CHEMISTRY

Macrocyclization of peptidoarylacetamides with self-assembly properties through late-stage palladium-catalyzed C(sp^2)–H olefination

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Peptide macrocycles often display diverse bioactivities and self-assembly properties, which lead to a variety of applications in medicinal and material sciences. Transition metal–catalyzed C–H activations are emerging strategies for site-selective functionalization of amino acids and peptides, as well as the construction of cyclic peptides. Here, we report the development of a peptide-directed method for the macrocyclization of peptidoarylacetamides by Pd(II)-catalyzed late-stage C(sp^2)–H olefination. In this protocol, peptide backbones act as internal directing groups and enable facile preparation of diverse cyclic peptides that are difficult to synthesize by conventional macrolactamization. Furthermore, we show that the incorporation of aryl-alkene cross-link in the backbone constrains cyclic peptides into conformations for self-assembly.

INTRODUCTION

Peptides and peptidomimetics are important compounds with broad applications in fields including medicinal chemistry (*1*–*3*) and material sciences (*4*). In particular, cyclic peptides have shown great success as therapeutics by combining favorable properties such as high binding affinity, target selectivity, and capability in regulating protein-protein interactions (*5*–*7*). In addition, cyclic peptides with self-assembly properties are important building blocks for organic nanotubes, which have a broad application in artificial ion channels, antimicrobial agents, and electronic materials (*4*, *8*, *9*). It is therefore highly desirable to develop efficient chemical methods to prepare peptide-based macrocycles. The strategy of transition metal–catalyzed C–H activation has recently been applied in the functionalization and macrocyclization of peptides, providing new peptide scaffolds that are not easily accessible from traditional methods (*10*, *11*). Seminal examples of late-stage C–H functionalization of peptides using Pd catalysts include β–C(sp^3)–H arylation and alkynylation at the N-terminal amino acid by Yu and colleagues (*12*, *13*), γ–C(sp^3)–H carbonylation of peptides by Carretero *et al.* (*14*), δ–C(sp^3)–H alkylation of peptides by Shi *et al.* (*15*), and cocatalyzed δ–C(sp^2)–H activation by Ackermann *et al.* (*16*). As a decent demonstration of the compatibility of C–H activation with functional motifs, Ackermann and co-workers (*17*, *18*) developed Pd-catalyzed β–C(sp^3)–H arylation that allows boron-dipyrromethene (BODIPY) labeling of peptides. In terms of peptide macrocyclization, classic methods include lactamization (*19*, *20*), disulfide formation (*21*), thioether cross-linking (*22*), ring-closing olefin metathesis (*23*–*26*), and Cu-catalyzed cycloaddition of azides to alkynes (*27*). As an important addition to the chemical toolbox, transition metal catalysis has achieved peptide stapling through Pd-catalyzed tryptophan C-2 arylation (*28*–*30*), β–C(sp^3)–H arylation (*31*, *32*), Mn-catalyzed C–H alkynylation (*33*), and δ–C(sp^2)–H olefination of phenylalanine (Phe) (*34*) and N-terminal arylsulfonamides (Fig. 1A) (*35*). Most recently, Chen *et al.* (*36*)reported a powerful strategy to synthesize cyclophane-braced peptide macrocycles through 8-aminoquinoline–directed arylation of C(sp^3)–H bonds (Fig. 1A).

As our continuous effort in developing chemical methods to synthesize cyclic peptides with high generality and atom economy, we use backbone amides as directing groups to promote Pd-catalyzed late-stage peptide functionalization. Here, we report a peptide-directed method for the functionalization and macrocyclization of arylacetamide-peptide conjugates (peptidoarylacetamides) by Pd(II)-catalyzed late-stage C–H activation. This reaction has a broad substrate scope and provides facile access to a variety of arylacetamide peptidomimetics. The *N*-acetylated peptides act as internal directing groups, and no external directing groups are required. In addition, this protocol allows macrocyclization of substrates bearing acrylates or unactivated alkenes to generate peptidoarylacetamide macrocycles with unique aryl-alkene cross-links, which are often found in cyclic peptide natural products and challenging to prepare by conventional macrolactamization. Furthermore, we show that the resulting peptide macrocycles containing unique aryl-alkene cross-links exhibit self-assembly properties in organic solvents.

RESULTS

We initiated our investigation by evaluating the utility of dipeptides as directing groups to enable the olefination of aryl acetamides. To establish optimal reaction conditions, we used an *ortho*-methylbenzylacetamide dipeptide conjugate **1a** and methyl acrylate **2a** as substrates (Fig. 2, entry **3aa**). Detailed optimization studies revealed that the reaction proceeds most efficiently with 1.2 equivalents of methyl acrylate **2a** in the presence of 10 mol% $Pd(OAc)_2$ and 2.0 equivalents of AgOAc in dichloroethane (DCE) at 80°C for 20 hours, affording the *ortho*-olefination product **3aa** in 94% yield (table S1). Nuclear magnetic resonance (NMR) analysis of product (3aa) indicated that the double bond was in *E*-configuration (fig. S1). Replacement of the dipeptide by tLeu methyl ester (fig. S2, substrate **1a′**) completely abolished the reaction under standard conditions, indicating that the dipeptide is required to enable the olefination reaction.

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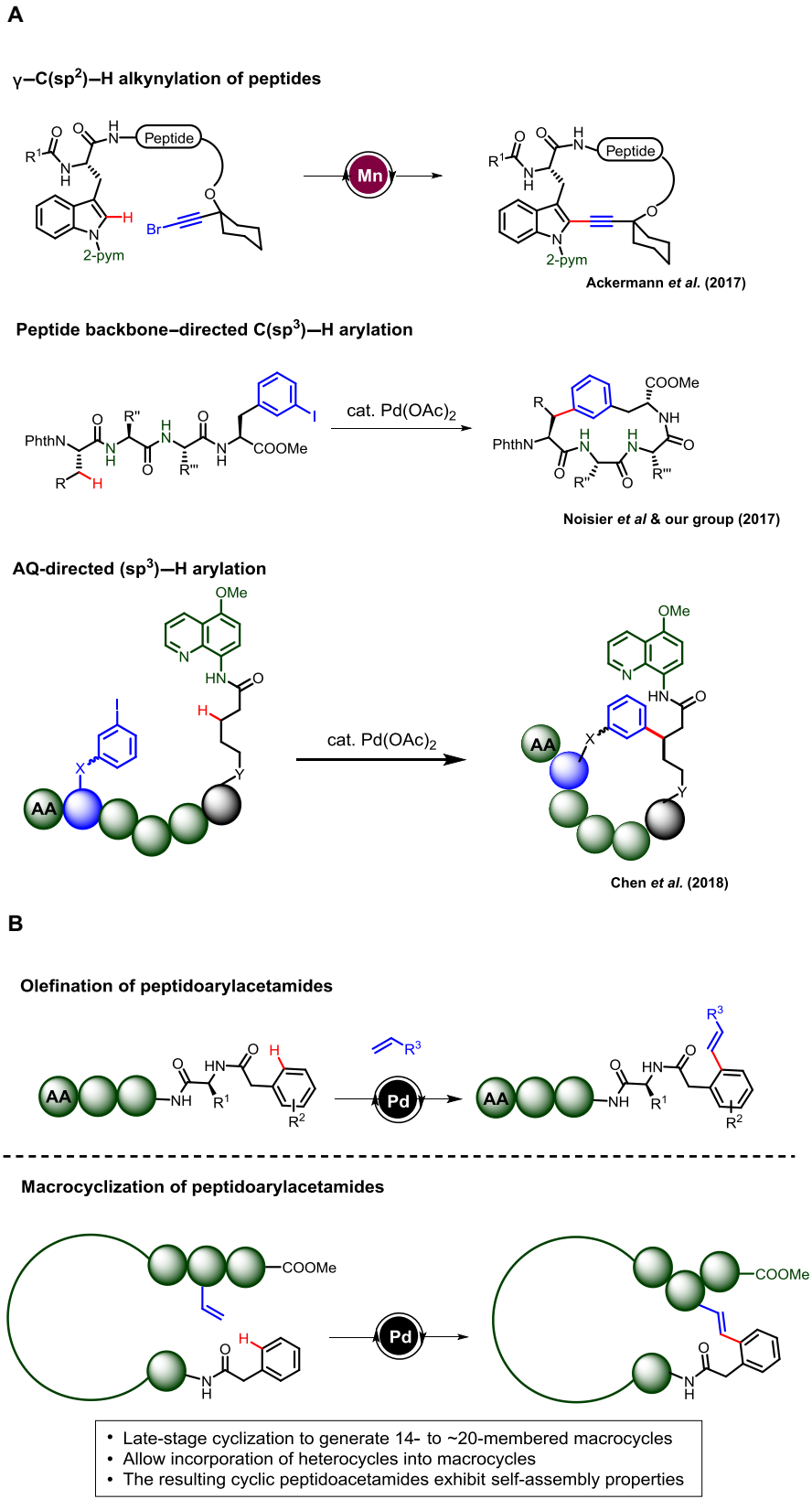

Fig. 1. Peptide-directed site-selective olefination and macrocyclization of peptidoarylacetamides through Pd-catalyzed late-stage C(sp^2)–H activation. (**A**) Macrocyclization of peptides via directed C–H activation. (**B**) This work: Peptide-enabled late-stage C(sp^2)–H macrocyclization of peptidoarylacetamides. AQ, 8-aminoquinoline.

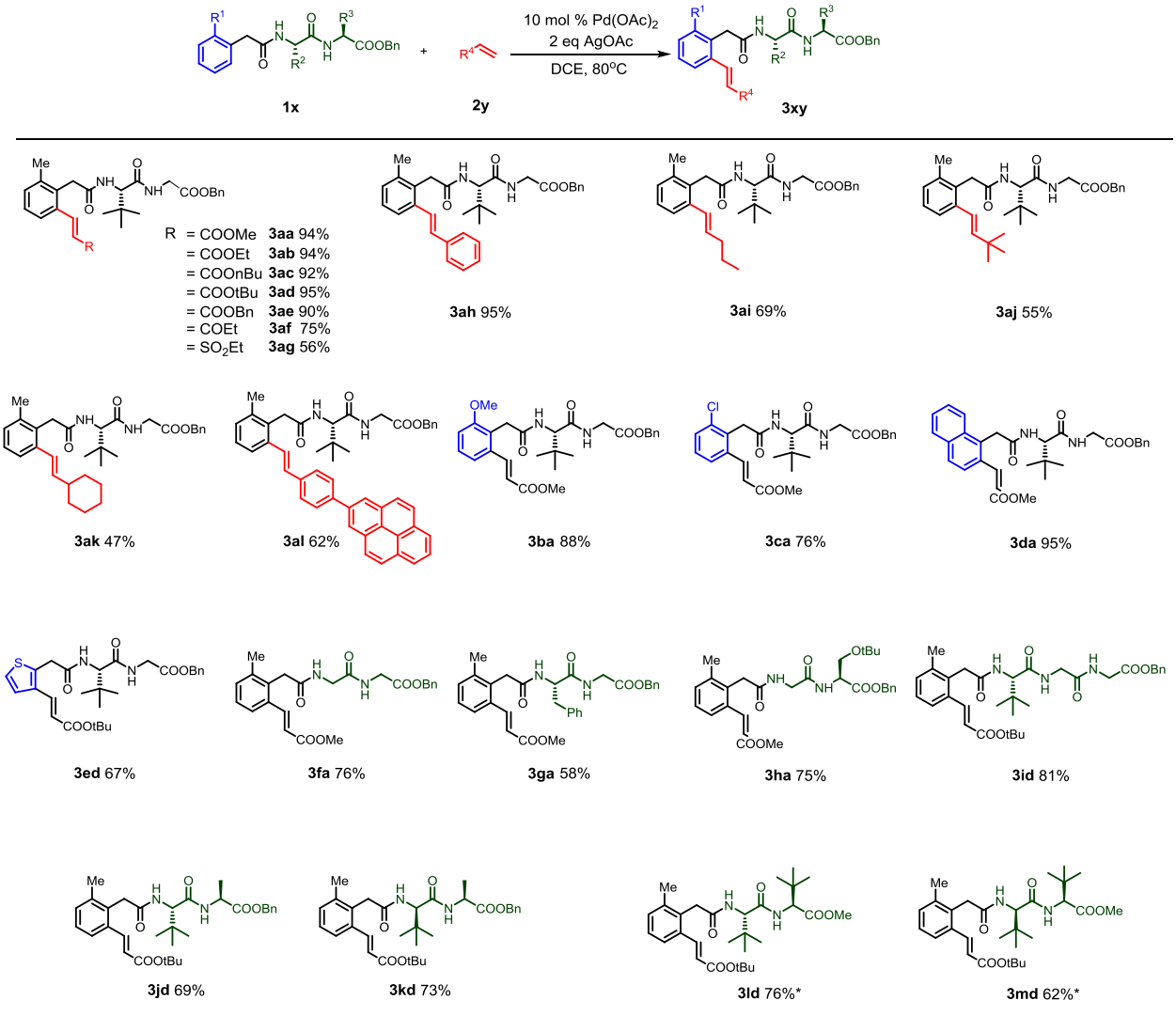

Fig. 2. Scope of the backbone-directed olefination reactions of *N*-arylacetamide peptides. *Reaction conversions determined by high-performance liquid chromatography analysis.

With the optimal conditions in hand, we proceeded to examine the substrate scope of this peptide-enabled reaction (Fig. 2). With dipeptide conjugate (**1a**) as the model substrate, we first evaluated a variety of alkenes as olefination reagents. Results showed that acrylate ethyl ester (**2b**) and acrylate butyl esters (**2c**) and (**2d**) all reacted with (**1a**) efficiently, yielding corresponding products in excellent yields [entries (**3ab**) to (**3ad**)]. Benzyl acrylate (**2e**) and pent-1-en-3-one (**2f**) are also good substrates, resulting in products (**3ae**) and (**3af**) in 90 and 75% yields, respectively. The reaction with (ethylsulfonyl) ethane (**2g**) proceeded with a moderate isolated yield of 56%. Pd-catalyzed olefination of arenes with unactivated alkenes is generally challenging (*37*–*39*). To our delight, substrate (**1a**) reacted smoothly with styrene (**2h**), pent-1-ene (**2i**), 3,3-dimethylbut-1-ene (**2j**), and vinylcyclohexane (**2k**) to generate the corresponding products in good yields [entries (**3ah**) to (**3ak**)]. Note that no branching products were observed during these reactions. Fluorescent labeling of peptides is highly useful in chemical biology and has been achieved by C–H activation methods (*18*, *40*). Our approach successfully installs a fluorescent 4-(4-vinylphenyl)pyrene (**2l**) onto dipeptide in 62% yield (entry **3al**), further demonstrating the broad scope of this protocol toward olefin donors. Next, we evaluated the impact of aryl substitutions on the reaction. Using methyl acrylate (**2a**) as the model alkene, substrates with *ortho*-substitutions, including methoxyl and chloro groups, underwent facile olefination in excellent yields [entries (**3ba**) and (**3ca**)]. 2-(naphthalen-1-yl)acetamide and thiophene dipeptide conjugates (**1d**) and (**1e**) also reacted with acrylates efficiently, resulting in products (**3da**) and (**3ed**) in 95 and 67% yields, respectively. The reaction was further shown to be compatible with various dipeptide and tripeptide substrates [entries (**3fa**) to (**3id**)]. To examine the impact of the chirality of peptide substrates, we synthesized (**1j**) and (**1k**) containing D-tLeu-Ala and L-tLeu-Ala dipeptides and subjected them to reactions with alkene (**2d**). Results showed that both reactions proceeded efficiently with similar yields [entries (**3jd**) to (**3kd**)]. To address the potential epimerization issue during the reaction, we synthesized substrates **1l** and **1m** and subjected them to reactions with acrylate **2d**. Results showed that crude reaction

mixtures of (**1l**) and (**1m**) gave distinct retention times when analyzed by high-performance liquid chromatography (fig. S3), indicating that the stereochemical integrity was retained and no epimerization occurred under the reaction conditions. Together, these results demonstrate the versatility of this peptide-directed olefination for peptidoarylacetamides.

On the basis of the high efficiency of this olefination reaction, we further used this method for the homologation of dipeptide (**1a**) with bis-functional alkenes (**4a** and **4b**) through twofold C–H olefination, delivering tetrapeptides (**5aa**) and (**5ab**) in good yields (Fig. 3A). The robustness of this reaction was further demonstrated by the site-selective ligation of amino acids and peptides to peptidoarylacetamides. Alkene-modified serine (**4c**) and dipeptide (**4d**) both reacted with substrate (**1n**) efficiently (Fig. 3B, entries **5nc** and **5nd**). These results indicate the potential application of this method in the preparation of complex peptide conjugates.

Encouraged by the success of intermolecular olefination of arylacetamide-peptide conjugates, we further explored the applicability of our method in generating cyclic peptides with aryl-alkene cross-links, which are found in bioactive cyclopeptide alkaloid natural products (*41*, *42*). We started our attempt with a dipeptide substrate (**6a**) containing an acrylate-modified Ser residue. Results showed that this reaction proceeded smoothly to yield a 14-membered cyclic peptide (**7a**) in 26% isolated yield (55% NMR yield; fig. S4). Structural analysis by NMR and x-ray crystallography revealed that the olefination reaction occurred at the *ortho* position of methylbenzylacetamide motif, and the resulting double bond is in *E*-configuration (Fig. 4 and fig. S5). Tripeptides (**6b**) cyclized with substantially increased efficiency, affording a 17-membered macrocycle (**7b**) in 65% isolated yield (92% NMR yield; fig. S6). In contrast, conventional macrolactamization of precursor **6b**′ under hexafluorophosphate azabenzotriazole tetramethyl uronium (HATU)/*N*,*N*-diisopropylethylamine (DIPEA) conditions only led to 12% conversion and trace isolated yield of (**7b**) (fig. S7). This result demonstrates that this Pd-catalyzed C–H activation method is superior to conventional amide condensation in generating constrained peptidoarylacetamide macrocycles. In addition, tripeptide substrates with Ala and Phe were positioned at the second residue to the N terminus (entries **7c** and **7d**). Tetrapeptide conjugates are also tolerated in this protocol by generating a 20-membered peptide macrocycles **7e** and **7f** in moderate yields. Next, we challenged our method by introducing an unactivated alkene in the substrates for macrocyclization. The C-terminal serine was therefore conjugated with pent-4-enoic acid at the side chain hydroxyl group in tripeptide substrates (**6g**) and (**6h**). To our delight, treatment of (**6g**) and (**6h**) with Pd catalyst resulted in a successful generation of 19-membered peptide macrocycles (**7g**) and (**7h**) in 43 and 38% isolated yields, respectively. The relatively low isolated yields of cyclic peptide products is mainly due to the loss of material during purification, because the NMR yields of (**7g**) and (**7h**) were 69 and 75%, respectively (figs. S8 and S9). Last,

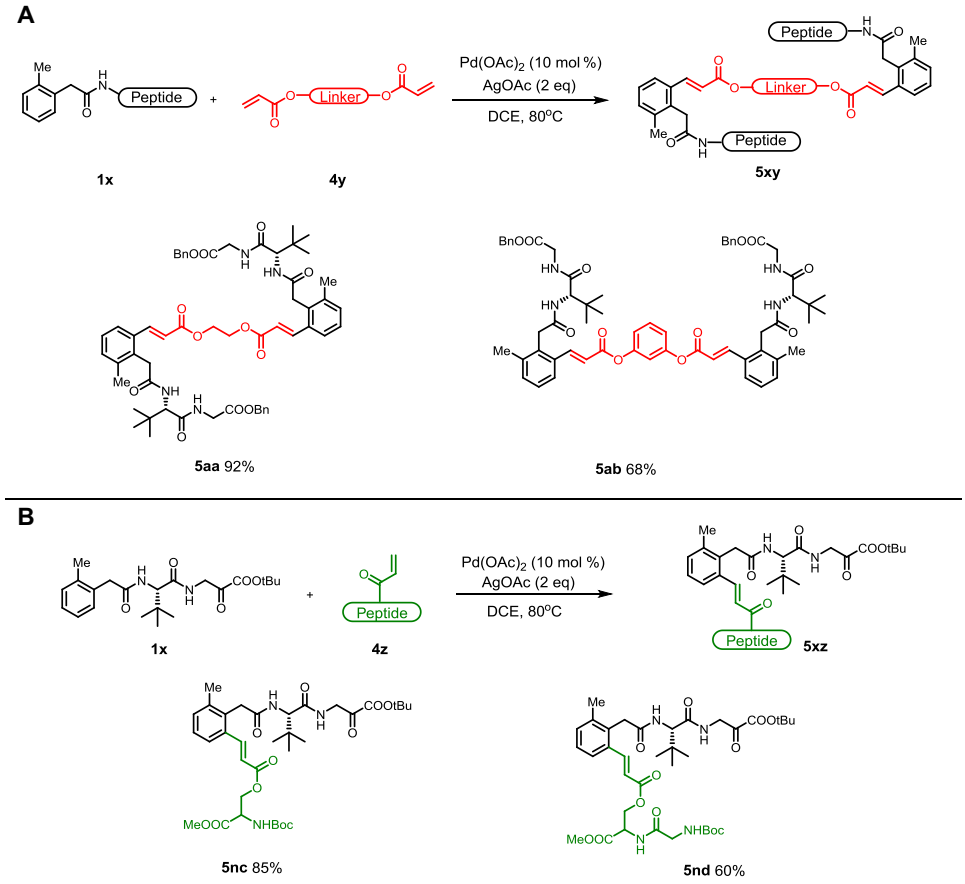

Fig. 3. Site-specific ligation of amino acids and peptides. (**A**) Two-fold C–H olefination for the homologation of dipeptides. (**B**) Ligation with amino acids and peptides bearing alkenes.

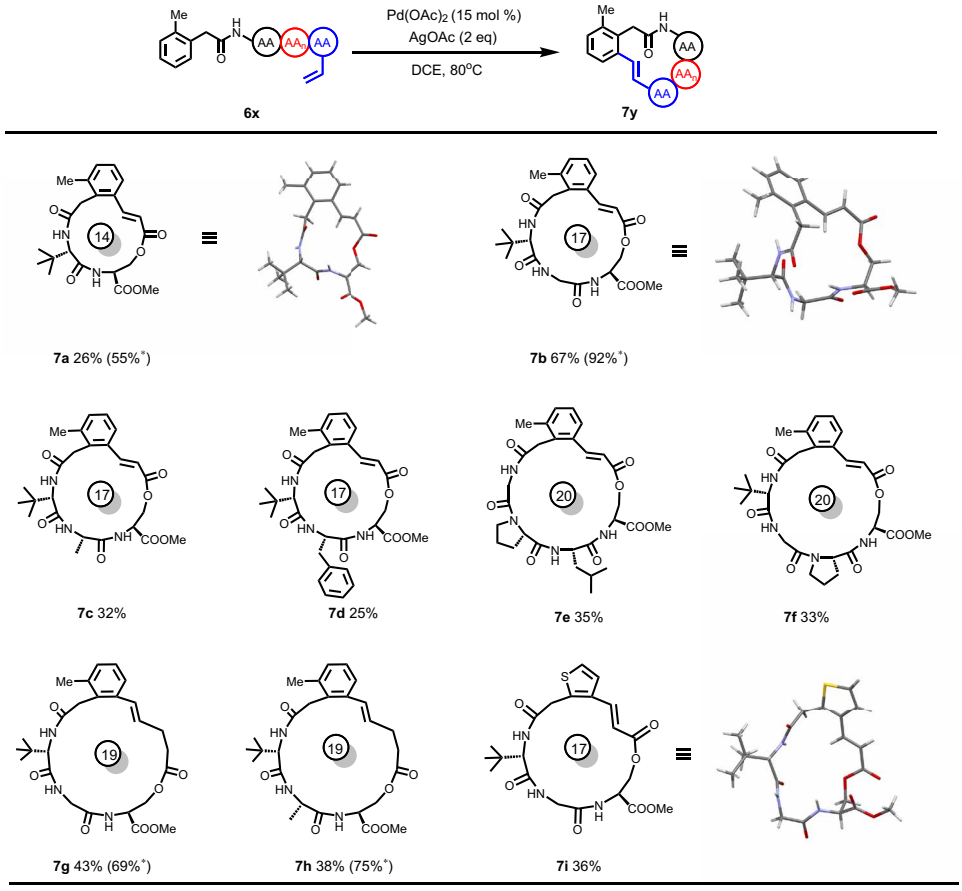

Fig. 4. Macrocyclization via late-stage Pd-catalyzed C(sp^2)–H olefination of peptidoarylacetamides. In the crystal structures, carbon, nitrogen, oxygen, sulfur, and hydrogen are shown in gray, blue, red, yellow, and white, respectively. NMR yields are provided in parentheses marked with * symbol.

we applied our method to incorporate heterocycles into cyclic peptides. Thiophene-acetamide peptide conjugate (**6i**) was therefore synthesized and subjected to cyclization conditions, leading to the production of macrocycle (**7i**) in 36% isolated yield, further demonstrating the versatility of this peptide-enabled macrocyclization (Fig. 4).

DISCUSSION

Crystal structures of peptidoarylacetamide macrocycles (**7a**) and (**7b**) reveal their unique self-assembly pattern. From the *A*-axis view of the crystal packing, macrocycle (**7b**) adopts a bent conformation (Fig. 5A), in which the carbonyl oxygen of tLeu forms hydrogen bonds with the Ha of NH(Gly) and the αH_d of tLeu from another molecule. From the *B*-axis view, molecules of (**7b**) align perfectly into channels with a width of 6.6 Å (Fig. 5A), where the carbonyl oxygen of Gly forms a hydrogen bond with H_f from the methoxyl group of the adjacent channel. To examine whether this self-assembly of compound **7b** is preserved in organic solvents, we performed a concentration-dependent NMR analysis in chloroform. Along with the increase of (**7b**) concentration, dramatic shift of H_a signal was observed, which is in good correlation with its direct involvement in the intermolecular hydrogen bonding (Fig. 5B). Similarly, the signals of H_d and H_e also exhibited a substantial shift when the concentration of (**7b**) was increased. In contrast, two other amide hydrogens, H_b and H_c, which are not directly involved in hydrogen bonding in the crystal structure, only displayed minor changes in their chemical shifts. Together, NMR analysis suggests that cyclic peptide (**7b**) has similar self-assembly behavior in organic solvent as in crystal packing. Compound (**7a**) displays a different packing pattern in the crystal structure by adopting a flat conformation. Molecules of (**7a**) associate in parallel through the intermolecular hydrogen bonding between amide bonds (Fig. 5C). The aromatic *o*-methylbenzyl groups stack on each other, presumably driven by π-π interactions. Along the assembly axis, macrocycle (**7a**) aligned to form a channel with a width of 6.5 Å. The concentration-dependent NMR analysis further suggested their self-assembly in organic solvent, as indicated by the change of chemical shifts of amide NH hydrogens, H_m and H_i (Fig. 5D). Thus, we demonstrate that peptidoarylacetamide macrocycles containing aryl-alkene cross-links have self-assembly properties in organic solvent.

CONCLUSION

In conclusion, we have developed an efficient late-stage peptide functionalization method through Pd-catalyzed C(sp^2)–H olefination that is facilitated by the internal peptide backbone. This method can be applied in the ligation and macrocyclization of peptidoarylacetamides. Furthermore, we demonstrate that the peptidoarylacetamide macrocycles containing aryl-alkene cross-links display unique self-assembly properties in organic solvents and therefore

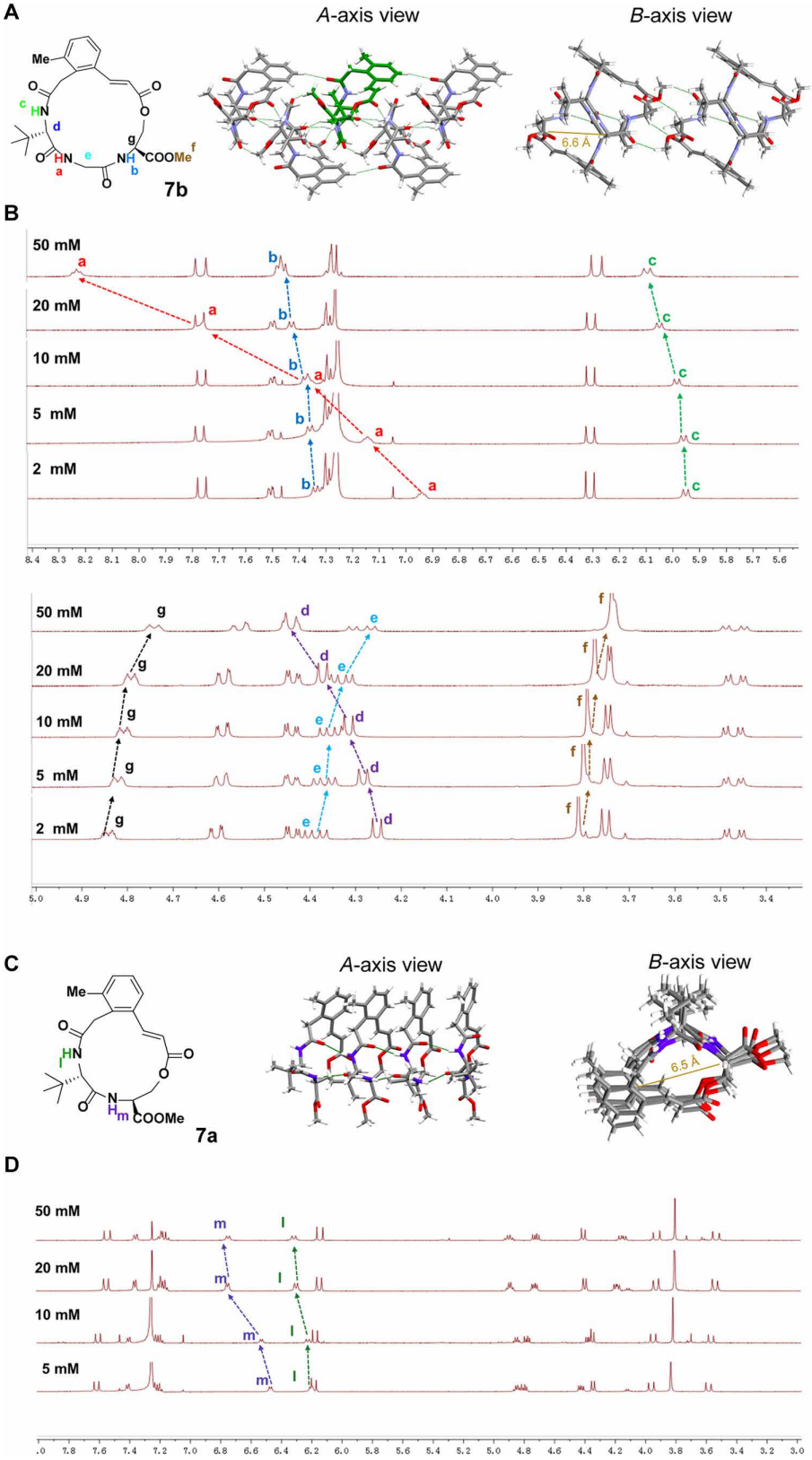

Fig. 5. Self-assembly of cyclic peptidoarylacetamides containing aryl-alkene cross-links. (**A**) Chemical and crystal structures of compound (**7b**). (**B**) Concentration-dependent ^{1}H NMR analysis of compound (**7b**) in chloroform. (**C**) Chemical and crystal structures of compound (**7a**). (**D**) Concentration-dependent ^{1}H NMR analysis of compound (**7a**) in chloroform. In the crystal structures, carbon, nitrogen, oxygen, sulfur and hydrogen are shown in grey, blue, red, yellow, and white, respectively. The contacts with a distance less than 3.0 nm are connected in green lines.

have potential applications in peptide-based material science. Our studies highlight the potency of the peptide backbone as an efficient directing group to facilitate site-selective functionalization of peptides and peptidomimetics through palladium catalysis.

MATERIALS AND METHODS

General procedure for the synthesis of peptidoarylacetamides

Oligopeptides were prepared by standard liquid-phase peptide synthesis. N-Boc oligopeptides were then subjected to 4 M HCl/dioxane for 4 hours at 0°C. Upon completion, the reaction mixture was concentrated and extracted with dichloromethane three times. The organic layer was combined, dried over anhydrous Na_2SO_4, and concentrated under vacuum. The resulting residue was purified with column chromatography to yield oligopeptide with a free amine at the N terminus. The oligopeptides were then coupled with 2-(o-tolyl) acetic acid to yield corresponding peptidoarylacetamides.

General procedure for Pd-catalyzed olefination of peptidoarylacetamides

To a 15-ml sealed reaction tube, dipeptide substrate (0.2 mmol) and olefin (0.24 mmol) were added to the reaction mixture containing $Pd(OAc)_2$ (0.02 mmol) and AgOAc (0.4 mmol) in DCE (2 ml). The reaction was conducted at 80°C for 20 hours and cooled to room temperature upon completion. The resulting sample was diluted with ethyl acetate (5.0 ml), filtered through a Celite pad, concentrated under reduced pressure, and purified by column chromatography.

General procedure for Pd-catalyzed macrocyclization of peptidoarylacetamides

Peptidoarylacetamides substrates containing an acrylate-modified Ser residue (0.2 mmol) were mixed with $Pd(OAc)_2$ (0.03 mmol) and AgOAc (0.4 mmol) in DCE (4 ml) in a 15-ml sealed reaction tube. The reaction mixture was stirred at 80°C for 20 hours. Upon completion, the reaction mixture was diluted with ethyl acetate (5.0 ml), filtered through a Celite pad, concentrated under reduced pressure, and purified by column chromatography.

SUPPLEMENTARY MATERIALS

Supplementary material for this article is available at http://advances.sciencemag.org/cgi/content/full/5/3/eaaw0323/DC1

Table S1. Optimization of reaction conditions for dipeptide conjugate **1a** and methyl acrylate **2a**.
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Table S3. Fractional atomic coordinates ($\times 10^4$) and equivalent isotropic displacement parameters ($Å^2 \times 10^3$) for compound **7a**.
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Table S5. Bond lengths for compound **7a**.
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Fig. S11. ^{1}H NMR spectrum of macrocycles **7a** at various concentrations.
Fig. S12. X-ray structure of compound **7a**.
Fig. S13. X-ray structure of compound **7b**.
Fig. S14. X-ray structure of compound **7i**.

REFERENCES AND NOTES

1. A. Zorzi, K. Deyle, C. Heinis, Cyclic peptide therapeutics: Past, present and future. *Curr. Opin. Chem. Biol.* **38**, 24–29 (2017).
2. L. J. Walport, R. Obexer, H. Suga, Strategies for transitioning macrocyclic peptides to cell-permeable drug leads. *Curr. Opin. Biotechnol.* **48**, 242–250 (2017).
3. A. A. Kaspar, J. M. Reichert, Future directions for peptide therapeutics development. *Drug Discov. Today* **18**, 807–817 (2013).
4. I. W. Hamley, Peptide nanotubes. *Angew. Chem. Int. Ed.* **53**, 6866–6881 (2014).
5. A. Russo, C. Aiello, P. Grieco, D. Marasco, Targeting "undruggable" proteins: Design of synthetic cyclopeptides. *Curr. Med. Chem.* **23**, 748–762 (2016).
6. A. Sandomenico, S. M. Monti, M. Sabatella, A. de Capua, L. Tornatore, N. Doti, F. Viparelli, N. A. Dathan, C. Pedone, M. Ruvo, D. Marasco, Protein-protein interactions: A simple strategy to identify binding sites and peptide antagonists. *Chem. Biol. Drug Des.* **73**, 483–493 (2009).
7. Z. Qian, P. G. Dougherty, D. Pei, Targeting intracellular protein-protein interactions with cell-permeable cyclic peptides. *Curr. Opin. Chem. Biol.* **38**, 80–86 (2017).
8. J. Montenegro, M. R. Ghadiri, J. R. Granja, Ion channel models based on self-assembling cyclic peptide nanotubes. *Acc. Chem. Res.* **46**, 2955–2965 (2013).
9. R. Chapman, M. Danial, M. L. Koh, K. A. Jolliffe, S. Perrier, Design and properties of functional nanotubes from the self-assembly of cyclic peptide templates. *Chem. Soc. Rev.* **41**, 6023–6041 (2012).
10. W. Wang, M. M. Lorion, J. Shah, A. R. Kapdi, L. Ackermann, Late-stage peptide diversification by position-selective C-H activation. *Angew. Chem. Int. Ed.* **57**, 14700–14717 (2018).
11. A. F. M. Noisier, M. A. Brimble, C-H functionalization in the synthesis of amino acids and peptides. *Chem. Rev.* **114**, 8775–8806 (2014).
12. W. Gong, G. Zhang, T. Liu, R. Giri, J.-Q. Yu, Site-selective C(sp^3)-H functionalization of di-, tri-, and tetrapeptides at the N-terminus. *J. Am. Chem. Soc.* **136**, 16940–16946 (2014).
13. T. Liu, J. X. Qiao, M. A. Poss, J.-Q. Yu, Palladium(II)-catalyzed site-selective C(sp^3)-H alkynylation of oligopeptides: A linchpin approach for oligopeptide-drug conjugation. *Angew. Chem. Int. Ed.* **56**, 10924–10927 (2017).
14. E. Hernando, J. Villalva, Á. M. Martínez, I. Alonso, N. Rodríguez, R. Gómez Arrayás, J. C. Carretero, Palladium-catalyzed carbonylative cyclization of amines via γ-C(sp^3)-H activation: Late-stage diversification of amino acids and peptides. *ACS Catal.* **6**, 6868–6882 (2016).
15. B.-B. Zhan, Y. Li, J.-W. Xu, X.-L. Nie, J. Fan, L. Jin, B.-F. Shi, Site-selective δ-C(sp^3)–H alkylation of amino acids and peptides with maleimides via a six-membered palladacycle. *Angew. Chem. Int. Ed.* **57**, 5858–5862 (2018).
16. M. M. Lorion, N. Kaplaneris, J. Son, R. Kuniyil, L. Ackermann, Late-stage peptide diversification by cobalt-catalyzed C–H activation: Sequential multicatalysis for stapled peptides. *Angew. Chem. Int. Ed.* **58**, 1684–1688 (2019).
17. M. Bauer, W. Wang, M. M. Lorion, C. Dong, L. Ackermann, Internal peptide late-stage diversification: Peptide-isosteric triazoles for primary and secondary C(sp^3)–H activation. *Angew. Chem. Int. Ed.* **57**, 203–207 (2018).
18. W. Wang, M. M. Lorion, O. Martinazzoli, L. Ackermann, BODIPY peptide labeling by late-stage C(sp^3)–H activation. *Angew. Chem. Int. Ed.* **57**, 10554–10558 (2018).
19. G. K. T. Nguyen, X. Hemu, J.-P. Quek, J. P. Tam, Butelase-mediated macrocyclization of D-amino-acid-containing peptides. *Angew. Chem. Int. Ed.* **55**, 12802–12806 (2016).
20. J. S. Davies, The cyclization of peptides and depsipeptides. *J. Pept. Sci.* **9**, 471–501 (2003).

21. M. Góngora-Benítez, J. Tulla-Puche, F. Albericio, Multifaceted roles of disulfide bonds. Peptides as therapeutics. *Chem. Rev.* **114**, 901–926 (2014).
22. H. Jo, N. Meinhardt, Y. Wu, S. Kulkarni, X. Hu, K. E. Low, P. L. Davies, W. F. DeGrado, D. C. Greenbaum, Development of α-helical calpain probes by mimicking a natural protein-protein interaction. *J. Am. Chem. Soc.* **134**, 17704–17713 (2012).
23. H. E. Blackwell, J. D. Sadowsky, R. J. Howard, J. N. Sampson, J. A. Chao, W. E. Steinmetz, D. J. O'Leary, R. H. Grubbs, Ring-closing metathesis of olefinic peptides: Design, synthesis, and structural characterization of macrocyclic helical peptides. *J. Org. Chem.* **66**, 5291–5302 (2001).
24. H. E. Blackwell, R. H. Grubbs, Highly efficient synthesis of covalently cross-linked peptide helices by ring-closing metathesis. *Angew. Chem. Int. Ed.* **37**, 3281–3284 (1998).
25. L. D. Walensky, A. L. Kung, I. Escher, T. J. Malia, S. Barbuto, R. D. Wright, G. Wagner, G. L. Verdine, S. J. Korsmeyer, Activation of apoptosis in vivo by a hydrocarbon-stapled BH3 helix. *Science* **305**, 1466–1470 (2004).
26. C. E. Schafmeister, J. Po, G. L. Verdine, An all-hydrocarbon cross-linking system for enhancing the helicity and metabolic stability of peptides. *J. Am. Chem. Soc.* **122**, 5891–5892 (2000).
27. V. D. Bock, R. Perciaccante, T. P. Jansen, H. Hiemstra, J. H. van Maarseveen, Click chemistry as a route to cyclic tetrapeptide analogues: Synthesis of cyclo-[Pro-Val-psi(triazole)-Pro-Tyr]. *Org. Lett.* **8**, 919–922 (2006).
28. L. Mendive-Tapia, A. Bertran, J. García, G. Acosta, F. Albericio, R. Lavilla, Constrained cyclopeptides: Biaryl formation through Pd-catalyzed C-H activation in peptides-structural control of the cyclization vs. cyclodimerization outcome. *Chem. Eur. J.* **22**, 13114–13119 (2016).
29. L. Mendive-Tapia, S. Preciado, J. García, R. Ramón, N. Kielland, F. Albericio, R. Lavilla, New peptide architectures through C–H activation stapling between tryptophan-phenylalanine/tyrosine residues. *Nat. Commun.* **6**, 7160 (2015).
30. H. Dong, C. Limberakis, S. Liras, D. Price, K. James, Peptidic macrocyclization via palladium-catalyzed chemoselective indole C-2 arylation. *Chem. Commun.* **48**, 11644–11646 (2012).
31. J. Tang, Y. He, H. Chen, W. Sheng, H. Wang, Synthesis of bioactive and stabilized cyclic peptides by macrocyclization using C(sp^3)-H activation. *Chem. Sci.* **8**, 4565–4570 (2017).
32. A. F. M. Noisier, J. García, I. A. Ionuț, F. Albericio, Stapled peptides by late-stage C(sp^3)-H activation. *Angew. Chem. Int. Ed.* **56**, 314–318 (2017).
33. Z. Ruan, N. Sauermann, E. Manoni, L. Ackermann, Manganese-catalyzed C-H alkynylation: Expedient peptide synthesis and modification. *Angew. Chem. Int. Ed.* **56**, 3172–3176 (2017).
34. Z. Bai, C. Cai, Z. Yu, H. Wang, Backbone-enabled directional peptide macrocyclization through late-stage palladium-catalyzed δ-C(sp^2)-H olefination. *Angew. Chem. Int. Ed.* **57**, 13912–13916 (2018).
35. J. Tang, H. Chen, Y. He, W. Sheng, Q. Bai, H. Wang, Peptide-guided functionalization and macrocyclization of bioactive peptidosulfonamides by Pd(II)-catalyzed late-stage C–H activation. *Nat. Commun.* **9**, 3383 (2018).
36. X. Zhang, G. Lu, M. Sun, M. Mahankali, Y. Ma, M. Zhang, W. Hua, Y. Hu, Q. Wang, J. Chen, G. He, X. Qi, W. Shen, P. Liu, G. Chen, A general strategy for synthesis of cyclophane-braced peptide macrocycles via palladium-catalysed intramolecular sp^3 C-H arylation. *Nat. Chem.* **10**, 540–548 (2018).
37. M.-Z. Lu, X.-R. Chen, H. Xu, H.-X. Dai, J.-Q. Yu, Ligand-enabled *ortho*-C-H olefination of phenylacetic amides with unactivated alkenes. *Chem. Sci.* **9**, 1311–1316 (2018).
38. A. S. Tsai, M. Brasse, R. G. Bergman, J. A. Ellman, Rh(III)-catalyzed oxidative coupling of unactivated alkenes via C-H activation. *Org. Lett.* **13**, 540–542 (2011).
39. C. S. Sevov, J. F. Hartwig, Iridium-catalyzed oxidative olefination of furans with unactivated alkenes. *J. Am. Chem. Soc.* **136**, 10625–10631 (2014).
40. A. Schischko, H. Ren, N. Kaplaneris, L. Ackermann, Bioorthogonal diversification of peptides through selective ruthenium(II)-catalyzed C-H activation. *Angew. Chem. Int. Ed.* **56**, 1576–1580 (2017).
41. M. Toumi, F. Couty, G. Evano, Total synthesis of paliurine F. *Angew. Chem. Int. Ed.* **46**, 572–575 (2007).
42. M. Toumi, F. Couty, G. Evano, Total synthesis of the cyclopeptide alkaloid paliurine E. insights into macrocyclization by ene-enamide RCM. *J. Org. Chem.* **73**, 1270–1281 (2008).

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