COURSE : SC202 (CHEMISTRY)

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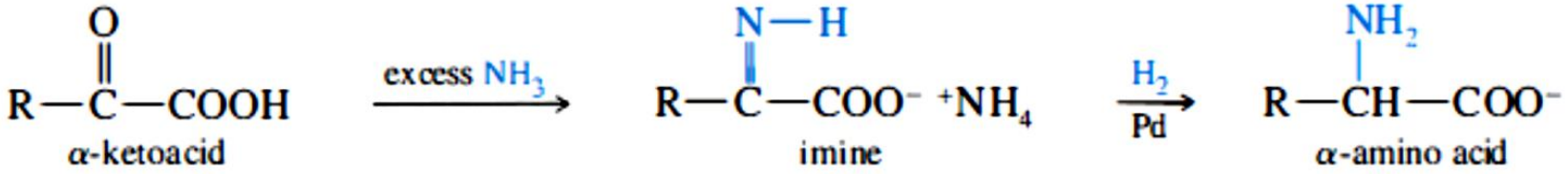
LECTURE-8

DATE: 25.1.2021

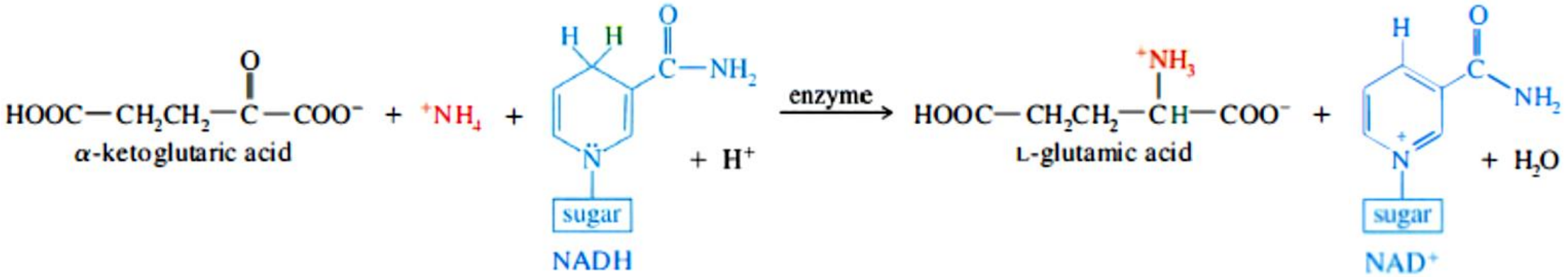
Synthesis of Amino Acids

1. Reductive Amination

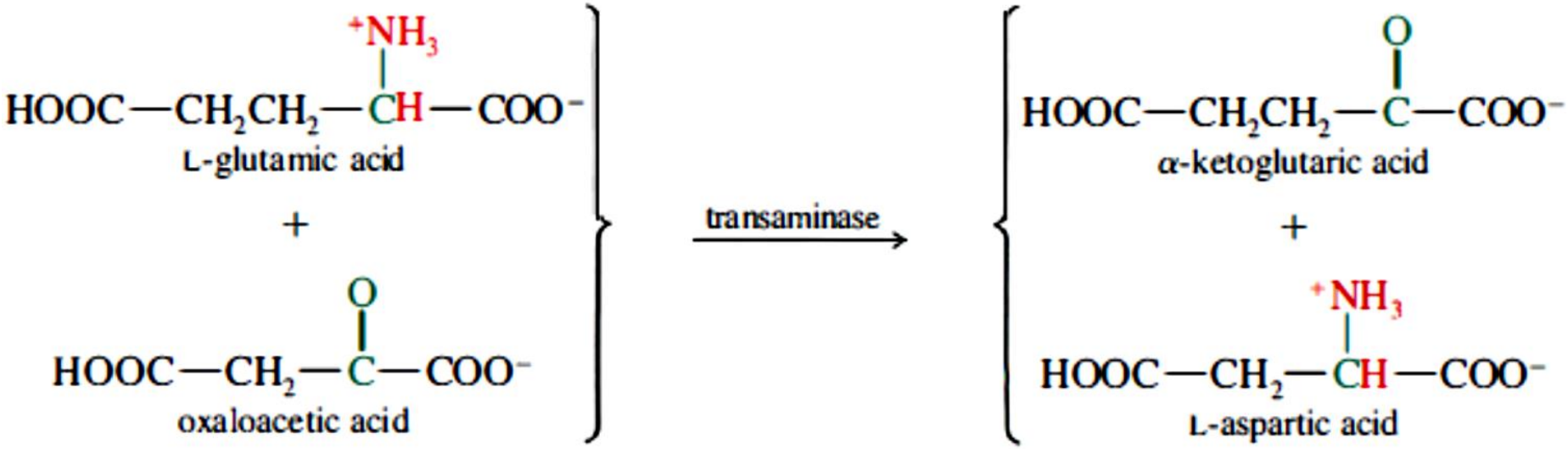
When an α-ketoacid is treated with ammonia, the ketone reacts to form an imine. The imine is reduced to an amine by hydrogen and a palladium catalyst. Under these conditions, the carboxylic acid is not reduced



The biosynthesis begins with reductive amination of a-ketoglutaric acid (an intermediate in the metabolism of carbohydrates), using ammonium ion as the aminating agent and NADH as the reducing agent. The product of this enzyme-catalyzed reaction is the pure L-enantiomer of glutamic acid.

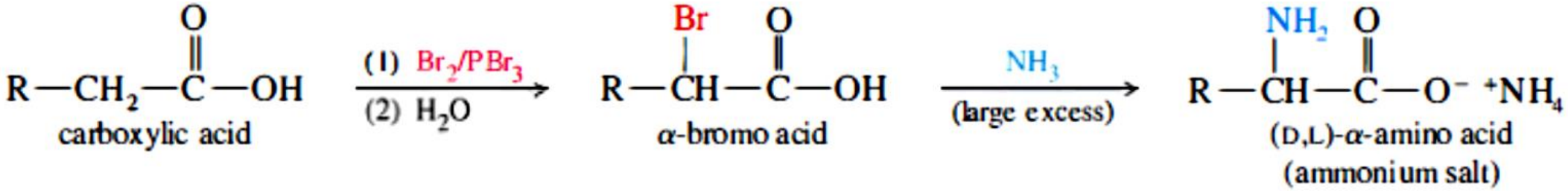


Biosynthesis of other amino acids uses L-glutamic acid as the source of the amino group. Such a reaction, moving an amino group from one molecule to another, is called a transamination, and the enzymes that catalyze these reactions are called transaminases. For example, the following reaction shows the biosynthesis of aspartic acid using glutamic acid as the nitrogen source. Once again, the enzyme-catalyzed biosynthesis gives the pure L-enantiomer of the product.



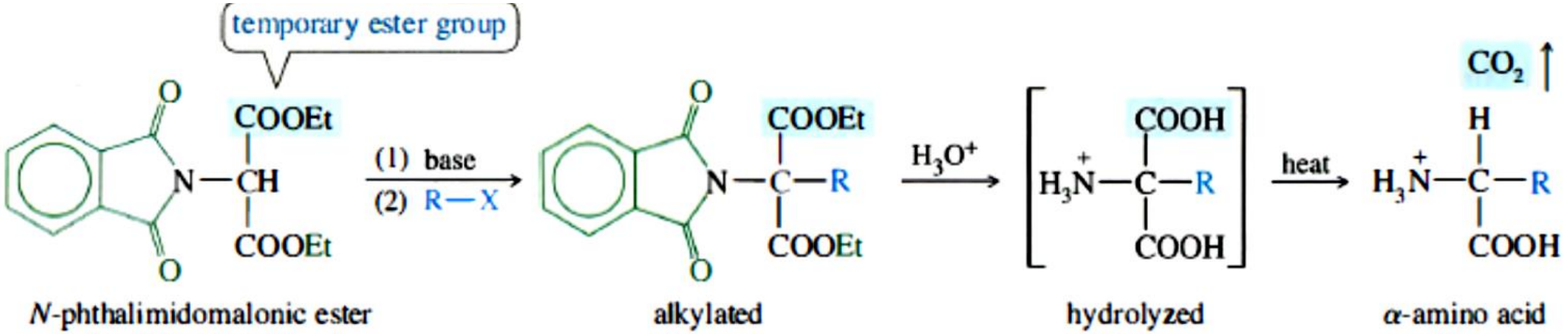
Amination of an α-Halo Acid

The Hell-Volhard-Zelinsky reaction is an effective method for introducing bromine at the α position of a carboxylic acid. The racemic α-bromo acid is converted to a racemic α-amino acid by direct amination, using a large excess of ammonia.



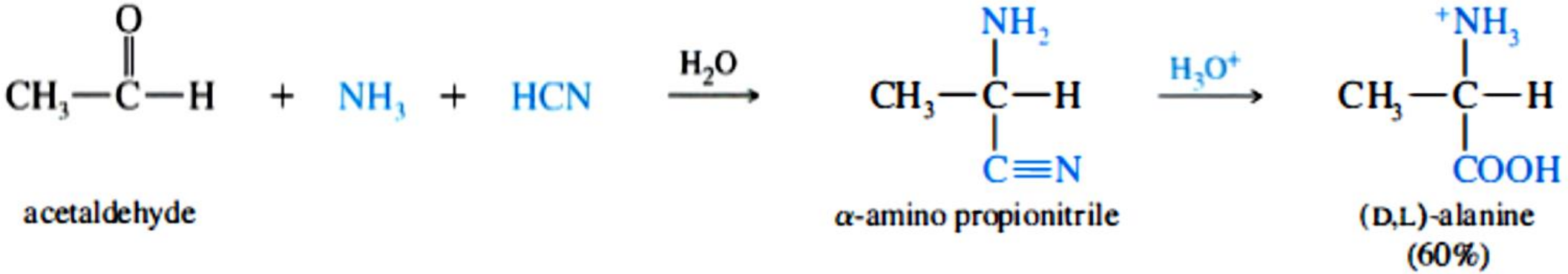
The Gabriel-Malonic Ester Synthesis

One of the best methods of amino acid synthesis is a combination of the Gabriel synthesis of amines with the malonic ester synthesis of carboxylic acids



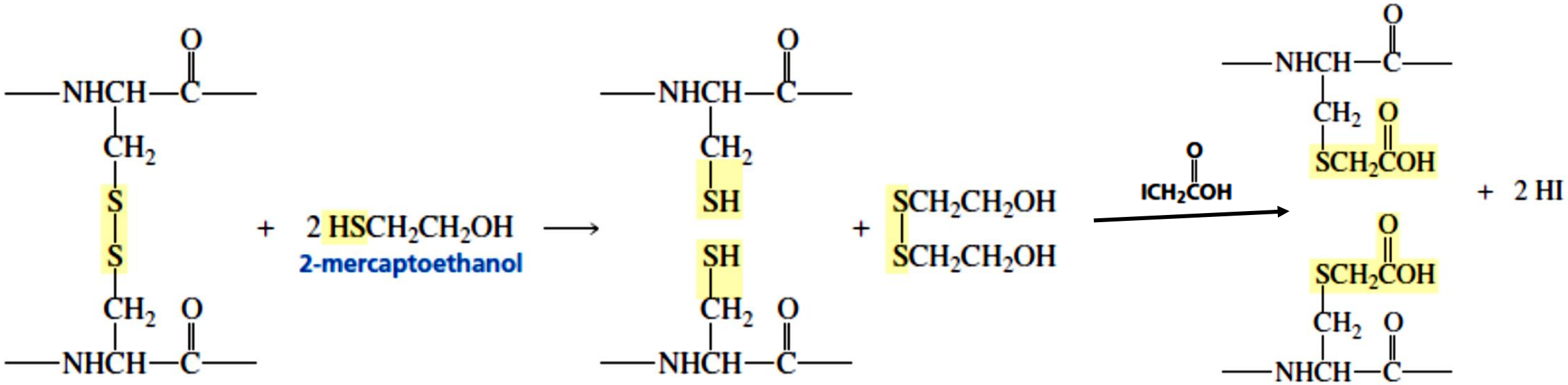
The Strecker Synthesis

Strecker added acetaldehyde to an aqueous solution of ammonia and HCN. The product was α-amino propionitrile, which Strecker hydrolyzed to racemic alanine.

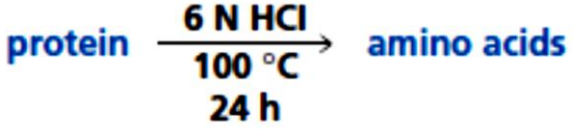


Determining the Primary Structure of a Protein

1. The first step is to reduce any disulfide bridges in the peptide or protein. A commonly used reducing agent is 2-mercaptoethanol, which is oxidized to a disulfide. Reaction of the protein thiol groups with iodoacetic acid prevents the disulfide bridges from reforming as a result of oxidation by O_2.



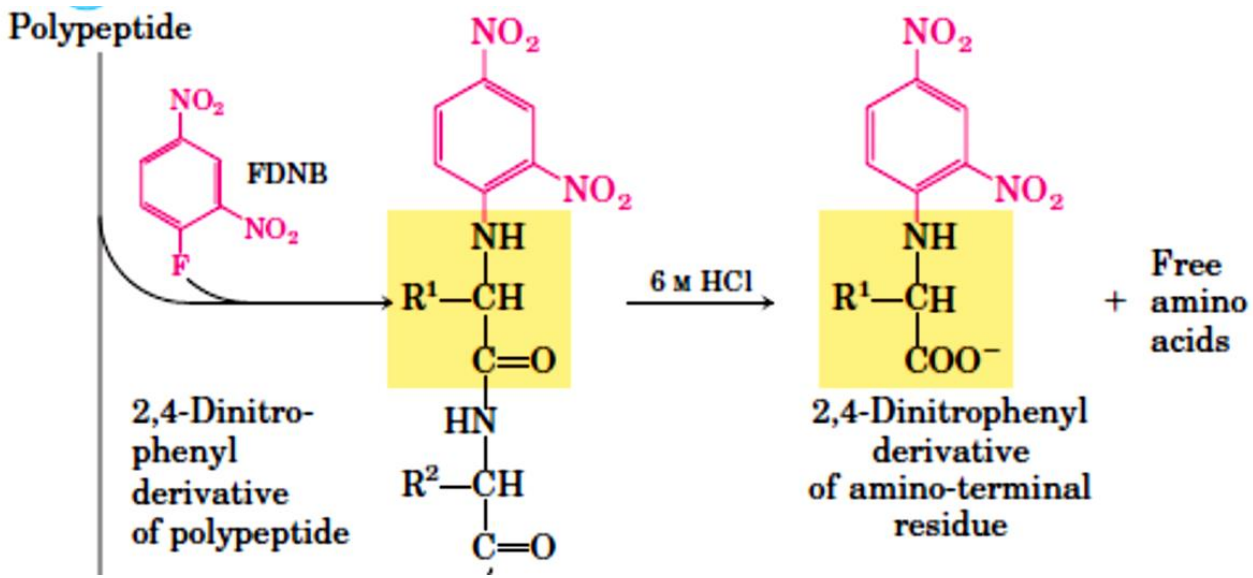
2.The next step is to determine the number and kinds of amino acids in the peptide or protein. To do this, a sample of the peptide or protein is dissolved in 6 N HCl and heated at 100 °C for 24 hours. This treatment hydrolyzes all the amide bonds in the protein, including the amide bonds of asparagine and glutamine.

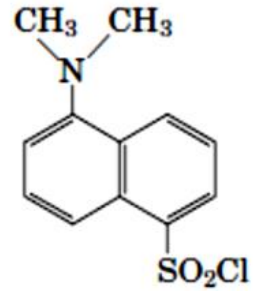


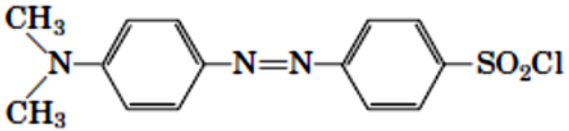
Diadvantages:

1. Because all the asparagine and glutamine residues have been hydrolyzed to aspartate and glutamate residues, the number of aspartate or glutamate residues in the amino acid mixture tells us the number of aspartate plus asparagine—or glutamate plus glutamine—residues in the original protein.
2. The strongly acidic conditions used for hydrolysis destroy all the tryptophan residues because the indole ring is unstable in acid. The tryptophan content of the protein can be determined by hydroxide-ion-promoted hydrolysis of the protein.

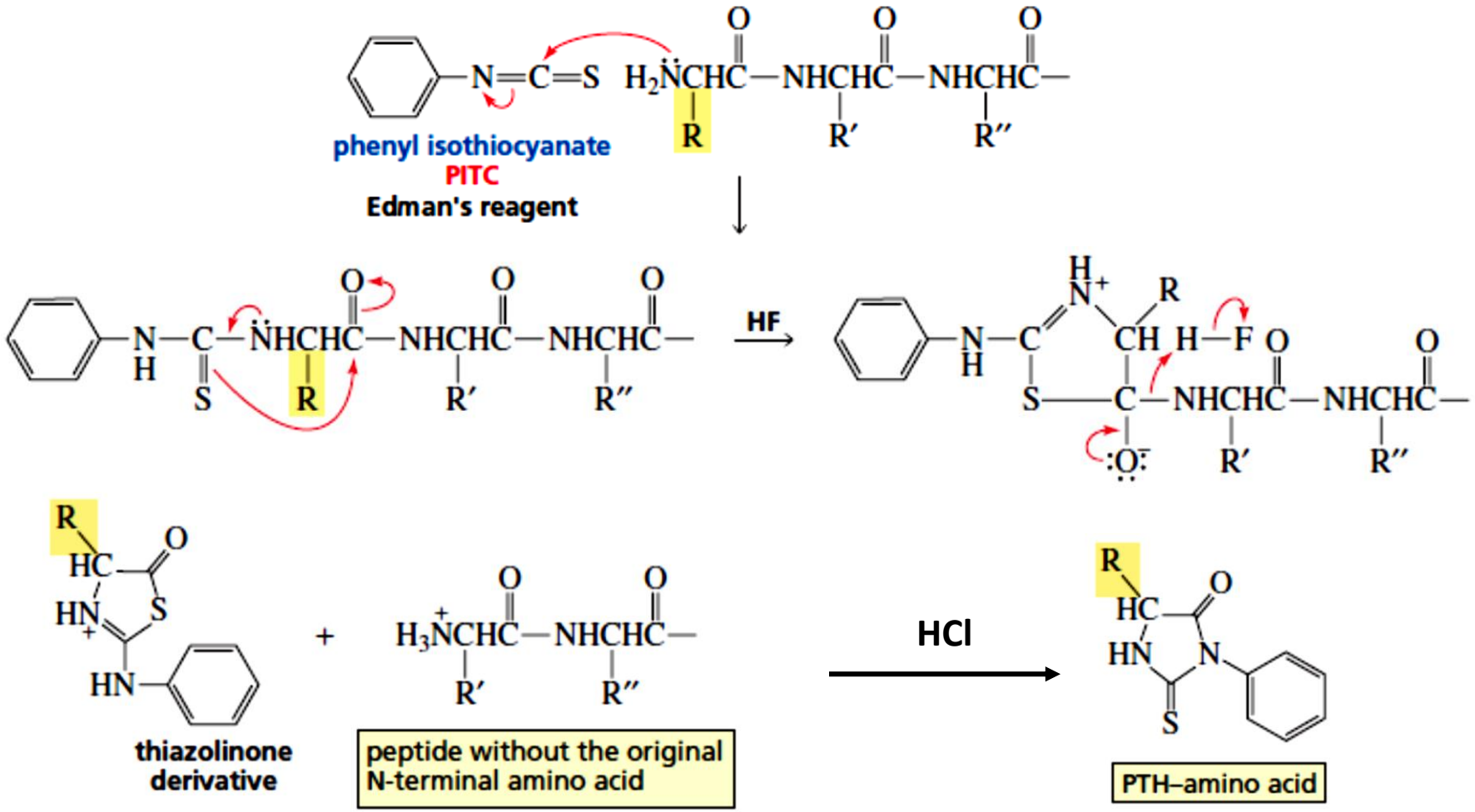
To label and identify the amino-terminal amino acid residue, Sanger developed the reagent 1-fluoro-2,4-dinitrobenzene (FDNB). Other reagents used to label the amino-terminal residue, dansyl chloride and dabsyl chloride, yield derivatives that are more easily detectable than the dinitrophenyl derivatives. After the amino-terminal residue is labeled with one of these reagents, the polypeptide is hydrolyzed to its constituent amino acids and the labeled amino acid is identified. Because the hydrolysis stage destroys the polypeptide, this procedure cannot be used to sequence a polypeptide beyond its amino-terminal residue.







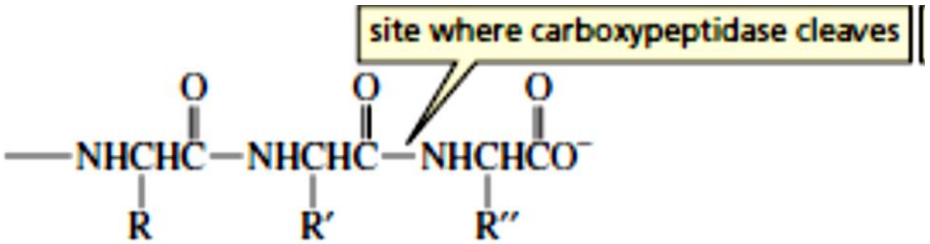
To sequence an entire polypeptide, a chemical method devised by Pehr Edman is usually employed. The **Edman degradation** procedure labels and removes only the amino-terminal residue from a peptide, leaving all other peptide bonds intact. In this method, protein is treated with phenyl isothiocyanate (PITC), more commonly known as **Edman's reagent**.This reagent reacts with the N-terminal amino group, and the resulting thiazolinone derivative is cleaved from the protein under mildly acidic conditions. The thiazolinone derivative is extracted into an organic solvent and in the presence of acid, rearranges to a more stable phenylthiohydantoin (PTH).



After removal and identification of the amino terminal residue, the *new* amino-terminal residue so exposed can be labeled, removed, and identified through the same series of reactions. This procedure is repeated until the entire sequence is determined. The Edman degradation is carried out on a machine, called a **sequenator**

C-Terminal Residue Analysis

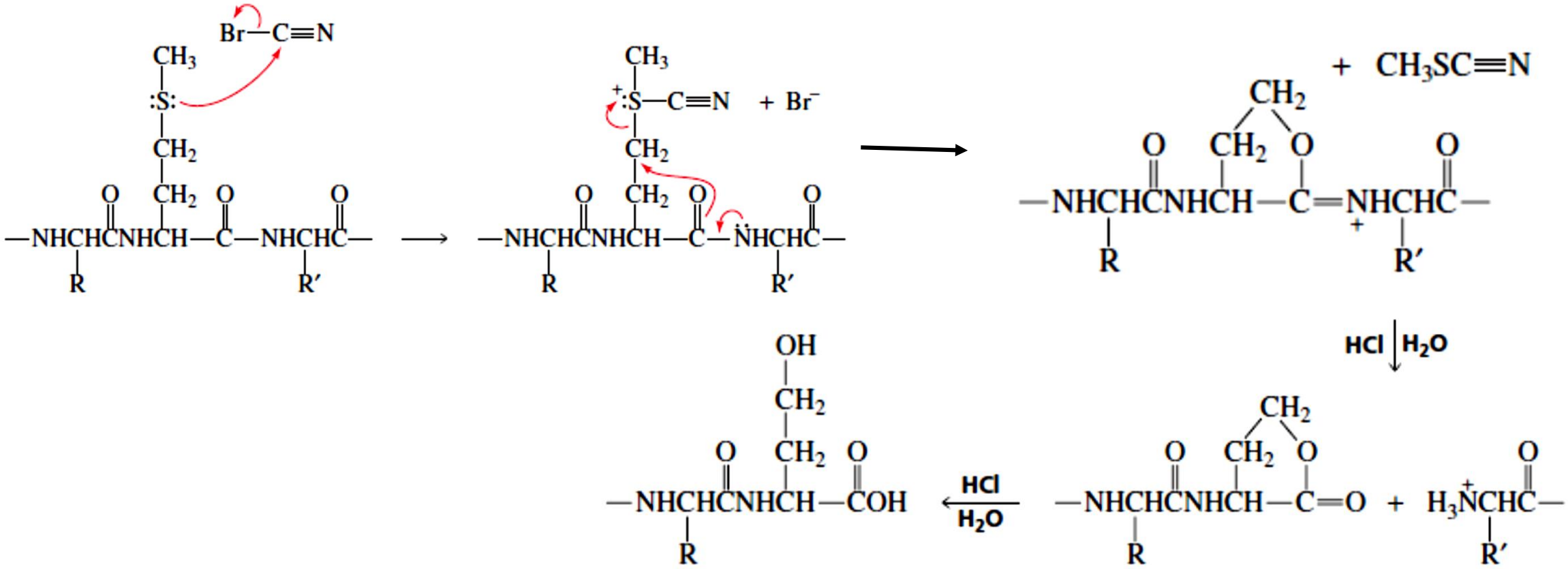
The C-terminal amino acid of the peptide or protein can be identified by treating the protein with carboxypeptidase A. Carboxypeptidase A cleaves off the C-terminal amino acid as long as it is *not* arginine or lysine. On the other hand, carboxypeptidase B cleaves off the C-terminal amino acid *only* if it is arginine or lysine. Carboxypeptidases are exopeptidases. An **exopeptidase** is an enzyme that catalyzes the hydrolysis of a peptide bond at the end of a peptide chain.



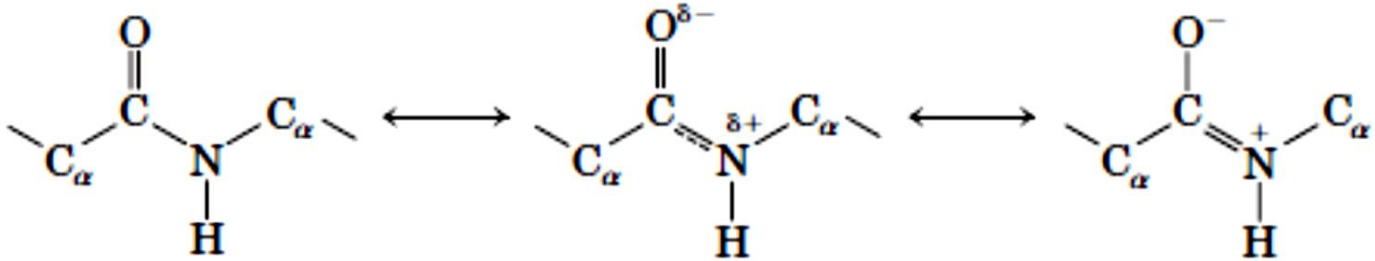
Once the N-terminal and C-terminal amino acids have been identified, a sample of the protein is hydrolyzed with dilute acid. This treatment, called **partial hydrolysis**, hydrolyzes only some of the peptide bonds. The resulting fragments are separated, and the amino acid composition of each is determined. The N-terminal and C-terminal amino acids of each fragment can also be identified. The sequence of the original protein can then be determined by lining up the peptides and looking for points of overlap.

The peptide or protein can also be partially hydrolyzed using endopeptidases. An **endopeptidase** is an enzyme that catalyzes the hydrolysis of a peptide bond that is not at the end of a peptide chain. Trypsin, chymotrypsin, and elastase are endopeptidases that catalyze the hydrolysis of only the specific peptide bonds

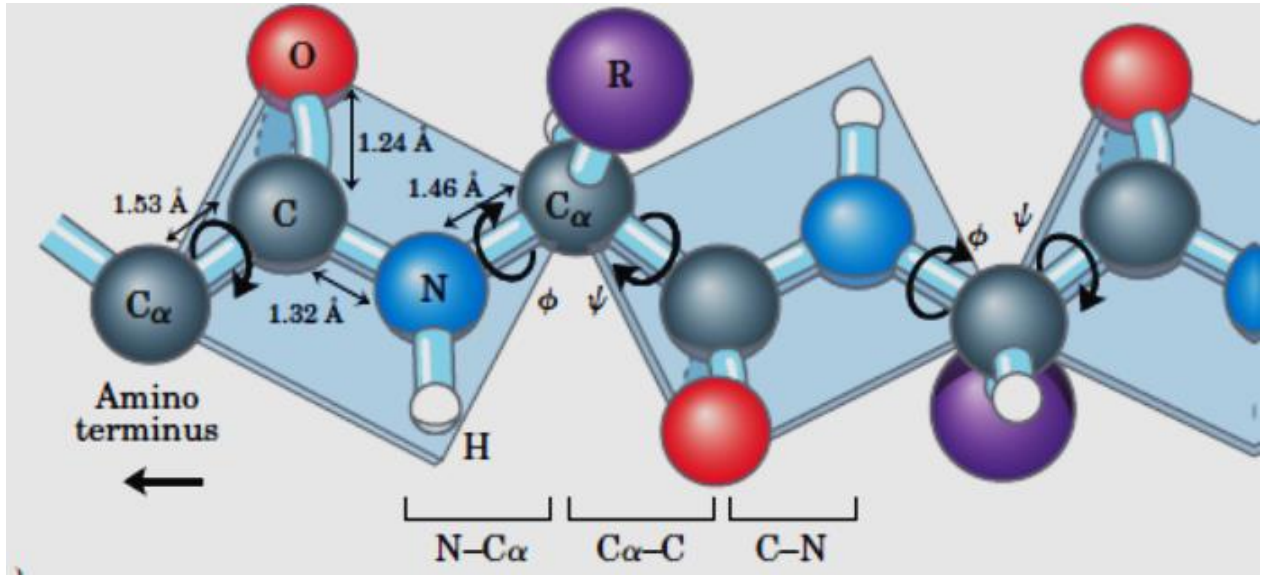
Cyanogen bromide causes the hydrolysis of the amide bond on the C-side of a methionine residue. Cyanogen bromide is more specific than the endopeptidases about what peptide bonds it cleaves, so it provides more reliable information about the primary structure. The first step in the mechanism for cleavage of a peptide bond by cyanogen bromide is attack by the highly nucleophilic sulfur of methionine on cyanogen bromide. Formation of a five-membered ring with departure of the weakly basic leaving group is followed by acid-catalyzed hydrolysis, which cleaves the protein.



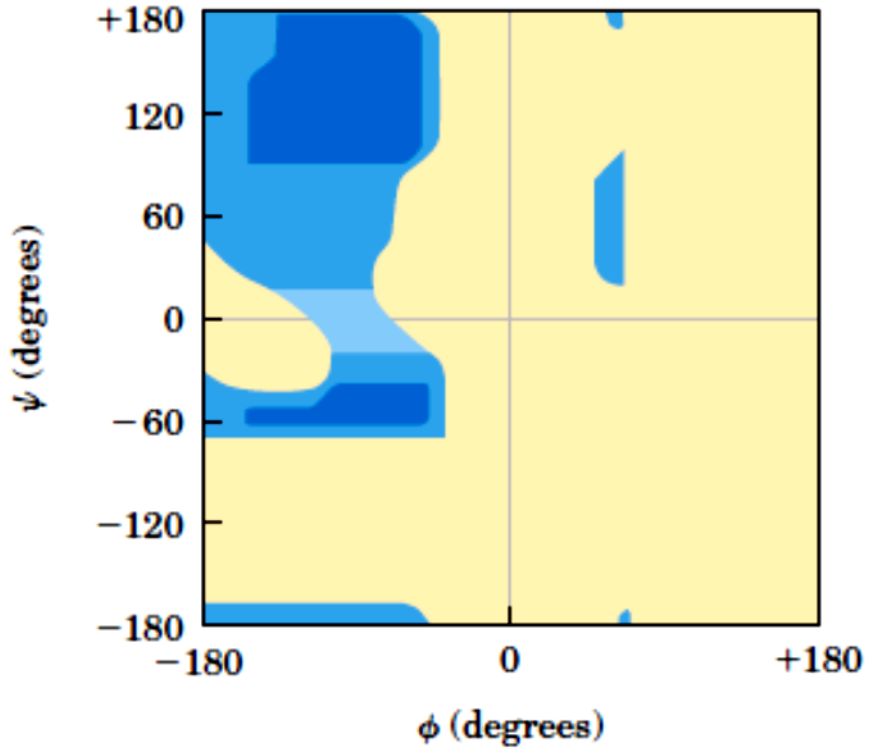
Ramachandran plot



The α carbons of adjacent amino acid residues are separated by three covalent bonds, arranged as C_α-C-N- C_α. Pauling and Corey concluded that the peptide C-N bonds are unable to rotate freely because of their partial double-bond character. Rotation is permitted about the N-C and the C-C bonds. The backbone of a polypeptide chain can thus be pictured as a series of rigid planes with consecutive planes sharing a common point of rotation at C.



By convention, the bond angles resulting from rotations at C are labeled ϕ (phi) for the N-C bond and ψ (psi) for the C-C bond. Again by convention, both ϕ and ψ are defined as 180° when the polypeptide is in its fully extended conformation and all peptide groups are in the same plane. In principle, ϕ and ψ can have any value between -180° and +180°, but many values are prohibited by steric interference between atoms in the polypeptide backbone and amino acid side chains. The conformation in which both ϕ and ψ are 0° is prohibited for this reason; this conformation is used merely as a reference point for describing the angles of rotation. Allowed values for ϕ and ψ are graphically revealed when ψ is plotted versus ϕ in a **Ramachandran plot**, introduced by G. N. Ramachandran.

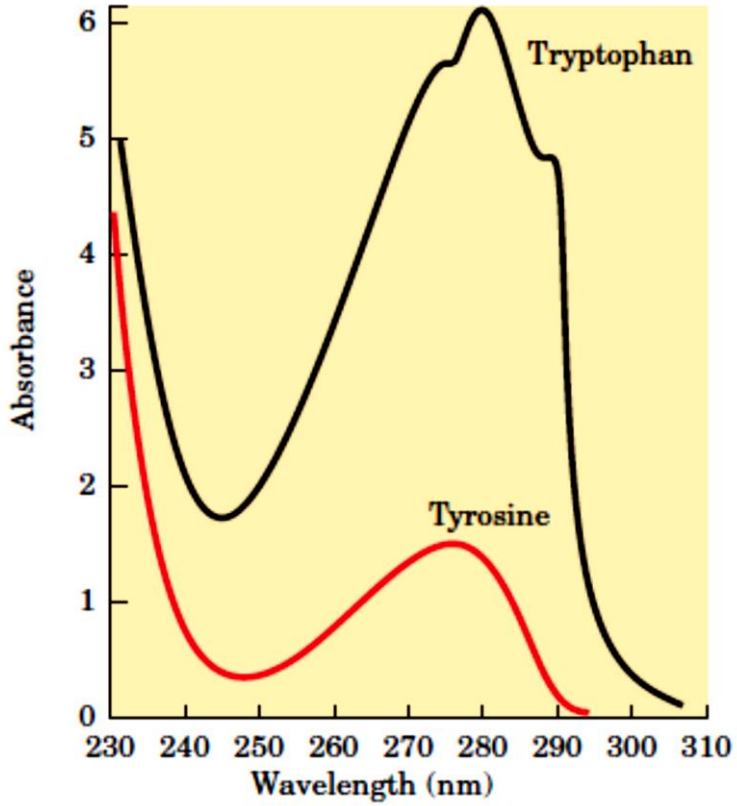


Ramachandran plot for L-Ala residues.

Conformations deemed possible are those that involve little or no steric interference, based on calculations using known van der Waals radii and bond angles. The areas shaded dark blue reflect conformations that involve no steric overlap and thus are fully allowed; medium blue indicates conformations allowed at the extreme limits for unfavorable atomic contacts; the lightest blue area reflects conformations that are permissible if a little flexibility is allowed in the bond angles.

UV absorbance properties of the Aromatic Amino Acids

Tryptophan and tyrosine, and to a much lesser extent phenylalanine, absorb ultraviolet light. This accounts for the characteristic strong absorbance of light by most proteins at a wavelength ofm280 nm, a property exploited by researchers in the characterization of proteins.



Absorption of ultraviolet light by aromatic amino acids.

Comparison of the light absorption spectra of the aromatic amino acids tryptophan and tyrosine at pH 6.0. The amino acids are present in equimolar amounts under identical conditions. The measured absorbance of tryptophan is as much as four times that of tyrosine.

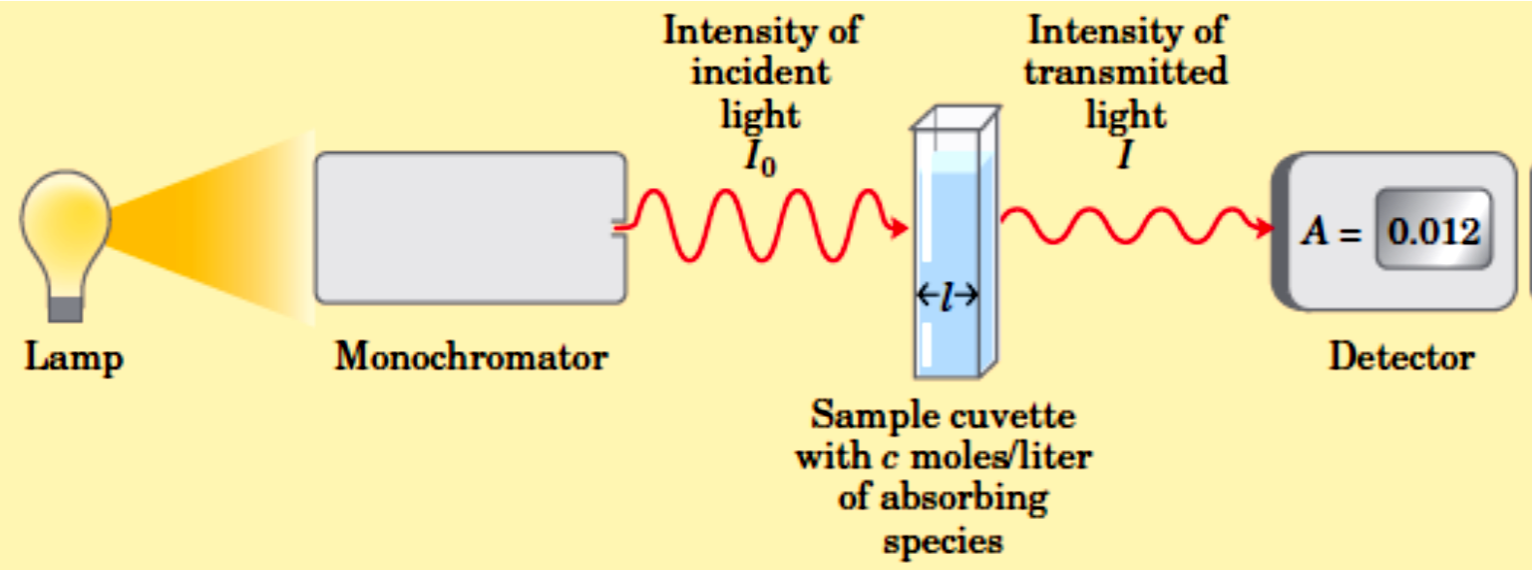
A wide range of biomolecules absorb light at characteristic wavelengths, just as tryptophan absorbs light at 280 nm. Measurement of light absorption by a spectrophotometer is used to detect and identify molecules and to measure their concentration in solution.

The fraction of the incident light absorbed by a solution at a given wavelength is related to the thickness of the absorbing layer (path length) and the concentration of the absorbing species. These two relationships are combined into the Lambert-Beer law,

$$\log (I_o / I) = \varepsilon c l$$

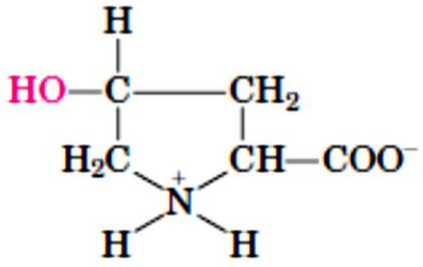
where I_0 is the intensity of the incident light, I is the intensity of the transmitted light, is the molar extinction coefficient (in units of liters per mole-centimeter), c is the concentration of the absorbing species (in moles per liter), and l is the path length of the light absorbing sample (in centimeters). The Lambert-Beer law assumes that the incident light is parallel and monochromatic (of a single wavelength) and that the solvent and solute molecules are randomly oriented. The expression $\log (I_0/I)$ is called the **absorbance,** designated A

*the absorbance,*A, *is directly proportional to the concentration of the absorbing solute*. The molar extinction coefficient varies with the nature of the absorbing compound, the solvent, and the wavelength, and also with pH if the light-absorbing species is in equilibrium with an ionization state that has different absorbance properties.

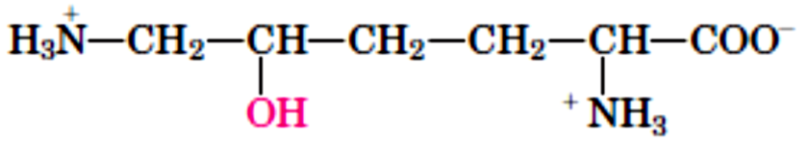


Uncommon Amino Acids

4-hydroxyproline, a derivative of proline, and **5-hydroxylysine,** derived from lysine. The former is found in plant cell wall proteins, and both are found in collagen, a fibrous protein of connective tissues

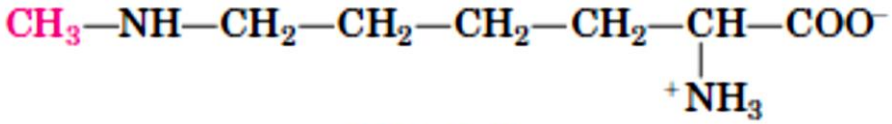


4-Hydroxyproline

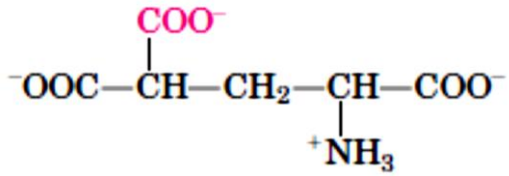


5-Hydroxylysine

6-*N* Methyllysine is a constituent of myosin, a contractile protein of muscle.
Another important uncommon amino acid is **γ-carboxyglutamate,** found in the bloodclotting protein prothrombin and in certain other proteins that bind Ca^{2+} as part of their biological function.

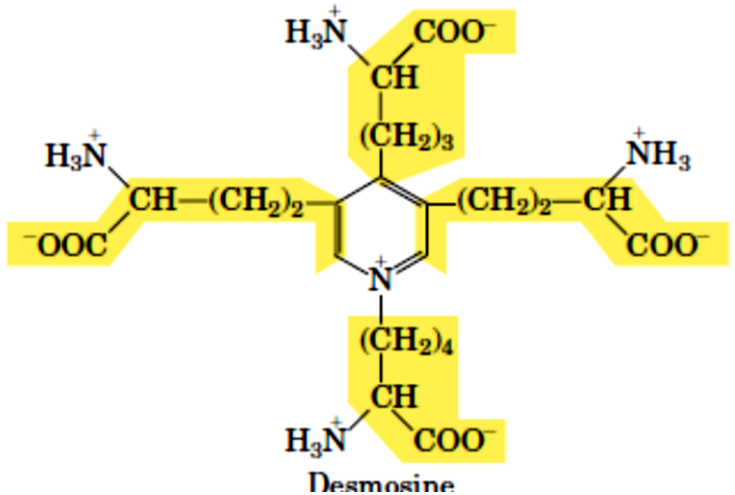


6-*N*-Methyllysine

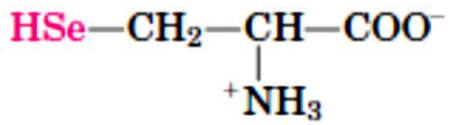


γ-Carboxyglutamate

Desmosine, a derivative of four Lys residues, which is found in the fibrous protein elastin.
Selenocysteine, a rare amino acid residue is introduced during protein synthesis. It contains selenium rather than the sulfur of cysteine.

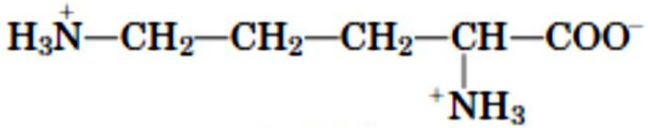


Desmosine

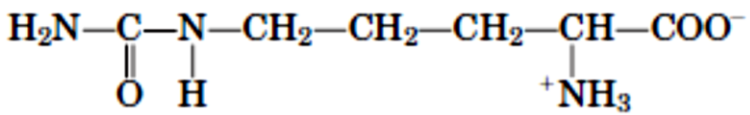


Selenocysteine

Ornithine and **citrulline** deserve special note because they are key intermediates (metabolites) in the biosynthesis of arginine and in the urea cycle



Ornithine



Citrulline

Enzymes

Enzymes are proteins that catalyse biological reactions. Some enzymes require no chemical groups for activity other than their amino acid residues. Others require an additional chemical component called a **cofactor**—either one or more inorganic ions or a complex organic or metalloorganic molecule called a **coenzyme**. Some enzymes require *both* a coenzyme and one or more metal ions for activity.

A coenzyme or metal ion that is very tightly or even covalently bound to the enzyme protein is called a **prosthetic group.**

A complete, catalytically active enzyme together with its bound coenzyme and/or metal ions is called a **holoenzyme.**

The protein part of such an enzyme is called the **apoenzyme** or **apoprotein.**

International Classification of Enzymes

No.	*Class*	*Type of reaction catalyzed*
1	Oxidoreductases	Transfer of electrons (hydride ions or H atoms)
2	Transferases	Group transfer reactions
3	Hydrolases	Hydrolysis reactions (transfer of functional groups to water)
4	Lyases	Addition of groups to double bonds, or formation of double bonds by removal of groups
5	Isomerases	Transfer of groups within molecules to yield isomeric forms
6	Ligases	Formation of C—C, C—S, C—O, and C—N bonds by condensation reactions coupled to ATP cleavage

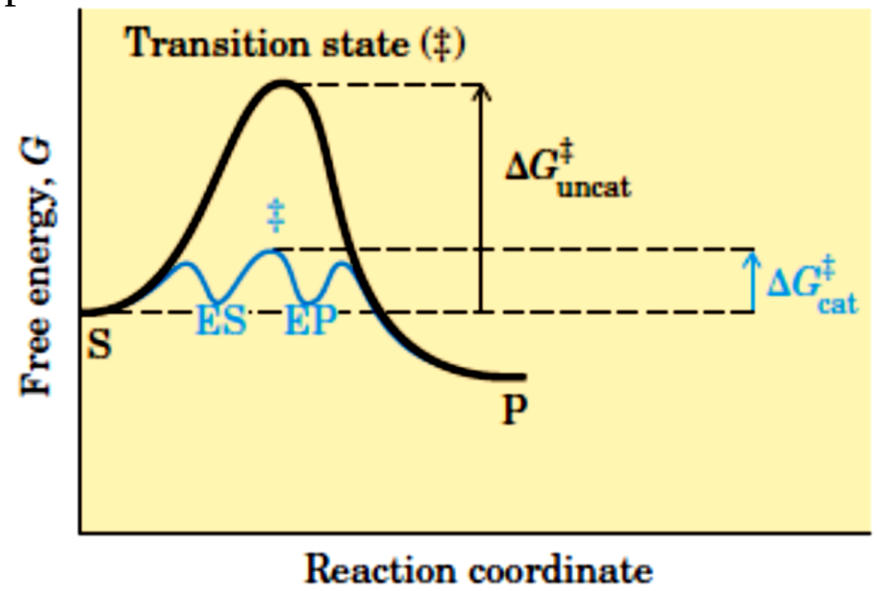
How Enzymes Work

Enzyme-catalyzed reaction takes place within the confines of a pocket on the enzyme called the **active site**. The molecule that is bound in the active site and acted upon by the enzyme is called the **substrate**

A simple enzymatic reaction might be written

$$E + S \rightleftharpoons ES \rightleftharpoons EP \rightleftharpoons E + P$$

where E, S, and P represent the enzyme, substrate, and product; ES and EP are transient complexes of the enzyme with the substrate and with the product.



Reaction coordinate diagram comparing enzyme catalyzed and uncatalyzed reactions. In the reaction S⟶P, the ES and EP intermediates occupy minima in the energy progress curve of the enzyme-catalyzed reaction. The terms $G^{\ddagger}_{uncat}$ *a*nd $G^{\ddagger}_{cat}$ correspond to the activation energy for the uncatalyzed reaction and the overall activation energy for the catalyzed reaction, respectively. The activation energy is lower when the enzyme catalyzes the reaction. The equilibrium of a reaction is unaffected by the enzyme.