Denaturation of proteins, protein sequencing, protein synthesis

University of Anbar/College of Pharmacy

Second semester 2021-2022 / Biochemistry I / 3rd stage

References :

1- Harper’s Illustrated Biochemistry
2- Lehninger Principles of Biochemistry

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Determination of primary structure:

The primary structure comprises the identification of component amino acids with regard to their quality, quantity and sequence in a protein structure. A pure sample of a protein or a polypeptide is essential for the determination of primary structure which involves 4 steps :

1- Determination of amino acid composition.

2- Identification of N-terminal and C-terminal amino acids

3- Degradation of protein or polypeptide into smaller fragments.

4- Determination of the amino acid sequence.

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1- Determination of amino acid composition:

In a protein : The protein or polypeptide is completely hydrolysed to liberate the amino acids which are quantitatively estimated. The hydrolysis may be carried out either by acid or alkali treatment or by enzyme hydrolysis. Treatment with enzymes, however results in smaller peptides rather than amino acids.

Pronase is a mixture of non-specific proteolytic enzymes that causes complete hydrolysis of proteins.

Separation and estimation of amino acids :

The mixture of amino acids liberated by protein hydrolysis can be determined by chromatographic techniques.

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2- Degradation of protein into smaller fragments :

Protein is a large molecule which is sometimes composed of individual polypeptide chains. Separation of polypeptides is essential before degradation.

(a) Liberation of polypeptides : Treatment with urea or guanidine hydrochloride disrupts the non-covalent bonds and dissociates the protein into polypeptide units. For cleaving the disulfide linkages between the polypeptide units, treatment with performic acid is necessary.

(b) Number of polypeptides : The number of polypeptide chains can be identified by treatment of protein with dansyl chloride. It specifically binds with N-terminal amino acids to form dansyl polypeptides which on hydrolysis yield N-terminal dansyl amino acid. The number of dansyl amino acids produced is equal to the number of polypeptide chains in a protein.

(c) Breakdown of polypeptides into fragments : Polypeptides are degraded into smaller peptides by enzymatic or chemical methods.

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Breakdown of polypeptides by chemical methods

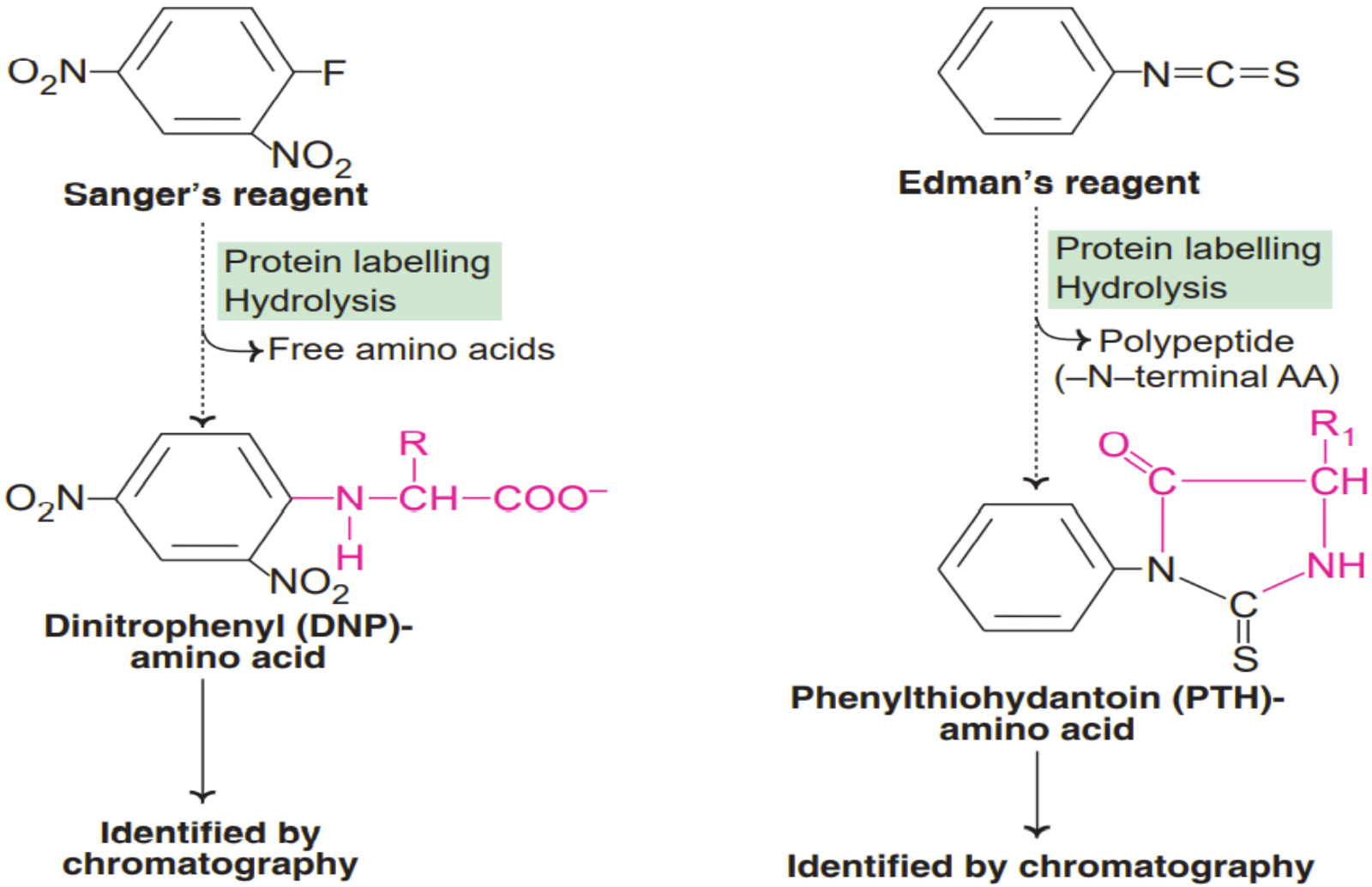


Fig. 4.8 : *Sanger's reagent (1-fluoro 2,4-dinitrobenzene) and Edman's reagent (Phenyl isothiocyanate) in the determination of amino acid sequence of a protein (AA-Amino acid).*

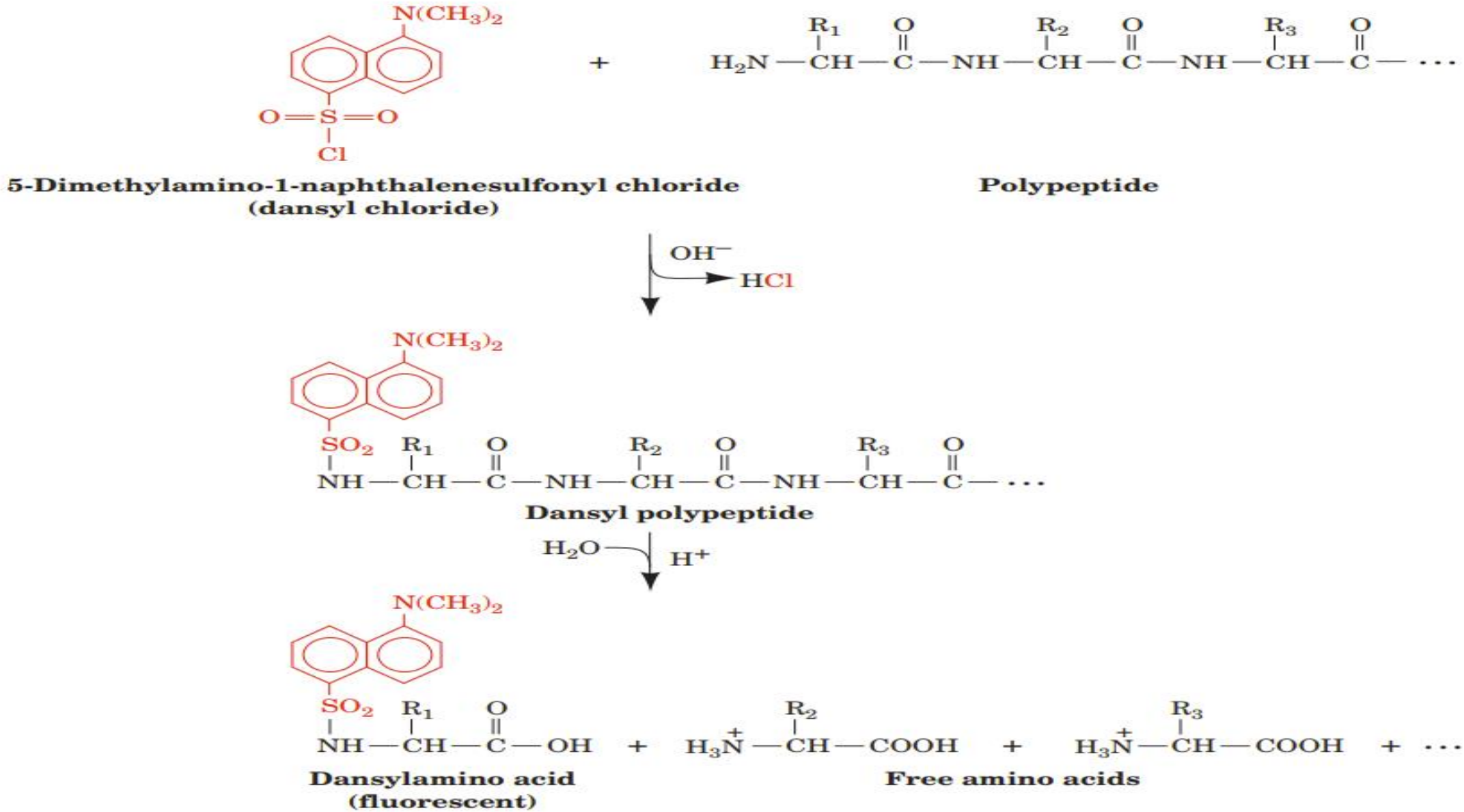
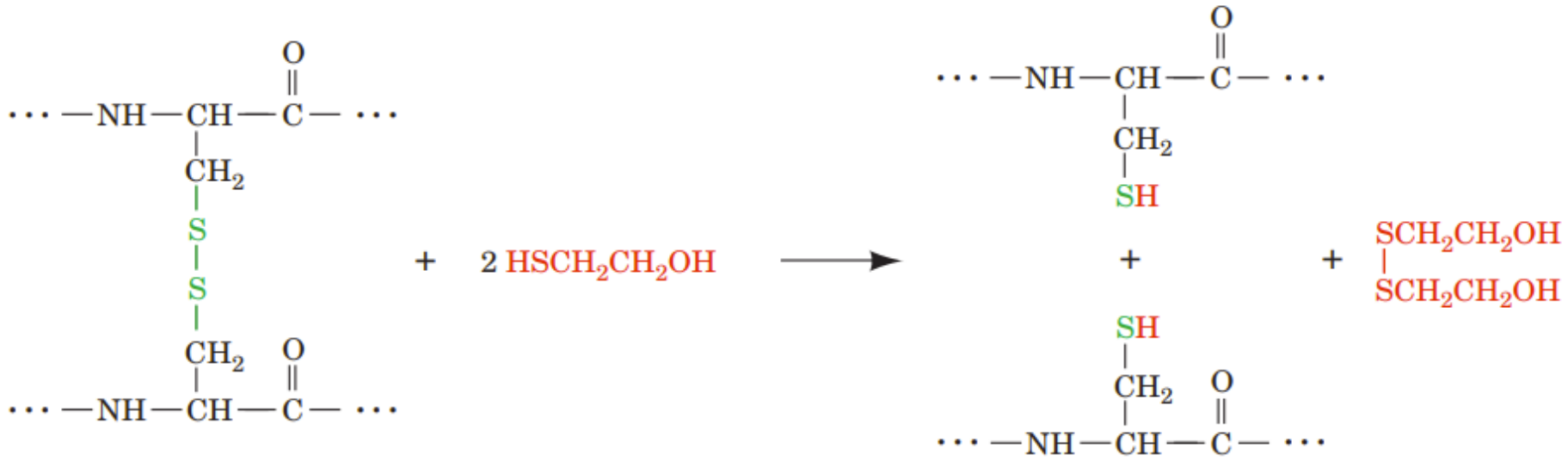
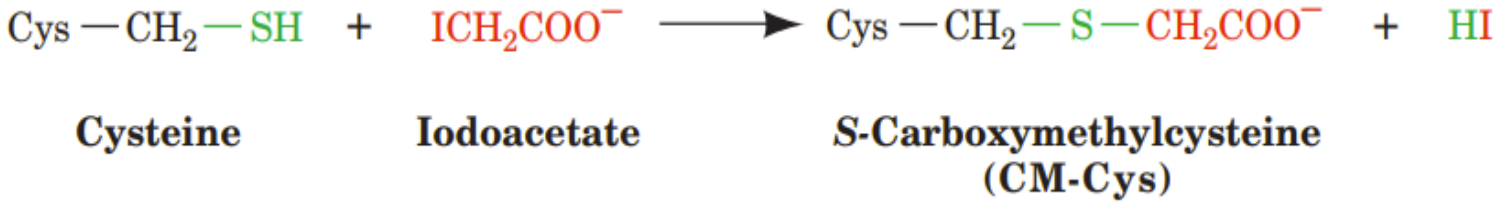


FIG. 5-14 The dansyl chloride reaction. The reaction of dansyl chloride with primary amino groups is used for end group analysis.

Disulfide bonds can be reductively cleaved by treating them with 2-mercaptoethanol or another mercaptan (compounds that contain an ¬SH group):



The resulting free sulfhydryl groups are then alkylated, usually by treatment with iodoacetate, to prevent the re-formation of disulfide bonds through oxidation by O2.



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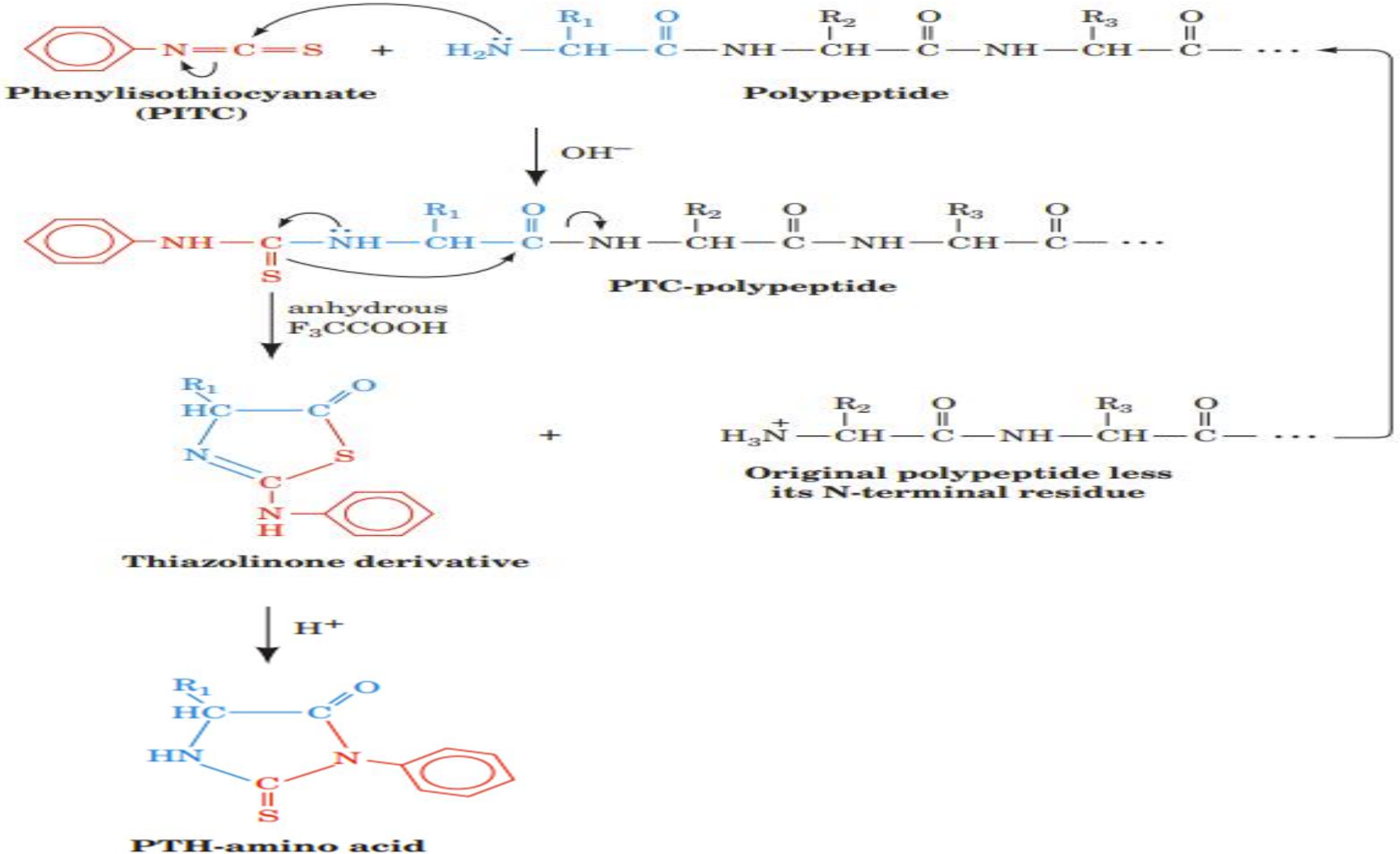

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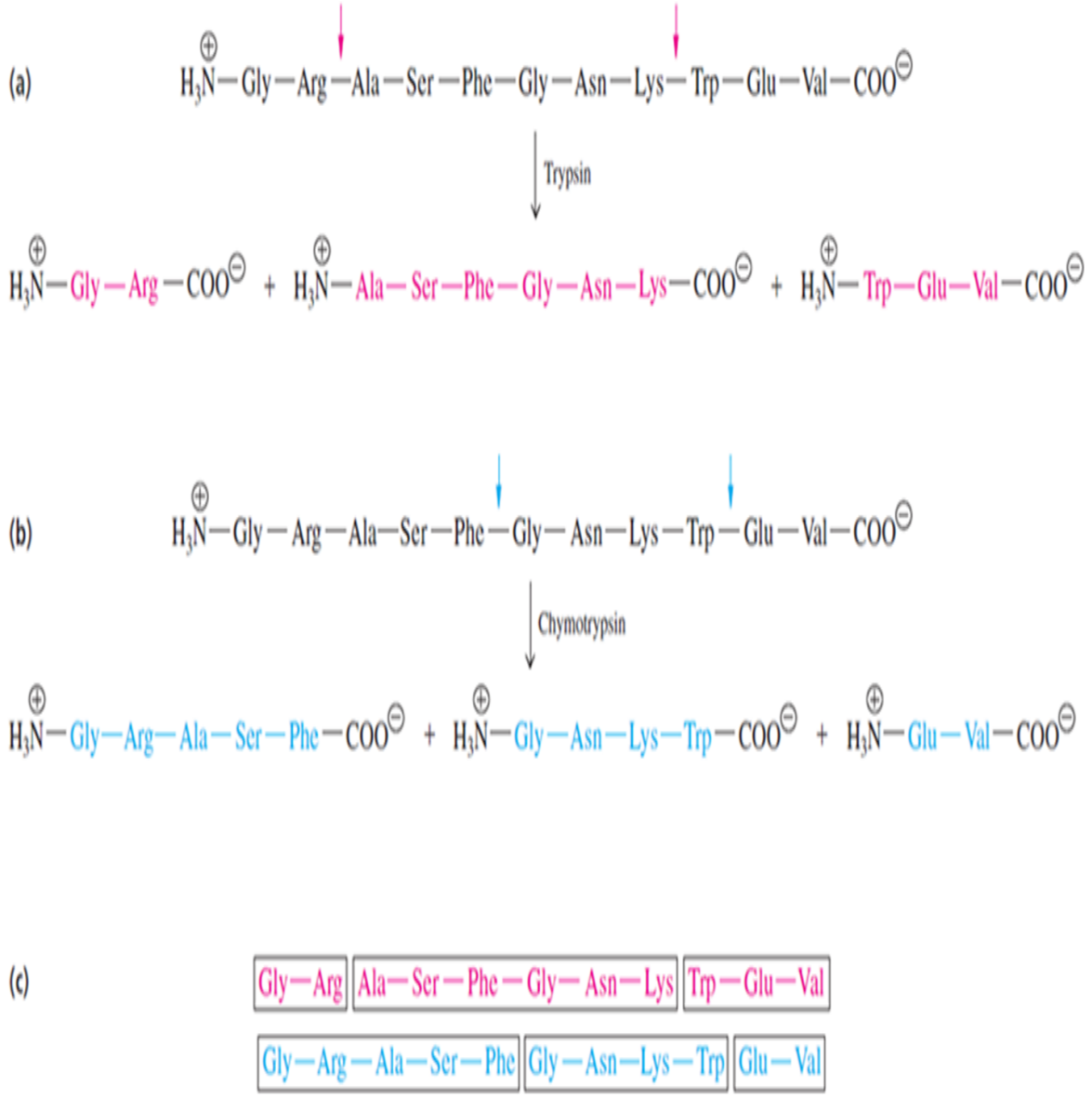
Breakdown of polypeptides enzymatic

TABLE 3–7 The Specificity of Some Common Methods for Fragmenting Polypeptide Chains

*Reagent (biological source)**	*Cleavage points†*
Trypsin (bovine pancreas)	Lys, Arg (C)
Submaxillarus protease (mouse submaxillary gland)	Arg (C)
Chymotrypsin (bovine pancreas)	Phe, Trp, Tyr (C)
Staphylococcus aureus V8 protease (bacterium *S. aureus*)	Asp, Glu (C)
Asp-*N*-protease (bacterium *Pseudomonas fragi*)	Asp, Glu (N)
Pepsin (porcine stomach)	Phe, Trp, Tyr (N)
Endoproteinase Lys C (bacterium *Lysobacter enzymogenes*)	Lys (C)
Cyanogen bromide	Met (C)



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Protein Sequencing Strategy:

1- If the protein contains more than one polypeptide chain, the chains are separated and purified.

2- Intrachain S-S (disulfide) cross-bridges between cysteine residues in the polypeptide chain are cleaved.

3-The amino acid composition of each polypeptide chain is determined.

4-The N-terminal and C-terminal residues are identified.

5- Each polypeptide chain is cleaved into smaller fragments, and the amino acid composition and sequence of each fragment are determined.

6- Step 5 is repeated, using a different cleavage procedure to generate a different and therefore overlapping set of peptide fragments .

7- The overall amino acid sequence of the protein is reconstructed from the sequences in overlapping fragments.

8-The positions of S-S cross-bridges formed between cysteine residues are located.

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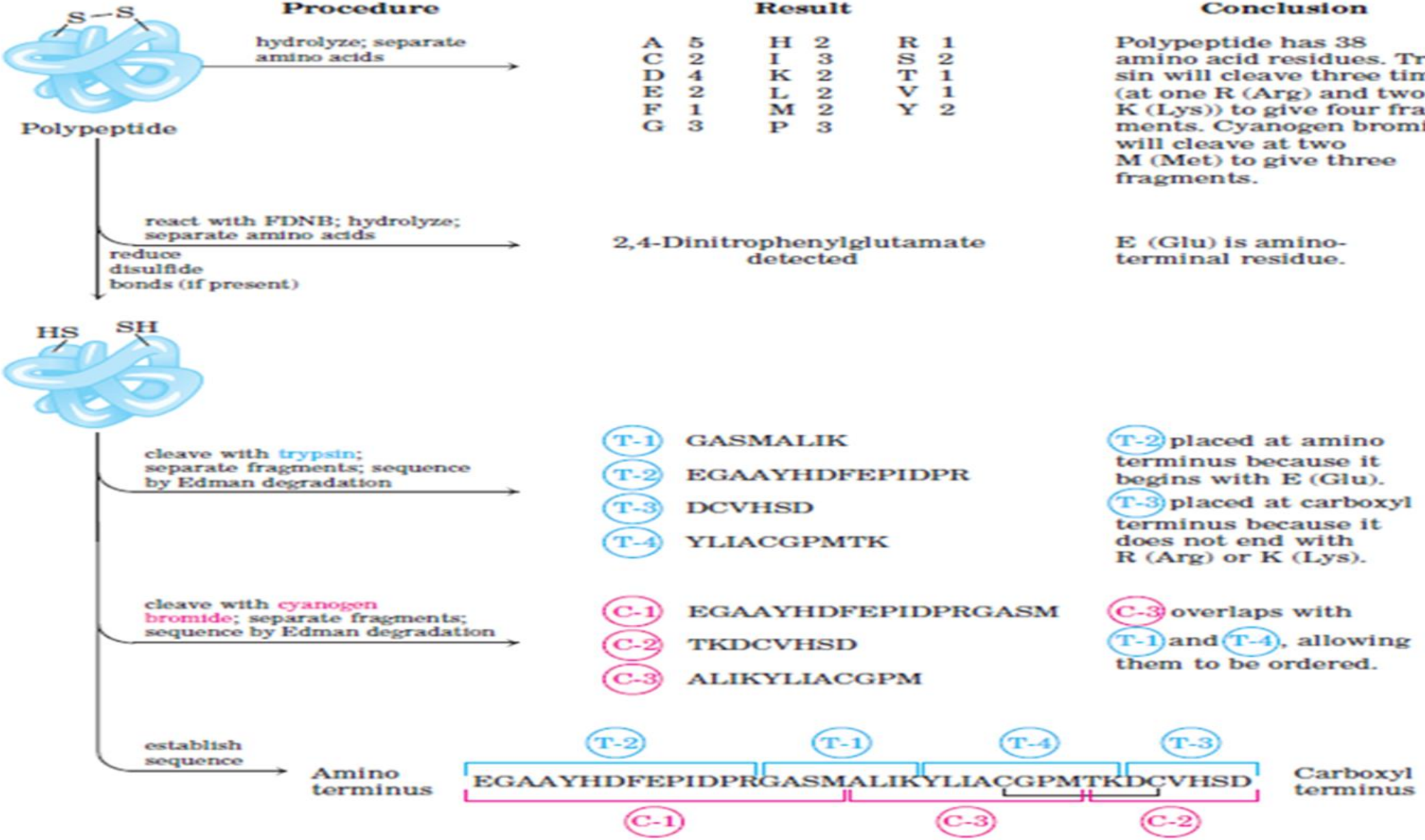

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Sequence Determination by Mass Spectrometry (MS):

Mass spectrometers exploit the difference in the mass-to-charge (m/z) ratio of ionized atoms or molecules to separate them from each other.

Macromolecular Ionization Methods in Mass Spectrometry	
Electrospray ionization (ESI-MS)	A solution of macromolecules is sprayed in the form of fine droplets from a glass capillary under the influence of a strong electrical field. The droplets pick up charge as they exit the capillary; evaporation of the solvent leaves highly charged molecules.
Fast-atom bombardment (FAB-MS)	A high-energy beam of inert gas molecules (argon or xenon) is directed at a solid sample, knocking molecules into the gas phase and ionizing them.
Laser ionization (LIMS)	A laser pulse is used to knock material from the surface of a solid sample; the laser pulse creates a microplasma that ionizes molecules in the sample.
Matrix-assisted desorption ionization (MALDI)	MALDI is a LIMS method capable of vaporizing and ionizing large biological molecules such as proteins or DNA. The biological molecules are dispersed in a solid matrix that serves as a carrier. Nicotinic acid is a commonly used matrix substance.

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MASS SPECTROMETRY CAN DETECT COVALENT MODIFICATIONS

MS is significantly more sensitive and tolerant of variations in sample quality. Moreover, since mass and charge are common properties of a wide range of biomolecules, MS can be used to analyze metabolites, carbohydrates, and lipids, and to detect posttranslational modifications such as phosphorylation or hydroxylation that add readily identified increments of mass to a protein

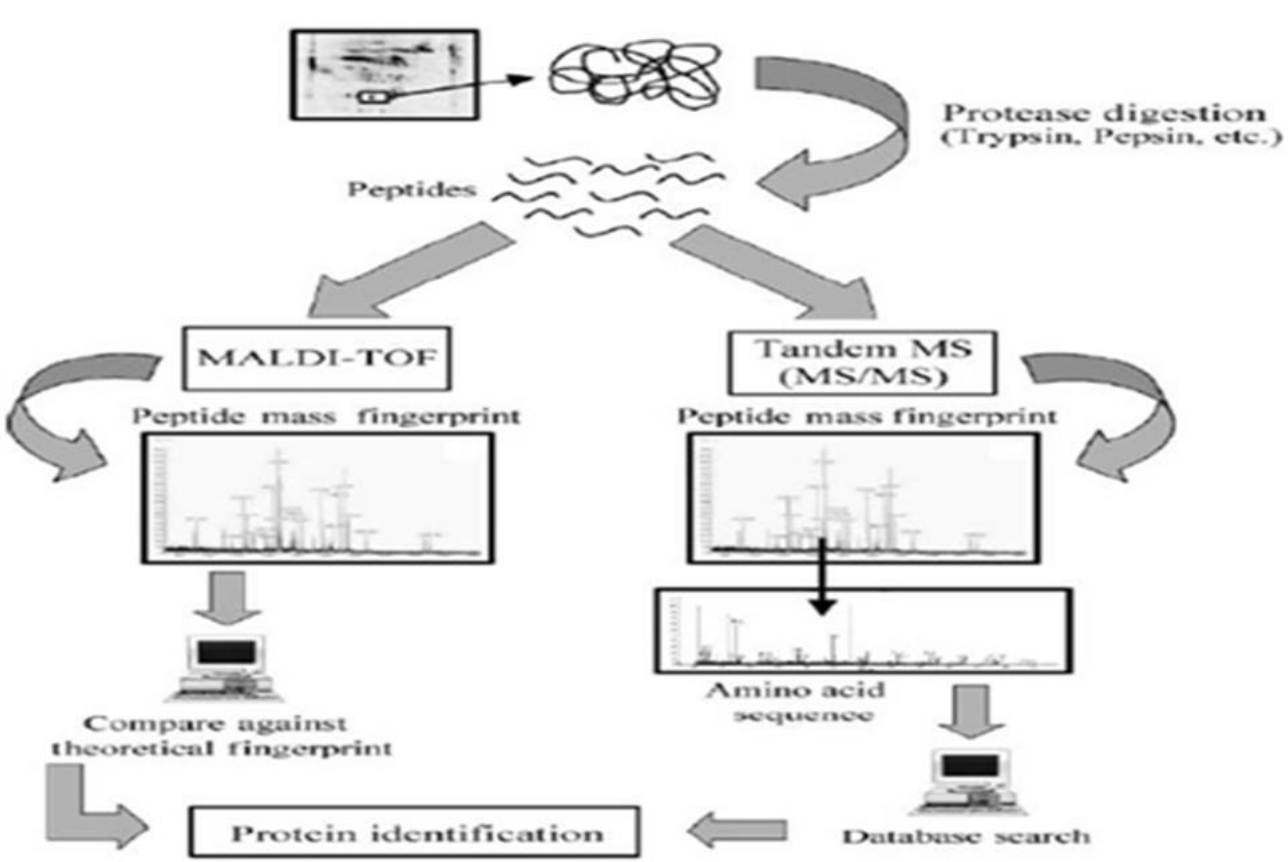
TABLE 4–1 Mass Increases Resulting From Common Posttranslational Modifications

Modification	Mass Increase (Da)
Phosphorylation	80
Hydroxylation	16
Methylation	14
Acetylation	42
Myristylation	210
Palmitoylation	238
Glycosylation	162

Proteomic:

The term proteomics was introduced in 1995 for analysis of the complete protein complements expressed by a genome in a cell or a tissue type. By analogy with genomics, the term proteomics refers to studies of a gene's function at the protein level.

Proteomics is the study of these proteins their identity, their biochemical properties and functional roles, and how their quantities, modifications, and structures change during development and in response to internal and external stimuli.



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Proteome for drugs design and other application:

There are several theoretical differences studying the human proteome as compared with human genome and they all analytical challenges in proteome.

- There is not a one relationship between the number of gens and the number of protein as proteins come in different splice variants and in addition undergo post-translational modification ,where sugars, phosphates ,and other molecules are added to the protein structure.

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- The process of drug discovery and development ,it has been postulated that scientists could use genome information to identify and validate a host of new drug targets and specific drugs based on an individual's detailed genetic makeup.

- The broad field of proteomics has as its primary goal the understanding of structure, function, expression cellular localization interaction partners, biomarkers, and regulation of every protein produced from a complete genome . For drugs design of specific protein modifying reagents that can be used for functional.

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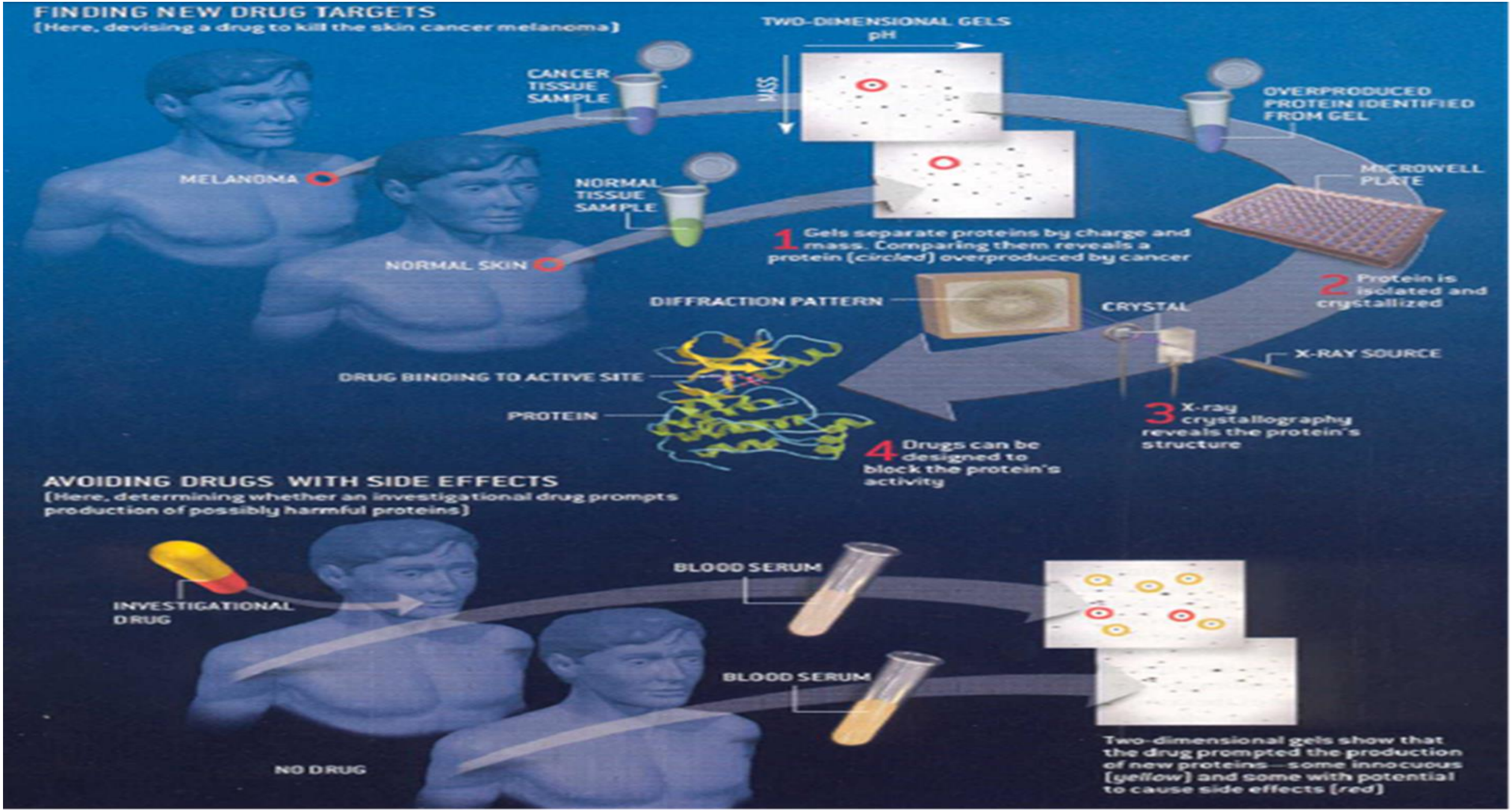

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Bioinformatics Assists Identification of Protein Functions

Bioinformatics is the application of computer technology to the management and analysis of biological data. The result is that computers are being used to gather, store, analyze and merge biological data.

Data mining through bioinformatics permits researchers to compare amino acid sequences of unknown proteins with those whose functions have been determined. This provides a means to uncover clues to their potential properties, physiologic roles, and mechanisms of action.
There are three central biological processes around which bioinformatics tools must be developed:

1-DNA sequence determines protein sequence
2-Protein sequence determines protein structure
3-Protein structure determines protein function

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Small Peptides and Proteins Can Be Chemically Synthesized:

Many peptides are potentially useful as pharmacologic agents, and their production is of considerable commercial importance. There are three ways to obtain a peptide:

(1) Purification from tissue, a task often made difficult by the vanishingly low concentrations of some peptides.

(2) Genetic engineering .

(3) Direct chemical synthesis.

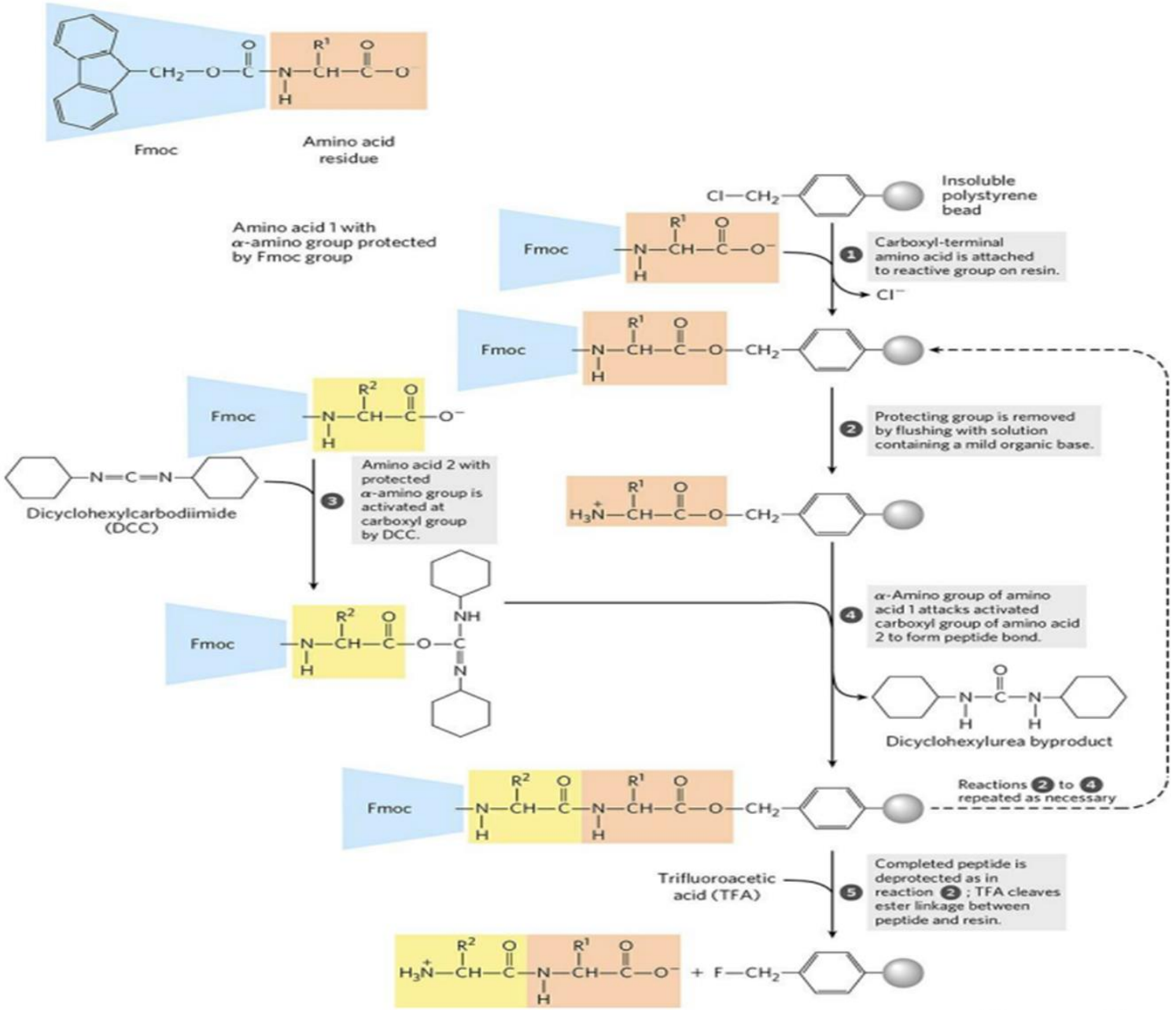

FIGURE 3-32 Chemical synthesis of a peptide on an insoluble polymer support. Reactions 1 through 4 are necessary for the formation of each peptide bond. The 9-fluorenylmethoxycarbonyl (Fmoc) group (shaded blue) prevents unwanted reactions at the α-amino group of the residue (shaded light red). Chemical synthesis proceeds from the carboxyl terminus to the amino terminus, the reverse of the direction of protein synthesis in vivo (Chapter 27).

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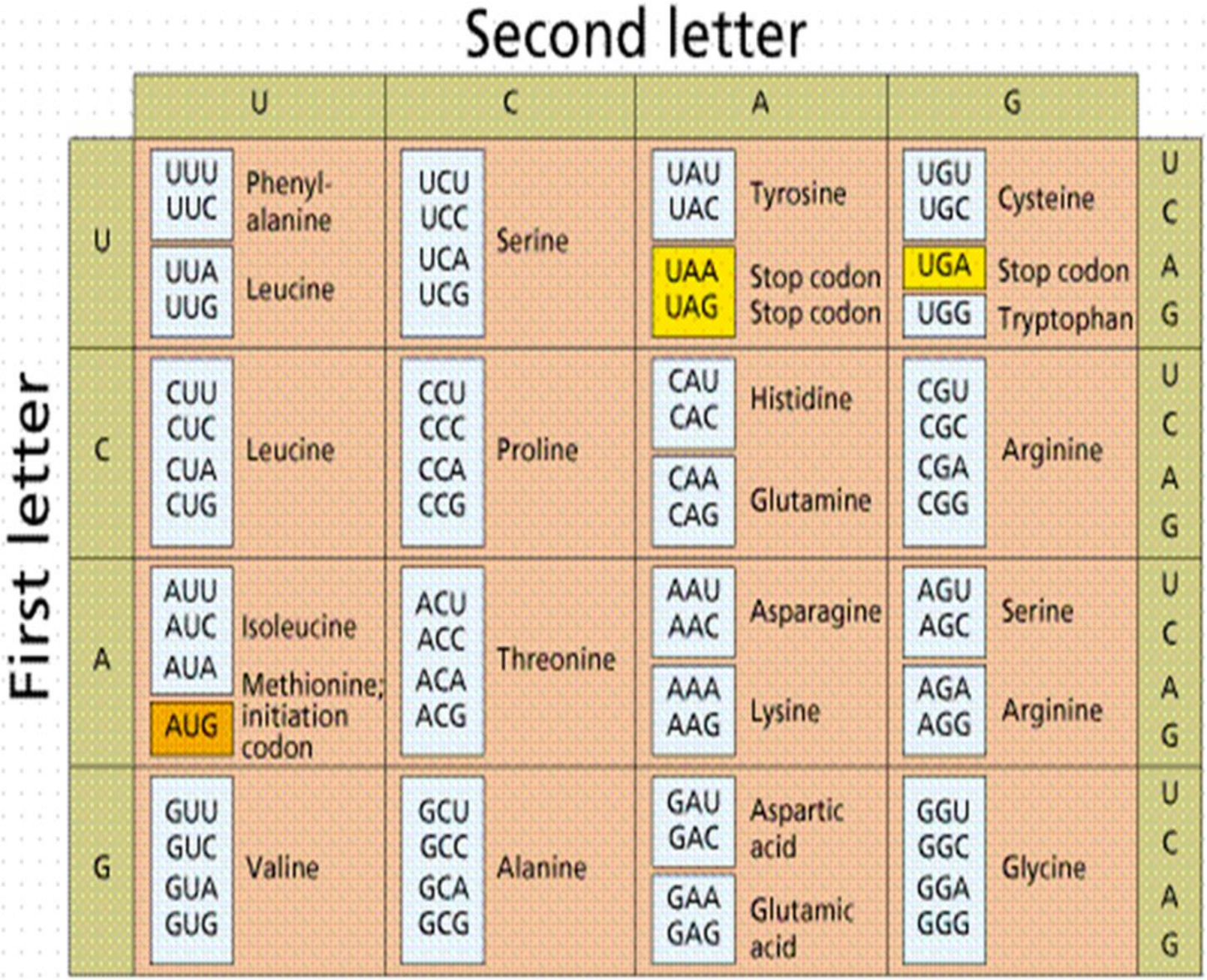
Protein synthesis:

Genetic information, stored in the chromosomes and transmitted to daughter cells through DNA replication, is expressed through transcription to mRNA and, in the case of subsequent translation into polypeptide chains. The pathway of protein synthesis is called translation because the "language" of the nucleotide sequence on the is translated into the language of an amino acid sequence.

The process of translation requires a genetic code, through which the information contained in the nucleic acid sequence is expressed to produce a specific sequence of amino acids.

Codon:

Codons are usually presented in the messenger RNA language of adenine (A), guanine (G), cytosine (C), and uracil (U). Their nucleotide sequences are always written from the 5'-end to the 3'-end. The four nucleotide bases are used to produce the three-base codons. There are, therefore, 64 different combinations of bases, taken three at a time.



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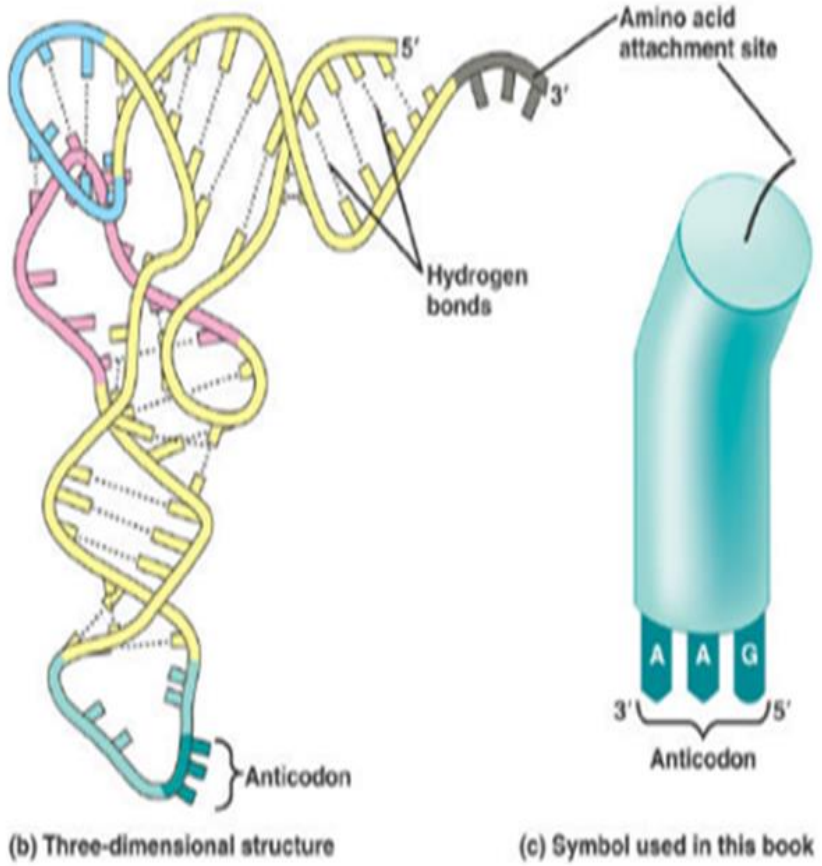
Translation: transfer RNA (tRNA)

In a typical eukaryotic cell, protein synthesis takes place in the cytoplasm while transcription and RNA processing take place in the nucleus. Eukaryotic mRNAs are monocistronic, capped at the 5' end and polyadenylated at the 3' end ribosomes dissociate into 40S and 60S subunits.



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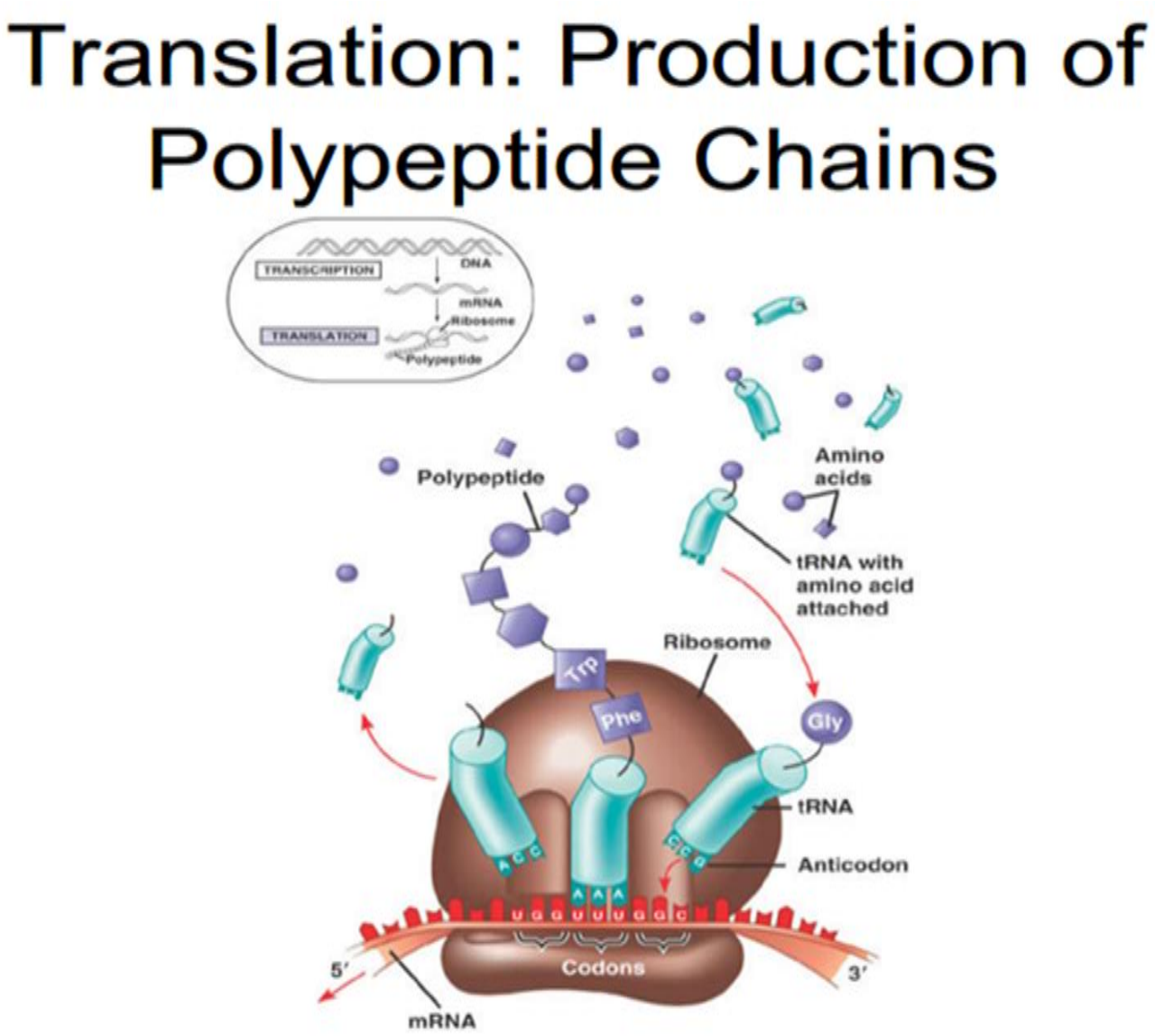
Conclusion:

1. Proteins are produced by the process of translation which involves three main steps:

- A start codon (AUG) complements with the Methionine (Met) tRNA in the ribosome, constituting the translation initiation complex.
- A new anticodon will land in the A site, and its amino acid will join Met. The tRNA will slide to the P site leaving the A site free for another anticodon.
- A stop codon (UAG, UAA, or UGA) signals the end of the mRNA molecule. A release factor activates the disassembling of the two ribosomal units and the mRNA molecule.



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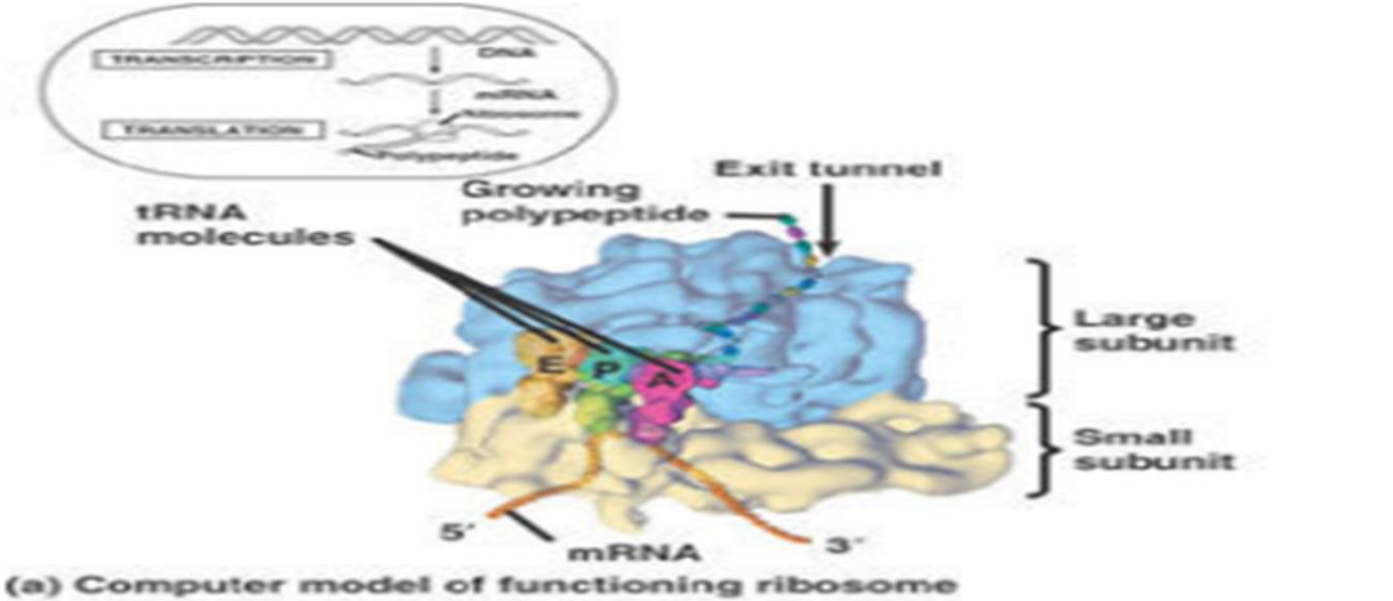
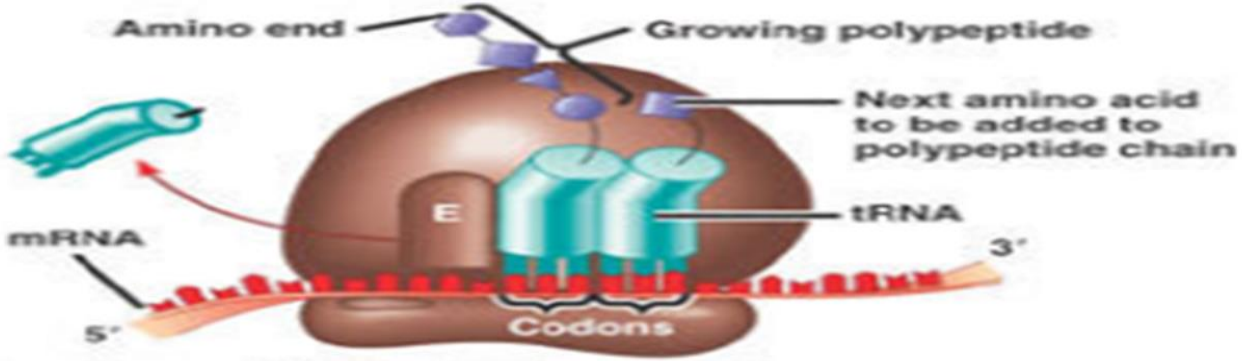


(a) Computer model of functioning ribosome

(b) Schematic model showing binding sites



(c) Schematic model with mRNA and tRNA

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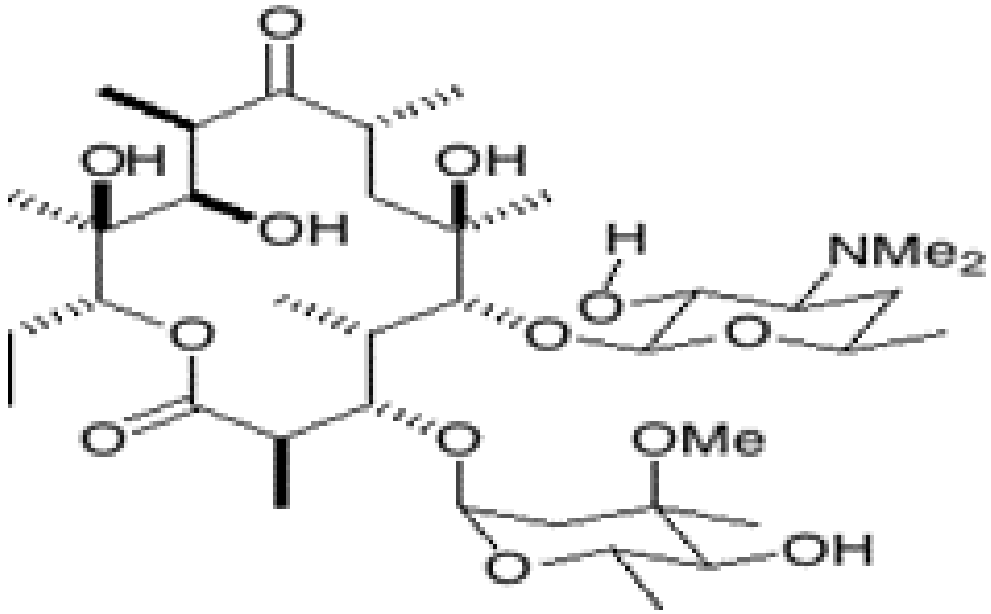
Drugs that inhibits protein synthesis:

1-Erythromycin

Mechanism of Action Erythromycin inhibits protein synthesis by binding to the 23S rRNA molecule (in the 50S subunit) of the bacterial ribosome blocking the exit of the growing peptide chain. (Humans do not have 50 S ribosomal subunits, but have ribosomes composed of 40 S and 60 S subunits).



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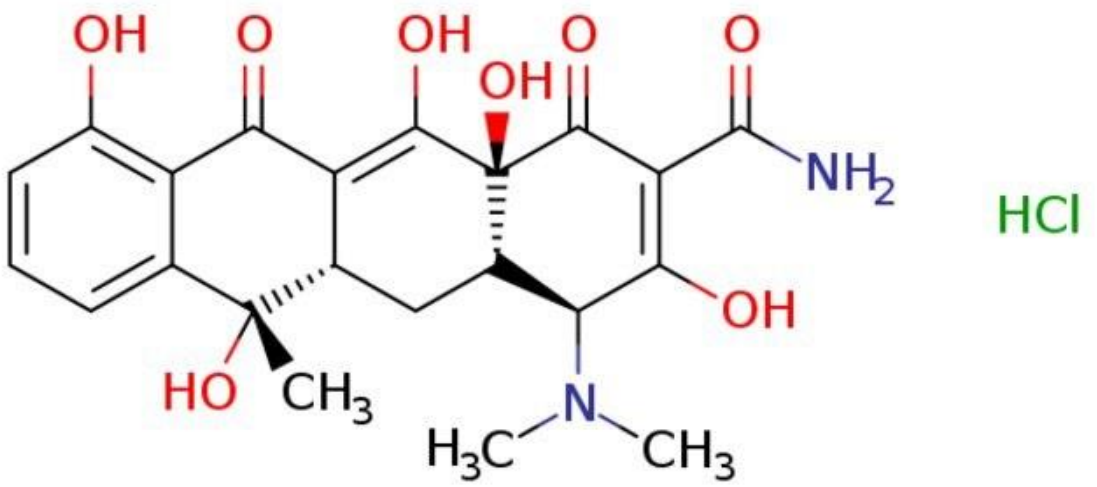
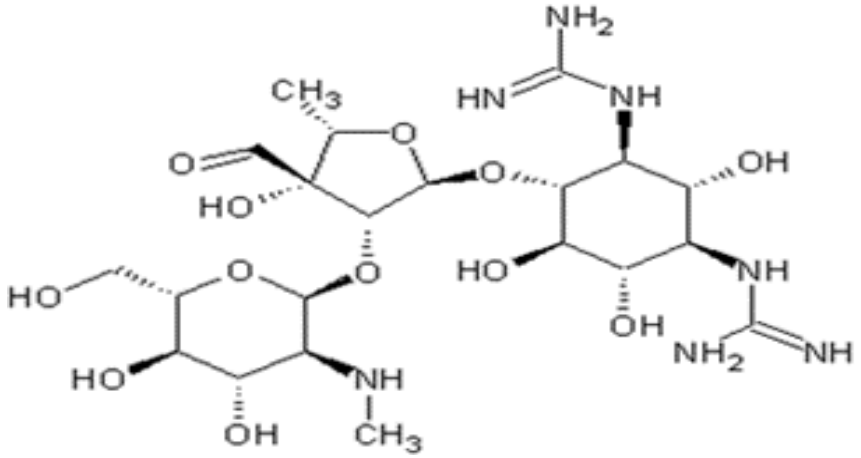
2-Tetracyclines

Tetracyclines have the broadest spectrum of antimicrobial activity Tetracyclines inhibit bacterial protein synthesis by blocking the attachment of the transfer RNA-amino acid to the ribosome. More precisely they are inhibitors of the codon-anticodon interaction..



3-Streptomycin:

Streptomycin binds to the 30S ribosome and changes its shape so that it and inhibits protein synthesis by causing a misreading of messenger RNA information.



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Thanks for listening