

O-Acyl isopeptide method for peptide synthesis: Application of O-acyl isodipeptide units to the synthesis of bioactive peptides

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Introduction

Solid-phase peptide synthesis (SPPS) has been routinely used for chemical synthesis of peptides. However, the synthesis of “difficult sequence”-containing peptides is one of the most problematic areas in peptide chemistry, and these peptides often have low solid-phase synthetic yield and purity due to their hydrophobic and aggregative properties.

In 2003, we discovered that the presence of an O-acyl instead of N-acyl hydroxyamino acid residue within the peptide backbone significantly changed the secondary structure of the native peptide, and thereby decreased the unfavorable nature derived from difficult sequences. This O-acyl isopeptide subsequently afforded the corresponding

target peptide via an O–N intramolecular acyl migration reaction. These findings led to the development of an “O-acyl isopeptide method” for the synthesis of peptides containing difficult sequences (Fig. 1a) [1, 2]. Several other research groups have used similar principles, and thus suggesting that the method is widely advantageous for peptide preparation [3–5].

However, epimerization at the esterified residue occurred during esterification on the resin in the synthesis of O-acyl isopeptides. To solve this problem, we designed an “O-acyl isodipeptide unit” (Fig. 1b) to omit the epimerization-inducing esterification on the resin [6]. Additionally, using the unit, fully automated protocols could take advantage of the routine amide bond formation in SPPS of O-acyl isopeptides. Thus, the O-acyl isodipeptide unit would be an important building block to enable the routine use of the O-acyl isopeptide method.

Here, we report the synthesis of forty kinds of O-acyl isodipeptide units Boc–Ser/Thr(Fmoc–Xaa)–OH with all naturally coded amino acids [7]. Moreover, we successfully applied synthesized isodipeptide units to synthesize bioactive peptides containing difficult sequences such as influenza A virus-related peptide **1** [7] and Alzheimer’s disease-related amyloid β peptide (A β) 1–42 (**2**) [8], optimizing the coupling conditions of the corresponding isodipeptide unit.

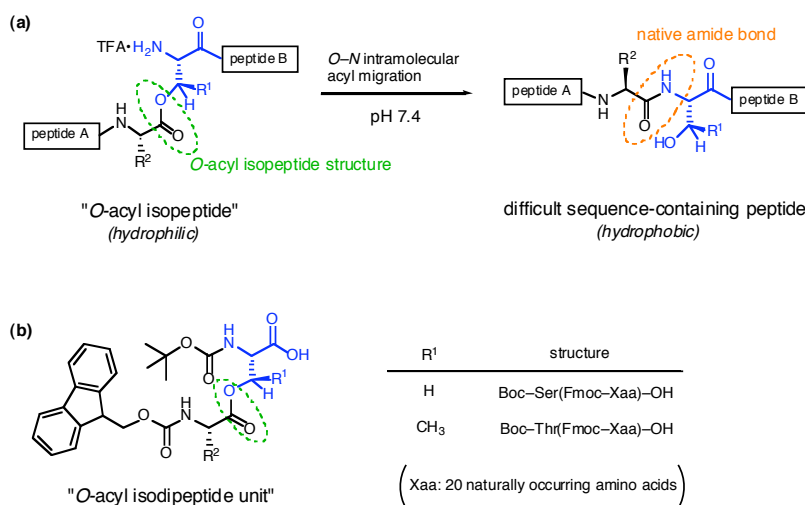
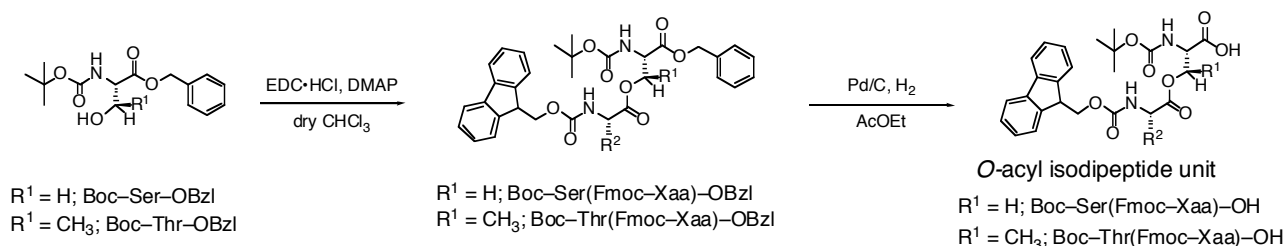


Fig. 1. (a) O-Acyl isopeptide method: Synthetic strategy for difficult sequence-containing peptides via the O–N intramolecular acyl migration reaction of O-acyl isopeptides, and (b) general structure of O-acyl isodipeptide units.

Results and Discussion

Forty kinds of the O-acyl isodipeptide units were synthesized in solution-phase synthesis according to Scheme 1 [7]. An Fmoc-amino acid was coupled to the



Scheme 1. Solution-phase synthesis of O-acyl isodipeptide units.

β -hydroxyl group of Boc-Ser/Thr-OBzl using EDC·HCl (*N*-ethyl-*N'*-(3-dimethylaminopropyl)-carbodiimide)-DMA P method in dry CHCl_3 to obtain the corresponding Boc-Ser/Thr(Fmoc-Xaa)-OBzl. Subsequently, the *O*-Bzl esters of the protected dipeptides were removed using Pd/C under H_2 atmosphere to afford the corresponding Boc-Ser/Thr(Fmoc-Xaa)-OH. Interestingly, we did not observe any epimerized *D*-derivative in each ester bond formation, possibly because of the faster coupling rate in solution as compared to that on solid support.

Synthesized *O*-acyl isodipeptide unit Boc-Thr(Fmoc-Phe)-OH (**3**) was applied to SPPS of influenza A virus matrix M1 58-66 (H-GILGFVFTL-OH, **1**) containing a difficult sequence [7]. HPLC analysis of the crude peptide, synthesized using *O*-acyl isodipeptide unit **3**, exhibited a high purity of the desired product with no by-product derived from the difficult sequence or epimerization (Fig. 2). The synthesis of **1** using unit **3** did not lead to any additional side reaction.

On the other hand, in the synthesis of A β 1-42 isopeptide (26-*O*-acyl isoA β 1-42, **4**) using *O*-acyl isodipeptide unit Boc-Ser(Fmoc-Gly)-OH (**5**), in which a native Gly²⁵-Ser²⁶ bond was modified to a β -ester bond, the deletion of Ser²⁶ in the *O*-acyl isopeptide structure was noticed during the coupling of unit **5** (Fig. 3a). We optimized the coupling conditions of the *O*-acyl isodipeptide unit. Non-polar solvent CH_2Cl_2 was superior to DMF in suppressing the deletion. Based on this result, using CH_2Cl_2 as solvent for the coupling of unit **5**, we synthesized A β 1-42 isopeptide **4** with nearly no Ser²⁶ deletion (Fig. 3b) [8]. By choice of solvent, A β 1-42 isopeptide **4** would be efficiently synthesized using fully automated protocols. A β 1-42 (**2**) was prepared from isopeptide **4** via the *O*-*N* intramolecular acyl migration reaction [2].

In summary, Boc-Ser/Thr(Fmoc-Xaa)-OH with all naturally occurring amino acids were synthesized in a two-step solution-phase synthesis without epimerization, starting from Boc-Ser/Thr-OBzl. Interestingly, we did not observe any epimerization in all ester bond-forming reactions between Fmoc-Xaa-OH and Boc-Ser/Thr-OBzl. Additionally, synthesized isodipeptide units were successfully applied in synthesizing bioactive peptides with difficult sequences such as influenza A virus-related peptide **1** and Alzheimer's disease-related A β 1-42 (**2**), optimizing the coupling conditions of the corresponding isodipeptide units. With the expansion of the use of the *O*-acyl isodipeptide units, *O*-acyl isopeptide method can be applied to wide ranges of peptide chemistry.

Acknowledgments

This research was supported in part by the "Academic Frontier" Project for Private Universities: matching fund subsidy from MEXT (Ministry of Education, Culture, Sports, Science and Technology) of the Japanese Government, and the 21st Century COE Program from

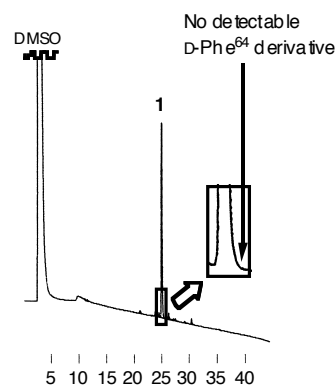


Fig. 2. HPLC profile of crude peptide **1** synthesized using *O*-acyl isopeptide method with Boc-Thr(Fmoc-Phe)-OH (**3**).

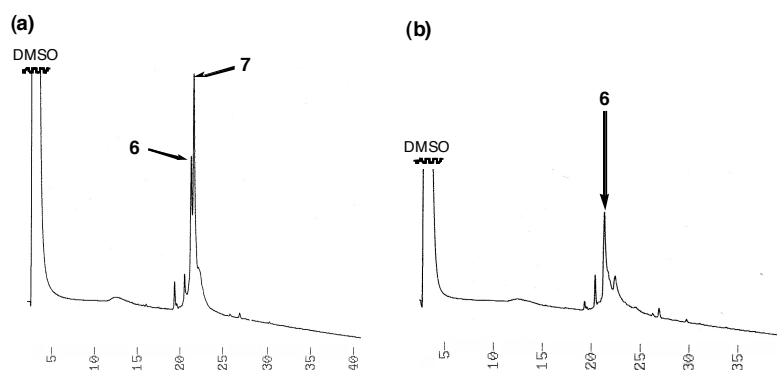


Fig. 3. HPLC profiles of the crude peptide after TFA treatment in the synthesis of A β 1-42 isopeptide **4** using *O*-acyl isodipeptide unit **5**. (a) **5** was coupled using DIPCDI-HOBT method in DMF, and (b) in CH_2Cl_2 . **6**: [Met(O)³⁵]-26-*O*-acyl isoA β 1-42, **7**: des-(Ser²⁶)-[Met(O)³⁵]-A β 1-42.

MEXT. A. T. and Y. S. are grateful for Research Fellowships of JSPS for Young Scientists.

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