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# Isoacylpeptide Method for Long-Chain and Difficult Sequence-Containing Peptide Preparation

*Yoshio Hamada*

## Abstract

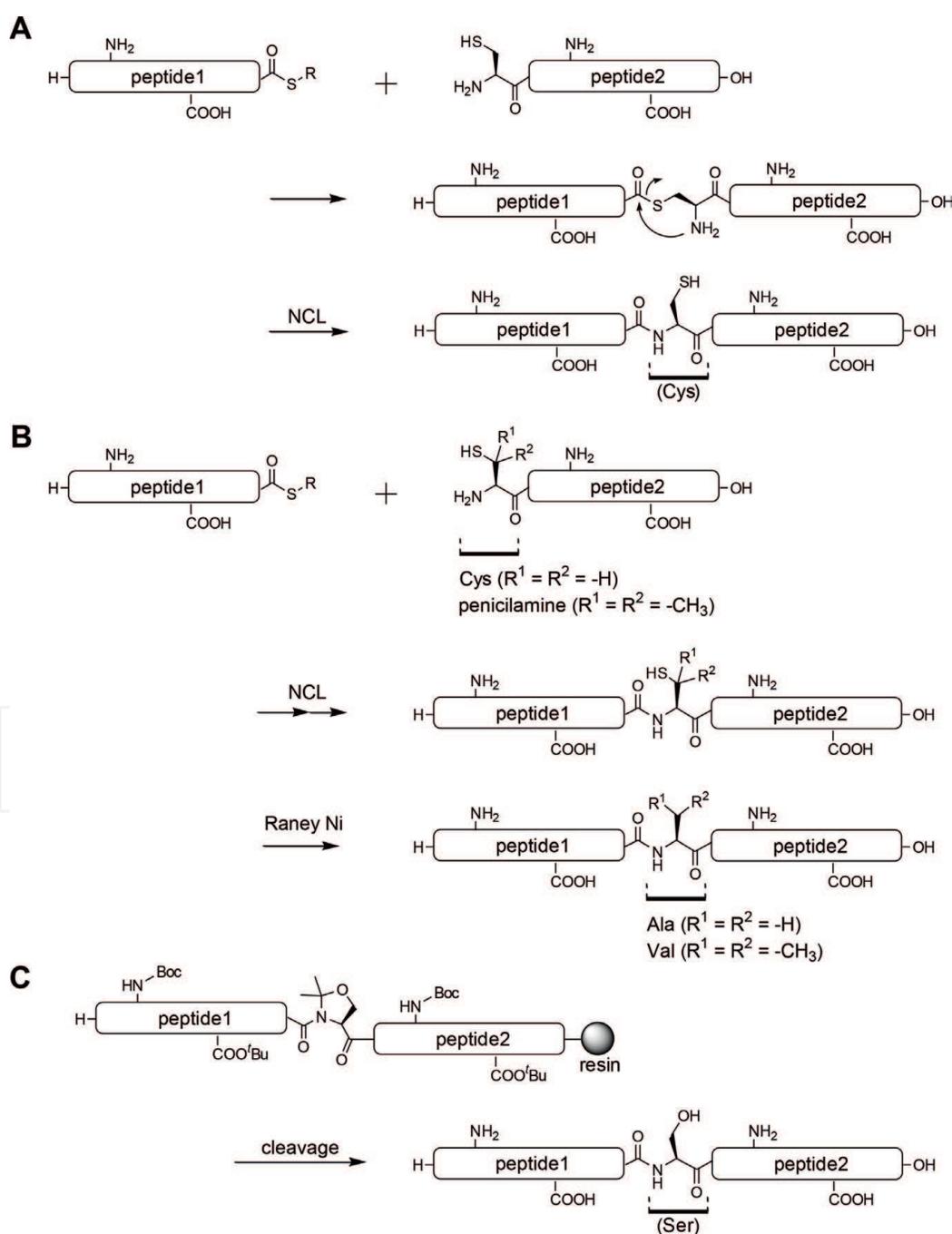
Peptides or small-size proteins are important substances for medicines, diagnosis, and molecular biology research. In organic synthesis, the peptide bonds formation is performed in an organic solution (liquid-phase peptide synthesis, LPPS), or on a resin (solid-phase peptides synthesis, SPPS). LPPS can prepare a high volume of peptides, but it is generally required long processes and high cost for peptides preparation and is not appropriate for long-chain peptides. SPPS can prepare long-chain peptides until 40 residues in a short time. However, it is difficult to obtain the pure peptides because of no purification of its intermediates. For a solution of these problems, Kent et al. reported native chemical ligation (NCL) method for the preparation of long-chain peptides. Because peptides with a long chain or difficult sequence formed  $\beta$ -sheet structure within a molecule, these peptides have high aggregability and low solubility, and their preparation and purification are generally difficult. Mutter et al. reported 'pseudoproline' method for difficult sequence-containing peptide preparation. We previously reported a series of prodrugs based on *O-N* intramolecular acyl migration. We reported '*O*-isoacylpeptide' method for the preparation of difficult sequence-containing peptides using the prodrug strategy based on *O-N* intramolecular acyl migration.

**Keywords:** difficult sequence-containing peptide, long-chain peptide, *O-N* intramolecular acyl migration, peptide synthesis, isoacylpeptide

## 1. Introduction

Peptides or small-size proteins are important substances for medicines, diagnosis, and molecular biology research, such as enzyme inhibitors, antagonists/agonists against receptors, antigenic peptides for antibody preparation, and peptide probes that detect a protein-peptide interaction. There are two general methodologies for peptide preparation—organic synthesis and genetic engineered synthesis. In organic synthesis, the peptide bonds formation is performed in an organic solution (liquid-phase peptide synthesis, LPPS), or on a resin (solid-phase peptides synthesis, SPPS). LPPS can prepare a high volume of peptides, but it is generally required long processes and high cost containing labor cost and is not appropriate for long-chain peptides. SPPS can prepare long-chain peptides until 40 residues in a short time.

However, it is difficult to obtain the pure peptides because of no purification of their intermediates. For a solution of these problem, Kent et al. reported native chemical ligation (NCL) method for the preparation of long-chain peptides as shown in **Figure 1A** [1–3]. In NCL reaction, a peptide possessing thioester at the C-terminus and a peptide possessing Cys residue at the N-terminus are prepared by SPPS. Next, both peptides are condensed by nucleophilic attack of thiol group at the N-terminus Cys residue in an aqueous solution, and then the condensed peptide with a thioester bond is spontaneously transformed into the peptide in which both peptides are connected with an amide bond. Although NCL allowed to preparing the long-chain peptides, this method is only available for the preparation of peptides with one or more Cys residues. Yan and Dawson [4] reported a modified NCL method for preparation of the peptides with one or more Ala residues (**Figure 1B**,  $R^1 = R^2 = -H$ ). In this reaction, the Cys-containing peptides that were obtained by NCL reaction are reduced into the Ala-containing peptides using Raney-Nickel catalyst. Haase et al. [5]



**Figure 1.** (A) Native chemical ligation. (B) Expanded native chemical ligation. (C) Pseudoproline method.

reported other modified NCL method for preparation of the Val-containing peptides from peptides with one or more penicillamine ( $\beta$ -mercaptovaline) as shown in **Figure 1B** ( $R^1 = R^2 = \text{—CH}_3$ ) in a manner similar to Haase et al.

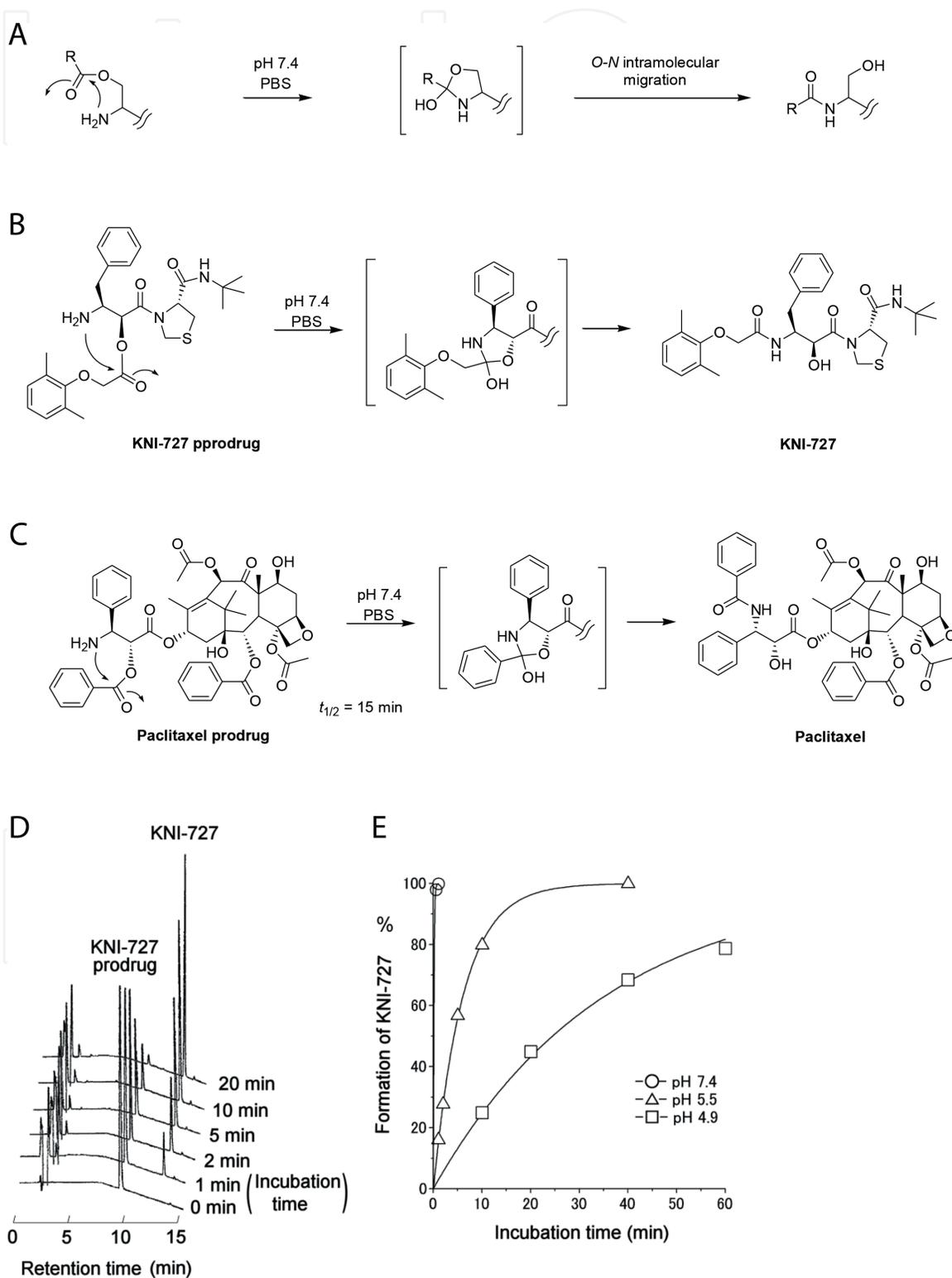
There are synthetic difficult peptides containing specific amino acid sequences. Because the difficult sequence-containing peptides and long-chain peptides formed  $\beta$ -sheet structure within a molecule, these peptides have high aggregability and low solubility in aqueous and organic solvents, and their preparation and purification are generally difficult. Although we can use various resin for preparation of difficult sequence-containing peptides, such as Tentagel™ (RAPP Polymere, Germany) with a PEG moiety on the polystyrene (PS) bead and HDODA resin with a flexible crosslinker [6], some game-changing technologies for the preparation of difficult sequence-containing peptides has been reported. Mutter et al. reported 'pseudoproline' method for the preparation of difficult sequence-containing peptides and long-chain peptides [7, 8] as shown in **Figure 1C**. Mutter et al. synthesized peptides on a resin using an oxazolidine-containing amino acid (pseudoproline) in which  $\alpha$ -amino and hydroxyl groups of Ser or Thr are cyclized by acetonidation. The deprotection and cleavage of peptides on the resin by a strong acid can convert form peptides with cyclic amino acid residue into peptides with Ser or Thr residue. Because the cyclic amino acids have a structure similar to Pro and peptides with a cyclic amino acid residue have greatly different structure compared with original peptides, peptides containing a pseudoproline are prevented to form  $\beta$ -sheet structure within a molecule. Because the oxazolidine-containing amino acids are labile in acid media, the dipeptide units, which consist of Fmoc-protected amino acid and oxazolidine-containing amino acid are commercially available.

We previously reported a series of prodrugs based on *O-N* intramolecular acyl migration [9–13]. Furthermore, we reported '*O*-isoacylpeptide' method for the preparation of difficult sequence-containing peptides using the prodrug strategy based on *O-N* intramolecular acyl migration [14, 15].

## 2. *O-N* intramolecular acyl migration and prodrugs

Previously, we reported a series of water-soluble prodrugs, such as human immunodeficiency virus type-1 (HIV-1) protease inhibitors and anti-cancer drugs [10–12, 15]. HIV-1 is a retrovirus that causes the acquired immunodeficiency syndrome (AIDS). Since HIV-1 encodes HIV-1 protease that is responsible for the processing of viral precursor proteins such as gag and gag-pol polyproteins to form mature structural proteins and some enzymes required in the production of infective viral particles, HIV-1 protease is an attractive target for the design of anti-AIDS drugs. HIV-1 protease is an aspartic protease that consists of a C2-symmetric homodimer, and its active site has some hydrophobic pockets. Thus most of the HIV-1 protease inhibitors that are optimized for the active site have high hydrophobicity and are sparingly water soluble. Many HIV-1 protease inhibitor formulations contain some solubilizers such as polyethylene glycol derivatives that often lead to unwanted side effects in clinical use. A water-soluble prodrug of amprenavir, fosamprenavir [16, 17], was approved by the US Federal Drug Administration (FDA) in 2003, and amprenavir was discontinued by the manufacture in 2004. Our previously reported HIV-1 protease inhibitors [18–21] also showed poorly water-solubility similar to amprenavir. Hence, we designed a series of water-soluble prodrug of HIV-1 protease inhibitor using a novel prodrug strategy. An acyl migration on the  $\beta$ -hydroxy- $\alpha$ -amino acid residue such as Ser and Thr in strong acids was well-known in peptide chemistry [22]. An *N*-acyl  $\beta$ -hydroxy- $\alpha$ -amino acid residue isomerizes into the *O*-acyl form in strong acids, and *O*-isoacylpeptide can regenerate the original peptide in weak

alkaline media *via* *O-N* intramolecular acyl migration (**Figure 2A**). Since our HIV-1 protease inhibitors contain a  $\beta$ -hydroxy- $\alpha$ -amino acid residue, (2*S*,3*S*)-3-amino-2-hydroxy-4-phenylbutyric acid (Apsn), we designed and synthesized the *O*-acyl isomer of KNI-727 as a water-soluble prodrug. The prodrug of KNI-727 was stable as an HCl salt in unbuffered aqueous solutions and in strong acidic solution such as gastric juice, and could be rapidly converted to the parent compound, KNI-727, *via* *O-N* intramolecular acyl migration reaction under the physiological condition



**Figure 2.** Prodrugs based on *O-N* intramolecular acyl migration (A) *O-N* intramolecular acyl migration. (B) Prodrug of HIV-1 protease inhibitor, KNI-727. (C) Paclitaxel prodrug. (D) HPLC profile of the *O-N* intramolecular acyl migration of KNI-727 prodrug in PBS (pH 5.5) at 37°C. (E) Time course of the migration reaction of KNI-727 prodrug.

(pH 7.4 PBS, 37°C) as shown in **Figure 1B**. KNI-727 could be purified by preparative HPLC using a reverse-phase C18 column and a linear gradient system of acetonitrile and 0.1% TFA. HPLC charts and Time course of the migration reaction in KNI-727 prodrug were shown in **Figure 2D** and **E** respectively. The rate constant and  $t_{1/2}$  were calculated using fitting Eq. (1).

$$[A]_t = A_{\text{MAX}} \times (1 - \text{Exp}(-k \times t)) \quad (1)$$

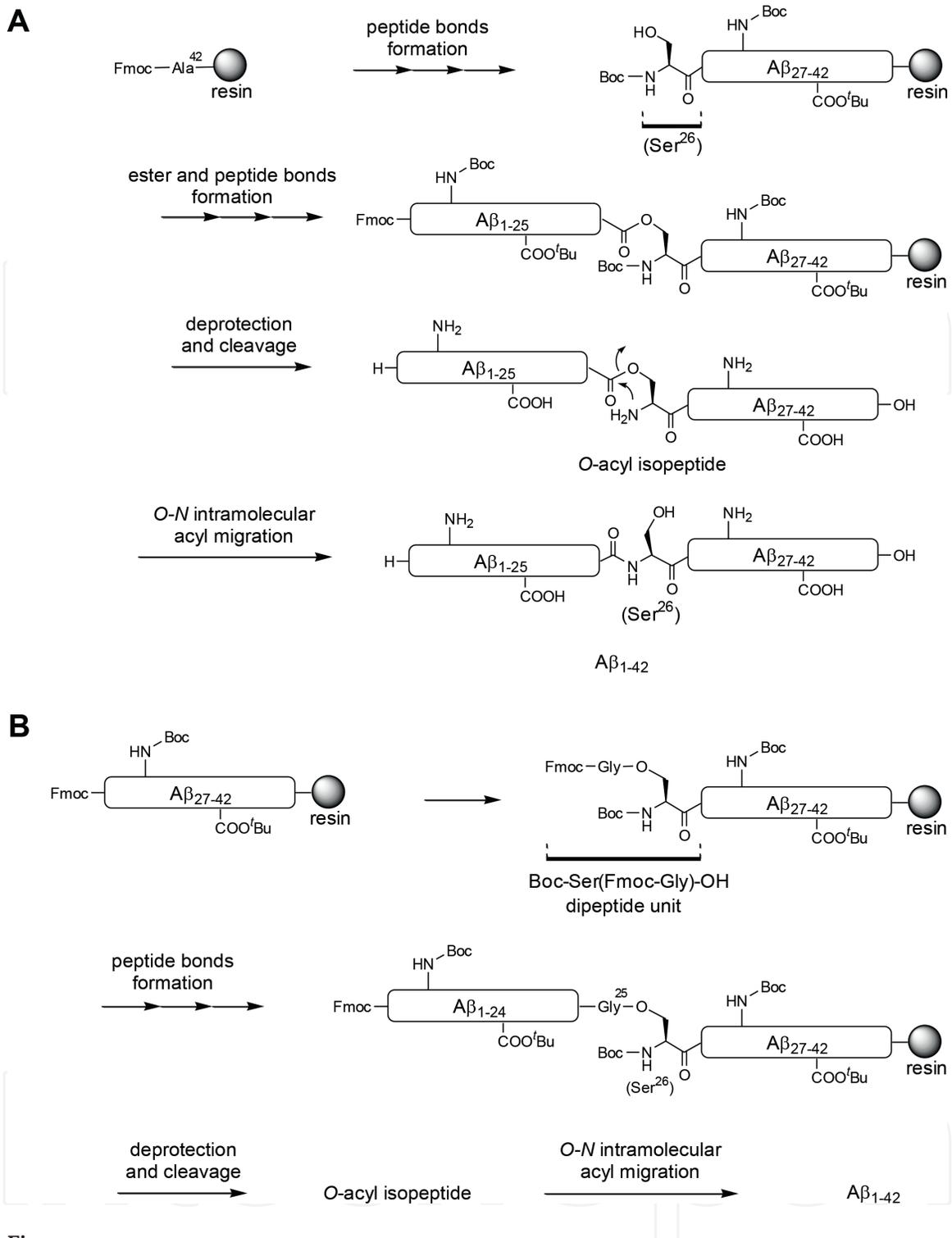
[ $t$ , incubation time;  $k$ , rate constant of migration;  $A_{\text{MAX}}$ , maximum concentration of the parent compound (initial concentration of prodrug);  $[A]$ , concentration of the parent compound].

*O-N* intramolecular acyl migration of KNI-727 prodrug did not involve any by-product as shown in **Figure 2D** and **E** using the fitting Eq. (1) showed typical first order reaction. Hence, this water-soluble prodrug is suitable as an administrated drug for the AIDS therapy.

Next, we designed and synthesized water-soluble paclitaxel prodrug [12, 13]. Paclitaxel is an anti-cancer agent that was extracted from the Pacific yew tree *Taxus brevifolia* with antineoplastic activity. Since paclitaxel binds to tubulin, it can inhibit the disassembly of microtubules, thereby resulting in the inhibition of cell division, and also induces apoptosis by binding to the apoptosis inhibitor protein, B-cell leukemia 2 (Bcl-2). As paclitaxel are labile in acidic media such as gastric fluid, the paclitaxel formulations had been used as an injectable drug. The paclitaxel is representative of poorly water-soluble drug, and its injectable formulations require some detergents, such as Cremophor EL, which has been suggested to cause hypersensitivity. By focusing on the  $\beta$ -hydroxy- $\alpha$ -amino acid moiety in the chemical structure of paclitaxel, we designed the prodrug, *O*-benzoyl isopaclitaxel, in which the benzoyl group on the amino group of the paclitaxel was moved to its hydroxyl group. This paclitaxel prodrug is stable in water as a salt, and can be rapidly converted to the parent drug under physiological conditions ( $t_{1/2} = 15$  min, pH 7.4 PBS, 37°C) as shown in **Figure 2C**. The paclitaxel prodrug seems to be suitable as an injectable drug.

### 3. *O*-isoacylpeptide method

Since two natural amino acids, Ser and Thr, have a  $\beta$ -hydroxyl- $\alpha$ -amino acid structure, we designed the precursors of biomolecules, so-called '*O*-isoacylpeptides', using our prodrug strategy *via O-N* intramolecular acyl migration [14–16]. *O*-isoacylpeptides that have an *O*-acyl ester bond instead of amide bond in the Ser/Thr residue of biomolecules are promptly converted to the corresponding biomolecules under physiological conditions. It is known that some biomolecules are aggregate in aqueous solutions because of their  $\beta$ -sheet structure formation. Among them, A $\beta$ s show strong water-insolubility and aggregability, making their handling in biochemical research potentially complicated. Hence, chemical synthesis on resins and purification of A $\beta$ s, especially A $\beta_{1-42}$ , is particularly challenging. To solve these problems, we designed *O*-isoacyl-A $\beta_{1-42}$  that has an ester bond instead of the amide bond at Gly<sub>25</sub>-Ser<sub>26</sub> in A $\beta_{1-42}$ . Synthesis of A $\beta_{1-42}$  using *O*-isoacylpeptide method showed in **Figure 3A**. Peptide bonds formation on the PS-resin was performed by 9-fluorenylmethoxycarbonyl (Fmoc)-based SPPS using diisopropylcarbodiimide (DIPCDI) as a coupling reagent in the presence of 1-hydroxybenzotriazole (HOBT). The hydroxy group in N-terminal Ser residue of the protected A $\beta_{27-42}$  that was prepared on a resin was esterified by Fmoc-Gly-OH using DIPCDI in the presence of catalytic amount of *N,N*-dimethyl-4-aminopyridine (DMAP). Next, remaining peptide bonds formation using the Fmoc-based SPPS similar to the former

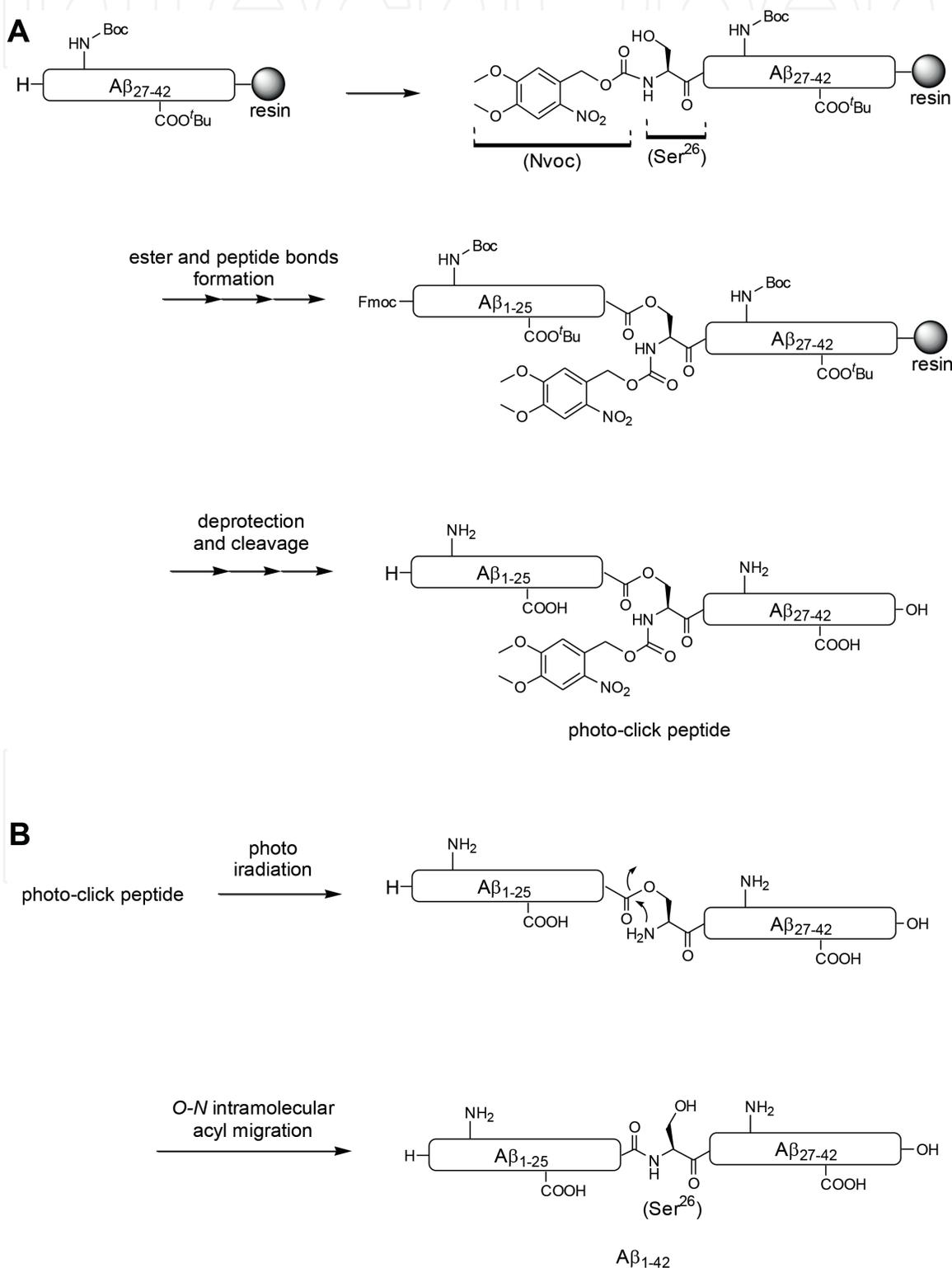


**Figure 3.** *O*-isoacylpeptide method. (A)  $A\beta_{1-42}$  synthesis via *O*-*N* intramolecular acyl migration. (B)  $A\beta_{1-42}$  synthesis using an dipeptide unit.

procedure formed the protected *O*-isoacyl- $A\beta_{1-42}$ , and then *O*-isoacyl- $A\beta_{1-42}$  was obtained by deprotection and cleavage from a resin. *O*-isoacyl- $A\beta_{1-42}$  was stable in acidic aqueous solution and unbuffered aqueous solution, and could rapidly release the native  $A\beta_{1-42}$  peptide. Because *O*-isoacylisopeptides showed good stability in acidic media, *O*-isoacylisopeptides can be easily isolated and purification in acidic solution [23]. In this manner, *O*-isoacylpeptide method has the advantage over the ‘pseudoproline’ method. *O*-isoacyl- $A\beta_{1-42}$  could be easily synthesized on a resin and purified by preparative HPLC using a reverse phase C18 column in acidic eluent solvents, and could release native  $A\beta_{1-42}$  that consists of  $A\beta_{1-42}$  monomers. However, because ester bond formation on a resin often involves a racemization of protected

amino acid, *O*-isoacyl-dipeptide units such as Boc-Ser(Fmoc-Gly)-OH have been commercial available from some chemical suppliers. *O*-isoacylpeptide method using an *O*-isoacyl-dipeptide unit was shown in **Figure 3B**. Use of Fmoc-*O*-isoacyl-dipeptide allows to synthesize the isoacylpeptides by the conventional Fmoc-based SPPS without use of DMAP catalyst.

Furthermore, we designed and synthesized photo cleavable-protected *O*-isoacylpeptide, so called 'photo-click peptide'. Because *O*-isoacylpeptides are rapidly converted to the biomolecules by stimuli such as pH changes and photo irradiation, as with the click of a button, we term 'click peptide'. Click peptides that can

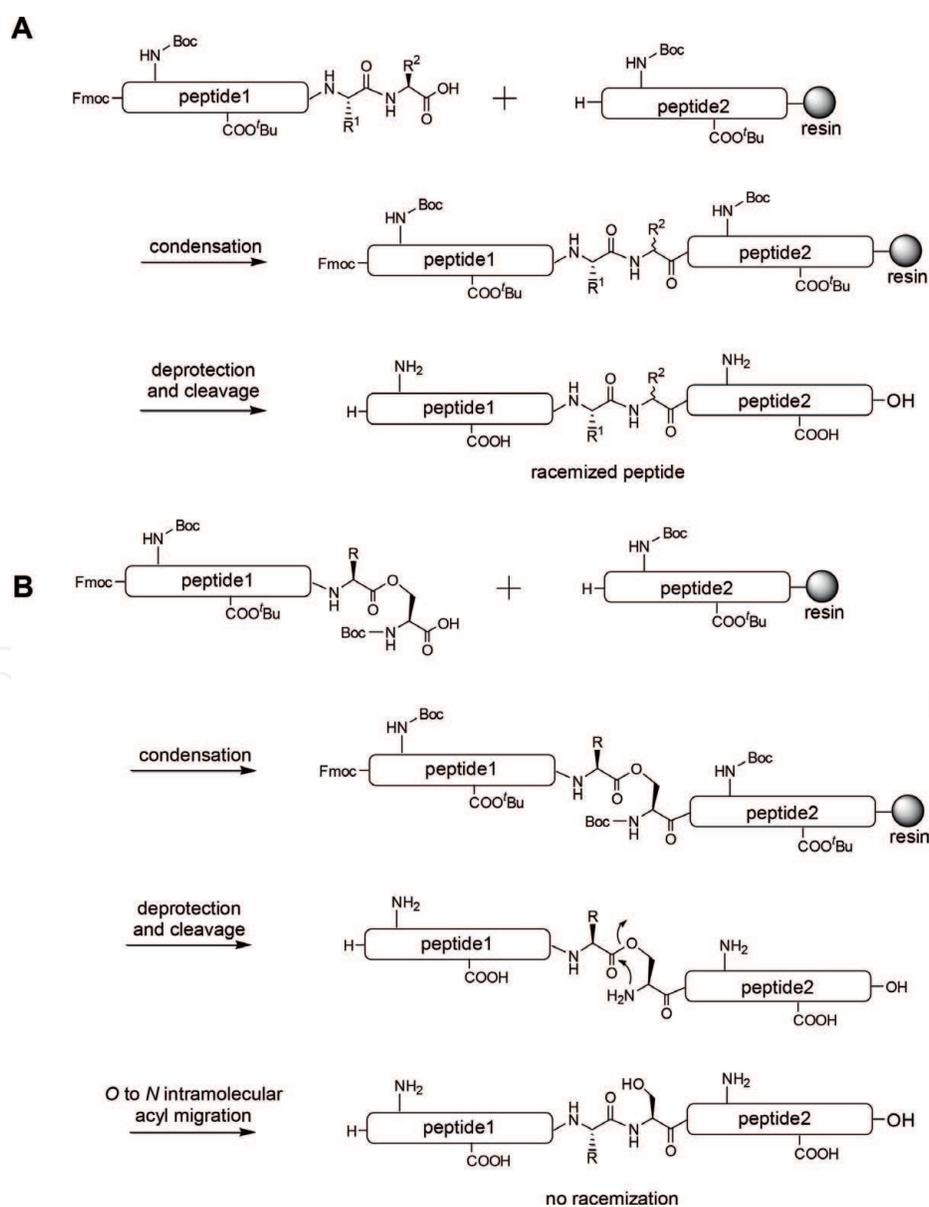


**Figure 4.** Photo-click peptide. (A) Synthesis of photo-click  $\text{A}\beta_{1-42}$ . (B) Photo-click reaction.

generate bioactive molecules in situ *via* a 'click' appear to be useful tools for chemical biology research. Synthesis of photo-click peptide was shown in **Figure 4A**. Photo-click  $A\beta_{1-42}$  could prepare on a resin in a similar manner showed in **Figure 3A**, using photo-cleavable protected amino acid instead of Boc-Ser-OH. Photo-cleavable protected amino acid, 6-nitroveratryloxycarbonyl (Nvoc)-Ser-OH, was coupled to protected  $A\beta_{27-42}$  on the resin after Fmoc-deprotection, and then Fmoc-Gly-OH was coupled on the hydroxyl group of Ser<sub>26</sub> using DIPCPI in the presence of catalytic amount of DMAP. The remaining peptide bonds formation using the Fmoc-based SPPS formed the protected *O*-isoacyl- $A\beta_{1-42}$ . Deprotection and cleavage from resin released *O*-isoacyl- $A\beta_{1-42}$ . Photo-click  $A\beta_{1-42}$  could rapidly release the native  $A\beta_{1-42}$  peptide by photo-irradiation and subsequent *O*-*N* intramolecular acyl migration under physiological condition (**Figure 4B**).

#### 4. Segment condensation of peptides using *O*-isoacylpeptides

Although NCL by Kent et al. allowed to preparing the long chain peptides, in general, conventional segment condensation other than NCL often involves the



**Figure 5.** Segment condensation of peptides. (A) Conventional segment condensation between peptides. (B) Segment condensation using *O*-isopeptide method.

racemization of amino acid as shown in **Figure 5A**. Especially, it is a serious problem in SPPS because of no purification of intermediates. It is well-known that the urethane structure, such as Boc and Fmoc protecting group, on the  $\alpha$ -amino group of amino acid can prominently reduce the racemization of amino acid in peptide bond coupling reaction. Hence, segment condensation between peptides other than NCL must not be in peptide chemistry. We noticed the protected *O*-isoacylpeptide structure that possesses a urethane structure on the  $\alpha$ -amino group of Ser or Thr residue. When an *O*-isoacylpeptide possessing a urethane-type protecting group at the C-terminus was coupled with another peptide, we speculated that the condensed peptide may be not racemized at the C-terminal amino acid of *O*-isoacylpeptide. Hence we designed segment condensation method as shown in **Figure 5B**. The segment condensation based on the *O*-isoacylpeptide method showed no racemization [24], and could release the original peptide similar to the other *O*-isoacylpeptides under physiological condition. This method appears to be alternative choice to NCL method.

## 5. Conclusion

Recently, some important synthesis methods such as NCL and pseudoproline method for preparation of long chain and difficult sequence-containing peptides had been reported. Although these approaches allow to preparing some long chain peptides without a genetic engineered approach. However, these methodologies are not a panacea for a long chain and difficult sequence-containing peptides. We supply alternative solution for the long chain and difficult sequence-containing peptide preparation. Namely, we have developed the *O*-isoacylpeptide method for peptide preparation. *O*-isoacylpeptides that have a/some ester bonds can be converted into the parent peptides under physiological condition *via* the *O*-*N* intramolecular acyl migration. Moreover, we developed segment condensation with no racemization using the *O*-isoacylpeptide method. The segment condensation based on *O*-isoacylpeptides may be alternative choice to NCL method. We desire to apply to these methodologies of various peptide preparations for drug, diagnosis, and molecular research.

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## Conflict of interest

We confirmed independence from the funding source.

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## References

- [1] Dawson PE, Muir TW, Clark-Lewis I, et al. Synthesis of proteins by native chemical ligation. *Science*. 1994;**266**:776-779. DOI: 10.1126/science.7973629
- [2] Johnson ECB, Kent SBH. Insights into the mechanism and catalysis of the native chemical ligation reaction. *Journal of the American Chemical Society*. 2006;**128**:6640-6646. DOI: 10.1021/ja058344i
- [3] Kent SBH. Total chemical synthesis of proteins. *Chemical Society Reviews*. 2009;**38**:338-351. DOI: 10.1039/b700141j
- [4] Yan LZ, Dawson PE. Synthesis of peptides and proteins without cysteine residues by native chemical ligation combined with desulfurization. *Journal of the American Chemical Society*. 2001;**123**:526-533. DOI: 10.1021/ja003265m
- [5] Haase C, Rohde H, Seitz O. Native chemical ligation at valine. *Angewandte Chemie International Edition in English*. 2008;**47**:6807-6810. DOI: 10.1002/anie.200801590
- [6] Varkey JT, Pillai VNR. Merrifield resin and 1,6-hexanediol diacrylate-crosslinked polystyrene resin for solid-phase peptide synthesis: A comparative study. *Journal of Applied Polymer Science*. 1999;**71**:1933-1939. DOI: 10.1002/(SICI)1097-4628(19990321)71:12<1933::AID-APP1>3.0.CO;2-C
- [7] Mutter M, Nefzi A, Sato T, et al. Pseudo-prolines (psi Pro) for accessing "inaccessible" peptides. *Peptide Research*. 1995;**8**:145-153
- [8] Wöhr T, Wahl F, Nefzi A, et al. Pseudo-prolines as a solubilizing, structure-disrupting protection technique in peptide synthesis. *Journal of the American Chemical Society*. 1996;**118**:9218-9227. DOI: 10.1021/ja961509q
- [9] Hamada Y, Ohtake J, Sohma Y, et al. New water-soluble prodrugs of HIV protease inhibitors based on O→N intramolecular acyl migration. *Bioorganic & Medicinal Chemistry*. 2002;**10**:4155-4167. DOI: 10.1016/S0968-0896(02)00322-X
- [10] Hamada Y, Matsumoto H, Kimura T, et al. Effect of the acyl groups on O→N acyl migration in the water-soluble prodrugs of HIV-1 protease inhibitor. *Bioorganic & Medicinal Chemistry Letters*. 2003;**13**:2727-2730. DOI: 10.1016/S0960-894X(03)00576-6
- [11] Hamada Y, Matsumoto H, Yamaguchi S, et al. Water-soluble prodrugs of dipeptide HIV protease inhibitors based on O→N intramolecular acyl migration: Design, synthesis and kinetic study. *Bioorganic & Medicinal Chemistry*. 2004;**12**:159-170. DOI: 10.1016/j.bmc.2003.10.026
- [12] Hayashi Y, Skwarczynski M, Hamada Y, et al. A novel approach of water-soluble paclitaxel prodrug with no auxiliary and no byproduct: Design and synthesis of isotaxel. *Journal of Medicinal Chemistry*. 2003;**46**:3782. DOI: 10.1021/jm034112n
- [13] Skwarczynski M, Sohma Y, Noguchi M, et al. No auxiliary, no byproduct strategy for water-soluble prodrugs of taxoids: Scope and limitation of O–N intramolecular acyl and acyloxy migration reactions. *Journal of Medicinal Chemistry*. 2005;**48**:2655-2666. DOI: 10.1021/jm049344g
- [14] Sohma Y, Hayashi Y, Skwarczynski M, et al. O–N intramolecular acyl migration reaction in the development of prodrugs and the synthesis of difficult sequence-containing bioactive peptides. *Biopolymers*. 2004;**76**:344-356. DOI: 10.1002/bip.20136

- [15] Hamada Y. Recent progress in prodrug design strategies based on generally applicable modifications. *Bioorganic & Medicinal Chemistry Letters*. 2017;**27**:1627-1632. DOI: 10.1016/j.bmcl.2017.02.075
- [16] Hamada Y, Kiso Y. Aspartic protease inhibitors as drug candidates for treating various difficult-to-treat diseases. In: *Amino Acids, Peptides and Proteins*. Vol. 39. London: Royal Society of Chemistry; 2015. pp. 114-147
- [17] Strickley RG. Solubilizing excipients in oral and injectable formulations. *Pharmaceutical Research*. 2004;**21**:201-230. DOI: 10.1023/B:P HAM.0000016235.32639.23
- [18] Panov I, Drabina P, Hanusek J, et al. Stereoselective synthesis of the key intermediates of the HIV protease inhibitor fosamprenavir and its diastereomer. *Synlett*. 2013;**24**:1280-1282. DOI: 10.1055/s-0033-1338803
- [19] Nguyen J-T, Hamada Y, Kimura T, et al. Design of potent aspartic protease inhibitors to treat various diseases. *Archiv der Pharmazie—Chemistry in Life Sciences*. 2008;**341**:523-535. DOI: 10.1002/ardp.200700267
- [20] Hamada Y, Kiso Y. The application of bioisosteres in drug design for novel drug discovery: Focusing on acid protease inhibitors. *Expert Opinion on Drug Discovery*. 2012;**7**:903-922. DOI: 10.1517/17460441.2012.712513
- [21] Hamada Y, Kiso Y. New directions for protease inhibitors directed drug discovery. *Biopolymers*. 2016;**106**: 563-579. DOI: 10.1002/bip.22780
- [22] Hurley TR, Colson CE, Hicks G, et al. Orally active water-soluble N,O-acyl transfer products of  $\alpha,\beta,\gamma$ -bishydroxyl amide containing renin inhibitor. *Journal of Medicinal Chemistry*. 1993;**36**:1496-1498. DOI: 10.1021/jm00062a024
- [23] Taniguchi A, Sohma Y, Kimura M, et al. "Click peptide" based on the "O-acyl isopeptide method": Control of A $\beta_{1-42}$  production from a photo-triggered A $\beta_{1-42}$  analogue. *Journal of the American Chemical Society*. 2006;**128**:696-697. DOI: 10.1021/ja057100v
- [24] Yoshiya T, Sohma Y, Hamada Y, et al. Racemization-free segment condensation based on the O-acyl isopeptide method: Toward a chemical protein synthesis on solid support. *Advances in Experimental Medicine and Biology*. 2009;**611**:161-162. DOI: 10.1007/978-0-387-73657-0\_73