# Solid-Phase Peptide Synthesis Mechanisms

## Overall Scheme:

Solid-Phase Peptide Synthesis (SPPS) occurs on a solid-support resin as shown in **Figure 1**. Amino acids with protecting groups are introduced sequentially from C-terminus to N-terminus. First, activation of the carboxylic acid of the amino acid occurs utilizing coupling reagents for reaction with a free amine on the resin. This is the first amino acid coupling. Note that the resin structure was simplified in **Figure 1** to just show the free amine for simplicity. For Rink amide resin, a deprotection step occurs before the first coupling. After the initial coupling, the base-labile fluorenylmethoxycarbonyl (Fmoc) group is removed with 20% piperidine in N,N-Dimethylformamide (DMF). This deprotection reaction leaves a free amine on the amino acid that is already attached to the resin. These coupling and deprotection steps are repeated for each amino acid in the desired peptide sequence. Once completed, the peptide is cleaved off resin utilizing acid to also remove acid-labile side-chain protecting groups from the amino acids.

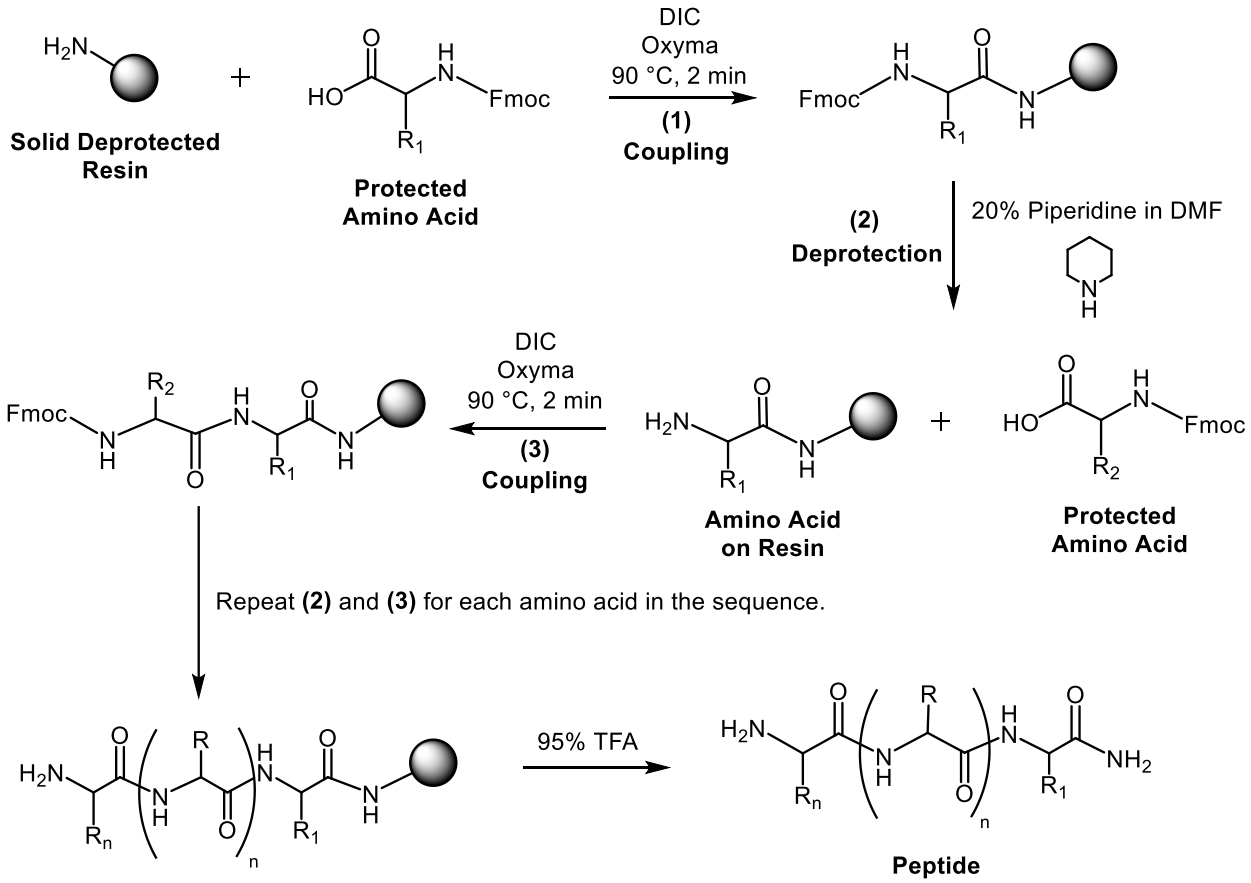


**Figure 1** – The overall reaction scheme for the solid-phase peptide synthesis (SPPS) workflow.[1–4]

## Scheme 1:

This scheme (**Figure 2**) depicts the activation of an amino acid carboxylic acid by diisopropylcarbodiimide (DIC). Oxyma then reacts to form a soluble urea side product, as well as create a good site for nucleophilic attack of the free amine associated with the resin. This allows for the coupling of the amino acid to the solid support resin. In this step, Oxyma is regenerated. Other coupling reagents can be utilized; however, the overall concept is similar.

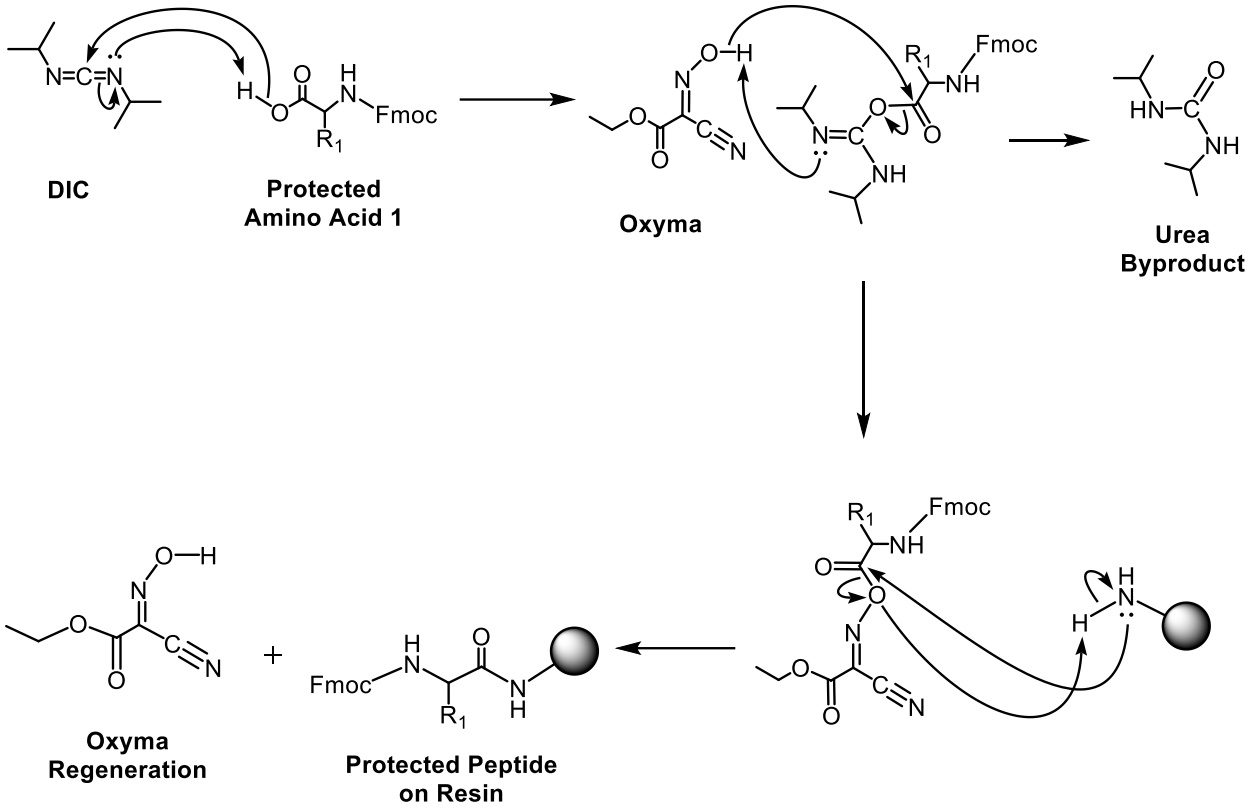


**Figure 2** – The reaction mechanism for the activation of the carboxylic acid of a protected amino acid by diisopropylcarbodiimide (DIC), which is subsequently reacted with Oxyma Pure to create a good site for nucleophilic attack and coupling of the amino acid to the free amine on the resin.[2,5]

## Scheme 2:

The electron withdrawing fluorene ring system of the Fmoc group renders the lone hydrogen on the beta-carbon very acidic and susceptible to removal by weak base. Therefore, during deprotection (**Figure 3**), beta elimination of benzofulvene occurs as piperidine deprotonates the Fmoc protecting group, which ultimately allows for the Fmoc group to leave the growing peptide. This leaves a free amine for another coupling. Typically, 20% piperidine in DMF is used for this deprotection. Additionally, the piperidine then scavenges the benzofulvene, leading to the formation of a benzofulvene-piperidine adduct in solution.

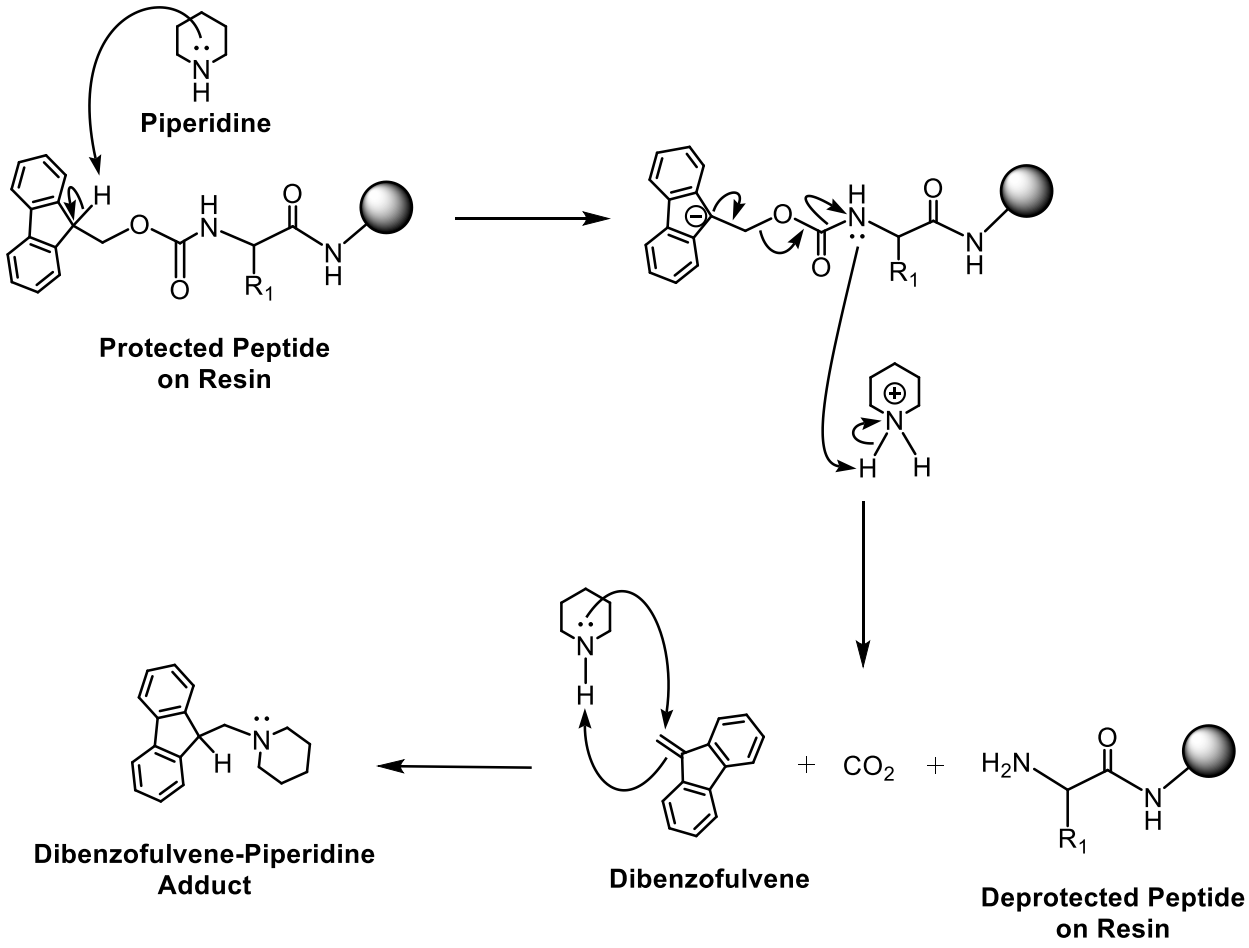


**Figure 3** – The reaction mechanism of fluorenylmethoxycarbonyl (Fmoc) deprotection and scavenging with piperidine.[2,3,5]

## Scheme 3:

When another amino acid is added, the same steps as earlier (**Figure 2**) for amino acid activation and coupling take place. This process is repeated in a cycle with deprotection steps to lead to peptide formation (**Figure 4**). Peptide on resin can then be modified in other reactions, or it can be cleaved using trifluoroacetic acid (TFA).

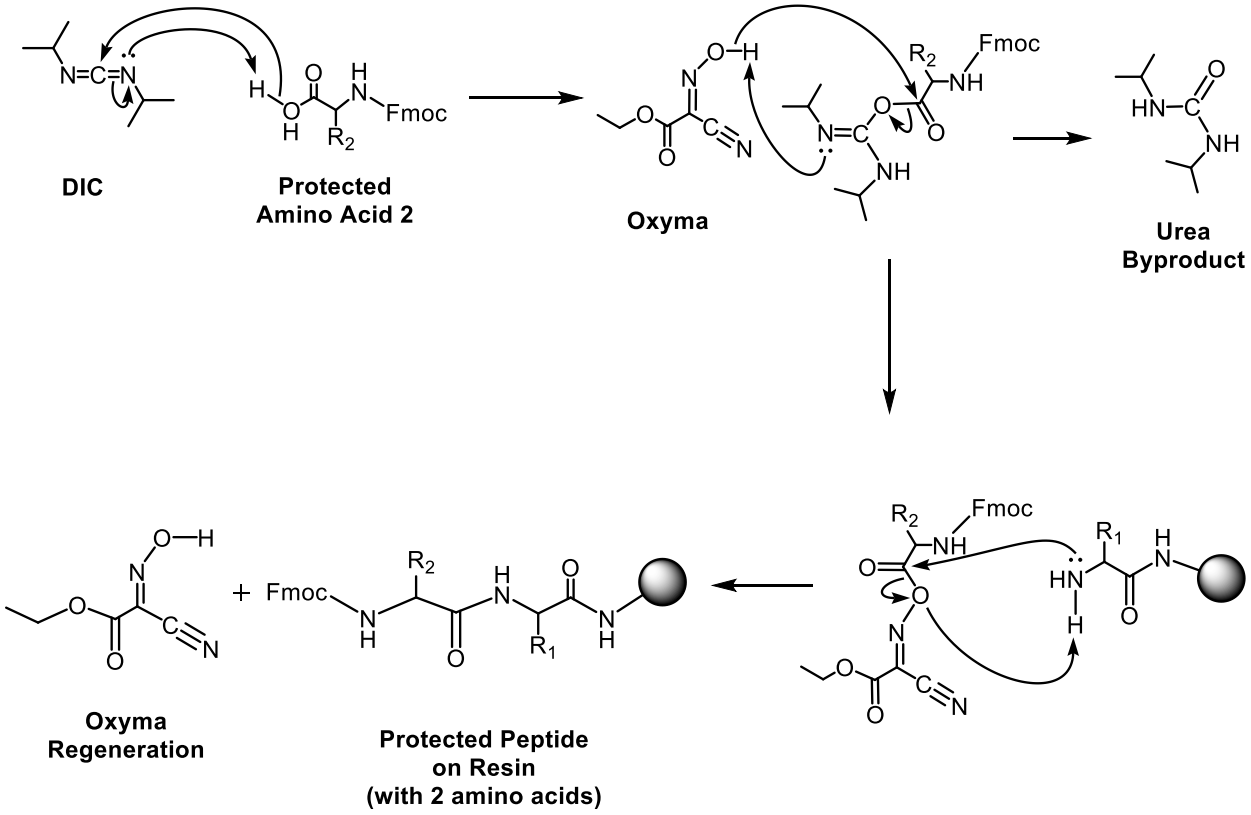


**Figure 4** – The reaction mechanism for the coupling of a second amino acid to resin. This reaction mechanism is the same as that used in **Figure 2**.[2,5]

## References:


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(2) Collins, J. M.; Porter, K. A.; Singh, S. K.; Vanier, G. S. High-Efficiency Solid Phase Peptide Synthesis (HE-SPPS). *Org. Lett.* **2014**, *16* (3), 940–943. https://doi.org/10.1021/ol4036825.

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