Synthesis of Peptide Amides on Safety-catch Resin with Microwave Irradiation

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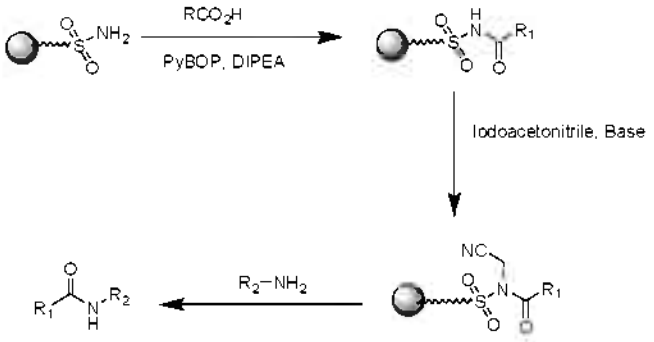
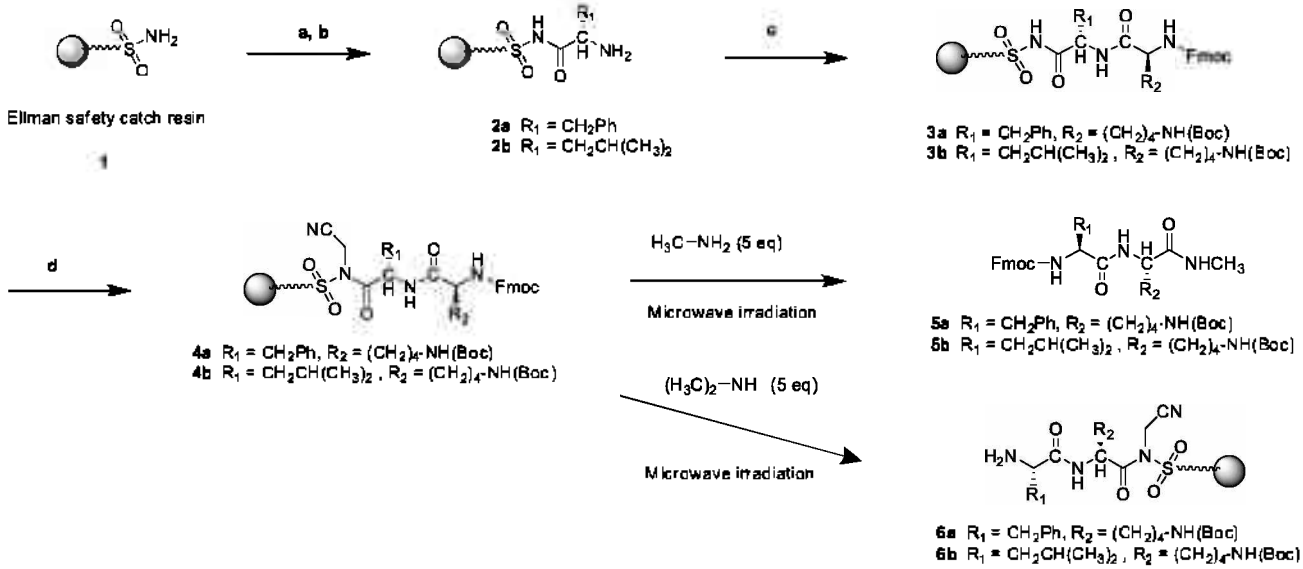
As the demand of small chemical and peptide libraries has increases, new solid phase synthesis methods for generating those libraries have received attention. Since Kenner reported the first safety catch resin using *N*-acylsulfonamide linker for synthesis of primary amides,[1] Ellman *et al.* published an alkylsulfonamide safety catch linker that had more improved coupling yield of acylation reaction (Scheme 1).[2] The alkylsulfonamide safety-catch linker has been widely used for solid-phase synthesis of peptide amides and peptide thioesters.[3,4] As the alkylsulfonamide linker is stable in acidic and basic condition, Fmoc and Boc chemistry can be applied in the preparation of C-terminal modified peptides using this linker.[5-7] After activation is accomplished with iodoacetonitrile to provide the activated cyanomethyl alkylsulfonamide resin, which is more reactive to nucleophiles, the addition of nucleophiles such as amines and thiols cause the release of peptide amides and peptide thioesters from the resins (Scheme 1). The addition of primary amines into the activated resin resulted in the release of target peptide amides in considerable yields with high purities.[3,5-7] If the primary amines can be removed in simple purification step such as precipitation or evaporation, target peptide amides can be isolated without further purification. However, if less nucleophilic primary amines were reacted with the activated resin, excess of nucleophiles, elevated reaction temperature (~ 90 °C), and longer reaction times (1 - 4 days) were required to obtain target peptide amides.[3,8-10]



Scheme 1. General reaction scheme of alkanesulfonamide "Safety-Catch" Linker for Solid-Phase Synthesis.

During the past decades, microwave-assisted solid phase peptide synthesis (SPPS) has received attention because microwave irradiation mostly accelerated reaction rates and improved the purities and yields in SPPS.[11] Microwave irradiation is an



Scheme 2. Synthesis of peptide amides on safety catch resin. Conditions: (a) Fmoc-Leu-OH (5 equiv) or Fmoc-Phe-OH (5 equiv), PyBOP (5 equiv), DIPEA (10 equiv), Triple coupling; (b) 50% piperidine in DMF; (c) Fmoc-Lys(Boc)-OH (3 equiv), DIC (3 equiv), HOBt (3 equiv); (d) ICH_2CN (25 equiv), DIPEA (5 equiv).

alternative way to conventional heating for providing energy into reactions. There are several successful publications of microwave-assisted solid phase synthesis of various unnatural biopolymers such as peptoids, pseudopeptides, and beta polymers.[12-15] The enhancement of solid phase synthesis under microwave irradiation can be explained by both thermal and non-thermal microwave effects. Microwave irradiation may result in the deaggregation of peptides on the resin due to the high dipole moment of amide bonds. However, microwave irradiation has not been used for the cleavage reaction of the activated alkylsulfonamide resin by nucleophiles. If microwave irradiation will help to rapidly synthesize diverse organic compounds as well as peptide amides on safety catch resins, this is of significant benefit for library generation as well as C-terminal modified peptide synthesis.

In the present study, we investigated the effect of microwave irradiation on solid phase synthesis of peptide amides with safety catch resin for the first time. As shown in Scheme 2, dipeptide loaded safety catch resins were subjected to the short peptide amide synthesis with microwave irradiation. Fmoc-Leu-OH or Fmoc-Phe-OH was introduced on the commercially available alkylsulfonamide linker, respectively. Triple coupling with 5 equiv of amino acid in each step was carried out for loading of first amino acid into the resin according to the literature procedure.[3] Fmoc group of the loaded amino acid on the resin was deprotected with 50% piperidine in DMF for 20 min. Fmoc-L-Lys(Boc)-OH was coupled with **2a**, **2b** using DIC, HOBt activation in DMF until no color change of the resin was observed by the ninhydrin test. The loading yields were determined by piperidine treatment of small aliquots of resin followed by absorbance analysis of Fmoc-piperidine adduct in solution. Activation of **3a**, **3b** with iodoacetonitrile (25 equiv) and DIPEA in NMP for 24 hrs provided the activated resin **4a**, **4b**.

We investigated cleavage reactions of the resin bound dipeptides by methyl amine with and without microwave irradiation. The release amount of **5a**, **5b** from the resins was measured on the basis of the absorbance of the product solution at 301 nm for Fmoc group. C_{18} reverse phase HPLC analysis at 214 nm and ESI mass spectrum analysis were used to gauge the purity of the peptide amides. THF was frequently used as solvent in the synthesis of peptide amides on safety catch resin.[2,3,7] However, THF was not used as solvent for this microwave irradiation reaction due to its low boiling point. As shown in Table 1, the nucleophilic displacement reaction under microwave irradiation was carried out in DMF as solvent due to its high boiling point and high resin swelling property. After adding 5 equiv of methyl amine into the activated resin **4a**, the cleavage reaction under microwave irradiation was studied. The reactions were carried out under power controlled irradiation (100 W, 130 °C; 170 W, 130 °C). Figure 1 showed that the temperature under microwave irradiation (100 W) reached to 130 °C within 10 min. However, the cleavage yield (61%) was not improved in comparison to that obtained (59%) at room temperature without microwave irradiation. However,

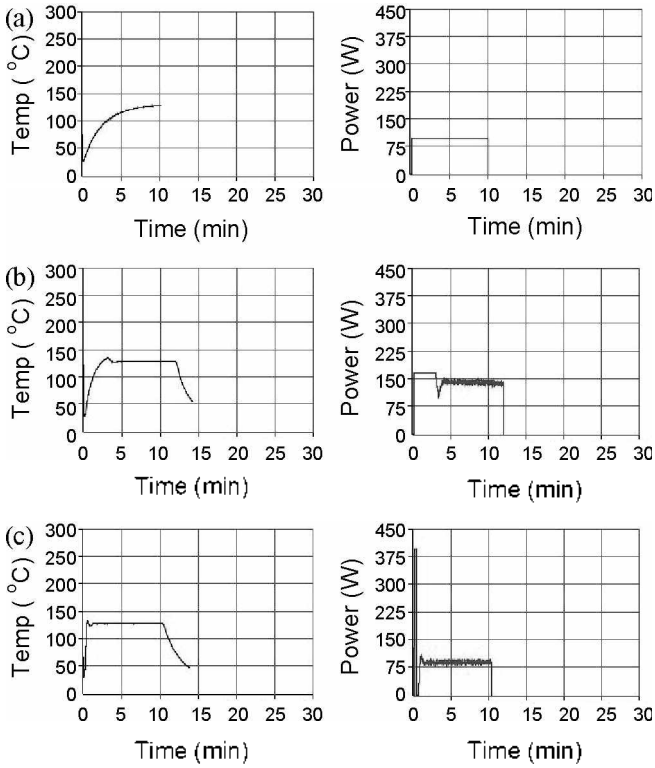


Figure 1. Reaction temperature in cleavage reaction of **4a** with methyl amine under microwave irradiation (a) (100 W, 130 °C), (b) (170 W, 130 °C), and (c) (400 W, 130 °C).

Table 1. Cleavage reactions on the activated safety catch resin with methylamine under microwave irradiation.

Entry	Name	Methylamine (equiv)	Microwave power (W)	Temp (°C)	Time (min)	Yield[a] (%)	Purity[b] (%)
1	**5a**	5	100	130	10	61	> 95
2	**5a**	5	170	130	12	65	> 95
3	**5a**	5	0	25	10	58	> 95
3	**5a**	5	0	25	100	83	> 95
4	**5a**	5	400	130	5	94	93
5	**5a**	5	400	130	10	96	92
7	**5b**	5	400	130	5	82	> 95
8	**5b**	5	400	130	10	92	> 95
9	**5b**	5	0	25	100	59	> 95

[a]Yields determined by Fmoc absorbance of product solution and by HPLC analysis. [b]C_{18} reverse phase HPLC analysis with detection at 214 nm.

long reaction time (≥ 100 min) without microwave irradiation provided a 83% yield of **5a** with a high purity. Thus, microwave irradiation power (170 W) and reaction time (12 min) were increased. However, the yield (65%) was not significantly improved. The cleavage reaction was performed under high microwave irradiation power (400 W, 130 °C). In this case, reaction temperature reached to 130 °C after an initial 20 seconds of microwave irradiation (Figure 1). The high yield of **5a** (96%) was achieved in this condition. HPLC analysis at 214 nm and ESI mass spectrum analysis of the product solution indicated that the purity of **5a** was over 95% (Figure 2). **5a** was not directly ionized by electrospray ionization. After Boc group of **5a** was removed by treatment of TFA :

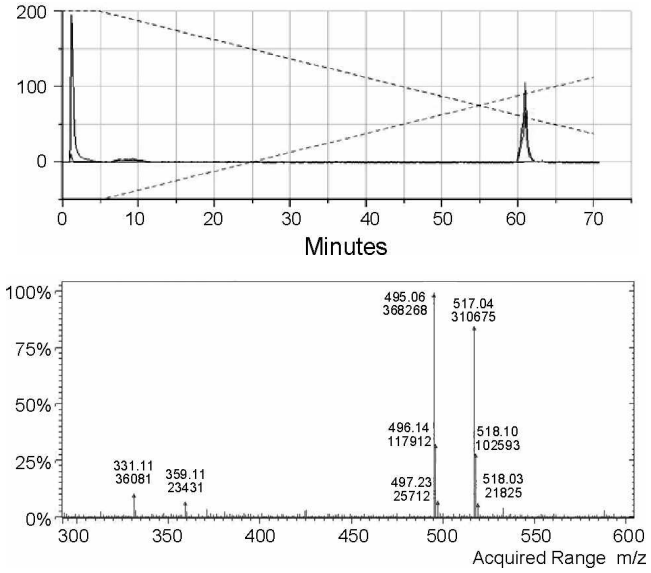


Figure 2. HPLC and mass spectrum of cleavage reaction solution of **4a** with methyl amine under microwave irradiation (400 W, 130 °C). The identity of the target peptide amide was established by ESI-mass. Fmoc-Lys-Leu-NHCH$_3$, $[M+H]^+$ = 495.06, $[M+Na]^+$ = 517.04.

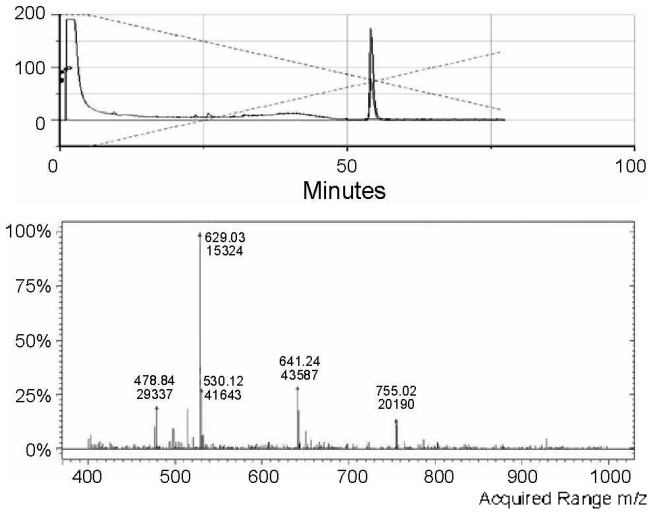


Figure 3. HPLC and mass spectrum of cleavage reaction solution of **4b** with methyl amine under microwave irradiation (400 W, 130 °C). The identity of the target peptide amide was established by ESI-mass. Fmoc-Lys-Phe-NHCH$_3$, $[M+H]^+$ = 529.03, $[M-TFA]^+$ = 641.24, $[M+2TFA]^+$ = 755.02.

H_2O (v/v, 95 : 5) solution, the resulting solution was analyzed using HPLC-ESI mass spectrometer. Interestingly, racemization product and Fmoc deprotected **5a** were not observed in this condition. To improve cleavage yield, the same reaction was performed in NMP as solvent under 400 W microwave irradiation at 150 °C because NMP has higher boiling point than DMF. However, HPLC analysis and ESI mass spectrum analysis indicated the purity of **5a** was low due to the side reactions (data not shown). The overall results indicated that high microwave irradiation power (400 W) seemed to be critical to achieve high yield of **5a**.

The cleavage reaction of **5b** by methyl amine was also investigated under the same microwave irradiation condition (DMF, 400 W, 130 °C). The release yield of **5b** (92%) was better than that (59%) achieved without microwave irradiation. Analysis of the product solution using HPLC-ESI mass spectrometer revealed that the purity of **5b** was over 95% (Figure 3). Table 1 summarized the reaction condition, yield, and purity of the cleavage reactions of **4a** and **4b** by methyl amine under microwave irradiation.

The cleavage reaction of **4a** and **4b** by dimethylamine was carried out under the same microwave irradiation condition (400 W, 130 °C) for 10 min. HPLC analysis and ESI mass spectrum analysis of the product solution revealed that the only deprotection reaction occurred instead of nucleophilic displacement reaction. This is due to the fact that dimethyl amine is less nucleophilic and more basic than methylamine.

In summary, we studied microwave-assisted synthesis of peptide amides on safety catch resin. In comparison to the conventional cleavage reaction, microwave-assisted cleavage reaction of the activated safety catch resin with primary amines was more efficient, providing peptide amides with a higher yield and similar purity in considerable shorter reaction times. Considering the recent demand for the generation of peptide libraries and peptide amides, the microwave-assisted procedure would be of great utility in the synthesis of peptide amides and peptide libraries.

Experimental Section

Loading procedure for Fmoc-amino acids into safety catch resin. To a 10 mL reaction bottle were added resin **1** (90 mg, 0.10 mmol), DMF (3 mL), *i*-Pr$_2$EtN (1 mmol), and an Fmoc-amino acid (0.50 mmol). The reaction mixture was stirred for 10 min and then PyBop (258 mg, 0.5 mmol) was added to the reaction mixture. The reaction mixture was stirred for 8 h, filtered, and washed with DMF (3 × 5 mL), MeOH (3 × 5 mL), and DMF (3 × 5 mL). The same coupling reaction repeated three times. A 50% piperidine in DMF solution (2.5 mL) was added to the reaction bottle and the solution was stirred for 20 min. The resin was then filtered and washed with DMF (3 × 5 mL), MeOH (3 × 5 mL), and DMF (3 × 5 mL). To a separate 10 mL flask were added Fmoc-amino acid (0.5 mmol), HOBt (0.50 mmol), DIC (0.50 mmol), and DMF (2.5 mL). After a 10 min premix period, the solution was added to the resin-containing reactor followed by agitation for 10 min under microwave irradiation (100 W, 80 °C). The resin was then filtered and washed with DMF (3 × 5 mL), MeOH (3 × 5 mL), and

DMF (3 × 5 mL). A ninhydrin test was employed to determine reaction completion. The resin **3a-b** was washed with NMP. The loading yield of the resin was determined by Fmoc analysis.[16] To the resin were added NMP (4 mL) and *i*-Pr$_2$EtN (1 mmol). Iodoacetonitrile (70 μL, 1 mmol) was added to the reaction mixture and the reaction vessel was shielded from light and stirred for 24 hr. The resin was filtered and washed with DMF (3 × 5 mL), MeOH (3 × 5 mL), and DMF (3 × 5 mL).

Cleavage procedure for the dipeptide amides from the activated resin by amines. All microwave irradiation experiments described herein were performed using a Biotage AB initiator with 10 mL solid phase reaction vessels. A stock solution of methylamine in THF (5 equiv) was added to resins **4a-b** (10 mg, 0.01 mmol) in 2 mL DMF. The reaction mixture was stirred under microwave irradiation. The coupling yield was determined by taking the aliquots of the solution followed by measuring absorbance at 301 nm. The product solution was analyzed by HPLC with a waters C_{18} column using a water (0.1% TFA)-acetonitrile (0.1% TFA) gradient and HPLC-ESI mass spectrometry (Mass1200L Quadruple LC/MS system, Varian).

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