Organic LETTERS

Letter

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Liquid-Phase Synthesis of Bridged Peptides Using Olefin Metathesis of a Protected Peptide with a Long Aliphatic Chain Anchor

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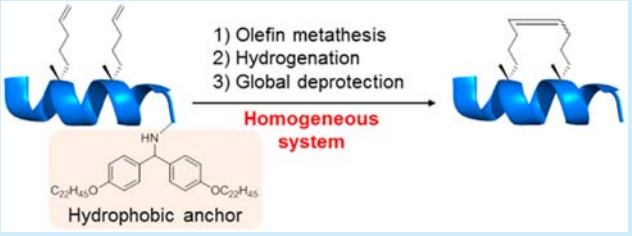
Supporting Information

ABSTRACT: Bridged peptides including stapled peptides are attractive tools for regulating protein−protein interactions (PPIs). An effective synthetic methodology in a heterogeneous system for the preparation of these peptides using olefin metathesis and hydrogenation of protected peptides with a long aliphatic chain anchor is reported.



Fixation of peptide conformation relevant to biological activity represents an entry indispensable for the development of peptide-based therapeutics.[1] Such a fixation includes the formation of covalent bonds between side-chain functionalities via disulfide[2] or lactam bridges.[3] Recently, peptides possessing an all-hydrocarbon staple (stapled peptides) have gained increasing attention because their features are suitable as drug leads.[4,5] Stapling of the pitch of α-helical peptides by a hydrocarbon chain allows the opposing nonstapled face to interact more efficiently with target proteins than parent peptides. Additionally, such α-helix stabilization has been reported to reduce the risk of degradation by proteases and to enhance the efficiency of cell membrane permeability, although these phenomena are case-by-case issues. The most common way to synthesize the stapled peptide starts from the pairwise incorporation of an olefin-bearing amino acid at *i* and *i* + 3 or *i* and *i* + 4 positions by solid-phase peptide synthesis (SPPS), followed by Ru-catalyzed ring-closing metathesis (RCM) toward the completed protected peptide on the resin.[6] However, the reaction efficiency of the heterogeneous system during the RCM step can be quite low, and the efficiency is dependent on the sequence and conformation of the peptides (Figure 1A).[7] Microwave irradiation at the RCM step has been used to overcome this potential inefficiency.[8] In addition, deprotection of the protected RCM-complete peptide resin yields a mixture of *cis*- and *trans*-alkene isomers, which complicates the downstream experimental operations, including separation and characterization of isomers.[9] Hydrogenation of the alkene part resolves this problem; however, reduction of alkene peptides remains an issue to be examined in full, regardless of whether the peptide exists in solution or on the resin.[10]

In terms of the problems mentioned above, we envisaged that the RCM step in a homogeneous solution phase should enhance the efficacy of the RCM reaction, and incidentally the most convenient hydrogenation catalysts such as Pd/C could be used in the reduction of the olefin unit of the RCM product to afford nonisomeric peptides (Figure 1B). Recently, Chiba's[11] (Molecular hiving) and our groups[12] (AJIPHASE) have

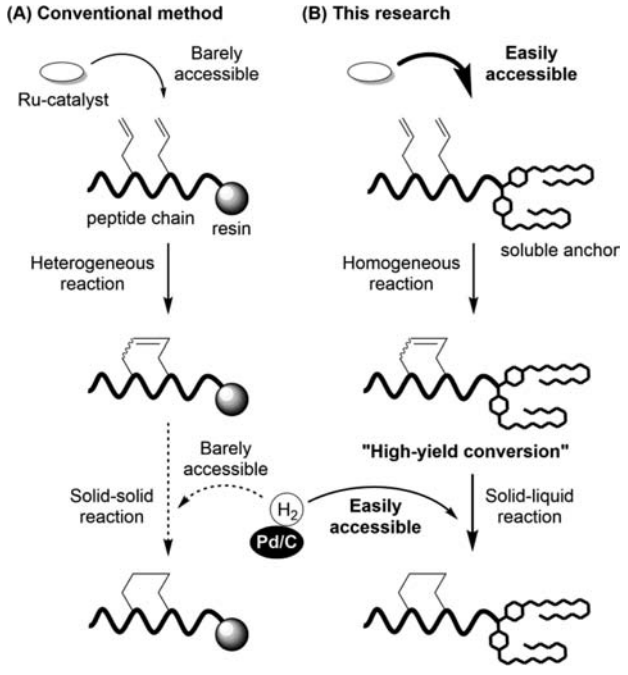


Figure 1. Synthetic strategy of the stapled peptide: (A) conventional method; (b) our method.

Received: December 25, 2014
Published: January 28, 2015

DOI: 10.1021/ol503718j
Org. Lett. 2015, 17, 696−699

independently reported a next-generation liquid-phase peptide synthesis (LPPS) method bearing operational merits seen in SPPS and conventional LPPS. This system features the use of C-terminal hydrophobic anchors that are soluble in halogenated solvents such as $CHCl_3$ or in THF but insoluble in MeOH or MeCN where coupling of Fmoc amino acids and removal of the Fmoc group in $CHCl_3$ are conducted in a homogeneous manner. After completion of the reactions, the addition of poor solvents such as MeOH or MeCN into the reaction mixture induces precipitation of the desired peptides bound on the hydrophobic anchor, whereas excess reagents and a cleaved Fmoc groups remain in solution.

We envisioned that the next-generation LPPS system should facilitate RCM for bridging in halogenated solvents and removal of a Ru catalyst with MeOH or MeCN after precipitation of the RCM product. Moreover, the bis(alkoxyphenyl)methyl-type anchor reported by us is tolerant to hydrogenation conditions for the reduction of the olefin moiety of the RCM product by the Pd/C−H_2 system.[13] Feasibility of the envisioned synthetic scheme for bridged peptides was examined by the synthesis of oxytocin analogues. Oxytocin (**1**) plays important roles in the neuroanatomical field and has been used in a clinical setting to induce labor and control postpartum hemorrhage (Figure 2).[14] Moreover, the

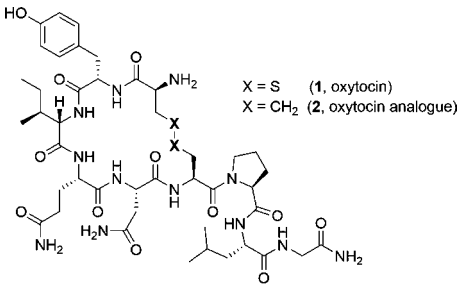
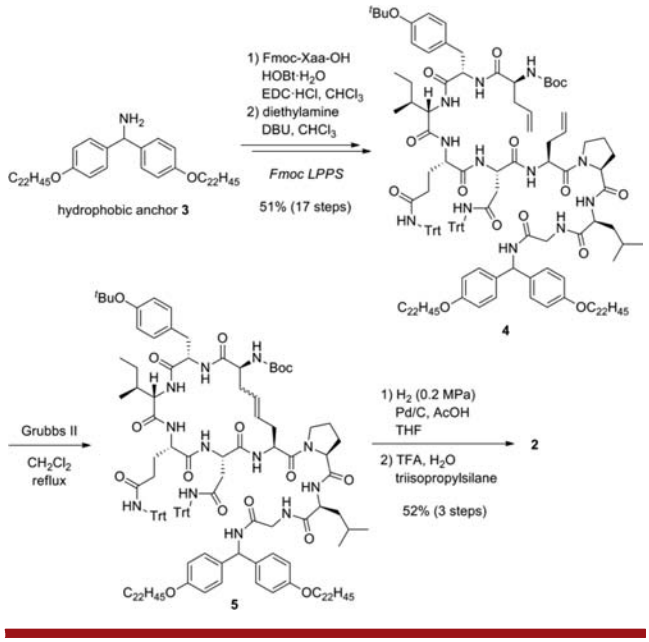


Figure 2. Oxytocin (**1**) and the oxytocin analogue **2**.

finding that an oxytocin analogue where the disulfide bond was replaced with an alkyl cross-linker showed enhanced biological activity as well as metabolic stability prompted us to synthesize the oxytocin analogue **2** as a model peptide in our envisioned scheme (Figure 1B).[15] Construction of the protected peptide on the bis(alkoxyphenyl)methyl type anchor **3** using next-generation LPPS afforded the CH_2Cl_2-soluble, but MeOH- or MeCN-insoluble, anchor-linked protected peptide **4** in 51% isolated yield over 17 steps from the starting anchor **3** (Scheme 1). The resulting protected peptide was refluxed with Grubbs second-generation catalyst (Grubbs II) in CH_2Cl_2 for 1 h, and the resulting anchor-bound peptide **5** was precipitated by addition of a mixture of MeOH and CH_3CN into the reaction mixture. The formed precipitate was washed with the mixed solvent to remove the catalysts. ESI-TOF MS analyses of the samples before and after the RCM reaction indicated that the linear peptide **4** was completely converted to the cyclized peptide **5** (Figure 3). Additionally, reversed-phase HPLC analyses of samples resulting from deprotection of **4** and **5** with TFA−triisopropylsilane−H_2O (95:2.5:2.5 (v/v)) for 1.5 h at room temperature followed by ESI-TOF MS analysis proved that the RCM reaction proceeded efficiently to afford a mixture of olefin isomers in a 1:1 ratio (Figure 3). Having the anchor-bound cyclized protected peptides **5** as mentioned above, we next attempted the reduction of the olefin peptides on the

Scheme 1. Synthesis of the Oxytocin Analogue



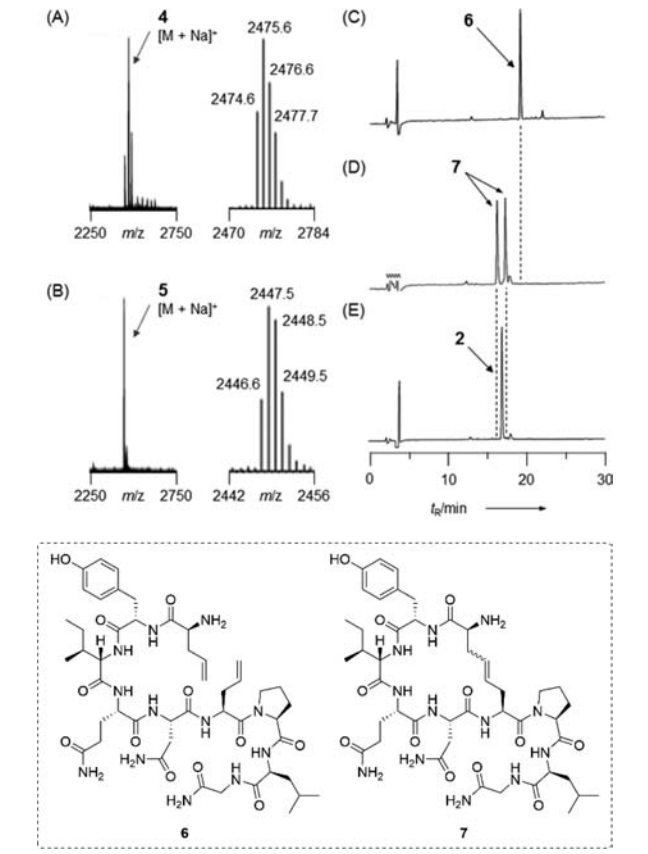


Figure 3. ESI-TOF MS (A and B) and HPLC chromatograms of the reaction mixtures (C−E): (A) before RCM; (B) after RCM. (C) Crude material generated by deprotection of **4**. (D) Crude material generated by deprotection of **5**. (E) Crude material after hydrogenation and global deprotection of **5**.

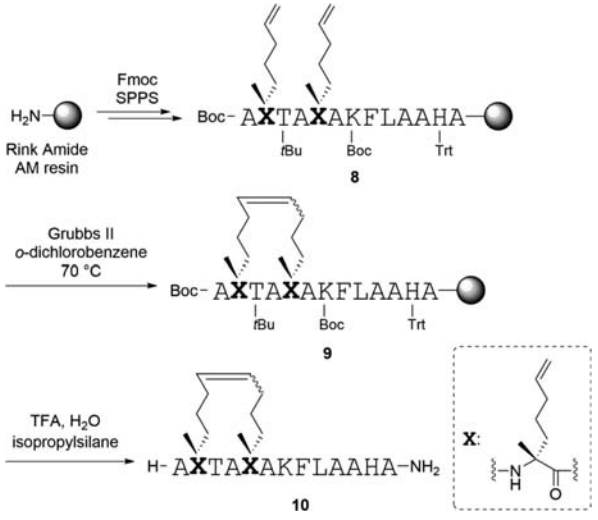
hydrophobic anchor. A solution of the cyclized protected peptides **5** in AcOH−THF was hydrogenated over Pd/C under H_2 gas for 34 h. After the reaction mixture was filtered and concentrated, the residues were treated with TFA−triisopro-

DOI: 10.1021/ol503718j
Org. Lett. 2015, 17, 696−699

pylsilane−H_2O (95:2.5:2.5 (v/v)) at room temperature for 1.5 h to yield the oxytocin analogue **2** with high purity in 52% isolated yield over three steps without HPLC purification.

In order to examine the practicality of the developed method, we next tried to synthesize an *i*, *i* + 3 type stapled peptide (Scheme 2).[16] In the case of the standard protocol using on-

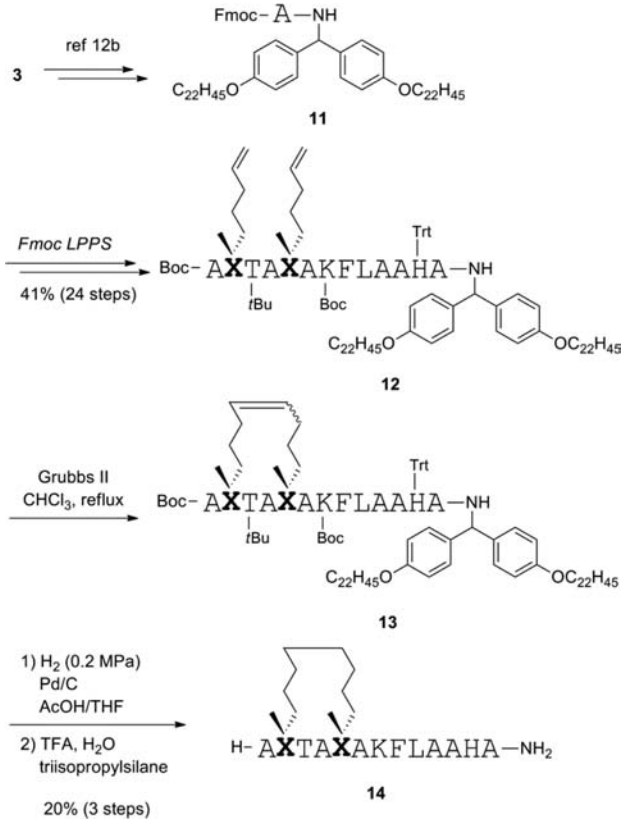
Scheme 2. Synthesis of the Stapled Peptide Using Conventional SPPS



resin RCM of **8** for 1 h, the reaction proceeded moderately and gave **9** in 52% conversion yield calculated from the HPLC peak area of the stapled peptide **10** and linear peptide **15**. This result suggested that the reactivity of the protected peptide resin in the two-phase RCM reaction was not high enough for efficient stapling. Therefore, we prepared protected peptide **12** requisite for the homogeneous RCM reaction in 41% yield using the next-generation Fmoc LPPS on the Fmoc-Ala-incorporated hydrophobic anchor **11** (Scheme 3). Anchor-bound protected peptide **12** was refluxed with Grubbs II catalyst in $CHCl_3$ for 1 h, and the homogeneous RCM reaction proceeded smoothly to yield the corresponding anchor-bound protected peptide **13**. Progress of the reaction was confirmed by both ESI-TOF MS analysis of **13** and HPLC analysis of samples resulting from acidic deprotection (Figure 4). Hydrogenation in the two-phase system of **13** in AcOH−THF over Pd/C followed by treatment with TFA−triisopropylsilane−H_2O (95:2.5:2.5 (v/v)) yielded the desired stapled peptide **14** in 20% isolated yield over three steps after HPLC purification. Moreover, we showed that homogeneous diimine reduction using excess *o*-nitrobenzenesulfonylhydrazide (NBSH)[17,18] and triethylamine in $CHCl_3$ can also be used for the reduction of the olefin instead of Pd/C. In this case, after the reaction the completed protected peptide can be precipitated with MeOH and excess reagents can be removed readily. (see the Supporting Information).

In conclusion, we have established a novel synthetic method for bridged peptides using a homogeneous RCM reaction of hydrophobic aliphatic anchor-bound protected peptides with practical application to the synthesis of an oxytocin analogue and an *i* and *i* + 3 type stapled peptide. The most characteristic feature of this method is that the use of the hydrophobic anchor-bound peptide allows the RCM reaction for bridging peptides to proceed in a homogeneous manner, which improves the low reactivity seen in conventional RCM-mediated bridging of protected peptide resins. Moreover, in

Scheme 3. Synthesis of the Stapled Peptide Using LPPS



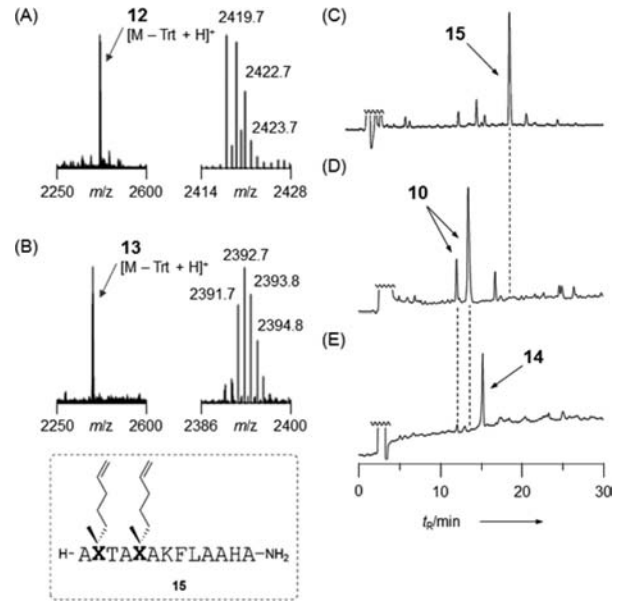


Figure 4. ESI-TOF MS (A, B) and HPLC (C−E) monitoring of the reactions: (A) before RCM; (B) after RCM. (C) Crude materials cleaved from **12**. (D) Crude materials cleaved from **13**. (E) Crude materials after hydrogenation and global deprotection. HPLC conditions are described in the Supporting Information.

both synthetic examples, no detectable amount of cross-metathesis products was found. The newly developed protocol should provide significant performance in the preparation of various bridged and stapled peptides that represent potential therapeutic agents.

DOI: 10.1021/ol503718j
Org. Lett. 2015, 17, 696−699

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures for key compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This research was supported in part by a Grant-in-Aid for Scientific Research (KAKENHI). AJINOMOTO Co., Inc., and Nagase & Co., Ltd., are also acknowledged. K.A. is grateful for a JSPS fellowship.

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