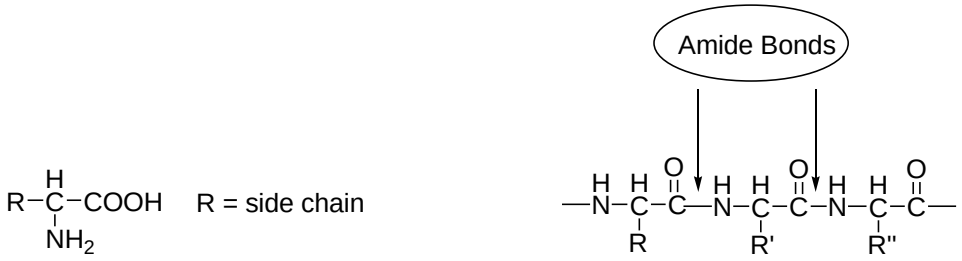
Module 12 Amino Acids, Peptides and Proteins

Lecture 32 Amino Acids

12.1 Introduction

In nature three kinds of polymers occur: (i) polysaccharides, (ii) proteins and (iii) nucleic acids. This section discusses proteins and peptides that are polymers of α-amino acids linked together by amide bonds. Scheme 1 shows representation for an α-amino acid and peptide. The repeating units in peptide is called amino acid residue. Proteins are polypeptides that are made up of 40-100 amino acids.



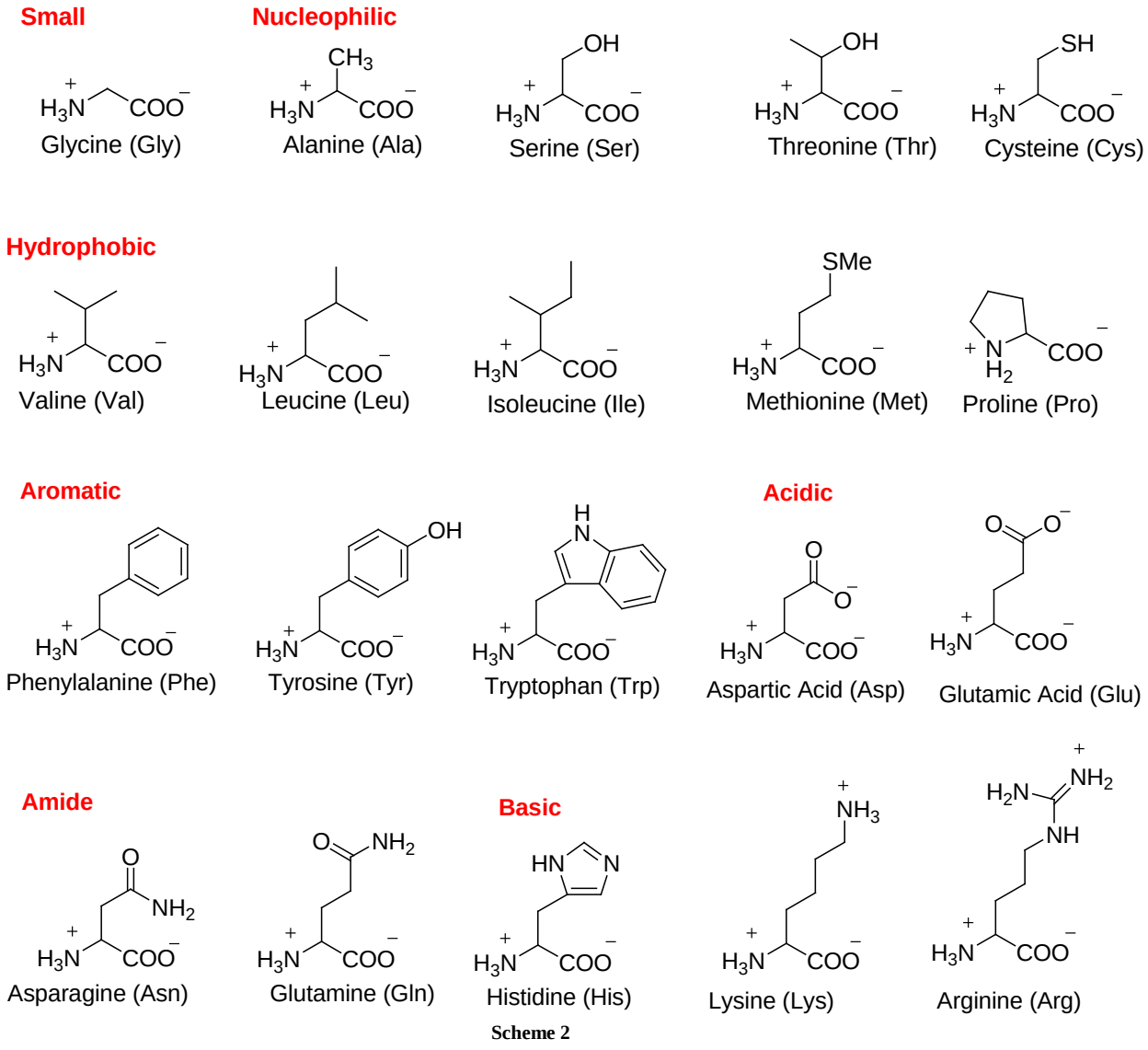
Representation for an α-amino acid

Representation for a tripeptide

Scheme 1

12.2 α-Amino Acids

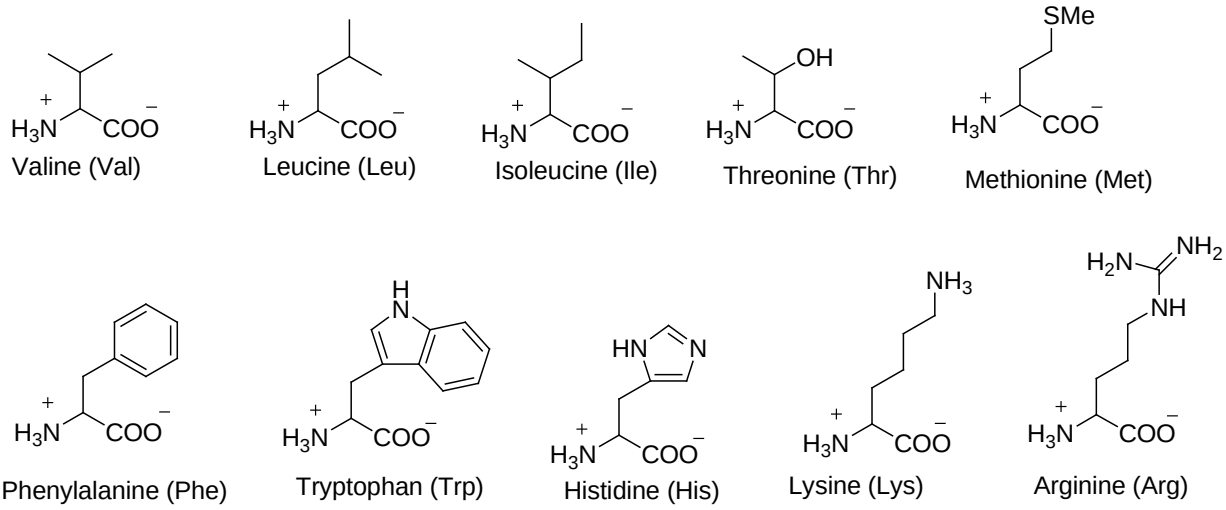
The structures of the 20 most common naturally occurring amine acids are shown in Scheme 2. They differ only in the side chain attached to the α-carbon. Among them, ten are essential amino



Scheme 2

acids (Scheme 3). These amino acids are to be obtained from diets because we either can not synthesize them at all or can not synthesize them in adequate amounts.

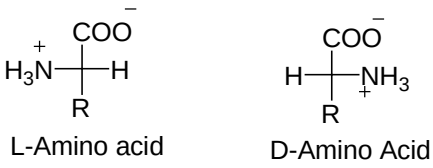
Essential Amino Acids



Scheme 3

12.3 Configuration α-Amino Acids

In 19 of the 20 naturally occurring α-amino acids, except glycine, the α-carbon is an asymmetric center. Thus, they can exit as enantiomers, and the most amino acids found in nature have L-configuration. Scheme 4 shows the Fischer projection of an amino acid with a carboxyl group on the top and the R group on the bottom of the vertical axis is an L-amino acid if the amino group is on the left and a D-amino acid if the amino group is one the right.

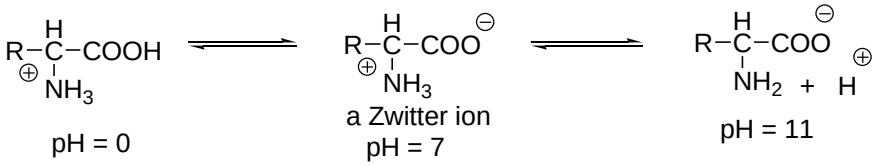


Scheme 4

12.4 Acid-Base Properties α-Amino Acids

Amino acid has a carboxyl group and amino group, and each group can exist in an acidic or basic form, depending on the pH of the solution in that the amino acid is dissolved. In addition, some amino acids, such as glutamate, also contain ionizable side chain.

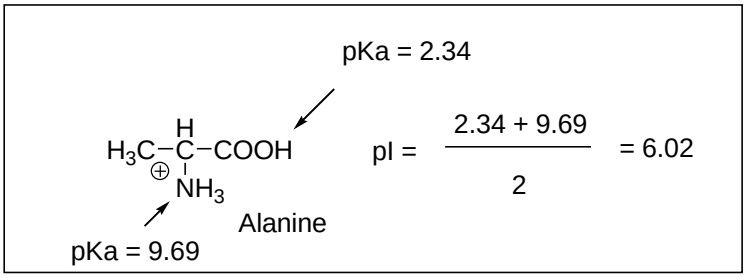
The pKa values of the carboxyl group and the protonated amino group of the amino acids approximately are 2 and 9, respectively (Scheme 5). Thus, both groups will be in their acidic forms in highly acidic medium (pH ~ 0). At pH 7, the pH of the solution is greater than the pKa of the carboxyl group, but less than the pKa of the protonated amino group. Hence, the carboxyl group will be in its basic form and the amino group in its acidic form (called Zwitter ion). In strongly basic medium (pH 11), both groups will be in basic form. Thus, an amino acid can never exist as an uncharged compound, regardless of the pH of the medium.



Scheme 5

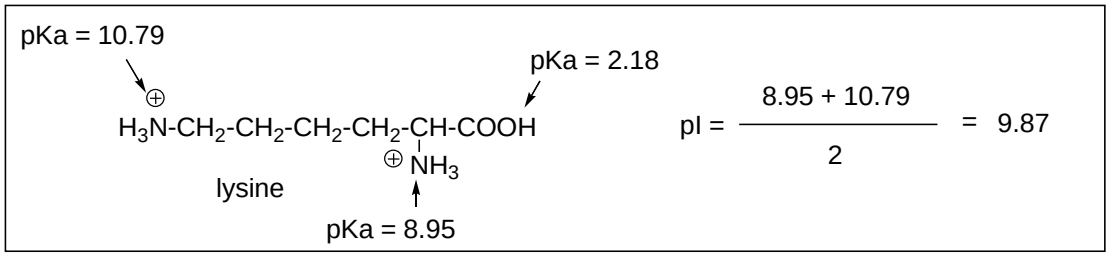
12.5 The Isoelectric Point (pI)

The isolectric point (pI) of an amino acid is the pH where it has no net charge. For example, the pI of an amino acid that does not possess an ionizable side chain is midway between its two pKa values (Scheme 6).



Scheme 6

In case of an amino acid that contains an ionizable side chain, the pI is the average of the pKa values of the similarly ionizing groups. For example, see pI of lysine (Scheme 7).

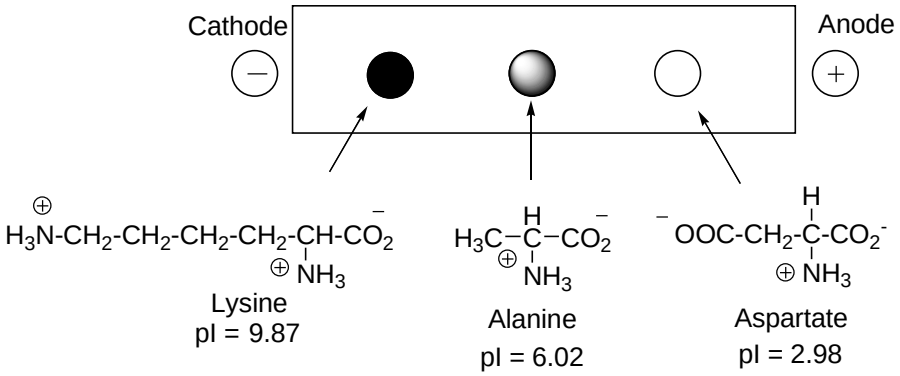


Scheme 7

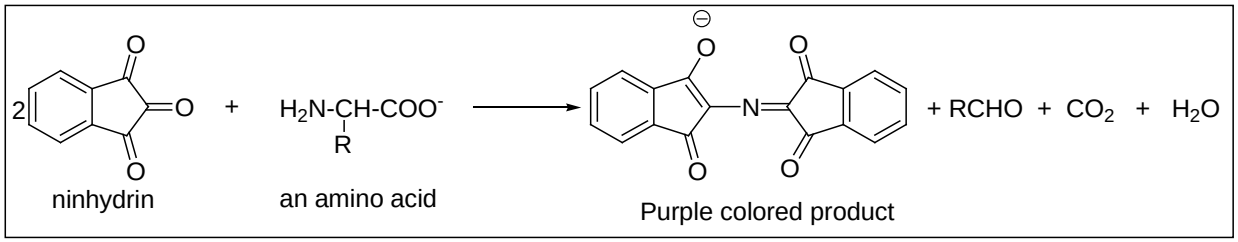
12.6 Separation of Amino Acids

A. Electrophoresis

In this method, the amino acids can be separated on the basis of their pI values (Scheme 8). A few drops of a solution of amino acid mixture are applied to the middle of the piece of filter paper or to a gel. When this paper or gel is placed in a buffered solution between two electrodes and an electric field is applied, an amino acid having a pI greater than the pH of the medium will have an overall positive charge and will move toward the cathode. While an amino acid with a pI less than the pH of the buffer will have an overall negative charge and will move toward anode. In case of the molecules have the same charge, the larger one will migrate more slowly compared to that of the smaller one during the electrophoresis. After the separation, the filter paper is sprayed with ninhydrin and dried in a warm oven to give purple colored spot. The amino acids are identified by their location on the paper comparing with a standard.



Separation of lysine, alanine and aspartate by electrophoresisat pH = 5.

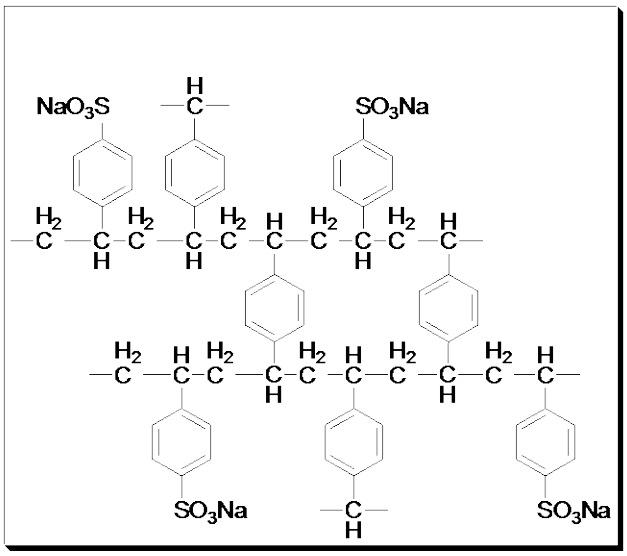


The reaction of ninhydrin with an amino acid to form a purple colored product.

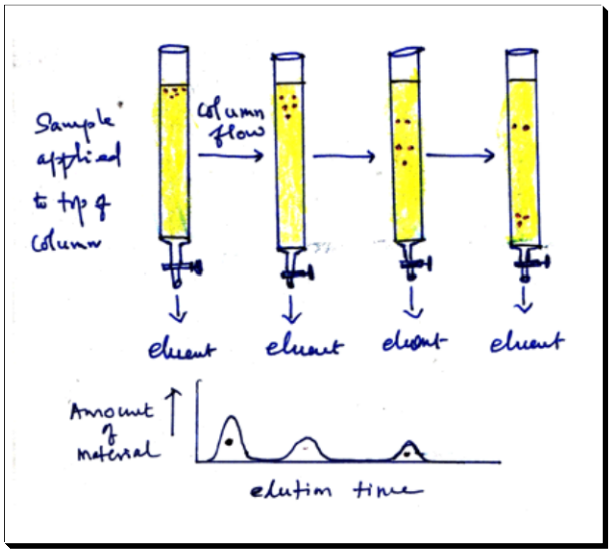
Scheme 8

B. Ion-Exchange Chromatography:

In this technique, a column is packed with an insoluble ion-exchange resin. Then, a solution of a mixture of amino acids is loaded on the top of the column, and eluted with aqueous solutions of increasing pH. Since the amino acids bind with the resin at different extent, during the elution, the weakly bound amino acid can flow faster compared to that bound strongly, which can be collected as different fractions. Scheme 9 presents the structure of section of a commonly used resin and diagram of the ion-exchange chromatography separation.



A section of a cation-exchange resin



Separation of amino acids by ion-exchange chromatography

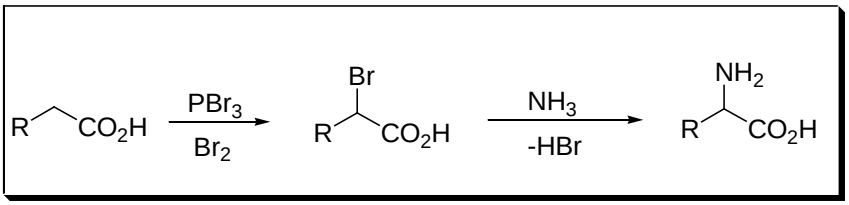
Scheme 9

12.7 The Synthesis of α–Amino Acids

Some of the common methods employed for the synthesis of α-amino acids follow:

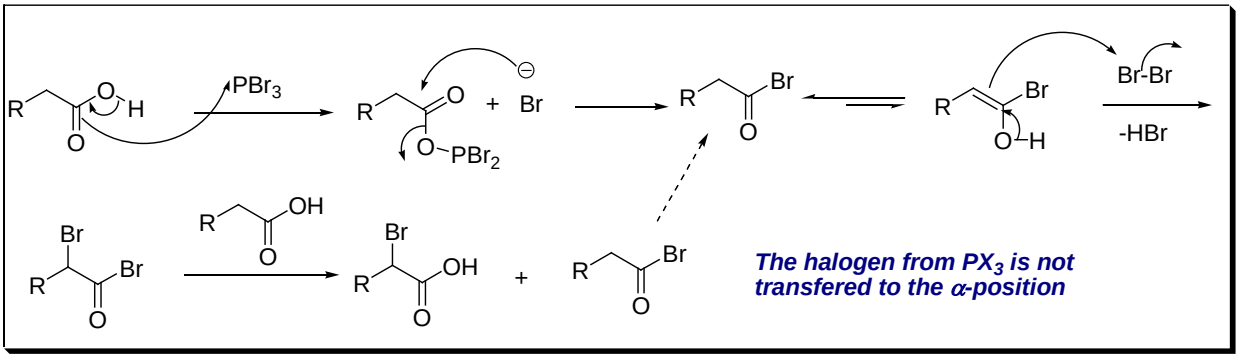
12.7.1From α-Halo acids

The simplest method is the conversion of carboxylic acid into it's α-bromo-derivative that can be reacted with ammonia to give α-amino acid (Scheme 10).

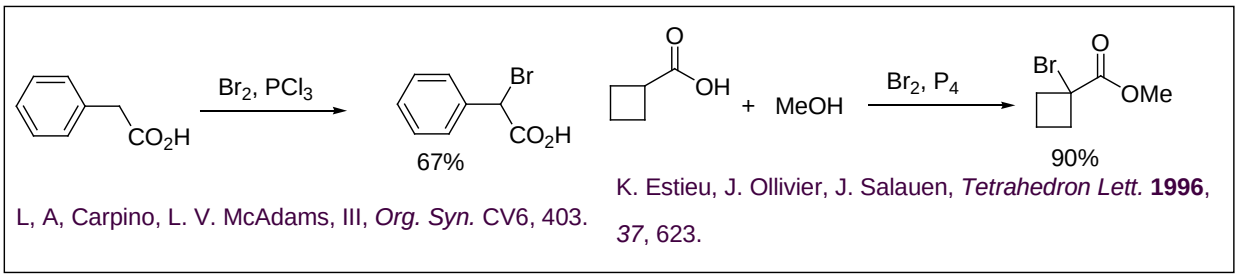


Scheme 10

Hell-Volhard-Zelinski reaction is generally used to prepare α-bromo acid (Scheme 11). The treatment of the acid with bromine in the presence of a small amount of phosphorus gives acid bromide which undergoes (electrophilic) bromination at the α-position *via* its enol tautomer. The resulting product exchanges with more of the acid to give α-bromo acid together with more acid bromide for the further bromination.

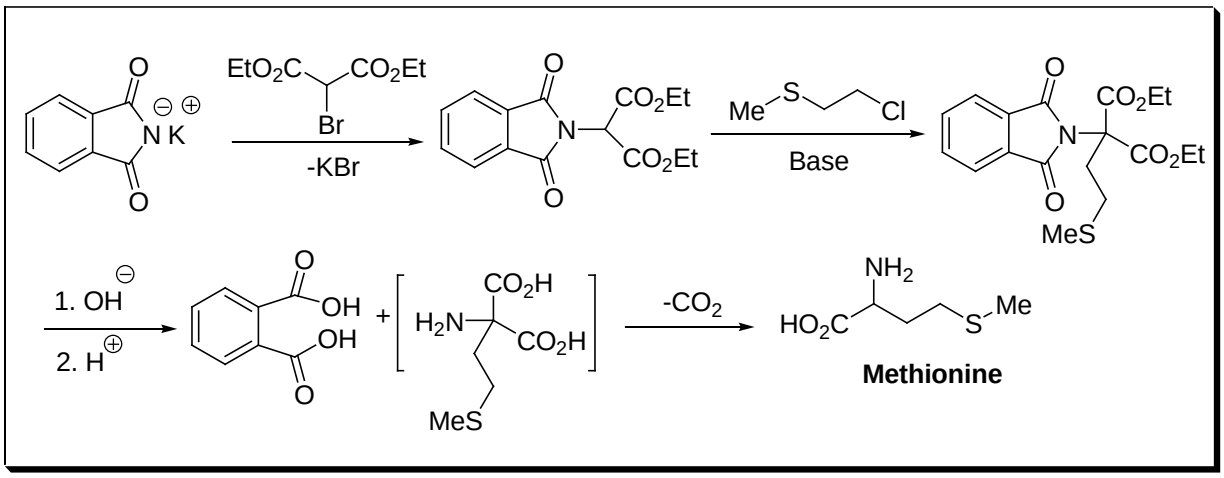


For example:



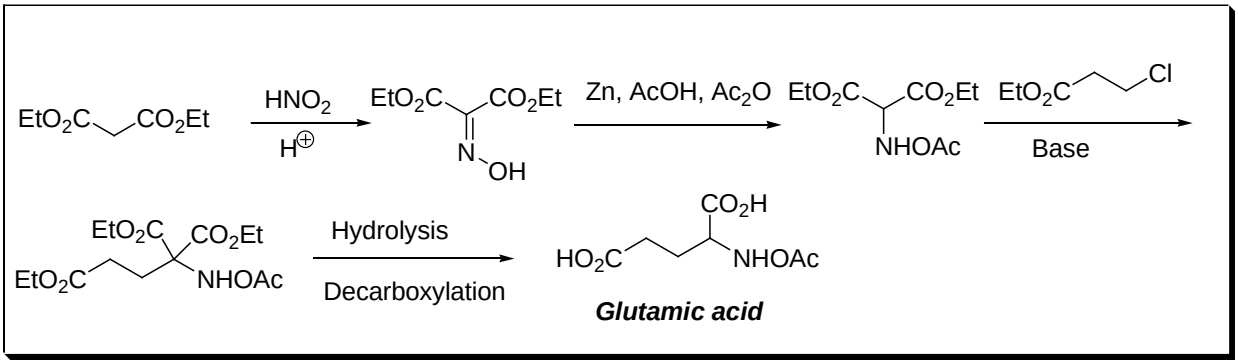
Scheme 11

The amino group can also be introduced by ***Gabriel procedure*** to give better yield compared to that of the above described reaction with ammonia as an aminating agent (Scheme 12).



Scheme 12

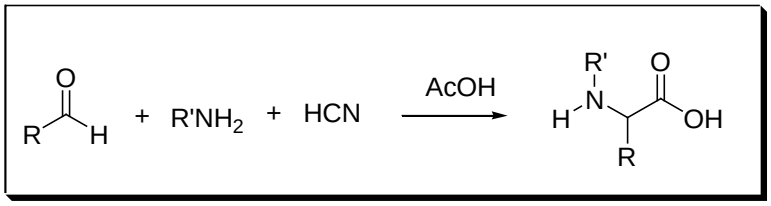
The amino group can also readily be introduced *via* ***nitrosation*** followed by reduction and hydrolysis processes (Scheme 13).



Scheme 13

12.7.2 The Strecker Synthesis

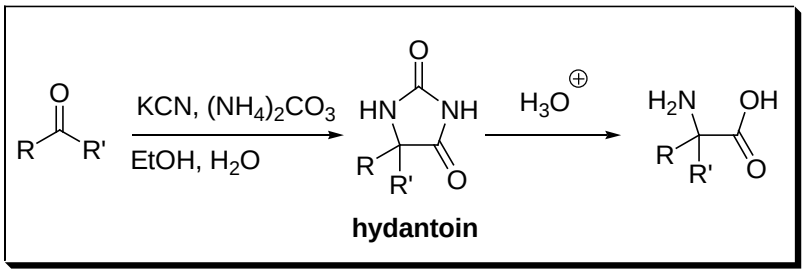
The condensation of aldehydes with amine gives imine that reacts with cyanide ion *in situ* to give an α-aminonitrile. The latter on hydrolysis gives α-amino acids (Scheme 14).



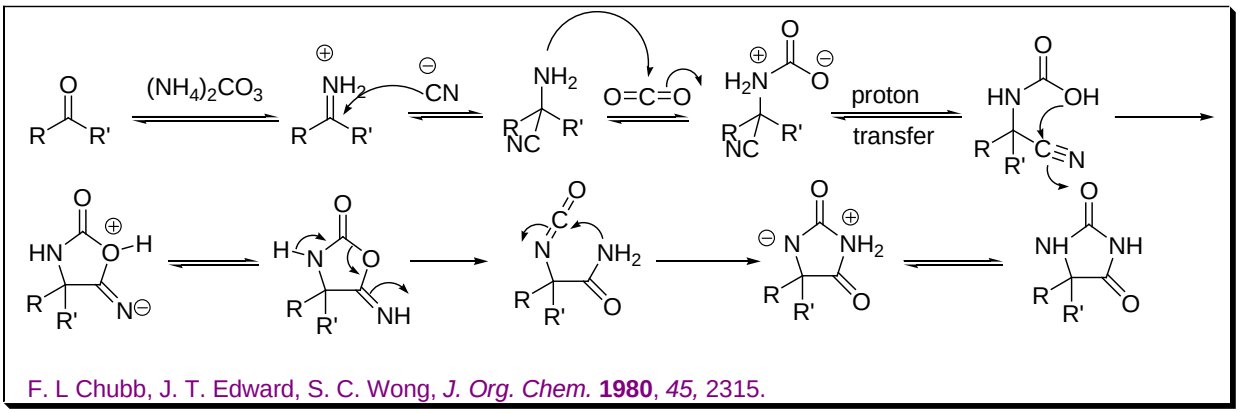
Scheme 14

12.7.3 Bucherer-Bergs Reaction

Ketones react with ammonium carbonate in presence of cyanide ion to afford hydantoin that can be hydrolyzed to α-amino acid (Scheme 15).



Mechanism



F. L Chubb, J. T. Edward, S. C. Wong, *J. Org. Chem.* **1980**, *45*, 2315.

Scheme 15

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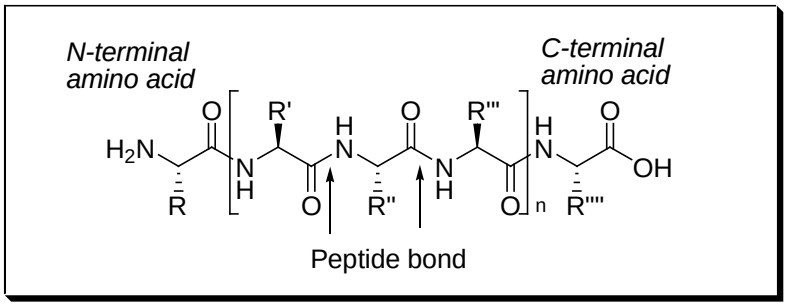
Module 12 Amino Acids, Peptides and Proteins

Lecture 33 Peptides and Proteins

12.8 Peptides and Proteins

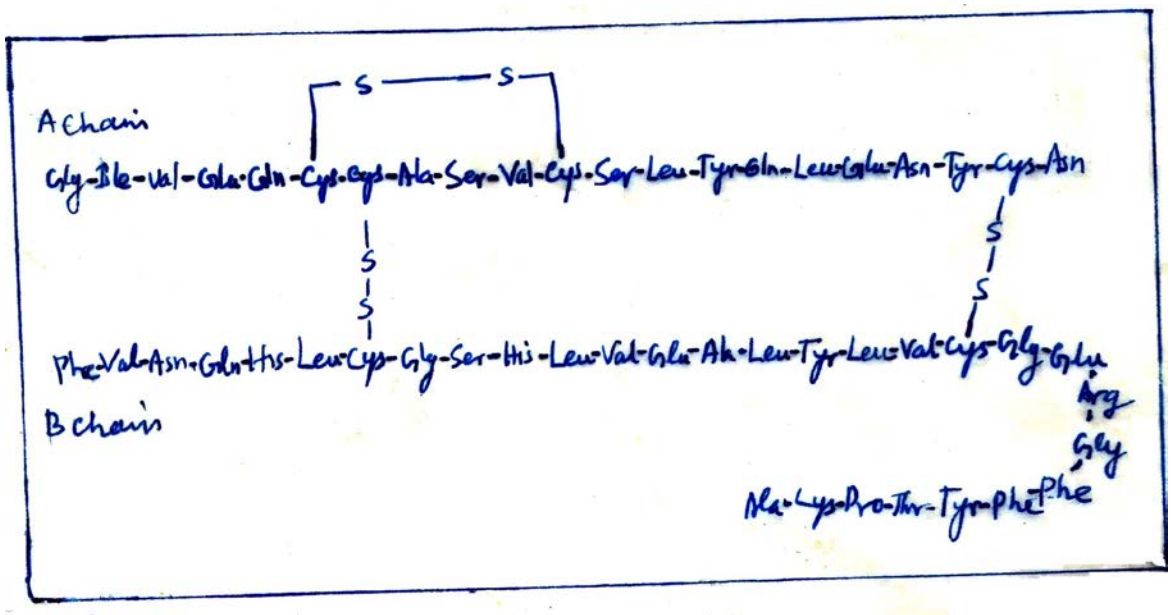
These are naturally occurring polymers in living systems. The polymers with molecular weights less than 10000 are termed as peptides and those with higher molecular weights are termed as proteins. The acid-catalyzed hydrolysis of peptides and proteins affords the constituent α-amino acids.



Scheme 1

12.8.1 Primary Structure of Protein

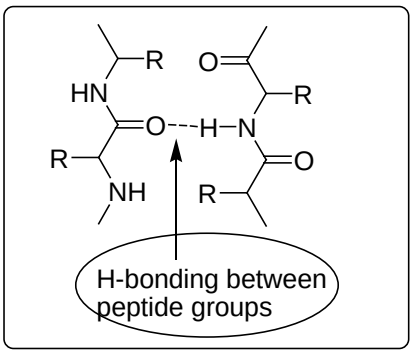
The primary structure of a protein describes the sequence of amino acids in the chain. Insulin is the first protein whose amino acid sequence was determined. Scheme 2 presents the primary structure of insulin.

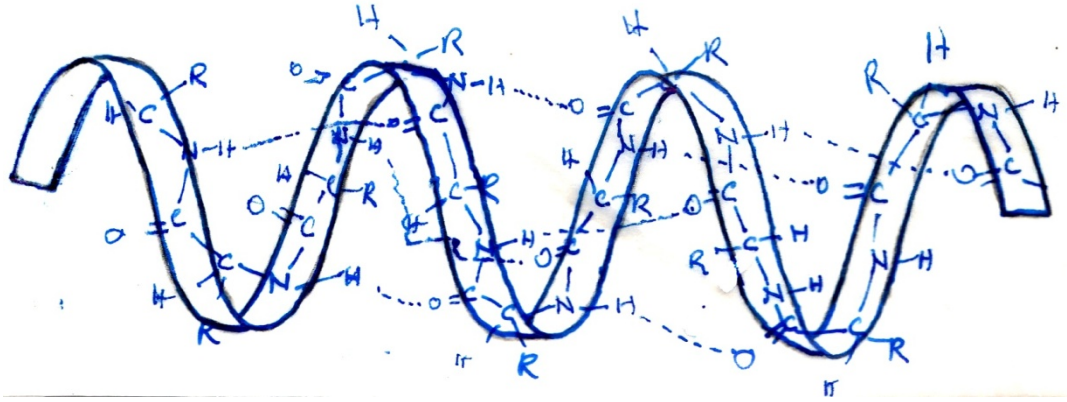


Scheme 2. Primary Structure of Insulin

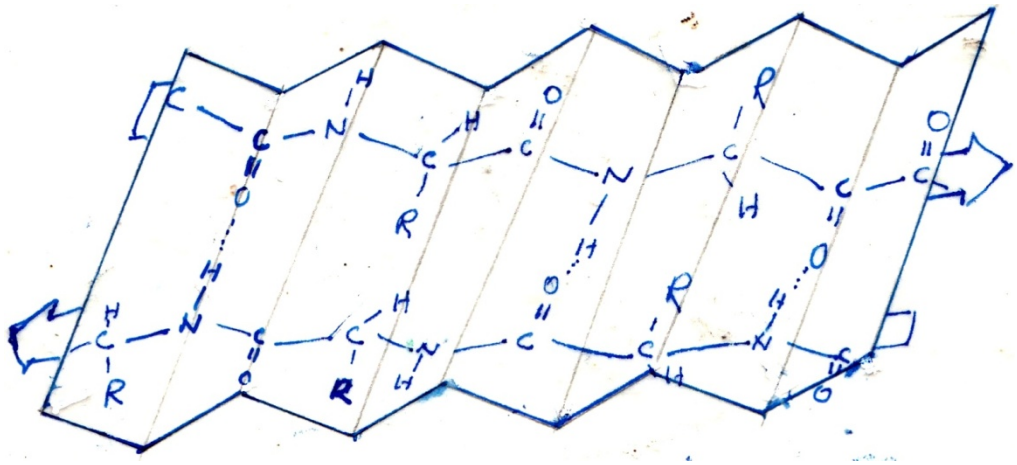
12.8.2 Secondary Structure of Protein

The secondary structure describes how the segments of the backbone chain fold (Scheme 3). These conformations are stabilized by H-bonding between the peptide groups- between NH of one amino acid residue and C=O group of another.





(a) α-Helix



(b) β-Pleated Sheet

Scheme 3. A segment of a protein in: (a) an α-helix; (b) β-pleated sheet.

α-Helix:

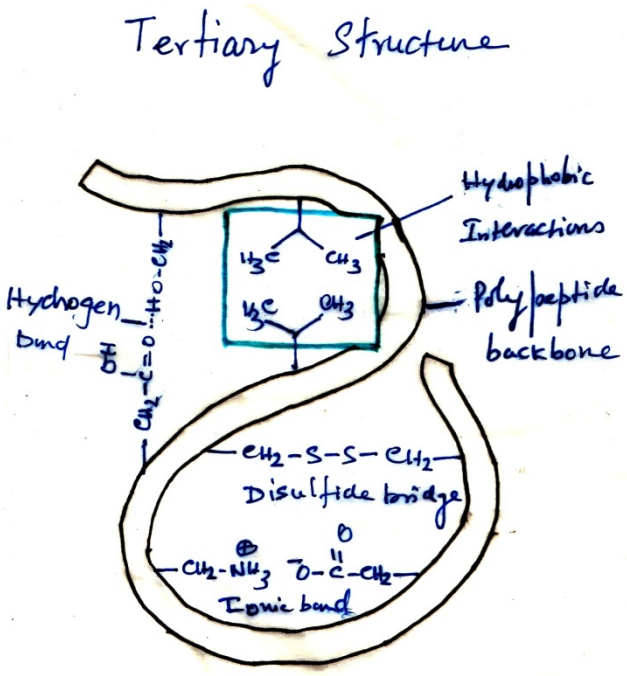
The first type of secondary structure is α-helix, where the backbone coils around the long axis of the protein molecule. The substituents on the α-carbon of the amino acids protrude outward from the helix to minimize the steric hindrance. The H attached to amide nitrogen makes H-bonding with the carbonyl oxygen of an amino acid.

β-Pleated Sheet

The second type of secondary structure is the β-pleated sheet, in which the backbone is extended in a zigzag structure resembling pleats. The H-bonding in a β-pleated sheet occurs between the adjacent peptide chains.

12.8.3 Tertiary Structure of Protein

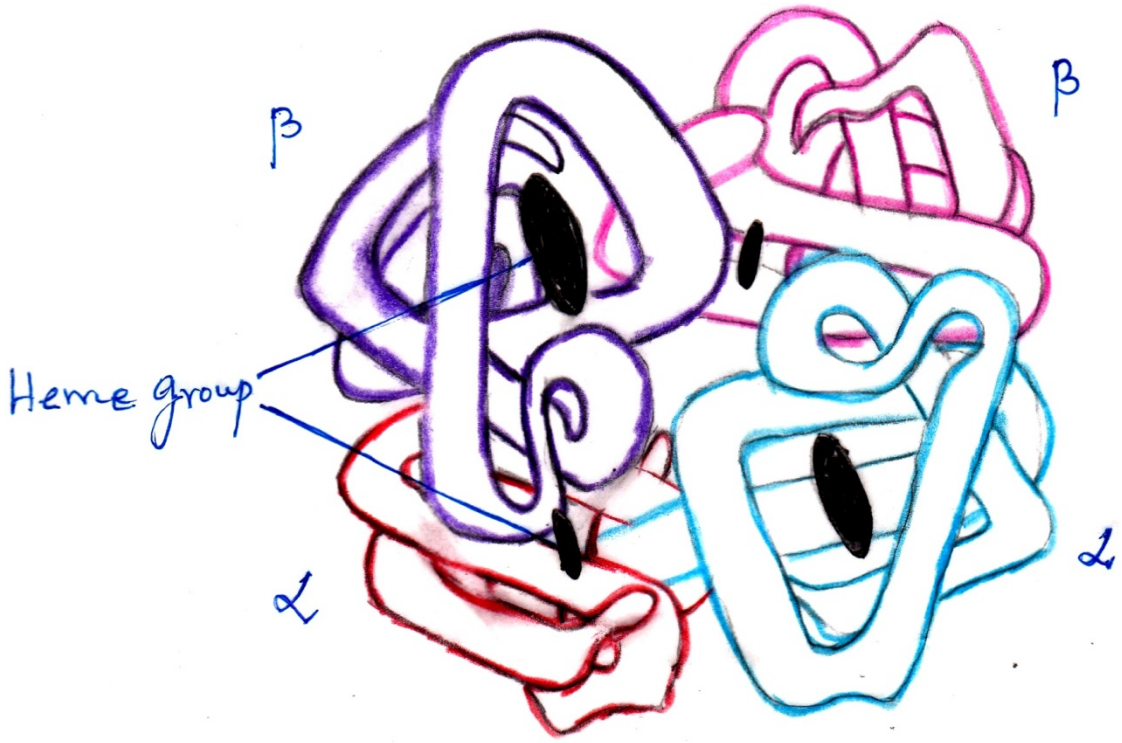
The tertiary structure of a protein describes the three-dimensional arrangement of all the atoms. In solution, proteins fold to maximize their stability through interactions include disulfide bonds, hydrogen bonds, electrostatic attractions and hydrophobic interactions (Scheme 4).



Scheme 4. Stabilizing interactions for the tertiary structure of protein.

12.8.4 Quaternary Structure of Protein

Some proteins have more than one peptide chain and the individual chain is called a subunit. The subunits are held together by intractions such as hydrophobic intraction, H-bonding, and electrostatic attractions. The quarternary structure of a protein describes the way the subunits are arranged in space. Scheme 5 shows the structure of hemoglobin which is a tetrameric structural protein comprising two α and two β subunits.



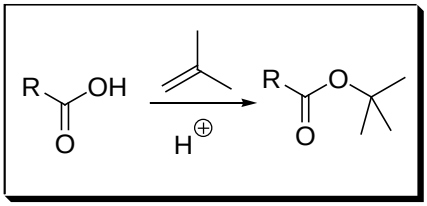
Scheme 5. Quaternary Protein Structure: Three-Dimensional Arrangement of Subunits.

12.8.5 Synthesis of Peptides

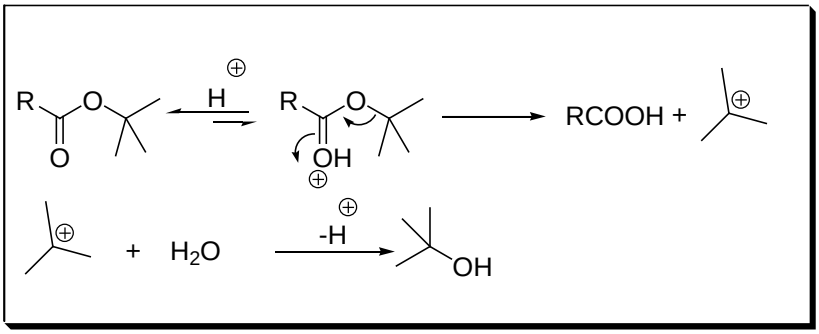
In peptide synthesis, one amino acid is protected at its amino end with group Y and the second is protected at its carboxyl end with a group Z. The condensation of these two protected amino acids using DCC generates a peptide bond. As per the requirement, one of the protecting groups can then be removed and a third protected amino acid can be introduced to the second peptide bond. Repetition of the procedure leads to the desired polypeptide. For example:

Protection and Deprotection of Carboxyl Group

- The carboxyl group can be protected by converting it into its *t*-butyl ester using isobutylene in the presence of sulfuric acid.

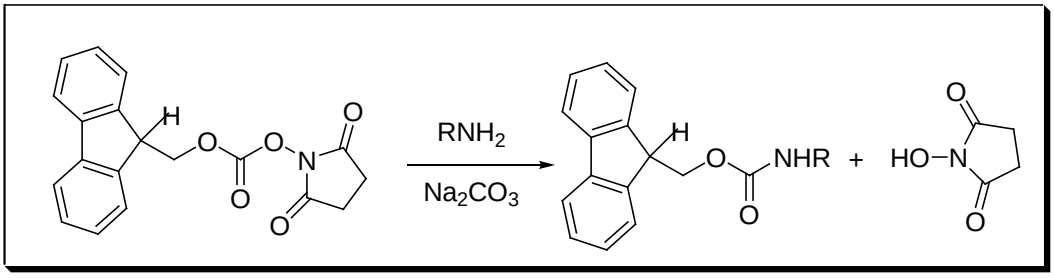


- The protecting group can also be easily removed using mild acid hydrolysis.

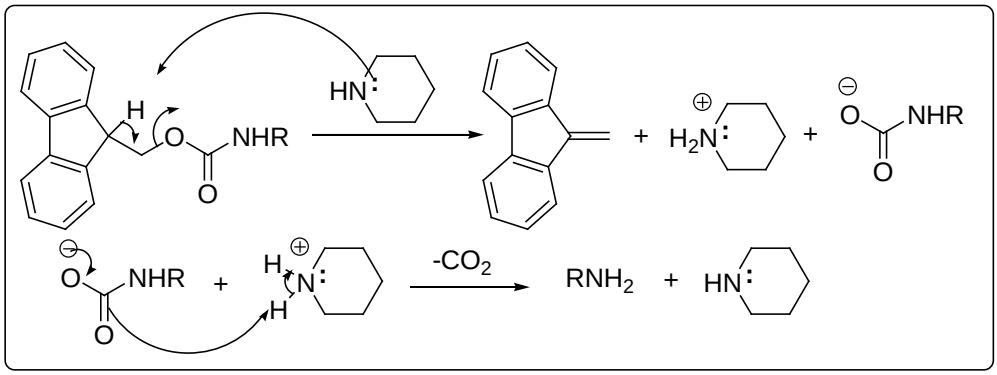


Protection and Deprotection of Amino Group

- (9-Fluorenyl)methyoxycarbonyl group (Fmoc) is commonly used for the protection of the amino group of amino acid.

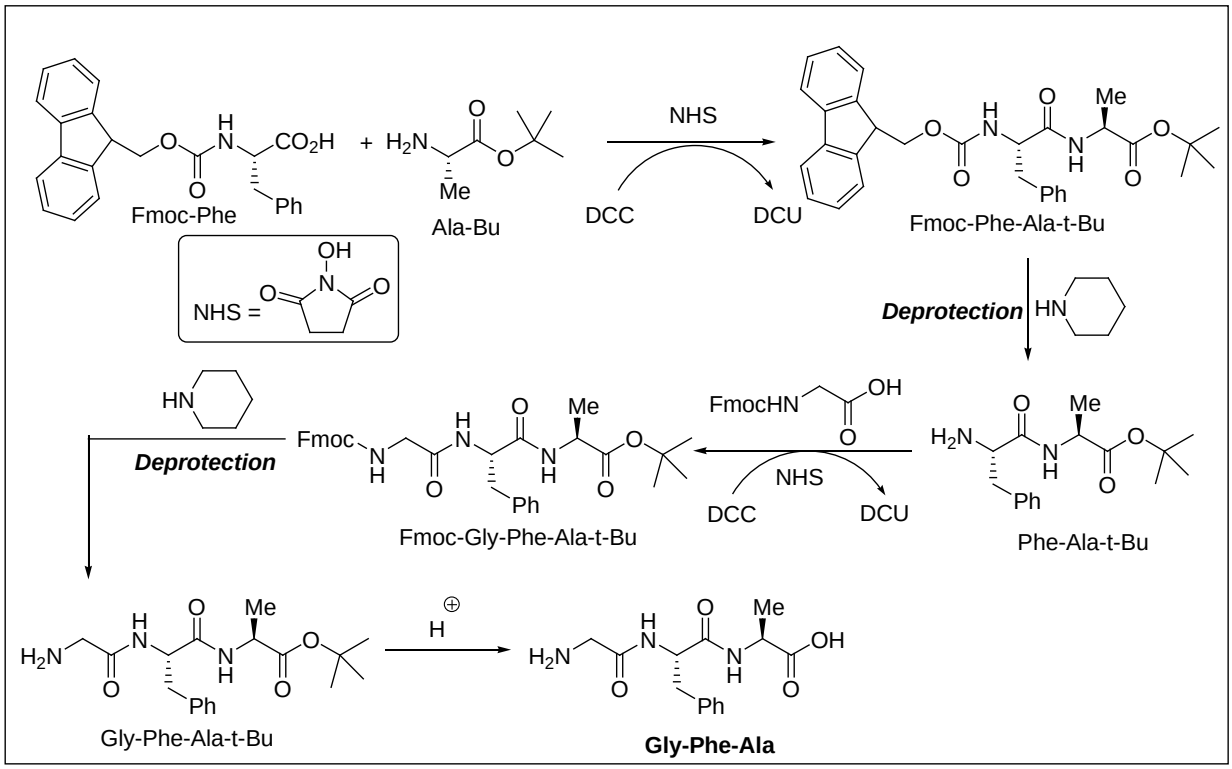


- The protecting group can also be easily removed by treatment with amine base, such as piperidine.



Synthesis of Tripeptide

Scheme 5 illustrates the synthesis of Gly-Phe-Ala sequence. The condensation of the Fmoc-phenylalanine with t-butyl protected alanine using 1,3-dicyclohexylcarbodiimide (DCC) and N-



hyroxysuccinimide (NHS) can give the protected dipeptide, Fmoc-Phe-Ala-^{t}Bu. The latter, after Fmoc deprotection using piperdine, can be coupled with Fmoc-glycine to give the protected tripeptide Fmoc-Gly-Phe-Ala-^{t}Bu. The protecting groups can be then deprotected using weak base (Fmoc) and mild acid (^{t}Bu) to afford the target tripeptide, Gly-Phe-Ala.

The Role of DCC and NHS in Peptide Synthesis