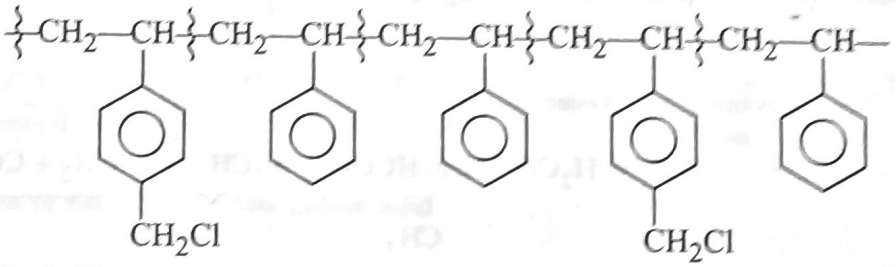
Further, the new peptide made in each cycle must be isolated and thoroughly purified. This requires enormous time even for a small peptide containing 8-9 amino acids. Therefore, the process is very tedious and time consuming.

(*ii*) **Low yield.** Since the process involves number of steps, the overall yield goes on decreasing after each extension even though each individual step has an excellent yield of 80%. Thus, if the dipeptide is formed in 80% yield, the yield of the tripeptide will be 80% of 80 = 64% and that of the tetrapeptide would be 80% of 64 = (80/100) × 64 = 51·2%. If a decapeptide is to be synthesized, the overall yield would be 13·4% and yield of still higher peptides would be still lower.

It is, therefore, evident that the classical method cannot be used for the synthesis of higher peptides in reasonable yields.

3.15. MERRIFIELD SOLID–PHASE PEPTIDE SYNTHESIS

The difficulties encountered in the classical peptide synthesis were overcome by R.B. Merrifield (Rockefeller University, USA) who in 1964 devised a brilliant method for peptide synthesis called the *solid phase peptide synthesis*. In this method, the peptide synthesis is carried out on the surface of an insoluble **solid support.** The solid support consists of a polystyrene polymer cross-linked with about 2% *p*-divinylbenzene. Further, about 5% of the benzene rings of this polymer carry chloromethyl (—CH_2Cl) substituents.

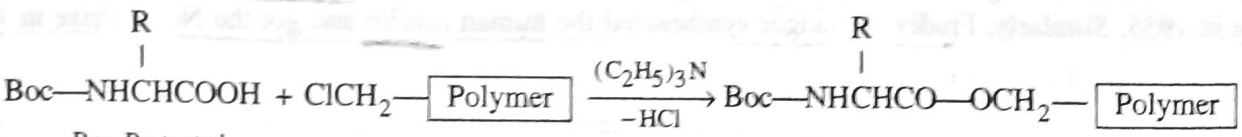


Chloromethylated polystyrene polymer

This chloromethylated polystyrene polymer is porous in nature and has gel like structure. It is used in form of small beads through which the various reagents and solvents used can easily percolate.

Let us now illustrate the main points of this synthesis by considering a general example. In this general example, the chloromethylated polystyrene polymer is shown as Polymer—CH_2Cl.

Step 1. The amino group of the C-terminal amino acid of the desired peptide is first protected by Boc (*tert*-butoxycarbonyl chloride) or any other suitable protecting group. The Boc-protected amino acid is then attached to the polystyrene polymer by heating it in presence of triethylamine in a suitable solvent when a benzyl ester linkage is formed between the COOH of the amino acid and CH_2Cl of the polymer as shown below :

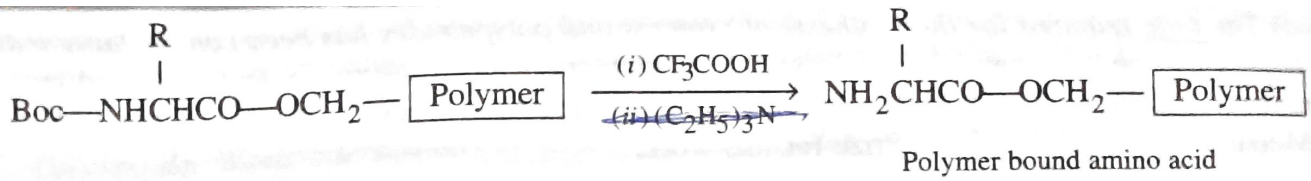


Boc-Protected amino acid

Ester linkage with the polymer

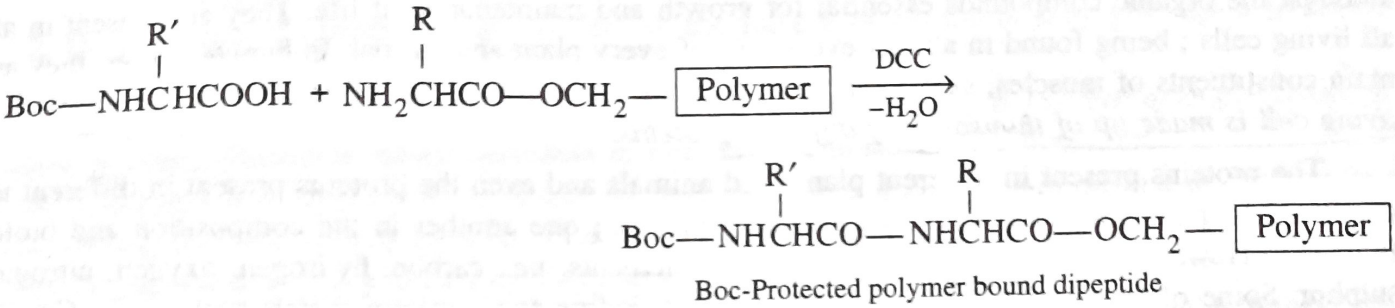
After the ester linkage has been formed, the excess reagents are removed by washing with suitable solvents.

Step 2. The Boc protecting group is then removed by treatment with trifluoroacetic acid (CF_3COOH). The salt of the polymer bound amino acid thus formed is converted into the free acid by treatment with a solution of excess of triethylamine and purified by washing.



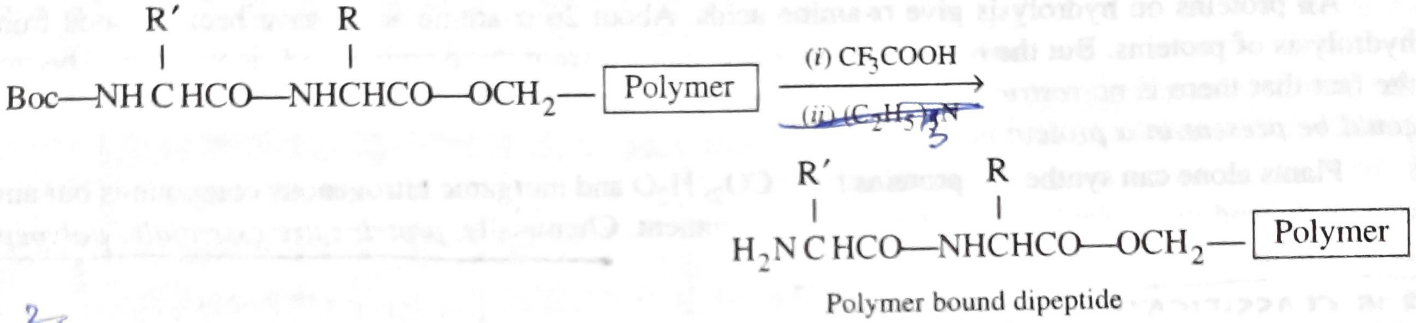
Polymer bound amino acid

Step 3. A second Boc-protected amino acid is then coupled with the above polymer bound amino acid by means of dicyclohexylcarbodiimide (DCC). The excess reagents and by-products (such as DCU) are washed away



Boc-Protected polymer bound dipeptide

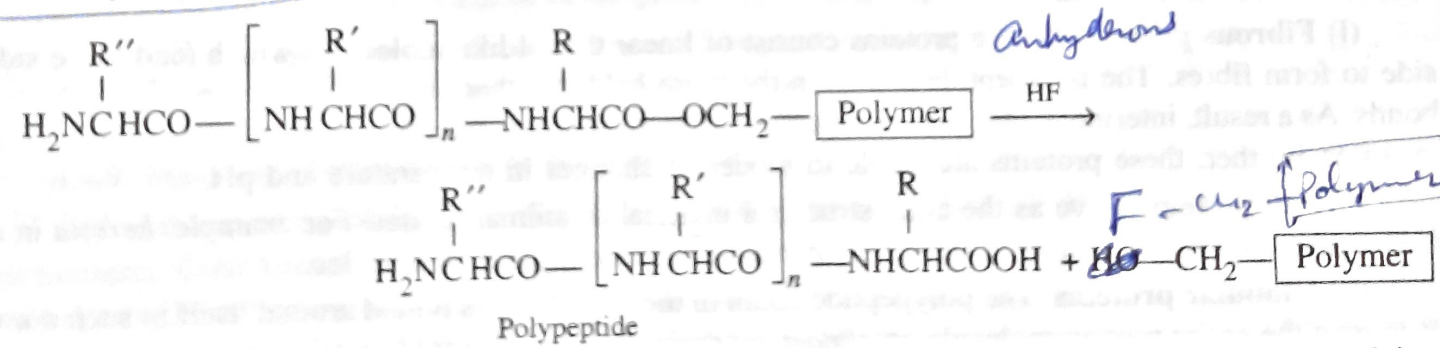
Step 4. The Boc-protecting group is removed as in **step 2.**



Polymer bound dipeptide

Step **3** and **4** are repeated to add another amino acid. In this way, dozens or even 100 of amino acids can be linked to one another in a specific order to synthesize the desired polymer bound peptide. The last acid to be attached is the N-terminal amino acid.

Step 5. At the end of the synthesis, the polypeptide is removed from the polymer by treatment with anhydrous hydrogen fluoride.



Polypeptide

This solid phase technique has now been automated, *i.e.*, computer controlled **peptide synthesizing machines** are now available in which coupling and removal of protecting groups with different amino acids is automatically carried out as many times as desired. Some advantages of this technique are :

(*i*) *Yields are extremely high.*

(*ii*) *Purification of the products after each coupling is not necessary since the insoluble polymer bound amino acid or the peptide is thoroughly washed with suitable solvents to remove the excess of the reagents.*

(*iii*) *The time required for the synthesis of proteins and polypeptides has been considerably reduced.* For example, bovine pancreatic ribonuclease, an enzyme containing 124 amino acid units and involving 369 chemical reactions and 11931 steps was synthesized in just six weeks in an overall yield of 17%. In 1984, Merrified received the Nobel Prize for this work.

PROTEINS

Proteins (Greek, proteois meaning *first*) are extremely complex natural polymers and are rated first amongst the organic compounds essential for growth and maintenance of life. They are present in almost all living cells ; being found in almost every part of every plant and animal. In human beings, they are the main constituents of muscles, skin, hair, nails, tendons, arteries and connective tissues. In a nutshell, *each living cell is made up of thousands of different proteins.*

The proteins present in different plants and animals and even the proteins present in different tissues of a particular part of plant or animal are different from one another in the composition and biological functions. However, all proteins contain the same elements, *i.e.*, carbon, hydrogen, oxygen, nitrogen and sulphur. Some of these may also contain phosphorus, iodine and traces of metals such as Fe, Cu, Zn and Mn.

All proteins on hydrolysis give α-amino acids. About 26 α-amino acids have been isolated from the hydrolysis of proteins. But the number of proteins obtained from these amino acids is very large because of the fact that there is no *restriction on the number, types and specific sequence in which the α-amino acids could be present in a protein molecule.*

Plants alone can synthesize proteins from CO_2, H_2O and inorganic nitrogenous compounds but animals