Organic Chemistry, 7th Edition
L. G. Wade, Jr.

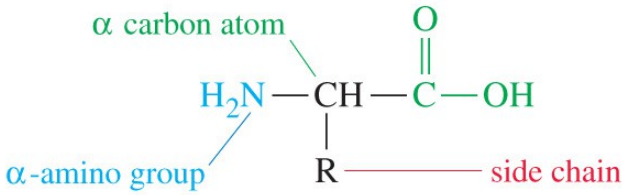
Chapter 24

Amino Acids, Peptides, and Proteins

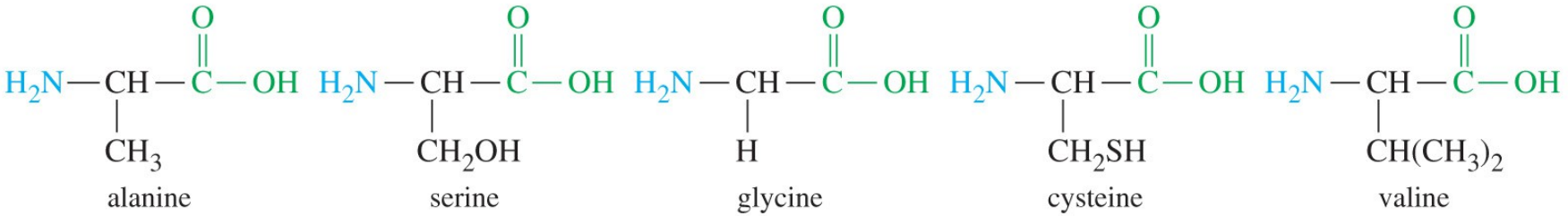
Proteins

- Biopolymers of -amino acids.
- Amino acids are joined by peptide bond.
- They serve a variety of functions:
  - Structure
  - Enzymes
  - Transport
  - Protection
  - Hormones

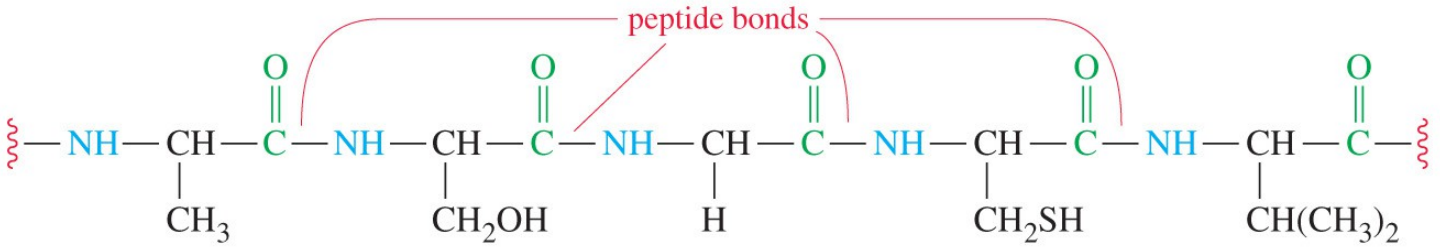
Structure of Proteins



an α-amino acid



several individual amino acids

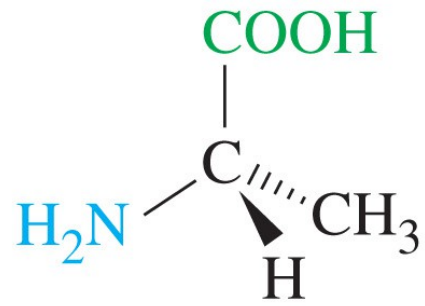


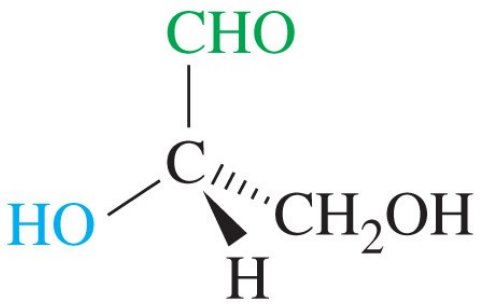
a short section of a protein

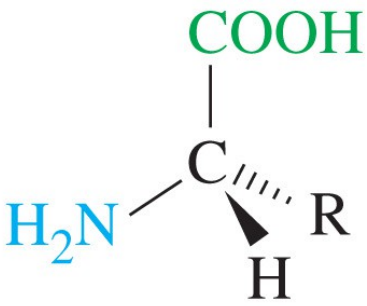
Amino Acids

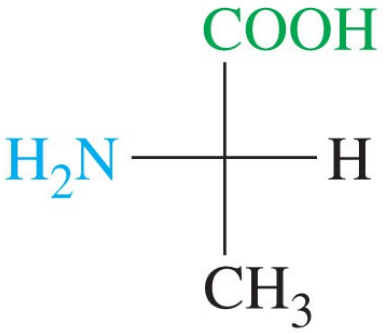
- —NH_2 on the carbon next to —COOH.
- Glycine, NH_2—CH_2—COOH, is simplest.
- With —R side chain, molecule is chiral.
- Most natural amino acids are L-amino acids, related to L-(-)-glyceraldehyde.
- Direction of optical rotation, (+) or (-), must be determined experimentally.

Stereochemistry of -Amino Acids

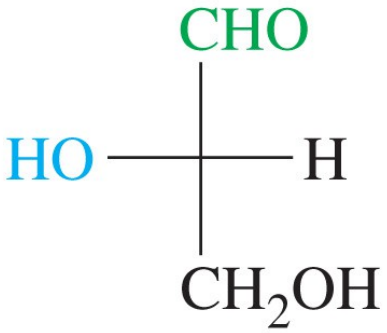




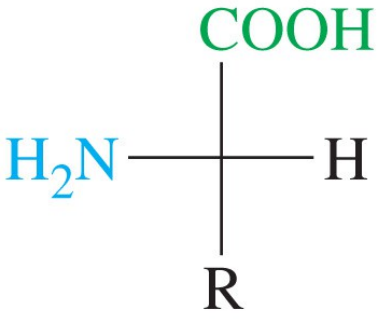




L-alanine
(*S*)-alanine



L-(–)-glyceraldehyde
(*S*)-glyceraldehyde



an L-amino acid
(*S*) configuration

Standard Amino Acids

- Twenty standard -amino acids.
- Differ in side-chain characteristics:
  - —H or alkyl
  - Contains an —OH
  - Contains sulfur
  - Contains a nonbasic nitrogen
  - Has —COOH
  - Has a basic nitrogen

Essential Amino Acids

- Arginine (Arg)
- Threonine (Thr)
- Lysine (Lys)
- Valine (Val)
- Phenylalanine (Phe)
- Tryptophan (Trp)
- Methionine (Met)
- Histidine (His)
- Leucine (Leu)
- Isoleucine (Ile)

TABLE 24-2

The Standard Amino Acids

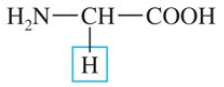
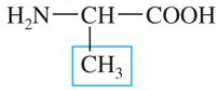
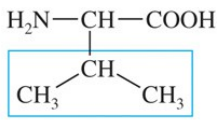
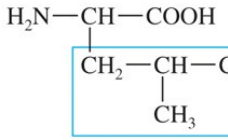
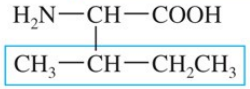
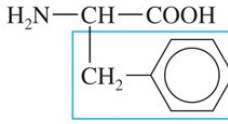
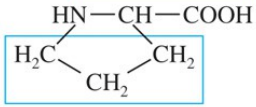
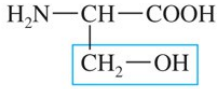
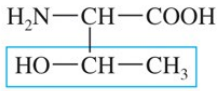
Name	Symbol	Abbreviation	Structure	Functional Group in Side Chain	Isoelectric Point
side chain is nonpolar, H or alkyl					
glycine	G	Gly		none	6.0
alanine	A	Ala		alkyl group	6.0
*valine	V	Val		alkyl group	6.0
*leucine	L	Leu		alkyl group	6.0
*isoleucine	I	Ile		alkyl group	6.0
*phenylalanine	F	Phe		aromatic group	5.5
proline	P	Pro		rigid cyclic structure	6.3
side chain contains an —OH					
serine	S	Ser		hydroxyl group	5.7
*threonine	T	Thr		hydroxyl group	5.6

TABLE 24-2

The Standard Amino Acids (*continued*)

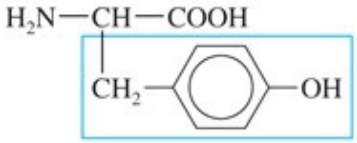
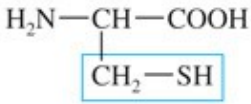
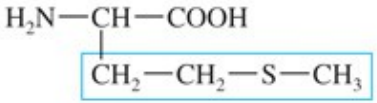
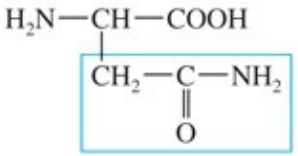
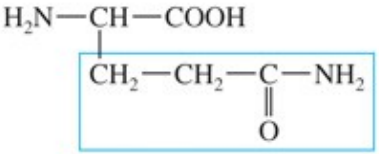
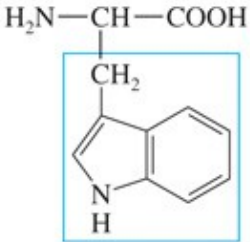
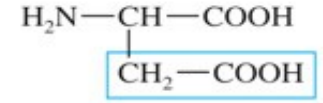
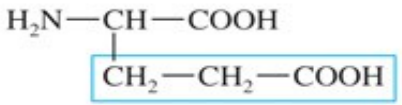
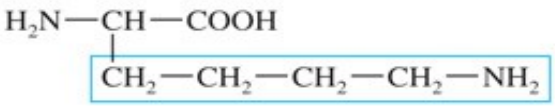
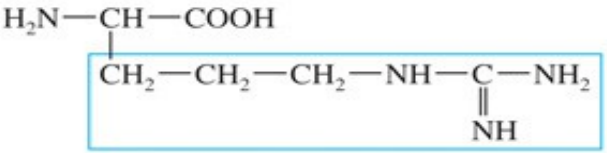
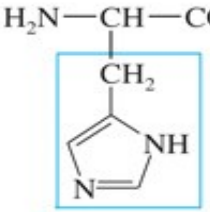
Name	Symbol	Abbreviation	Structure	Functional Group in Side Chain	Isoelectric Point
tyrosine	Y	Tyr		phenolic—OH group	5.7
side chain contains sulfur					
cysteine	C	Cys		thiol	5.0
*methionine	M	Met		sulfide	5.7
side chain contains nonbasic nitrogen					
asparagine	N	Asn		amide	5.4
glutamine	Q	Gln		amide	5.7
*tryptophan	W	Trp		indole	5.9

TABLE 24-2

The Standard Amino Acids (*continued*)

Name	Symbol	Abbreviation	Structure	Functional Group in Side Chain	Isoelectric Point
side chain is acidic					
aspartic acid	D	Asp		carboxylic acid	2.8
glutamic acid	E	Glu		carboxylic acid	3.2
side chain is basic					
*lysine	K	Lys		amino group	9.7
*arginine	R	Arg		guanidino group	10.8
*histidine	H	His		imidazole ring	7.6

*essential amino acid

Complete Proteins

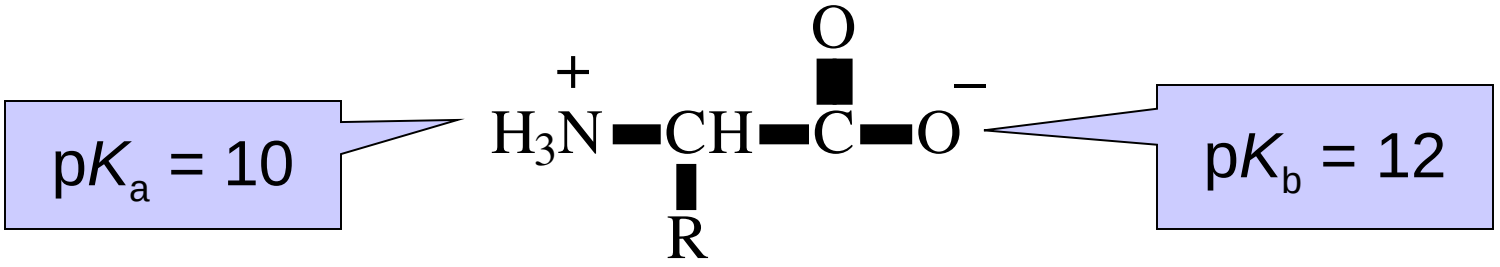
- Provide all the essential amino acids.
- Examples: Those found in meat, fish, milk, and eggs.
- Plant proteins are generally incomplete.
- Vegetarians should eat many different kinds of plants, or supplement their diets with milk and/or eggs.

Rare Amino Acids

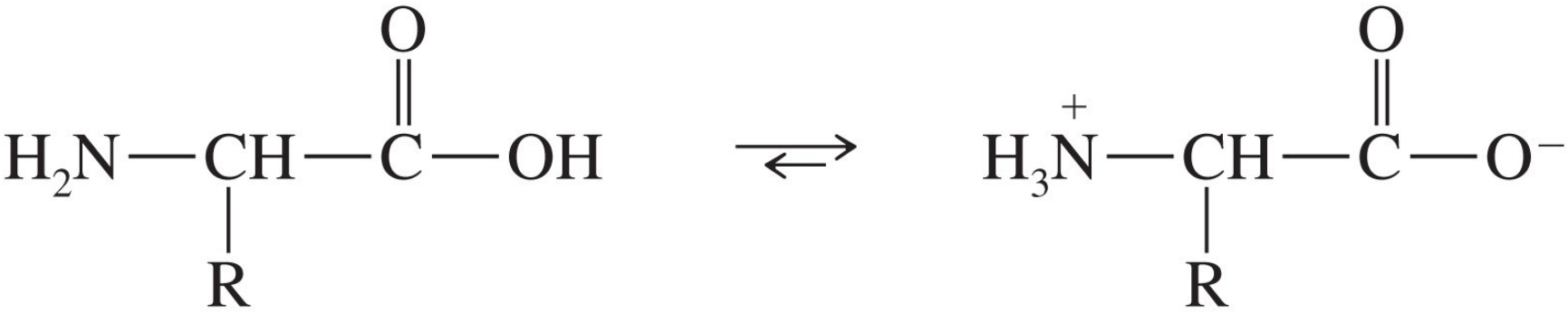
- 4-Hydroxyproline and 5-hydroxylysine is found in collagen.
- D-Glutamic acid is found in cell walls of bacteria.
- D-Serine is found in earthworms.
- -Aminobutyric acid is a neurotransmitter.
- -Alanine is a constituent of the vitamin pantothenic acid.

Properties of Amino Acids

- High melting points, over 200 C.
- More soluble in water than in ether.
- Larger dipole moments than simple acids or simple amines.
- Less acidic than most carboxylic acids; less basic than most amines.

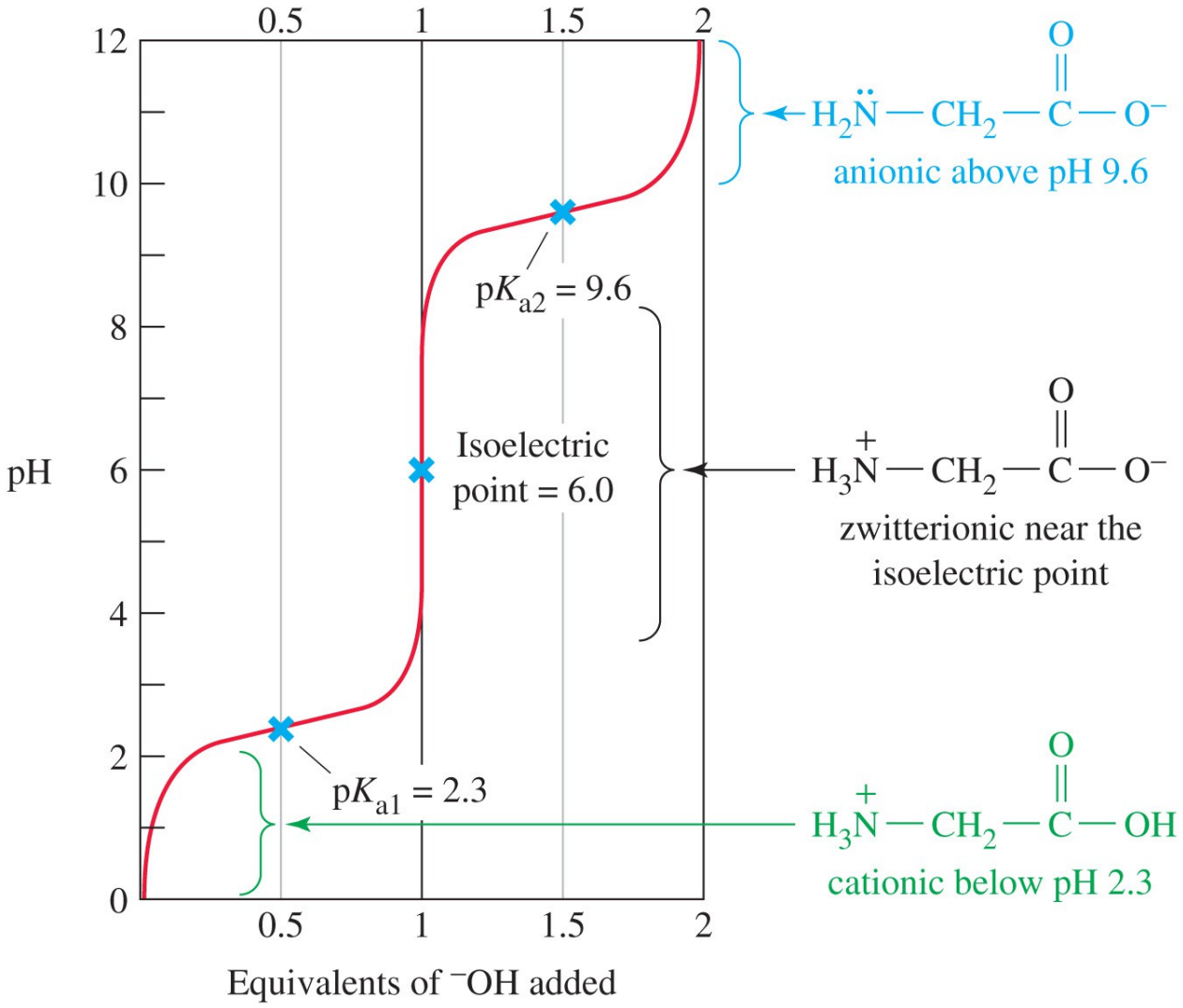


Zwitterion Formation



- Amino acid exists as a dipolar ion.
- —COOH loses H^+, —NH_2 gains H^+.
- Actual structure depends on pH.

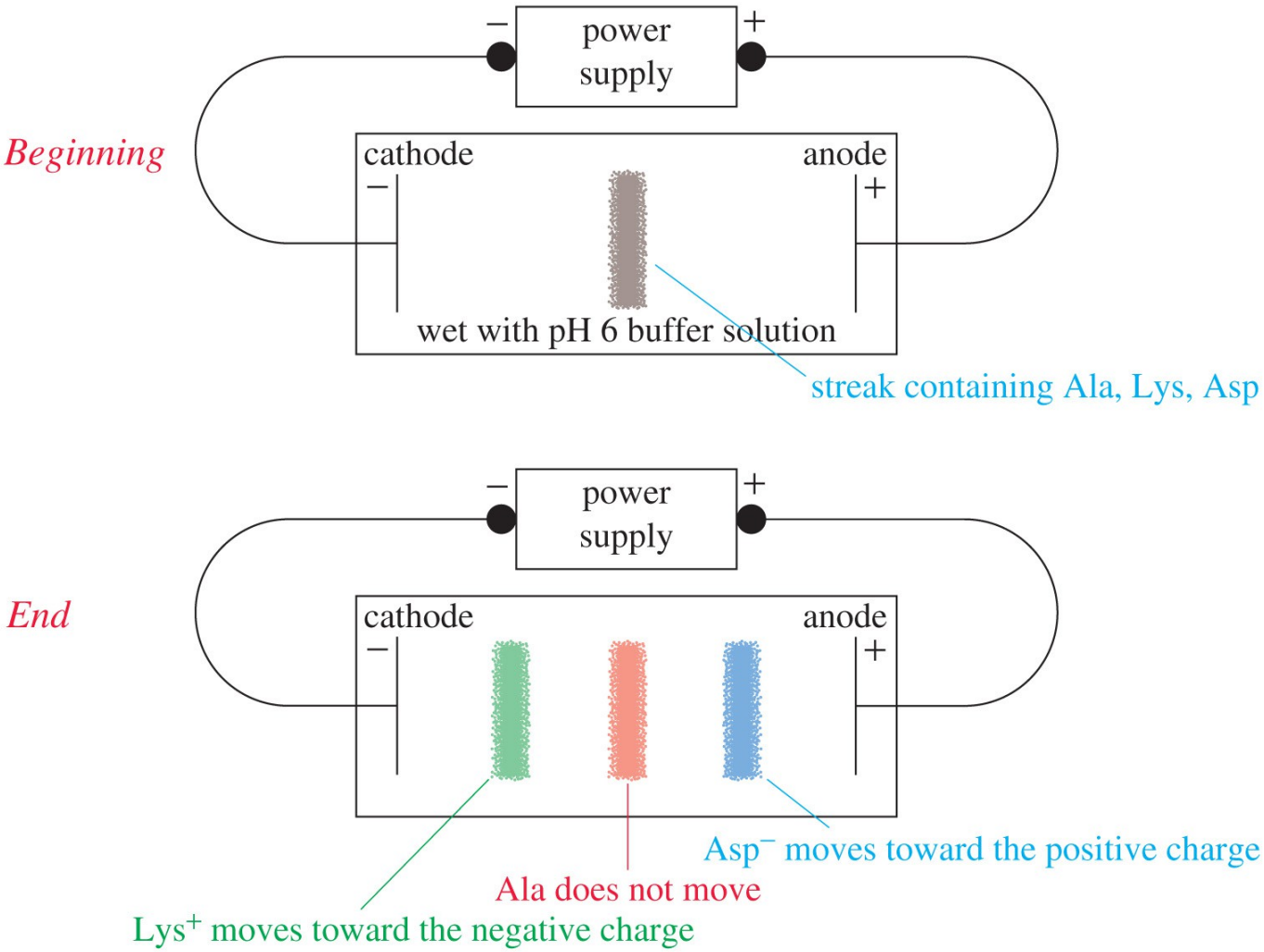
Structure and pH



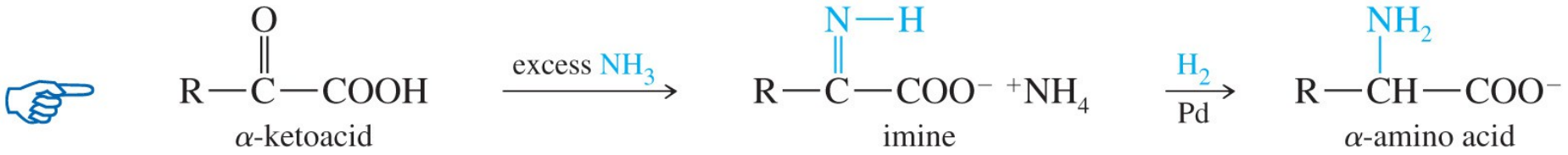
Isoelectric Point of Amino Acids

- ***Isoelectric point*** (pI) is defined as the pH at which amino acids exist as the zwitterion (neutral charge).
- The pI depends on structure of the side chain of the amino acid.
- Acidic amino acids, isoelectric pH ~3.
- Basic amino acids, isoelectric pH ~9.
- Neutral amino acids, isoelectric pH is slightly acidic, 5–6.

Electrophoresis

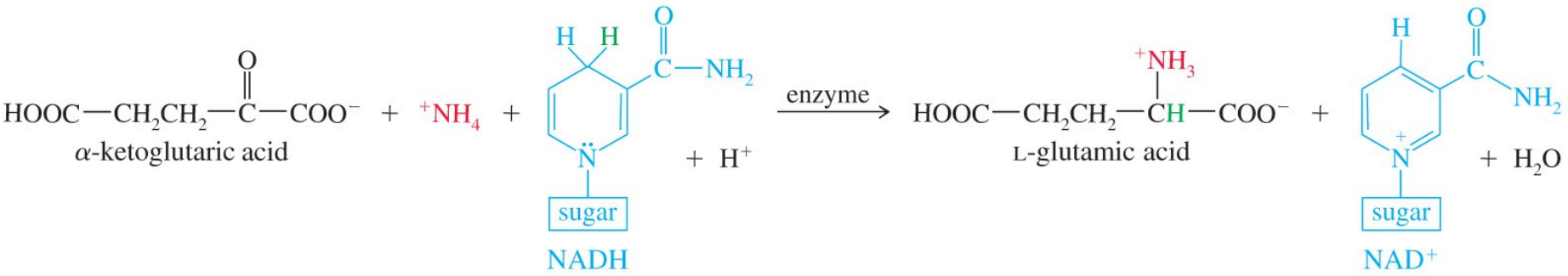


Reductive Amination



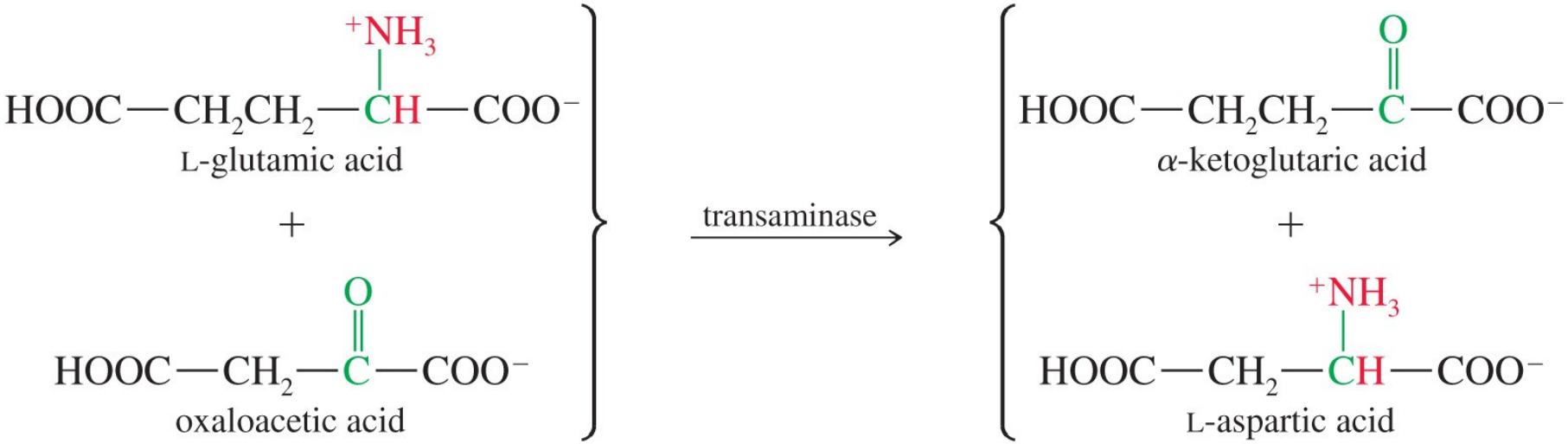
- This method for synthesizing amino acids is ***biomimetic***, mimics the biological process.
- React an -ketoacid with ammonia, then reduce the imine with H_2/Pd.
- Racemic mixture is obtained.

Biosynthesis of Amino Acids



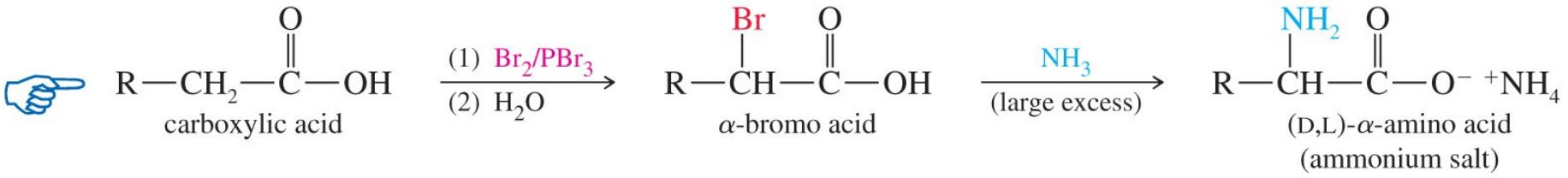
- The biosynthesis begins with reductive amination of -ketoglutaric acid (an intermediate in the metabolism of carbohydrates), using the ammonium ion as the aminating agent and NADH as the reducing agent.
- The product of this enzyme-catalyzed reaction is the pure L–enantiomer of glutamic acid.

Transamination



- 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***.

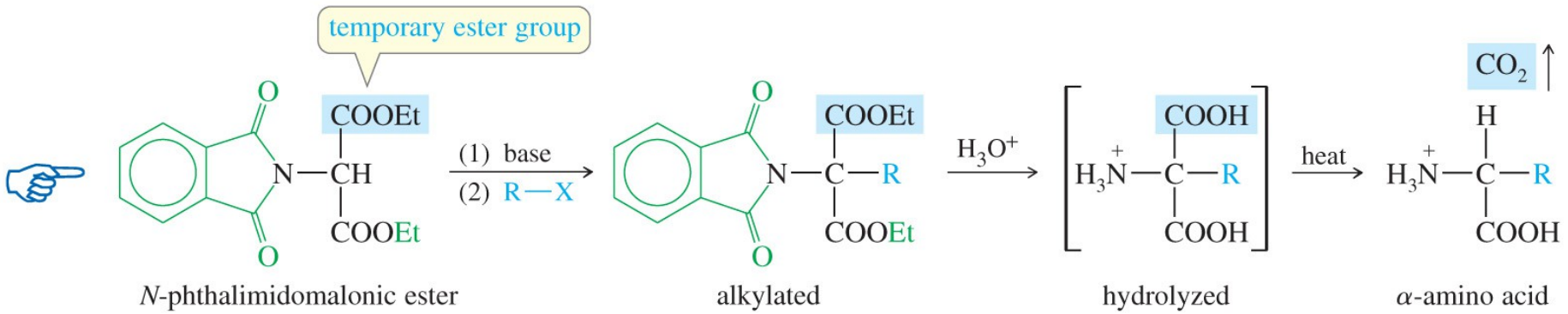
Synthesis from -Halo Acid



- Hell–Volhard–Zelinsky reaction places a bromine on the carbon of a carboxylic acid.
- Bromine is then replaced by reaction with excess ammonia.
- A racemic mixture is obtained.

Gabriel–Malonic Ester Synthesis

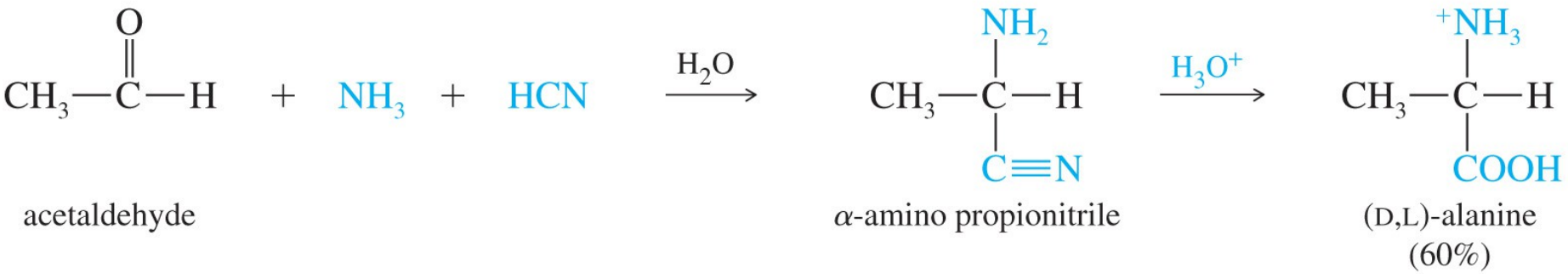
The Gabriel–malonic ester synthesis



- The amino group is protected as amide.
- The carboxylic acid group is protected as an ester.
- The -position is further activated by the additional temporary ester group.

Strecker Synthesis

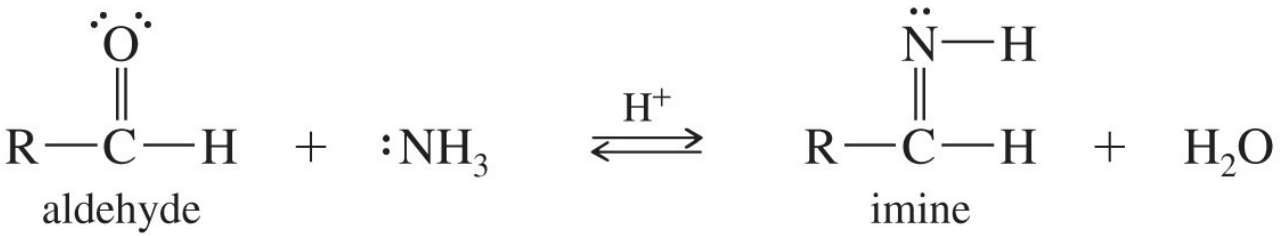
The Strecker synthesis of alanine



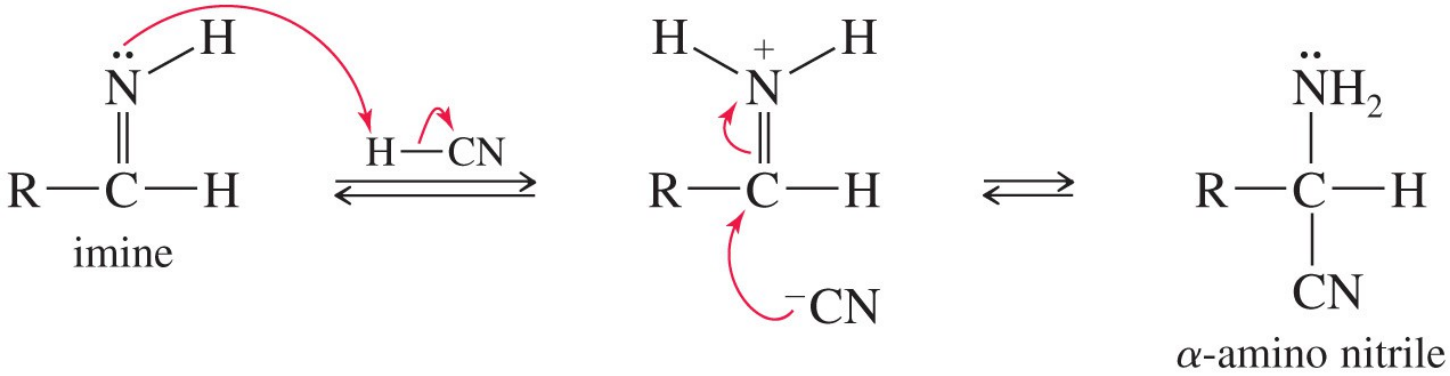
- First known synthesis of amino acid occurred in 1850.
- Aldehyde reaction with NH_3 yields imine.
- Cyanide ion attacks the protonated imine.
- Resulting -amino nitrile is hydrolyzed to a carboxylic acid.

Strecker Mechanism

Step 1: The aldehyde reacts with ammonia to form the imine (mechanism in Section 18-16)



Step 2: Cyanide ion attacks the imine.

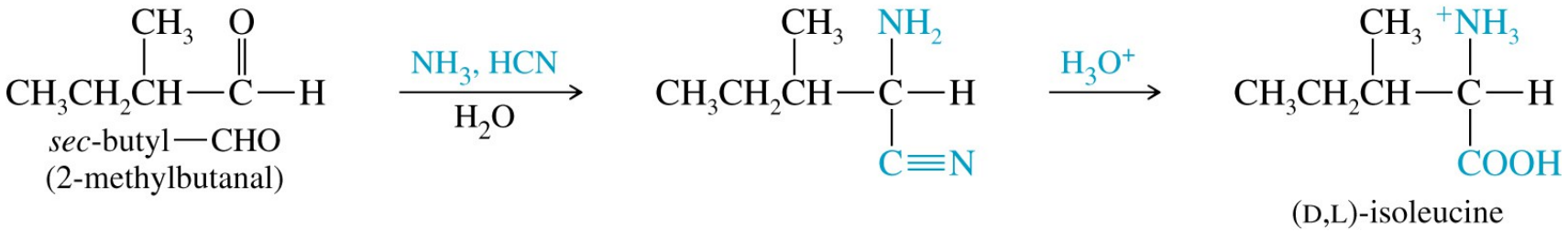


Solved Problem 1

Show how you would use a Strecker synthesis to make isoleucine.

Solution

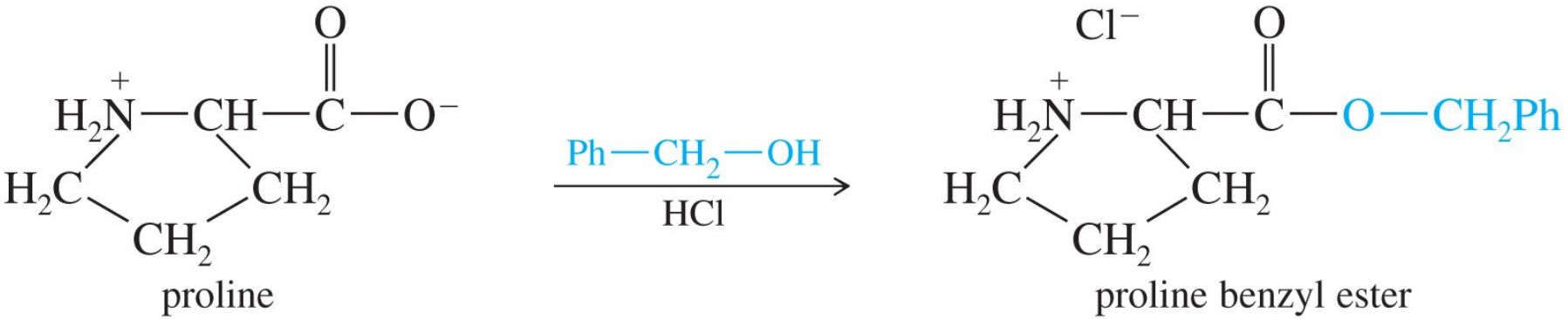
Isoleucine has a *sec*-butyl group for its side chain. Remember that CH_3–CHO undergoes Strecker synthesis to give alanine, with CH_3 as the side chain. Therefore, *sec*-butyl–CHO should give isoleucine.



Resolution of Amino Acids

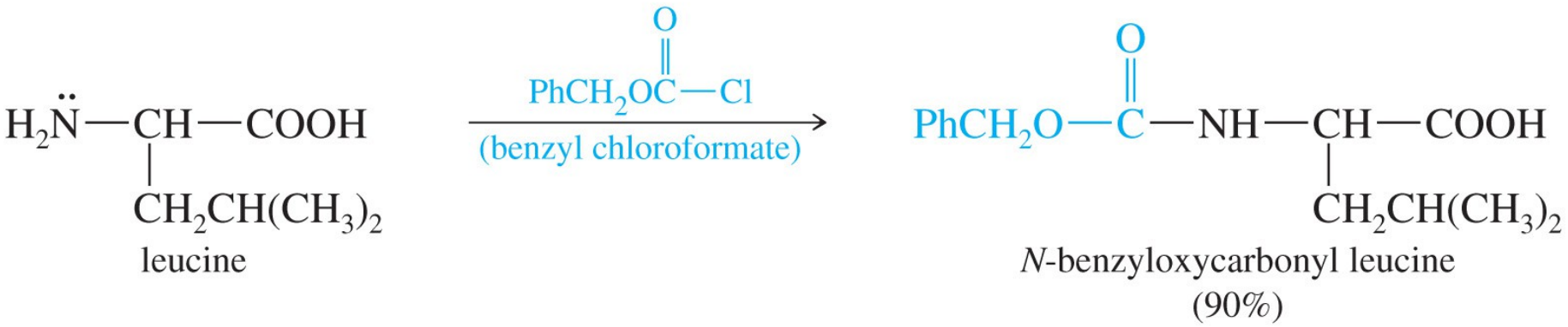
- Usually, only the L–enantiomer is biologically active.
- Convert the amino acid to a salt, using a chiral acid or base. The result is a mixture of diastereomeric salts that can be separated by chromatography.
- Use an enzyme, such as acylase, that will react with only one enantiomer.

Esterification of the Carboxyl Group



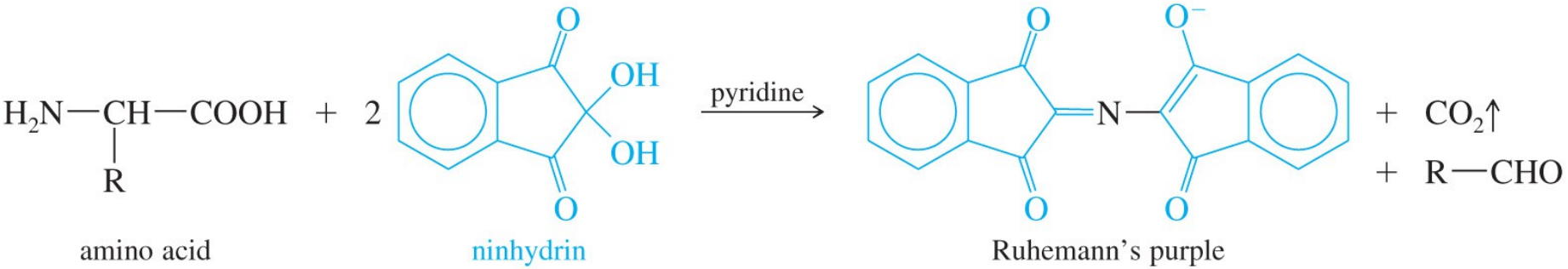
- Use a large excess of alcohol and an acidic catalyst.
- Esters are often used as protective derivatives.
- Aqueous hydrolysis regenerates the acid.

Acylation



- The amino group is converted to an amide.
- Acid chlorides and anhydrides are the acylating agents.
- Benzyl chloroformate, $PhCH_2OCOCl$, is commonly used because it is easily removed.

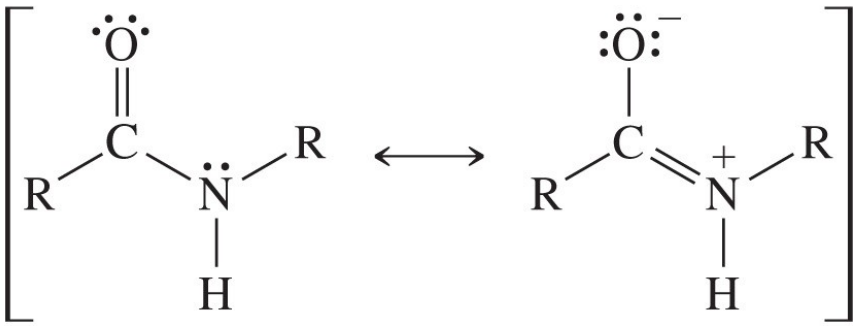
Reaction with Ninhydrin

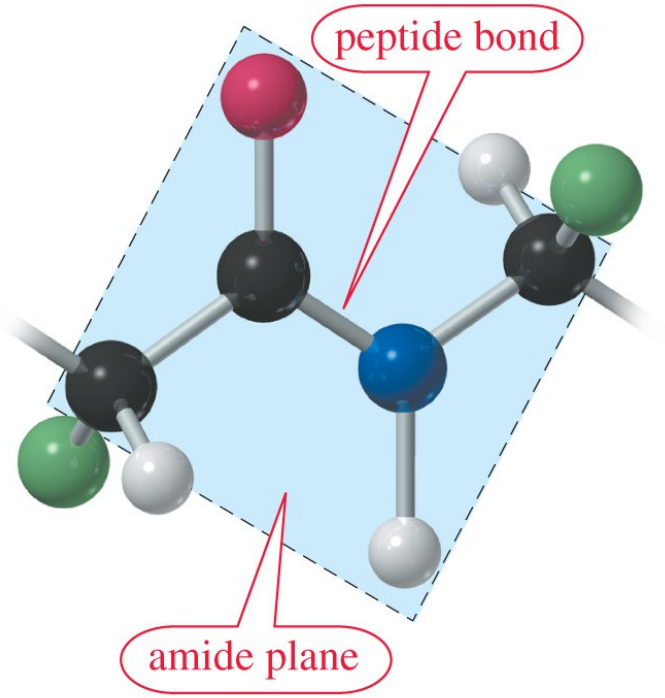


- Used to visualize spots or bands of amino acids separated by chromatography or electrophoresis.
- Deep purple color formed with traces of any amino acid.

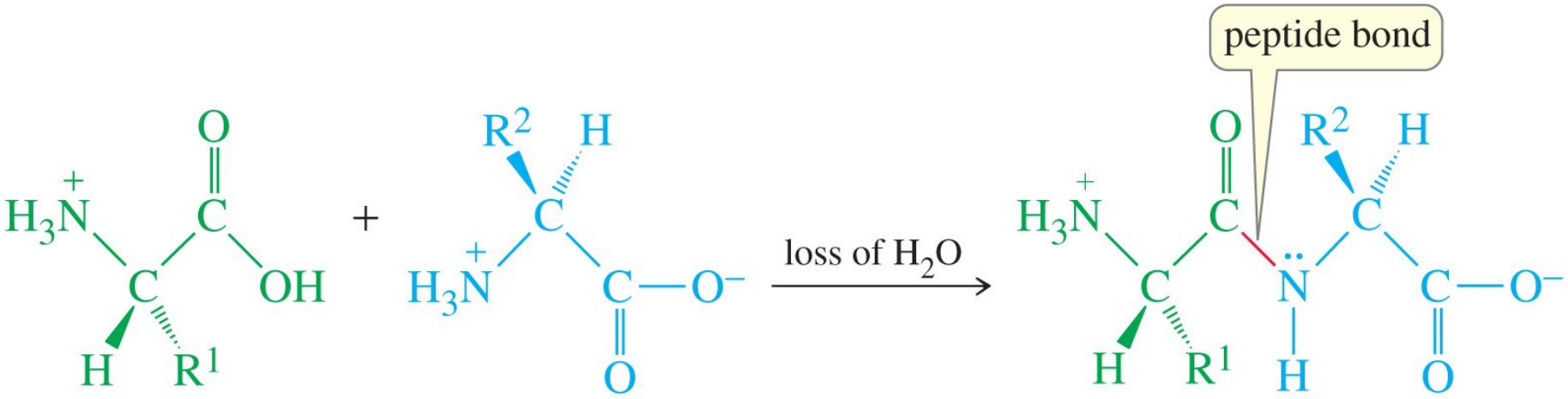
Resonance Stabilization

- The peptide bond is an amide bond.
- Amides are very stable and neutral.



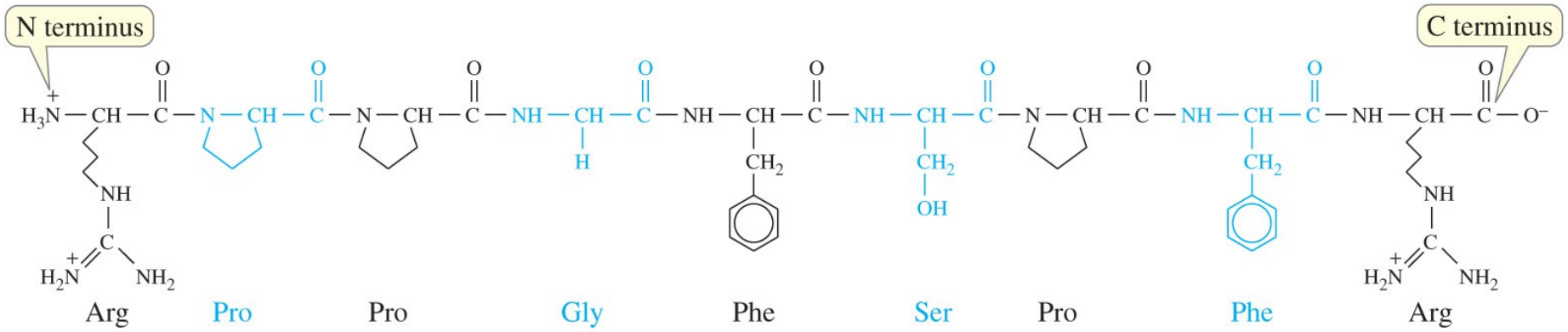


Peptide Bond Formation



- The amino group of one molecule condenses with the acid group of another.
- Polypeptides usually have molecular weight less than 5,000.
- Protein molecular weight is 6,000–40,000,000.

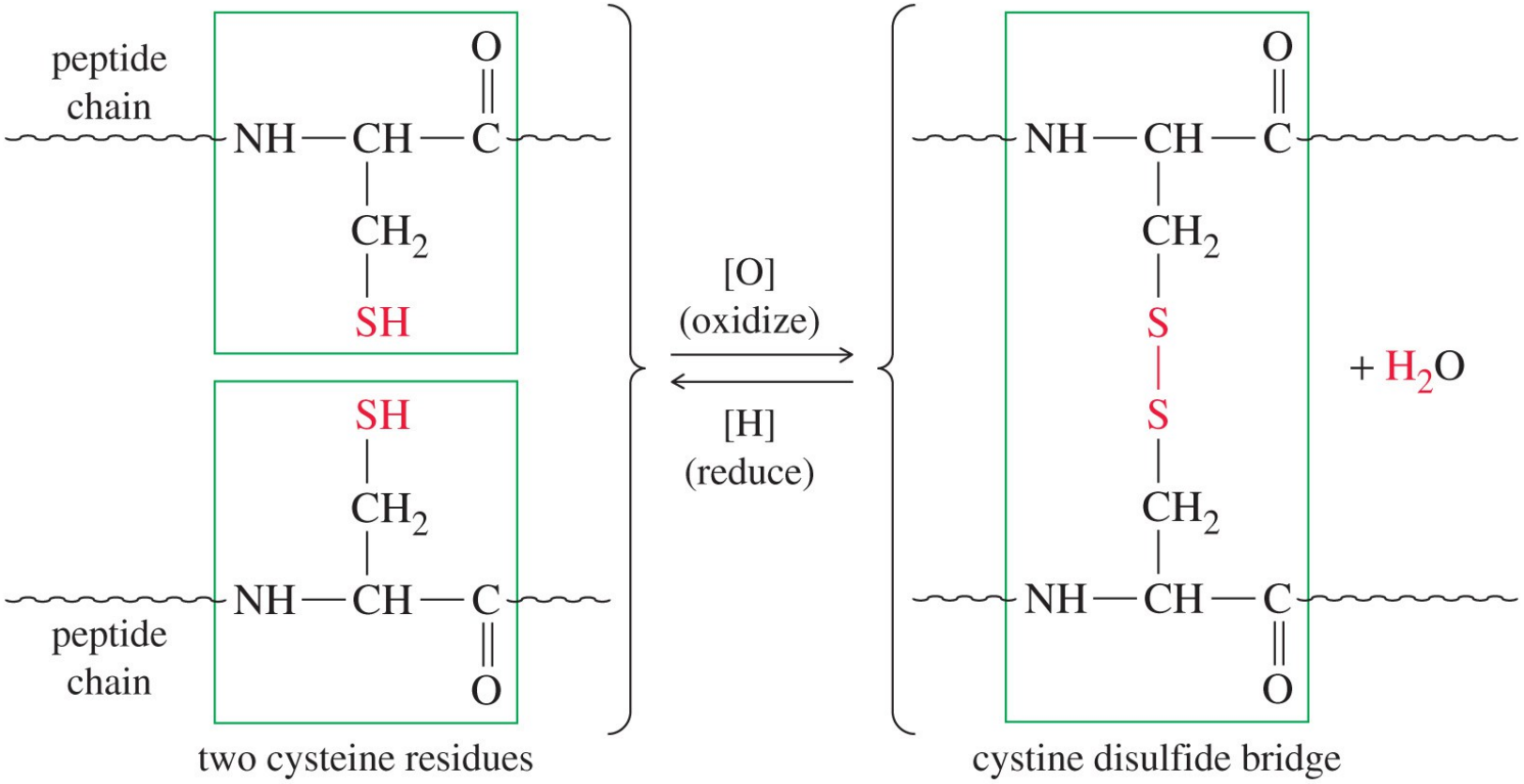
Human Hormone Bradykinin



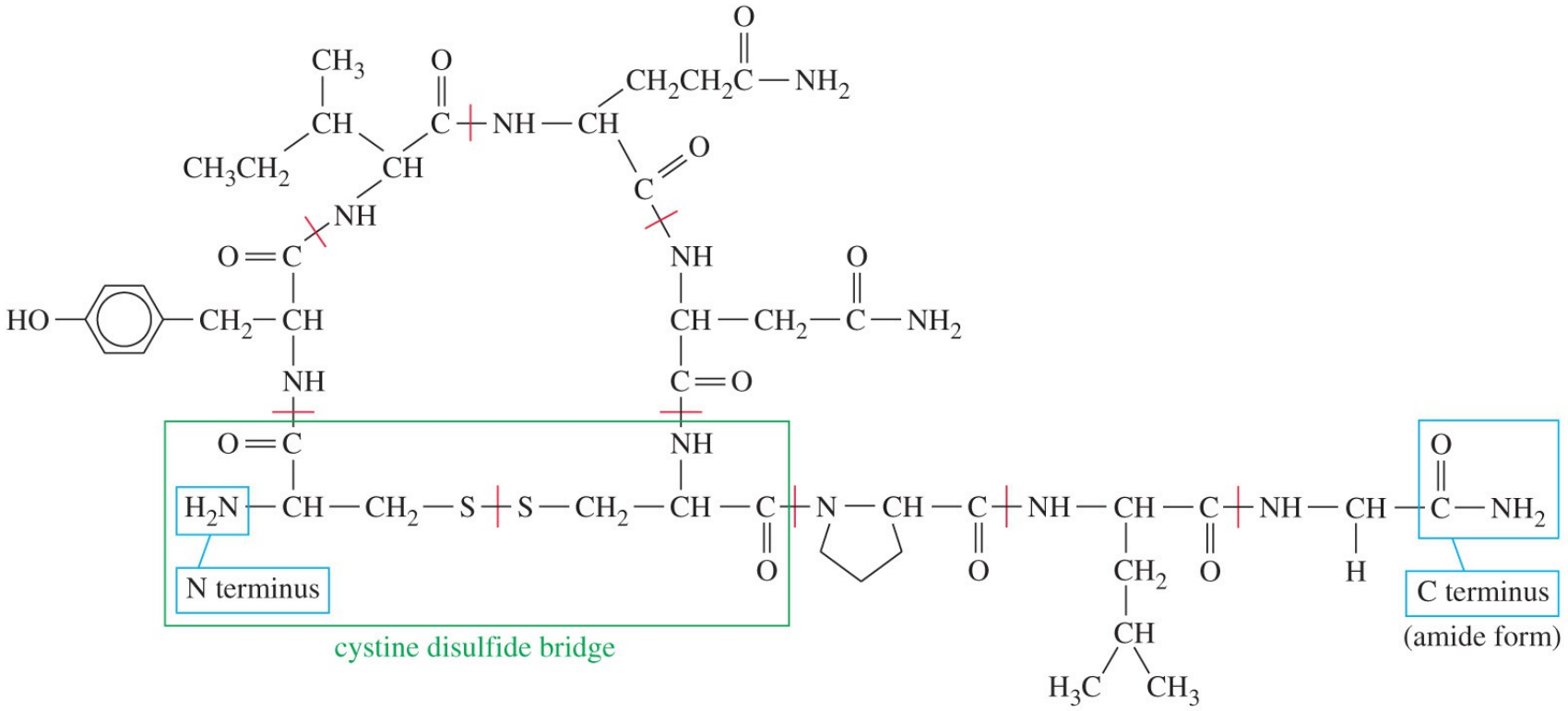
- An oligopeptide is made out of four to ten amino acids.
- Peptide structures are drawn with the N-terminal end at the left.
- Peptides are named from left to right: arginylprolylprolyl……arginine.

Disulfide Linkages

- Cysteine can form disulfide bridges.

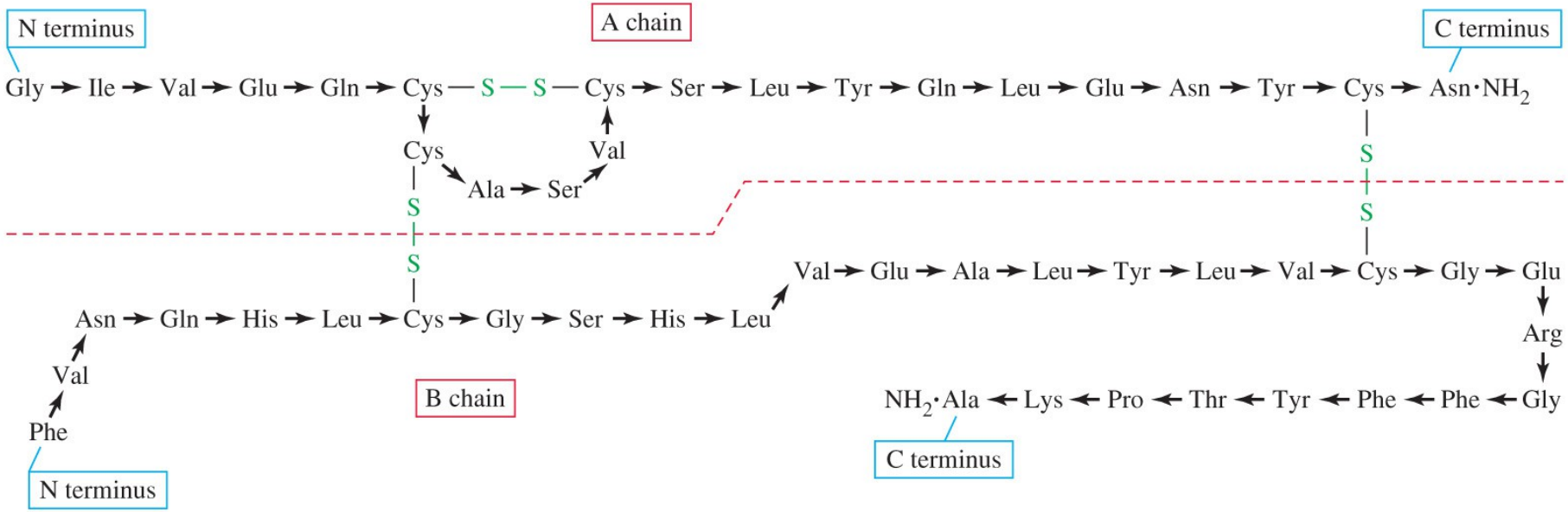


Human Oxytocin



- Oxytocin is a nonapeptide with two cysteine residues (at Positions 1 and 6) linking part of the molecule in a large ring.

Bovine Insulin

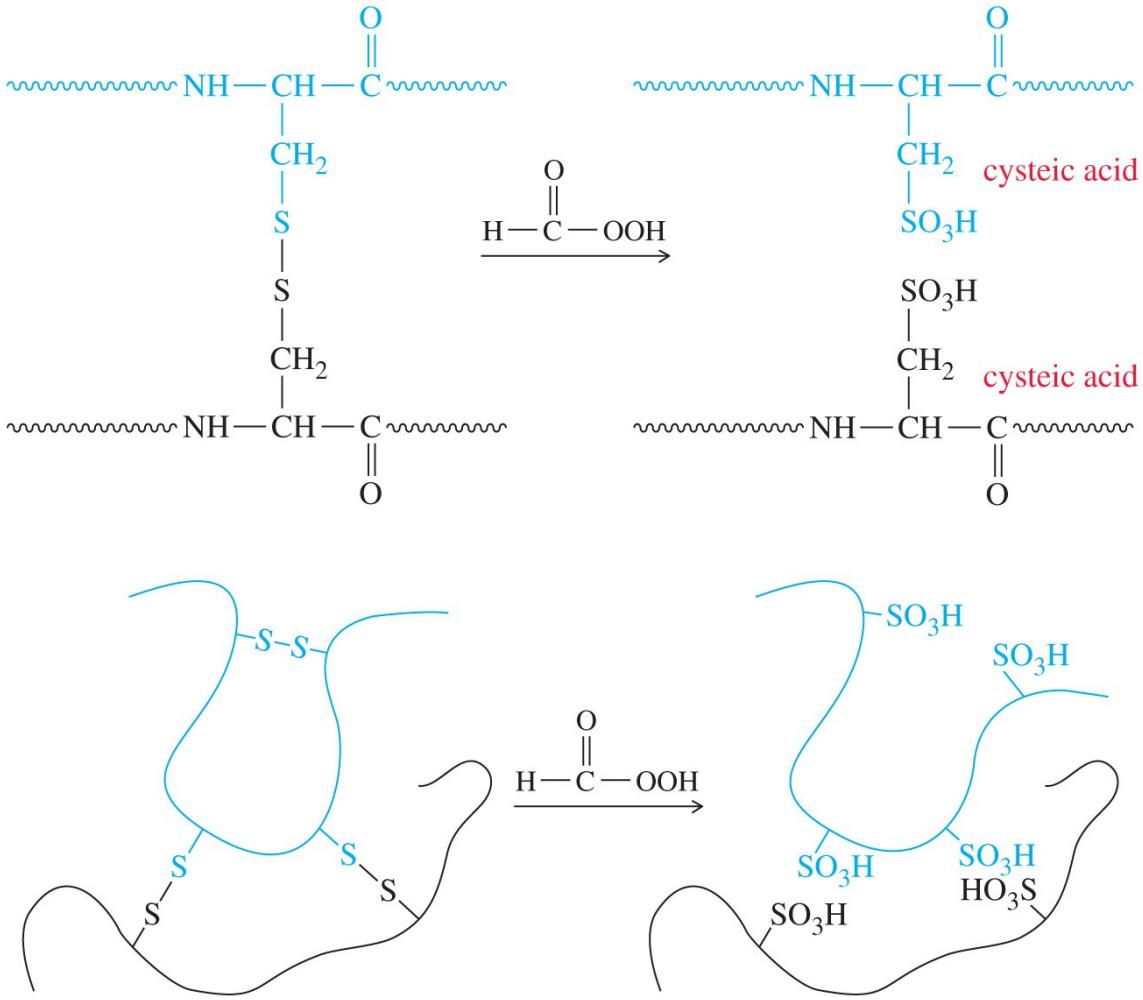


- Insulin is composed of two separate peptide chains, the A chain containing 21 amino acid residues, and the B chain containing 30.

Peptide Structure Determination

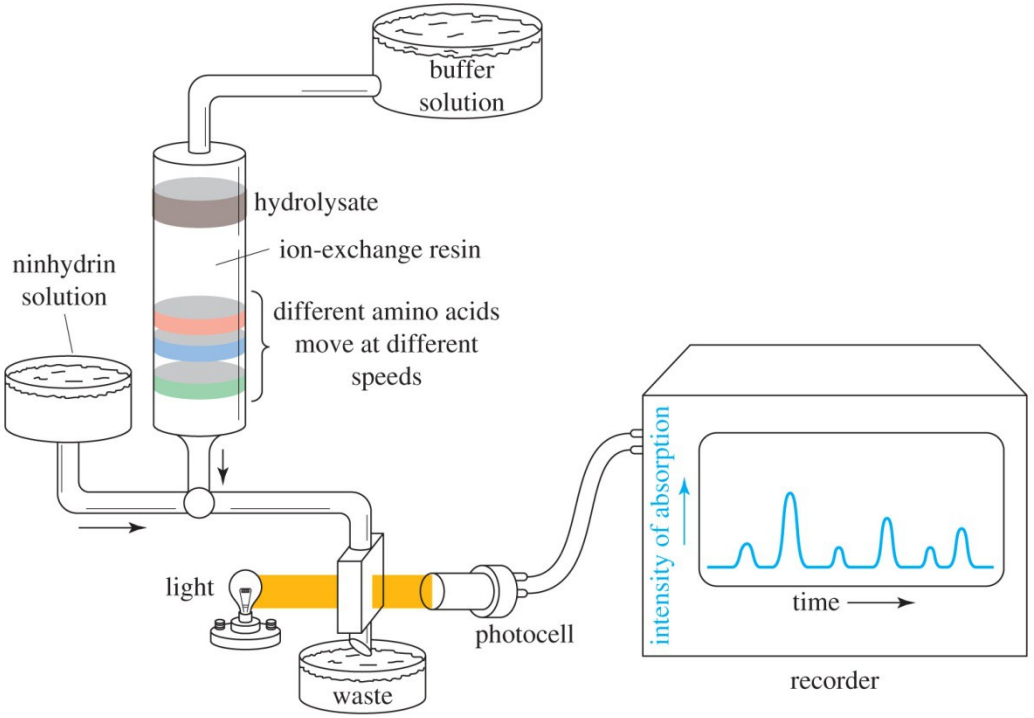
- Cleavage of disulfide linkages.
- Determination of amino acid composition.
- Sequencing from the N terminus.
- C-terminal residue analysis.
- Partial hydrolysis.

Disulfide Cleavage

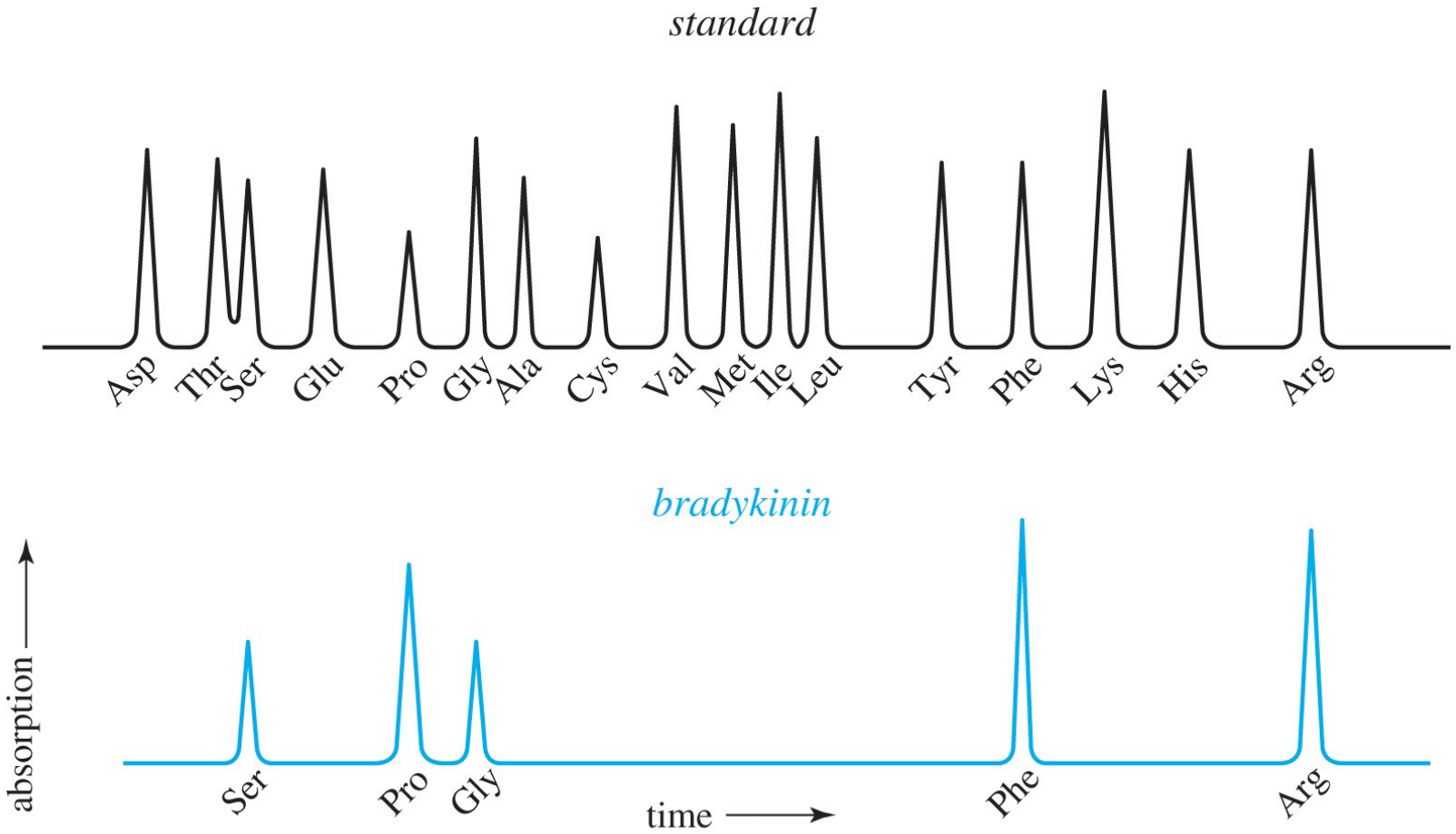


Amino Acid Composition

- Separate the individual peptide chains.
- Boil with 6 M HCl for 24 hours.
- Separate in an amino acid analyzer.



Composition of Human Bradykinin

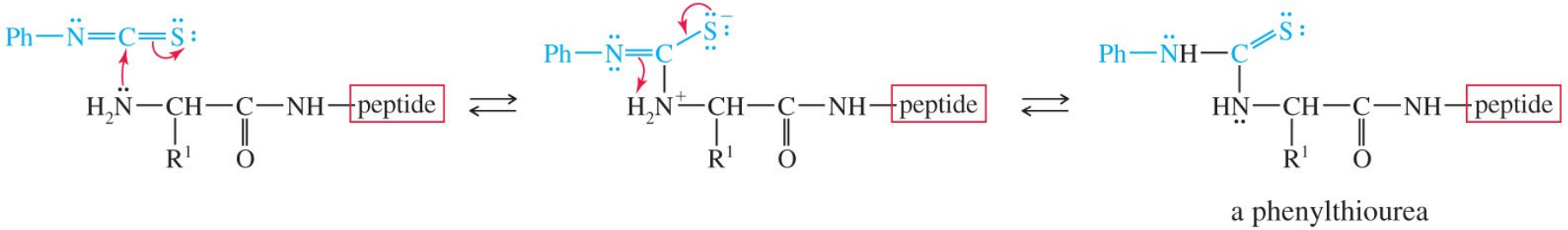


Sequencing from the N Terminus

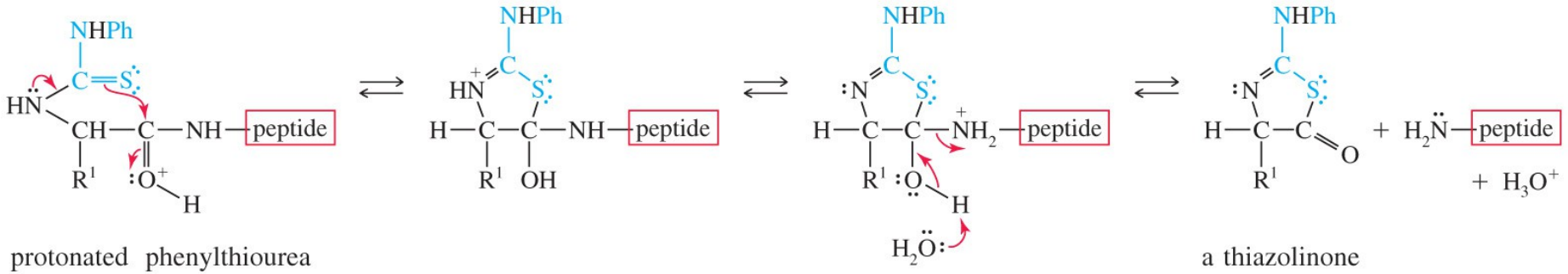
- ***Edman degradation***: The reaction with phenyl isothiocyanate followed by hydrolysis removes the N terminus amino acid.
- The phenylthiohydantoin derivative is identified by chromatography.
- Use for peptides with < 30 amino acids.

Edman Degradation

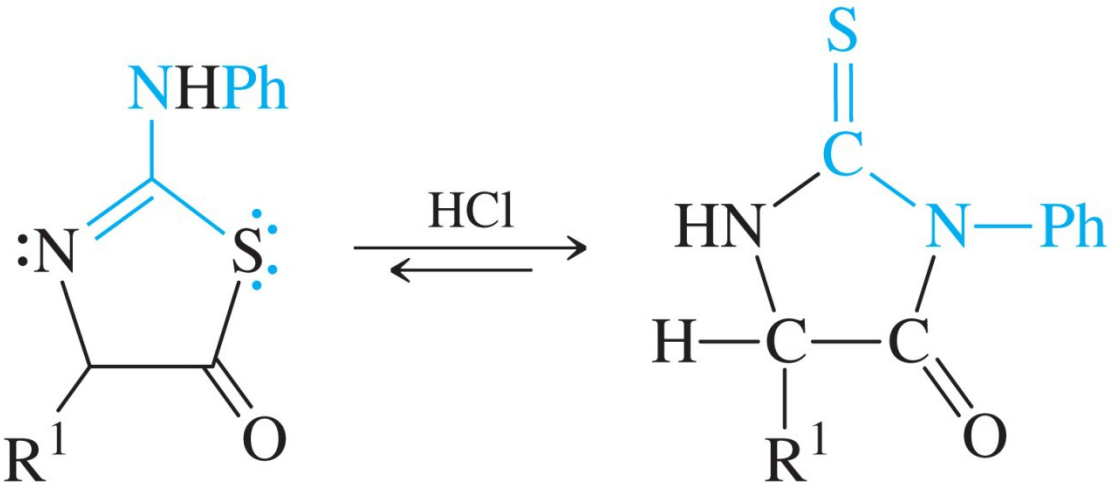
Step 1: Nucleophilic attack by the free amino group on phenyl isothiocyanate, followed by a proton transfer, gives a phenylthiourea.



Step 2: Treatment with HCl induces cyclization to a thiazolinone and expulsion of the shortened peptide chain.

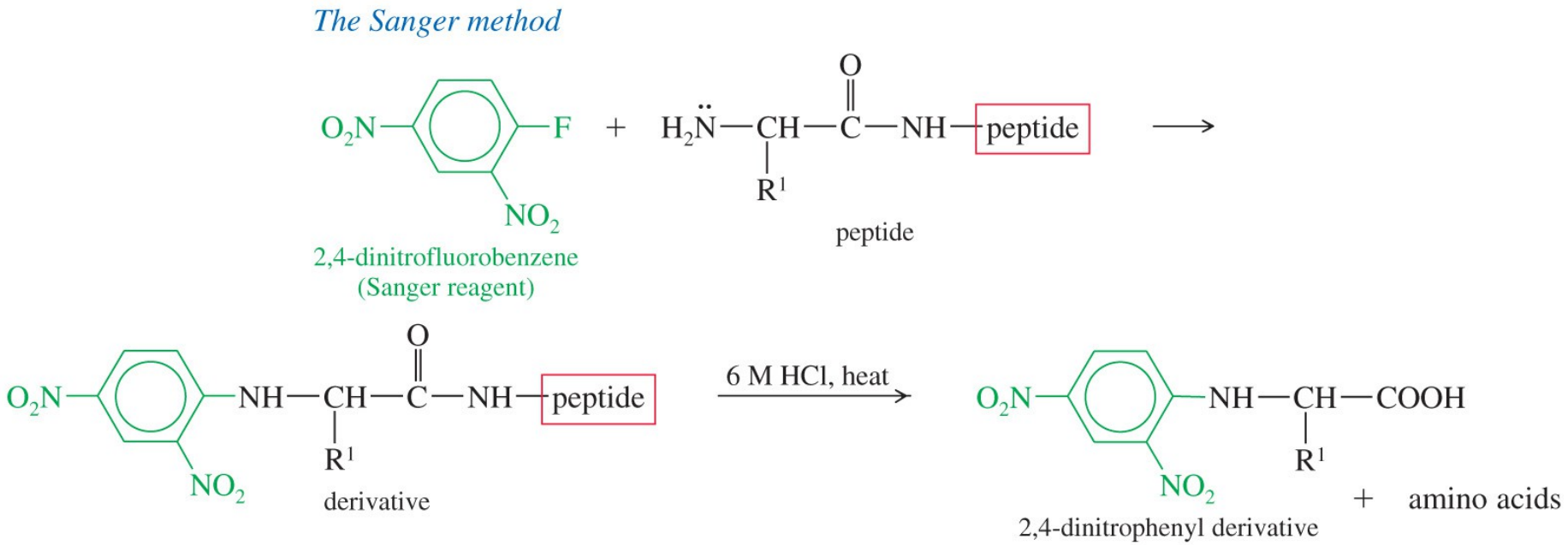


Edman Degradation (Continued)



- In the final step (step 3) the thiazoline isomerizes to the more stable phenylthiohydantoin.

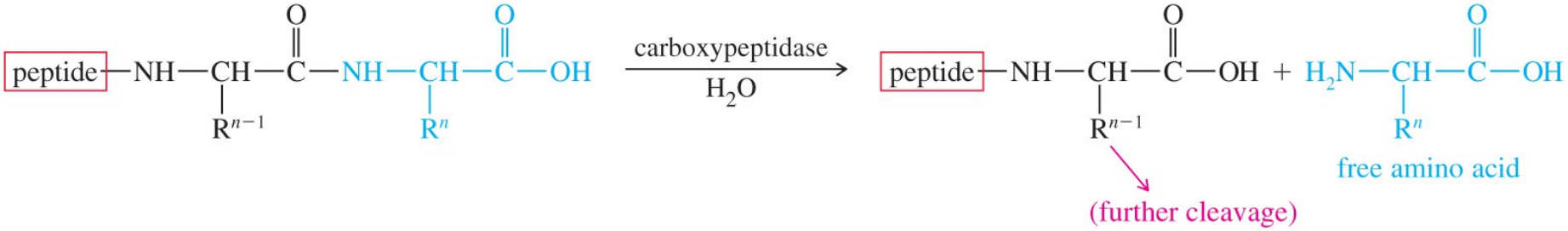
The Sanger Method



Sequencing from the C Terminus

- The enzyme carboxypeptidase cleaves the C-terminal peptide bond.
- However, since different amino acids react at different rates, it’s difficult to determine more than the original C-terminal amino acid.

C-Terminal Residue Analysis



- The C-terminal amino acid can be identified using the enzyme carboxypeptidase, which cleaves the C-terminal peptide bond.
- Eventually, the entire peptide is hydrolyzed to its individual amino acids.

Partial Hydrolysis

- Break the peptide chain into smaller fragments.
  - Trypsin cleaves at the carboxyl group of lysine and arginine.
  - Chymotrypsin cleaves at the carboxyl group of phenylalanine, tyrosine, and tryptophan.
- Sequence each fragment, then fit them together like a jigsaw puzzle.

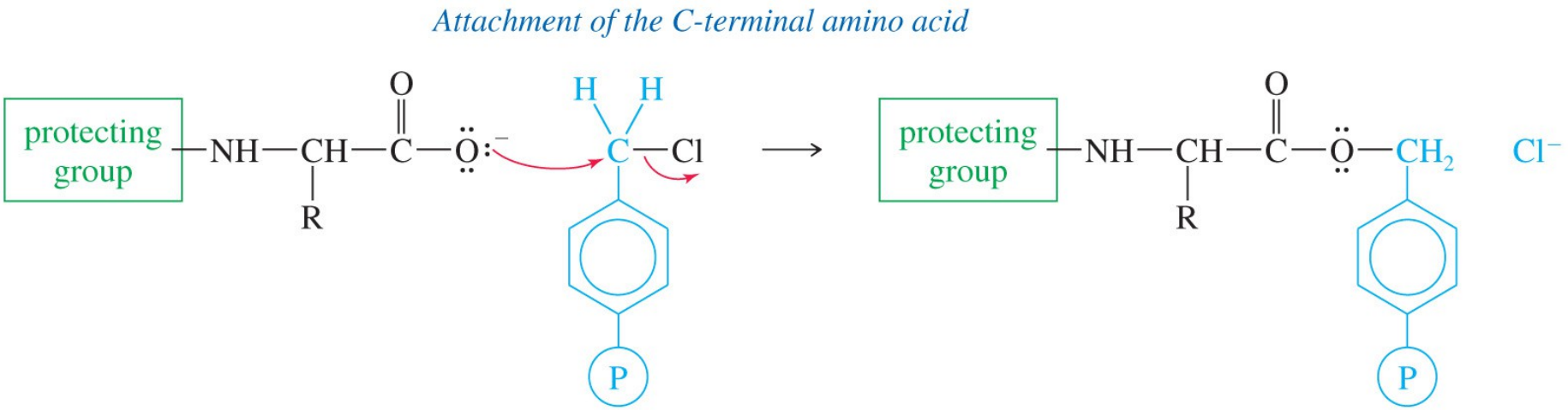
Solution Phase Peptide Synthesis

- First, protect the amino group at the N terminus with benzyl chloroformate.
- Activate the carboxyl group with ethyl chloroformate to form anhydride of carbonic acid.
- Couple the next amino acid.
- Repeat activation and coupling until all amino acids needed have been added.
- Remove the protecting group.

Advantages of Solid Phase Synthesis

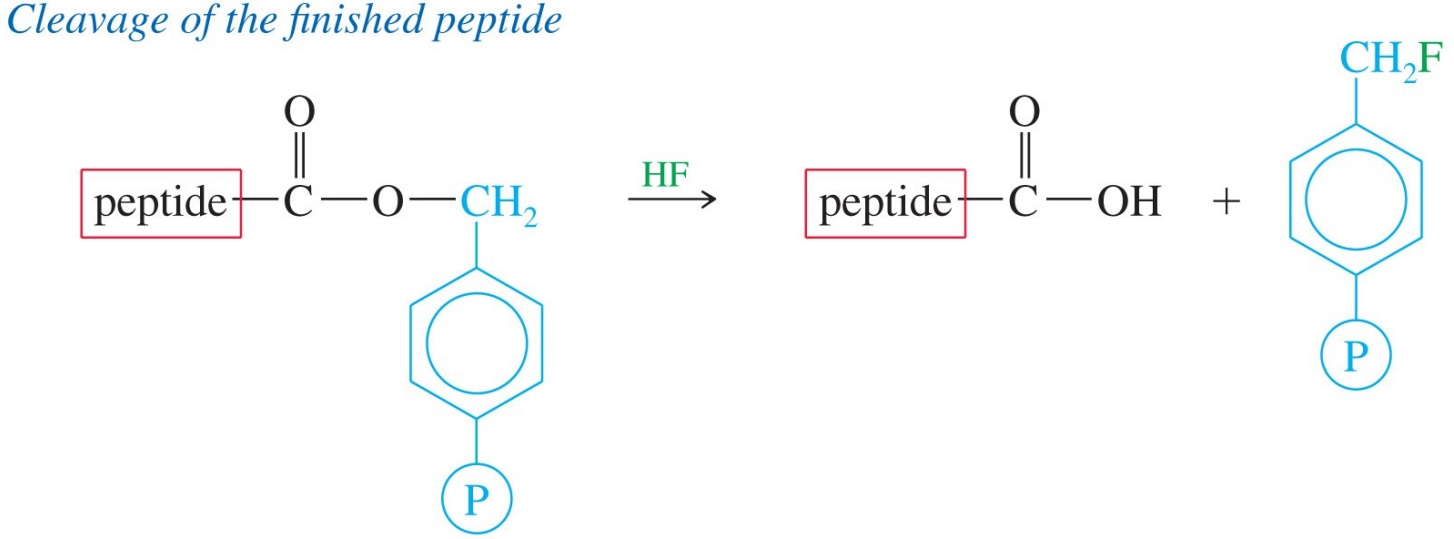
- Growing chain, built from C to N terminus, is attached to polystyrene beads.
- Intermediates do not have to be purified.
- Excess reagents are washed away with a solvent rinse.
- Process can be automated.
- Larger peptides can be constructed.

Attachment of the C-Terminal Amino Acid



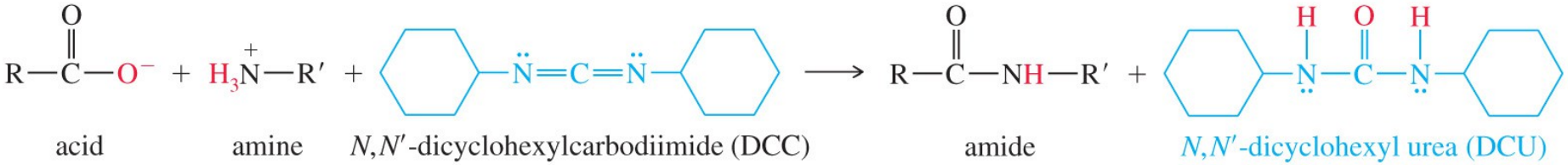
- Once the C-terminal amino acid is fixed to the polymer, the chain is built on the amino group of this amino acid.

Cleavage of the Finished Peptide



- At the completion of the synthesis, the ester bond to the polymer is cleaved by anhydrous HF.
- Because this is an ester bond, it is more easily cleaved than the amide bonds of the peptide.

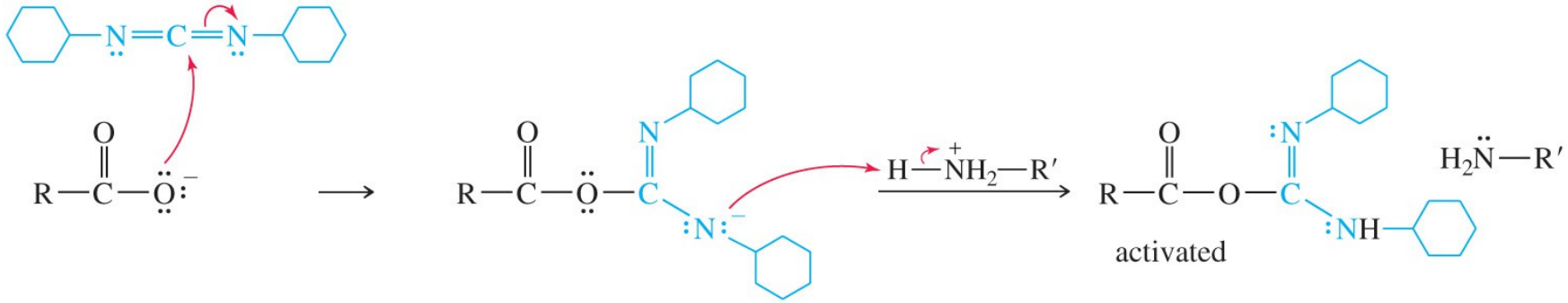
N,N’-Dicyclohexylcarbodiimide (DCC) Coupling



- When a mixture of an amine and an acid is treated with DCC, the amine and the acid couple to form an amide.

DCC-Activated Acyl Derivative

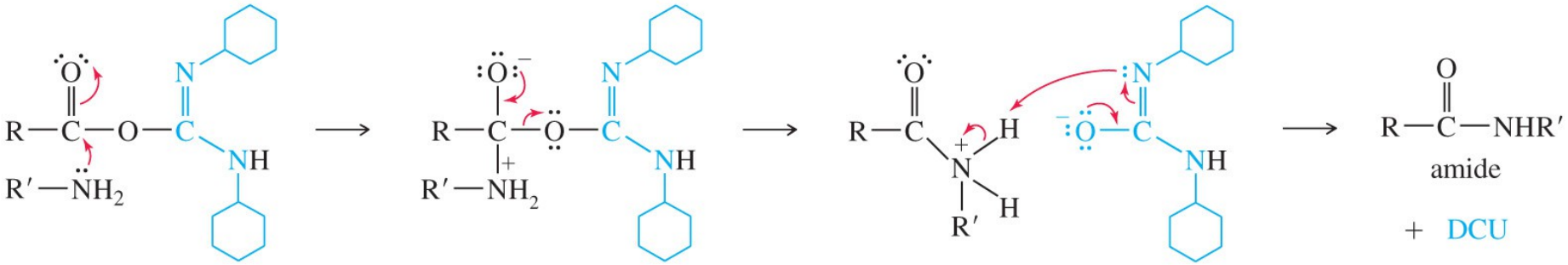
Formation of an activated acyl derivative



- The carboxylate ion adds to the strongly electrophilic carbon of the diimide, giving an activated acyl derivative of the acid.

Coupling

Coupling with the amine and loss of DCU



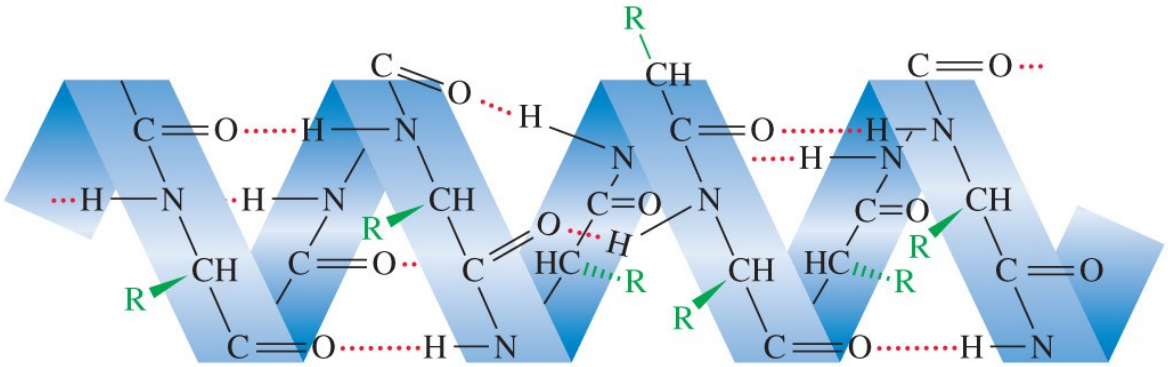
Classification of Proteins

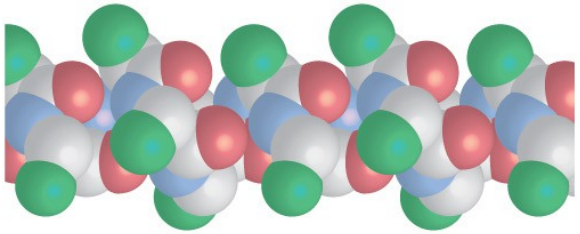
- ***Simple***: Hydrolyze to amino acids only.
- ***Conjugated***: Bonded to a nonprotein group, such as sugar, nucleic acid, or lipid.
- ***Fibrous***: Long, stringy filaments, insoluble in water; function as structure.
- ***Globular***: Folded into spherical shape; function as enzymes, hormones, or transport proteins.

Levels of Protein Structure

- ***Primary***: The sequence of the amino acids in the chain and the disulfide links.
- ***Secondary***: Structure formed by hydrogen bonding. Examples are - helix and pleated sheet.
- ***Tertiary***: Complete 3-D conformation.
- ***Quaternary***: Association of two or more peptide chains to form protein.

Alpha Helix



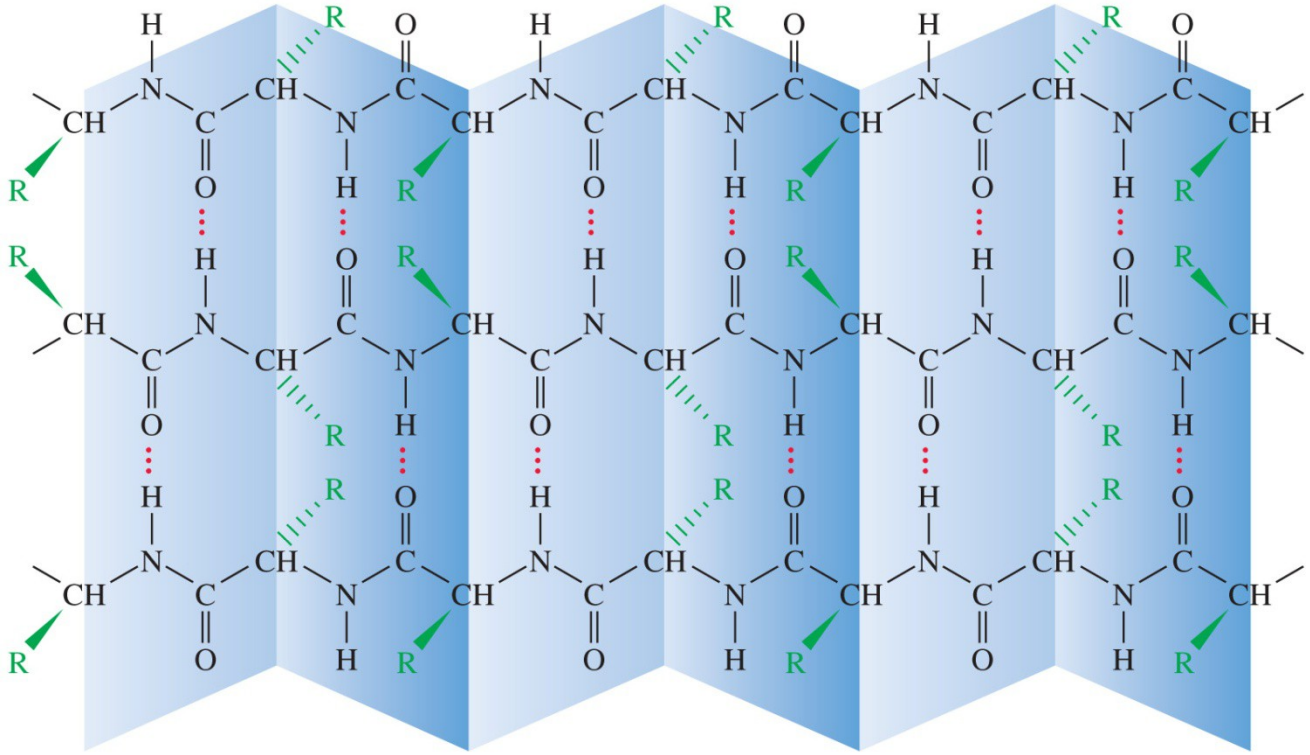


C = gray
N = blue
O = red
R = green

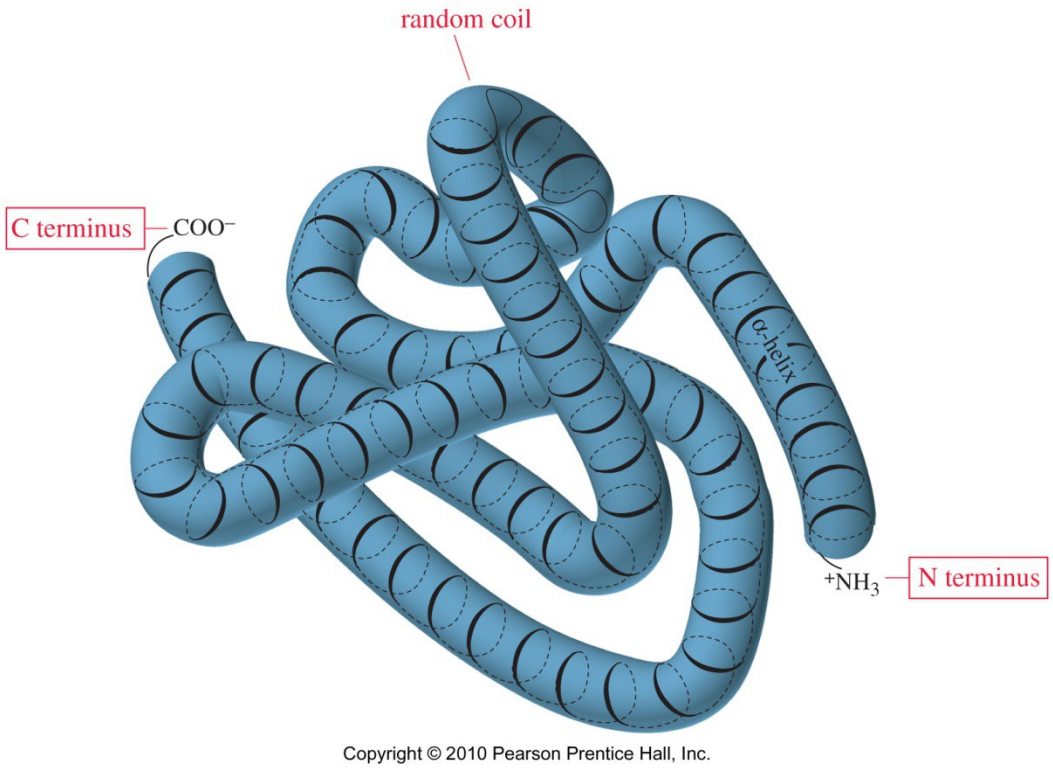
- Each carbonyl oxygen can hydrogen bond with an N—H hydrogen on the next turn of the coil.

Pleated Sheet Arrangement

Each carbonyl oxygen hydrogen bonds with an N—H hydrogen on an adjacent peptide chain.

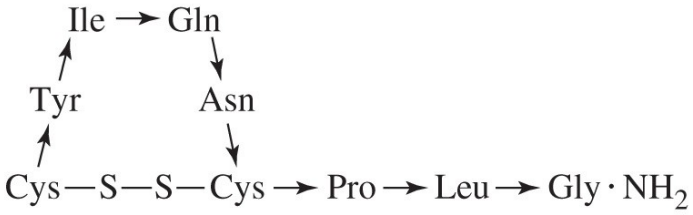


Tertiary Structure of Globular Proteins

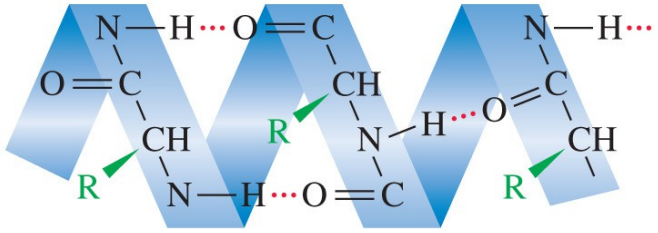


- The tertiary structure of a typical globular protein includes segments of -helix with segments of random coil at the points where the helix is folded.

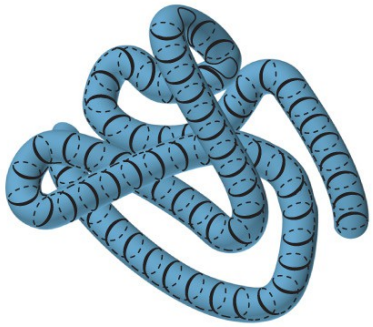
Summary of Structures



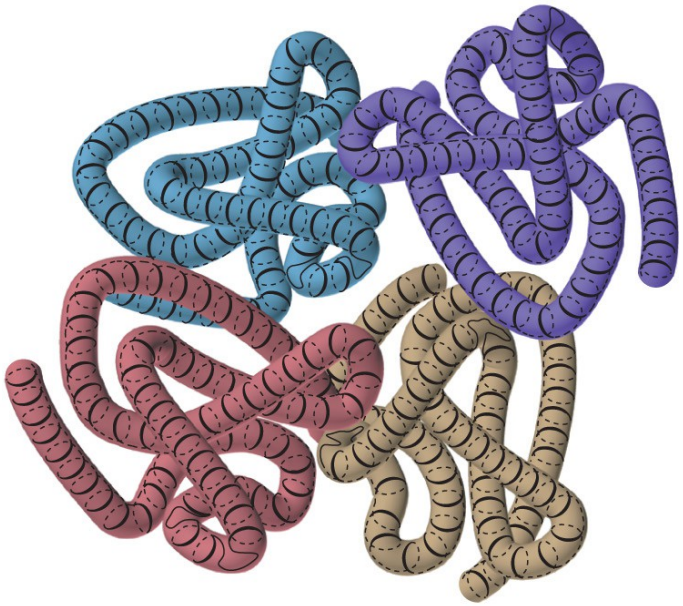
primary structure



secondary structure



tertiary structure



quaternary structure

Denaturation

- ***Denaturation*** is defined as the disruption of the normal structure of a protein, such that it loses biological activity.
- Usually caused by heat or changes in pH.
- Usually irreversible.
  - A cooked egg cannot be “uncooked”.