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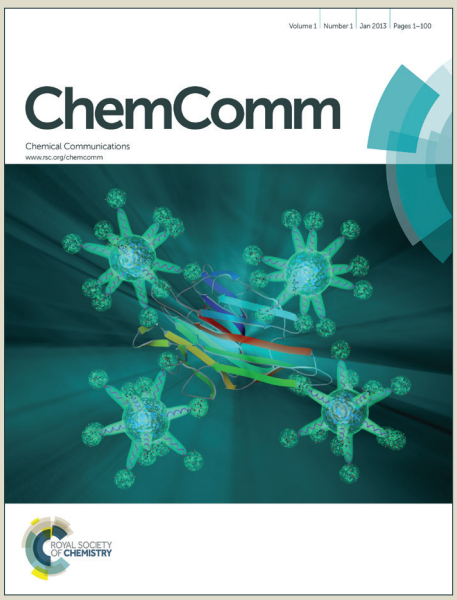

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COMMUNICATION

Site-specific Indolation of Proline-based Peptides via Copper(II)-Catalyzed oxidative coupling of tertiary amine *N*-oxides †

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The first site-specific and purely chemical method for modifying proline-based peptides was developed via a convenient, copper-catalyzed oxidative coupling of tertiary amine *N*-oxides with indoles. This novel approach features high regioselectivity and diastereoselectivity, mild conditions, and compatibility with various functional groups. In addition, a simplified process was realized in one pot and two steps via the in situ oxidative coupling of tertiary amine and indoles.

Natural and unnatural peptides occur widely in proteomics and pharmaceuticals. There have been considerable studies on peptide-based drugs in the pharmaceutical community.[1] However, the most difficult challenge in the development of peptide drug candidates lies in solving the stability issues.[2] Recently, the site-specific chemical modification of peptides has emerged as an intriguing approach to construct a library of unnatural peptide, thus providing a powerful tool to change the conformations and stabilities of biologically active peptides.[3] Among the various developed synthetic methods, C–H bond functionalization stand out as an efficient and atom-economical tool for preparation of α-substituted amino acid or peptide derivatives. However, the site-specific modification of peptides via C-H bond functionalization is still challenging and limited to only a few examples.[3b,4] Proline-based peptides have shown a variety of biological activities and are prevalent in pharmaceutical ingredients (Figure 1).[5]

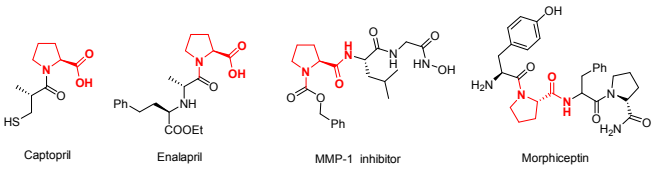

Figure 1. Proline derivatives and proline-based peptides.

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Li's group established a copper-catalyzed method for the direct arylation, alkynylation of glycine derivatives and short peptides by using oxidative C-H/C-H cross-coupling. Their method allows for the site-specific modification of the glycine residue- via the introduction of N-PMP (p-methoxyphenyl)-protected groups. However, there is still no precedent for the direct functionalization of proline-based peptides. Given that proline-based peptides have shown a variety of biological activities and are prevalent in pharmaceutical ingredients, we desired to develop an efficient protocol for the modification of proline-based peptides (Figure 2). Herein, we present a copper-catalyzed, highly C-5 site-specific indolation of proline in peptides. This method features high diastereoselectivty and good to excellent yield under mild and convenient conditions.

We chose N-benzyl-protected proline-based dipeptide N-oxide **1a** as a benchmark substrate to screen reaction conditions. We envisaged that in the presence of copper catalysts, the proline N-oxide would readily generate iminium, which could be captured by nucleophiles in situ to achieve the direct site-specific modification of **1a**. Furthermore, the N-benzyl group should be readily removed, allowing for further modification. With these considerations in mind, we set out to screen various copper catalysts with indole **2a** as a nucleophile. It was found that the counter ion of copper had a remarkable effect on the yield (entries 1-3). $CuBr_2$ was shown to be the optimal catalyst; it gave a good yield (68%) and excellent diastereoselectivity (d.r. = 94:6). Performing the reaction in other chlorinated solvents such as $CHCl_3$ (entry 4) and PhCl (entry 5) resulted in decreased yield. Further

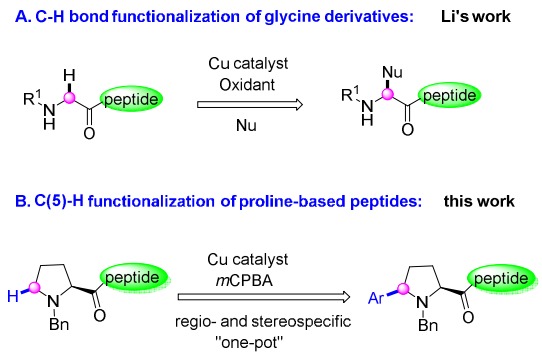
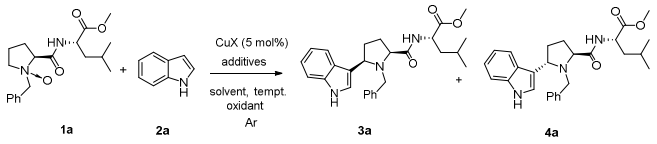


Figure 2. C-5 site specific modification of proline in peptides.

Table 1. Optimization of the reaction conditions.[a]



Entry	Cat.	Additives	Solvent	Yield (%)[b]	d.r.[c]
1	$Cu(OAc)_2$		DCE	11	nd
2	$Cu(OTFA)_2$		DCE	17	nd
3	$CuBr_2$		DCE	68	94:6
4	$CuBr_2$		$CHCl_3$	62	93:7
5	$CuBr_2$		PhCl	55	nd
6[d]	$CuBr_2$	TsOH	DCE	76 (68)	92:8
7[d]	$CuBr_2$	TsOH	$CHCl_3$	75 (70)	93:7
8[d,e]	$CuBr_2$	TsOH	$CHCl_3$	75	93:7
9[d]		TsOH	$CHCl_3$	trace	nd

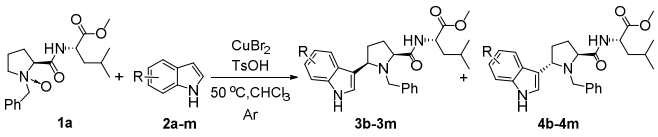
[a]Reaction conditions: **1a** (0.2 mmol), **2a** (0.6 mmol), CuX (5 mol%), solvent (3 mL), 50 °C, 36h, Ar. [b] NMR yields using CH_2Br_2 as an internal standard, combined yields of isolated crude product **3a/4a** are given in parentheses. [c] Determined by LC/MS analysis of the crude product **3a/4a**. nd = not determined. [d] 20 mol% of TsOH was added. [e] The reaction time was 48h, the temperature was 40 °C.

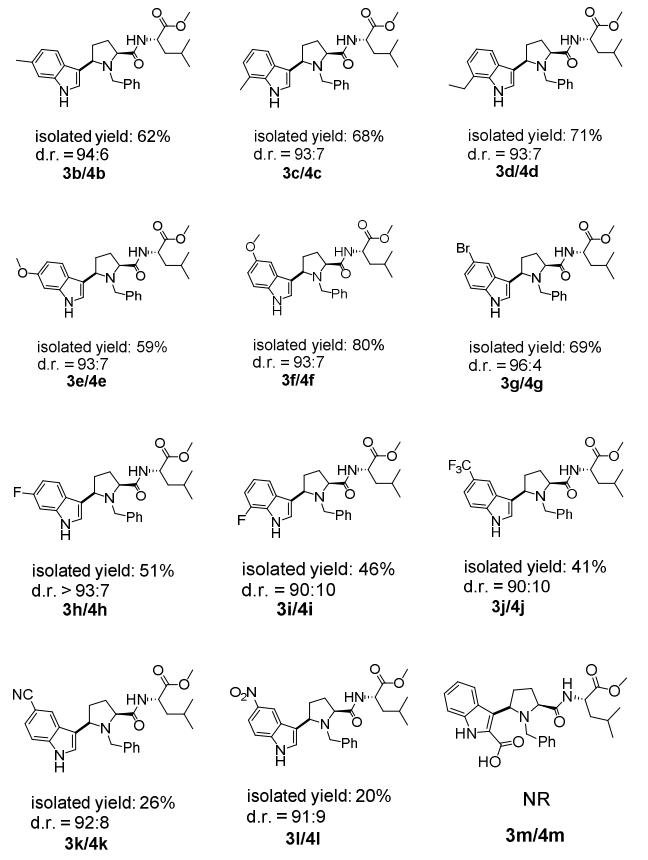
screening of additives demonstrated that the addition of 0.2 equiv. TsOH was beneficial for the reaction (entries 6-7). Elongating the reaction time and lowering the reaction temperature resulted in comparable yield and diastereoselectivity (entry 8). Additionally, this reaction doesn't proceed well in the absence of copper catalyst (entry 9). Taken together, the reaction with $CuBr_2$ as a metal catalyst and TsOH as a bronsted acid catalyst in chloroform gave the most satisfactory yield and dr (entry 7), and the absolute configuration of the major product **3a** was unarguably determined by X-ray analysis (see the Supporting Information).[6]

With the optimized reaction conditions established, a variety of functionalized indoles were tested for the site specific chemical modification of dipeptide **1a** to explore the scope of the reaction shown in Table 2. In general, the desired products **3a-i**/**4a-i** were obtained in acceptable to excellent yields (46%-80%) and with high diastereoselectivity (d.r. > 90:10) regardless of whether alkyl, alkoxy or halogen groups were introduced at the 6, 7, or 8-positions of indole, respectively. The introduction of electron-donating groups (-Me, -Et, and -OMe) at the C5, C6, or C7 positions of the indoles gave good yields. In contrast, substrates bearing electron-withdrawing substituents such as -F, and especially -CF_3, -CN, and -NO_2 at the 5-position of indole resulted in significantly decreased yields (**3j**-**3l**), likely due to the lower nucleophilicity of the resultant indoles. Finally, it is necessary to report an unsuccessfully example; the indole with -COOH substituted at the C-2 position did not yield the desired product **3m**, presumably due to the strong electron-withdrawing effect and poor solubility in $CHCl_3$. Notably, various functionalized indoles bearing cyano (**3k**), nitro (**3l**), and halides such as bromine (**3g**), and fluorine (**3h-3i**) were tolerated under the standard reaction conditions, which guarantees further transformation or derivatization.

Subsequently, to further explore the selectivity and

Table 2. Substrate scope of the reactions of various indoles with compound **1a**.[a]

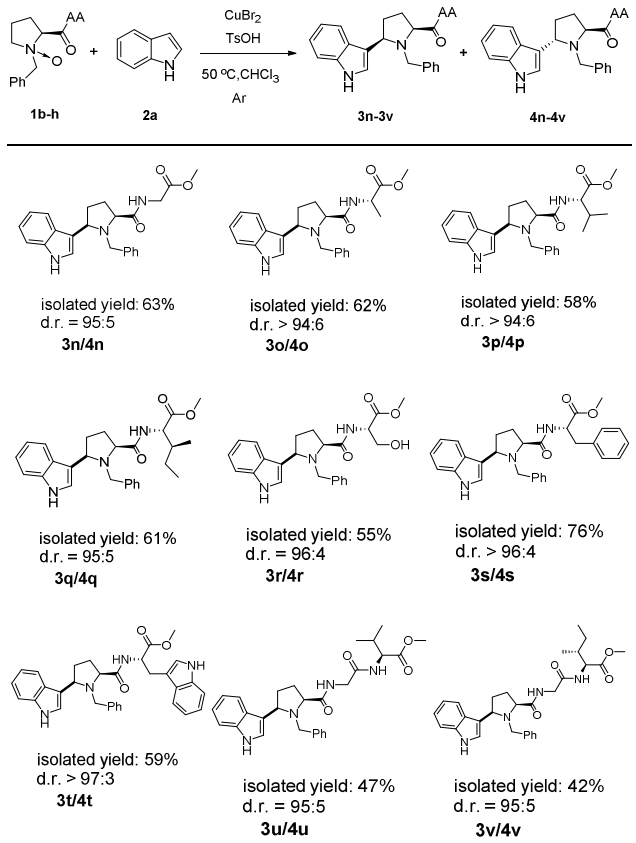




[a] Reaction conditions: **1a** (0.2 mmol), **2a**-**m** (0.6 mmol), $CuBr_2$(5 mol%), TsOH(20 mol%), $CHCl_3$ (3 mL), 50 °C, 36 h, Ar; Combined isolated yields of products. [b] Determined by LC/MS analysis of the crude products. NR = not reaction.

compatibility of this method for peptide modification, we also synthesized a set of various proline-based dipeptides and tripeptides to investigate the effect site specific peptide indolation. To our delight, various proline-based peptide derivatives were compatible with standard reaction conditions, giving the coupling products in good yields and with excellent dr values (table 3). Dipeptides with an amino acid moiety such as glycine, alanine, valine, and isoleucine afforded the desired products (**3n**-**3q**) with satisfactory yield and high d.r. 95:5 irrespective of the effects of peptide steric hindrance. Interestingly, excellent diastereoselectivities with d.r. values up to 97:3 were observed when incorporating phenylalanine and tryptophan moieties in peptides. It is worth mentioning that the peptide based on serine with a hydroxyl group was tolerated well, affording the desired product (**3r**) and ensuring

Table 3. Investigation of the scope of peptides.[a]

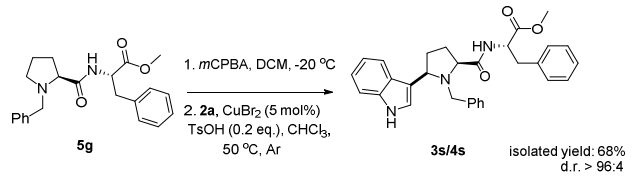


[a] Reaction conditions: **1a** (0.2 mmol), **2a**-**m** (0.6 mmol), $CuBr_2$ (5 mol%), TsOH(20 mol%), $CHCl_3$ (3 mL), 50 °C, 36h, Ar; Combined isolated yields of products. [b] Determined by LC/MS analysis of the crude products.

further transformation. Additionally, high diastereoselectivity (d.r. = 95:5) with moderate yield could be achieved even for the modification of tripeptides (**3u**-**3v**).

Moreover, we found that the desired the indolation product **3s**/**4s** can be obtained directly via in situ oxidative coupling of tertiary amine **5g** and indoles. This process gives good yield and high dr from C-H and C-H coupling in a one-pot, two-step sequential manner under the standard conditions (Scheme 1). The advantages above further demonstrate the utility of our method.

This reaction doesn't proceed well in the absence of copper catalyst, which suggests the copper salt is very important to this transformation. A plausible mechanism[7] for the direct indolation of the *N*-benzyl protected proline-based dipeptide *N*-oxide is proposed. The first step is most likely the formation of the imine type intermediate (coordinated to



Scheme 1. Simplified process through one pot by two steps.

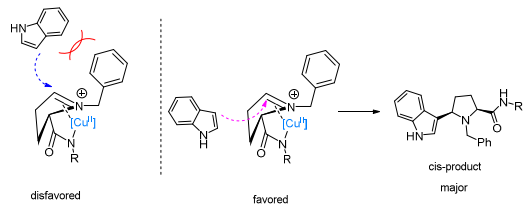


Figure 3. Mechanistic proposals for stereocontrol

copper) catalyzed by $CuBr_2$, then the reaction is followed by an *in situ* Friedel-Crafts-type reaction[7e] of indoles and imine type intermediates to give the desired products. The indole prefers to attack the imine type intermediate from the sterically unblocked *si*-face of the imine C=N bond (Figure 3), which rationalizes why the major diastereomer was observed in this reaction. Formation of the minor diastereomer would suffer from severe repulsion between the substrate and the phenyl residue of proline-based peptide.

In conclusion, we have developed a direct, specific-site, and purely chemical modification of peptides via the copper and acid co-catalyzed oxidative coupling of proline-based peptides and indoles. This method enables the efficient preparation of potentially bioactive peptides with high regio- and stereo-selectivity. This approach is also compatibile with various peptides and a wide range of substrates and can be carried out under mild operational conditions.

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