

# Racemization-free segment condensation based on isopeptide method: Toward an efficient preparation of peptides/proteins on solid support

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## Introduction

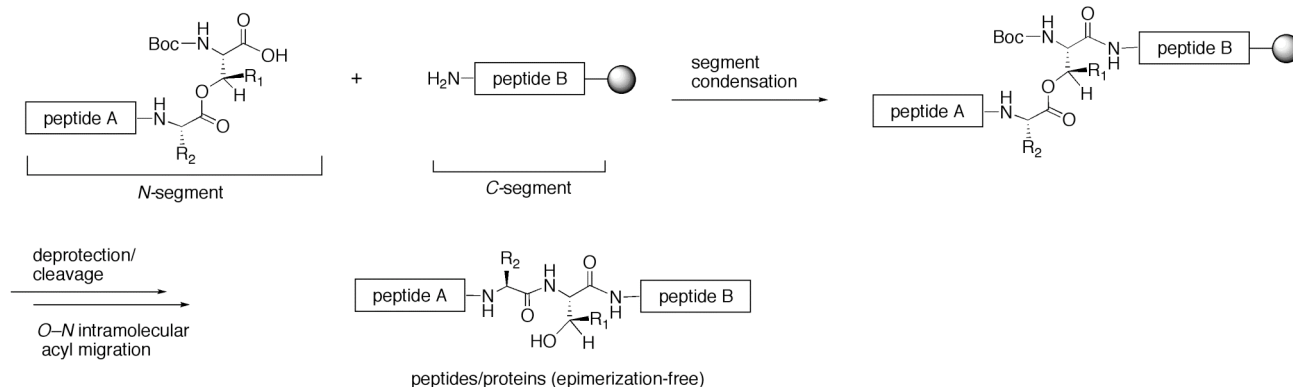
The convergent synthetic method has greatly facilitated the assembly of peptides and proteins. However, a fundamental drawback of convergent synthesis is that epimerization at the C-terminal residue of an *N*-segment may occur during the condensation reaction with a C-segment. Particularly, in solid phase segment condensation, a large amount of epimerization is generally involved, thereby limiting the *N*-segment to contain either a C-terminal Gly or Pro residue.

We have developed a “racemization-free segment condensation” based on the “*O*-acyl isopeptide method” (Fig. 1) [1]. This method allows the use of an *N*-segment possessing a C-terminal Ser/Thr residue for segment condensation without any epimerization, as a result of the C-terminal *O*-acyl isopeptide structure with a urethane-protected Ser/Thr residue. Thus, in the synthesis of long peptides/proteins, racemization-free segment condensation becomes possible at not only the C-terminal Gly/Pro but also Ser/Thr of the *N*-segment.

Here, we report the application of the *O*-acyl isopeptide method-based racemization-free segment condensation method in the synthesis of a model pentapeptide.

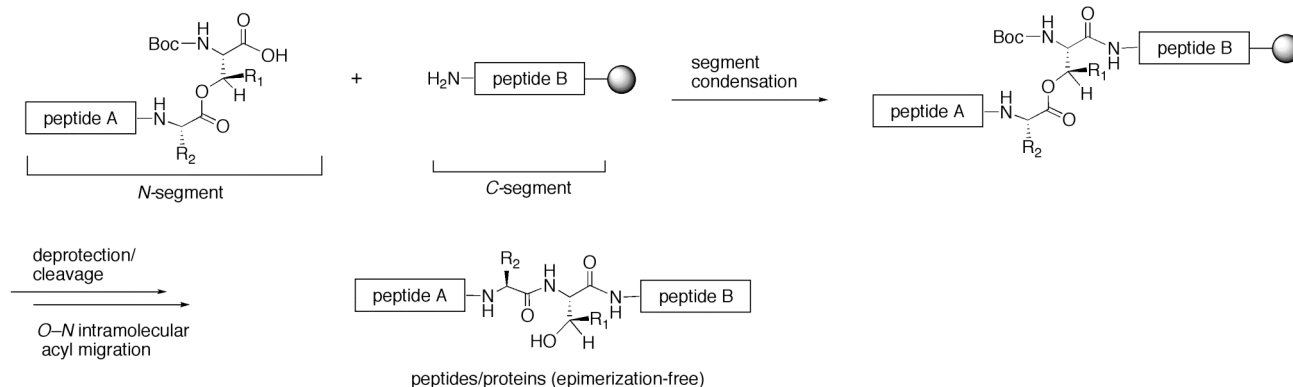
## Results and Discussion

As a model peptide, pentapeptide Ac-Val-Val-Thr-Val-Val-NH<sub>2</sub> (**1**) was adopted. In the condensation of Ac-Val-Val-Thr(*t*Bu)-OH with H-Val-Val-NH-resin (as a standard segment condensation), a large amount of epimerization (37.5%) at the activated Thr residue occurred in a DIPCDCI (1,3-diisopropylcarbodiimide)–HOBt



(1-hydroxybenzotriazole) method, which was confirmed by an independent synthesis of Ac-Val-Val-D-*allo*-Thr-Val-Val-NH<sub>2</sub>.

On the other hand, in the *O*-acyl isopeptide method-based segment condensation (Scheme 1), *N*-segment Boc-Thr(Ac-Val-Val)-OH (**5**), which was synthesized using “*O*-acyl isodipeptide unit” [2] Boc-Thr(Fmoc-Val)-OH, was coupled to C-segment H-Val-Val-NH-resin (**2**) by the DIPCDCI (2.5 eq)–HOBt (2.5 eq) method in DMF (2 h). After TFA treatment, *O*-acyl isopeptide **4**-TFA was obtained with an isolated



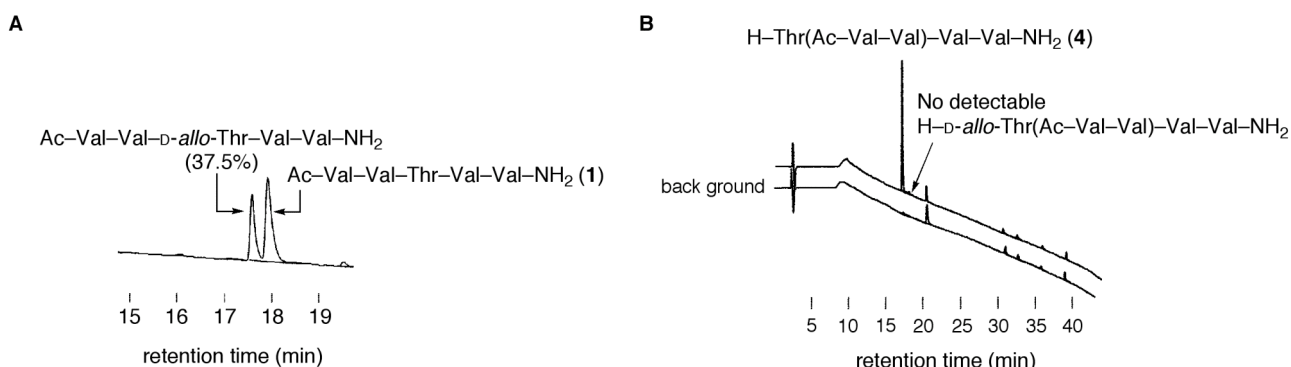


Fig. 2. HPLC profile of (A) crude 4 synthesized using a standard segment condensation, (B) crude isopeptide 7 synthesized using the “O-acyl isopeptide method”-based segment condensation. The retention time of H-D-allo-Thr(Ac-Val-Val)-Val-Val-NH<sub>2</sub>, which was synthesized independently, was 17.8 min. Analytical HPLC was performed using a C18 reverse phase column (4.6 × 150 mm; YMC Pack ODS AM302) with a binary solvent system: a linear gradient of CH<sub>3</sub>CN (0–100% CH<sub>3</sub>CN, 40 min) in 0.1% aqueous TFA at a flow rate of 0.9 mL min<sup>-1</sup> (40°C), detected at 230 nm.

yield of 69%. HPLC analysis of crude 4 exhibited high purity of the desired product without any byproduct derived from epimerization at Thr, which was confirmed by an independent synthesis of H-D-allo-Thr-(Ac-Val-Val)-Val-Val-NH<sub>2</sub> (Fig. 2). Isopeptide 4 was quantitatively converted to 1 in phosphate buffered saline (pH 7.4) [3].

In summary, we report a racemization-free segment condensation based on the O-acyl isopeptide method with a successful synthesis of a model pentapeptide. This method allows the use of an N-segment possessing a C-terminal Ser/Thr residue for segment condensation without any epimerization, as a result of the C-terminal O-acyl isopeptide structure with a urethane-protected Ser/Thr residue. Thus, in the synthesis of long peptides/proteins, racemization-free segment condensation becomes possible at not only the C-terminal Gly/Pro but also Ser/Thr residue of the N-segment. Additionally, final deprotected peptides/proteins synthesized using the O-acyl isopeptide method-based segment condensation are effectively purified by HPLC, because a simple isomerization to an O-acyl isopeptide changed the physicochemical properties of the native peptide [3].

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