BIOMOLECULES

AMINO ACIDS AND PROTEINS
SEM-5, CC-12
PART-1, PPT-21

Part-1: Amino Acids-I, Synthesis

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BIOMOLECULES (PART-1, PPT-21)

Amino Acids-I

Amino Acids and Proteins: Introduction

The name protein is taken from the Greek *proteios*, which means 'primary' or 'holding the first place'. Of all chemical compounds, proteins must almost certainly be ranked first, for they are the substance of life. Proteins make up a large part of the animal body, they hold it together, and they run it. They are found in all living cells. They are the principal material of skin, muscle, tendons, nerves, blood, enzymes, antibodies, and many hormones.

Chemically, proteins are high polymers. They are polyamides, and the monomers from which they are derived are the α-amino carboxylic acids. A single protein molecule contains hundreds or even thousands of amino acid units; these units can be of twenty-odd different kinds. The number of different combinations, that is, the number of different protein molecules that are possible, is almost infinite.

When hydrolyzed by acids, alkalis or enzymes, protein yield a mixture of amino acids. Acid hydrolysis destroys certain amino acids, particularly tryptophan. On the other hand, alkaline hydrolysis causes complete racemization and also the destruction of a number of amino acids, e.g., serine, threonine, cysteines, etc.

Enzymatic hydrolysis has also difficulties, particularly the long time that is usually needed and the fact that the hydrolysis is often incomplete. Thus, acid hydrolysis is the most satisfactory, but enzymatic hydrolysis is very useful for the isolation of tryptophan.

The number of amino acids so far obtained from proteins appears to be about twenty-five, all of which except two are α-amino acids. The two exceptions are proline and hydroxyproline, which are imino-acids. The amino acids are classified in several ways. The letters *g*, *l* and *e* which follow the name of the acids indicate that the acid is, respectively, of general occurrence, lesser occurrence and essential to human.

Amino acids are derivatives of the carboxylic acids in which a hydrogen atom in the carbon chain has been replaced by an amino group. Therefore, amino acids contain both a carboxyl group ($-CO_2H$) and an amino group ($-NH_2$) in the same molecule.

The amino group may occupy the α-, or β-, γ-… positions. There may also be two or more amino groups present in the chain of the same molecule. The structural representation of α-amino acid is shown in Figure 1. All these structures are the same and have the same stereochemistry. Except for glycine, the α-amino acids from proteins have at least one *chiral* centre.

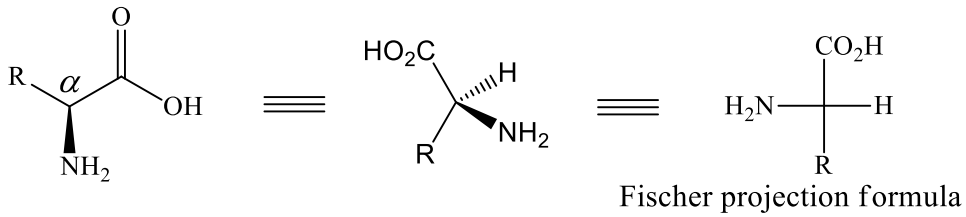


Figure 1: α-Amino acids in different projections

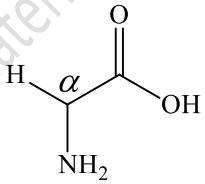
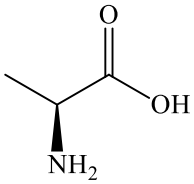
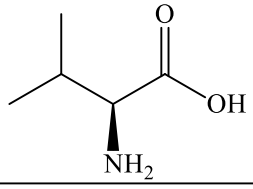
Tables 1-3 gives the structures and names of twenty-two amino acids that have been found in proteins. Ten of these are the essential amino acids (marked *e*), i.e., a deficiency in any one prevents growth in young animals, and may even cause death. All these are *alpha*-amino carboxylic acids. However, in two cases (proline and hydroxyproline) the amino group forms part of a pyrrolidine ring. This common feature gives the amino acids a common set of chemical properties, one of which is the ability to form the long polyamide chains that make up proteins.

In other respects, the structures of these compounds vary rather widely. In addition to the carboxyl group and the amino group *alpha* to it, some amino acids contain a second carboxyl group (e.g., aspartic acid or glutamic acid), or a potential carboxyl group in the form of a carboxamide (e.g., asparagine). These amino acids are called *acidic amino acids*. Some contain a second basic group, which may be an amino group (e.g., lysine), a guanidino group (arginine), or the imidazole ring (histidine) which are called *basic amino acids*. Some of the amino acids contain benzene or heterocyclic ring systems, phenolic or alcoholic hydroxyl groups, halogen or sulfur atoms. Each of these ring systems or functional groups undergoes its own typical set of reactions.

Structures and Names of α-Amino Acids

The 22 α-amino acids that can be obtained from proteins are subdivided into three different groups on the basis of the structures of their side chains, R. These are given in Tables 1-3.

Table 1: Neutral α-Amino acids

Structure	Name	Abbereviation	pK_{a1} α-CO_2H	pK_{a2} α-NH_3^+	pK_{a3} R-group	*pI*
	Glycine (*g*)	G or Gly	2.3	9.6	-	6.0
	Alanine (*g*)	A or Ala	2.3	9.7	-	6.0
	Valine (*g*, *e*)	V or Val	2.3	9.6	-	6.0

Neutral α-Amino Acids Found in Proteins

Table 1: Neutral *a*-Amino acids

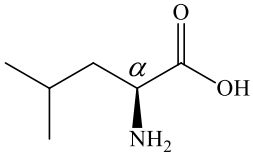
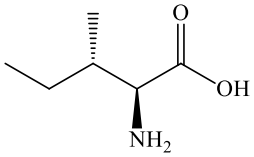
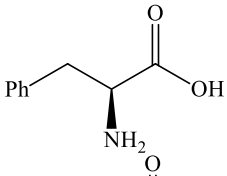
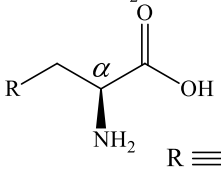
Structure	Name	Abbereviation	pK_{a1} α-CO_2H	pK_{a2} α-NH_3^+	pK_{a3} R-group	*pI*
	Leucine (*g*, *e*)	L or Leu	2.4	9.6	-	6.0
	Isoleucine (*g*, *e*)	I or Ile	2.4	9.7	-	6.1
	Phenyl-alanine (*g*, *e*)	F or Phe	1.8	9.1	-	5.5
	Tyrosine (*g*)	Y or Tyr	2.2	9.1	10.1	5.7

Table 1: Neutral α-Amino acids

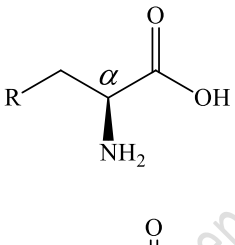
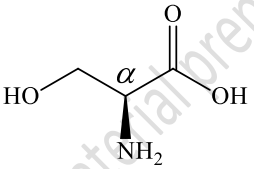
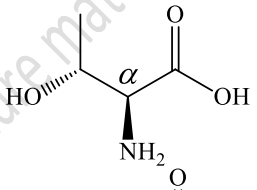
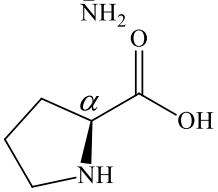
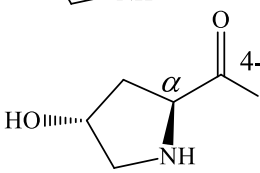
Structure	Name	Abbereviation	pK_{a1} α-CO_2H	pK_{a2} α-NH_3^+	pK_{a3} R-group	*pI*
	Tryptophan (*g*, *e*)	W or Trp	2.4	9.4	-	5.9
	Serine (*g*)	S or Ser	2.2	9.2	-	5.7
	Threonine (*g*, *e*)	T or Thr	2.6	10.4	-	6.5
	Proline (*g*)	P or Pro	2.0	10.6	-	6.3
	4-hydroxyproline (*cis* or *trans*) (*g*)	O or Hyp	1.9	9.7	-	6.3

Table 1: Neutral α-Amino acids

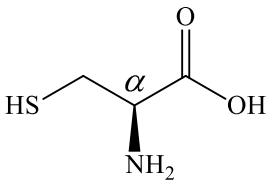
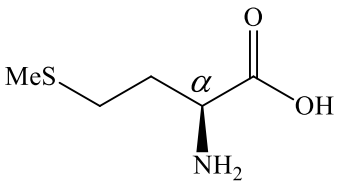
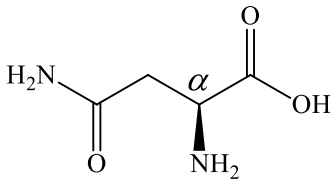
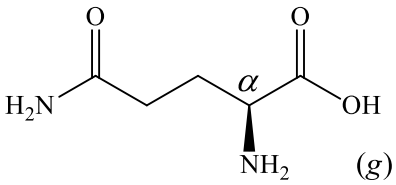
Structure	Name	Abbereviation	pK_{a1} α-CO_2H	pK_{a2} α-NH_3^+	pK_{a3} R-group	pI
	Cysteine (*g*)	C or Cys	1.7	10.8	8.3	5.0
	* Cystine (*g*)	Cys-Cys				
	Methionine (*g*, *e*)	M or Met	2.3	9.2	-	5.8
	Asparagine (*l*)	N or Asn	2.0	8.8	-	5.4
 (*g*)	Glutamine	Q or Gln	2.2	9.1	-	5.7

Table 2: Acidic α-Amino acids

Side Chains Containing an Acidic (Carboxyl) Group

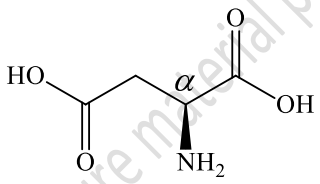
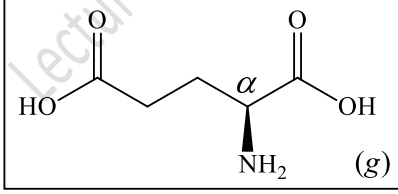
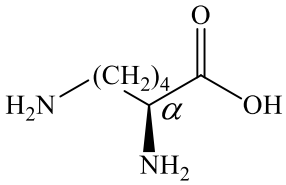
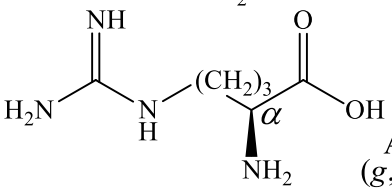
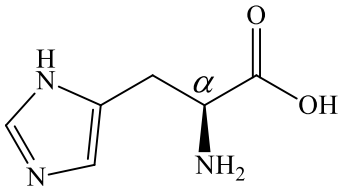
Structure	Name	Abbereviation	pK_{a1} α-CO_2H	pK_{a2} α-NH_3^+	pK_{a3} R-group	pI
 (*g*)	Aspartic acid	D or Asp	2.1	9.8	3.9	3.0
 (*g*)	Glutamic acid	E or Glu	2.2	9.7	4.3	3.2

Table 3: Basic α-Amino acids

Side Chains Containing a Basic (Amino) Group

Structure	Name	Abbereviation	pK_{a1} α-CO_2H	pK_{a2} α-NH_3^+	R-group	pI
	Lysine (*g*, *e*)	K or Lys	2.2	9.0	10.5[a]	9.8
	Arginine (*g*, *e*)	R or Arg	2.2	9.0	12.5[a]	10.8
	Histidine (*g*, *e*)	H or His	1.8	9.2	6.0[a]	7.6

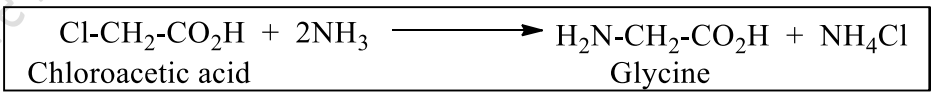
[a]pKa is of protonated amine of R group

General Methods of Preparation of the α-Amino Acids

There are many general methods for preparing α-Amino Acids, but usually each method applies to a small number of particular acids. Many acids are also synthesized by methods special to an individual. It should also be noted that very often a synthesis is a more convenient way of preparing an amino acid than preparing it from natural sources.

Method I: α-Amino Acids by amination of α-halogenated acids

An α-chloro- or bromo acid on treatment with concentrated ammonia is converted into an α-amino acid. The necessary α-halo acid can be prepared through the H. V. Z. reaction.



This method is convenient for the preparation of glycine, alanine, serine, threonine, valine, and leucine.

Method II: α-Amino Acids by Gabriel's Method

The yields obtained by the direct amination of α-halogenated acids are variable because of side reactions. Better yields are obtained by using Gabriel's phthalimide synthesis with α-halogeno acids or the corresponding esters.

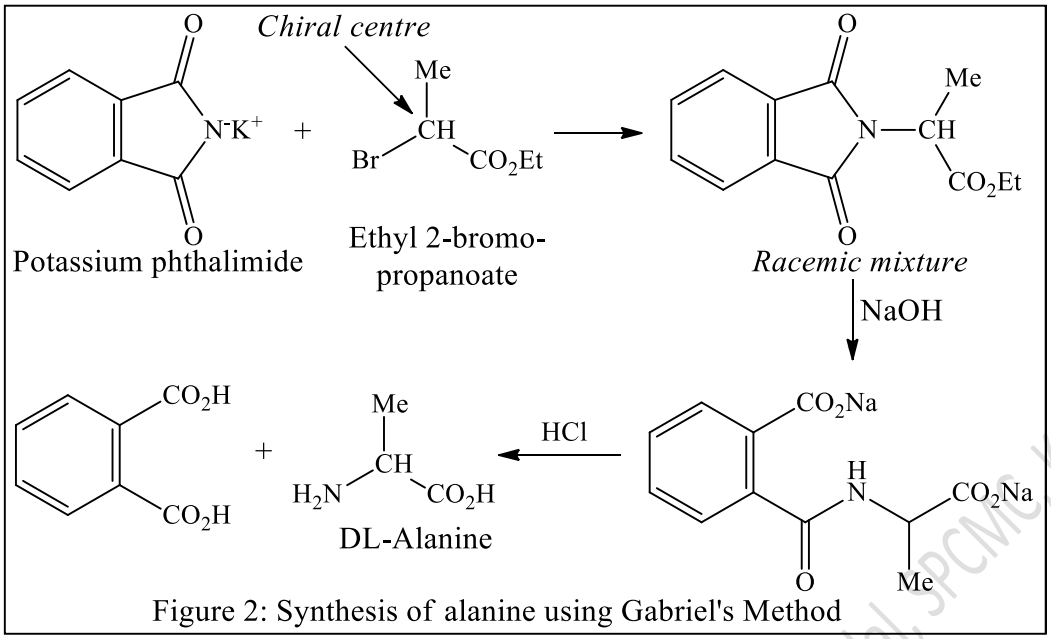


Figure 2: Synthesis of alanine using Gabriel's Method

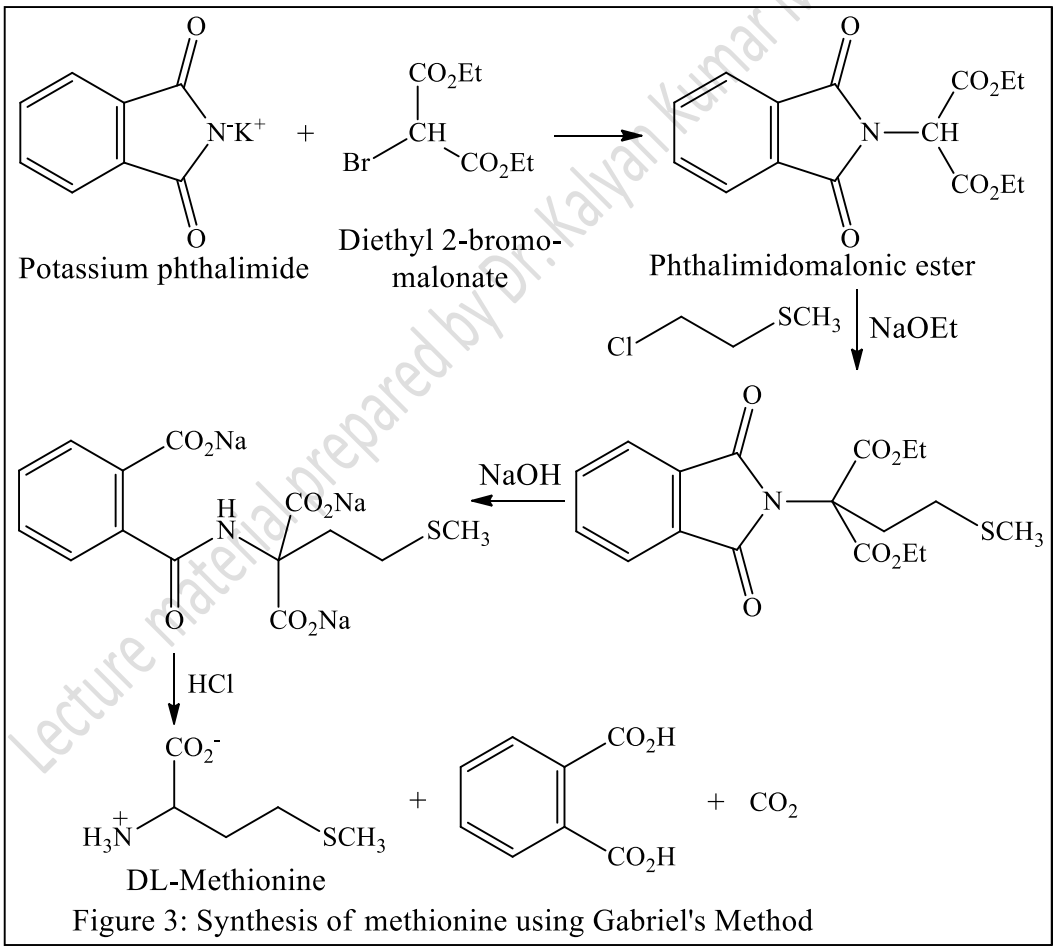


Figure 3: Synthesis of methionine using Gabriel's Method

Method III: The Strecker Synthesis

Treating an aldehyde with concentrated ammonia and hydrogen cyanide produces an α-aminonitrile. Hydrolysis of the nitrile group of the α-aminonitrile converts the latter to an α-amino acid. This synthesis is called the Strecker synthesis (Figure 4).

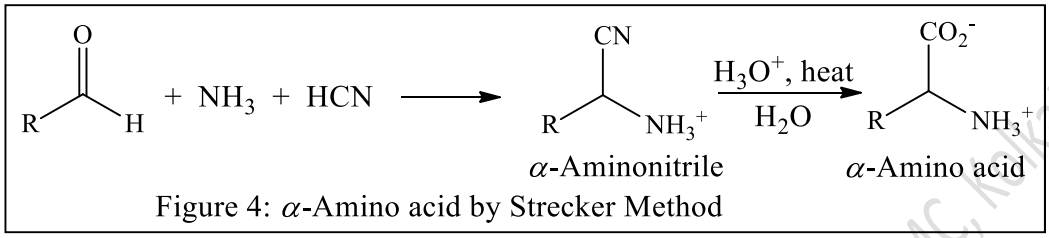


Figure 4: α-Amino acid by Strecker Method

The first step of this synthesis involves the initial formation of an imine from the aldehyde and ammonia followed by the addition of hydrogen cyanide. In practice, the aminonitrile is usually prepared from the oxo compound in one step by treating the latter with an equimolecular mixture of ammonium chloride and potassium cyanide (this mixture is equivalent to ammonium cyanide).

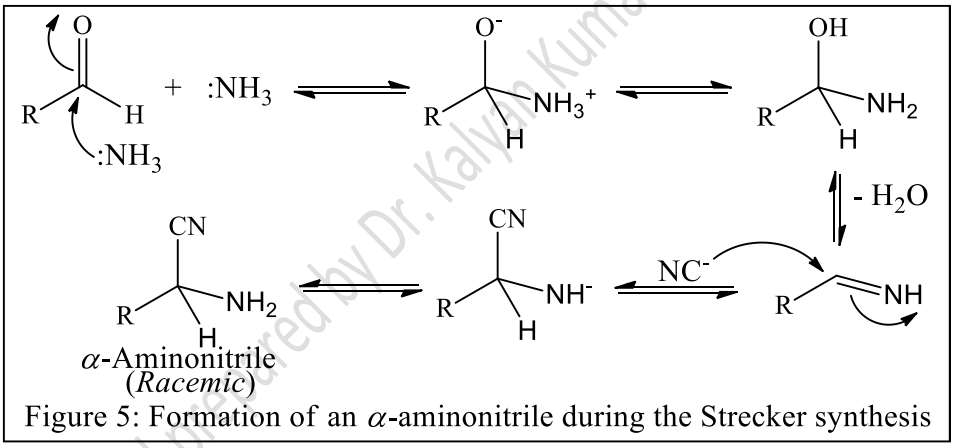


Figure 5: Formation of an α-aminonitrile during the Strecker synthesis

Optically active α-amino acids have been prepared when the reaction is carried out in the presence of an optically active base, e.g., an alkaloid.

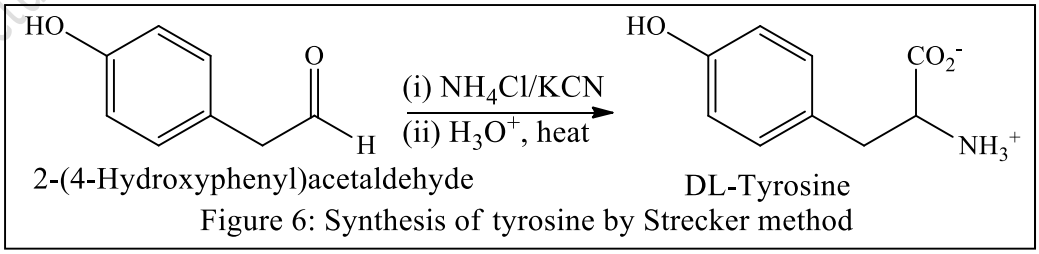


Figure 6: Synthesis of tyrosine by Strecker method

This method is useful for preparing the following amino acids: glycine, alanine, serine, methionine, glutamic acid, and phenylalanine.

Method IV: Malonic Ester Synthesis

This method is practically an extension of Method I. It offers a means of preparing α-halogeno acids as shown in Figure 7.

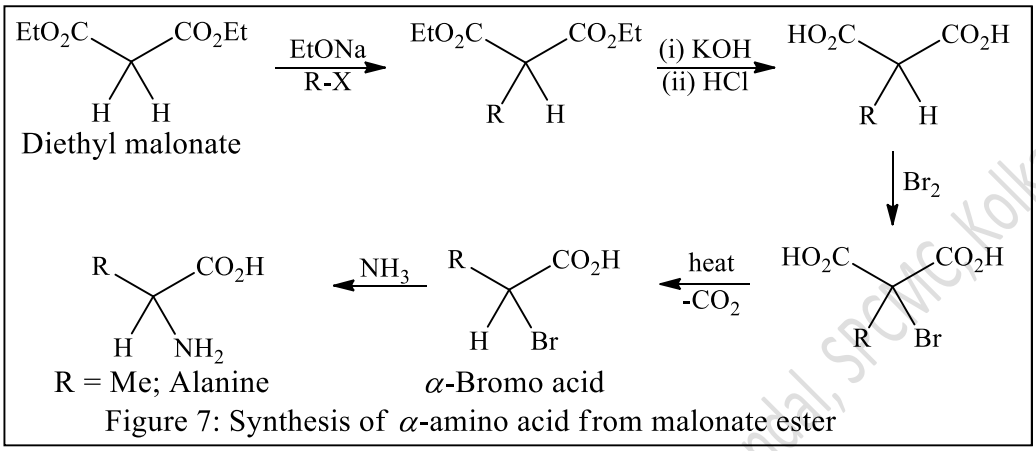


Figure 7: Synthesis of α-amino acid from malonate ester

This method offers a means of preparing, from readily accessible materials, the following amino acids: phenylalanine, proline, leucine, isoleucine, and methionine.

Synthesis Involving Phthalimido Derivative of Malonic Ester

The malonic ester synthesis may also be obtained with the Gabriel phthalimide synthesis to prepare phenylalanine, tyrosine, proline, cysteine, serine, aspartic acid, lysine, and methionine. Phthalimido derivative of malonic ester is conveniently prepared by the reaction between potassium phthalimide and α-bromo ester as shown in Figure 8. Synthesis of *racemic*-cystine and racemic-proline are shown in Figures 9 and 10.

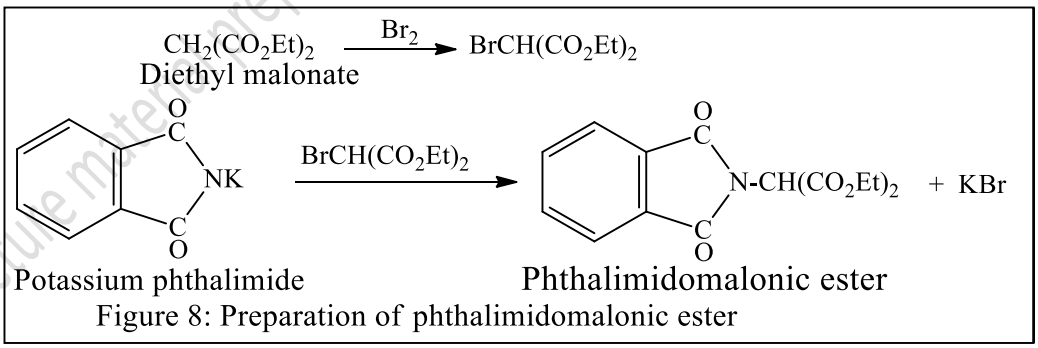


Figure 8: Preparation of phthalimidomalonic ester

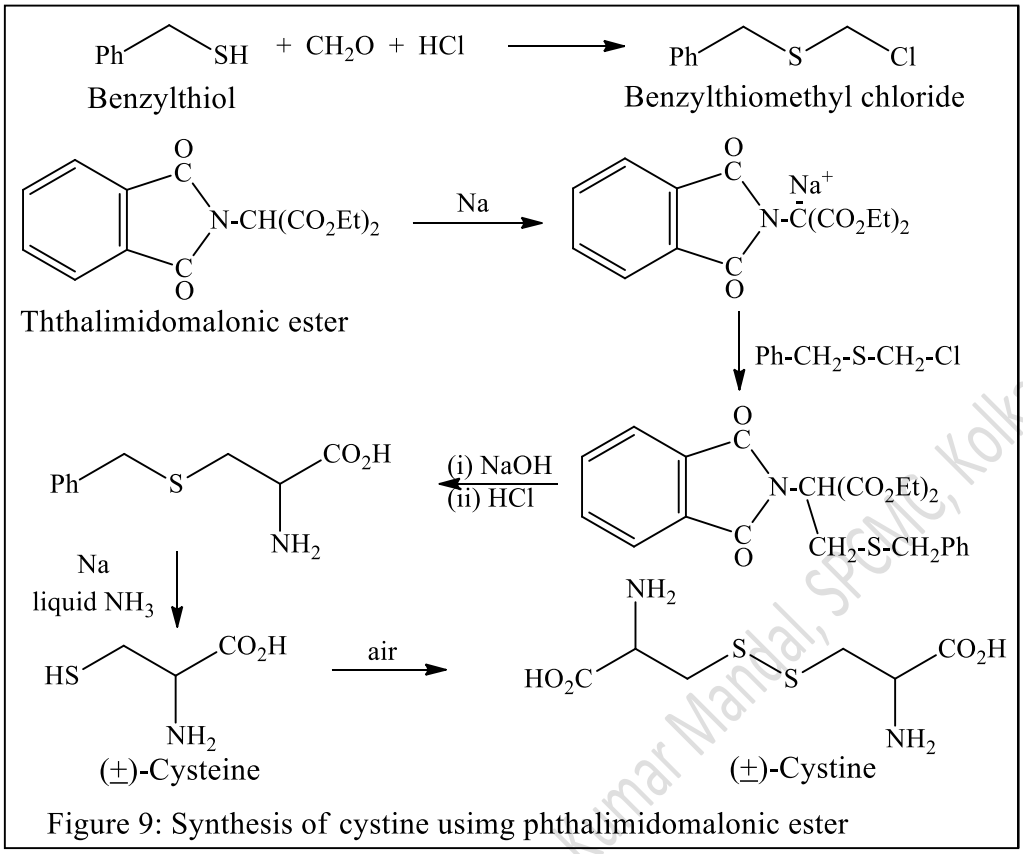


Figure 9: Synthesis of cystine usimg phthalimidomalonic ester

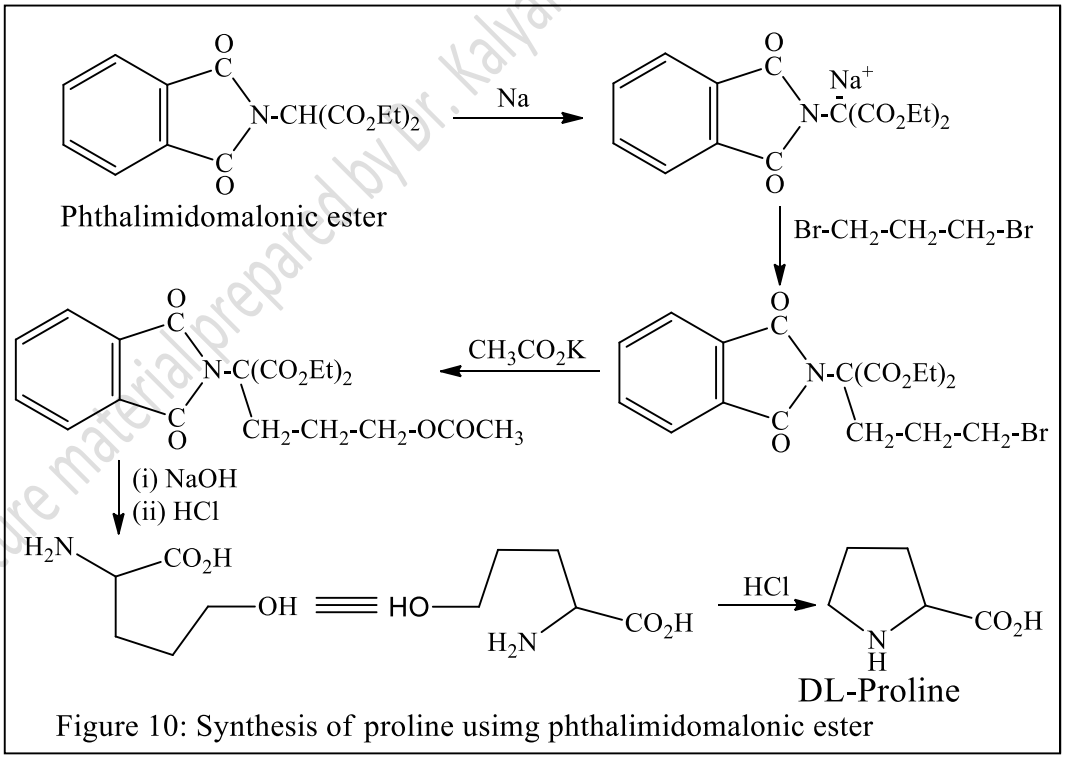


Figure 10: Synthesis of proline usimg phthalimidomalonic ester

Synthesis Involving Acylamido Derivative of Malonic Ester

Acylamido derivatives of malonic ester may also be used to synthesize amino acids. The usual derivative employed is ethyl acetamidomalonate whose preparation is shown in Figure 11.

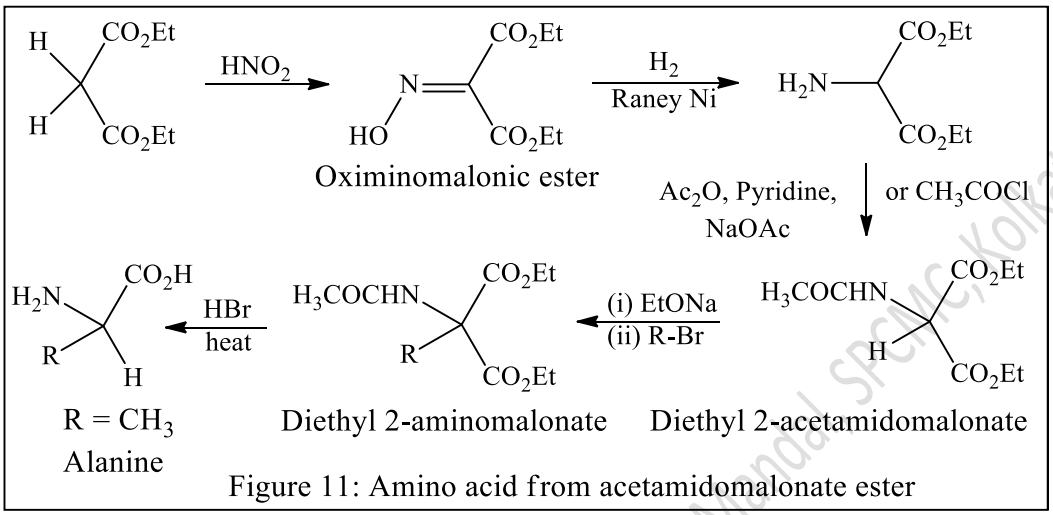


Figure 11: Amino acid from acetamidomalonate ester

The following acids may be prepared by this method: serine, leucine, valine, methionine, lysine, and glutamic acid. An application of this method is the preparation of DL-tryptophan from benzamidomalonic ester and gramine methosulfate (Figure 12).

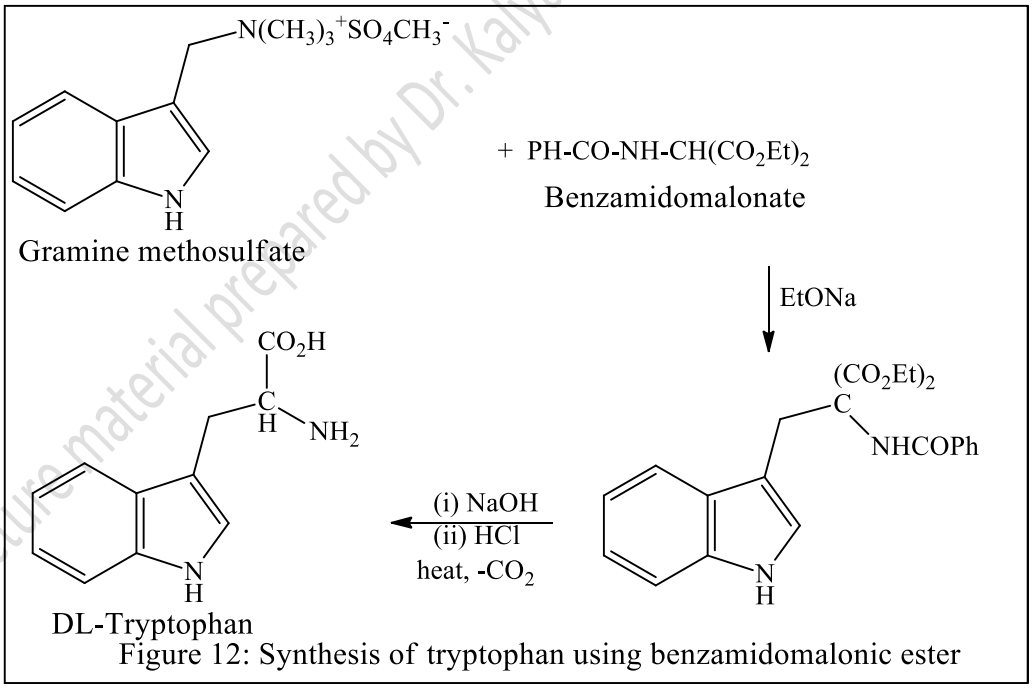


Figure 12: Synthesis of tryptophan using benzamidomalonic ester

Method V: Erlenmeyer Azlactone Synthesis

Azlactones are usually prepared by heating an aromatic aldehyde, with hippuric acid (benzoylglycine) (B) in the presence of acetic anhydride and sodium acetate, e.g., benzaldehyde (A) forms benzoyl-*α*-aminocinnamic azlactone (4-benzylidene-2-phenyloxazol-5-one) (C). This reaction is usually referred to as the Erlenmeyer azlactone synthesis.

Aceturic (acetylglycine) may also be used instead of hippuric acid. Again, it has been found that aliphatic aldehydes may condense with hippuric acid to form azlactones if lead acetate is used instead of sodium acetate. When azlactones (C) are warmed with sodium hydroxide (1%) solution, the ring is opened, and if the product is reduced with sodium amalgam by hydrolysis with acid, an *α*-amino acid is produced (Figure 13).

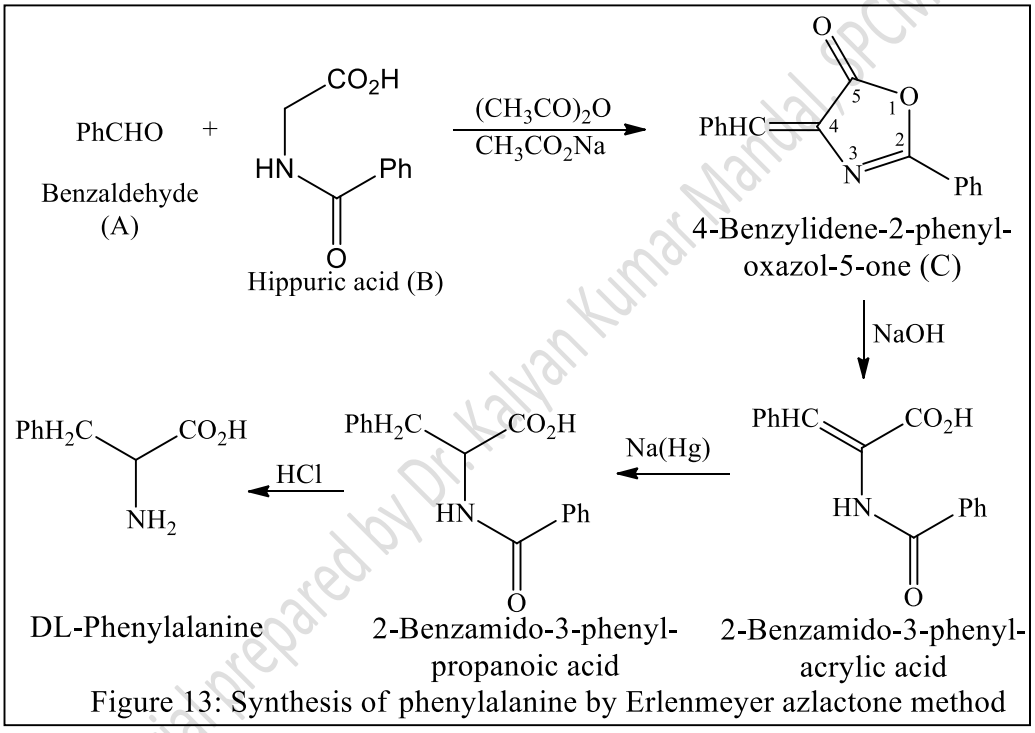


Figure 13: Synthesis of phenylalanine by Erlenmeyer azlactone method

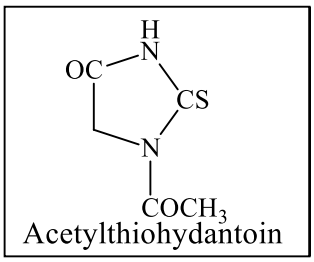
The azlactone synthesis offers a convenient means of preparing phenylalanine, tyrosine, and tryptophan.

Method VI: *α*-Amino Acids Using Hydantoins

Aromatic aldehydes condense with hydantoin, and reduction of the product with sodium amalgam or ammonium hydrogen sulfide, followed by hydrolysis, gives an *α*-amino acid. Tryptophan may be prepared by first converting indole into indole-3-aldehyde (A) by means of the Reimer-Tiemann-reaction.

Indole-3-aldehyde (A) on reacting with hydantoin followed by reduction with sodium (amalgam) and subsequent hydrolysis gives *racemic*-tryptophan (E). The reactions are illustrated in Figure 14. This method has been improved by using acetylthiohaydantoin

instead of hydantoin. This method may be used to prepare phenylalanine, tyrosine, tryptophan and methionine.



Acetylthiohydantoin

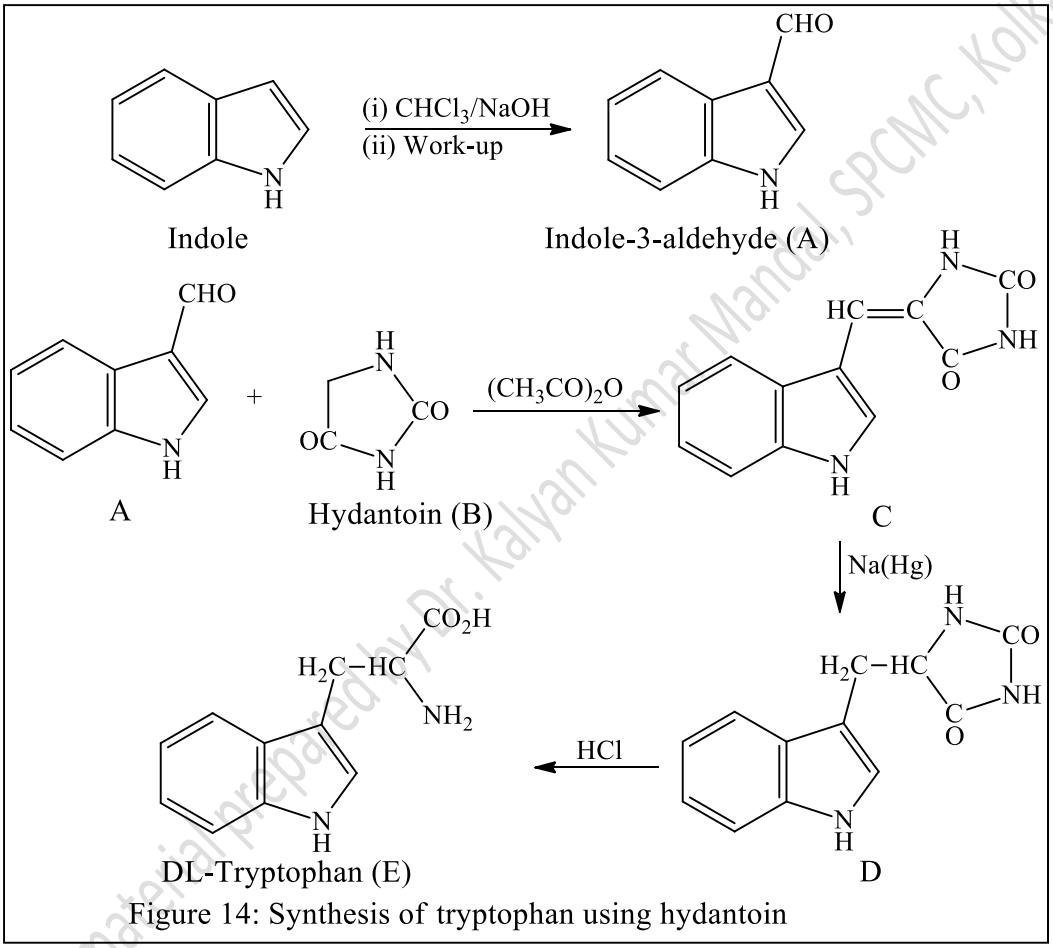


Figure 14: Synthesis of tryptophan using hydantoin

Method VII: Bücherer Hydantoin Synthesis

A modification of the hydantoin synthesis is the Bücherer hydantoin synthesis. In this method an oxo compound is converted into the cyanohydrin and this, on treatment with ammonium carbonate, produces a 5-substituted hydantoin which, on hydrolysis, gives an α-amino acid (Figure 15). Therefore, Bucherer-Bergs reaction is the chemical reaction of carbonyl compounds (aldehydes or ketones) or cyanohydrins with ammonium carbonate and potassium cyanide to give hydantoins.

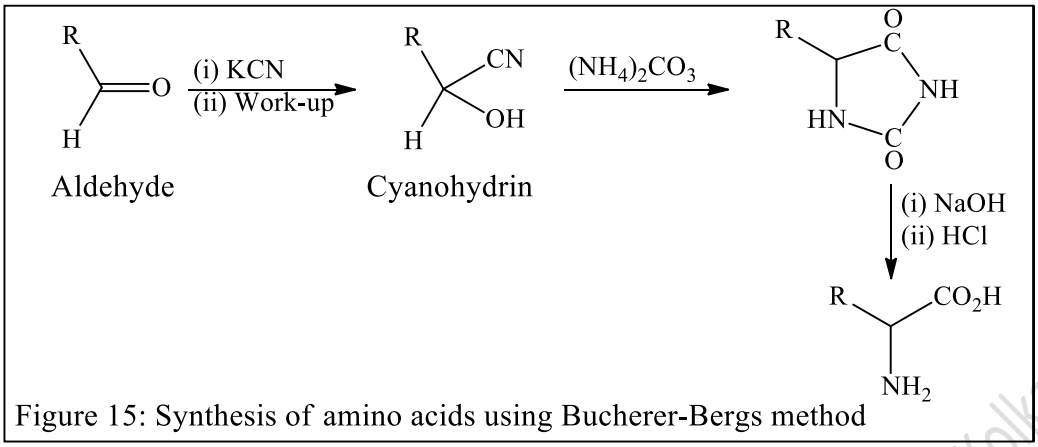


Figure 15: Synthesis of amino acids using Bucherer-Bergs method

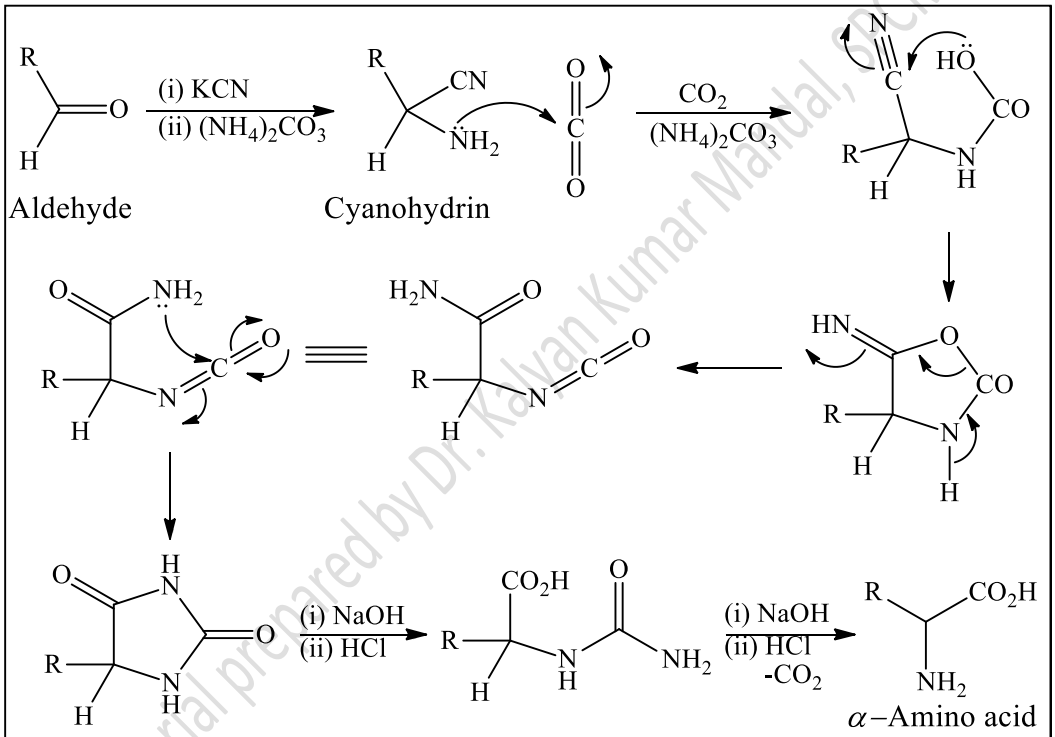


Figure 16: Mechanism of Bucherer-Bergs method

Method VIII: Synthesis of *α*-Amino Acid Using 2,5-Diketopiperizine

Aromatic aldehydes may be condensed with diketopiperazine, and the product converted into an amino acid by heating with hydroiodic acid and red phosphorous.

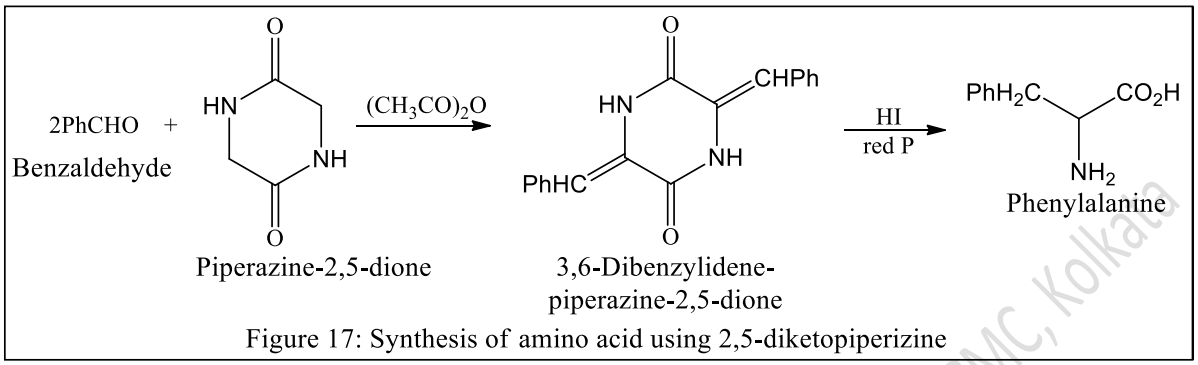


Figure 17: Synthesis of amino acid using 2,5-diketopiperizine

Resolution of DL-Amino Acids Enzymatic Method

With the exception of glycine, which has no chirality centre, the *α*-amino acids that are produced by the methods outlined so far are all produced as *racemic* forms. In order to obtain the naturally occurring L-amino acid, the *racemic* mixture obtained using different methods is to be resolved. This can be done in a variety of ways, including the enzymatic method.

One especially interesting method for resolving amino acids is based on the use of enzymes called *deacylases.* These enzymes catalyze the hydrolysis of *N-acylamino acids* in living organisms. Since the active site of the enzyme is *chiral*, it hydrolyzes only *N*-acylamino acids of the L configuration. When it is exposed to a *racemic* mixture of *N*-acylamino acids, only the derivative of the L-amino acid is affected and the products, as a result, are separated easily as shown in Figure 18.

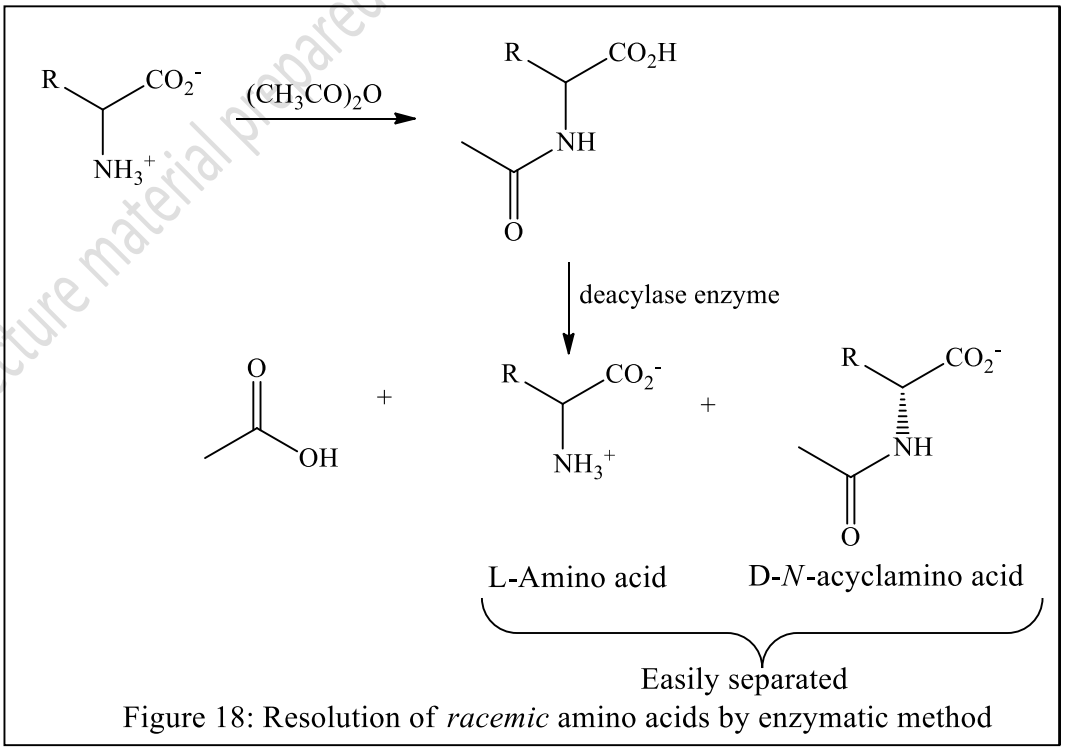


Figure 18: Resolution of *racemic* amino acids by enzymatic method