AMINO ACIDS, PEPTIDES, AND PROTEINS

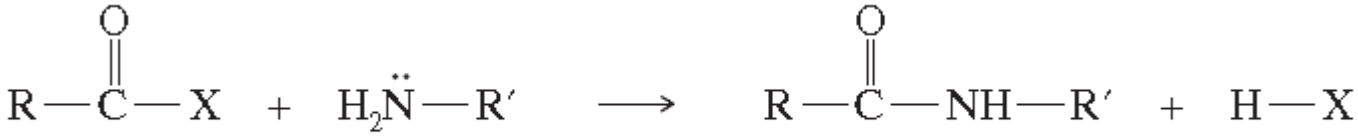
Dr. Tarek Bedair

Lecturer at Chemistry Department, Faculty of Science,
Minia University

Laboratory Peptide Synthesis

10. LABORATORY PEPTIDE SYNTHESIS

- Peptide synthesis requires the formation of amide bonds between the proper amino acids in the proper sequence. With simple acids and amines, we would form an amide bond simply by converting the acid to an activated derivative (such as an acyl halide or anhydride) and adding the amine.

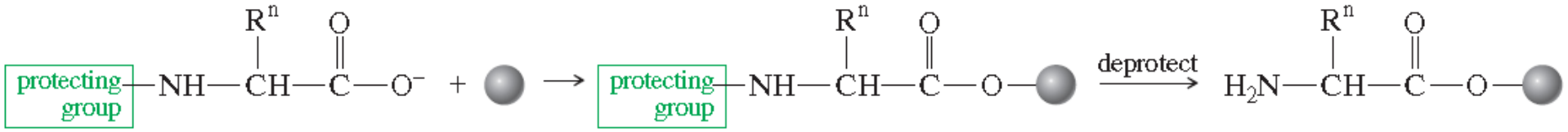


(X is a good leaving group, preferably electron-withdrawing)

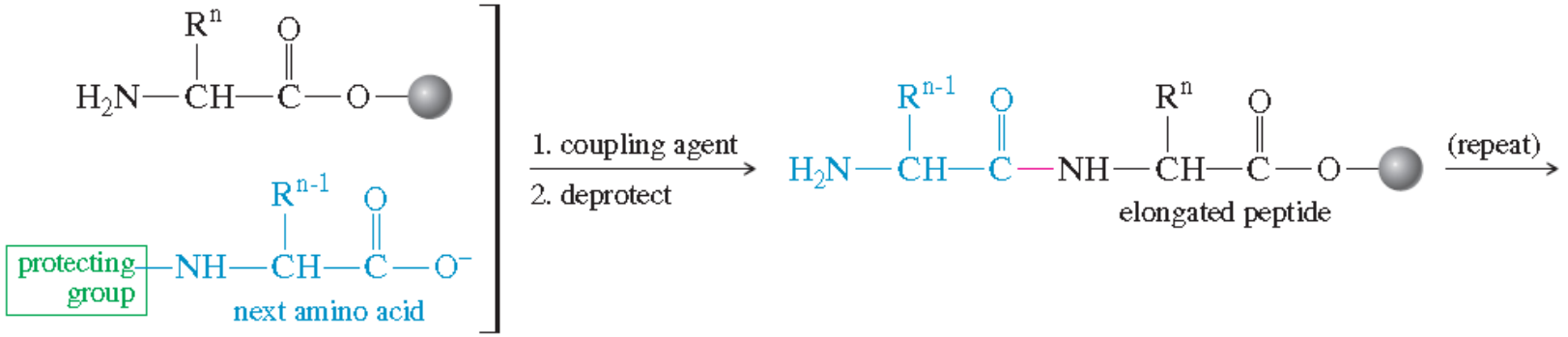
- Amide formation is not so easy with amino acids, however. Each amino acid has both an amino group and a carboxyl group. If we activate the carboxyl group, it reacts with its own amino group. If we mix some amino acids and add a reagent to make them couple, they form every conceivable sequence. Also, some amino acids have side chains that might interfere with peptide formation. For example, glutamic acid has an extra carboxyl group, and lysine has an extra amino group. As a result, peptide synthesis always involves both activating reagents to form the desired peptide bonds and protecting groups to block the formation of undesired bonds.
- These *solution-phase methods* involved adding reagents to solutions of growing peptide chains and purifying the products as needed. The chemists would apply protecting groups, add activating groups, couple an amino acid to the growing peptide, and then laboriously purify the product.

- Merrifield's method (*solid-phase method*) synthesizes the peptide starting from the C terminus and working toward the N terminus, which is the opposite of the way we usually draw peptides. First, the protected C-terminal amino acid is attached to the polymer bead. For this reaction, its ¬NH2 group must be protected so that it does not react. The protecting group is removed, and then the next protected amino acid is coupled to the first. Many more deprotection and coupling reactions occur until the entire peptide is formed. At that point, it is cleaved from the polymer bead. Here is a summary of the process:

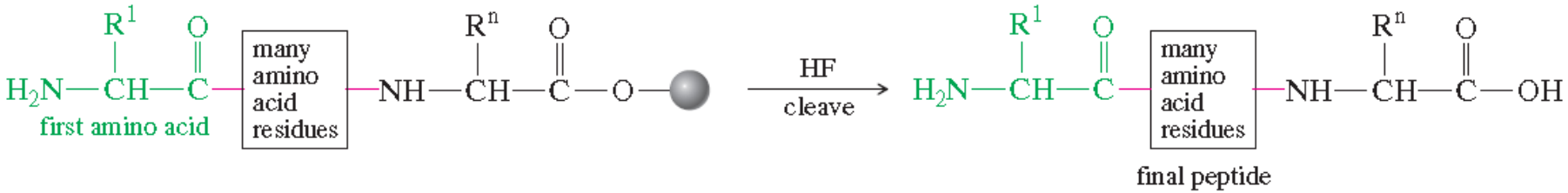
1. *Attach the protected C-terminal amino acid to the bead, and then deprotect.*



2. *Couple the next protected amino acid, and then deprotect.* (Repeat many times.)



3. *After adding all the residues, cleave the finished peptide from the bead.*

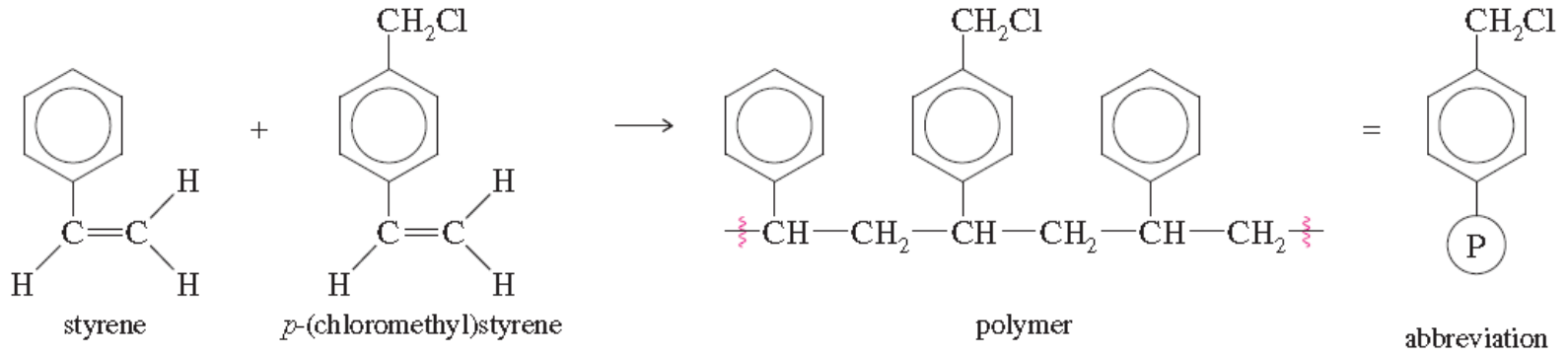


10A. The Individual Reactions

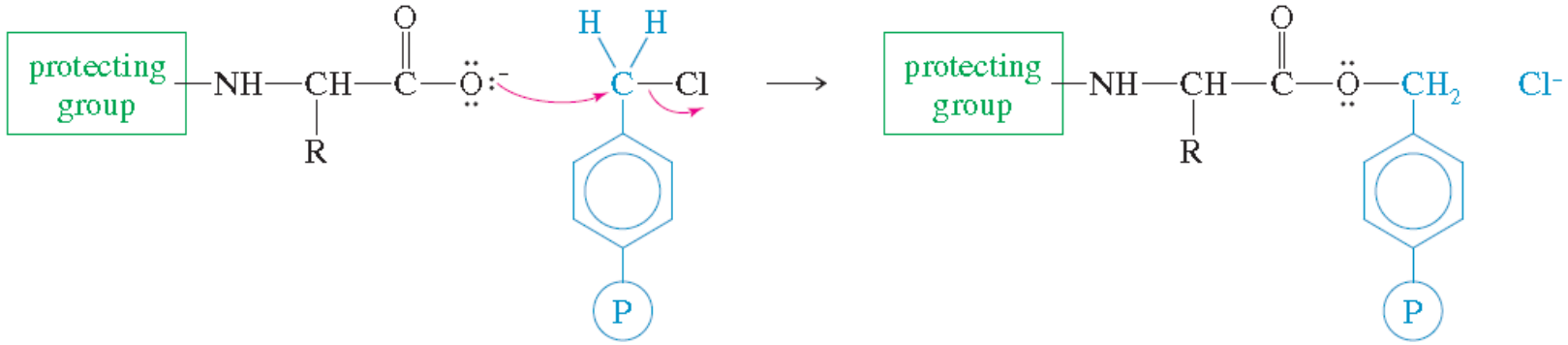
- **Attaching the Peptide to the Solid Support**

The solid-phase synthesis is done in the reverse direction, right-to-left as we draw the peptide. It starts with the C terminus and builds toward the N terminus. The first step is to attach the last amino acid (the C terminus) to the solid support.

Formation of the Merrifield resin

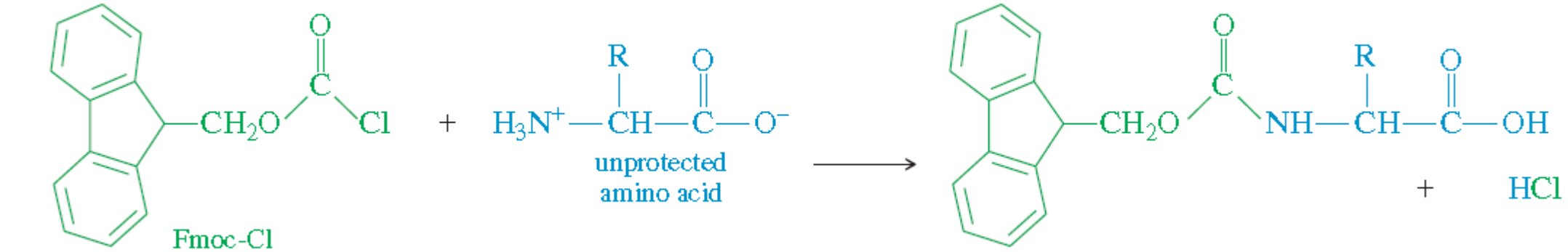


Attachment of the C-terminal amino acid



- **Using the 9-Fluorenylmethoxycarbonyl (Fmoc) Protecting Group**

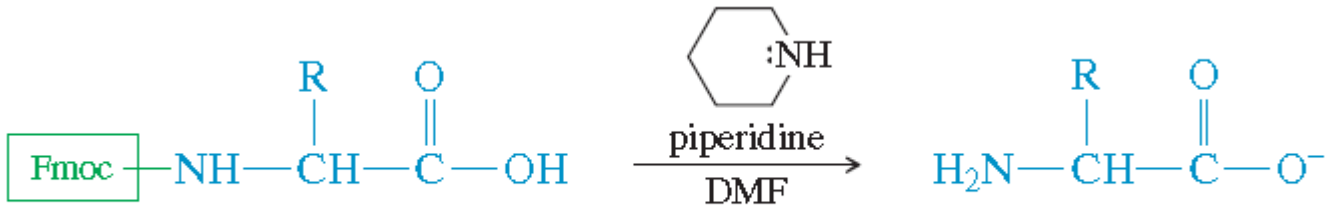
The most common protecting group for the ¬NH_2 groups of amino acids and peptides is currently the Fmoc group. Its structure is shown below, with the structure of a protected amino acid. We will generally refer to it by its abbreviation, Fmoc.



Fmoc-Cl
(9-fluorenylmethoxycarbonyl chloride)

protected Fmoc-amino acid

The protected amino acid no longer has a nucleophilic ¬NH_2 group; that group is now an amide, part of a urethane (carbamate ester). The Fmoc protecting group is easily removed by very mildly basic conditions. The most common reagent for its removal is a solution of piperidine in DMF (N,N-dimethylformamide).

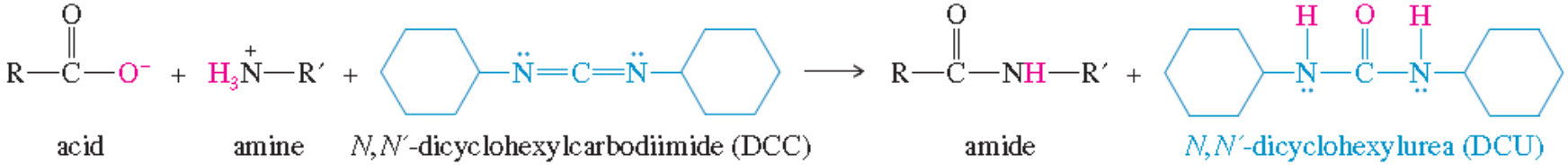


protected Fmoc-amino acid

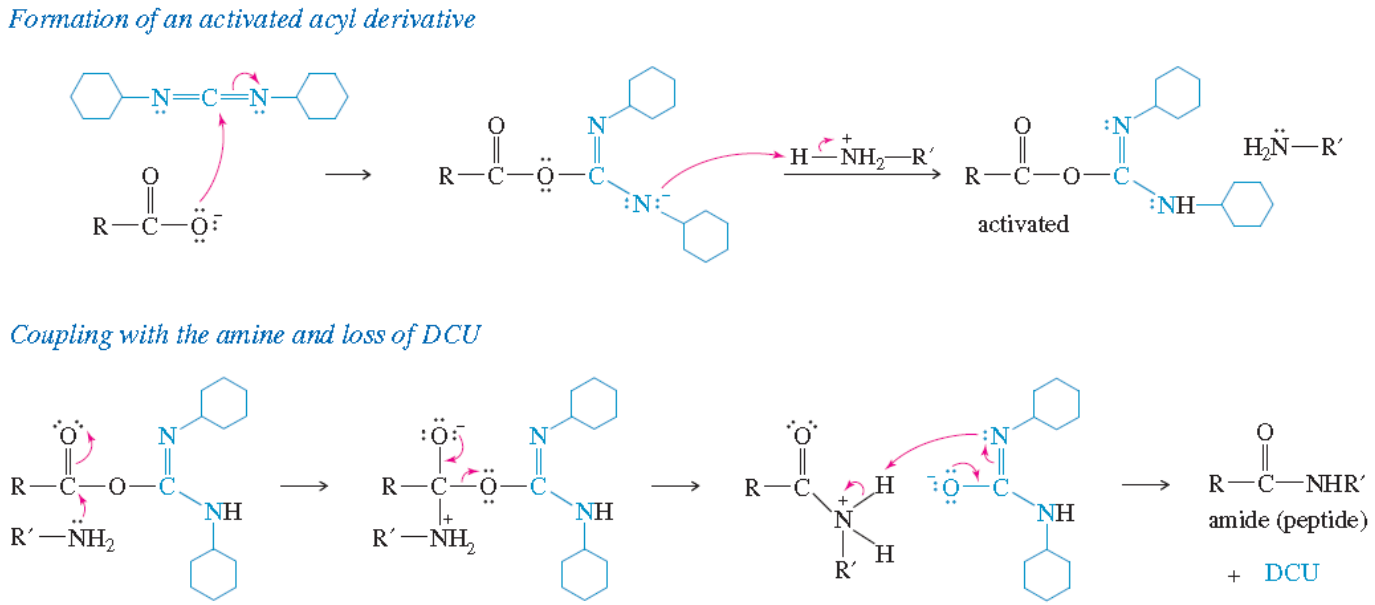
deprotected amino acid

• Use of DCC as a Peptide Coupling Agent

- The final reaction needed for the Merrifield procedure is the peptide bond-forming condensation. When a mixture of an amine and an acid is treated with N,N′@dicyclohexylcarbodiimide (abbreviated DCC), the amine and the acid couple to form an amide. The molecule of water lost in this condensation converts DCC to N,N′-dicyclohexylurea (DCU).

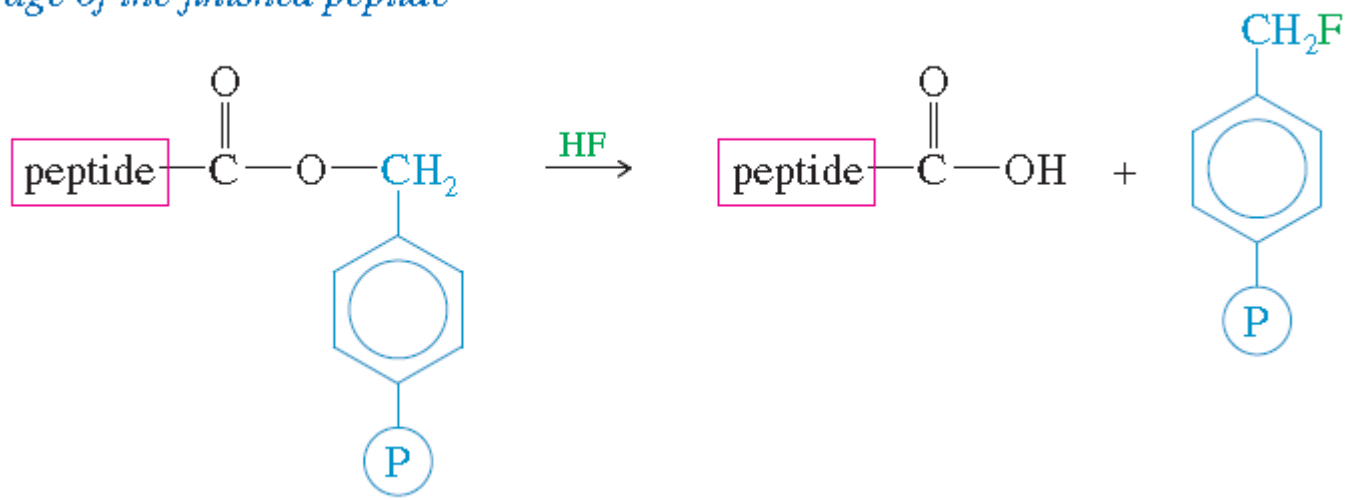


- The mechanism of the reaction: The carboxylate ion adds to the strongly electrophilic carbon of the diimide, giving an activated acyl derivative of the acid. This activated derivative reacts readily with the amine to give the amide. In the final step, DCU serves as an excellent leaving group. The cyclohexane rings are miniaturized for clarity.



- At the completion of the synthesis, the ester bond to the polymer is cleaved by anhydrous HF. Because this is an ester bond, it is more easily cleaved than the amide bonds of the peptide.

Cleavage of the finished peptide



PROBLEM 24-24

Propose a mechanism for the coupling of acetic acid and aniline using DCC as a coupling agent.

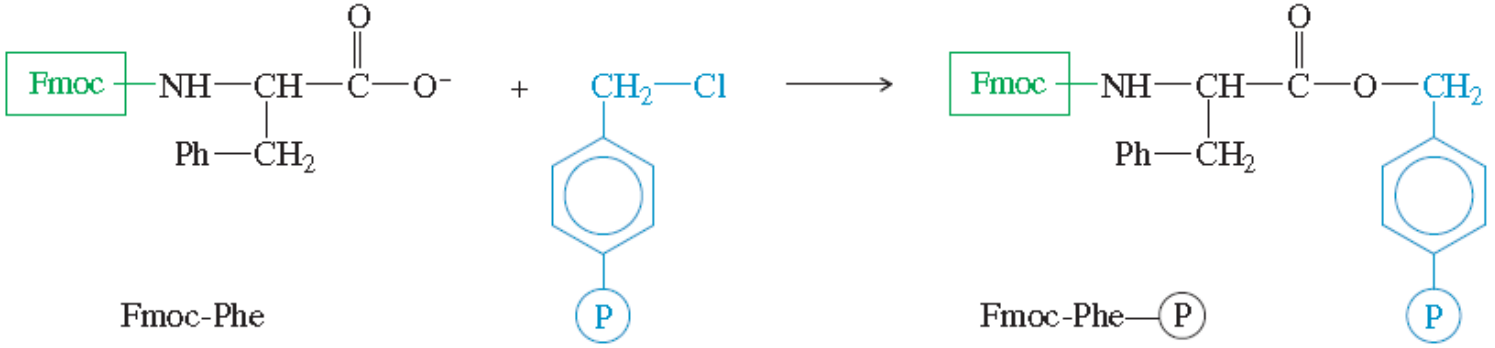
PROBLEM-SOLVING HINT

Remember that solid-phase peptide synthesis goes C → N.

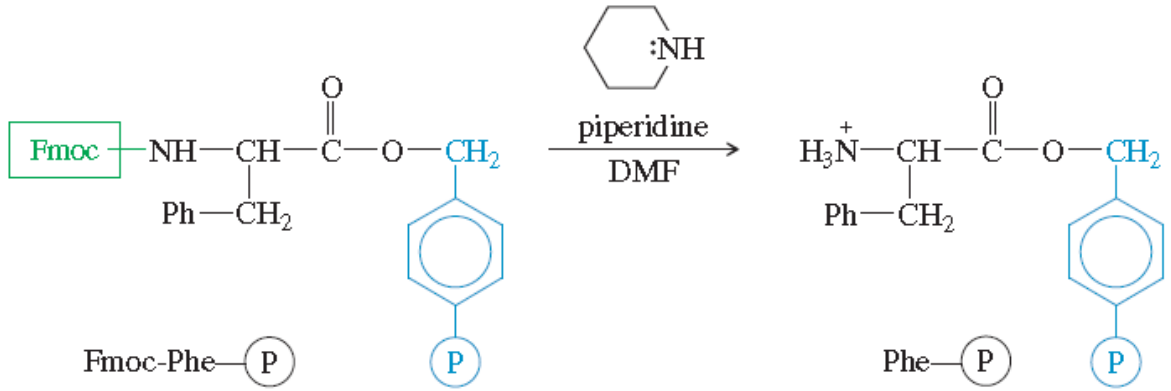
1. Attach the Fmoc-protected C terminus to the bead first.
2. Couple each amino acid by removing the Fmoc group from the N terminus, and then add the next Fmoc-protected amino acid with DCC.
3. Cleave (HF) the finished peptide from the bead.

10B. An Example of Solid-Phase Peptide Synthesis

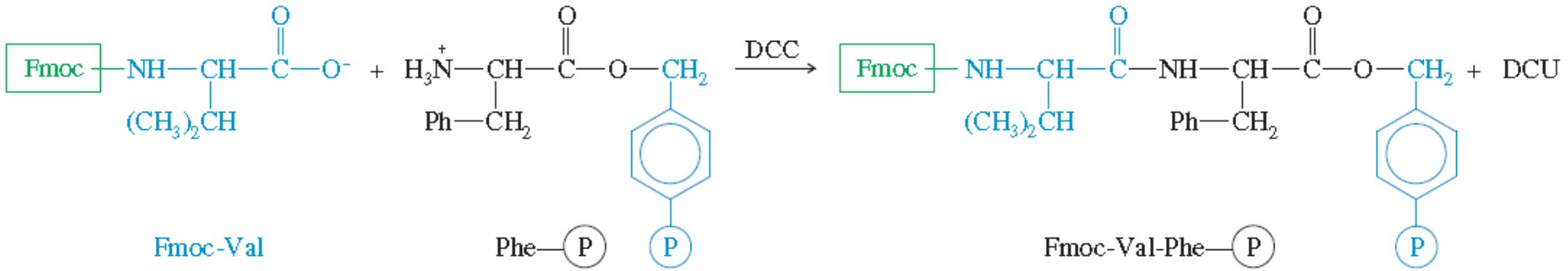
- The solid-phase synthesis proceeds in the opposite direction from the way we write the peptide structure. The first step is attachment of the N-protected C-terminal amino acid (Fmoc-phenylalanine) to the polymer.



- Piperidine cleaves the Fmoc protecting group of phenylalanine so that its amino group can be coupled with the next amino acid.

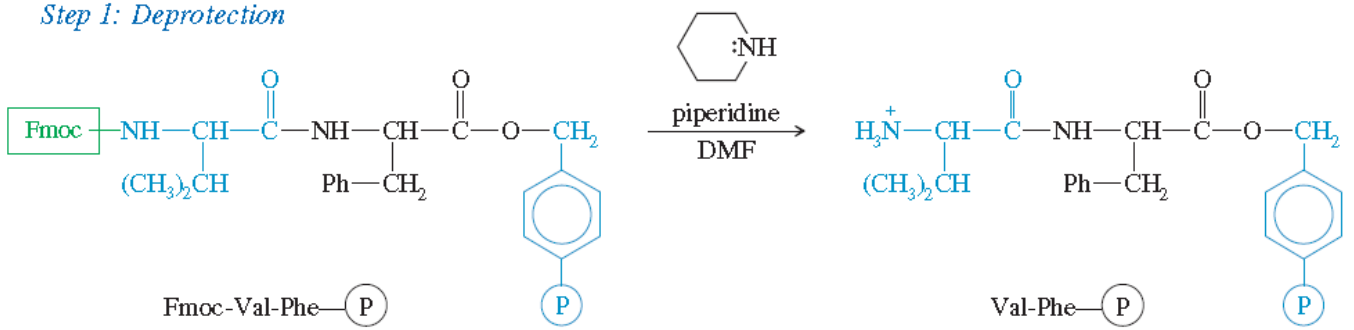


- The second amino acid (valine) is added in its N-protected Fmoc form so that it cannot couple with itself. Addition of DCC couples the valine carboxyl group with the free ¬NH_2 group of phenylalanine.

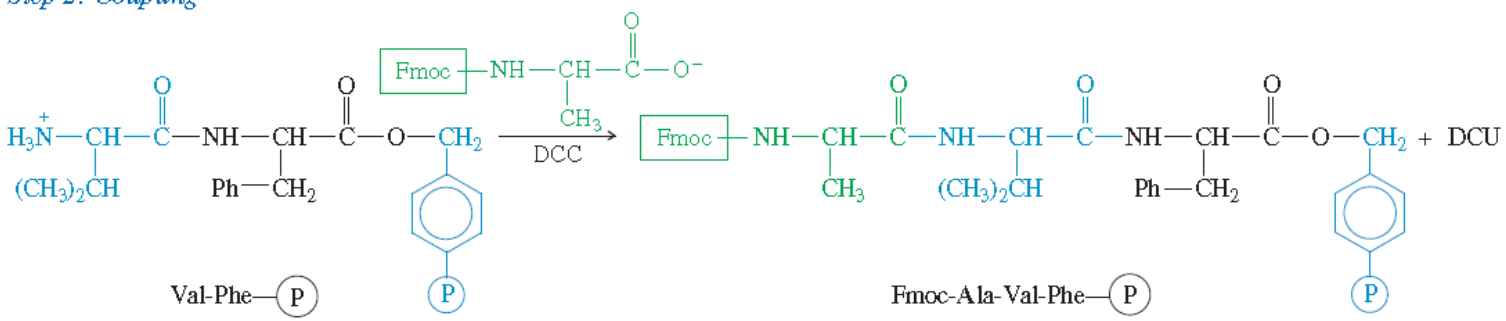


- To couple the final amino acid (alanine), the chain is first deprotected by treatment with piperidine. Then the N-protected Fmoc-alanine and DCC are added.

Step 1: Deprotection



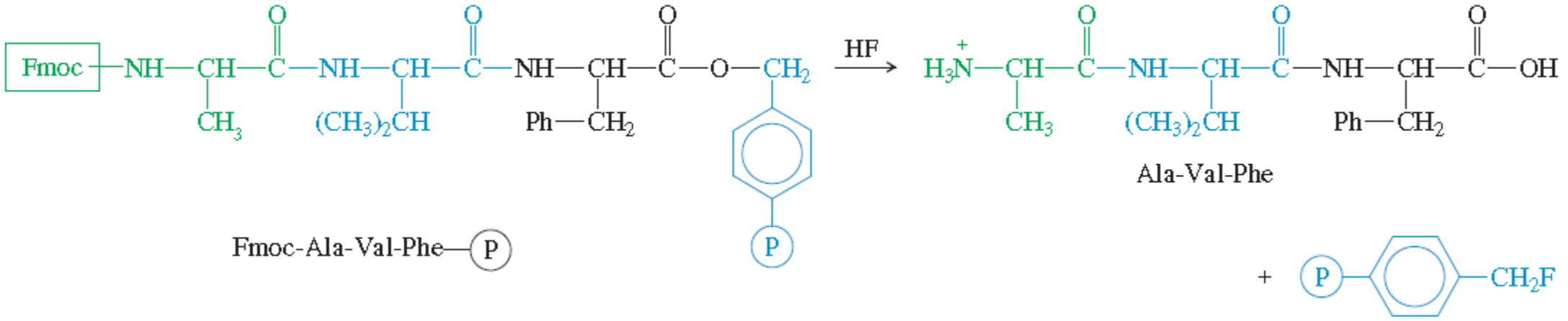
Step 2: Coupling



- If we were making a longer peptide, the addition of each subsequent amino acid would require the repetition of two steps:

1. Use piperidine in DMF to deprotect the amino group at the end of the growing chain.
2. Add the next Fmoc–amino acid, using DCC as a coupling agent

- Once the peptide is completed, the final Fmoc protecting group must be removed, and the peptide must be cleaved from the polymer. Anhydrous HF cleaves the ester linkage that bonds the peptide to the polymer, and it also removes the Fmoc protecting group. In our example, the following reaction occurs:



PROBLEM 24-25

Show how you would synthesize Leu-Gly-Ala-Val-Phe starting with Fmoc-Ala-Val-Phe—(P).

PROBLEM 24-26

Show how solid-phase peptide synthesis would be used to make Ile-Gly-Asn.