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Macrocyclization of Biaryl-Bridged Peptides through Late-Stage Palladium-Catalyzed C(sp^2)−H Arylation

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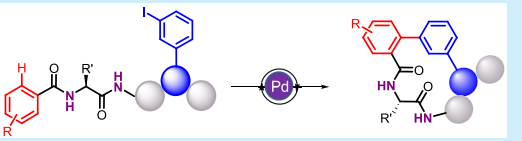
Supporting Information

ABSTRACT: Macrocyclic peptides are promising scaffolds of bioactive compounds and clinical therapeutics. Herein, we develop a strategy for the macrocyclization of biaryl-bridged peptides through late-stage Pd-catalyzed C(sp^2)−H arylation. This method displays broad substrate scope and high efficiency in the synthesis of peptide conjugates with various bioactive molecules. Furthermore, we applied this method to prepare peptide macrocycles with aryl−aryl cross-links. Our results show the effectiveness of backbone amide groups as directing groups in Pd-catalyzed C−H functionalization of peptides.



Peptides are a major class of biologically active compounds with molecular weights poised between small molecules and proteins. In particular, cyclic peptides with chemical diversity and unique molecular topologies have attracted attention from fields including medicinal chemistry and chemical biology.[1] Peptide macrocycles have shown high potency and specificity as regulators of protein−protein interactions, which are associated with numerous biological processes and challenging to intervene with small molecules.[2] In addition, conjugation of small molecule drugs to bioactive peptides usually results in improved target specificity, which further expands the application of peptides. Natural products are rich sources of bioactive peptide macrocycles.[3] For example, arylomycins are broad-spectrum cyclic peptide antibiotics with inhibitory activity toward type I signal peptidase.[4] The characteristic feature of arylomycins is 14-membered, biaryl-bridged macrocyclic core formed between a tyrosine and a 4-hydroxyphenylglycine residue (Figure 1A). Recently, G0775, a synthetic arylomycin derivative, was discovered with potent antibacterial activity against Gram-negative pathogens through a systematic optimization of this natural scaffold.[5] The synthesis of cyclic peptides with constrained biaryl-cross-links is chemically challenging, and current strategies are limited to macrolactamization, cross-coupling, and oxidative phenol coupling (Figure 1B).[6−8]

Recently, peptide functionalization by transition-metal-catalyzed C−H activation has shown great potential in the synthesis of cyclic peptides with diverse structures.[9−11] Seminal examples include peptide macrocyclization through tryptophan (Trp) C(sp^2)−H arylation at the C-2 position,[12−14] β-C(sp^3)−H arylation,[15,16] Mn-catalyzed C(sp^2)−H alkynylation,[17] as well as δ-C(sp^2)−H olefination of phenylalanine (Phe)[18] and *N*-terminal arylsulfonamides.[19] Using proper directing groups, Chen group developed methods to prepare cyclophane-braced peptide macrocycles through Pd-catalyzed C−H activation.[20,21] Our group have developed methods that utilizes amide bonds of peptide backbone as directing groups for palladium catalyst to achieve site-selective macrocyclization of peptides.[22,23] Herein, we report a backbone-directed method

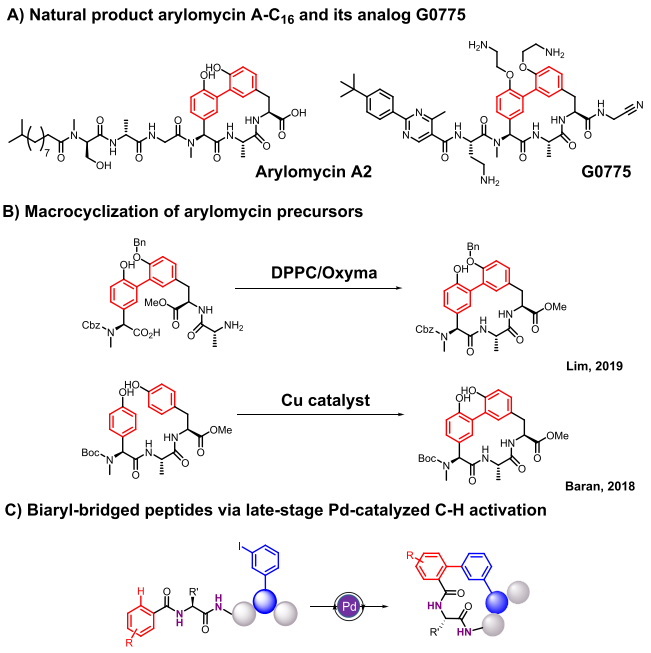


Figure 1. Macrocyclization of biaryl-bridged peptides via late-stage Pa-catalyzed C−H activation. (A) Arylomycin and its bioactive analogue G0775. (B) Macrocyclization of arylomycins through macrolactamization and Cu-catalyzed C−H activation. (C) Backbone-directed macrocyclization of biaryl-bridged peptides through late-stage Pd(II)-catalyzed C−H arylation.

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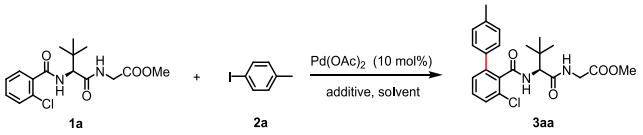
for arylation of peptidomimetics, as well as the synthesis of biaryl-bridged cyclic peptides (Figure 1C). This method displays broad substrate scope and are compatible with bioactive molecules to generate corresponding peptide conjugates. Furthermore, we applied this method for the preparation of biaryl-bridged peptide macrocycles with complex structures.

We initiated our studies with dipeptide **1a** bearing *N*-terminal benzamide group and 1-iodo-4-methylbenzene **2a** as model substrates (Table 1). Detailed optimization of reaction

Table 1. Optimization of the Reaction Conditions[a,b]



entry	additives	solvent	T (°C)	time (h)	yield[c] (%)
1	AgOAc (2.0 equiv)	DCE	100	16	6[d]
2	AgOAc (2.0 equiv)	$PhCF_3$	100	16	22
3	Ag_2CO_3 (1.0 equiv)	$PhCF_3$	100	16	30
4	Ag_2CO_3 (1.0 equiv)	$PhCF_3$	120	16	47
5	Ag_2CO_3 (1.0 equiv) + Na_2CO_3 (2.0 equiv)	$PhCF_3$	120	24	59
6	NMe_4Cl (2.0 equiv) + Na_2CO_3 (3.0 equiv)	$PhCF_3$	120	24	34
7	NMe_4Cl (3.0 equiv) + KOAc (4.0 equiv)	$PhCF_3$	120	24	67[e]

[a]Reaction conditions: **1a** (0.1 mmol), **2a** (2.0 equiv), $Pd(OAc)_2$ (10 mol %), and additives in solvent (1 mL). [b]Optimization studies are provided in the Supporting Information. [c]Reaction yields were determined by ^1H NMR using 1,2-dibromoethane as the internal standard. [d]**1a** (0.05 mmol). [e]**2a** (3 equiv).

conditions led to the standard conditions as follows: 3.0 equiv of substrate **2a**, 10 mol % of $Pd(OAc)_2$ as the catalyst, 3.0 equiv of NMe_4Cl, and 4.0 equiv of KOAc in trifluorotoluene ($PhCF_3$) at 120 °C for 24 h, affording *ortho*-arylated product **3aa** in 67% yield (Table 1, entry 7). Alteration of additives and solvents decreased the yield of the reaction significantly (entries 1−5).

First, we evaluated the substrate scope of the arylation method toward the aryl iodides (Figure 2). Using dipeptide **1o** as a substrate, iodobenzene, *p*-iodomethylbenzene, and *m*-iodomethylbenzene all reacted efficiently by generating the corresponding products in high isolated yields (Figure 2, **3oa**−**3oc**). Substrate **2d** and **2e** with *p*-butyl and dimethyl substitutions, respectively, both reacted with **1o** in excellent yields (entries **3od** and **3oe**). Substitution of a methoxyl group at the *para*- and *meta*-positions does not affect the reaction yields (entries **3of** and **3og**). Chloro and fluoro substitutions are also well-tolerated in this protocol (entries **3oh** and **3oi**). Substrates with electron-withdrawing groups, such as trifluoromethyl and nitro groups, led to high reaction yields (entries **3oj** and **3ok**). Finally, iodonaphthalene **2l** was shown to be an excellent substrate in this chemistry to yield product **3ol** in 94% yield. In addition, detailed NMR analysis revealed that the arylation occurs at the *ortho*-position (Supporting Information section 5). However, when *ortho*-substituted aryl iodides are employed as substrates, reaction yields are generally low. We reasoned that the steric hindrance at the *ortho*-position decreases reaction efficiency. Together, our results show that

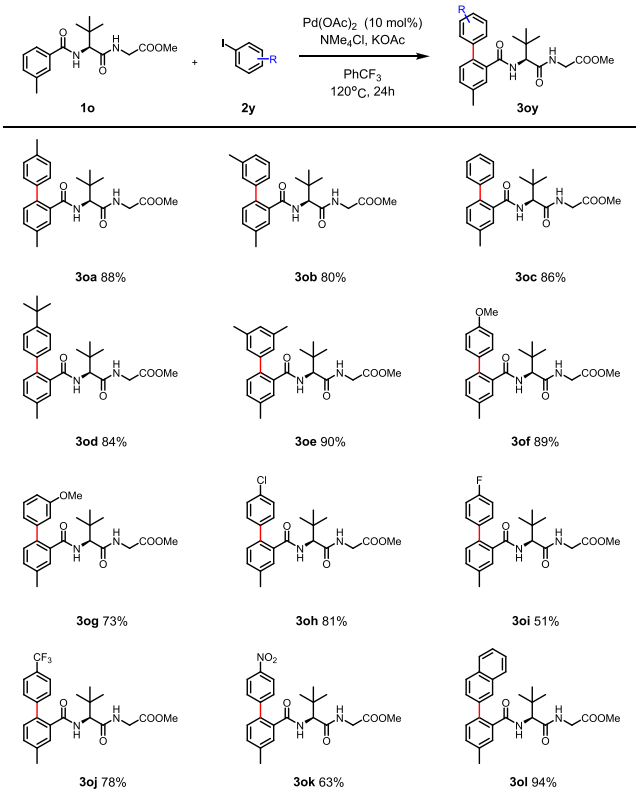


Figure 2. Substrate scope with respect to iodobenzene derivatives of the arylation of dipeptide **1o**. Reaction yields represent isolated yields.

para- and *meta*-substituted aryl iodide compounds are well tolerated by this method.

We next explored the substrate scope of this chemistry with *N*-terminal benzamides of peptide substrates using iodobenzene **2a** as the aryl donor (Figure 3). Peptide substrate **1j** reacted with **2a** efficiently by generating mono- and diarylated products in 40% and 43% yields, respectively (entry **3ja**). Halo substitutions on the *N*-terminal benzamides of dipeptide substrates are tolerated in this reaction, resulting in the corresponding products in moderate to good yields (entries **3la**−**3na**). Substrates containing *meta*-methyl substitutions were arylated at the *ortho*-position exclusively (entries **3oa** and **3ra**), whereas *para*-methylated dipeptide **1p** generated both mono- and diarylated products in moderate yields (entry **3pa**). Notably, when the methyl substitution is at the *ortho*-position, arylation at $C(sp^2)$−H and $C(sp^3)$−H were both observed (entries **3qa** and **3qa′**), further demonstrating the versatility of this reaction. In addition, methoxyl-substituted and *N*-naphthamide-protected substrates (**1s** and **1t**) were both good substrates for this chemistry. Overall, our results showed that this method is compatible with peptide substrates with various *N*-terminal benzamide groups.

To address the impact of peptide sequences to the reaction, a variety of dipeptide substrates with an *N*-terminal *o*-chlorobenzamide group were synthesized and subjected to reaction with iodobenzene **2a** (Figure 4). The results showed that incorporation of hydrophobic amino acids at the N-terminus of peptide substrates, such as tLeu, Leu, Ile, and Val, does not affect their reaction with **2a**, resulting in the corresponding products in moderate to good yields (entries **3aa**−**3da**). However, dipeptide **1e** containing a Gly residue at the N-terminus exhibited lower reaction efficiency (entry **3ea**).

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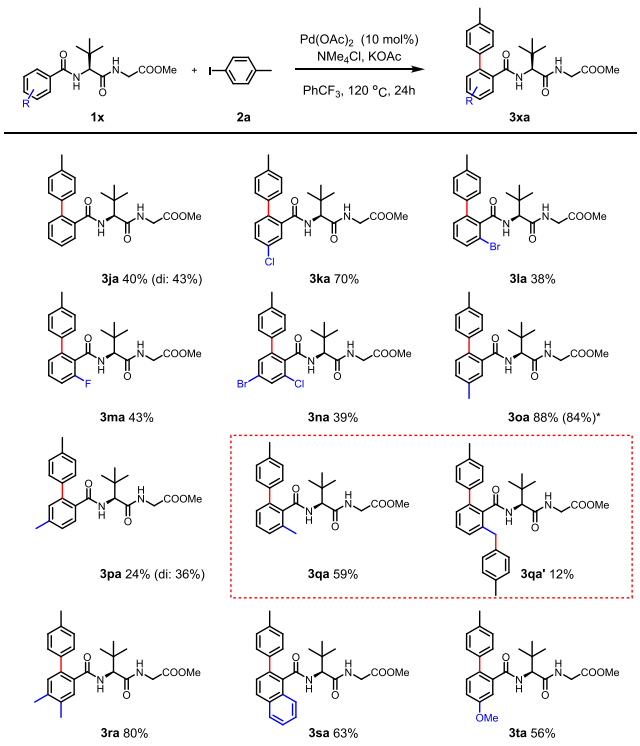

Figure 3. Scope of the arylation reaction with respect to *N*-terminal benzamides of peptide substrates.

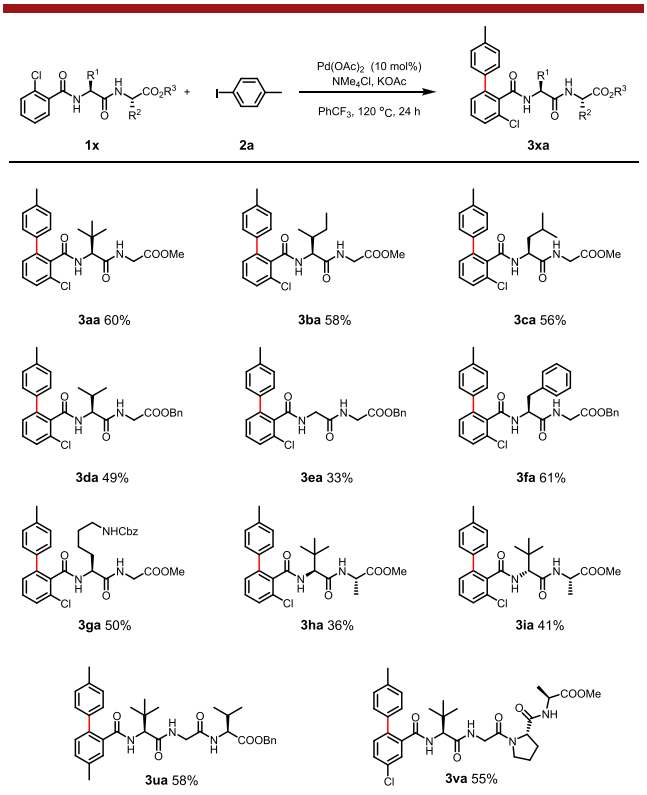

Figure 4. Scope of the arylation reaction with various oligopeptides.

In contrast, incorporation of Phe and protected Lys residues at the N-terminus resulted in good arylation efficiency (entries **3fa** and **3ga**). Substrates **1h** and **1i** both reacted efficiently although the chirality of their C-terminal amino acids is different, indicating that D- or L-amino acids in peptide substrates have little impact on their arylation (entries **3ha** and **3ia**). Furthermore, we examined our method on tripeptide and tetrapeptide substrates **1u** and **1v**, respectively. The results showed that both substrates are modified efficiently by yielding corresponding products (entries **3ua** and **3va**). Thus, this method demonstrates good substrate scope regarding peptides of various sequence and length.

The success of this method on peptide arylation prompted us to further explore its applicability in the synthesis of complex peptide derivatives. First, we synthesized iodobenzene derivatives of bioactive molecules containing a hydroxyl group, including (−)-menthol, cholesterol, and diacetone-D-galactose. Under optimized conditions, these derivatives were efficiently conjugated to peptide substrate **1o** in good yields (Figure 5A),

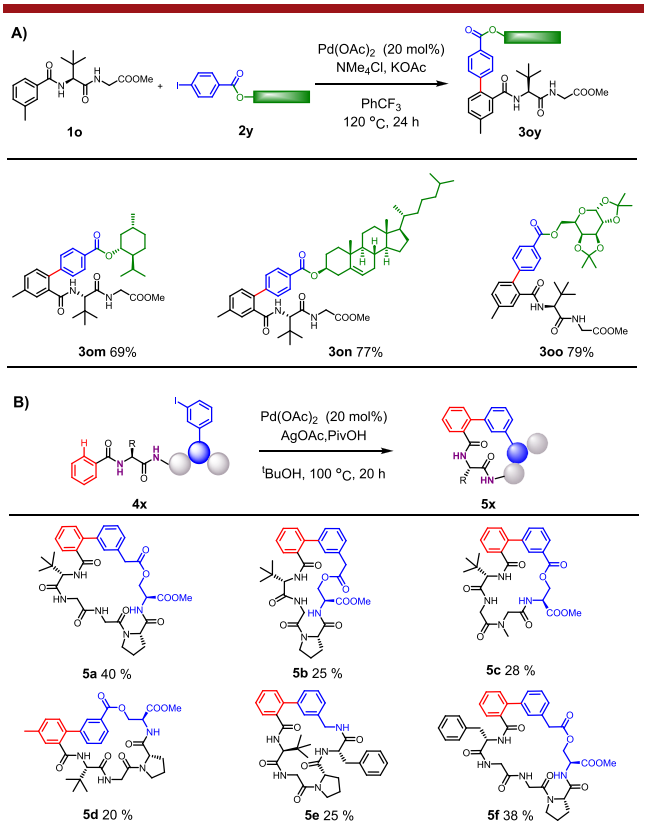

Figure 5. Pd-catalyzed C−H activation to build complex peptide derivatives. (A) Ligation of peptides with bioactive molecules. (B) Macrocyclization of biaryl-bridged cyclic peptides.

demonstrating the versatility of this method. Furthermore, we challenged this method in the construction of peptide macrocycles with biaryl-cross-links (Figure 5B). We first prepared precursor pentapeptide **4a** by incorporating iodobenzene-modified Ser. Under the optimized conditions, substrate **4a** underwent macrocyclization smoothly through *ortho*-arylation, affording the 24-membered macrocycle **5a** in with 80% conversion, as determined by HPLC analysis of the crude reaction mixture (Figure S5), and 40% isolated yield. Finally, the macrocyclization of peptides **4b**−**4f** were examined. Gratifyingly, all substrates cyclized smoothly, and corresponding products were purified in reasonable yields (entries **5b**−**5f**). Thus, this peptide arylation method is efficient in the synthesis of peptide conjugates and macrocycles with unique biaryl cross-links.

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To sum up, we have developed a versatile method for the modification and macrocyclization of short peptides with *N*-terminal benzamide groups via Pd-catalyzed C(sp^2)−H arylation. Amide groups of the peptide backbone behave as directing groups and facilitate the Pd-catalyzed and site-specific arylation. Moreover, our protocol allows direct conjugation of bioactive molecules to peptide substrates as well as direct preparation of peptide macrocycles with aryl−aryl bridges. This chemistry serves as a valuable addition to the chemical toolbox in synthesizing peptide derivatives with complex architectures.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.9b02945.

Experimental procedures and ^{1}H and ^{13}C NMR spectra for all new compounds (PDF)

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Notes

The authors declare no competing financial interest.

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