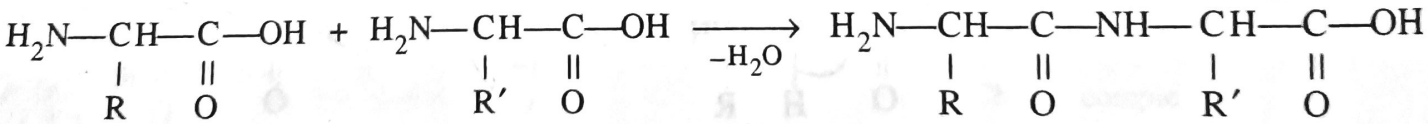
Peptides are amides formed by condensation of amino group of one α-amino acid with the carboxyl group of the other amino acid with the elimination of a molecule of water. For example,



The amide linkage  is called the **peptide bond or the peptide linkage.**

3.14. SYNTHESIS OF PEPTIDES

One of the most remarkable achievements in the field of protein chemistry is the synthesis of some of the naturally occurring polypeptides in the laboratory in recent times. Theoretically, it appears very simple to synthesize polypeptides by stepwise condensation in which amino group of one amino acid is condensed with the free carboxyl group of the other, until after several operations, the desired polypeptide chain is built up. But in actual practice, it is not so simple as it appears because of certain problems. We shall now briefly discuss what were these problems and how were these problems overcome in the earlier methods called **classical synthesis** or the **solution phase synthesis** of peptides. This will be followed up by description of modern methods developed for the synthesis of peptides.

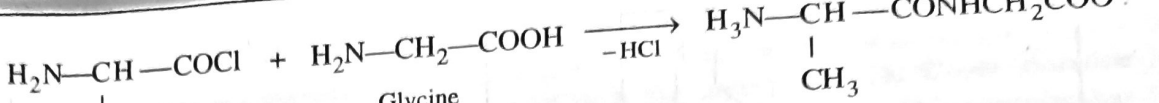
3.14.1. Classical Peptide Synthesis

Normally, when two different amino acids are allowed to react in presence of a dehydrating agent, besides the desired reaction between – NH_2 group of one amino acid and – COOH group of the other, there are equal chances that the reaction may also occur between the – COOH and – NH_2 groups of two molecules of the same amino acid, thereby giving a mixture of dipeptides. For example, when glycine and alanine are heated together in presence of a dehydrating agent, a mixture of four different dipeptides is formed.

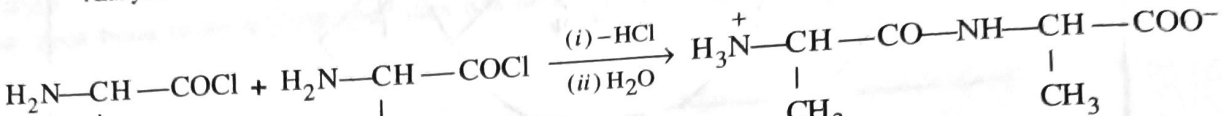
$$\text{Glycine + Alanine} \xrightarrow{-H_2O} \text{Gly-Gly + Ala-Ala + Gly-Ala + Ala-Gly}$$

Further, the condensation reaction between the two amino acid molecules to form a peptide bond is an *endothermic reaction* and hence does not take place easily. To overcome the low reactivity of the COOH group towards nucleophilic substitution reaction by the NH_2 group of the other amino acid, *the carboxyl group of one amino acid molecule is* **activated** by converting it into its acid chloride and then allowing it to react with the second amino acid. The problem that still remains is that the activated acid chloride can also react with another molecule of its own as well as the other amino acid. To understand this problem, let us consider the synthesis of alanylglycine (Ala-Gly). To do so, the carboxyl group of alanine is first activated by converting it into its acid chloride and is then allowed to react with glycine. In principle, alanyl chloride can react not only with the amino group of glycine to give the dipeptide, Ala-Gly but can also react with the amino group of another molecule of alanyl chloride to give the dipeptide, Ala-Ala. As a result, the yield of the desired dipeptide (Ala-Gly) will decrease. Furthermore, it will have to be separated from the other dipeptide Ala-Ala which itself is quite difficult.

(Contd)



Alanyl chloride — Glycine — Ala-Gly



Alanyl chloride — Alanyl chloride — Ala-Ala

To overcome this problem, *the amino group of the amino acid is first* **protected** *before its carboxyl group is* **activated** by converting it into its acid chloride.

The main points of peptide synthesis are discussed below :

3.14.2. Protecting groups used in peptide synthesis

A protecting group is a group which otherwise makes a reactive group unreactive and thus prevents the molecule from undergoing an unwanted reaction.

There are many methods available for the protection of both the amino and the carboxyl groups but any protecting agent used must fulfil the following criteria :

(*i*) *It should be relatively easy to introduce.*

(*ii*) *It should be stable under the given reaction conditions.*

(*iii*) *It should be easily removable under mild conditions leaving the newly formed peptide bond intact.*

1. Amino acid protecting groups. These groups convert the $—NH_2$ group into some other group of low nucleophilicity which would not react with the acid chloride to form the peptide bond. The usual method of protecting the amino group by acetylation or benzoylation cannot be used here. The reason being that the attempts to remove these protecting (acetyl or benzoyl) group from the acylated peptides will cleave the peptide bonds as well.

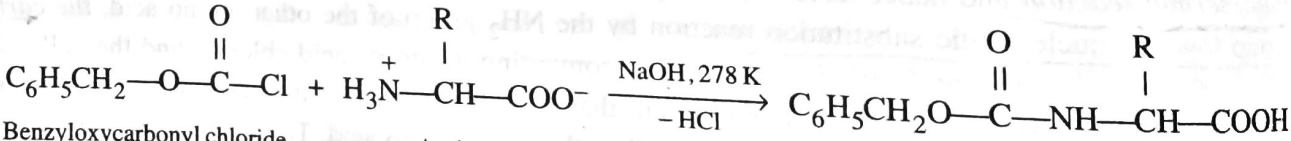
The three most widely used groups to protect the amino group are :—

(*i*) Benzyloxycarbonyl or carbobenzyloxy group $C_6H_5CH_2—O—CO—$.

(*ii*) *tert*-Butoxycarbonyl group, $(CH_3)_3C—O—CO—$.

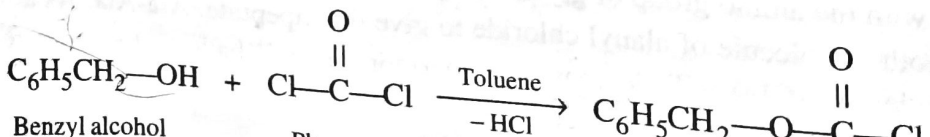
(*iii*) Phthaloyl group,

(*i*) **Benzyloxycarbonyl group** is given the symbol **Z** according to IUPAC system. It can be introduced by reacting the amino acid with benzyloxycarbonyl chloride (earlier known as carbobenzyloxy chloride) in presence of NaOH at 278 K.



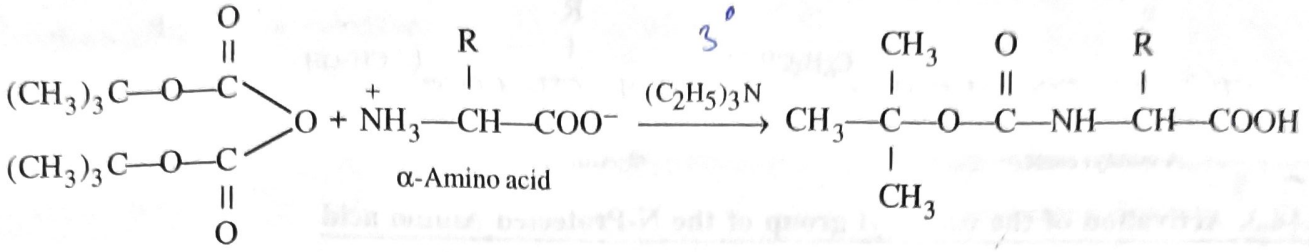
Benzyloxycarbonyl chloride — α-Amino acid — Benzyloxycarbonylamino acid or Z-Amino acid

The reagent benzyloxycarbonyl chloride itself can be prepared by passing carbonyl chloride (phosgene) through a solution of benzyl alcohol in toluene.



Benzyl alcohol — Phosgene — Benzyloxycarbonyl chloride or Benzylchloroformate

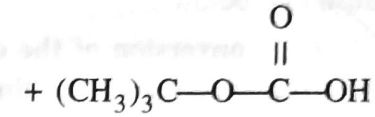
(*ii*) **tert-Butoxycarbonyl, $[(CH_3)_3C—O—CO—]$ group** is generally abbreviated as **Boc.** It is introduced by treating the amino acid with di-*tert*-butyl dicarbonate in presence of triethylamine. For example,



Di-*tert*-butyl dicarbonate

α-Amino acid

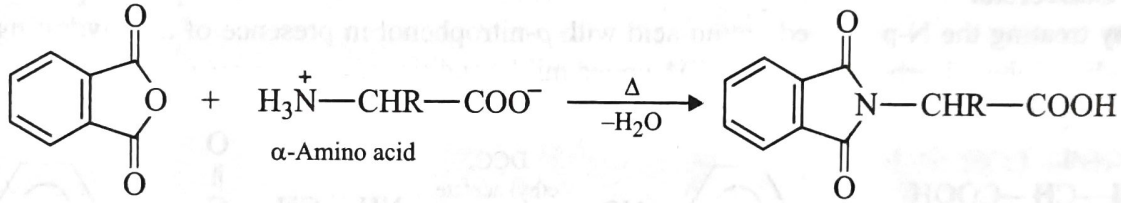
tert-Butoxycarbonylamino acid or Boc-amino acid



tert-Butoxyformic acid

tert-Butoxycarbonyl group can also be introduced by using *t*-butylazidoformate, *i.e.*, $(CH_3)_3C—O—CON_3$.

(*iii*) **Phthaloyl group.** Phthaloyl group was used by Sheehan *et al.* for protecting the NH_2 group of amino acids. To do this, amino acid is heated with phthalic anhydride. For example,

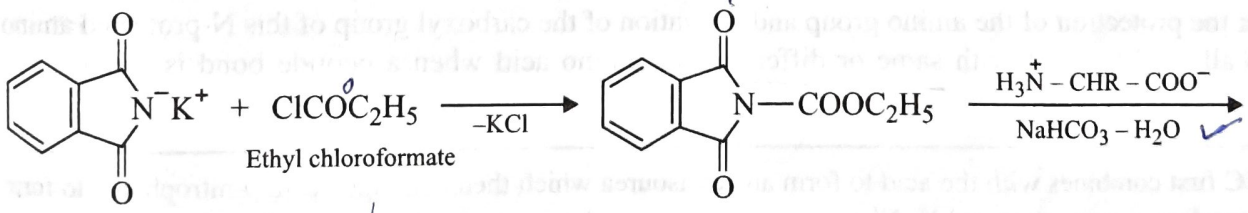


Phthalic anhydride

α-Amino acid

N-Phthaloyl α-amino acid

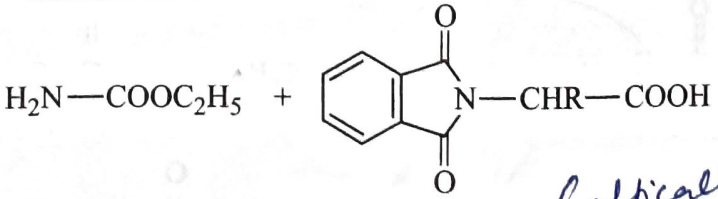
Phthaloylation occurs without recemization provided the temperature does not exceed 423 K (or 150°C). To completely eliminate recemization, a much milder method of phthaloylation uses N-carbethoxyphthalimide prepared from potassium phthalimide and ethyl chloroformate. This reagent reacts with α-amino acids in aq. $NaHCO_3$ solution at room temperature to form the optically pure phthaloyl derivative in excellent yield,



Pot. phthalimide

Ethyl chloroformate

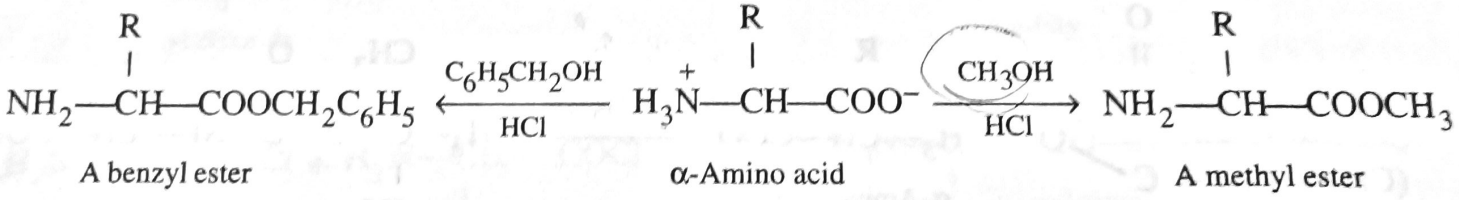
N-Carbethoxyphthalimide



N-Phthaloyl protected α-amino acid

(optically active)

2. Carboxyl protecting groups. Carboxyl groups are usually protected by forming their methyl, ethyl or benzyl esters. These groups are easily introduced by standard methods of ester formation as illustrated below :



A benzyl ester | α-Amino acid | A methyl ester