

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

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### Introduction

With the advancement in the combinatorial chemistry, microwave enhanced synthesis has received much attention these days. During the last one decade, an increasing number of reports have been published that support the advantages and use of microwave irradiation to carry out several reactions in solid phase organic synthesis [1-4]. Significant increase in reaction rate, coupling yield and purity of the product have frequently been observed employing this method in comparison to that of the conventional heating method. Solid phase synthesis has been extensively used for the preparation of peptides, pseudopeptides and organic compounds. This technique offers many advantages over conventional synthesis in terms of efficiency as well as 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, resulting in slow reaction and degradation of the resin under long reaction conditions, and requirements of excess reagents. Recently, significant increase in reaction rate, improving yield and high purity of the product have frequently been observed in solid phase synthesis with microwave irradiation. Several successful attempts for the application of microwave irradiation for the solid phase synthesis of peptides, peptoid and beta polymer have been published [5-7]. However, the microwave irradiation method has not extensively been applied for the solid phase synthesis of pseudopeptides.

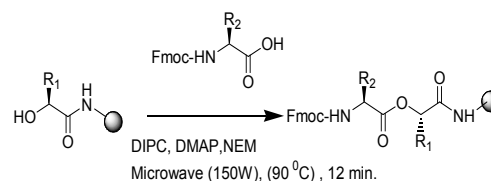
Pseudopeptides have received much attention due to their better bioavailability than peptides and the easy development of non-peptide drugs. Thus, recently many classes of pseudopeptides have been reported. We are interested in the solid phase synthesis of pseudopeptides containing ester bond as following reasons. The amide and ester bonds are very much similar in terms of structural and conformational preferences. Thus, replacement of amide bond with ester bond is well-known strategy for investigating the role of hydrogen bonding of amide bond in proteins and peptides for their structures and biochemical interactions. In addition, pseudopeptides containing ester bond have better bioavailability, resulting from higher resistance against protease and increasing hydrophobicity. Even though pseudopeptides containing ester bond exhibited unique properties, pseudopeptides containing ester bond were rarely synthesized because the synthesis of the pseudopeptides is not straightforward and very often poses a synthetic challenge due to the low nucleophilicity of hydroxyl group compared to amino group. Since the reaction times using the conventional

conditions have been reported to be rather long (4-16 hr) and obtained yield and purity was not significant [8-10].

In the era of the proteomics, the time required for the synthesis of the target pseudopeptides becomes an important issue and it would be beneficial to increase the yield and reaction rate for the synthesis of the pseudopeptides. Considering this, we developed a microwave irradiation procedure for the purpose of reducing the reaction time and increasing the reaction yield for the synthesis of pseudopeptides containing ester bond in solid phase synthesis.

### Results and Discussion

We chose the dipeptide containing ester bond (Fmoc-Lys-[COO] Leu-NH<sub>2</sub>) as a model system and optimized microwave irradiation procedures for solid phase synthesis. The (S)-2-Hydroxy-4-methylpentanoic acid ( $\alpha$ -hydroxy-Leu) and (S)-6-[[Phenylmethoxy] carbonyl] amino]-2-hydroxy hexanoic acid ( $\alpha$ -hydroxy-Lys (z)) were synthesized by diazotization method in acidic condition with sodium nitrite from the respective amino acid. DIPC among various coupling reagents provided the best yield in the previous esterification reaction for pseudopeptides in solid phase synthesis. We chose diisopropylcarbodiimide (DIPC) as a coupling reagent and DMAP/NEM as base and investigated yields of the esterification reaction with microwave irradiation in various temperatures and solvents for the optimization of this model reaction. In this model reaction, the



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

pseudopeptide was synthesized by using Rink-amide MBHA resin as shown in scheme 1.

The  $\alpha$ -hydroxy acid was coupled to the resin by activation of 3 eq. of  $\alpha$ -hydroxy acid with the same equivalent of DIPC and HOBT in the presence of DMF with microwave irradiation. The coupling reaction was repeated until no color was observed in ninhydrin test. Then, the esterification reaction was performed using activated Fmoc-amino acid with DIPC/DMAP in DMF (3 mL) and mixed it to the hydroxy Leu attached resin followed by NEM. The reaction mixture was placed in microwave vial and irradiated at 90°C, supplying 150-watt

power for 12 min. in Biotage Microwave Initiator System. After completing the reaction, the reaction was cooled with N<sub>2</sub> gas and washed with DMF/MeOH (3 mL 3 times each). As hydroxyl group is not sensitive with ninhydrin color test; the coupling yield for esterification step was measured by Fmoc quantitation assay based on the absorbance observed at 301 nm for Fmoc-piperidine adduct. Deprotection was achieved by treatment with a mixture of trifluoroacetic acid: water (95:5v/v) at room temperature for 4 hr. The crude peptide was analyzed by HPLC with a waters C<sub>18</sub> 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).

As shown in Table 1, the esterification reaction with microwave irradiation was performed in NMP as solvent due to its high boiling point. However the yield was not significantly improved 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 base. We selected DMF as solvent and

**Table 1:** Coupling yield for model dipeptide at different time under microwave irradiation

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

investigated esterification reaction with microwave irradiation.

Table 1 indicates that the reaction reached a level of 80% at 12 min. To improve coupling yield, we increased reaction time but the yield was not improved. We performed the esterification reaction in DMF solvent with microwave irradiation (150W) at high temperature (120 °C) however the yield (80%) was not improved. Thus, the reaction condition with microwave irradiation (Temp. 90°C, power 150 watt, time 12 min.) was kept constant for further

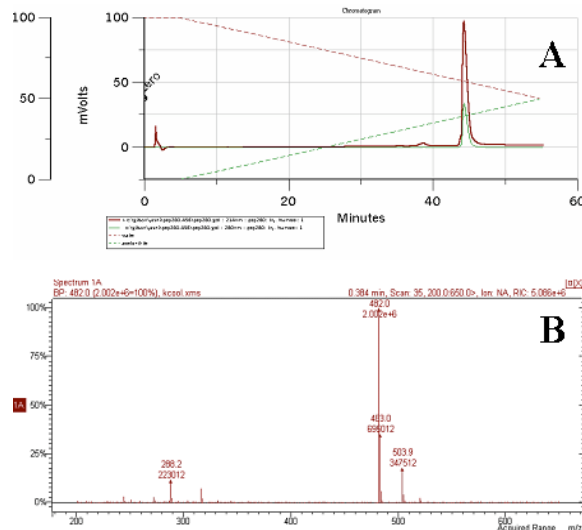
**Table 2:** Coupling yield of various pseudodipeptides containing ester bond

No.	Sequence	Yield (%)	
		Microwave (12min)	Without microwave (480 min)
1	Fmoc-Kψ[COO]L-NH <sub>2</sub>	80	75
2	Fmoc-Eψ[COO]L-NH <sub>2</sub>	83	72
3	Fmoc-Sψ[COO]L-NH <sub>2</sub>	78	68
4	Fmoc-Fψ[COO]L-NH <sub>2</sub>	78	68
5	Fmoc-Aψ[COO]L-NH <sub>2</sub>	78	64

synthesis of pseudopeptides in this study and further applied for the synthesis of various pseudopeptides.

As shown in Table 2, the higher yield was observed in all dipeptides by utilizing microwave method with compare to normal methods. This indicates the significance of this method for the synthesis of pseudopeptides containing ester bond. By applying this

microwave irradiation method, the reaction time was significantly reduced as compare to Normal methods. This is due to the rapid heating in microwave, which accelerates the reaction rate. Energy transfer to the reaction vessel in microwave irradiation method can provide impressive improvement of coupling efficiency in SPPS. Moreover, the microwave reactor has capacity to sustain the target temperature while subjecting the reaction mixture to



**Figure 1:** HPLC and ESI mass spectra of dipeptide [Fmoc-Kψ(COO)L-NH<sub>2</sub>], synthesized by microwave method: (a) HPLC and (b) ESI mass (Calculated (M+H<sup>+</sup>) 481.5 and observed (M+H<sup>+</sup>) 482.0.

continuous irradiation with microwaves by cooling the reaction vessel with a stream of nitrogen gas.

As shown in Figure 1, the HPLC spectra of dipeptide [Fmoc-Kψ(COO)L-NH<sub>2</sub>], synthesized by utilizing microwave method has one peak as major product and measured mass is identical to that of the calculated mass. From HPLC and ESI mass analyses, it is clear that the microwave heating method provided the target compound as a major product and no racemization occurred during the microwave assisted reaction.

**Table 3:** HPLC retention time and measured mass of the pseudopeptides synthesized by both microwave and normal methods.

S. No.	Sequence	Calcd. / Obs. Mass (M+H <sup>+</sup> )	HPLC Ret. Time (Min.)	
			Normal	Microwave
1	Fmoc-Kψ[COO]L-NH <sub>2</sub>	481.5/482.0	42.81	43.16
2	Fmoc-Eψ[COO]L-NH <sub>2</sub>	482.23/482.95	48.08	48.28
3	Fmoc-Sψ[COO]L-NH <sub>2</sub>	440.13/439.93	44.26	44.37
4	Fmoc-Fψ[COO]L-NH <sub>2</sub>	500.23/500.03	53.94	53.37
5	Fmoc-Aψ[COO]L-NH <sub>2</sub>	424.22/424.99	53.93	52.23
6	Fmoc-Lψ[COO]K-NH <sub>2</sub>	481.5/482.0	44.46	44.13
7	Fmoc-Lψ[COO]K-KLLK-NH <sub>2</sub>	963.62/963.12	42.96	43.68
8	Kψ[COO]LLL-KWLK-KLLK-NH <sub>2</sub>	1524.06/1524.09	51.55	52.67

The HPLC retention time and observed mass for all peptides were tabulated in Table 3.

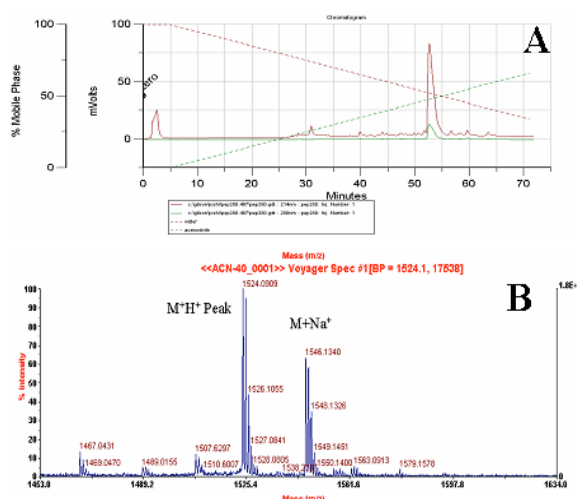
Both methods provided the target pseudopeptide as a major product with identical retention time and mass, and the purity achieved by using microwave irradiation was

similar or better than that obtained by normal method. After optimizing the microwave-assisted procedure in dipeptides containing various amino acids, we chose  $\alpha$ -helical 6-mer and 12-mer peptides, which are known as a difficult sequence for synthesis in solid phase synthesis as model peptides and synthesized the corresponding pseudopeptides containing the ester bond by using the microwave irradiation procedure.

Similarly, the same peptides were synthesized by using normal method at room temperature with continuous stirring for 8 hr and compared the yield with that obtained by microwave methods. The coupling yield achieved with the microwave-assisted method was better than that

**Table 4:** Coupling yield of 6-mer and 12-mer pseudopeptide containing ester bond using microwave and without microwave methods.

No.	Sequence	Yield (%)	
		Microwave (12 min)	Without microwave (480 min)
1	Fmoc-L $\psi$ [COO]K-KLLK-NH <sub>2</sub>	86	45
2	K $\psi$ [COO]LLL-KWLK-KLLK-NH <sub>2</sub>	79	44



**Figure 2:** HPLC and mass spectra of 12-mer pseudopeptide synthesized by microwave method: (a) HPLC and (b) ESI mass (Calculated ( $M+H^+$ ) 1524.06 and observed ( $M+H^+$ ) 1524.09).

obtained by the esterification reaction without microwave irradiation. (Table 4) The HPLC and ESI mass spectra of 12-mer pseudopeptide was shown in Figure 2.

In conclusion, the use of microwave irradiation allowed a rapid and high yield preparation of pseudopeptides containing ester bond for Fmoc based solid phase synthesis. In a direct comparison of the optimized protocol with conventional methods, the dipeptides, 6-mer peptides and 12-mer peptides synthesized by microwave irradiation method were achieved in high yield and significant purity. This study has demonstrated that controlled microwave heating would be advantageous for the synthesis of pseudopeptides containing ester bond.

## Acknowledgments

This work was supported by the Grant (R01-2006-000-10956-0) from the Basic Research Program of the Korea Science & Engineering Foundation and the Grant (C00344) from the basic research program of the Korean Research Foundation.

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