INTRODUCTION

Peptide structures with repetitive sequences have been found in many kinds of peptides [1-13] and proteins [14] such as collagen [1-3], elastin [4-7], resilin [7,8], silks [9,10] and others [11-14]. Synthetic methods [3,15-20] for such repetitive sequences may be useful tools and some perspective in clarifying the relationship between the sequences and their functions or the higher-order structures. The conventional methods for polypeptide synthesis have not always been suitable for preparing repetitive sequential polypeptides. The polymerization reactions (Fig. 1) from \( N \)-carboxy-amino acid anhydride (NCA) [21] have been useful for the preparation of homo-polypeptides and co-polypeptides but not useful for that of repetitive sequential polypeptides. The polymerization reactions of \( N \)-Boc-tripeptide derivatives such as Boc-Pro-Pro-Gly-OH, Boc-Pro-Pro-Gly-NH₂, Boc-Pro-Pro-Gly-OCH₃ and Boc-Ala-Ala-Ala-OH with a carboxy group at C-terminal. Thermal reactions were carried out at a constant temperature near their melting points for 1 to 24 h to afford polypeptides whose average molecular weight reached 2,500 Da.

**Keywords:** Thermal reaction, Sequential peptides, \( N \)-Boc-tripeptides, Proline, Alanine.
from the free acid structure has been suggested for facilitating the polymerization reactions. Based on these suggestions, N-Boc-dipeptides (Boc-Gly-Asp-OH, Boc-Ala-Asp-OH and Boc-Val-Asp-OH) and an N-Boc-tripeptide Boc-Gly-Gly-Asp-OH were used for thermal reactions [27,31] to afford sequential polypeptides in a previous study (Fig. 3). The obtained polypeptide structures were determined as the sequential polypeptide with molecular weight up to 7,800 Da [31].

![Fig. 1. Polypeptide formation from N-carboxy-amino acid anhydride](image1)

![Fig. 2. Polypeptide formation from N-t-butyloxycarbonyl-aspartic acid upon thermal treatment](image2)

\[ n = (l_1 + m_1) + (l_2 + m_2) + \cdots + (l_i + m_i) \]

The higher molecular weight fractions after gel permeation chromatography were hydrolyzed to amino acids with the amino acid composition of the initial structure of Boc-peptides. However, such polypeptide structures with sequential Asp residues cannot always be found in the natural polypeptides. Whether the intermediate possessing anhydride structure at the C-terminal is necessary has not been determined for polymerization. The reaction activity of amide group (-C(=O)-NH-)
of N-Boc-asparagine (Boc-Asn-OH) was used to give polypeptides up to 4,900 Da and the results were reported [30].

In the different studies, peptides derivatives having not active side chains like N-t-butyloxycarbonyl-prolyl-prolyl-glycine (Boc-Pro-Pro-Gly-OH) were heated to give polypeptides [28]. However, the detailed mechanisms and reaction conditions were not clarified. This article reports the thermal reactions of Boc-Pro-Pro-OH (4) and Boc-Pro-Pro-Gly-OH (5) which were prepared from Boc-Pro-OH (1) as shown in Fig. 4. In addition, polymerization reactions of N-Boc-tripeptides: Boc-Pro-Pro-OH (4), Boc-Pro-Gly-OH (5), Boc-Pro-Pro-Gly-OH (7), Boc-Pro-Pro-Gly-NH2 (8), Boc-Pro-Pro-Gly-OCH3 (9) and Boc-Ala-Ala-Ala-OH (17) in Fig. 6 were performed. The products of the thermal reaction of sequential polypeptides like H-(Pro-Pro-Gly)5-OH have a similar sequence to collagen [1-3]. These N-Boc-oligopeptides possess no polar reactive side chains. Boc-Ala-Ala-Ala-OH has a typically simple structure without reactive side chains, but might give water-soluble polypeptides H-(Ala-Ala-Ala)5-OH under thermal treatment, whereas polypeptides H-(Gly-Gly-Gly)5-OH has a much lower solubility than H-(Ala-Ala-Ala)5-OH.

The six N-Boc-peptides viz. Boc-Pro-Pro-OH (4), Boc-Pro-Pro-Gly-OH (5), Boc-Pro-Pro-Gly-OH (7), Boc-Pro-Pro-Gly-NH2 (8), Boc-Pro-Pro-Gly-OCH3 (9) and Boc-Ala-Ala-Ala-OH (17) were separately heated without solvents at constant temperatures. During the thermal reactions, the weight change and the change in the heat formation were measured. The reaction mixtures were analyzed in relation to the molecular weight spread. From these results, the thermal reactions from N-Boc-tripeptides resulted in polypeptides. This study describes the detailed results and reveals a simple new thermal reaction for the polypeptide formation.

**EXPERIMENTAL**

A Jeol JLC-300 Amino Acid Analyzer was used for determining the amino acid composition in the hydrolysates of peptides (Jeol, Japan). A Hitachi 200-10 spectrophotometer was used for spectrophotometry measurements (Hitachi, Japan). A Jasco DIP-181 digital polarimeter (Jasco, Japan) was used for the measurement of the optical rotation of the peptide derivatives. A DT-40 thermal analyzer (Shimadzu, Japan) was used for thermal analysis. A Jasco Trimotor-V as the flow pump and a Jasco UVIDEC-100-IV spectrophotometer (Jasco, Japan) as the detector were used for the HPLC system equipped with a gel permeation column G-3000 PW (TSK, Yamaguchi, Japan). Analysis of the evolved gases from the thermal analyzer was performed with a Shimadzu GCMS-QP1000A. A nuclear magnetic resonance (NMR) (Jeol FX-100 NMR system (Jeol, Japan)) was used for the collection of 1H NMR spectra.

**Standard polypeptides:** Standard peptide compound H-(Pro-Pro-Gly)5-OH (m.w. = 1,274 Da) and H-(Pro-Pro-Gly)10-OH (m.w. = 2,531 Da) were purchased from The Peptide Institute, Inc.

**Preparation of peptide derivatives:** Several N-t-butyloxycarbonyl-peptides (N-Boc-peptides) were prepared by the coupling of the corresponding N-Boc-amino acids and amino acids. The procedures are shown in Figs. 4 and 5.

**Amino acid derivatives and other reagents:** Proline (Pro-OH (1)), glycine (Gly-OH), alanine (Ala-OH) and di-t-butyldicarbonate ((Boc)2O) were purchased from Peptide Institute, Inc., Minoh-shi, Osaka, Japan. Glycinamide hydrochloride (HCl·Gly-NH2) and glycine benzyl ester p-tosylate (Tos-Gly-OBzl) were purchased from Wako Pure Chemical Industries,
Boc-Pro-OH (2) and Boc-Pro-ONSu (3): Boc-Pro-OH (2) was prepared by protection of Pro (1) with ((Boc)2O in a dioxane-water mixture. From Boc-Pro-OH (2) (10.8 g, 50 mmol) and HONSU [33] (5.75 g, 50 mmol) in presence of dicyclohexylcarbodiimide (DCC, 10.3 g, 50 mmol) in 50 mL ethyl acetate to obtain Boc-Pro-ONSu (3) (15.6 g, 100%) white crystal, which was recrystallized with 2-propanol (40 mL) to give white crystal (14.1 g, 90%, m.p.: 133-134 ºC (lit. [Ref. 33] 135-136 ºC).

Boc-Pro-Gly-OH (5): Boc-Pro-OH (2) (4.31 g, 20 mmol) was coupled with N-hydroxysuccinimide (HONSU, 2.7 g, 24 mmol) in the presence of DCC (2.53 g, 22 mmol) in 50 mL THF for 2 h at 0 ºC to give a suspension. After filtration of the suspension, the resulted filtrate solution of an active ester Boc-Pro-ONSu (3) was added in an aqueous solution including Gly-OH (2.25 g, 30 mmol) and triethylamine (6.3 mL, 45 mmol) at room temperature. After 2 days’ reaction, the reaction solution after filtration was evaporated and dissolved in ethyl acetate. The resulted ethyl acetate solution was combined with ethyl acetate (100 mL) to give white crystal (7.83 g, 70 mL to Boc-Pro-OH (9.95 g, 80%), which was recrystallized with 2-propanol (40 mL) to give white crystal (14.1 g, 90%, m.p.: 133-134 ºC (lit. [Ref. 33] 135-136 ºC).

Boc-Pro-Gly-OH (6): Boc-Pro-ONSu (3) (12.5 g, 40 mmol) and Pro-OH (1) (6.91 g, 60 mmol) in the presence of triethylamine (NETz, 60 mmol, 8.4 mL) in water 30 mL and dioxane 70 mL to Boc-Pro-Gly-OH (9.95, 80%), which was recrystallized with ethyl acetate (100 mL) to give white crystal (7.83 g, 63%, m.p.: 176-177 ºC). Elementary analysis: calcd. (found) % for C16H21O4N2: C, 57.46 (57.40); H, 7.47 (7.45); N, 9.17 (9.14). 

Boc-Pro-Gly-OBzl (7): Boc-Pro-Gly-OH (6) (4.60 g, 10 mmol) was hydrogenated in 30 mL ethanol in the presence of Pd-C for 45h. The reaction solution was filtered, and the resulting solution was evaporated and crystallized to give Boc-Pro-Gly-OH (7), 3.33 g (90%), m.p.: 72-74 ºC. Elementary analysis: calcd. (found) % for C17H26N2O4: C, 59.68 (59.64); H, 7.98 (7.96); N, 10.64 (10.59).

Tos-Ala-OBzl (13): Ala (8.91 g, 100 mmol), benzyl alcohol (52.5 g, 485 mmol), p-toluene sulphonic acid (20.9 g, 110 mmol) and benzene (100 mL) were refluxed in a Dean-Stark apparatus for 20 h. The reaction mixture was evaporated to an
Thermal Synthesis of Polypeptides from N-t-Butyloxycarbonyl-tripeptide Derivatives

**Fig. 6.** Preparation procedure of Boc-Ala-Ala-Ala-OH (17) [Ala: L-alanine, Boc-: t-butyloxycarbonyl-, HONSu: N-hydroxysuccinimide, DCC: dicyclohexylcarbodiimide, NEt3: triethylamine, Tos-Ala-OBzl (13): alanine benzyl ester p-tosylate, 4 M HCl: 4 mol/L HCl in dioxane, H2/Pd-C: hydrogenation over palladium on charcoal]

The results that the thermal reactions of compound 4 (1.0 mmol) at 170 ºC for 4 h, the resulting product was recrystallized using THF and n-hexane to give crystalline solid (48%, m.p. 133-135 ºC). The ninhydrin reaction of the product was not detected. The 1H NMR analysis of this compound in CDCl3 showed as follows: δ = 4.10-4.20 (t, J = 8.1 Hz, 2H), 3.45-3.60 (m, 5H), 2.50-2.55 (m, 4H), 1.85-2.45 (m, 8H), 1.30-1.50 (m, 4H), 0.80-1.00 (m, 3H). The data showed that the product was diketopiperazine (cyclo-(Pro-Asp)). The thermal reaction of compound 4 for 24 h at 170 ºC produced 38% decrease in mass, which corresponds to the total of the complete Boc group deprotection and dehydration between two prolines to cyclo-(Pro-Asp).

The Boc-Pro-Gly-Asp (5) (1.0 mmol) was heated at 150 ºC for 24 h to reveal 43% decrease in mass, which corresponds the complete Boc-group deprotection and dehydration between a proline molecule and glycine molecule to cyclo-(Pro-Gly). The results that the thermal reactions of N-Boc-tripeptides afforded cyclic dipeptides were not clearly observed in the thermal reactions of Boc-Gly-Asp. The compound having a reactive side chain at C-terminal like Boc-Gly-Asp can make β-peptide bond after melting and deprotection of Boc-group. However, N-Boc-tripeptides having no reactive side chains may cyclize to diketopiperazines. Therefore, these results suggested that longer N-Boc-peptides like N-Boc-tripeptides are necessary to reveal polypeptides for thermal reaction.

RESULTS AND DISCUSSION

**Thermal reactions of N-Boc-dipeptides:** Depending on the angle from which structure of N-Boc-tripeptide substrate is viewed, they are composed of N-Boc-amino acids, N-Boc-dipeptides and amino acid segments. Because these segments may differently react according to their different structures, these compounds having similar segmental structure were heated separately. The amino acid derivatives, Boc-Ala-OH, Boc-Val-OH, Boc-Asp-OH were separately heated at 130-140 ºC to give free amino acid with the yield of more than 80% [25].

**N-Boc-dipeptides:** Boc-Pro-Pro-OH (4) and Boc-Pro-Gly-Oh (5) were heated in the same type but in different glass test tubes. After the thermal reactions of each compound at 170 ºC for 4 h, the resulting products was recrystallized using THF and n-hexane to give crystalline solid (48%, m.p. 133-135 ºC). The ninhydrin reaction of the product was not detected. The 1H NMR analysis of this compound in CDCl3 showed as follows: δ = 4.10-4.20 (t, J = 8.1 Hz, 2H), 3.45-3.60 (m, 5H), 2.50-2.55 (m, 4H), 1.85-2.45 (m, 8H), 1.30-1.50 (m, 4H), 0.80-1.00 (m, 3H). The data showed that the product was diketopiperazine (cyclo-(Pro-Asp)). The thermal reaction of compound 4 for 24 h at 170 ºC produced 38% decrease in mass, which corresponds to the total of the complete Boc group deprotection and dehydration between two prolines to cyclo-(Pro-Pro).

The Boc-Pro-Gly-OH (5) (1.0 mmol) was heated at 150 ºC for 24 h to reveal 43% decrease in mass, which corresponds the complete Boc-group deprotection and dehydration between a proline molecule and glycine molecule to cyclo-(Pro-Gly). The results that the thermal reactions of N-Boc-tripeptides afforded cyclic dipeptides were not clearly observed in the thermal reactions of Boc-Gly-Asp [31]. The compound having a reactive side chain at C-terminal like Boc-Gly-Asp can make β-peptide bond after melting and deprotection of Boc-group. However, N-Boc-tripeptides having no reactive side chains may cyclize to diketopiperazines. Therefore, these results suggested that longer N-Boc-peptides like N-Boc-tripeptides are necessary to reveal polypeptides for thermal reaction.

**Gel permeation chromatography of thermal reaction mixtures:** Each reaction mixture after thermal treatment in the glass tube was cooled to room temperature and dissolved in 5.0 M acetic acid (5 mL). The resulted solution was loaded onto a gel permeation chromatography (91 cm x 1.6 cm, G-25F). Elution was carried out with 0.5 M acetic acid and the eluted solution was collected with a fraction collector. Each solution collected in the test tubes was monitored at 230 nm absorption.
Thermal analysis of Boc-Pro-Pro-Gly-X (7, 8 and 9) derivatives: The peptide derivatives Boc-Pro-Pro-Gly-OH (7) as well as Boc-Pro-Pro-Gly-NH₂ (8) were chosen as the substrates for the thermal syntheses. A small amount of sample was heated in a thermal analyzer (Shimadzu DT-40) under helium stream to monitor the thermal gravimetry (TG) and differential thermal analysis (DTA). Figs. 7 and 8 show the thermal analysis data (TG and DTA) from compounds 7 and 8, respectively. However, the thermal analysis of the derivative Boc-Pro-Pro-Gly-OCH₃ (9) was not carried out, because of the much lower melting point and the difficulty of sample making for the thermal analysis.

![Fig. 7. TG (thermal gravimetry, green line) and DTA (differential thermal analysis, red line) of Boc-Pro-Pro-Gly-OH (7)](image)

![Fig. 8. TG (thermal gravimetry, green line) and DTA (differential thermal analysis, red line) of Boc-Pro-Pro-Gly-NH₂·H₂O (8)](image)

Boc-Pro-Pro-Gly-OH (7) (3.10 mg) was heated from 50 to 300 °C at a rate of 5 °C/min. The first endothermic peak between 50 and 100 °C corresponds to the melting point of Boc-Pro-Pro-Gly-OH (72-74 °C). The second endothermic peak corresponds to the decomposition of Boc-group and the following dehydration between Pro-Pro-Gly-OH molecules to give polypeptides. At a little lower temperature (150 to 160 °C) before the second endothermic peak, the decrease in weight started. The results suggested that the decomposition of compound 7 started at 150 to 160 °C. Such degrees will be proper for polymerization of Boc-Pro-Pro-Gly-OH (7).

Boc-Pro-Pro-Gly-NH₂·H₂O (8) (3.10 mg) was also heated from 50 to 300 °C at a rate of 5 °C/min. A small endothermic peak at a little higher temperature than 100 °C is according to the melting point of Boc-Pro-Pro-Gly-NH₂·H₂O (109-111 °C). The second endothermic peak corresponds to the decomposition of Boc-group and the following dehydration between H-Pro-Pro-Gly-NH₂ to give polypeptides. At a little lower temperature (150 to 160 °C) before the second endothermic peak, the decrease in weight of Boc-Pro-Pro-Gly-NH₂·H₂O was also started, which suggests the thermal reactions.

Mass spectrometry of the gases from compounds 7 and 8 upon thermal: Figs. 9 and 10 show the MS data emitted during the thermal analysis of compounds 7 and 8, respectively. The gas mixture generated during the thermal reaction was directly introduced to a mass spectrometry (Shimadzu QP-1000A). Water (m/z = 18), carbon dioxide (m/z = 44), and isobutene (m/z = 56) were detected by selective ion monitoring. In the thermal reaction of Boc-Pro-Pro-Gly-OH (7), ammonia was not detected at higher temperature, although in the reaction of Boc-Pro-Pro-Gly-NH₂·H₂O (8), ammonia (m/z = 17) was detected but water was not detected. Water molecule of the compound 7 is a kind of crystal water, which is estimated to have been evaporated at a lower temperature. The generated gases during the thermal analysis suggested that deprotection and condensation proceeded in the thermal reaction.

Thermal reactions of compounds 7, 8, 9 and 17: N-Boc-Pro-Pro-Gly-X derivatives (7, 8 and 9, 0.50 mmol) were heated for a constant reaction time at a constant temperature. The weight recovery of substrate 7 during the thermal at 130, 140 and 150 °C was plotted against the reaction time as shown in Fig. 11. After 16-24 h reaction, the rate of weight decrease became slow. The weight loss after 24 h reaction at 150 °C was larger than the decrease (32% loss: 68% recovery) equivalent to the total deprotection and the condensation between peptides. This observation might be explained by substrate sublimation.

The weight recovery of substrates 8, 9 and 17 during the thermal is shown in Figs. 12-14, respectively. The thermal reactions of Boc-Pro-Pro-Gly-OH (7) at 140 °C, Boc-Pro-Pro-Gly-NH₂ (8) at 160 °C, Boc-Pro-Pro-Gly-OH (9) at 160 °C Boc-Ala-Ala-Ala-OH (17) at 170 °C were estimated to be complete in 1 or 2 days. However, the recovery data may not reflect sublimation from the reaction mixture might happen in the thermal reactions. This research contributed to analyzing the remaining products after the thermal reactions, whereas sublimation of the peptide derivatives will be discussed in other work. The molecular weight feature of the remaining products was analyzed by gel permeation chromatography.

Gel permeation chromatography of compounds 7, 8, 9 and 17: The reaction mixture after thermal reaction at 150 °C was dissolved in 0.5 M acetic acid and the solution was loaded onto a Sephadex G-25 column. The gel permeation chromatogram (GPC) of the reaction mixture from Boc-Pro-Pro-Gly-OH (7) is shown in Fig. 15.

With an increase in the reaction time, the higher molecular weight fractions moved to the further higher molecular weight
fraction area. Comparing the chromatograms with those of standards: (a) H-(Pro-Pro-Gly)₁₀-OH (m.w. = 2,531) and (b) H-(Pro-Pro-Gly)₅-OH (m.w. = 1,274), the highest molecular fractions of the chromatogram of the reaction mixture for 16 h was in the range between standards (a) and (b).

The higher molecular-weight fractions than H-(Pro-Pro-Gly)₅-OH (m.w. = 1,274) were combined and lyophilized after GPC to give amorphous powder. The yield was 5.3 mg from the reaction at 150 °C for 16 h. The other reaction mixtures were purified in the same manner to give higher molecular
Fig. 11. Weight recovery of Boc-Pro-Pro-Gly-OH (7) during the thermal reaction [the dashed line indicates the estimated level after complete deprotection and peptide formation]

Fig. 12. Weight recovery of Boc-Pro-Pro-Gly-NH$_2$ (8) during the thermal reaction [the dashed line indicates the level after complete deprotection and peptide formation]

Fig. 13. Weight recovery of Boc-Pro-Pro-Gly-OCH$_3$ (9) during the thermal reaction [the dashed line indicates the level after complete deprotection and peptide formation]

Fig. 14. Weight recovery during the thermal reaction of Boc-Ala-Ala-Ala-OH (17) [the dashed line indicates the level after complete deprotection and peptide formation]

Fig. 15. Gel permeation chromatography of the thermal reaction mixtures from Boc-Pro-Pro-Gly-OH (7) at 150 ºC.; (a) H-(Pro-Pro-Gly)$_{10}$-OH, (m.w. = 2,531); (b) H-(Pro-Pro-Gly)$_{5}$-OH, (m.w. = 1,274)
weight (1-5 mg). The average molecular weight of the purified product was estimated at the range of 1,200-2500 Da by HPLC analysis using gel permeation chromatography (TSK-GEL 300PW). The IR spectrum of the product appeared similarly to that of the standard H-(Pro-Pro-Gly)₅-OH. The higher molecular weight fractions were hydrolyzed in 6 M HCl and the amino acids in the hydrolysate were analyzed. The molar ratio of Pro and Gly in the hydrolysate of the higher molecular weight fraction was almost 2:1. The results of the above mentioned data indicate that the higher molecular weight product would be a sequential polypeptide like H-(Pro-Pro-Gly)₅-OH.

The GPCs from Boc-Pro-Pro-Gly-NH₂ (8) at 160 °C, Boc-Pro-Pro-Gly-OCH₃ (9) at 160 °C, Boc-Ala-Ala-Ala-OH (17) at 170 °C and Boc-Ala-Ala-Ala-OH (17) at 180 °C are shown in Figs. 16-19, respectively. The GPCs of the reaction mixtures of compounds 8, 9 and 17 showed the similar pattern to the chromatogram of the reaction mixture of compound 7. The higher molecular weight fractions increased but the lower molecular weight fractions decreased with the reaction time. Although these compounds have different leaving groups (-OH for 7 and 17; -NH₂ for 8; -OCH₃ for 9), the reaction rate seems to be at a similar level.

The higher molecular-weight fractions than H-(Pro-Pro-Gly)₅-OH (m.w. = 1,274) were combined and lyophilized after GPCs from the reactions to give amorphous powder products. The yield in several cases were 0.7 mg (from 8, 160 °C, 8 h), 0.8 mg (from 9, 160 °C, 30 h), 0.5 mg (from 17, 170 °C, 16 h), 8.1 mg (from 17, 180 °C, 8 h) and 15.5 mg (from 17, 180 °C, 16 h). Comparing the yields above cited with that 5.3 mg from Boc-Pro-Pro-Gly-OH (7) at 150 °C for 16 h, the compounds 7

![Fig. 16. Gel permeation chromatograms of the thermal reaction materials from Boc-Pro-Pro-Gly-NH₂ (8) at 160 °C. (a) H-(Pro-Pro-Gly)₁₀-OH (m.w. = 2,531) and (b) H-(Pro-Pro-Gly)₅-OH (m.w. = 1,274)](image)

![Fig. 17. Gel permeation chromatograms of the thermal reaction materials from Boc-Pro-Pro-Gly-OCH₃ (9) at 160 °C. (a) H-(Pro-Pro-Gly)₁₀-OH (m.w. = 2,531) and (b) H-(Pro-Pro-Gly)₅-OH (m.w. = 1,274)](image)
and 17 having C-terminal -OH gave higher yields than other leaving groups (-NH₂ (8) and -OCH₃ (9)).

Although peptide bond formation proceeded according to the kinds of leaving group, the reaction procedure may consist of three steps. The first step is assumed to be the melting of the compounds, whereas the second step would be the deprotection of the Boc-group, which emits carbon dioxide and isobutene to afford free amino group. The deprotection reaction upon thermal is confirmed in the measurement with mass spectrometry measurement (Figs. 9 and 10). The third step would be peptide formation by the attack of a free amino group to a carboxy group of the different molecules. The third step results in emitting H₂O (from 7), NH₃ (from 8) and CH₃OH (from 9) to give polypeptides. The reaction mechanism is explained in Fig. 20.

The thermal reaction of Boc-Ala-Ala-Ala-OH (17) is also estimated to proceed with a similar mechanism to that in Fig. 21 as for the reaction of Boc-Pro-Pro-Gly-X (X, -OH (7); X: -NH₂ (8); X: OCH₃ (9)).

Conclusions

Based on the results and discussion, it is concluded the reactivity of N-Boc-amino acids, N-Boc-dipeptides and N-Boc-tripeptides in the thermal reactions without solvents as follows:

1. N-Boc-amino acids (Boc-Ala-OH, Boc-Val-OH, Boc-Pro-OH, etc.) without reactive side chains change to free α-amino acids in the thermal reactions for several hours at the temperatures near melting points.

2. N-Boc-dipeptides (Boc-Pro-Pro-OH, Boc-Pro-Gly-OH) without reactive side chains change to cyclic dipeptides (diketopiperazines: cyclo-(Pro-Pro), cyclo-(Pro-Gly)) in the thermal reactions for several hours at the temperatures near melting points.

3. N-Boc-tripeptides: Boc-Pro-Pro-Gly-OH (7), Boc-Pro-Pro-Gly-NH₂ (8), Boc-Pro-Pro-Gly-OCH₃ (9) and Boc-Ala-
Fig. 20. A proposed reaction mechanism of thermal polymerization of Boc-Pro-Pro-Gly-X (X: -OH (7), -NH₂ (8), -OCH₃ (9))

Fig. 21. A proposed reaction mechanism of thermal polymerization of Boc-Ala-Ala-Ala-OH (17)
Ala-Ala-OH (17) produce repetitive polypeptides in the thermal reactions for several hours at the temperatures near melting points.

4. The highest molecular masses of polypeptides obtained from N-Boc-tripeptides: compounds 7, 8, 9 and 17 were in the range of about 1,272 to 2,531 g/mol.

5. If the thermal reactions of N-Boc-tripeptides: compounds 7, 8, 9 and 17 were carried out at a higher temperature for a long time, the mass of N-Boc-tripeptides decreased much more than expected for the total decrease due to deprotection and dehydration. Sublimation from the reaction mixtures may be necessary for future work.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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