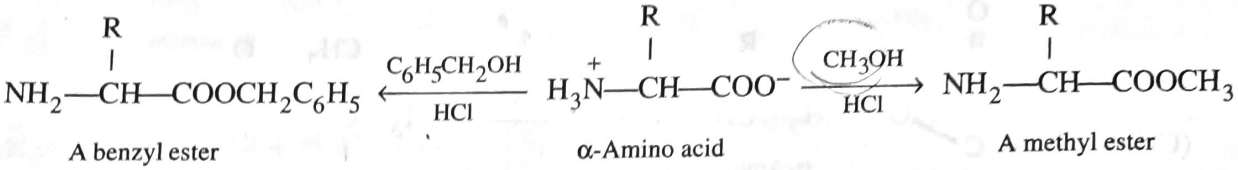
2. Carboxyl protecting groups. Carboxyl groups are usually protected by forming their methyl, ethyl or benzyl esters. These groups are easily introduced by standard methods of ester formation as illustrated below :

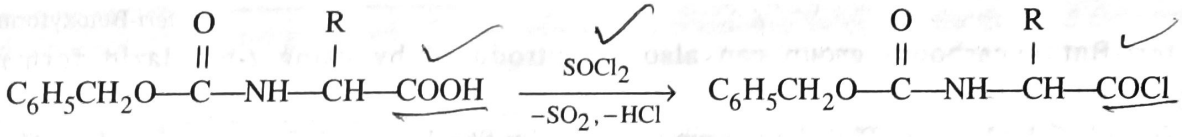


A benzyl ester — α-Amino acid — A methyl ester

3.14.3. Activation of the carboxyl group of the N-Protected Amino acid

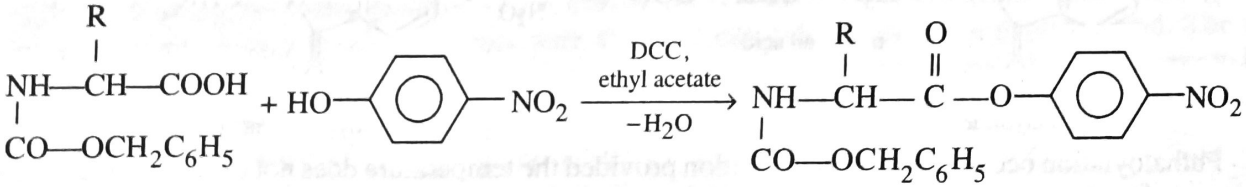
This can be achieved in many ways. The essential feature of all these methods being the conversion of – OH of – COOH group into a better leaving group. Two of the most commonly employed methods are illustrated below :

(*a*) **Conversion of the carboxyl group into the acid chloride.** This is achieved by treating the N-protected amino acid with thionyl chloride or phosphorus pentachloride.



Benzyloxycarbonylamino acid — Benzyloxycarbonylamino acid chloride

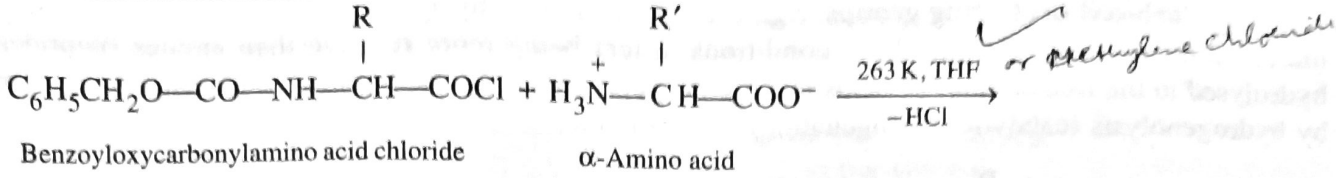
(*b*) **Conversion of the carboxyl group into a very reactive ester such as p-nitrophenyl ester.** This is done by treating the N-protected amino acid with *p*-nitrophenol in presence of a dehydrating agent such as N, N′-dicyclohexylcarbodiimide (DCC)* under mild conditions,



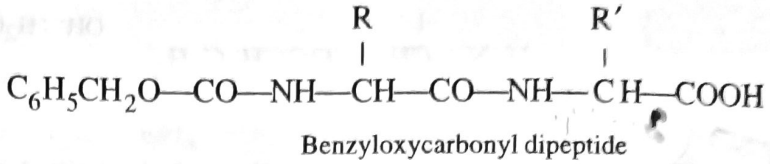
Benzyloxycarbonyl-amino acid — *p*-Nitrophenol — *p*-Nitrophenyl ester of benzyloxycarbonylamino acid

3.14.4. Formation of the peptide bond

After the protection of the amino group and activation of the carboxyl group of this N-protected amino acid, it is allowed to react with same or different free amino acid when a peptide bond is formed. For example,



Benzoyloxycarbonylamino acid chloride α-Amino acid



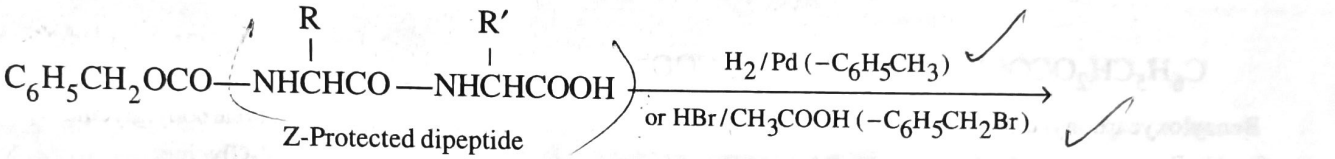
Benzyloxycarbonyl dipeptide
(A Z-protected dipeptide)

The reaction is usually carried out at 263 K in solvents of low polarity such as methylene chloride or tetrahydrofuran (THF). These mild conditions do not cause racemization.

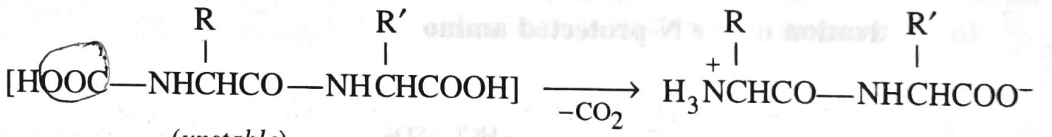
3.14.5. Removal of the protecting groups

The final step in the synthesis of peptides is the removal of the protecting groups. Depending upon the nature of the protecting groups used, the following methods are commonly used.

(*i*) *Benzyloxycarbonyl group* can be easily removed either by catalytic hydrogenation (H_2/Pd) or by hydrolysis with HBr in cold CH_3COOH.

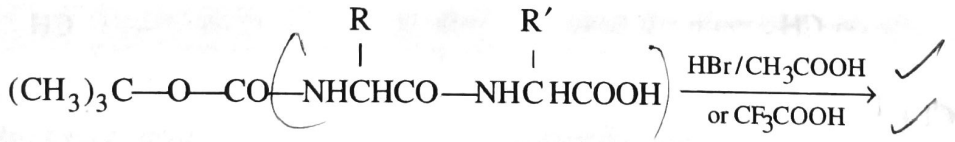


Z-Protected dipeptide

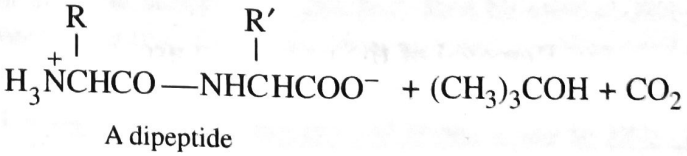


(*unstable*) A dipeptide

(*ii*) *t-Butoxycarbonyl group* can be readily removed by treating the protected dipeptide with HBr/CH_3COOH or by brief treatment with a strong acid such as trifluoroacetic acid (CF_3COOH)

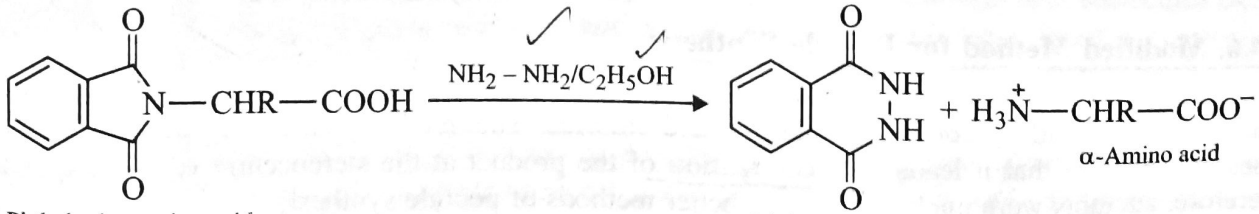


Boc-Protected dipeptide



A dipeptide

(*iii*) The **phthaloyl group** can be easily removed by hydrazinolysis, *i.e.*, treatment with hydrazine



N-Phthaloyl α-amino acid Phthalhydrazide α-Amino acid

(*iv*) **Carboxyl protecting groups, e.g., esters** are removed by hydrolysis with an aqueous acid or base under mild conditions. Under these conditions, esters being more reactive than amides (peptides) are hydrolysed to the free carboxylic acids leaving the peptide bonds intact. Benzyl esters can also be removed by hydrogenolysis (catalytic hydrogenation). For example,

$$H_2N{-}\underset{\displaystyle R}{\underset{|}{CH}}{-}COOCH_2C_6H_5 \xrightarrow[\text{or } H_2/Pd]{OH^-/H_2O} H_3\overset{+}{N}{-}\underset{\displaystyle R}{\underset{|}{CH}}{-}COO^- + C_6H_5CH_2OH \text{ or } C_6H_5CH_3$$

Sum up Summary

From the above discussion, it follows that a typical peptide synthesis involves the following steps :

(*i*) *Protection of the amino group.*

(*ii*) *Activation of the carboxyl group of the N-protected amino acid.*

(*iii*) *Formation of the peptide bond.*

(*iv*) *Removal of the protecting groups.*

Let us now illustrate the above synthetic scheme by taking up the synthesis of a simple dipeptide, *i.e.*, glycylalanine.

(*i*) **Protection of the amino group of glycine**

$$\underset{\text{Benzyloxycarbonyl chloride}}{C_6H_5CH_2OCOCl} + \underset{\text{Glycine}}{H_3\overset{+}{N}CH_2COO^-} \xrightarrow[-HCl]{} \underset{\text{N-Benzyloxycarbonylglycine or Z-Glycine}}{C_6H_5CH_2OCO{-}NHCH_2COOH}$$

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(*ii*) **Activation of the N-protected amino acid**

$$C_6H_5CH_2OCO{-}NHCH_2COOH \xrightarrow[-HCl, -SO_2]{SOCl_2} \underset{\text{Acid chloride of N-benzyloxycarbonylglycine}}{C_6H_5CH_2OCO{-}NHCH_2COCl}$$

(*iii*) **Formation of the peptide bond**

$$C_6H_5CH_2OCO{-}NHCH_2COCl + \underset{\text{Alanine}}{H_3\overset{+}{N}\underset{\displaystyle CH_3}{\underset{|}{CH}}COO^-} \xrightarrow[-HCl]{263\,K,\,THF} \underset{\text{N-Benzyloxycarbonylglycylalanine or Z-Gly-Ala}}{C_6H_5CH_2OCO{-}NHCH_2CO{-}\underset{\displaystyle CH_3}{\underset{|}{NHCH}}COOH}$$

(*iv*) **Removal of the protecting group.**

$$C_6H_5CH_2OCO{-}NHCH_2CO{-}\underset{\displaystyle CH_3}{\underset{|}{NHCH}}COOH \xrightarrow{H_2/Pd} \underset{\text{Glycylalanine (Gly-Ala)}}{H_3\overset{+}{N}CH_2CO{-}\underset{\displaystyle CH_3}{\underset{|}{NHCH}}COO^-} + C_6H_5CH_3 + CO_2$$

3.14.6. Modified Method for Peptide Synthesis

The method of peptide synthesis discussed above involves the activation of the carboxyl group of a N-protected amino acid by conversion into its acid chloride. This method has certain drawbacks. The most series drawback is that it leads to **racemization** of the product at the stereocentre α-to the COCl group. Therefore, attempts were made to discover better methods of peptide synthesis. One such method involves the protection of the – NH_2 group of N-terminal amino acid and the protection of the – COOH group of the C–terminal amino acid. These two protected amino acids are then condensed together through their free – COOH and – NH_2 groups by means of dicyclohexylcarbodiimide (DCC) to form the desired peptide bond.

Using this approach glycylalanine may be synthesized as follows :

(*i*) $(t\text{–BuOCO})_2O + H_3\overset{+}{N}CH_2COO^- \longrightarrow t\text{–BuOCO—NHCH}_2\text{COOH}$

Di-tert-butyl dicarbonate — Glycine — *tert*-Butoxycarbonylglycine or Boc-Glycine

(*ii*) $C_6H_5CH_2OH + H_3\overset{+}{N}CHCOO^- \xrightarrow{\text{Dry HCl}} H_2N\text{—}\underset{\displaystyle CH_3}{\underset{|}{CH}}\text{—}COOCH_2C_6H_5$

Benzyl alcohol — Alanine — Alanine benzyl ester

(*iii*) $t\text{–BuOCO—NHCH}_2\text{COOH} + H_2N\text{—}\underset{\displaystyle CH_3}{\underset{|}{CH}}\text{—}COOCH_2C_6H_5 \xrightarrow{DCC} -DCU$

Boc-Glycine (*N-Protected amino acid*) — Alanine benzyl ester (*C-Protected amino acid*)

$t\text{-BuCO—NHCH}_2\text{CO—NHCH}\underset{\displaystyle CH_3}{\underset{|}{}}\text{—}COOCH_2C_6H_5 \xrightarrow[CH_3COOH]{\text{HBr in cold}}$

Boc- Glycylalanyl benzyl ester

$H_3\overset{+}{N}CH_2CO\text{—}NHCH\underset{\displaystyle CH_3}{\underset{|}{}}COO^- + (CH_3)_2C = CH_2 + CO_2 + C_6H_5CH_2Br$

Glycylalanine

DCC is a mild dehydrating agent. It removes –OH from the –COOH group of one amino acid and –H from the $-NH_2$ group of the other amino acid to form a peptide bond while it itself is converted into N, N′-dicyclohexylurea (DCU).

The above method can be easily used to convert the dipeptide into a tripeptide and a tripeptide into a tetrapeptide and so on. To do this, one of the protecting groups in the resulting dipeptide is selectively removed and the peptide is built from this end.

By repeating the above steps, one amino acid at a time can be added from either end of the precursor to the growing peptide chain. Alternatively, a number of suitable simple peptides may first be synthesized and then linked together to give the required polypeptide or the protein. Using the above procedure, *Vigneaud* (Cornell Medical College, USA) achieved the synthesis of the harmone oxytocin* and recieved the Nobel Prize in 1955. Similarly, Frederick Sanger synthesized the human insulin and got the Nobel Prize in 1958.

3.14.7. Drawbacks of Classical Peptide Synthesis

The classical peptide synthesis which involves stepwise condensation of amino acid molecules can be successfully used to synthesize oligopeptides (containing 2-10 amino acid residues). But the synthesis of large peptides (polypeptides) containing hundreds of molecules of amino acids by this method is a very difficult task as explained below :

(*i*) **Time consuming.** The classical method of peptide synthesis requires several steps involving protection, activation, condensation and deprotection for each new peptide bond to be formed.

*Oxytocin is a peptide consisting of nine amino acids. It occurs in the posterior pituitary gland and is responsible for contraction of uterus after the child birth and produces lactation in the mammary glands.

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