Chapter 24
Proteins

♦ Introduction

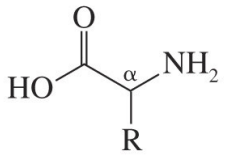
➔ **The three major groups of biological polymers are polysaccharides, proteins and nucleic acids**

➔ **Proteins have many diverse functions; they are major components of the following biomolecules**

- **Enzymes and hormones which catalyze and regulate biological reactions**
- **Muscles and tendons which provide the body with means for movement**
- **Hemoglobin which carries oxygen to all parts of the body**
- **Antibodies they are integral parts of the immune system**

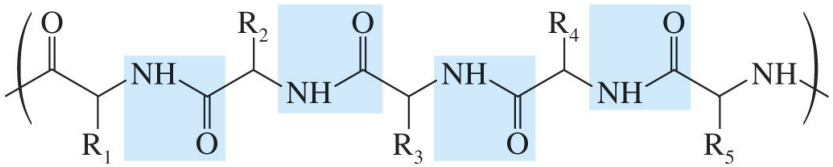
➔ **All proteins are polyamides**

- **Their monomeric units are one of about 20 α- amino acids**



An α-amino acid

R is a side chain at the α carbon that determines the identity of the amino acid (Table 24.1).



A portion of a protein molecule

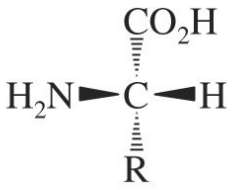
Amide (peptide) linkages are shaded.

R_1–R_5 may be any of the possible side chains.

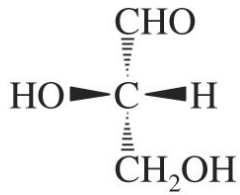
➔ **Proteins have several levels of structure**

- ***Primary structure*** **refers to the exact sequence of amino acids along a protein chain**
- ***Secondary*** **and** ***tertiary structures*** **refer to the further bending and folding of the primary structure**
- ***Quaternary structure*** **refers to the aggregation of more than one polyamide chain**

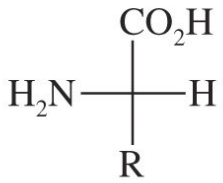
➔ **All amino acids except glycine are chiral and have the L configuration (as related to glyceraldhyde) at the α carbon**

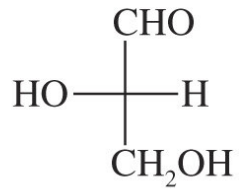


An L-α-amino acid [usually an (*S*)-α-amino acid]



L-Glyceraldehyde [(*S*)-glyceraldehyde]





Fischer projections for an L-α-amino acid and L-glyceraldehyde

♦ Amino acids

● Structure and Names

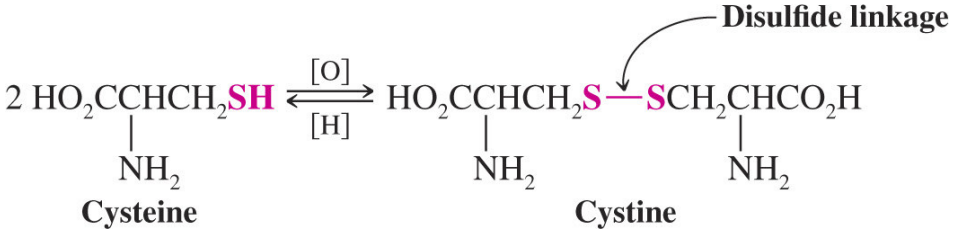
➔22 amino acids but only 20 amino acids comprise the building blocks for synthesis of proteins

➔The remaining 2 amino acids are derived by modification after biosynthesis of the protein

- Hydroxyproline and cystine are synthesized from proline and cysteine, respectively, after the protein chain has been synthesized

➔Cysteine is oxidized under mild conditions to the dissulfide cystine

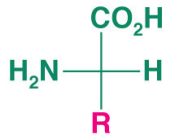
- The reaction is reversible
- This linkage is important in maintaining the overall shape of a protein

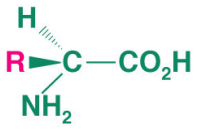


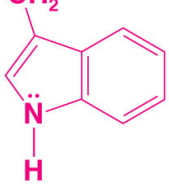
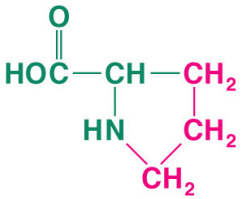
● Essential Amino Acids

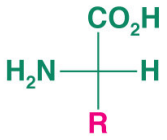
➔Essential amino acids are not made by higher animals and must be part of the diet

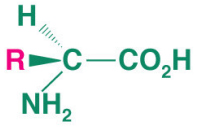
- There are 8 essential amino acids for adult humans (see Table 24.1)

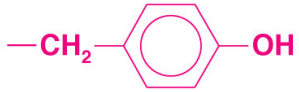
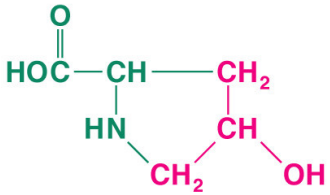


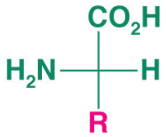


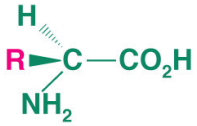
Structure of R	Name	Abbreviations[a]	pK_{a_1} α-CO_2H	pK_{a_2} α-NH_3^+	pK_{a_3} R group	p*I*
Neutral Amino Acids						
—H	Glycine	G or Gly	2.3	9.6		6.0
—CH_3	Alanine	A or Ala	2.3	9.7		6.0
—$CH(CH_3)_2$	Valine[b]	V or Val	2.3	9.6		6.0
—$CH_2CH(CH_3)_2$	Leucine[b]	L or Leu	2.4	9.6		6.0
—$CHCH_2CH_3$ CH_3	Isoleucine[b]	I or Ile	2.4	9.7		6.1
—CH_2 	Phenylalanine[b]	F or Phe	1.8	9.1		5.5
—CH_2CONH_2	Asparagine	N or Asn	2.0	8.8		5.4
—$CH_2CH_2CONH_2$	Glutamine	Q or Gln	2.2	9.1		5.7
—CH_2 	Tryptophan[b]	W or Trp	2.4	9.4		5.9
 (complete structure)	Proline	P or Pro	2.0	10.6		6.3





Structure of R	Name	Abbreviations[a]	pK_{a_1} α-CO_2H	pK_{a_2} α-NH_3^+	pK_{a_3} R group	p*I*
Neutral Amino Acids						
—CH_2OH	Serine	S or Ser	2.2	9.2		5.7
—CHOH CH_3	Threonine[b]	T or Thr	2.6	10.4		6.5
	Tyrosine	Y or Tyr	2.2	9.1	10.1	5.7
 (complete structure)	Hydroxyproline	Hyp	1.9	9.7		6.3
—CH_2SH	Cysteine	C or Cys	1.7	10.8	8.3	5.0
—CH_2—S —CH_2—S	Cystine	Cys-Cys	1.6 2.3	7.9 9.9		5.1
—$CH_2CH_2SCH_3$	Methionine[b]	M or Met	2.3	9.2		5.8

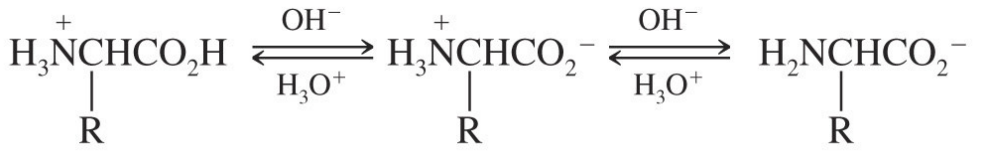




Structure of R	Name	Abbreviations[a]	pK_{a_1} α-CO_2H	pK_{a_2} α-NH_3^+	pK_{a_3} R group	p*I*
R Contains an Acidic (Carboxyl) Group						
$-CH_2CO_2H$	Aspartic acid	D or Asp	2.1	9.8	3.9	3.0
$-CH_2CH_2CO_2H$	Glutamic acid	E or Glu	2.2	9.7	4.3	3.2
R Contains a Basic Group						
$-CH_2CH_2CH_2CH_2NH_2$	Lysine[b]	K or Lys	2.2	9.0	10.5[c]	9.8
$-CH_2CH_2CH_2NH-C(=NH)-NH_2$	Arginine	R or Arg	2.2	9.0	12.5[c]	10.8
$-CH_2$-(imidazole ring)	Histidine	H or His	1.8	9.2	6.0[c]	7.6

[a]Single-letter abbreviations are now the most commonly used form in current biochemical literature.
[b]An essential amino acid.
[c]pK_a is of protonated amine of R group.

- **Amino Acids as Dipolar Ions**
  - ➔ **In the dry solid state amino acids exist as dipolar ions (zwitterions)**
  - ➔ **In aqueous solution an equilibrium exists between the dipolar ion, the cationic and the anionic forms of the amino acid**
    - **The predominant form depends on the pH of the solution**



Cationic form (predominant in strongly acidic solutions, e.g., at pH 0) | **Dipolar ion** | **Anionic form (predominant in strongly basic solutions, e.g., at pH 14)**

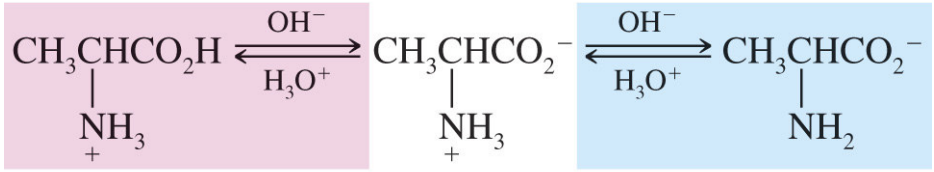
  - ➔ **At low pH the amino acid exists primarily in the cationic form**
  - ➔ **At high pH the amino acid exists primarily in the anionic form**
  - ➔ **At some intermediate pH called the p*I* (*isoelectric point*), the concentration of the dipolar ion is at a maximum and the concentrations of anionic and cationic forms are equal**
  - ➔ **Each individual amino acid has a characteristic p*I* (see Table 24.1)**
    - **Entire proteins also have a characteristic p*I***

➔ **The amino acid alanine has a neutral side chain and can be used to illustrate the fundamental behavior of an amino acid at various pHs**

- **At low pH alanine exist as the cation**
- **pK_{a1} of alanine (for ionization of the carboxylic acid proton) is 2.3, considerably lower than the pK_a of a normal carboxylic acid**

Cationic form of alanine $pK_{a_1} = 2.3$ **Propanoic acid** $pK_a = 4.89$

- **pK_{a2} of alanine (for ionization of a proton from the protonated amino group) is 9.7**



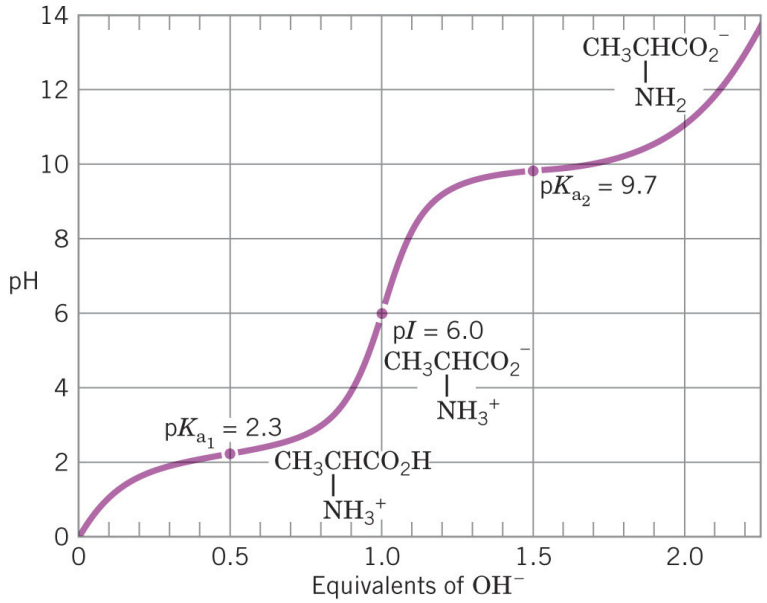
Cationic form ($pK_{a_1} = 2.3$) **Dipolar ion** ($pK_{a_2} = 9.7$) **Anionic form**

- **The isolectric point, p*I*, for alanine is the average of the two pK_a values *i.e.* (pK_{a1} + pK_{a2})/2**

- When base is slowly added to fully protonated alanine, a pH is reached where half of the carboxylic acid groups are deprotonated
- This pH of 2.3 is the value of pK_{a1}
- The Henderson-Hasselbach equation predicts this result

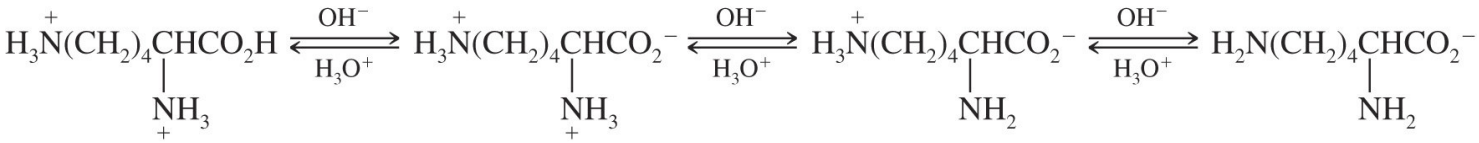
$$pK_a = pH + \log \frac{[HA]}{[A^-]} \qquad [HA] = [A^-] \quad \text{and} \quad \log \frac{[HA]}{[A^-]} = 0$$

- As more base is added, the pI is reached and the molecule is electrically neutral; this point is reached when exactly one equivalent of base is added
- As more base is added and pH 9.7 is reached, half of the the aminium groups will be deprotonated
- Addition of more base will eventually produce only the anionic amino acid



➔ Lysine, which contains a basic side-chain, has a more complex equilibrium

- The p*I* for lysine will be high because of the presence of two basic groups
- The p*I* for lysine is the average of the monocation (pK_{a2}) and the dipolar ion (pK_{a3})



Dicationic form of lysine ($pK_{a_1} = 2.2$)

Monocationic form ($pK_{a_2} = 9.0$)

Dipolar ion ($pK_{a_3} = 10.5$)

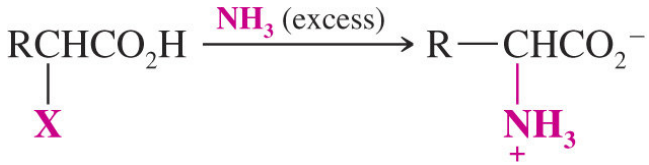
Anionic form

♦ Synthesis of α-Amino Acids

➔ The first three methods result in racemic mixtures of amino acids

- **Direct Ammonolysis of an α-Halo Acid**

  ➔ Yields tend to be poor in this reaction

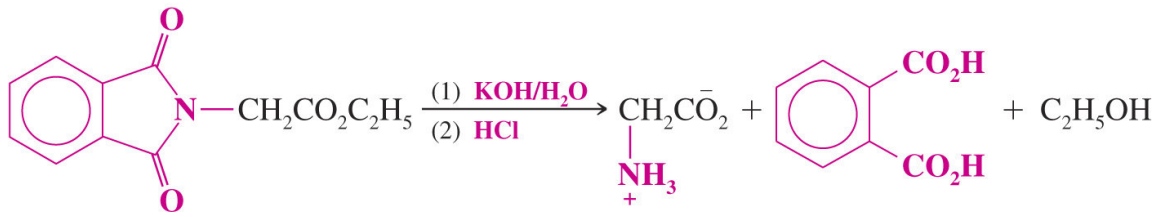


- **From Potassium Phthalimide**

  ➔ This is a variation of the Gabriel synthesis and yields are usually high

Potassium phthalimide

Ethyl chloroacetate

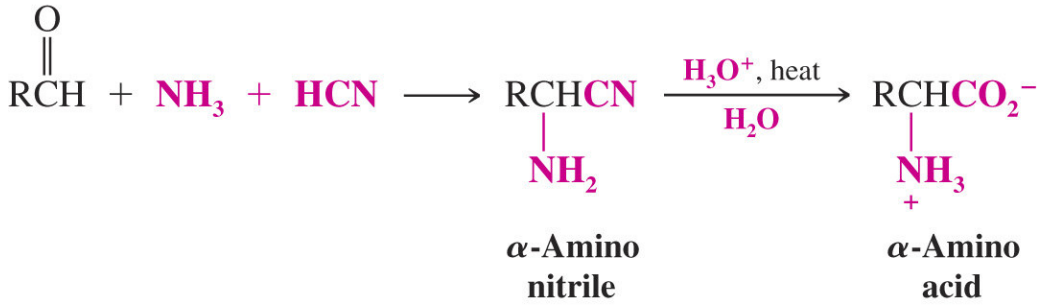


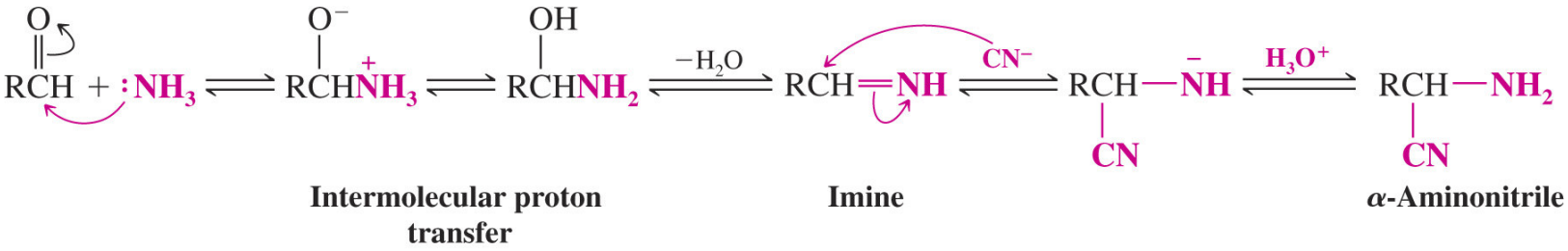
(97%)

Glycine (85%)

Phthalic acid

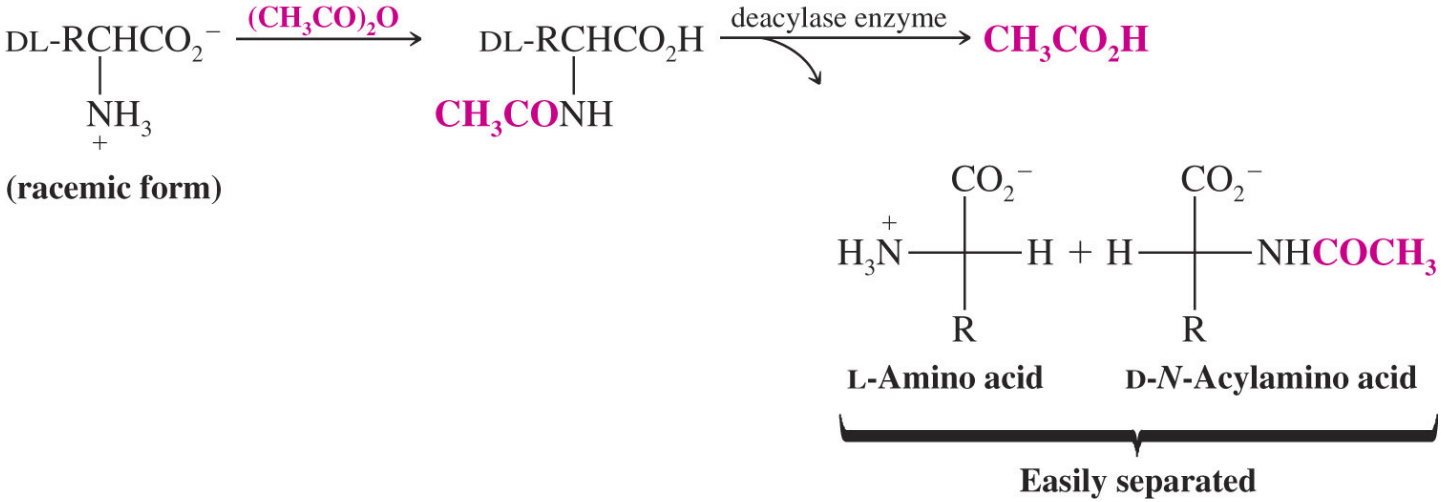
- **The Strecker Synthesis**
  - **➔Treatment of an aldehyde with ammonia and hydrogen cyanide yields an α-aminonitrile which is hydrolyzed to the α-amino acid**
    - **The reaction proceeds via an intermediate imine**





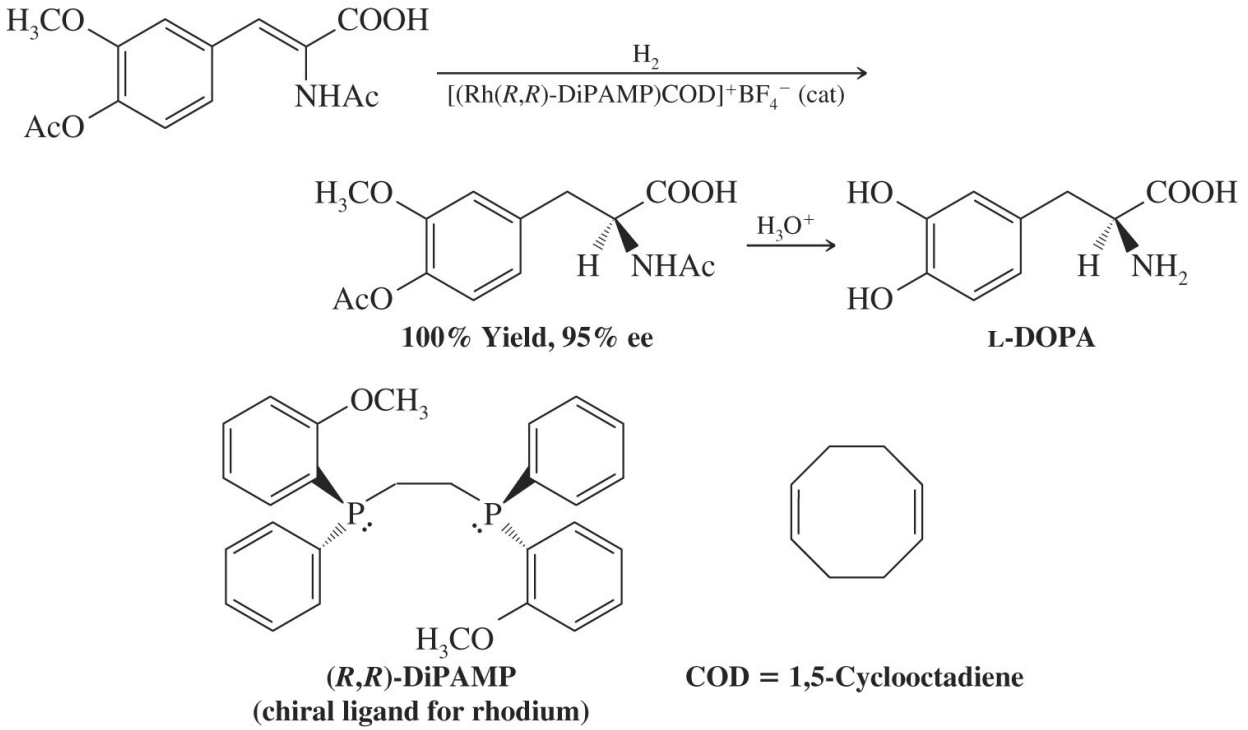
- **Resolution of DL-Amino Acids**

  ➔ A racemic amino acid mixture can be resolved by
  (1) conversion to a racemic mixture of *N*-acylamino acids, followed by
  (2) hydrolysis with a deacylase enzyme that selectively deacylates the L-acylamino acid



- **Asymmetric Synthesis of Amino Acids**
  - ➔ **Enantioselective syntheses that produce only the desired naturally occurring amino acid enantiomers are ideal**
  - ➔ **One important method involves asymmertic hydrogenation of an enamide using a chiral transition metal catalyst**
    - **This method was used to synthesize L-dopa, a chiral amino acid used in the treatment of Parkinson's disease**

Asymmetric Synthesis of L-DOPA



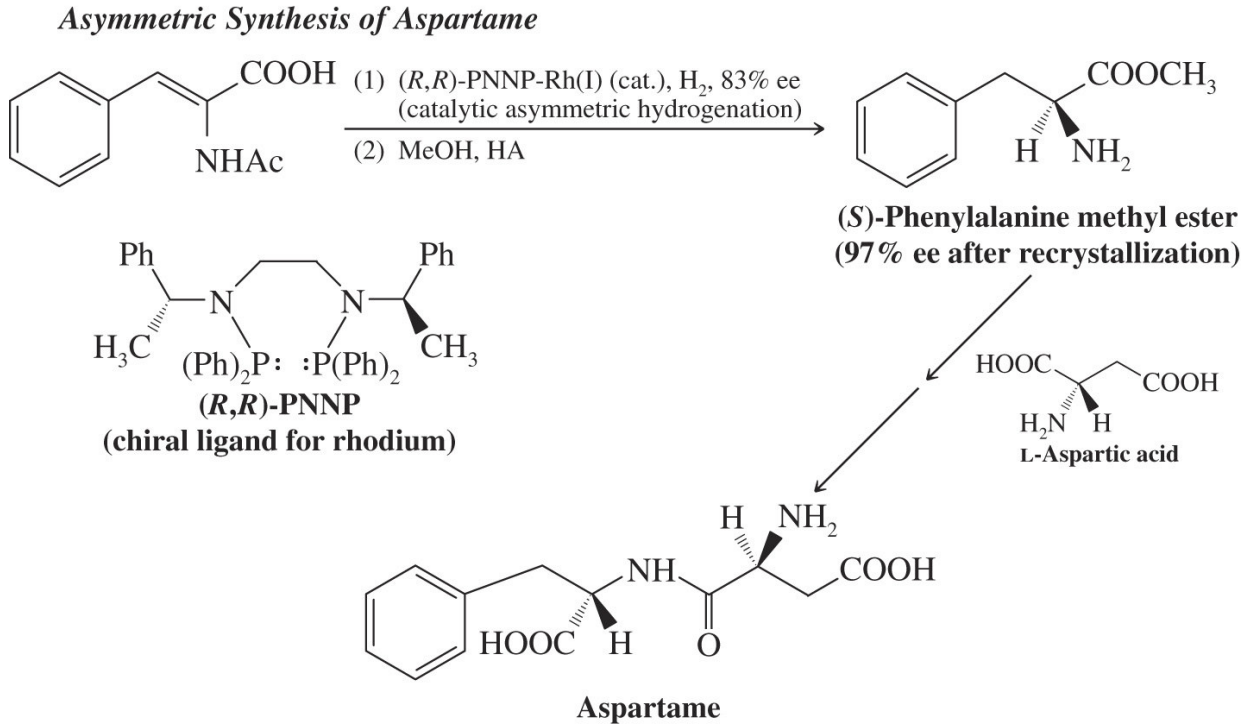
100% Yield, 95% ee

L-DOPA

(*R*,*R*)-DiPAMP
(chiral ligand for rhodium)

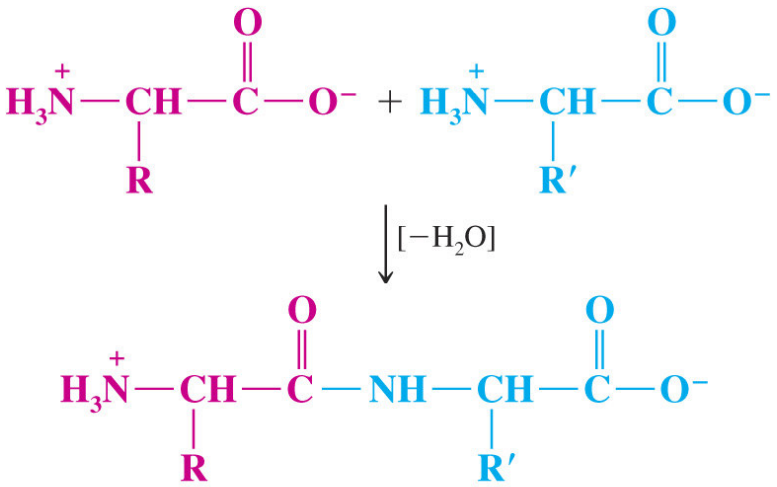
COD = 1,5-Cyclooctadiene

- **A similar method is used to synthesize (S)-phenylalanine, needed for preparation of Aspartame**



♦ Polypeptides and Proteins

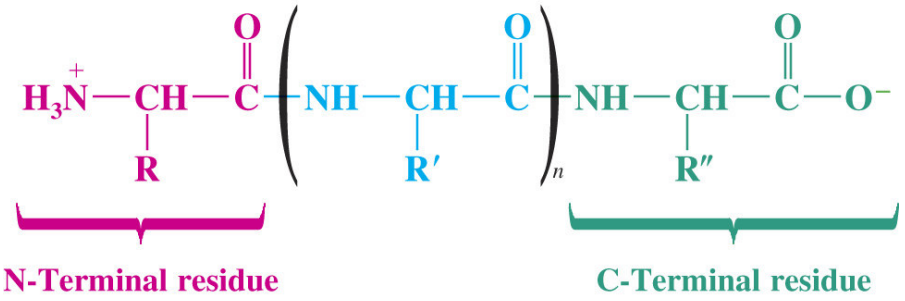
- ➔ Enzymes polymerize amino acids by forming amide linkages
- ➔ The polymer is called a *peptide* and the amide linkages are called *peptide bonds* or *peptide linkages*
- ➔ Each amino acid in the peptide is called an amino acid residue
- ➔ Proteins can contain one or more polypeptide chains and other associated molecules or metal ions

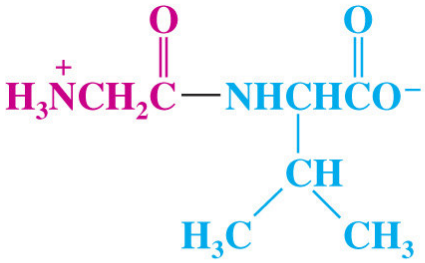


A dipeptide

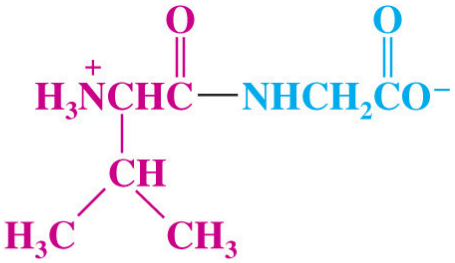
➔ **Polypeptides are customarily written with the *N*-terminal residue to the left**

- **Three letter or one letter abbreviations are usually used as a short hand to indicate the sequence of a polypeptide**



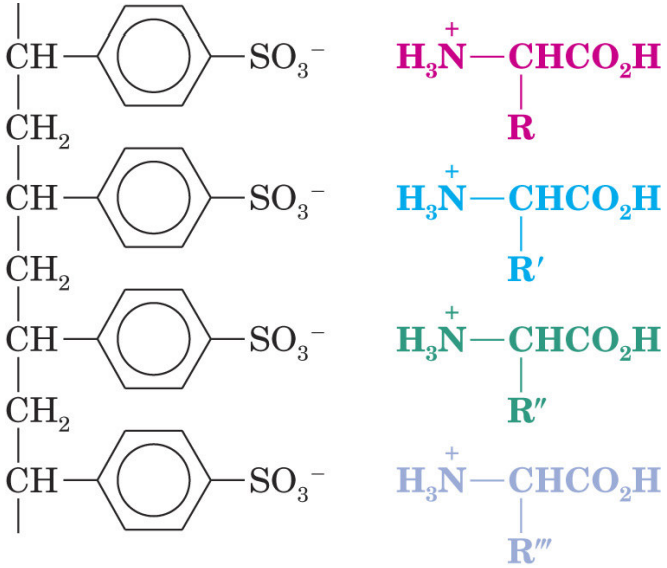


Glycylvaline
(Gly · Val)

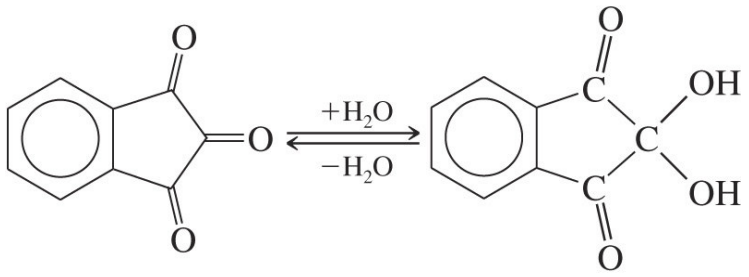


Valylglycine
(Val · Gly)

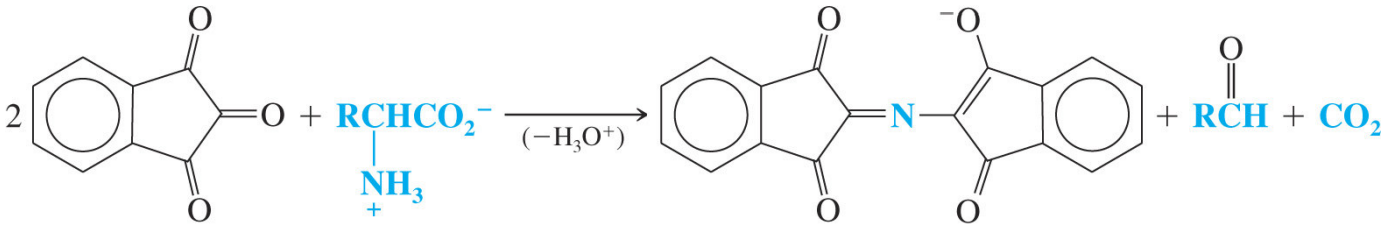
- **Hydrolysis**
  - ➔ **A polypeptide can be hydrolyzed by refluxing with 6M hydrochloric acid for 24h**
  - ➔ **The individual amino acids can be separated from each other using a cation-exchange resin**
    - **An acidic solution of the amino acids is passed through the cation-exchange column; the strength of adsorption varies with the basicity of each amino acid (the most basic are held most strongly)**
    - **Washing the column with a sequence of buffered solutions causes the amino acids to move through it at different rates**



➔ **In the original method, the column eluant is treated with ninhydrin, a dye used for detecting and quantifying each amino acid as it comes off the column**



Indane-1,2,3-trione **Ninhydrin**



Purple anion

➔ **In modern practice, analysis of amino acid mixtures is routinely accomplished using high performance liquid chromatography (HPLC)**

♦ Primary Structure of Polypeptides and Proteins

➔ **The sequence of amino acids in a polypeptide is called its primary structure**

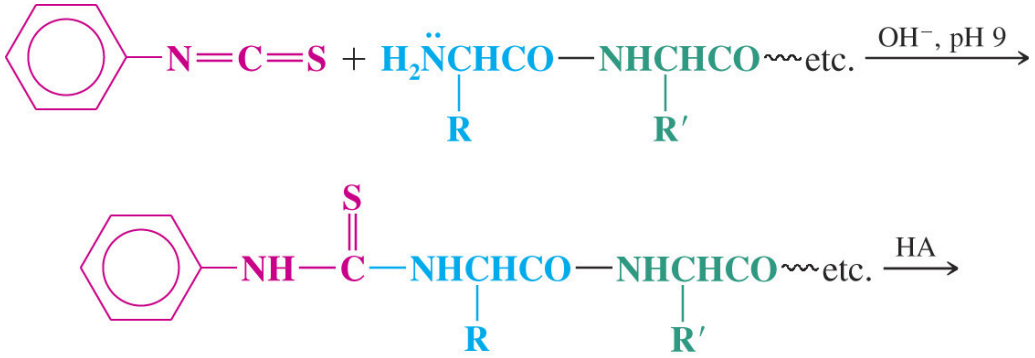
- **Several methods exist to elucidate the primary structure of peptides**

● Edman Degradation

➔ **Edman degradation involve sequential cleavage and identification of *N*-terminal amino acids**

➔ **Edman degradation works well for polypeptide sequence analyses up to approximately 60 amino acid residues**

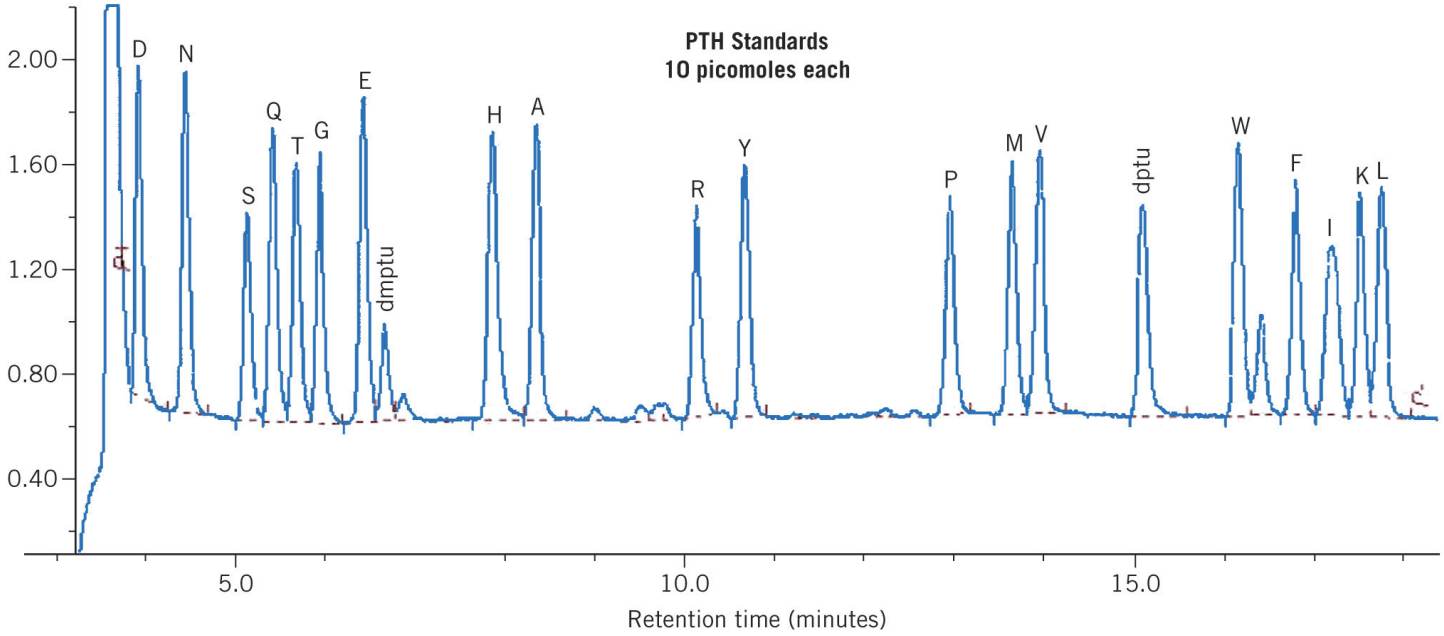
- **The N-terminal residue of the polypeptide reacts with phenyl isothiocyanate**
- **The resulting phenylthiocarbamyl derivative is cleaved from the peptide chain**
- **The unstable product rearranges to a stable phenylthiohydantoin (PTH) which is purified by HPLC and identified by comparison with PTH standards**



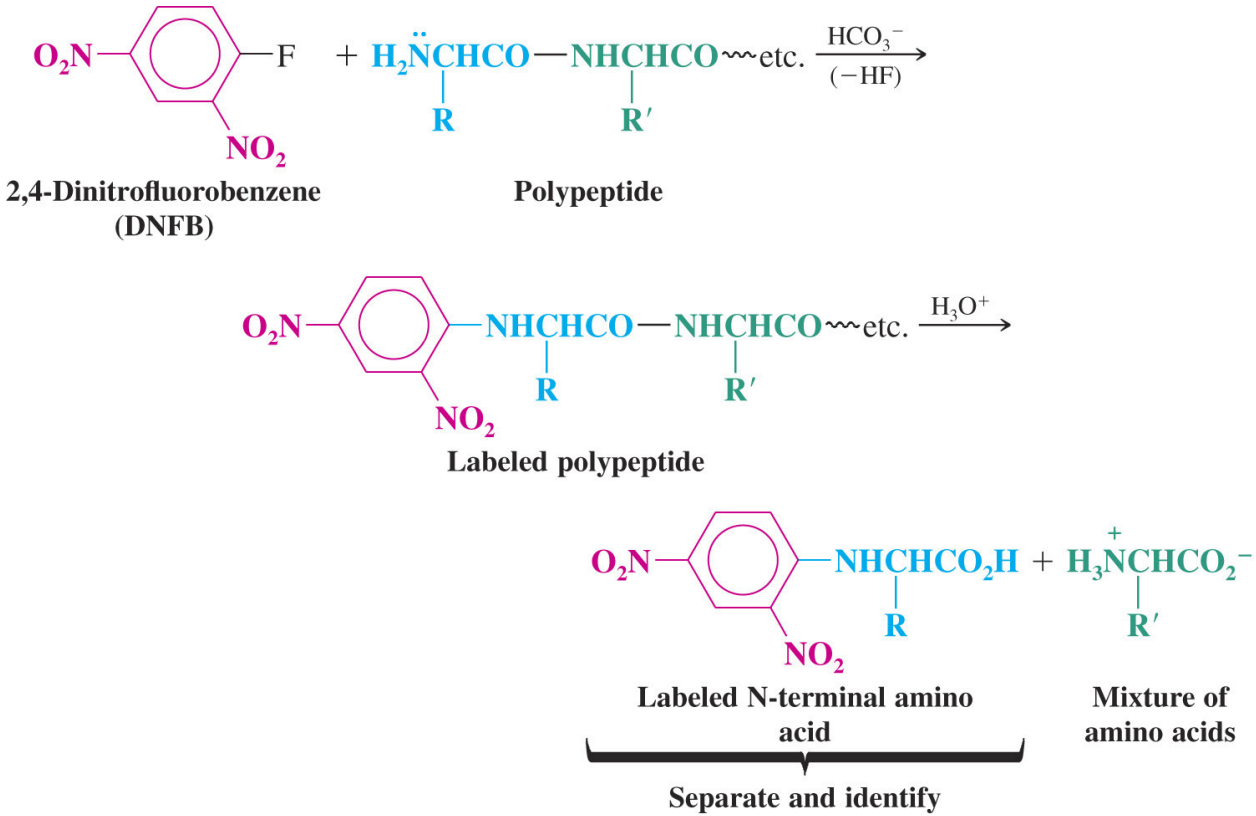
Labeled polypeptide

➔ Automated amino acid sequencing machines use the Edman degradation and high performance liquid chromatography (HPLC)

- One Edman degradation cycle beginning with a picomolar amount of polypeptide can be completed in approximately 30 minutes
- Each cycle results in identification of the next amino acid residue in the peptide



- **Sanger *N*-Terminal Analysis**
  - ➔ **The *N*-terminal end of the polypeptide is labeled with 2,4-dinitrofluorobenzene and the polypeptide is hydrolyzed**
    - **The labeled *N*-terminal amino acid is separated from the mixture and identified**



  - ➔ **The Sanger method is not as widely used as the Edman method**

- ***C*-Terminal Analysis**
  - **➔Enzymes called carboxypeptidases hydrolyze *C*-terminal amino acids selectively**
    - **The enzyme continues to release each newly exposed *C*-terminal amino acid as the peptide is hydrolyzed; it is necessary to monitor the release of *C*-terminal amino acids as a function of time to identify them**

- **Complete Sequence Analysis**
  - ➔ **The Sanger and Edman methods of analysis apply to short polypeptide sequences (up to about 60 amino acid residues by Edman degradation)**
  - ➔ **For large proteins and polypeptides, the sample is subjected to partial hydrolysis with dilute acid to give a random assortment of shorter polypeptides which are then analyzed**
    - **The smaller polypeptides are sequenced, and regions of overlap among them allow the entire polypeptide to be sequenced**
  - ➔ **Example: A pentapeptide is known to contain the following amino acids:**

$$Val_2, Leu, His, Phe$$

    - **Using DNFB and carboxypeptidase, the N-terminal and C-terminal amino acids are identified**

$$Val\ (Val, His, Phe)\ Leu$$

    - **The pentapeptide is subjected to partial hydrolysis and the following dipeptides are obtained**

$$Val \cdot His + His \cdot Val + Val \cdot Phe + Phe \cdot Leu$$

    - **The amino acid sequence of the pentapeptide must be:**

$$Val \cdot His \cdot Val \cdot Phe \cdot Leu$$

➔ Larger polypeptides can also be cleaved into smaller sequences using *site-specific* reagents and enzymes

- The use of these agents gives more predictable fragments which can again be overlapped to obtain the sequence of the entire polypeptide
- Cyanogen bromide (CNBr) cleaves peptide bonds only on the C-terminal side of methionine residues

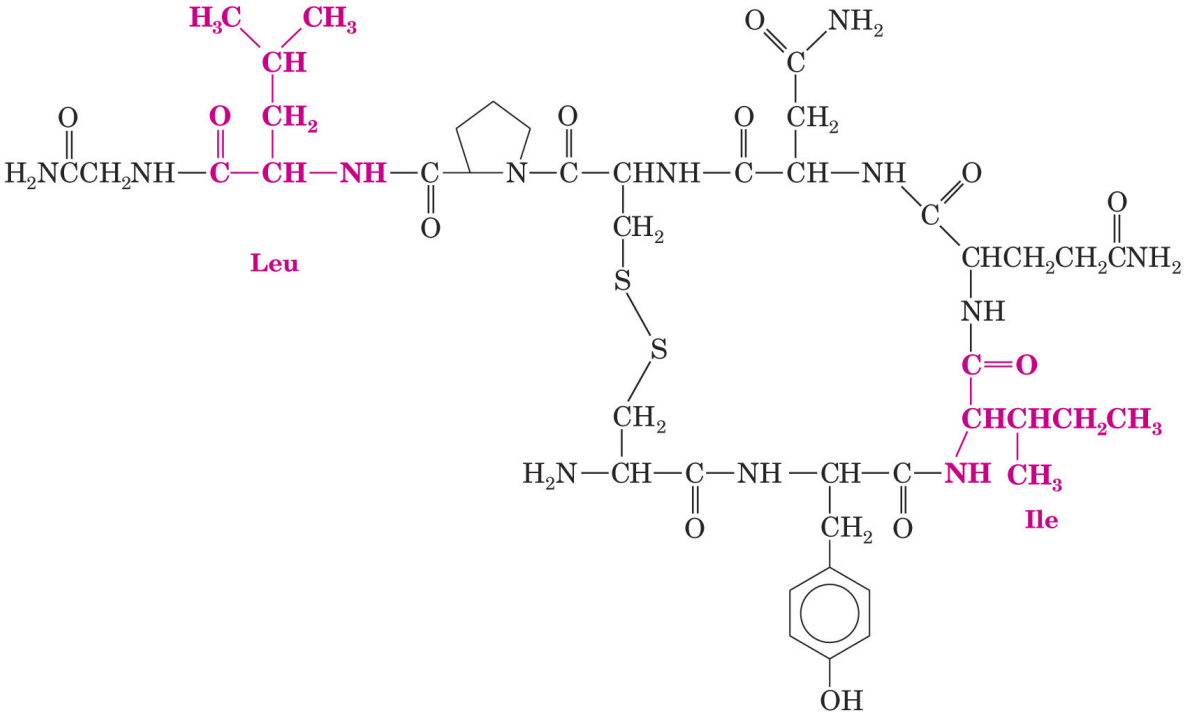
➔ Mass spectrometry can be used to determine polypeptide and protein sequences

- "Ladder sequencing" involves analyzing a polypeptide digest by mass spectrometry, wherein each polypeptide in the digest differs by one amino acid in length; the difference in mass between each adjacent peak indicates the amino acid that occupies that position in the sequence
- Mass spectra of polypeptide fragments from a protein can be compared with databases of known polypeptide sequences, thus leading to an identification of the protein or a part of its sequence by matching

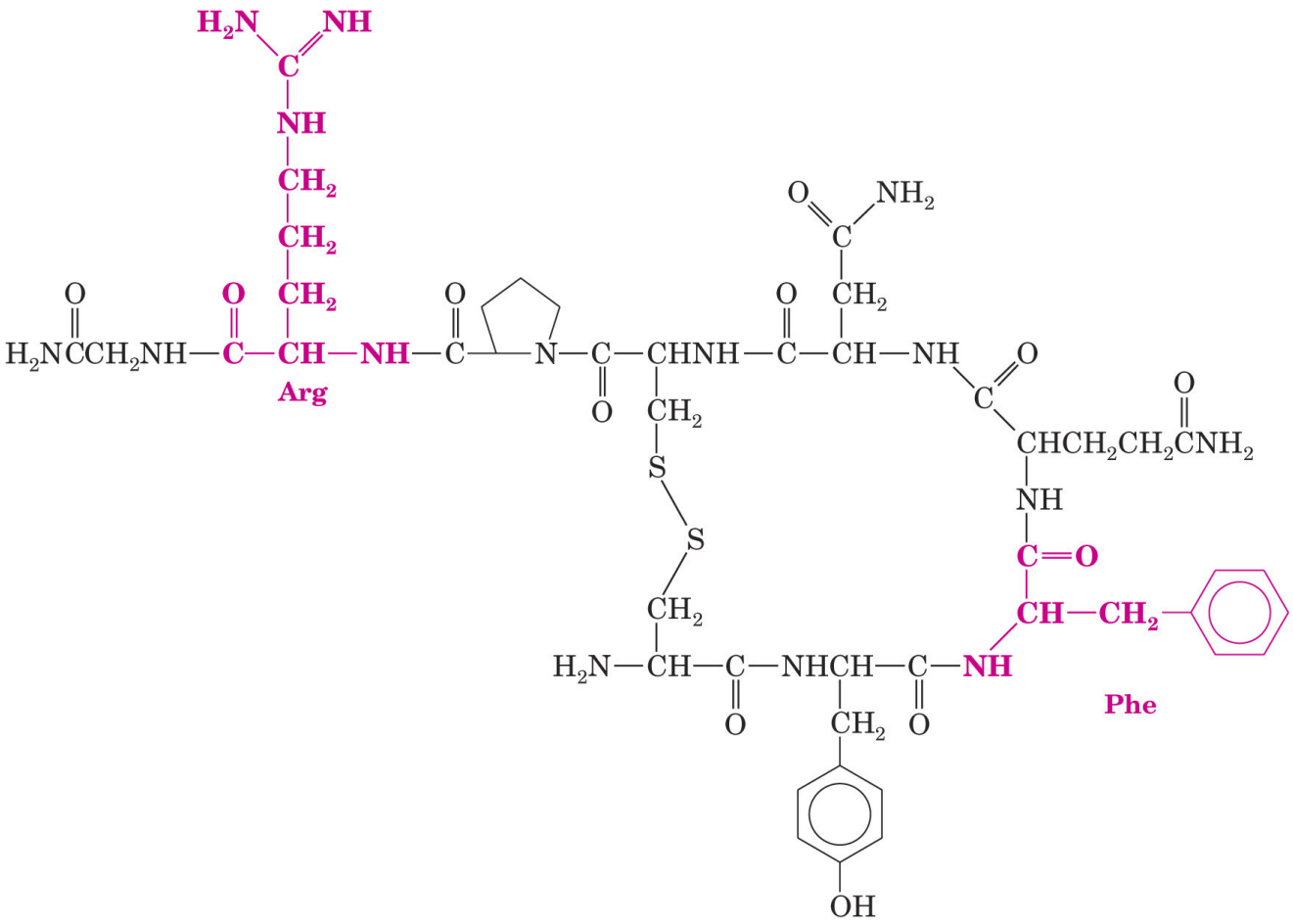
♦ Examples of Polypeptide and Protein Primary Structure

- **Oxytocin and Vasopressin**
  - ➔ **Oxytocin stimulates uterine contractions during childbirth**
  - ➔ **Vasopressin causes contraction of peripheral blood vessels and a resultant increase in blood pressure**
    - **The two polypeptides are nonapeptides and differ in only 2 amino acid residues**

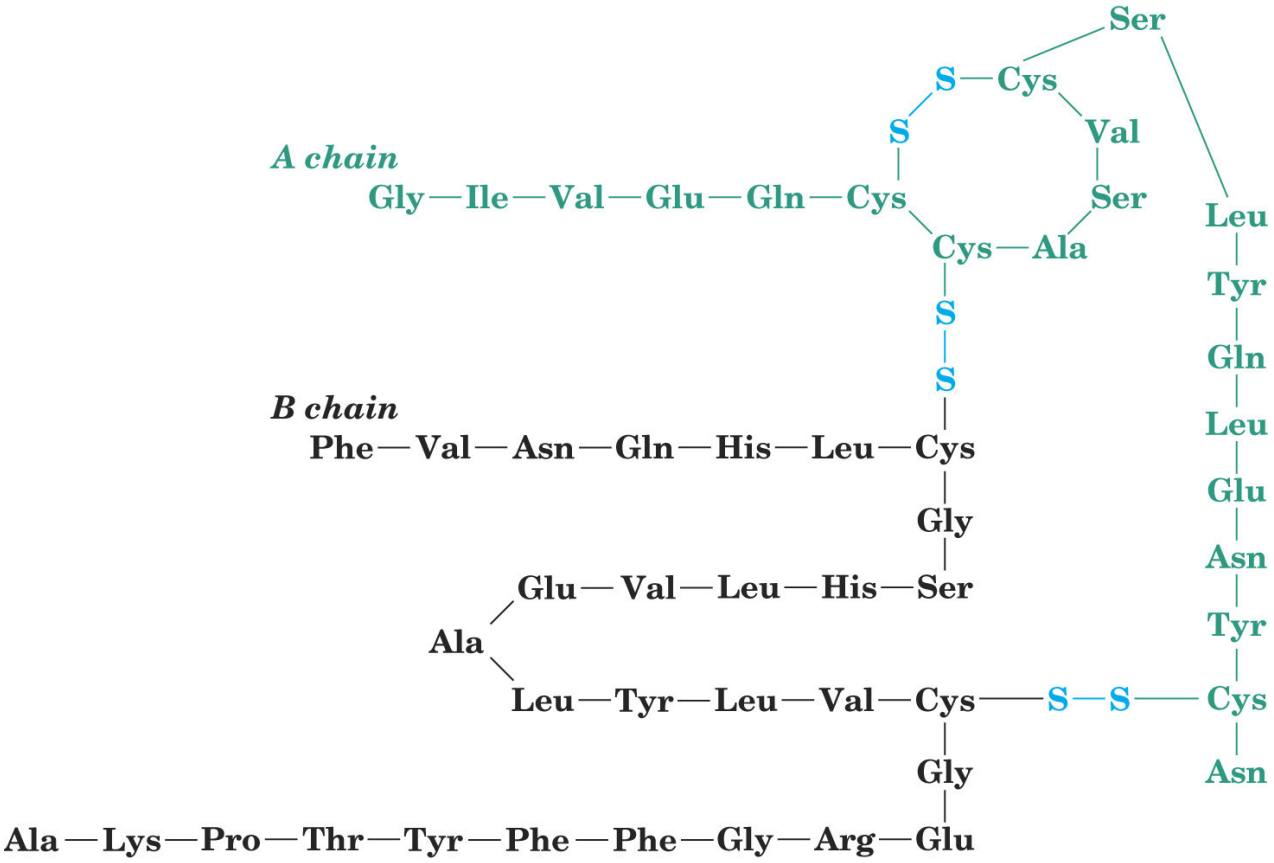
Oxytocin



Vasopressin



- **Insulin**
  - **➔Insulin is a hormone which regulates glucose metabolism**
    - **Insulin deficiency in humans is the major cause of diabetes mellitus**
    - **The structure of bovine insulin (shown below) was determined in 1953 by Sanger**
    - **Human insulin differs from bovine insulin at only three amino acids in its sequence**



♦ Polypeptide and Protein Synthesis

➔ **Laboratory synthesis of polypeptides requires orchestration of blocking and activating groups to achieve selective amide bond formation**

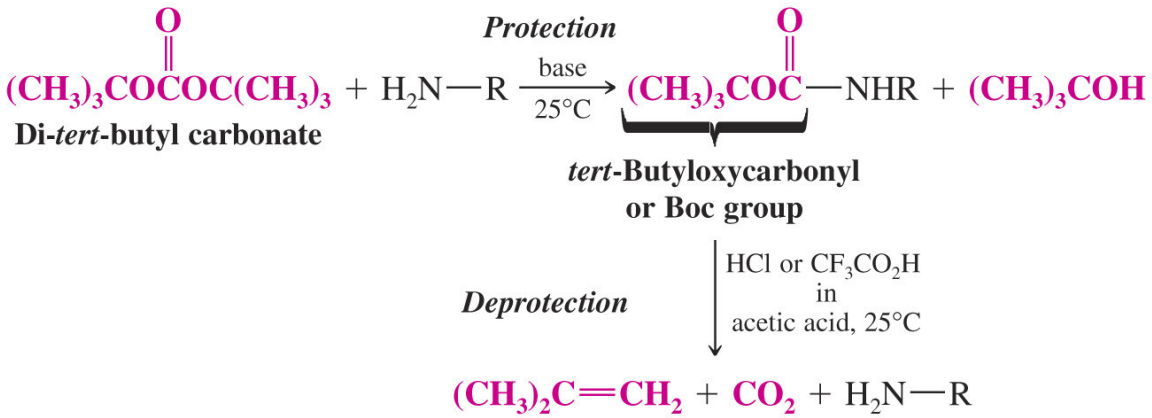
- **Amino groups must be blocked until their reactivity as a nucleophile is desired**
- **Carboxylic acid groups must be activated for acyl substitution at the appropriate time**

➔ **Amino groups are usually blocked using one of the following:**

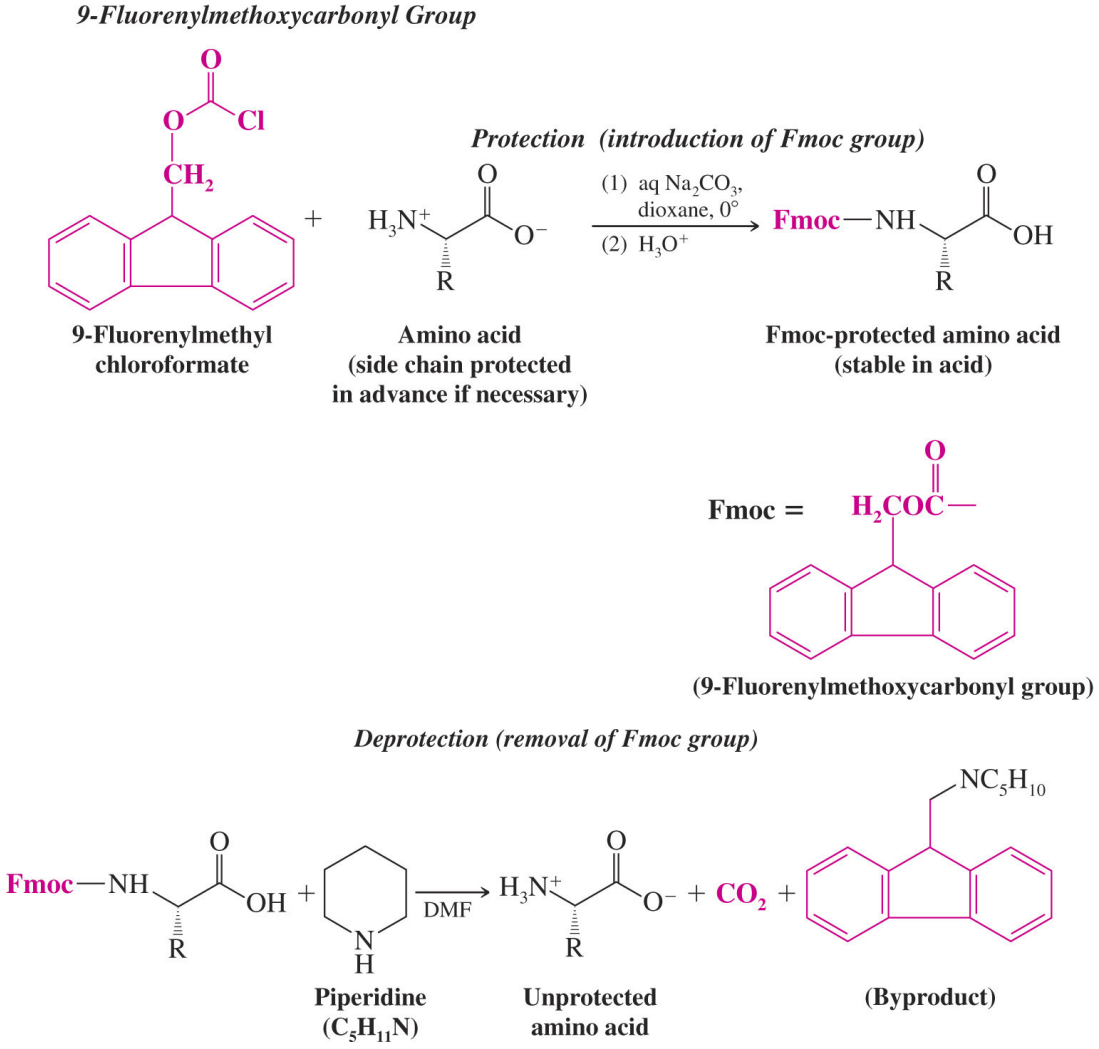
- **A benzyloxycarbonyl group (a "Z" group)**
- **A di-*tert*-butyloxycarbonyl group (a "Boc" group)**
- **An 9-fluorenylmethoxycarbonyl group (an "Fmoc" group)**

➔ **Methods for installing and removing Z, Boc, and Fmoc groups are shown below:**

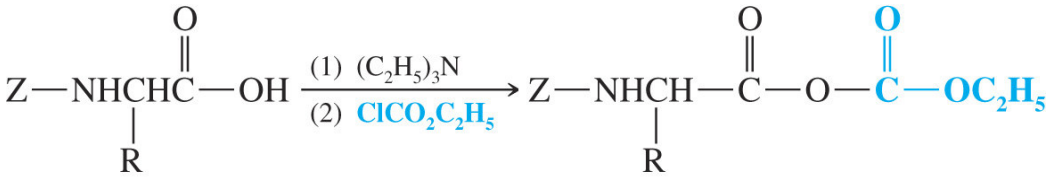
tert-Butyloxycarbonyl Group



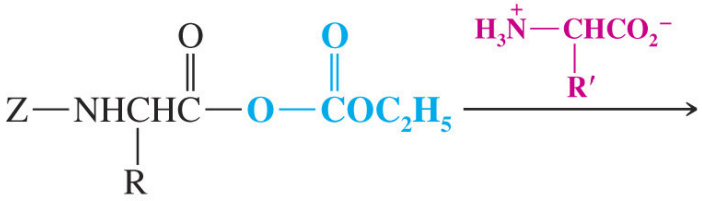
➔ **Methods for installing and removing Z, Boc, and Fmoc groups are shown below:**

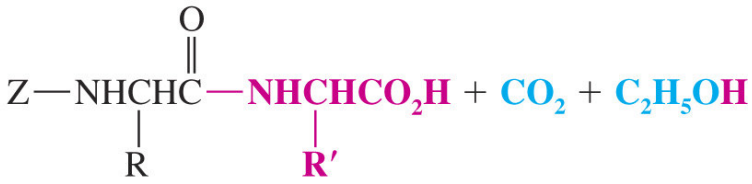


- **Carboxylic acid groups are usually activated by conversion to a mixed anhydride:**
  - **Ethyl chloroformate can be used**



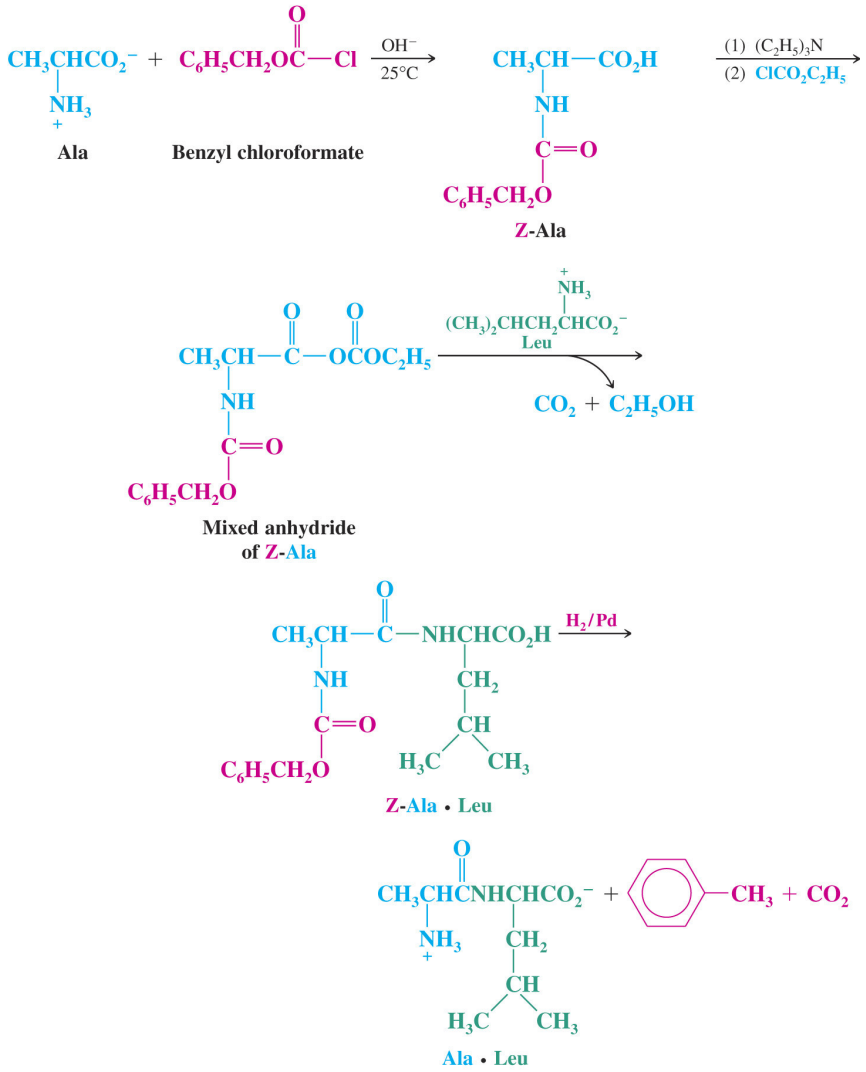
"Mixed anhydride"





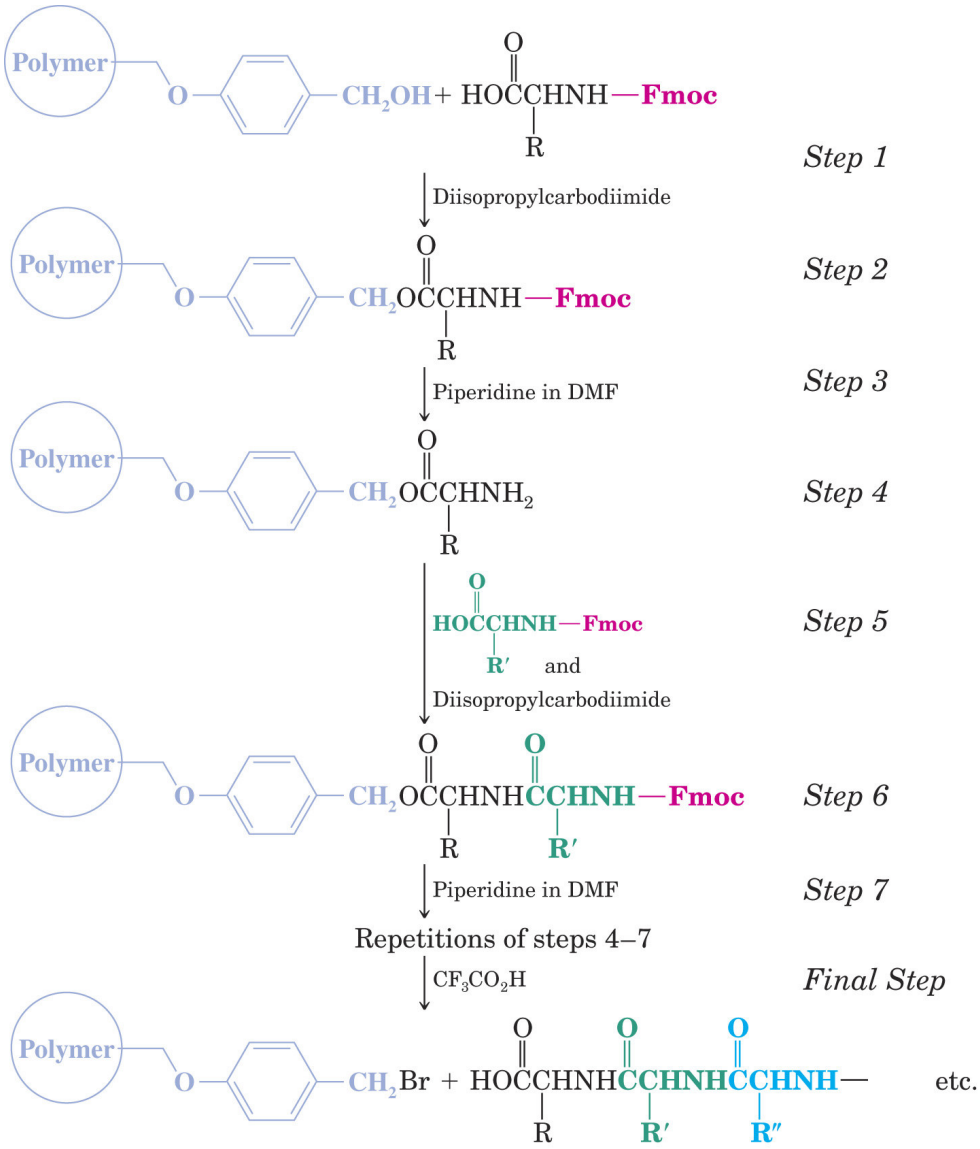
♦ An Example of Laboratory Peptide Synthesis:

➔Synthesis of Ala-Leu



♦ Automated Peptide Synthesis

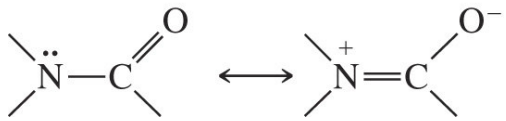
- ➔ **Solid Phase Peptide Synthesis (SPSS) was invented by R. B. Merrifield, for which he earned the Nobel Prize in 1984**
- ➔ **SPSS involves 'growing' a peptide on a solid polymer bead by sequential cycles of amide bond formation**
- ➔ **The peptide is cleaved from the bead when the synthesis is complete**
- ➔ **SPSS is used in commercial peptide synthesis machines**
  - **Peptides dozens of residues in length can be synthesized automatically**
  - **A landmark example is synthesis of ribonuclease, having 124 amino acid residues**


Polymer
O
CH2OH + HOCCHNH—Fmoc
R
Diisopropylcarbodiimide
CH2OCCHNH—Fmoc
CH2OCCHNH2
HOCCHNH—Fmoc
R′
and
Diisopropylcarbodiimide
CH2OCCHNHCCHNH—Fmoc
Piperidine in DMF
Repetitions of steps 4–7
CF3CO2H
CH2Br + HOCCHNHCCHNHCCHNH—
etc.
Step 1
Attaches C-terminal (Fmoc protected) amino acid residue to resin
Step 2
Purifies resin with attached residue by washing
Step 3
Removes Fmoc protecting group
Step 4
Purifies by washing
Step 5
Adds next (protected) amino acid residue
Step 6
Purifies by washing
Step 7
Removes protecting group
Final Step
Detaches completed polypeptide

♦ Secondary, Tertiary, and Quaternary Structures of Proteins

- **Secondary Structure**
  - ➔ **The secondary structure of a protein is defined by local conformations of its polypeptide backbone**
    - **These local conformations are specified in terms of regular folding patterns such as helices, pleated sheets, and turns**
  - ➔ **The secondary structure of a protein is determined by the sequence of amino acids in its primary structure**
  - ➔ **Key to secondary structure is that peptide bonds assume a geometry in which all 6 atoms of the amide linkage are trans coplanar**

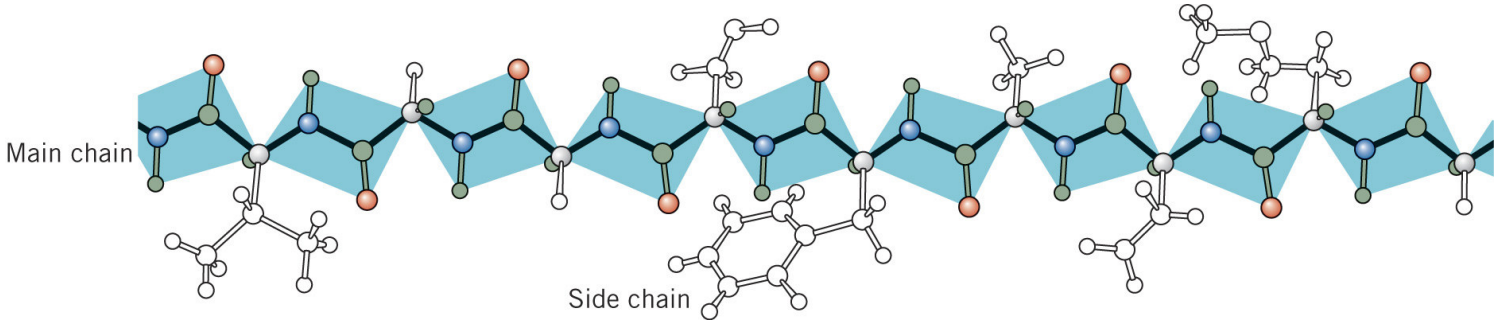
➔ **Coplanarity results from contribution of the second resonance form of amides, in which there is considerable N-C double bond character**



➔ **The carbon with attached R groups between the amide nitrogen and the carbonyl group has relatively free rotation and this leads to different conformations of the overall chain**

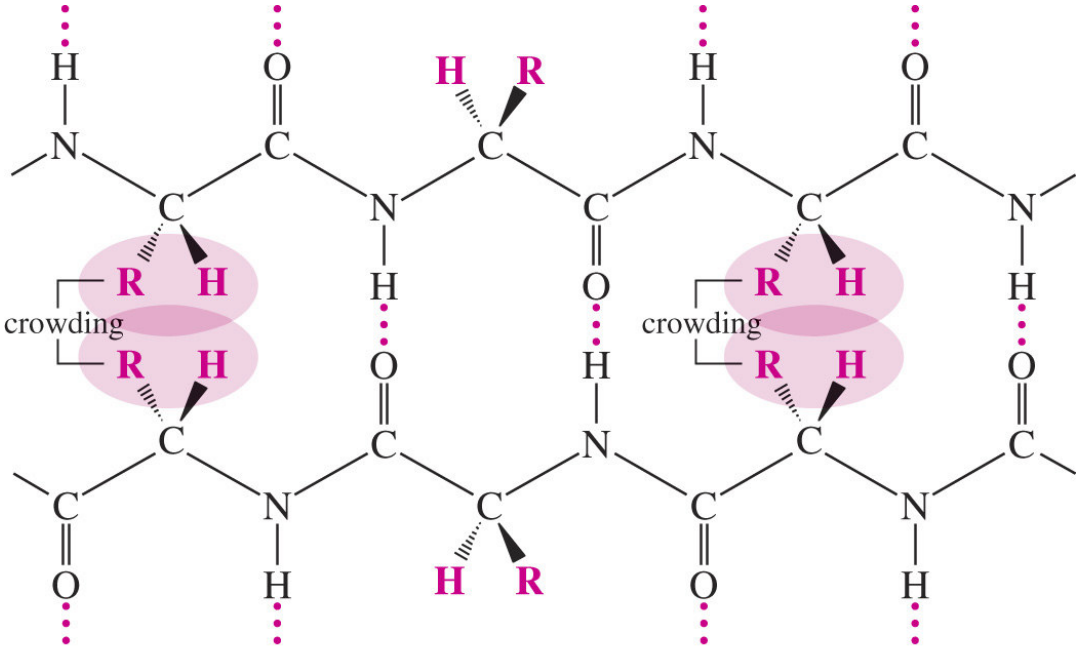
- **Two common secondary structure are the *β-pleated sheet* and the *α-helix***

  ➔ **In the β-pleated sheet, a polypeptide chain is in an extended conformation with groups alternating from side to side**



➔ The extended polypeptide chains in β-pleated sheets form hydrogen bonds to adjacent polypeptide chains

- Slight bond rotations are necessary between amide groups to avoid unfavorable steric interactions between peptide side chains, leading to the pleated structure
- The β-pleated sheet is the predominant structure in silk fibroin



Hypothetical flat-sheet structure
(not formed because of steric hindrance)

- **➔ The α-helix is the most important protein secondary structure**
- **➔ α-Helices in a polypeptide are right-handed with 3.6 amino acid residues per turn (See figure 24.11 page 1198)**
  - **The amide nitrogen has a hydrogen bond to an amino acid carbonyl oxygen that is three residues away**
  - **The R groups extend away from the axis of the helix**
- **➔ α-Helices comprise the predominant secondary structure of fibrous proteins such as myosin (in muscle) and α-keratin (in hair and nails)**
- **➔ There are other secondary structures that are more difficult to describe**
  - **Examples are coil or loop conformations and reverse turns or β bends**

- **Carbonic Anhydrase**
  - **➔The structure of the enzyme carbonic anhydrase is shown in Figure 24.12,page 1198**
    - **Alpha helices are in magenta and strands of β-pleated sheets are in yellow**
    - **The mechanism of carbonic anhydrase reaction was discussed in Chapter 3**

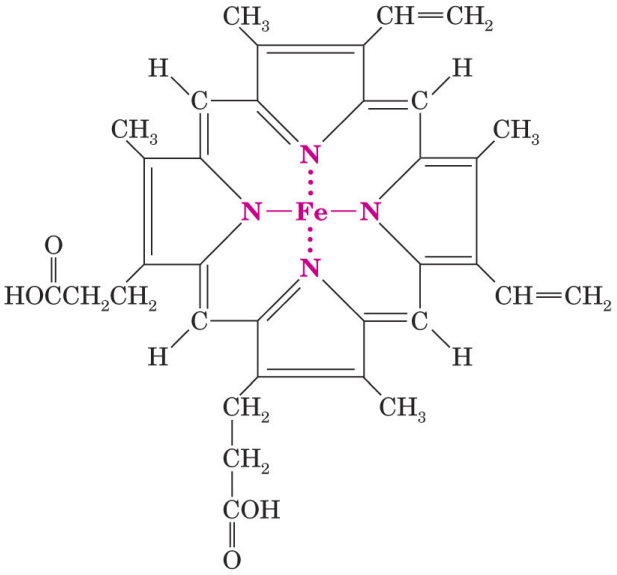
- **Tertiary Structure**
  - ➔ **The tertiary structure of a protein is the three-dimensional shape which results from further folding of its polypeptide chains**
    - **This folding is superimposed on the folding caused by its secondary structure**
  - ➔ **In globular proteins, the folding in tertiary structures exposes the maximum number of polar (hydrophilic) side chains to the aqueous environment, making most globular proteins water soluble**
    - **The folding also serves to enclose a maximum number of nonpolar (hydrophobic) side chains within the protein interior**
  - ➔ **Tertiary structures are stabilized by forces including hydrogen bonding, disulfide bonds, van der Waals forces, and ionic attractions**

- **Myoglobin**
  - **➔The globular protein myoglobin transports oxygen within muscle tissues (See Figure 24.13, page 1200)**
    - **Myoglobin has an associated non-polypeptide molecule called heme (shown in gray)**
    - **The heme group is the site of oxygen binding**

- **Quaternary Structure**
  - ➔ **The overall structure of a protein having multiple subunits is called its quaternary structure**
    - **Not all proteins have quaternary structure**
- **Hemoglobin**
  - ➔ **Hemoglobin is a globular protein that transports oxygen in the blood**
  - ➔ **Hemoglobin contains four polypeptide subunits (2 designated α, and 2 designated β) (See Figure 24.21, page 1210)**
    - **The α subunits are shown in blue and green; β subunits are shown in yellow and cyan**

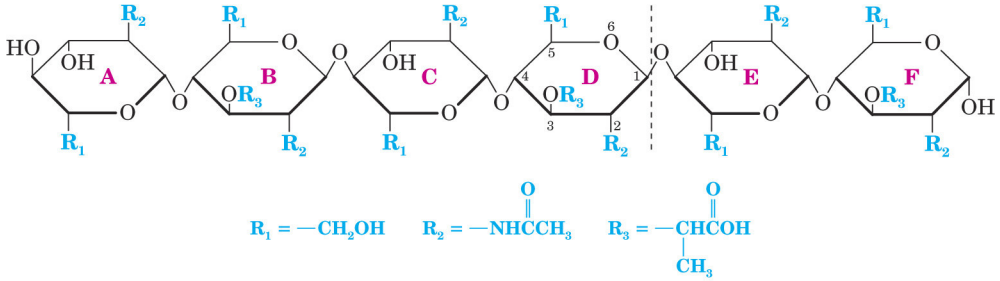
➔Each of the four protein subunits carries a heme group

- **The four heme groups are shown in purple**
- **Each heme group can bind one oxygen molecule in a reversible complex**



- **Introduction to Enzymes**
  - ➔ **Most enzymes are proteins**
  - ➔ **Enzymes can catalyze reactions by a factor of 10^{6}-10^{12}**
  - ➔ **Enzymes have very high specificity for their respective substrates (reactants)**
  - ➔ **Enzymatic reactions take place in the active site of each enzyme**
    - **The structure of the active site facilitates binding and catalysis**
  - ➔ **Enzymes sometimes require a cofactor or coenzyme**
    - **A cofactor can be a metal ion (e.g., Zn^{+2}, Mg^{+2}) bound at the active site**
    - **A coenzyme is a small organic molecule bound at the active site that becomes chemically changed during the enzymatic reaction (e.g., NAD^{+})**

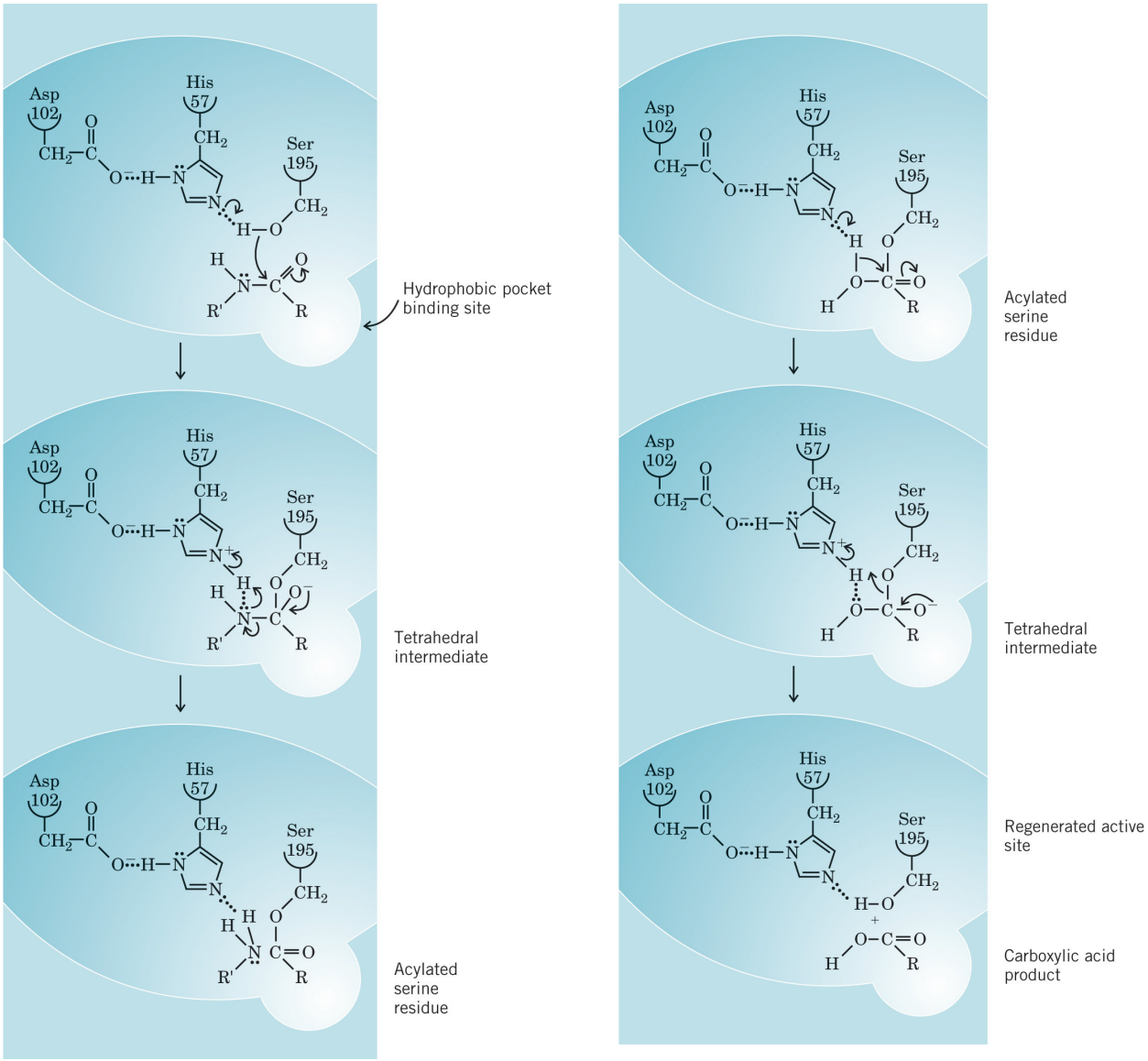
- **Lysozyme**
  - ➔ **Lysozyme catalyzes hydrolysis of a glycosidic linkage in the polysaccharide cell wall of bacteria**
    - **The mechanism of lysozyme involves acid-base reactions and S_N1 reaction**



  - ➔ **The mechanism of lysozyme is shown in Figure 24.16, page 1204**

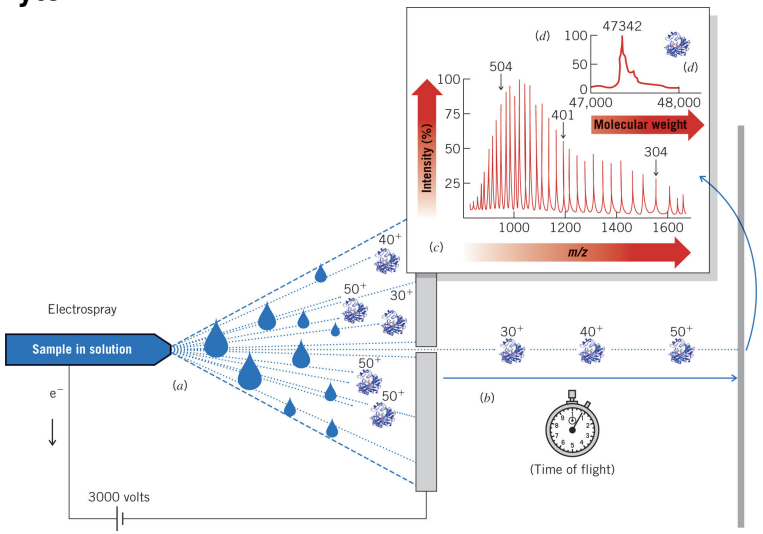
- **Serine Proteases**
  - **➔Proteases hydrolyze amide bonds in proteins**
  - **➔Chymotrypsin, trypsin, and elastin are serine proteases**
  - **➔Serine proteases have a serine hydroxyl group that is involved in the mechanism of amide bond hydrolysis**
    - **A "catalytic triad" involving the side chains of specific aspartic acid, histidine, and serine residues catalyze the amide hydrolysis**
    - **The serine hydroxyl attacks the amide carbonyl group, forming a tetrahedral intermediate**
    - **The aspartic acid and histidine side chains form an acid-base relay system to assist with protonation and deprotonation steps**
    - **The serine tetrahedral intermediate releases the amine, leaving an acylated serine**
    - **A water molecule attacks the carbonyl group of the acylated serine**
    - **A new tetrahedral intermediate forms**
    - **When this tetrahedral intermediate collapses to the carboxylic acid, the serine hydroxyl is released for a new catalytic cycle**
    - **See the following slide for the mechanism of trypsin**
- **The Active Site Catalytic Triad of Trypsin**
  - **This is shown figure 24.17, page 1205**

- **The Catalytic Mechanism of Trypsin**



- **Purification and Analysis of Polypeptides and Proteins**
  - ➔ **Proteins are purified initially by precipitation, column chromatography, and electrophoresis**
  - ➔ **HPLC is the method of choice for final purification of a protein**
- **Analysis of proteins**
  - ➔ **Molecular weight can be estimated by gel electrophoresis and size exclusion chromatography**
  - ➔ **Mass spectrometry is used to determine protein molecular weights with high accuracy and precision**
    - **Electrospray ionization (ESI) mass spectrometry is one way to create protein ions for mass spectrometry**
    - **Matrix-assisted laser desorption ionization (MALDI) mass spectrometry is another technique for generating protein ions for mass spectrometry**
    - **The 2002 Nobel Prize in Chemistry was awarded in part for development of ESI (by Fenn, et al) and MALDI (by Tanaku) for mass spectrometry**

- **Electrospray Ionization (ESI) Mass Spectrometry (MS)**
  - Multiply charged ions of the analyte (e.g., a protein sample) are formed by protonation in an acidic solvent
  - The protonated analyte may have one, several, or many positive charges
  - The charged analyte is sprayed through a high-voltage nozzle into a vacuum chamber
  - Molecules of the solvent evaporate, leaving 'naked' ions of the multiply charged analyte
  - The ions are drawn into a mass analyzer and detected according to mass-to-charge (m/z) ratio
  - Quadrupole and time of flight (TOF) mass analyzers are common methods for detecting and separating ions
  - The family of detected ions is displayed as a series according to m/z ratio
  - Computer deconvolution of the m/z peak series leads to the molecular weight of the analyte



- **Proteomics**
  - **➔Proteomics involves identification and quantification of all of the proteins expressed in a cell at a given time**
    - **Proteins expression levels vary in cells over time**
    - **Proteomics involves identification and quantification of all of the proteins expressed in a cell at a given time**
    - **Proteomics data can shed light on the health or life-cycle stage of a cell**
  - **➔Tools for Proteomics**
    - **Polyacrylamide gel electrophoresis (2D-PAGE) is a low resolution technique for separating protein mixtures**
    - **Two-dimensional (2D) microcapillary HPLC coupled with mass spectrometry is a high resolution technique for separating and identifying proteins in a cell extract**

- **Multidimensional Protein Identification Technology**
  - ➔ **MudPIT (Multidimensional protein identification technology) involves:**
    - **Lysis of intact cells**
    - **Digestion of the proteins to a mixture of smaller peptides**
    - **Separation of the peptide mixture by 2D HPLC using a strong cation exchange column in tandem with a reversed-phase (hydrophobic) column)**
    - **Direct introduction of the 2D HPLC eluent into a mass spectrometer**
    - **Comparison of mass spectra with a database of mass spectral data for known proteins**
    - **Data matching can lead to identification of >1000 proteins in one integrated analysis**