

# Peptide Synthesis in Aqueous Solution With a Reusable Solid Phase

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## ABSTRACT

A procedure has been developed for synthesizing peptides in an aqueous solution with a reusable solid phase. Specifically designed linker molecule is employed to attach peptides to hydrophilic solid phases, enabling Solid Phase Peptide Synthesis (SPPS) in aqueous solutions. The linker molecule is utilized to connect peptides to an anionic exchange resin during peptide synthesis in an aqueous solution. The general structure of the linker molecule is Fmoc-AA-CH<sub>2</sub>-Ph-Rx-SO<sub>3</sub><sup>-</sup>, the Fmoc (9-fluorenylmethoxycarbonyl) group serves as a protecting group for amino acids. Amino acids (AA) are linked to the solid phase through a structure of Methoxyphenylcarbonyl group, which is cleavable under strong acidic conditions. The sulfate group is present for forming an ionic bond with the solid resin in an aqueous solution. In this procedure, Fmoc-AA are utilized as building blocks for sequentially adding amino acids in peptide synthesis. Due to Fmoc-AA poor solubility in aqueous solutions, a procedure was developed to enhance the solubility of hydrophobic compounds, with a specific emphasis on dissolving Fmoc-protected Amino Acids (Fmoc-AA) in an aqueous solution. This enhancement facilitates SPPS in aqueous conditions with Fmoc-AA as building blocks. Cationic exchange resin, which is reusable, serves as the solid phase.

Our research objective is to shift from the use of organic solvents to an aqueous system while maintaining the existing SPPS practices in organic solvents as closely as possible. This transition involves minimal alterations, maintaining consistency with the organic solvent system, except for the utilization of novel peptide linker molecule to hydrophilic solid resins that are commercially available. This approach is designed to facilitate a more readily acceptable transition for the peptide synthesis industry from using organic solvents to aqueous solution, contributing to greener and more sustainable synthetic methodologies.

## INTRODUCTION

SPPS is a method for peptide synthesis. In SPPS, a peptide is tethered by its C-terminus to an insoluble solid phase, and the peptide is subsequently constructed through the sequential addition of protected amino acids. SPPS is predominantly carried out in organic solvents within the current peptide industry.

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Dimethylformamide (DMF) and dichloromethane ( $\text{CH}_2\text{Cl}_2$ ) are commonly used in organic solvents for peptide synthesis, despite their serious toxicity concerns and significant waste solvent generation. Given these issues, there is a pressing need for the development of more environmentally friendly and safer protocols within the field of peptide synthesis [1].

Aqueous-phase peptide synthesis typically employs protected amino acids, coupling reagents, and other essential additives that are soluble or dispersible in water. Several strategies have been devised to facilitate peptide bond formation and safeguard the reactive functional groups of amino acids in an aqueous environment [2].

Recently, certain researchers have suggested that propylene carbonate, recognized for its environmentally friendly and polar properties, could effectively replace dichloromethane and DMF in both solution and solid-phase peptide synthesis [3].

Another novel technology has surfaced in peptide synthesis, featuring a tandem deprotection/coupling sequence designed for solution-phase peptide synthesis in water. This process operates under micellar catalysis conditions, employing the unique surfactant TPGS-750-M. [4]

Another invention introduces a method for peptide synthesis, involving the steps of condensing an N-Fmoc protected amino acid with a peptide possessing C-terminal protection. This process employs a carrier that crystallizes in response to alterations in the solvent composition [5].

In aqueous-phase peptide synthesis, a prevalent strategy involves utilizing water-soluble coupling reagents like water-soluble carbodiimides or peptide coupling agents that operate effectively in an aqueous environment. Moreover, protective groups compatible with water-based conditions are employed to selectively shield functional groups prone to undesirable reactions during peptide bond formation.

Aqueous-phase peptide synthesis represents a vibrant field of research and development, continually striving to enhance the efficiency, scalability, and sustainability of peptide synthesis methods. Leveraging water as the primary solvent offers numerous advantages in terms of safety, environmental impact, and compatibility with downstream applications, rendering it a highly appealing option for peptide synthesis.

Our research aims to transition from the use of organic solvents to an aqueous system while preserving the current solid-phase peptide synthesis (SPPS) practices in organic solvents to the greatest extent feasible. This conversion entails minimal changes, with no significant differences from the organic system, except for the peptide linker molecules and hydrophilic solid resins which are commercially available. Our approach aims to facilitate a smoother transition that is readily acceptable for the industry. Our primary objective is to shift from traditional SPPS in organic solvents to an aqueous solution. Our goal is to maintain the established methodology of traditional SPPS as closely as possible, ensuring that SPPS in an aqueous solution becomes readily accepted in the peptide industry. This transition seeks to decrease organic solvent consumption, thereby fostering a greener environment.

Our work introduces a peptide synthesis method predominantly utilizing an aqueous solvent, incorporating Fmoc deprotection, coupling, and cleavage processes. By removing the necessity for organic solvents in these stages, our aim is to reduce organic solvent consumption and foster a greener environment.

## Results and Discussion

The present research specifically focuses on, the utilization of specifically designed linker molecules to attach peptides to solid resin in SPPS within an aqueous environment, while Fmoc-protected Amino Acids (Fmoc AAs) serving as building blocks to facilitate the peptide sequence assembly within the aqueous solution. Specifically designed a linkage molecule is used to establish bonds between the peptides and the solid phases in the aqueous phase. A tailored procedure has been devised to enhance the solubility of Fmoc-AAs in the aqueous phase. This innovative approach aims to minimize the utilization of organic solvents in peptide synthesis, thereby contributing to greener and more sustainable synthetic methodologies.

Linker molecules are often used for attaching peptides to solid phases in SPPS. Here anionic exchange resins are used as solid phase in SPPS in aqueous solution, a novel linker molecule is used for attaching peptides to anionic exchange resins.

In traditional SPPS in organic solvents, Wang Resin is widely favored for its effectiveness. The linker molecule within Wang Resin offers a reversible connection between the peptide chain and the solid support (resin). In Wang Resin, the linker molecule is tethered to the resin via a phenyl ether bond, while the amino acid (AA) is typically linked to the linker molecule through a benzylic ester bond. Trifluoroacetic acid is employed to cleave the ester bond in the linker molecule, thereby releasing the synthesized peptide at the end of the peptide synthesis process.

In this research, the amino acid (AA) is also linked to the linker molecule through a benzylic ester bond, that resembles of linker molecule in Wang Resin. The primary distinction lies in the fact that linker molecule in Wang Resin is affixed to the solid phase through a chemical bond. And here linker molecule is attached to a solid resin through an ionic bond in an aqueous solution. Similar to linker molecule in Wang Resin, the linker molecule is cleavable under acidic conditions to release the synthesized peptide upon the completion of peptide synthesis.

This novel Linker molecule has a general structure of Fmoc-AA-CH<sub>2</sub>-Ph-Rx-SO<sub>3</sub><sup>-</sup> (as illustrated in Figure 1). This linker molecule is specially designed for attaching peptides to anionic exchange resins, this linker molecule provides linkage between peptide and solid phase through ionic bond. The anionic group includes sulfonate and phosphonate groups, preferably sulfonate, its structure likes this: AA-CH<sub>2</sub>-Ph-Rx- SO<sub>3</sub><sup>-</sup>. Ph represents the benzene group, AA denotes the initial amino acid in the peptide, and - SO<sub>3</sub><sup>-</sup> signifies the sulfonate group. This sulfonate group is for forming ionic bonds, which enable the linker molecule to bind the anion exchange resin in aqueous solutions. The carboxyl group of the amino acid (AA) is linked to the phenyl group through a benzylic ester (-COO-CH<sub>2</sub>-Ph-). This linkage can be selectively cleaved under acidic conditions at the end of the peptide synthesis process. Consequently, the peptide will be liberated from linker molecule A in this acidic environment. Anion exchange resins can be effectively reused through standard anion resin regeneration procedures.

The Rx group offers diverse options, including the methoxyphenyl group (-O-CH<sub>2</sub>-Ph-), methylene group (-CH<sub>2</sub>-), carboxylate group (-OOC-), carboxymethylene group (-OOC- CH<sub>2</sub>-), amino group (-NH-), and ether linkage group (-O-). These chemical groups are utilized individually or in combinations of two or more groups in Rx structure. This Rx designed imparts flexible structure within the linker molecule, providing easier way to build connection between benzene group and sulfonate group. (Figure 2)

Linker molecule can be made from a precursor molecule Fmoc-AA -CH<sub>2</sub>--Ph-Rx-COOH, the precursor molecule is dissolved into aqueous solution, then coupling it with Taurine in by coupling agent EDC

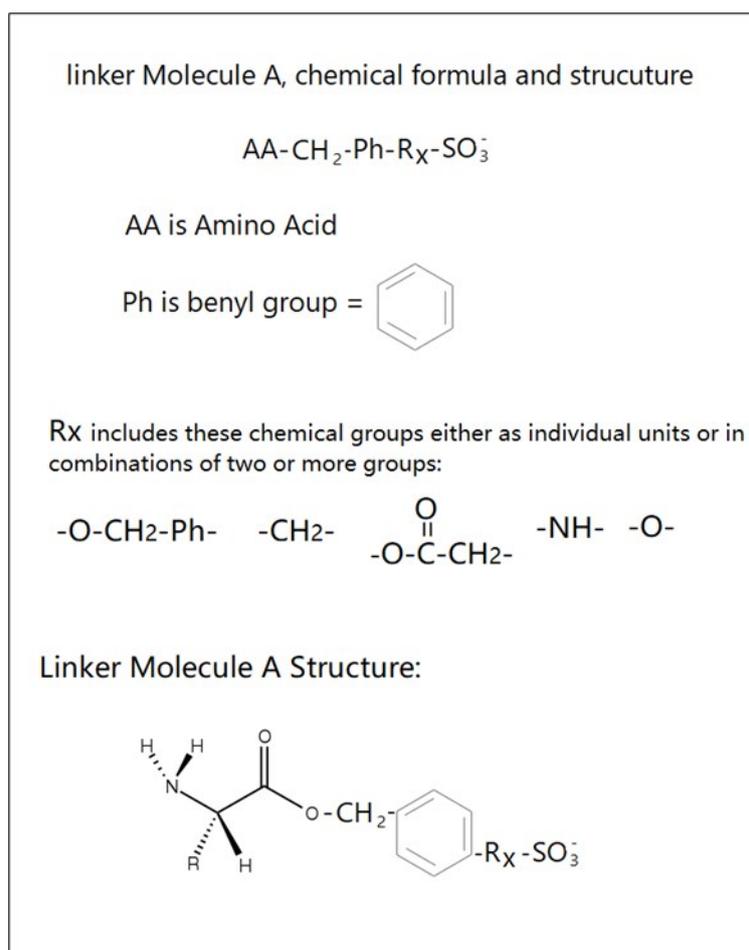


Figure 1. Linker molecule A Chemical Structure

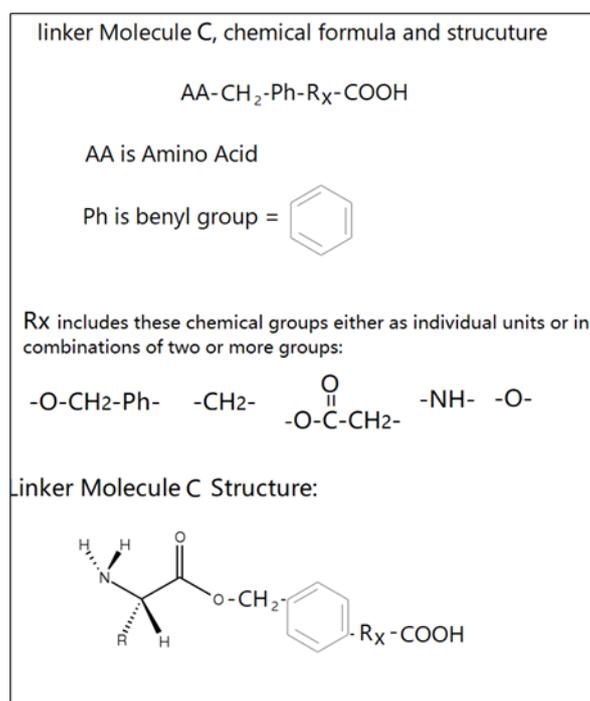


Figure 2. Precursor of Linker molecule Chemical Structure

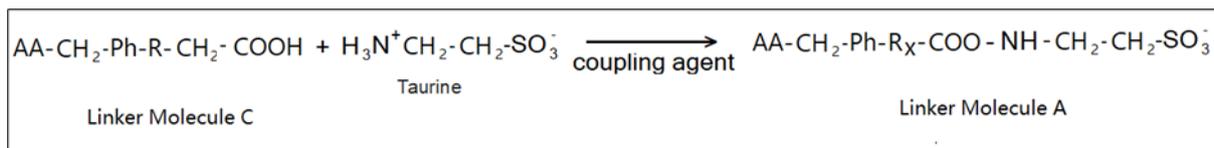
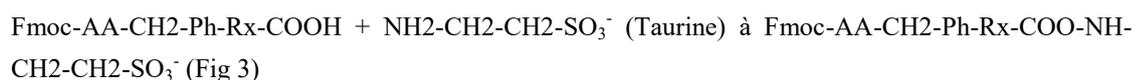


Figure 3. Making Linker molecule from its precursor molecule

EDC (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide) at a pH range of 4 to 5 in aqueous solution. The carboxyl group can form a covalent bond with the amino group (as shown in Fig 3) as follows:



In our scheme for synthesizing peptides via SPPS in an aqueous solution, similar to SPPS in organic solvents, Fmoc-protected amino acids also serve as the fundamental building blocks in this scheme. Fmoc-protected amino acids (Fmoc AAs) are extensively utilized in SPPS in organic solvent. Nevertheless, Fmoc AAs are inherently insoluble in water. Here presents a method for dissolving Fmoc AAs in water, enabling the synthesis of peptides in an aqueous phase while still using Fmoc AAs as the building blocks. By doing so, it allows the transition of many practices from traditional organic solvent-based SPPS to peptide synthesis in an aqueous environment.

#### *The Process for Enhancing the Solubility of hydrophobic molecules in Aqueous Solution*

In order to use Fmoc-AA in SPPS in aqueous solution, it is necessary to make Fmoc-AA soluble aqueous solution. We developed a Process for Enhancing the Solubility of Hydrophobic Molecules in Aqueous Solution, especially useful for dissolving Fmoc-AA in aqueous solution (as illustrated Fig 4). It is summarized as follows. First, a non-ionic surfactant is dissolved in a small amount of a water-miscible organic solvent to create solution A, and then secondly, Fmoc amino acid (Fmoc-AA) is dissolved in a small portion of a water-miscible organic solvent, creating solution B., Solution A is thoroughly mixed with Solution B to form even mixture. The resulting mixture is then vigorously added to an aqueous solution. The solubility of Fmoc-AA can be reached to 1-15% in aqueous solution. Solubility of Fmoc-AA may be various depending on solubility of AA in water solution and temperature, for example AA such as Glycine, Fmoc-Glycine needs much less surfactant than Fmoc-Phe to get soluble into water (as illustrated in table 1).

After attachment of the initial amino acids to solid resins via linker molecule, peptide synthesis is achieved by introducing additional Fmoc-AA that is the next amino acid to be added to the peptide chain, the additional Fmoc-AA is dissolved in an aqueous solution by using the process for enhancing the solubility of hydrophobic molecules in aqueous solution. Subsequently, a coupling agent like EDC is used to couple the Fmoc-AA to the previous AA which is bond to the solid resin.

Deprotection, coupling, and cleavage can be achieved in an aqueous solution. Fmoc deprotection can be accomplished using a basic solution with 10% Tween 80 at a pH of 9.5-10.5. For instance, a solution of 0.1% NaOH or 0.3% monoamine can be utilized to remove Fmoc from the peptide chain. Coupling can be conducted using EDC at pH 4-5 in a 10% Tween 80 solution. Upon completion of peptide synthesis, an acidic solution such as 0.1% HCl or 0.1% H<sub>3</sub>PO<sub>4</sub> can be employed to cleave the peptide chain from the solid phase.

#### *Dissolving Fmoc-AA in aqueous solution*

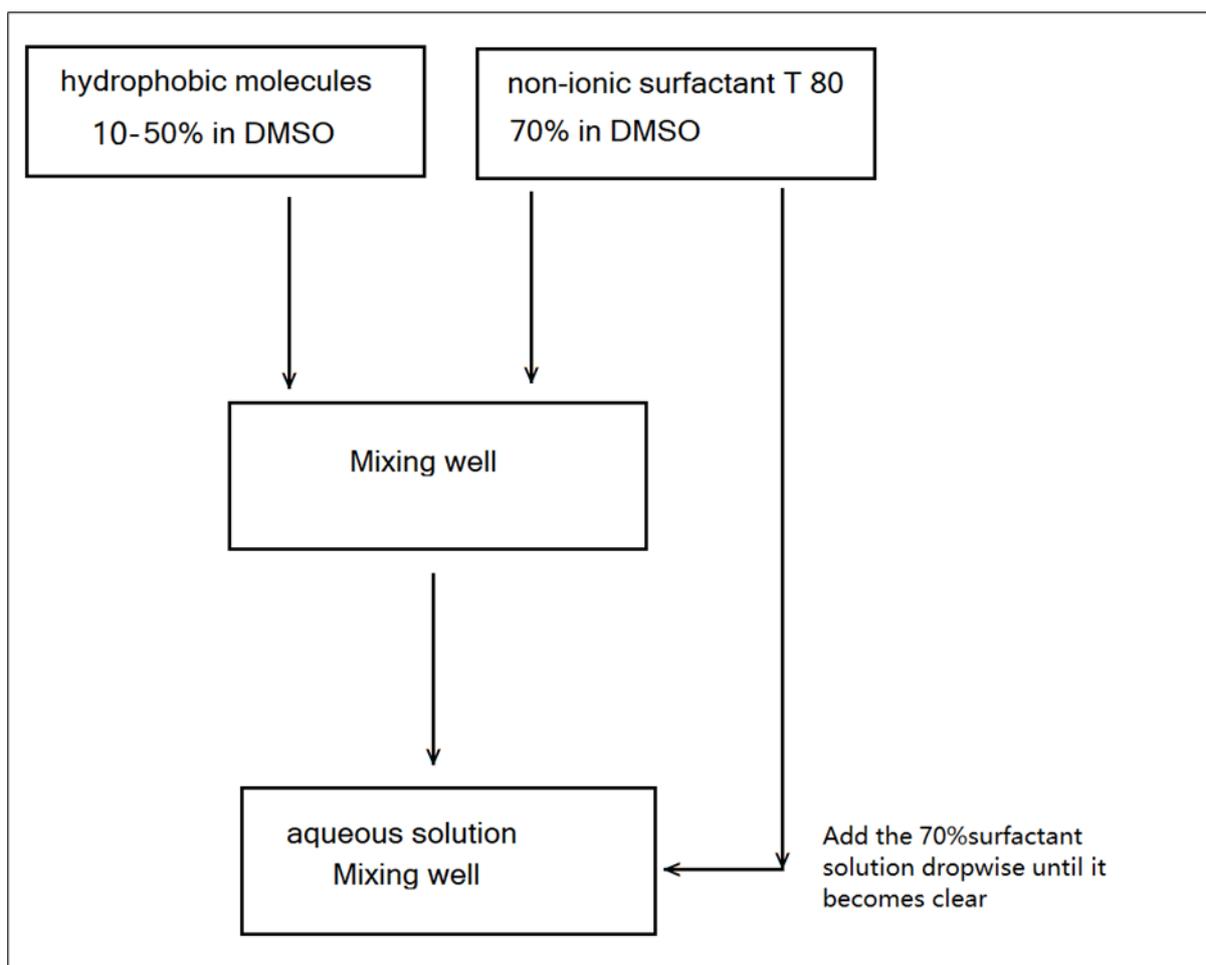


Figure 4. Diagram of dissolving hydrophobic molecules in aqueous solution

FAA=Fmoc Amino Acid, DMSO is water-miscible organic solution, 70% surf=70% Tween 80 in DMSO, Additional 70% surf is amount of 70% Tween needed to make the solution appears clear.

Here is the method for rendering Fmoc-AA soluble in a water solution: First, a non-ionic surfactant is dissolved in a small amount of water-miscible organic solvent, creating solution A. Secondly, Fmoc-AA is dissolved in a small amount of water-miscible organic solvent to create solution B. Solution A is thoroughly mixed with Solution B. The resulting mixture is then vigorously added to an aqueous solution, yielding an Fmoc-AA aqueous solution (Fig 4). To illustrate the procedure for dissolving Fmoc-AA in an aqueous solution, a simple example is provided here:

4 grams of Tween 80 are dissolved in 2 ml of Dimethyl sulfoxide (DMSO) to create solution A. Additionally, 1 gram of Fmoc-AA is dissolved in 5 ml of DMSO to create solution B. Solution A and solution B are thoroughly mixed, and then the resulting mixture is added to 88 ml of water to obtain a 1% concentration of Fmoc-AA in the aqueous solution.

To dissolve water-insoluble Fmoc-protected amino acids at room temperature, like Fmoc-Phenylalanine, it typically requires approximately eight times the amount of Tween 80 in an aqueous solution. Conversely, for water-soluble Fmoc-protected amino acids such as Fmoc-Glycine, it typically requires approximately four times the amount of Tween 80 in an aqueous solution.

The following steps outline the process by which two Fmoc-protected amino acids, Fmoc-Glycine (Fmoc-Gly), and Fmoc-Phenylalanine (Fmoc-Phe), achieve solubility in an aqueous solution.

Dissolving Fmoc-Gly in aqueous solution

1. Dissolve 1.08 grams of Fmoc-Gly in 6.13 ml of DMSO solvent. Next, add 6.00 milliliters (mL) of a 70% Tween 80 in DMSO solution to the Fmoc-Gly DMSO solution.
2. Pour this mixture into 87 mL of deionized (DI) water. Initially, the solution may appear slightly cloudy.
3. Gradually, add 70% Tween 80 dropwise until the solution becomes clear. After adding 0.01 mL of 70% T, the solution becomes clear.
4. This process yields a solution containing 1.07% Fmoc-Gly in an aqueous solution with 7.85% DMSO. The ratio of Tween 80 to Fmoc-Gly is approximately 4:1 (w/w), as depicted in table 1.

#### Dissolving Fmoc-Phe in aqueous solution

1. Dissolve 0.18 grams of Fmoc-Phe in 1.98 DMSO of solvent. Next, add 2.26 milliliters (mL) of a 70% Tween 80 in DMSO solution to Fmoc-Phe solution.
2. Transfer this mixture into 20.38 mL of deionized water. Initially, the solution appears cloudy.
3. Gradually, add 70% T dropwise until the solution becomes clear. An additional 0.11 mL of 70% Tween 80 is required for the solution becomes clear.
4. This process yields a solution containing 0.72% Fmoc-Phe in an aqueous solution with 10.80% DMSO. The ratio of Tween 80 to Fmoc-Phe is approximately 8:1 (w/w), as shown in Table 1.

Table 1. presents the solubility testing results for four Fmoc-protected amino acids: Fmoc-Gly, Fmoc-His, Fmoc-Thr, and Fmoc-Phe, as well as for Fmoc itself.

Sample	Sample weight (G)	Solvent DMSO mL	70% Tween 80 added	H2O added	Additional 70% Tween 80 added to clarity*	Total weight	Fmoc AA %	DMSO volume	Tween 80 containing %	DMSO contained %	Tween 80 to Fmoc-AA Ratio
Fmoc-Gly 1	1.08	6.13	6.05	8.08	0.01	101.26	1.07	7.94	4.23	7.85	3.9
Fmoc-Gly 2	0.59	3.64	1.36	40.9	2.06	48.55	1.22	4.66	2.39	9.61	4.1
Average	-	-	-	-	-	-	-	-	-	-	<b>4.0</b>
Fmoc-His 1	0.33	2.05	3.49	33.15	0.00	39.02	0.85	3.09	2.44	7.94	7.4
Fmoc-His 2	0.21	2.50	2.32	23.99	0.10	29.12	0.72	3.22	1.69	11.08	8.1
Average											<b>7.7</b>
Fmoc 1	0.58	3.18	3.03	41.95	2.86	51.6	1.12	4.94	4.12	9.59	7.1
Fmoc 2	0.70	3.47	3.67	50.28	2.61	60.73	1.15	5.35	4.39	8.82	6.3
Average	-	-	-	-	-	-	-	-	-	-	<b>6.7</b>
Fmoc-Thr 1	0.33	2.31	4.32	18.77	0	25.73	1.28	3.60	3.02	14.01	9.2
Fmoc-Thr 2	0.2	2.29	2.25	21.45	0.22	26.41	0.76	3.03	1.72	11.48	8.6
Average	-	-	-	-	-	-	-	-	-	-	<b>8.9</b>
Fmoc-Phe 1	0.18	1.98	2.26	20.38	0.11	24.91	0.72	2.69	1.65	10.80	9.2
Fmoc-Phe 2	0.17	2.12	1.71	22.86	0.2	27.06	0.63	2.69	1.33	9.95	7.9
Average	-	-	-	-	-	-	-	-	-	-	<b>8.5</b>

\* additional 70% Surf needed to reach the clarity solution

Table 1 presents test results of five different Fmoc amino acids' solubility. It indicates that for water-soluble amino acid Gly, the optimal ratio of Surfactant to Fmoc-AA is 4:1 (w/w) to achieve solubility in an aqueous solution at room temperature. It also indicates that for the non-soluble amino acid Phe, the required ratio of surfactant to Fmoc-AA is 8:1 (w/w) to achieve solubility in an aqueous solution.

While increasing the concentration of surfactants can enhance the Fmoc-AA concentration in an aqueous solution, higher concentrations may result in increased viscosity of the aqueous solution, potentially reducing coupling efficiency. In such cases, raising the temperature becomes necessary to mitigate solution viscosity and improve the efficiency of the coupling process. An increase in temperature can significantly enhance the solubility of Fmoc-AA in the water solution.

Testing results for the solubilities of four Fmoc-protected amino acids in aqueous solution: Fmoc-Gly, Fmoc-His, Fmoc-Thr, Fmoc-Phe, and Fmoc itself.

The solubility of the amino acid (AA) influences the solubility of the corresponding Fmoc-AA. Specifically, Fmoc-Gly is more soluble than Fmoc-Phe. Fmoc-Gly requires approximately four times the amount of surfactant to dissolve in water, while Fmoc-Phe requires about eight times the amount of surfactant to reach a solubility of 0.7%. Interestingly, Fmoc itself requires approximately six times the amount of surfactant to achieve a solubility of 1% in an aqueous solution at room temperature.

#### *Coupling and Deprotection and Cleaving from solid phase*

In our experiment, Fmoc deprotection, amino acid coupling, and peptide cleaving are all conducted in an aqueous solution. The addition of Fmoc-AA is the only step that necessitates a small amount of water-miscible organic solvent, which significantly reduces the overall consumption of organic solvents.

Deprotection of Fmoc in aqueous solution typically occurs under alkaline conditions with a pH range of 9.5-10.5 by using diluted NaOH or ethyl amine in 10% Tween 80, followed by a washing step in an approximately 10% tween 80, it is necessary to use surfactant aqueous solution since the removed Fmoc is not soluble in pure aqueous solution.

Coupling is facilitated by EDC within a pH range of 4-5. Initially, additional Fmoc-protected amino acid (FAA) dissolved in an aqueous solution is introduced, followed by the addition of a coupling agent such as EDC. The coupling reaction is conducted within the pH range of 4-5 for a duration of 20-30 minutes. It may be necessary to elevate the temperature for faster coupling, particularly when dealing with non-water-soluble amino acids. Additionally, the percentage of surfactant required may vary depending on the solubility of the amino acids. It's important to note that higher surfactant levels may increase viscosity, thus temperature adjustments may be necessary to reduce viscosity and enhance coupling efficiency.

#### *Procedure for Employing an anion exchange resin as the solid phase in Solid-Phase Peptide Synthesis*

The initial step involves binding the amino acid (AA) to an anion exchange resin through a linker molecule. A simpler approach to create the linker molecule is by utilizing a precursor molecule of Fmoc-AA-CH<sub>2</sub>-Ph-Rx-COOH to bind Taurine through the following reaction:



First, incubate a taurine water solution with an anion exchange resin to allow taurine to attach to the resin. This incubation typically occurs for several hours at room temperature to facilitate the attachment

of taurine to the anion exchange resin. Subsequently, the resin is washed with deionized (DI) water to prepare it for coupling with the precursor of the linker molecule.

Following the preparation of the taurine-attached anion exchange resin, the precursor molecule, dissolved in an aqueous solution, is introduced to the solution containing the taurine-attached anion exchange resin.

The precursor of linker molecule structure is:

Fmoc-AA-CH<sub>2</sub>-Ph-Rx-COOH,

After the attachment of taurine to the anion exchange resin, the initial amino acid in the linker molecule is Fmoc-protected. The next step involves removing the Fmoc protective group by exposing it to a solution with a pH range of 9.5-10.5 for 30-60 minutes in a 10% Tween 80 solution, followed by rinsing with DI water. At this point, the resin is ready for coupling with additional Fmoc-AA.

*Solid Phase Synthesis dipeptide His-Gly in aqueous solution with anionic exchange resin as solid phase*

1. 2-aminourinethanesulfonic acid (Taurine), 0.5 grams, is incubated with 2 grams of anionic exchange resin in 20 ml of H<sub>2</sub>O for one hour in an aqueous solution to ensure Taurine is attached to the anionic exchange resin. 1% Ninhydrin solution can be utilized to detect Taurine on the surface of the anionic exchange resin.
2. Wash the anionic exchange resin with DI water
3. Coupling taurine with the precursor molecule of the linker molecule is achieved using EDC under the condition of pH=4-5. This reaction involves the transformation of Fmoc-AA-CH<sub>2</sub>-Ph-Rx-COOH and NH<sub>2</sub>-R- SO<sub>3</sub><sup>-</sup> into Fmoc-AA-CH<sub>2</sub>-Ph-Rx-COO-NH-Rx- SO<sub>3</sub><sup>-</sup> (refer to Fig 3).
4. Wash the anionic exchange resin with a 10% Tween 80 water solution to remove any excess reagents.
5. Cap the unreacted amino group of taurine on the surface of the anionic resin by adding a small amount of acidic acid and additional EDC before introducing the next Fmoc amino acid. Subsequently, wash the resin with DI water.
6. The linker molecule Fmoc-AA-CH<sub>2</sub>-Ph-Rx-COO-NH-CH<sub>2</sub>-CH<sub>2</sub>- SO<sub>3</sub><sup>-</sup> is now attached to the anionic exchange resin.
7. To deprotect the Fmoc group, maintain a pH range of 9.5-10.5 using a diluted NaOH solution or a diluted monoethanolamine solution in 10% Tween 80, followed by sequential washing with 10% Tween 80 and DI water.
8. Wash the mixture with a 10% Tween 80 solution followed by rinsing with deionized (DI) water, respectively.
9. Following this, introduce 0.3 grams of an Fmoc-Histidine aqueous solution to the anionic exchange resin attached by linker molecule with Gly as the initial amino acid, ensuring continuous agitation through vibration and stirring.
10. Coupling Fmoc-Histidine with Gly in the linker A attached to the anionic exchange resin is achieved by using a coupling agent such as EDC at pH 4-5 and room temperature in a 10%

Tween 80 solution for 30 minutes.

11. Perform a thorough washing with a 10% Tween 80 solution followed by rinsing with deionized (DI) water.
12. To deprotect the Fmoc group, maintain a pH range of 9.5-10.5 using a diluted NaOH solution or a diluted monoethanolamine solution in 10% Tween 80, followed by sequential washing with 10% Tween 80 and DI water.
13. Now, we have a dipeptide His-Gly attached to the anionic exchange resin.
14. To release this dipeptide into an aqueous solution, cleave the dipeptide with diluted HCl about pH=1.
15. The anionic exchange resin can be regenerated through a standard regeneration process for ion exchange procedures.

### Conclusion

An efficient technology has been developed to facilitate the transition from the use of organic solvents to an aqueous system while maintaining current SPPS practices as much as possible. This conversion involves minimal changes, with no significant differences from the organic system, except for the peptide linker molecules and hydrophilic solid resins, which are commercially available. The goal of this approach is to make the transition more readily acceptable for the industry.

SPPS in organic solvents is currently the most widely used method in the industry, often requiring significant amounts of organic solvents. Our novel technology provides a practical scheme to facilitate the transition from traditional SPPS in organic solvents to an aqueous solution. Simultaneously, this novel technology preserves the established methodology of traditional SPPS as closely as possible, making SPPS in an aqueous solution easily acceptable to the peptide industry. This transition aims to reduce organic solvent consumption, thereby contributing to a greener environment.

Another advantage is to offer a peptide synthesis method mostly within an aqueous solvent, encompassing Fmoc deprotection, coupling, and cleavage processes, thereby eliminating the need for organic solvents in these stages. Consequently, this technology avoids the use of organic solvents in SPPS during the steps of deprotection, coupling, and cleavage.

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