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Application of microwave method to the solid phase synthesis of pseudopeptides containing ester bond

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Abstract—A new procedure was developed for reducing the reaction time and improving the yield of esterification reaction in solid phase synthesis of pseudopeptides containing an ester bond by utilizing microwave irradiation. We selected a pseudodipeptide (Fmoc-LysΨ[COO]Leu-NH_2) and optimized the microwave-assisted esterification reaction in solid phase synthesis using Fmoc chemistry. For this, microwave-assisted esterification reactions with different reaction time, temperature, and solvents were performed using 1,3-diisopropylcarbodiimide (DIC) as the coupling reagent. We synthesized several pseudodipeptides containing an ester bond by using the optimized microwave irradiation method. The purity and yield of the pseudodipeptides synthesized in this way were better than those obtained without microwave irradiation. Furthermore, we applied this methodology for synthesizing pseudopeptides (6- and 12-mer) corresponding to the α helical peptide. The microwave-assisted esterification reaction afforded the target pseudopeptides with high yield (~80%) and purity within 12 min, whereas the reaction without microwave irradiation afforded the target compound with poor yield (~45%) and low purity.

Solid phase synthesis has been extensively used for the preparation of peptides, pseudopeptides, and organic compounds.[1] This technique offers many advantages over conventional synthesis in terms of efficiency, convenient work up and purification procedures. However, the main problems with this method are the difficulties for the reagents to reach the active sites located in the solid phase, thereby slowing the reaction and degrading the resin under long reaction conditions, and the need for excess reagents. Recently, significantly increased reaction rate, improved yield and raised product purity have frequently been observed in solid phase synthesis with microwave irradiation.[2] Several successful attempts for the solid phase synthesis of peptides, peptoids and beta polymers using microwave irradiation have been published.[3] However, the microwave irradiation method has not been extensively applied for the solid phase synthesis of pseudopeptides.

Pseudopeptides have received much attention due to their better bioavailability than peptides and the easy development of non-peptide drugs. Thus, many classes of pseudopeptides have recently been reported.[3,4] We are interested in the solid phase synthesis of pseudopeptides containing an ester bond for the following reasons. The amide and ester bonds are very similar in terms of structural and conformational preferences.[5] Thus, replacement of the amide bond with an ester bond is a well-known strategy for investigating the role of the hydrogen bonding of the amide bond in proteins and peptides for their structures and biochemical interactions.[6] In addition, pseudopeptides containing an ester bond have better bioavailability, due to their higher resistance against protease and increasing hydrophobicity. Even though pseudopeptides containing an ester bond exhibited unique properties, pseudopeptides containing an ester bond were rarely synthesized because the synthesis of the pseudopeptides is not straightforward and very often possesses a synthetic challenge due to the low nucleophilicity of the hydroxyl group compared to the amino group. Pseudopeptides containing ester bonds were synthesized by using solid phase synthesis with α-hydroxy acids or unnatural α-hydroxy acids.[5b,6a,7] Compared to the solid phase peptide synthesis, the reaction time was relatively long (4–16 h) and even double coupling reaction afforded low yield (70%).[5b,6a,7] In the era of proteomics, the time required

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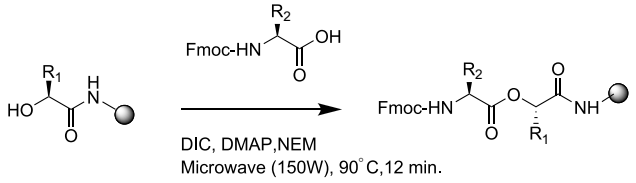
for the synthesis of the target pseudopeptides has become an important issue and it would be beneficial to increase the yield and reaction rate for the pseudopeptide synthesis. Considering this, we have developed a microwave irradiation procedure to reduce the reaction time and increase the reaction yield for the synthesis of pseudopeptides containing an ester bond in solid phase synthesis.

We chose a pseudodipeptide containing ester bond (Fmoc-LysΨ[COO]Leu-NH_2) as a model system and optimized the microwave irradiation procedures for solid phase synthesis. (*S*)-2-Hydroxy-4-methylpentanoic acid (α-hydroxy-Leu) was synthesized by diazotization method in acidic condition with sodium nitrite from Leu.[8] Previous research indicated that among various coupling reagents, 1,3-diisopropylcarbodiimide (DIC) afforded the best yield in the esterification reaction for pseudopeptides in solid phase synthesis.[2b,6,9] Thus, we chose DIC as the coupling reagent and DMAP/NEM as the base and investigated the esterification reaction with microwave irradiation at various temperatures and solvents for the optimization of this model reaction (Scheme 1). First, α-hydroxy-Leu was coupled to Rink-amide MBHA resin by the activation of alpha hydroxy acid (3 equiv) with the same equivalent of DIC and HOBt with microwave irradiation. The coupling reaction was repeated until no color was observed in ninhydrin test.[10] Then, the microwave-assisted esterification reactions were performed for solid phase synthesis of the pseudopeptide (Fmoc-LysΨ[COO]Leu-NH_2), as mentioned.[11] The coupling yield of the esterification reaction was measured by Fmoc quantitation assay.[12]



Scheme 1. Introduction of ester bond in the resin using microwave irradiation method.

Table 1. Coupling yield of the model pseudodipeptide at different time and different solvent under microwave irradiation[a]

Solvent	Power (W)	Temp (°C)	Time (min)	Yield (%)
NMP	200	150	12	58
DMF	150	90	7	50
			10	61
			12	80
			15	81
			20	78

[a] The reaction was performed under a closed vessel using microwave irradiation.

DCM has frequently been used as a solvent in esterification reaction. However, DCM was not considered as a solvent for the microwave irradiation method due to its low boiling point (40 °C) and because some Fmoc-protected amino acids have poor solubility in DCM. As shown in Table 1, the esterification reaction with microwave irradiation was performed in NMP as the solvent due to its high boiling point. However, the yield was not significantly improved[13] and Fmoc-deprotection of the conjugated Fmoc amino acid to the resin was observed in this condition, perhaps due to high temperature in the presence of the base. We selected DMF as the solvent and investigated the esterification reaction with microwave irradiation. Table 1 indicates that the reaction reached a level of 80% at 12 min. To improve the coupling yield, we increased the reaction time but the yield was not improved. We performed the esterification reaction in DMF solvent with microwave irradiation (150 W) at high temperature (120 °C) but the yield (80%) was not improved. In conventional solid phase synthesis, a different DCM/DMF ratio was frequently employed as the solvent for the coupling reaction.[14] The esterification reaction with microwave irradiation was also attempted using DMF/DCM (1:1, v/v); however, the obtained yield was low (56%). Thus, the reaction condition with microwave irradiation (temp 90 °C, power 150 W, time 12 min) was kept constant for further synthesis of pseudopeptides in this study.

Additionally, several coupling reagents were reconsidered for the esterification reaction by applying this microwave irradiation condition. The coupling yield with various coupling reagents is summarized in Table 2.

As shown in Table 2, the DIC-mediated coupling reaction afforded the higher yield. The activation of an Fmoc-amino acid with coupling reagents **2**, **4**, and **5** resulted in the formation of the corresponding OBt ester that was known to provide low racemic product. However, the OBt ester afforded low yield in this condition.

The microwave-assisted method by using DIC as the coupling reagent was applied for the synthesis of various dipseudopeptides (Table 3). Their yields and purities

Table 2. Microwave-assisted yields of Fmoc-LysΨ[COO]Leu-NH_2 with various coupling reagents[a]

No.	Coupling reagent	Base	Yield (%)
1	DIC (3 equiv)	NEM (1.2 equiv)/DMAP (0.1 equiv)	80
2	DIC (3 equiv)/HOBt (3 equiv)	NEM (1.2 equiv)/DMAP (0.1 equiv)	72
3	TFFH (3 equiv)	DIEA (8 equiv)	62
4	PyBop (3 equiv)/HOBt (3 equiv)	DIEA (6 equiv)	50
5	DCC (3 equiv)/HOBt (3 equiv)	DIEA (3 equiv)/DMAP (0.1 equiv)	54

[a] The resin (0.025 mmol) containing α-hydroxy-Leu was mixed with Fmoc-Lys(Boc)-OH (3 equiv) in the presence of the coupling reagent and the reaction was performed under microwave irradiation (temp 90 °C, power 150 W, time 12 min).

Table 3. Coupling yield of various pseudodipeptides containing an ester bond

No.	Sequence	Yield (%)	
		Microwave (12 min)	Normal (480 min)
1	Fmoc-KΨ[COO]L-NH_2	80	75
2	Fmoc-EΨ[COO]L-NH_2	83	72
3	Fmoc-SΨ[COOlL-NH_2	78	68
4	Fmoc-FΨ[COO]L-NH_2	78	68
5	Fmoc-AΨ[COO]L-NH_2	78	64

were compared with those obtained by the esterification reaction without microwave irradiation.[15]

The coupling yield of each reaction was measured by Fmoc quantitation assay.[12] After completion of the reaction, the pseudopeptides were cleaved and deprotected by treatment of TFA/H_2O (95:5) solution. The resulting product was analyzed by using C_{18} reverse phase HPLC and an ESI mass spectrometer. HPLC and ESI mass analyses of the product mixtures indicated that both methods afforded the target pseudopeptide as the major product (Figs. S1–S5). The presence of only one peak in the chromatogram suggested that no racemization occurred during the microwave-assisted reaction. Table 3 shows that the coupling yields for the various pseudodipeptides containing an ester bond synthesized were higher in the microwave irradiation method than those achieved without microwave irradiation. Specifically, the reaction time was significantly reduced by applying this microwave irradiation method, as compared to that achieved without microwave irradiation.

We chose the α helical peptide, which is known to be a difficult sequence,[16] and synthesized the corresponding pseudopeptides containing an ester bond by using the microwave irradiation procedure. The 6- and 12-mer pseudopeptides containing a hydroxy group at their N-terminus were synthesized on Rink-amide MBHA resin in solid phase synthesis. The esterification reaction was performed using the microwave-assisted esterification method.[11] The same pseudopeptides were synthesized by using the method without microwave irradiation at room temperature with continuous stirring for 8 h and the yields were compared with those achieved using microwave irradiation methods.

As shown in Table 4, the coupling yield achieved using the microwave-assisted method was about twice as high as that obtained by the esterification reaction without microwave irradiation. The HPLC and mass spectra of 6-mer pseudopeptide (Figs. S6 and S7) indicated that both methods afforded the target pseudopeptide as a major product, whereas the purity obtained using microwave irradiation methods was better than that obtained without microwave irradiation.

We did not use HOBt in this reaction. Racemic product may have been generated in the microwave-assisted reaction. Even though only one peak was observed in the HPLC spectrum of the product obtained using the microwave method, the peak seemed to be broad. Thus, we synthesized the D-form of 6-mer pseudopeptide (Fmoc-D-LeuΨ[COO]Lys-Lys-Leu-Leu-Lys-NH_2) and mixed L- and D-forms and analyzed them by HPLC. Two distinct peaks were observed with different retention time in this HPLC (Fig. S8). This result clearly indicates that no racemic product formation occurred during the microwave reaction.

The 12-mer pseudopeptide was also successfully synthesized by using the microwave-assisted procedure (Fig. 1). The HPLC and MALDI TOF mass spectrum (Figs. S9 and S10) indicated that the microwave method afforded the target molecule as the sole product, whereas the method without microwave irradiation afforded two major products. The mass spectrum (Fig. S10) indicated that the product corresponding to peak 1 in Figure 1B was the unreacted product (HO-LLL-KWLK-KLLK-NH_2), while the product corresponding to peak 2 was the target pseudopeptide. The presence of only one major peak in the HPLC chromatogram indicated that no detectable racemic product was synthesized in this microwave-assisted reaction.

In conclusion, we successfully developed a fast and efficient procedure for the solid phase synthesis of pseudopeptides using microwave irradiation. In a direct

Table 4. Coupling yield of 6- and 12-mer pseudopeptide containing an ester bond by using microwave irradiation method

No.	Sequence	Yield (%)	
		Microwave (12 min)	Without microwave (480 min)
1	Fmoc-LΨ[COO]K-KLLK-NH_2	86	45
2	KΨ[COO]LLL-KWLK-KLLK-NH_2	79	44

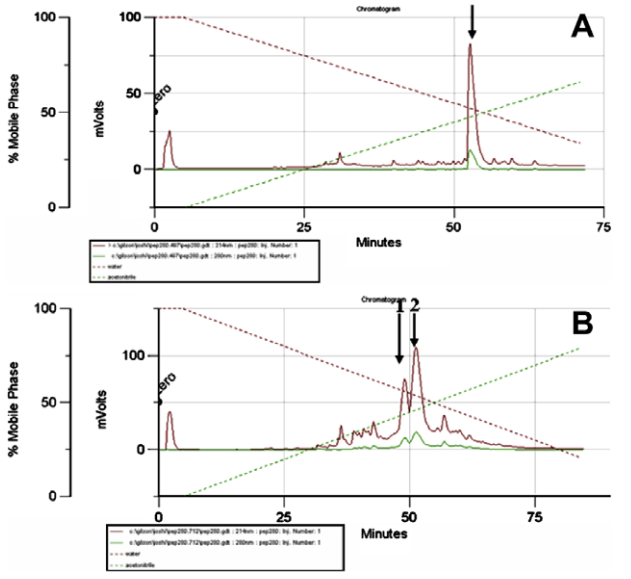


Figure 1. HPLC of 12-mer pseudopeptide KΨ[COO]-LLL-KWLK-KLLK-NH_2: (A) with microwave irradiation, (B) without microwave irradiation.

comparison of our optimized protocol with normal methods, the pseudodipeptides synthesized by the microwave irradiation method, 6- and 12-mer, were afforded at high yield and significant purity. Considering the recent demand for pseudopeptides in the era of proteomics, this microwave-assisted procedure has shown promising potential for the synthesis of pseudopeptides containing an ester bond.

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Supplementary data

HPLC condition and characterization data for pseudopeptides by HPLC and mass spectroscopy. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2007.11.004.

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11. The coupling reaction was accomplished as follows. Activated Fmoc-amino acid with DIC/DMAP in DMF (3 mL) was mixed with the hydroxy Leu attached resin followed by NEM. The reaction mixture was placed in a microwave vial and irradiated at 90 °C, supplying 150-W power for 12 min in the Biotage Microwave Initiator System. The reaction was cooled with N_2 gas and the resin was washed with DMF and MeOH, respectively (3 mL, three times each). Deprotection was achieved by treatment with a mixture of trifluoroacetic acid: water (95:5, v/v) at room temperature for 4 h. The crude peptide was analyzed by HPLC with a waters C_{18} column using a water (0.1% TFA) acetonitrile (0.1% TFA) gradient. The products were characterized by ESI mass spectrometry (Mass1200L Quadruple LC/MS system, Varian) and MALDI TOF mass spectrometry (Voyager-DE STR, Applied Biosystem).
12. The dry resin was weighed (3–5 mg) in an eppendorf tube and added to a 50% piperidine/DMF solution. The mixture was incubated for 30 min at room temperature with continuous stirring. Then, the absorbance of the Fmoc-piperidine adduct was measured at 301 nm by using a UV–vis spectrophotometer (Lambada 40, Perkin Elmer, UK). The blank titrations were performed without resin containing supernatant. The loading yield of the esterification reaction was compared to that of the loading yield of Fmoc-protected α-hydroxy leucine. The degree of substitution was calculated by using the following equation: (mmol/g) = absorbance at 301 nm × volume (mL)/7800 × weight (mg).
13. Fmoc-Lys (Boc)-OH (3.0 equiv) was activated with 3 equiv of DIC and HOBt in the presence of DMAP (0.1 equiv) followed by 1.2 equiv of NEM and added to a resin containing alpha hydroxy resin in 3 mL NMP. The mixture was placed in a microwave vial irradiated at 150 °C supplying 200-watt power for 12 min and the obtained yield was 58%.
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