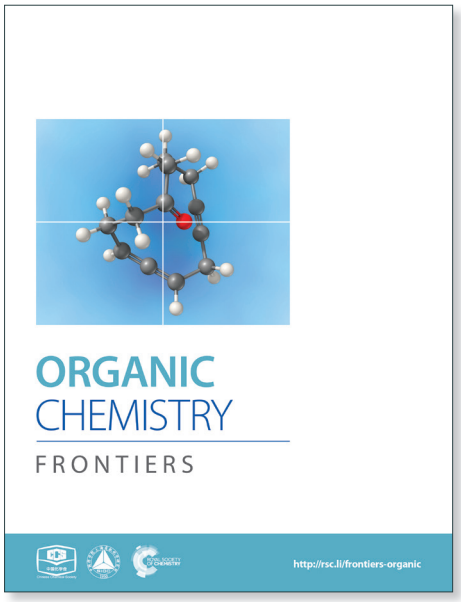

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Direct Amidation of Phenylalanine Moiety in Short Peptides via Pd-Catalyzed C–H Activation/C–N Formation

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Selective modifications on life's basic components, such as polypeptides, are of great importance in both chemistry and biology. Herein, we developed an unprecedented intramolecular amidation of phenylalanine moiety in dipeptides via Pd-catalyzed sp^2 C–H activation and C–N formation.

Selective modifications of life's basic components via chemical protocols lay the foundation for building designed organisms from ground up and achieving conventional chemical reactions *in vivo*, which are two of the ultimate aims of synthetic chemistry in life sciences. Since protein is one of the most vital and abundant biomacromolecules for life, its polypeptide structural feature keeps being an active research object aimed my synthetic chemists. However, tough challenges are encountered if planning to use peptide as substrate for purely chemical transformations without the help of enzymes due to the solubility and uncontrollable selectivity. For example, to realize the chemo- and regioselectivity of designed chemical transformation on complicated peptides containing manifold functional groups as well as various reactive sites is formidable[1]. As one of the tools in functionalizing the organic molecules, the transition metal catalysis might not be a good choice since transition metal catalysts are readily deactivated by detrimental chelation of heteroatoms on substrates if spatially matched with the peptide as a research subject[2]. The secondary, third and even higher ordered structures also hamper the selective functionalization of the peptides. Last but not least, the solubility and thermo stability of peptides under traditional chemical conditions also remain severe concerns.

In the past several years, the trials to in-situ functionalize the peptides, as well as some special proteins have been studied and some exciting chemistries have been developed by prominent researchers despite facing exceedingly difficulties. For example, suitable bioorthogonal ligation reactions, which could afford the covalent fusion on biomolecules bearing specific functional groups, provide excellent labelling tools[3,4].Besides the modification of the active functional groups in peptide, the inactive substituents in natural residue of peptide are hardly approached. Depicted as one of the most powerful methods to diversify the molecules, some of the methods via transition-metal catalysis have been adapted to modify the short peptides[5]. Therefore, other unexplored adaptations of conventional chemical reactions catalyzed by transition metals on peptides as well as the proteins are regarded promising research targets with high value. Herein we first presented the intramolecular amidated annulation to convert the phenylalanine derivatives of short peptides into the cyclic unnatural amino residue via Pd catalysis.

C–H functionalization has been a hot spot of research for years because of its attractive atom- and step- economy, synthetic efficiency and novelty of the fundamental chemical researches. Various chemical bonds, such as C–C[6], C–N[7], C–O[8], C–B[9]and C–Si[10], could be constructed readily and directly starting from C–H bonds. Undoubtedly, implementation of C–H functionalization strategy on life's substance possesses a great importance, for it will largely expand the application scope of C–H functionalization reactions, and enrich biologists' tool kits on peptide modification. Due to the peptidicinnate diversity and complexity, a large library of modified peptides with potential biochemical applications would be produced in a convenient and quick fashion through direct and selective C–H functionalization.

Pioneering methods on site-specific functionalization of short peptides have been reported scatteredly in literature over past decades. However, most of them only focused on the reaction of the most active α-position of glycine moiety[1,11]and 2-position of tryptophan ring[12].Indeed, a beautiful example of Pd-catalyzed direct C–H functionalization of tryptophan residue of the protein has been well studied. More complementary methods enabling selective modifications on different sites of peptide via C–H activation are in demand.

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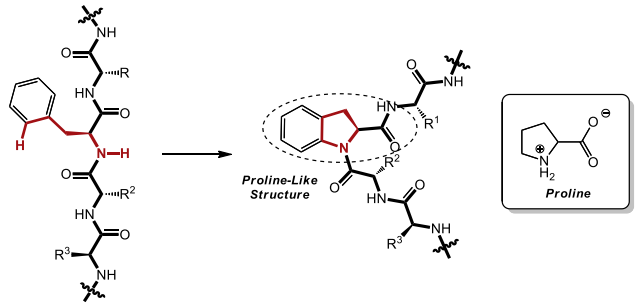
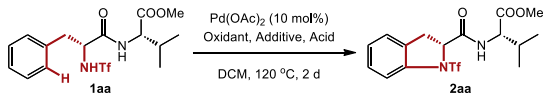


Scheme 1. A linear peptide chain would be twisted after envisioned selective intramolecular amination.

As a subfield of C–H functionalization, sp^2 C–H activation and intramolecular C–N formation sequence could furnish a variety of *N*-contained ring system in a swift manner, thus exhibiting a supplementary approach to the powerful Buchwald-Hartwig amination reaction[7a,13]. Enlightened by these elegant pioneering works and with an eye on the ubiquity of phenylalanine moiety on polypeptide chain in nature, we conceived that it is very possible while useful to perform the unprecedented intramolecular amidation of phenylalanine moiety in small peptides via palladium catalysis. This proposed transformation would serve as a latent instrument for chemical modification on peptides with excellent feasibility and practicability. Most importantly, a proline-like2,3-dihydroindolyl moiety could be constructed after envisioned amidation, which results in a beta-turn-type twist on linear chain to induce the completely change of the secondary structure of the peptide(Scheme 1)[14]. Therefore, this work might cast a light on a prospective method for the artificial and chemical adjustment on the conformation of polypeptide if applicable.

We started our research from the simple dipeptide **1aa** containing phenylalanine for reaction condition optimization. Primary screening on common oxidants in Pd chemistry unfortunately gave less than 5% of **2aa** (entries 1-7). To our delight, with higher loading of $Ce(SO_4)_2$, a modest yield of desired product was acquired (entries 8-9). However, further increase of equivalents of $Ce(SO_4)_2$ (8.0 equiv) is not beneficial for prompting the efficiency and yield (entry 10). Changes on ligands or equivalents of DMF failed giving better results, while the reaction was even impeded with excessive DMF (entries 11-14). Unluckily, inconsistent results were observed among multiple experiments when 6.0 equivalents of $Ce(SO_4)_2$ and 6.0 equivalents of DMF were added (entry 9). After many attempts to stabilize the result, we were pleased to find that addition of TsOH led to a lower but reproducible yield (entry 15). Further screening on acids revealed that MsOH was the best (entries16-19), and reducing the amount of MsOH had little impact on the reaction and even a slightly higher yield was obtained (entries 20-21).

Table 1. Condition screening for the amidation of phenylalanine moiety

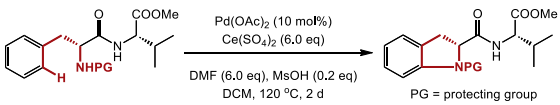


Entry	Oxidant	Additive	Acid	Yield (%)[a]
1	$PhI(OAc)_2$ (2.0 eq)	DMF (6.0 eq)	–[c]	10
2	$K_2S_2O_8$ (2.0 eq)	DMF (6.0 eq)	–[c]	14
3	$Cu(OAc)_2$ (2.0 eq)	DMF (6.0 eq)	–[c]	< 5
4	TBP (2.0 eq)	DMF (6.0 eq)	–[c]	< 5
5	Oxone (2.0 eq)	DMF (6.0 eq)	–[c]	< 5
6	NFSI (2.0 eq)	DMF (6.0 eq)	–[c]	16
7	$Ce(SO_4)_2$ (2.0 eq)	DMF (6.0 eq)	–[c]	37
8	$Ce(SO_4)_2$ (4.0 eq)	DMF (6.0 eq)	–[c]	49
9	$Ce(SO_4)_2$ (6.0 eq)	DMF (6.0 eq)	–[c]	56
10	$Ce(SO_4)_2$ (8.0 eq)	DMF (6.0 eq)	–[c]	48
11	$Ce(SO_4)_2$ (6.0 eq)	DMF (3.0 eq)	–[c]	55
12	$Ce(SO_4)_2$ (6.0 eq)	DMF (12.0 eq)	–[c]	29
13	$Ce(SO_4)_2$ (6.0 eq)	NMP (6.0 eq)	–[c]	27
14	$Ce(SO_4)_2$ (6.0 eq)	DMAc (6.0 eq)	–[c]	42
15	$Ce(SO_4)_2$ (6.0 eq)	DMF (6.0 eq)	TsOH (1.0 eq)	47
16	$Ce(SO_4)_2$ (6.0 eq)	DMF (6.0 eq)	**A**[d] (1.0 eq)	40
17	$Ce(SO_4)_2$ (6.0 eq)	DMF (6.0 eq)	TFA (1.0 eq)	45
18	$Ce(SO_4)_2$ (6.0 eq)	DMF (6.0 eq)	AcOH (1.0 eq)	23
19	$Ce(SO_4)_2$ (6.0 eq)	DMF (6.0 eq)	MsOH (1.0 eq)	57
20	$Ce(SO_4)_2$ (6.0 eq)	DMF (6.0 eq)	MsOH (0.5 eq)	60
21	**$Ce(SO_4)_2$ (6.0 eq)**	**DMF (6.0 eq)**	**MsOH (0.2 eq)**	**60 (53)[b]**

[a] ^1H NMR yield with CH_2Br_2 as internal standard; [b] Isolated yield in parentheses; [c] Without acid; [d] A is 2-Nitrobenzenesulfonic acid

Having the optimized reaction condition in hand, several substrates with common *N*-protecting groups other than Tf group were subjected to standard condition (Table 2). However, less than 5% of desired products were detected when using Ts, TFA, Boc or Ac protecting groups, manifesting the unique activity of triflamide[13c-e,15].

Table 2. The effect of protecting group in amidation



Entry	PG	Yield (%)[a]
1	Tf	60
2	Ac	< 5
3	Ts	< 5
4	TFA	< 5
5	Boc	< 5

[a] ^1H NMR yield with CH_2Br_2 as internal standard.

To test the effect of the relative stereochemistry in the substrates of dipeptide, all of 4 diastereoisomers of dipeptide **1aa** were synthesized and submitted to standard conditions (Table 3). In fact, similar results were obtained, showing little difference on their reactivities of these 4 isomers with different distereochemistry (**2aa-2ad**). This discovery substantiated negligible effect of substrate's configuration on reaction, and rationalized the utilization of several

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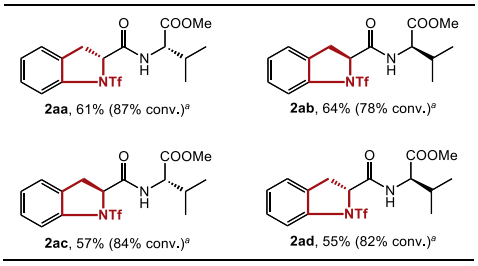
substrates with exceptional configuration other than that of **1aa** for further study.

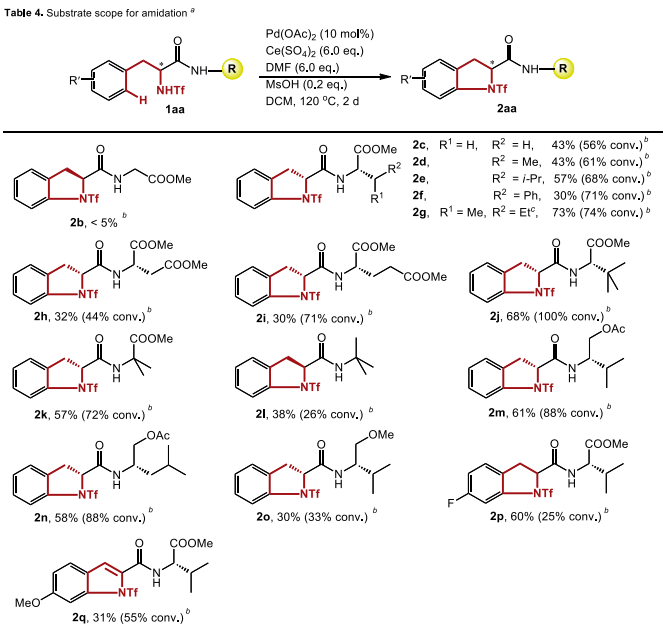
Table 3. The configuration effect in amidation reaction



[a] Yield based on isolated yield divided by percentage of conversion.

To determine the feasibility of this transformation, various dipeptides containing phenylalanine and another naturally occurring amino acids were studied (Table 4).To our interest, steric effect of R group presented a notable impact on the efficiency of amidation. For example, no product was detected when the chain of amino acid residue at *C*-terminus was glycine (**2b**). Along with the extension of amino acid side chain from methyl to *i*-butyl group, all the amidation occurred in comparable efficacy (**2c-2e**). Brunched structure on beta position of *C*-terminus further promoted the yield significantly (**2aa**, **2g**). A Phe-Phe dipeptide was also measured, and expected annulation took place exclusively at the side of triflamide (**2f**). It is worthy noting that although much lower yields were afforded, catalyst maintained its activity under the presence of two ester groups at *C*-terminus where excessive and deleterious coordination towards palladium center was possible (**2h-2i**).

Table 4. Substrate scope for amidation [a]



[a] Condition:**1** (0.1 mmol), $Pd(OAc)_2$ (2.2 mg, 0.1 equiv), $Ce(SO_4)_2$ (199.0 mg, 6.0 equiv) were placed in a vial under air. DCM (0.8 mL), DMF (47 μL, 6.0 equiv) and MsOH (0.2 mL of 0.1 M solution in DCM) were added sequentially, rt for 5 min and then was stirred for 2 d at 120 °C. [b] Yield based on isolated yield divided by percentage of convension. [c] Configuration at the carbon where methyl and ethyl group attached is S.

Many dipeptides with unnatural amino acids at *C*-terminus, which might serve as the key reactive part on peptide modification[16], were also well tolerated in our system, showing that a continuous and multiple modifications on peptides would be possible. Consistent with the results shown above, dipeptide with a large *t*-butyl group as side chain afforded a good yield (**2j**). To further prove this trend, 2-methylalanine was used as *C*-terminal amino acid, which may promote the efficiency by Thorpe-Ingold effect. Albeit larger steric hindrance was induced through the installation of two methyl groups, an unexpected descent of yield was discovered, which might be attributed to the increased steric strain and improper configuration of the substrate that deteriorated the inner stability of intermediate (**2k**). Substrates with *O*-esterificated 2-amino alcohols at *C*-terminus, which could be obtained readily by a simple reduction from amino acids, also gave good yields (**2m-2n**). Thus, a variety of potential substrates of the type **2m** were accessible in a straightforward way taking advantage of plentiful amino acids.

In comparison to the different substrate with the different *C*-terminal moieties (**2k**, **2l**, **2m**and **2o**), the results clearly illustrated the necessity of the presence of estergroup at *C*-terminus for satisfying reactivity, maybe arising from the possible coordination from carbonyl group of the ester of amino alcohol instead of ether group of dipeptides to stabilized the key intermediate. This conclusion is consistent with the similar "relay effect" of appropriately positioned heteroatoms in transition-metal-catalyzed transformations reported before[17]. Finally, experiments targeting on electronic effect of phenyl ring on this transformation were conducted. Surprisingly, only electron-neutral substrate underwent the amidation smoothly with high conversion and yield while both electron-donating group and electron-withdrawing group dramatically diminished the efficacy, and electron-rich substrate was over-oxidized to an indole structure(**2aa, 2p-2q**).

Another goal to modify the peptides is to keep the optical purity during the transformations. Traditional methods on functionalizing short peptides usually require the excessive use of strong base to form enolate intermediate, which lead to racemization at stereogeniccenters on peptides. In our developed method, the reaction condition is relatively mild in the absence of either strong base or acid. Indeed, to our satisfactory, under this developed condition, diverse optically pure *N*-triflated short peptides (except **2p**) were tested, and corresponding single diastereoisomers were isolated without any racemization, demonstrating the retention of the configuration of modified short peptides.

Conclusions

In summary, we for the first time developed the direct amidation of dipeptide through direct aryl C–H transformation of phenylalanine residue via Pd-catalysis in good efficacy. Such a cyclization exhibited the flexibility with different amino acid at *C*-terminus. In displacement of amino acid esters by the aminoalcohol acetates, the credible efficacy was obtained to extend the substrate scope. Under such mild conditions, the racemization of both amino acids of dipeptide did not observed. Further studies to promote the efficacy and explore such chemistry in long peptide and even in proteins is still on the way.

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Notes and references

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† Footnotes should appear here. These might include comments relevant to but not central to the matter under discussion, limited experimental and spectral data, and crystallographic data.

Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/c000000x/

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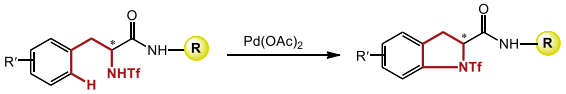

Direct amidation of dipeptide through direct aryl C-H transformation of phenylalanine residue via Pd-catalysis.

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