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Solid Phase Synthesis

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Overview

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Merrifield's solid-phase synthesis is a Nobel Prize winning invention where a reactant molecule is bound on a solid support and undergoes successive chemical reactions to form a desired compound. When the molecules are bound to a solid support, excess reagents and byproducts can be removed by washing away the impurities, while the target compound remains bound to the resin. Specifically, we will showcase an example of solid-phase peptide synthesis (SPPS) to demonstrate this concept.

Principles

Solid-phase synthesis is a method used to streamline the synthesis of molecules. It is often used in combinatorial chemistry(a technique used to prepare a large number of molecules in a short period of time), to generate libraries of compounds due to the ease of purification, and overall chemical synthesis. Solid-phase synthesis typically involves the use of a resin; a non-soluble, polymer-based material, which is pre-functionalized so the starting building blockcan easily bind. The building blocks are generally protected once they are added onto the resin, and they can be easily deprotected and treated with the next desired building block in solution (**Figure 1**). Once the desired molecule has been synthesized, it can easily be cleaved from the resin.

Because it is robust, solid-phase synthesis has been used to synthesize nucleic acids, oligosaccharides, and most commonly, peptides. Discovered and reported by Robert Bruce Merrifield in 1963, SPPS has become the most widely used method to generate libraries of peptides.Merrifield won the 1984 Nobel Prize for the invention of SPPS.SPPS can easily take advantage of Fmoc (base sensitive) or Boc (acid sensitive)*N*-protecting groups on the amino acids to build up libraries of peptides in a short amount of time. HBTU (coupling agent) and *i*-Pr_2EtN (base) activate the *C*-terminus of the amino acid for coupling with another amino acid. Fmoc protecting groups can be removed by 4-methylpiperidine, while Boc protecting groups can be removed by strong acids such as trifluoroacetic acid.In this experiment, we will demonstrate SPPS through the synthesis of a dipeptide. We will use the Kaiser test, a qualitative method to test for the presence of primary amines, to monitor the progress of the reaction.

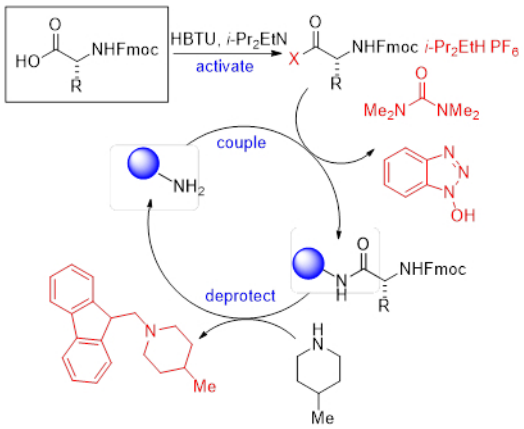

Figure 1. Concept behind the solid phase peptide synthesis (SPPS).

Procedure

1. Loading the Resin

1. To a 100mL peptide synthesis vessel, add 2-chlorotrityl chloride (CTC) resin (1.1 mmol/g, 0.360 g, 0.400 mmol). Add 20 mL DMF and allow them to swell for 30 min under N_2.
2. Drain the beads under vacuum and add 10 mL DMF.
3. Add 500 mg Fmoc-Ala-OH (1.60 mmol) and 2.5 mL *i*-Pr_2EtN, and mix under N_2 for 15 min.
4. Drain the solvent under vacuum and repeat the loading with Fmoc-Ala-OH for 15 min.
5. Drain the solvent under vacuum and wash the beads with 10 mL DMFunder N_2, and drain under vacuum 3x.

2. Deprotection of the Fmoc Group

1. Add 10 mL 20% 4-methylpiperidine in DMF and stir the beads under N_2 for 15 min.
2. Drain the solvent under vacuum and repeat the deprotection.
3. Wash the beads with 10 mL DMF under N_2 and drain under vacuum 3x.

3. Performing the Kaiser Test

1. Perform the Kaiser test by adding 1-2 drops of solution A (0.5 mL 0.01 M KCN, 24.5 mL pyridine), solution B (1 g ninhydrin, 20 mL *n*-butanol), and solution C (20 g phenol, 10 mL *n*-butanol) each into two test tubes. One test tube will be the control while the other will monitor the reaction.
2. Add a few beads of the resin from the reaction vessel to reaction test tube and heat up the two test tubes to 110 °C.
3. If the deprotection is complete, the contents of the test tube will turn a dark blue/purple color. If the deprotection is incomplete or failed, the solution will remain yellow. Compare the reaction test tube with the control test tube.

4. Coupling the Next Building Blocks

1. Drain the solvent under vacuum.
2. Wash the beads with 10 mL *N*-methyl-2-pyrrolidone under N_2 and drain the solvent under vacuum.
3. To begin the next coupling, add 10 mL NMP, 620 mgFmoc-Phe-OH (1.6 mmol), 610 mg HBTU (1.6 mmol), and 2.5 mL*i*-Pr_2EtN, and allow the resin to bubble under N_2 for 30 min.
4. Drain the solvent under vacuum.
5. Wash the beads with 10 mL DMF under N_2 and drain under vacuum 3x.
6. Perform the Kaiser test (see steps 3.1-3.3) to look for the completion of the coupling. The beads and solution in the test tube should be yellow.

5. Cleaving the Peptide Off the Resin

1. Cleave the remaining Fmoc group using steps 2.1-2.3.
2. After the solvent is drained under vacuum, add 40 mL cleavage solution (95% TFA, 2.5% H_2O, 2.5% TIPS) to the resin and bubble under N_2 for 3 h.
3. Place a new receiving flask on the peptide synthesizer and drain theTFA solution containing the desired peptide under vacuum into the new flask.

6. Precipitation and I solation of the Peptide

1. Separate the TFA solution into 4 conical vials and add 25 mL cold ether (−20 °C) to each vial to precipitate the peptide.
2. Centrifuge the vials (3,000 rpm, 0-4 °C) for 20 min. Decant the remaining TFA and ether solution from the conical vials and concentrate the peptide precipitate to afford the desired dipeptide as a white solid.

Results

Representative results for solid phase peptide synthesisfor Procedure 3.

Procedure Step	Color of solution
3.1	Control - Clear, light yellow Reaction – Clear, light yellow
3.2	Control - Clear, light yellow Reaction – Dark blue
3.3	Dark blue solution, beads blue – complete deprotection or coupling failed Colorless, beads yellow – deprotection failed or completing complete Colorless solution, beads red – incomplete coupling or incomplete deprotection

Table 1. Representative results for Procedure 3.

Applications and Summary

In this experiment, we have demonstrated an example of solid-phase synthesis via SPPS through the synthesis of a dipeptide.

Solid-phase synthesis is widely used in combinatorial chemistry to build up libraries of compounds for rapid screening. It has been commonly used to synthesize peptides, oligosaccharides, and nucleic acids. Moreover, this concept has been implemented in chemical synthesis. Because it is heterogeneous, these solid-supported reagents can often be recycled and reused in subsequent reactions.