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Pd(II)-Catalyzed Site-Selective C(sp³)–H Alkynylation of Oligopeptides: A Linchpin Approach for Oligopeptide-Drug Conjugation

Tao Liu^[a], Dr. Jennifer X. Qiao^[b], Dr. Michael A. Poss^[b], and Prof. Dr. Jin-Quan Yu^[a]

^[a]Department of Chemistry, The Scripps Research Institute (TSRI), 10550 North Torrey Pines Road, La Jolla, CA 92037 (USA)

^[b]Department of Discovery Chemistry, Bristol-Myers Squibb, P.O. Box 5400, Princeton, NJ 08543 (USA)

Abstract

The palladium(II)-catalyzed C(sp³)–H alkynylation of oligopeptides was developed with tetrabutylammonium acetate as a key additive. Through molecular design, the acetylene motif serves as a linchpin to introduce a broad range of carbonyl-containing pharmacophores onto oligopeptides, thus providing a chemical tool for the synthesis and modification of novel oligopeptide-pharmacophore conjugates via C–H functionalization. Dipeptide conjugates with coprostanol and estradiol has been synthesized using this method for potential application in targeted drug delivery to tumor cells with overexpressed nuclear hormone receptors.

Graphical Abstract

The palladium(II)-catalyzed C(sp³)–H alkynylation of oligopeptides was developed with tetrabutylammonium acetate as a key additive. Through molecular design, the acetylene motif serves as a linchpin to introduce a broad range of carbonyl-containing pharmacophores onto oligopeptides, thus providing a chemical tool for the synthesis and modification of novel oligopeptide-pharmacophore conjugates via C–H functionalization. Dipeptide conjugates with coprostanol and estradiol have been synthesized using this method for potential application in targeted drug delivery to tumor cells having overexpressed nuclear hormone receptors.

Keywords

palladium; C–H activation; alkynylation; pharmacophore; peptide

Correspondence to: Jin-Quan Yu.

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Substantial progress has been made in the field of selective post synthetic modification of oligopeptides and proteins through bioorthogonal modification of prefunctionalized amino acid residues and reactive side chains.^[1] Combining both chemical and genomics tools,^[1b] methods ranging from ones focusing on cysteine or lysine^[1c–d] to others utilizing metal-catalyzed C–C and C–N bond formation^[1e, 1g] have been developed, and successively utilized in medicinal chemistry in the past decade.

However, due to the inevitable restriction of 20 proteinogenic amino acids, and limited prefunctionalized amino acids and oligopeptides in the toolbox of peptide synthesis, new methods to functionalize unactivated side chains of oligopeptides are still in urgent need. Transition-metal-catalyzed C(sp²)–H functionalization on phenylalanine and tryptophan residues has been elegantly demonstrated on bioactive oligopeptides.^[2] C(sp³)–H arylation of amino acid residues at the *N*-terminus of oligopeptides was also developed recently.^[3] In the context of developing technologies for oligopeptide-drug conjugation, we became interested in developing C(sp³)–H alkynylation of side chains as alkynes can serve as a versatile linchpin for conjugation with various ketone molecules. These peptide-ketone conjugates offer a number of potential pharmaceutical applications (Figure 1). Despite a few recent reports on alkynylation of unactivated C(sp³)–H bonds,^[4] C(sp³)–H alkynylation of peptides has not been developed to date. Moreover, only silyl acetylene derivatives have proven to be suitable reactive coupling partners in C(sp³)–H alkynylation in general.

Herein, we report palladium(II)-catalyzed selective C(sp³)–H alkynylation of unactivated side chains of oligopeptides with alkyne coupling partners. Through this coupling reaction, a wide range of pharmaceutically relevant ketones including biologically active coprostanol, estradiol and fatty acid are covalently attached to peptides. Pharmacologically important amidyl *N*-methylated oligopeptides are also alkynylated using this protocol.

We initiated our investigation of Pd(II)-catalyzed C(sp³)–H alkynylation of oligopeptides with the commonly used coupling partner triisopropylsilane acetylene bromide **2a**, and found that dipeptide **1a** could be alkynylated in 79% NMR yield (entry 1, Table 1). In order to establish a broadly useful linchpin strategy for peptide-drug conjugation through this C(sp³)–H alkynylation, we proposed to covalently link the acetylene to ketones via a simple nucleophilic addition reaction. Hence, the conjugation of carbonyl-containing pharmacophores with oligopeptides can be accomplished via the C–H alkynylation. To test this hypothesis, we prepared propargyl silyl ether **2b** (Table 1, entry 2). Under the same optimized conditions as that for **2a** (Table 1, entry 1), alkynylation of dipeptide **1a** with the new coupling partner **2b** afforded the desired product **3b** only in 23% NMR yield (entry 2).

Subsequent investigation of additives led to a surprising finding. In the presence of tetrabutylammonium acetate (NBu₄OAc), the yield was significantly increased from 23% to 61% (Table 1, entry 4). Other quaternary ammonium salts generally showed lower reactivity (entries 5–9, 21%–39%), as did acetic acid or common acetate salts provided comparable low yields (entries 10–13, <5%–41%). We propose that the acetate anion in NBu₄OAc facilitates C–H activation, and the quaternary ammonium cation enhances the stability of the palladium species during C–H activation.^[5] The combined effects granted NBu₄OAc the

highest reactivity in the C(sp³)-H alkynylation of oligopeptides using propargyl silyl ether **2b** as the coupling partner.

After further optimization, we finalized the conditions for the alkynylation of dipeptide **1a** with the propargyl silyl ether coupling partner **2b**: 10 mol% of Pd(OAc)₂, 1.1 equivalent of AgOAc, 1.0 equivalent of NBu₄OAc, and *t*-butyl alcohol as the solvent (Table 1, entry 14).

With optimized conditions in hand, we investigated the synthesis of dipeptide conjugates from dipeptide **1a** and a wide range of coupling partners derived from ketones (Table 2). In the presence of NBu₄OAc, the isolated yield of the C(sp³)-H alkynylation of dipeptide **1a** with **2a** was increased to 84% (compared with 79% NMR yield without NBu₄OAc, Table 1, entry 1). Propargyl silyl ethers **2b–2l** were prepared from representative ketones of pharmaceutical interest. The oxane-containing coupling partner **2c** and fluoro-containing coupling partner **2d** showed good reactivity, offering the desired product in good isolated yield. Admantane (**2e**), monoterpenoid camphor (**2f**), and menthone (**2g**) are privileged structures in medicinal chemistry research. The corresponding propargyl silyl ethers and dipeptide conjugates were obtained in high yields (**2e–2g**, 74–82%). Cycloheptane **2h** and cyclooctane **2i** are widely found in bio-imaging agents, and alkynylation with each gave high yields of 84% and 87%, respectively. Alkynylation with diethyl substituted propargyl silyl ether **2j** afforded the desired product in 63% yield, while coupling with the highly sterically hindered diisobutyl propargyl silyl ether **2k** gave an excellent yield of 90%. We selected the benzophenone derivative **2l** as a model example of propargyl silyl ethers containing aromatic rings. To our delight, alkynylation of **2l** afforded the desired product in 79% yield.

Overexpressed nuclear hormone receptors have proven to be ideal targets for targeted drug delivery. For instance, coprostanol (**4m**, Figure 2) selectively binds overexpressed androgen receptor (AR) on prostate tumor cells,^[6a] while estradiol (**4n**) selectively binds overexpressed estrogen receptor (ER) on breast tumor cells.^[6b] We demonstrated application of the C(sp³)-H alkynylation method to synthesize the dipeptide-based conjugates **3m** and **3n** in good yields regardless of the steric hindrance introduced by the steroids. A cytotoxic payload can then be attached to either the *N*-terminus or *C*-terminus of the dipeptide in **3m** and **3n**. It is also known that long chain fatty acids are effective as pharmacokinetic enhancers.^[7] Oligopeptide-fatty acid conjugate **3o** was obtained in 68% yield while the free carboxylic acid group on oxostearic acid **4o** was tolerated during the synthesis. The dipeptide conjugates **3m–3o** can then be elongated to form linear or cyclic peptides of interest.

To extend the above protocol to other peptide substrates, dipeptides containing various amino acids at the *C*-terminus were prepared from the corresponding natural or unnatural amino acids (Table 3). Selective alkynylation proceeded smoothly on dipeptides with glycine at the *C*-terminus (**3p**, 67% yield). Dipeptides with alkyl side chains reacted with **2b** to give the desired products in high yields (**3q–3t**, 74%–78%). Desired product **3u** was obtained in 84% yield from the dipeptide containing threonine at the *C*-terminus. Furthermore, phenylalanine (**3v**), *para*-carboxy phenylalanine (**3w**) and tyrosine (**3x**) containing dipeptides showed good reactivity. Desired product **3y** was obtained in 67% yield from the dipeptide containing tryptophan at the *C*-terminus. Alkynylation of the dipeptide derived from 2-

aminoisobutyric acid also proceeded in high yield (**3z**, 84%) using only 5 mol% of Pd(OAc)₂.

Amidyl *N*-methylation has been used as a powerful tool to modify the pharmacological properties of oligopeptides.^[8] To test whether C(sp³)-H alkynylation method is capable of selectively modifying *N*-methylated alanine residues, we prepared the *N*-methylated dipeptide substrates in Table 3. *N*-Acyl was used to mimic *N*-terminus upstream amino acid residues in these peptides. To our delight, Pd-catalyzed alkynylation under slightly modified conditions gave the desired products selectively (**3aa–3ae**). It is worth noting that the extra coordinating group on the side chain of aspartic acid (**3ab**) did not impair proper coordination of the Pd-catalyst and subsequent C(sp³)-H alkynylation of the dipeptide. *N*-Methylated dipeptides derived from *para*-carboxy phenylalanine (**3ac**, **3ad**), as well as tyrosine (**3ae**), were alkynylated in reasonable yields.

Amino acid residues embedded in peptide backbones can potentially offer multiple amidyl coordinating sites to bind to the palladium catalyst in a *N,N*-biscoordinating mode. Tripeptide **5a** (Figure 3) containing three alanine residues was prepared to investigate the possibility of selective labeling of just one alanine. To our delight, the alanine residues at the *C*-terminus facilitated the selective activation of C(sp³)-H on the alanine at the *N*-terminus. Alkynylated tripeptide **6a** was obtained in 47% isolated yield.

In conclusion, we have developed a Pd(II)-catalyzed C(sp³)-H alkynylation of oligopeptides. Through molecular design, the acetylene motif serves as a linchpin to introduce a broad range of privileged carbonyl-containing pharmacophores onto oligopeptides. This straightforward C(sp³)-H functionalization offers rapid access to novel oligopeptide-pharmacophore conjugates, and has the potential to accelerate the development of peptide-conjugate based therapeutics. Further investigation is ongoing in our laboratory to modify native oligopeptides and cyclic peptides through direct C-H functionalizations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.
Linchpin approach for oligopeptide-drug conjugation.

Figure 2. Synthesis of oligopeptide-pharmacophore conjugates. AR: Androgen receptor. ER: Estrogen receptor.

Figure 3.
Pd-Catalyzed alkylation on tripeptide by *N,N*-biscoordination.

Table 1Optimization of reaction conditions.^[a,b]

Entry	Coupling Partner	Additive	NMR Yield
1	2a	K ₃ PO ₄	79%
2	2b	K ₃ PO ₄	23%
3	2b	-	8%
4	2b	NBu₄OAc	61%
5	2b	NBu ₄ I	39%
6	2b	NBu ₄ Br	34%
7	2b	NBu ₄ Cl	27%
8	2b	NBu ₄ BF ₄	35%
9	2b	NBu ₄ PF ₆	21%
10	2b	HOAc	19%
11	2b	LiOAc	<5%
12	2b	NaOAc	24%
13	2b	KOAc	41%
14 ^[c]	2b	NBu₄OAc	84%

^[a] Conditions: **1a** (0.1 mmol), **2a** or **2b** (1.5 equiv), Pd(OAc)₂ (10 mol%), AgOAc (1.1 equiv), additive (1.0 equiv), *t*-butyl alcohol (1.0 mL), 100 °C in air, 14 h.

^[b] NMR yields were determined with CH₂Br₂ as internal standard.

^[c] **2b** (3.0 equiv).

Table 2Alkynylation with different propargyl silyl ether coupling partners.^[a,b]

^[a] Conditions: **1a** (0.1 mmol), **2** (3.0 equiv), Pd(OAc)₂ (10 mol%), AgOAc (1.1 equiv), NBu₄OAc (1.0 equiv), *t*-butyl alcohol (1.0 mL), 100 °C in air, 14 h.

^[b] Isolated yield.

Table 3

Alkynylation of various dipeptides **1** with alkynyl bromide **2b**.^[a, b]

^[a] Conditions: **1** (0.1 mmol), **2b** (3.0 equiv), Pd(OAc)₂ (10 mol%), AgOAc (1.1 equiv), NBu₄OAc (1.0 equiv), *t*-butyl alcohol (1.0 mL), 100 °C in air, 14 h.

^[b] Isolated yield.

^[c] 5 mol% of Pd(OAc)₂ was used.

^[d] Conditions: 120 °C, 4 h. NMR yields in round brackets.

^[e] Using **2a** (3.0 equiv) instead of **2b** as the coupling partner.