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GOMEL STATE MEDICAL UNIVERSITY

Department of general and bioorganic chemistry

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BIOREGULATORS AND BIOPOLYMERS

LABORATORY MANUAL

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Ф 53

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Учебно-методическое пособие предназначено для организации лабораторного практикума и самостоятельной работы студентов, изучающих курс биоорганической химии на английском языке. Оно призвано помочь в формировании целостного представления о строении, роли биополимеров, об особенностях организации молекул белков, липидов и нуклеиновых кислот.

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CHAPTER 1
LIPIDS

After reading this chapter, you should be able to:

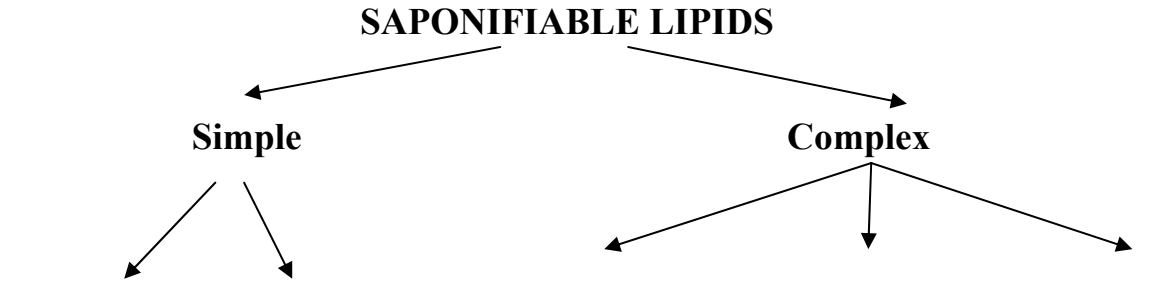
- **Define lipids and their classification.**
- **Describe structure of simple and complex saponifiable lipids.**
- **Discuss chemical properties of fats, oils and phospholipids.**
- **Outline classification, structure and biological functions of steroids.**

1.1. Classification of the lipids

Lipids (from *greek* "lipos" – fat) are constituents of plants and animals that are characterized by their solubility properties. In particular, they are insoluble in water but soluble in nonpolar organic solvents. Lipids can be extracted from cells and tissues by organic solvents. All lipids may be subdivided into two groups:

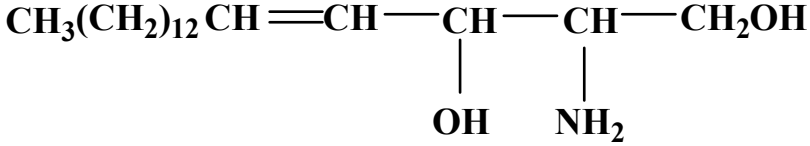
- which can be hydrolyzed or saponified;
- which can not be hydrolyzed.

CLASSIFICATION OF SAPONIFIABLE LIPIDS



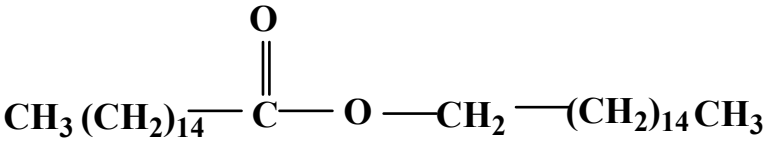
The structural base for all saponifiable lipids are alcohols:

- long-chain monohydric alcohols: mericyl alcohol $C_{30}H_{61}OH$ or cetyl alcohol $C_{16}H_{33}OH$
- trihadric alcohol glycerol,
- long-chain dihydric amino alcohol with one double bong, named sphingosine:

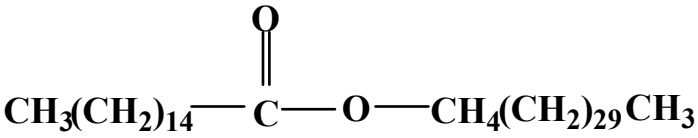


1.2. Saponifiable lipids

Waxes are simple monoesters that are composed of long-chain monohydric alcohols and fatty acids:



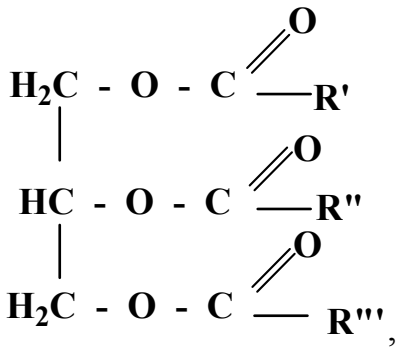
Cetyl palmitate is a component of spermaceti, a wax in sperm whale oil.



Mericyl palmitate is a component of a bee wax.

In nature, waxes act as protective coatings on fruit and leaves as well on fur, feathers and skin. They are used to make polishes, cosmetics, ointments and other pharmaceutical preparations as well as candles and phonograph records.

Fats and oils are familiar parts of daily life. Common fats include butter, lard, and the fatty portions of meat. Oils come mainly from plants and include corn, cottonseed, olive, peanut, and soybean oils. Although fats are solids and oils are liquids, they have the same basic organic structure. Fats and oils are tri-esters of glycerol and are called **triacylglycerols** or **triglycerides**. Their general formula is:



where R — radicals of fatty acids.

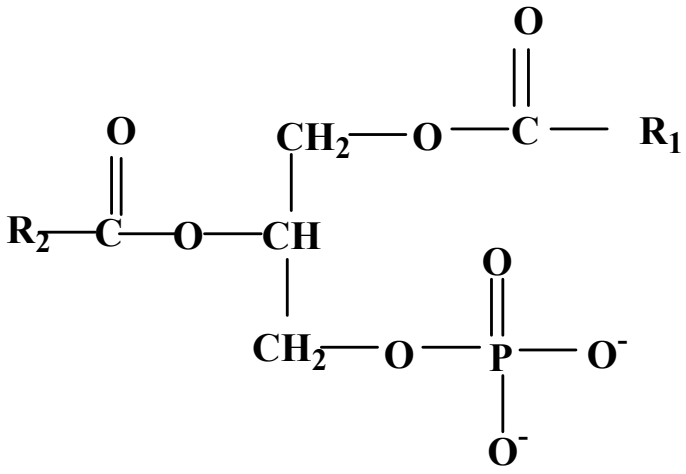
In general, a particular fat or oil consists, not of a single triglyceride, but of a complex mixture of triglycerides. Oils contain a much higher percentage of unsaturated fatty acids than do fats. The analytic characteristic for fats and oils is **iodine number** – a number of grams of I_2 that adds to 100 grams of a triglyceride. For oils iodine number is greater than 70, for fats – less than 70.

1.3. Phospholipids

Phospholipids are esters either of glycerol or sphingosine.

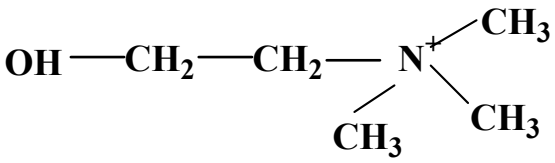
Phosphoglycerides constitute about 40% of cell membranes, the remaining 60% being proteins. They are related structurally to fats and oils, except that one of the three ester groups is replaced by a phosphatidylamine.

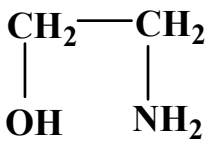
They may be considered to be the derivatives of L-phosphatidic acid:



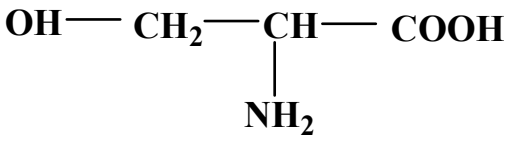
The three principal phosphoglycerides are esters that are formed between phosphatidic acid and either **choline, ethanolamine or serine** to give respectively:

- phosphatidylcholine (lecithin)**,**
- phosphatidylethanolamine (cephalin)
- phosphatidylserine



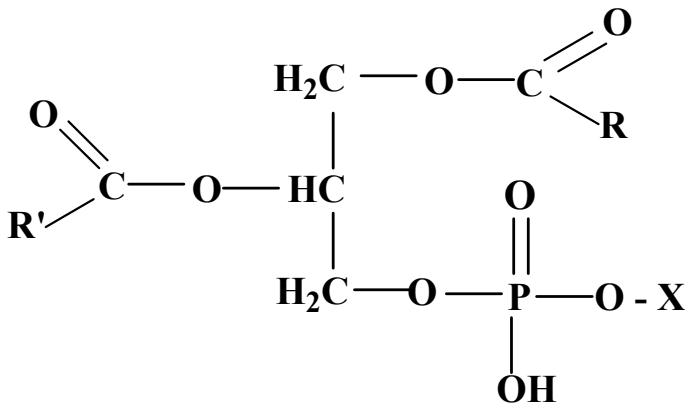


Choline **Ethanolamine (Calamine)**



Serine

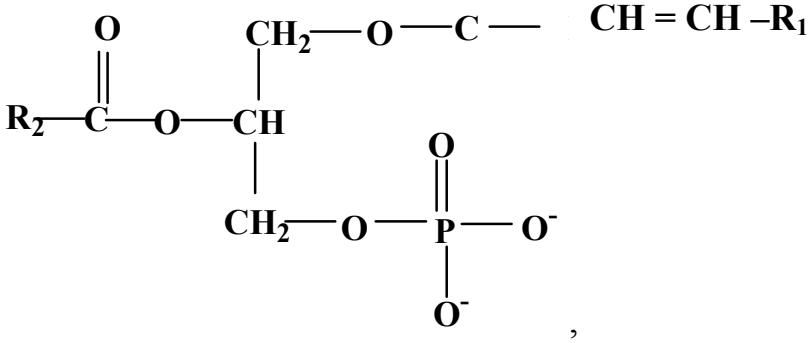
General formula for phospholipids



where X is a radical of amino alcohols, which formulas are given above.

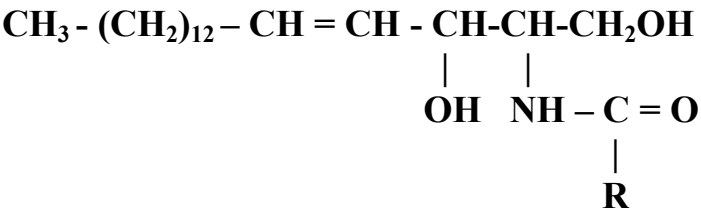
1.4. Plasmalogens

Plasmalogens make up another family of glycerol-based phospholipids, and they occur widely in the membranes of nerve cells and muscle cells. Their general formula is

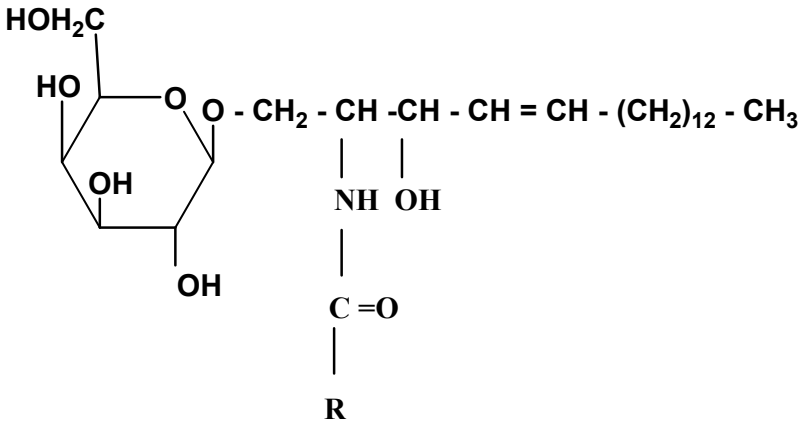


where phosphoric acid is estereficated by the following amino alcohols: choline, ethanolamine or serine.

Ceramide is a bright example of sphingolipids, which are accumulated in brain and nerve tissues:



Cerebroside is a widespread glycolipid, which is a constituent of nerve tissues:



1.5. Chemical properties of saponifiable lipids

Hydrolysis of lipids occurs in acidic and basic media. Acid catalyzed hydrolysis is reversible and gives a mixture of fatty acids and glycerol. Base catalyzed hydrolysis (**saponification)** is irreversible and gives glycerol and a mixture of fatty acids' salts.

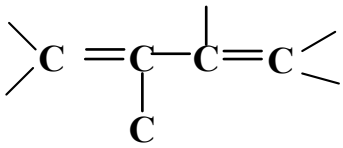
Addition. Lipids undergo addition reactions to double bonds of unsaturated acids' radicals. The most important reaction of this type is hydrogenation of vegetable oils. By hydrogenation vegetable oils are converted into solid fats.

Oxidation of lipids also involves double bonds. The most important type of oxidation is **beta (β)-oxidation** (or fatty acid cycle), which takes place in mitochondria. **Peroxide oxidation** of phospholipids takes place in cell membranes and is the main factor of their destruction. This process is initiated by γ -rays thus and its rate rapidly increases under the influence of even

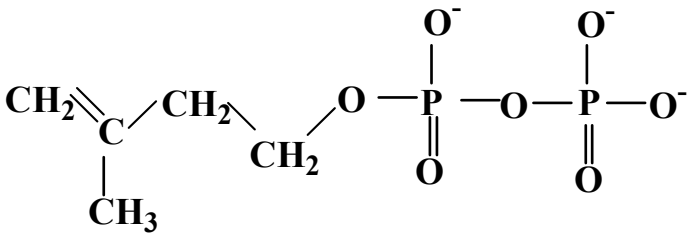
1.6. Terpenes

STEROIDS and **TERPENES** are nonsaponifiable lipids, contained in plants (terpenes) and animals (steroids).

Terpenes are compounds containing multiples of isoprene unit:



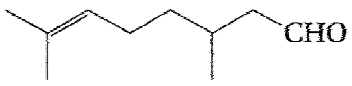
They are synthesized in the plant from acetate by way of an important biochemical intermediate, isopentenyl pyrophosphate:



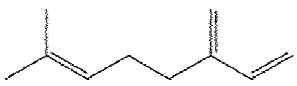
Most terpene structures can be broken down into multiples of isoprene units. Terpenes contain various functional groups (C=C, OH, C=O) as part of their structures and may be acyclic or cyclic.

Compounds with a single isoprene unit (C_5) are rare in nature, but compounds with two such units (C_{10}), called monoterpenes, are common

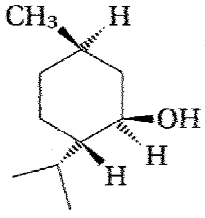
For example:



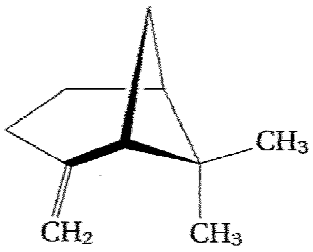
citronellal
(lemon oil)



myrcene
(bay leaves)



menthol
(peppermint oil)



β-pinene
(turpentine)

1.7. Steroids

Steroids are tetracyclic lipids derived from the acyclic triterpene squalene.

Steroids constitute a major class of lipids. The common structural feature of steroid is a system of four fused rings. The A, B, and C rings are 6-membered, and the D ring is five-membered, usually all fused in a trans manner (fig. 1).

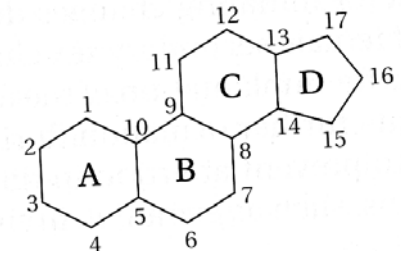


Fig. 1. The steroid ring system, showing the numbering

In most steroids, the 6-membered rings are not aromatic, although there are exceptions. Usually methyl substituents attached to C-20 and C-13 and some sort of side chain attached to C-17.

Perhaps the best known steroid is **CHOLESTEROL. C**holesterol (fig. 2) is present in all animal cells but is mainly concentrated in the brain and spiral cord.

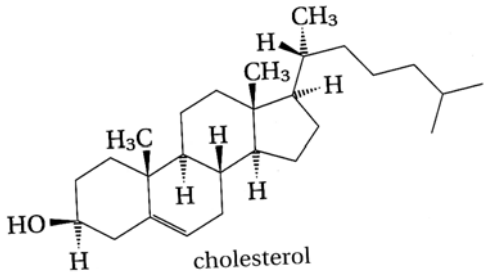


Fig. 2. Cholesterol

CHOLIC ACID occurs in the bile duct, where it is present mainly in the form of various amide salts. These salts function as emulsifying agents to facilitate the absorption of fats in the intestinal tract. They are, in a sense, biological soaps (fig. 3).

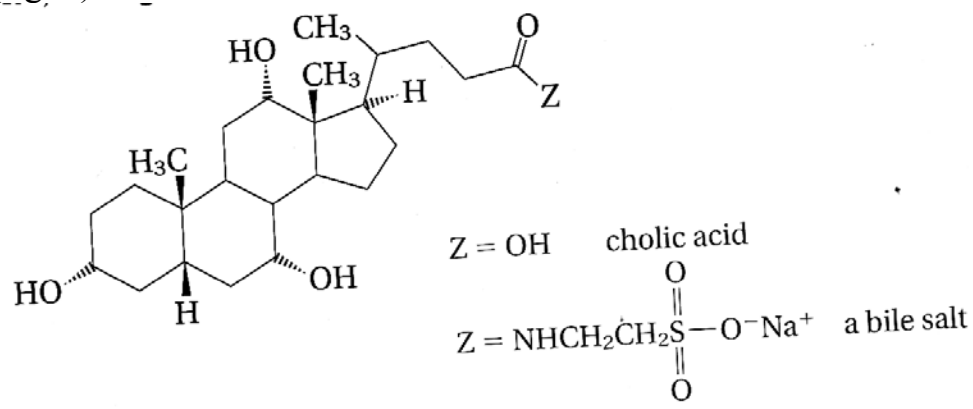


Fig. 3. Scheme of cholic acid and bile salt

The **SEX HORMONES** are compounds, produced in the ovaries and testes, that control reproductive physiology and secondary sex characteristics. Those sex hormones that predominate in females are of two types.

The **ESTROGENS**, of which the most plentiful is **ESTRADIOL,** are essential for initiating changes during the menstrual cyclic and for the development of female secondary sex characteristics. Progesterone, which prepares the uterus for implantation of the fertilized egg, also maintains a pregnancy and prevents further ovulation during that time (fig. 4).

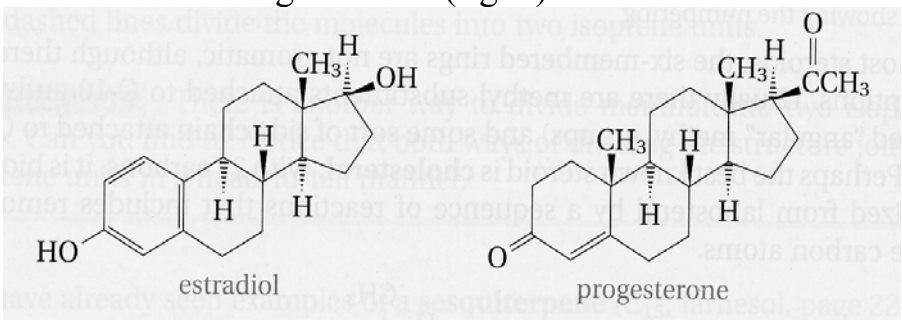


Fig. 4. The woman's hormones

Sex hormones that predominate in males are called androgens. Two important androgens are **TESTOSTERONE** and **ANDROSTERONE** (fig. 5).

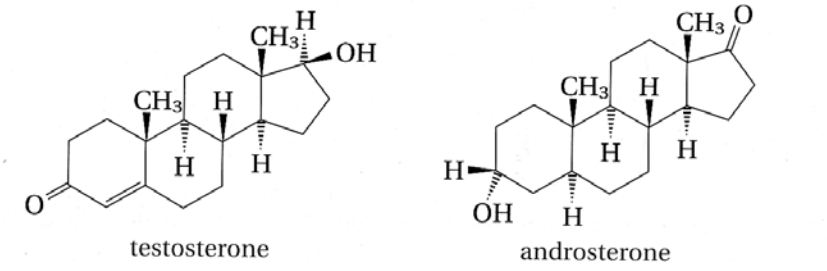


Fig. 5. The sex hormones

They regulate the development of male reproductive organs and secondary sex characteristics, such as facial and body hair, deep voice, and male musculature.

Anabolic steroid (muscle-building) is testosterone. Drugs based on its structure are sometimes administered to prevent withering, or similar trauma. If taken in high doses, they can have serious side effects, including sexual malfunctions and liver tumors.

Another steroid bearing a resemblance to testosterone and progesterone is CORTISONE, a drug used in the treatment of arthritis (fig. 6).

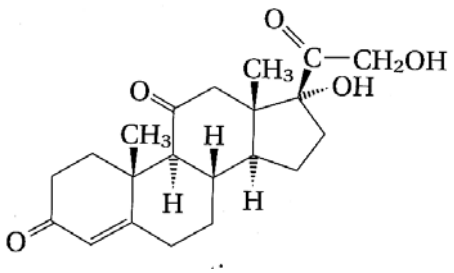


Fig. 6. Cortisone

1.8. Laboratory work

LIPIDS

Test №1. Solubility of fats and oils

Pour 5 drops of a vegetable oil into 6 test tubes and try to dissolve it in 1 ml of a solvent. Solvents are the following: water – in the first test tube, ethyl alcohol – in the second test tube, ethyl ether – in the third test tube, hexane – in the fifth test tube and benzene – in the sixth test tube. Compare solubility of vegetable oil in different solvents. Make a conclusion about fats and oils solubility.

Test №2. Emalgation of fats and oils

Pour 5 ml of distilled water into four test tubes and add 10 drops of vegetable oil. Treat a mixture in the first test tube with 5 drops of 1% sodium hydroxide solution. Treat a mixture in the second test tube with 2–5 drops of soap solution. Treat a mixture in the third test tube with 3–5 drops of soda solution. A mixture in the fourth test tube is given for comparison.

Compare stability of prepared emulsions. What substances are the best emulators?

Test №3 Preparing of saturated fatty acids

Treat 5 ml of soap solution with 1-2 ml of hydrochloric acid (1:1). Strong acids display saturated fatty acids from their salts. What can you see?

Write an equation for a chemical reaction when sodium stearate is treated with hydrochloric acid.

$C_{17}H_{35}COONa + HCl \rightarrow C_{17}H_{35}COOH\downarrow + NaCl$

Test №4. Test on unsaturated fatty acids

Pour 1-2 ml of vegetable oil into a test tube and dissolve it in 2-3 ml of hexane. You may use diethyl ether or chlorophorm as well. Treat a prepared solution with 1-2 drops of bromine water and stur a mixture.

What can you see? Write the equation for a given reaction.

$C_{17}H_{33}COOH + Br_2\ (\mathbf{aq}) \rightarrow CH_3(CH_2)_7 – CHBr – CHBr – (CH_2)_7 – COOH$

Test №5. Elaidinic test

Pour 1 ml of vegetable oil into a test tube and treat it with 10–15 drops of 20% nitric acid and 1-2 ml of 10% $NaNO_3$ solution. Stur a prepared mixture.

Under the influence of nitrogen oxides liquid oleic acid (cis-isomer) isomerises into solid elaidic acid (trans-isomer).

1.9. Exercises for the self-control

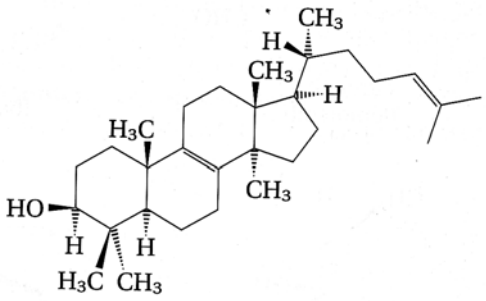
1. Write the formulas for the following lipids:

- gluceryl triolinolate;
- lecithin with the radicals of the palmitic and arachidonic acids;
- phosphatidylserine with the radicals of the oleic and linolenic acids;
- cephalin with the radicals of the stearic and palmitic acids;
- plasmalogen with the radicals of the calamine, oleic and linoleic acids.

2. Write the equations for chemical reactions of one cycle of the saturated acid's beta (β)-oxidation.

3. Write the equations for all steps of the phospholipids' peroxide oxidation.

4. How many stereogenic centers are present in lanosterol?



lanosterol (C_{30})

5. Steroids: chemical structure, biological functions. Steran and its conformations.

6. Steroid hormones: chemical structure, biological functions.

7. Bile acids: chemical structure, biological functions.

8. Cholesterol: chemical structure, biological functions.

CHAPTER 2

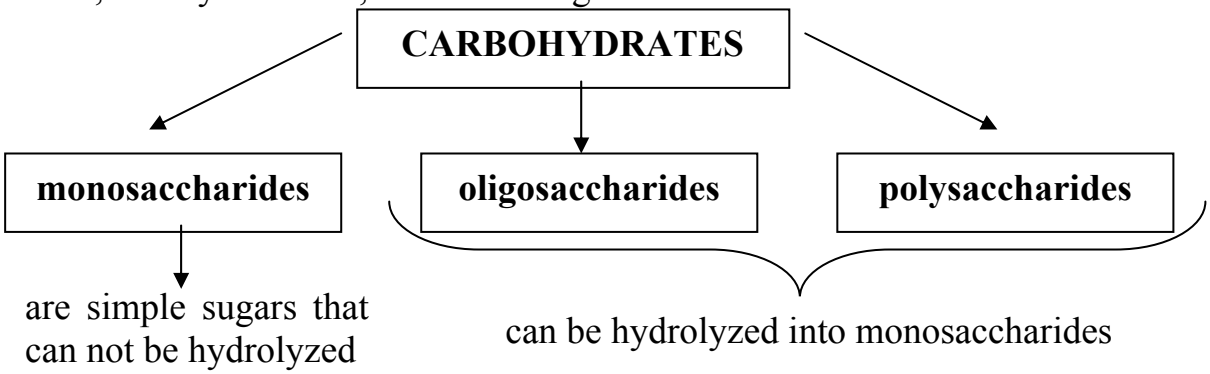
CARBOHYDRATES

After reading this chapter, you should be able to:

- **Define carbohydrates and discuss their classification;**
- **Describe structure, isomerism and chemical properties of monosaccharides;**
- **Discuss structure, chemical properties and biological functions of reducing and non-reducing disaccharides**
- **Describe structure and properties of most important homo- and heteropolysaccharides (starch, glycogen, cellulose, dextrans, chondroitin sulfate and hyaluronic acid)**

2.1. Classification of the carbohydrates

Carbohydrates occur in all plants and animals and are essential to life. Through photosynthesis, plants convert atmospheric carbon dioxide to carbohydrates, mainly cellulose, starch and sugars



Monosaccharides are polyhydroxyl aldehydes (aldoses) and polyhydroxy ketones (ketoses). According to the number of carbon atoms present they are: triose, tetrose, pentose, hexose. In nature pentoses and hexoses are most abundance.

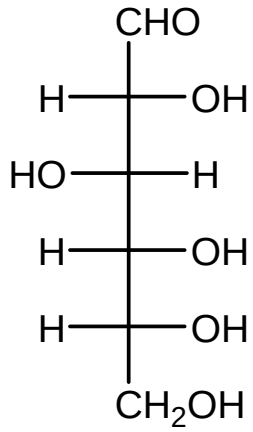
Oligosaccharides are condensation products of two to ten monosaccharide units. **Disaccharides** are condensation products of two monosaccharide units. (maltose, lactose, sucrose)

Polysaccharides are condensation products of more that ten monosaccharide units. They are:

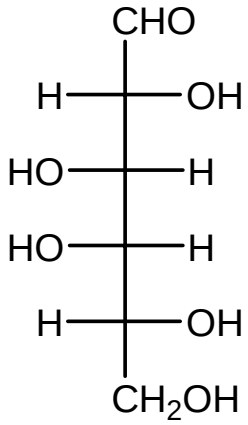
- Homopolysaccharides – consist of the identical monosaccharides units (starch, cellulose)
- Heteropolysaccharides – consist of the different monosaccharides units (hyalunic acid, chondroitin sulfate, heparin)

2.2. Monosaccharides

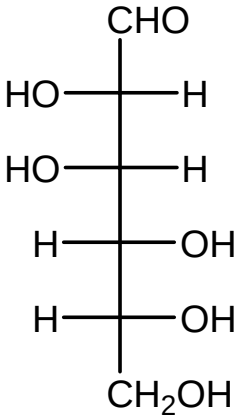
All monosaccharides except dihydroxyacetone contain stereogenic centre and form optical isomers. Thus aldohesozes a number of stereogenic centre is 4 hence there are $2^4 = 16$ optical isomer (8 pairs of enentiomers). Only d-monosacchirides are biologically active, take part in metabolism processes. In the chemistry of carbohydrates a special name is given to diastereomers that differ in configuration at only one stereogenic centre, they are called **EPIMERS.**



D-glucose



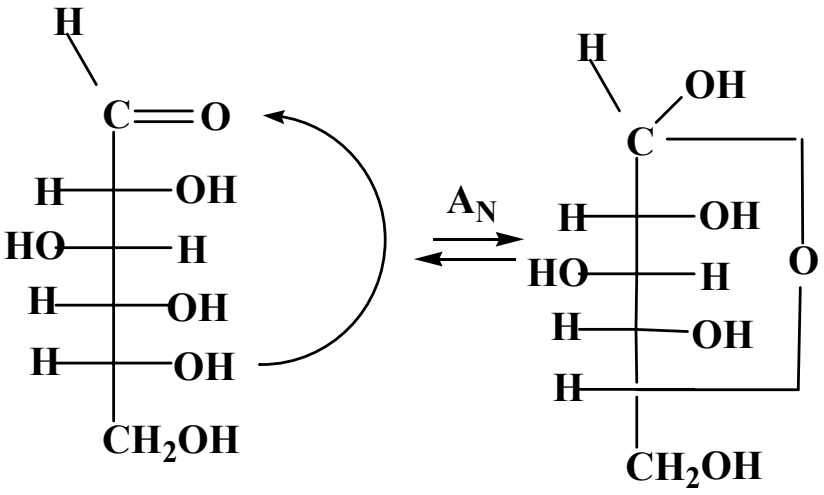
D-galactose



D-mannose

Epimer of glucose at **C-4** Epimer of glucose at **C-2**

Cyclization of monosaccharides is result of intramolecular interaction between a hydroxyl group and a carbonyl group. Formation of hemiacetals is a reversible process due to which an equilibrium is stated between open-chain and cyclic form of a substance in aqua solutions.

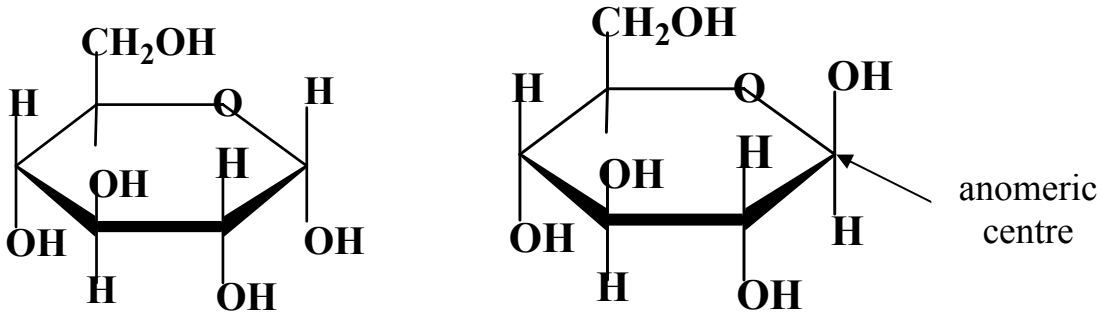


Open-chain (0.02%) form of D-glucose

Cyclic formula for D-glucose

The Fisher projection

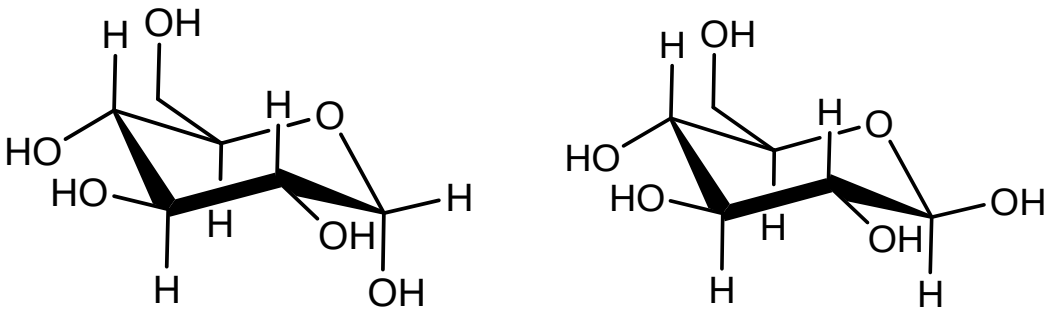
Howorth projection



α-D glucopyronose (36%)

β-D- glucopyronose (64%)

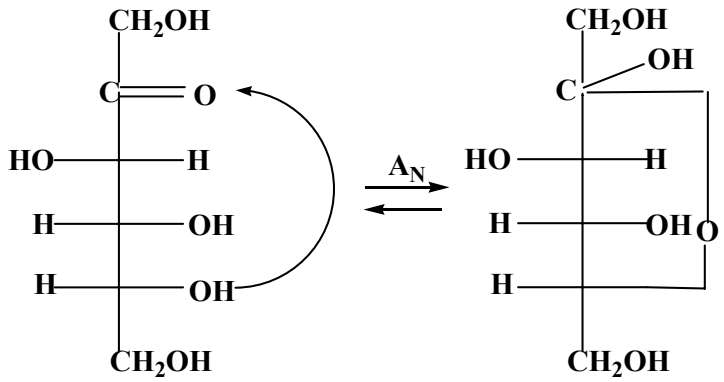
The more stable conformation for glucose is chair.



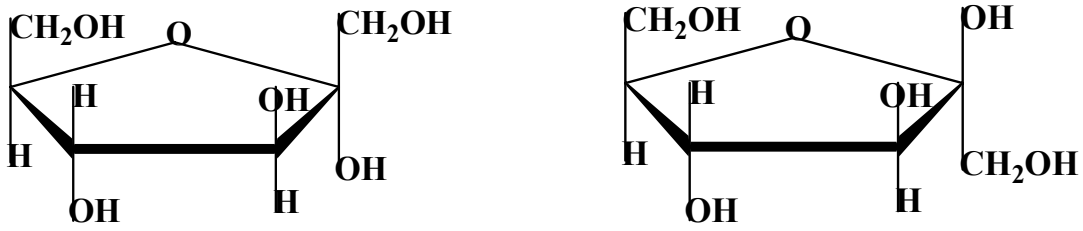
α-D glucopyronose

β-D- glucopyronose

FRUCTOSE is structural isomer of glucose. It is contained in honey and fruits. Fructose is a ketohexose and three its main forms are involved in the equilibrium in its aqueous solution.

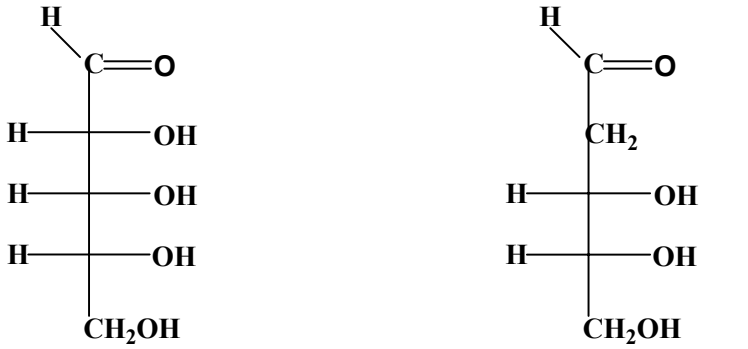


D-fructose Cyclic formula for D-fructose



α-D fructofuranose β-D- fructofuranose

RIBOSE and **DEOXYRIBOSE** are aldopentoses of nucleic acids.

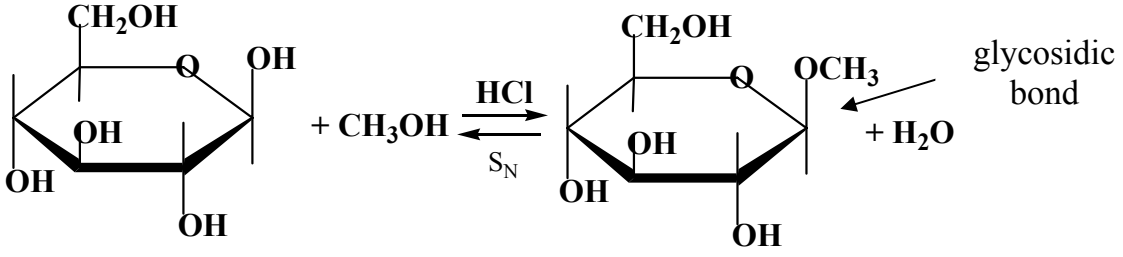


D –ribose **D - deoxyribose**

2.3. Chemical properties

Monosaccharides undergo reactions typical of alcohols and aldehydes (ketones).More over hemiacetal hydroxyl group is responsible for the specific reaction of **Glycosides formation**.

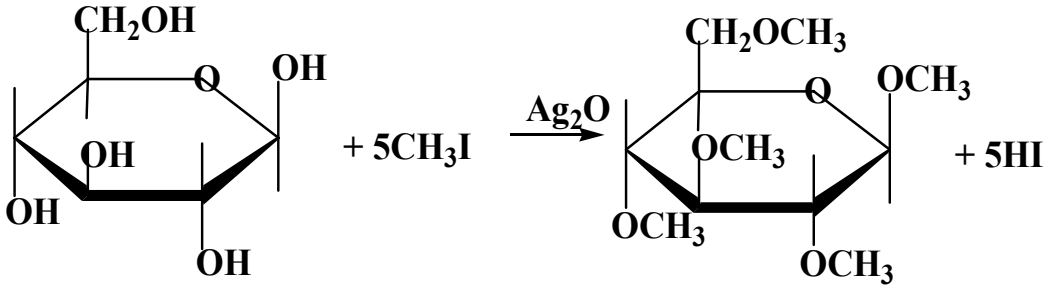
(a) Formation of Glycosides



O-metyl-β-D-glycopyranoside

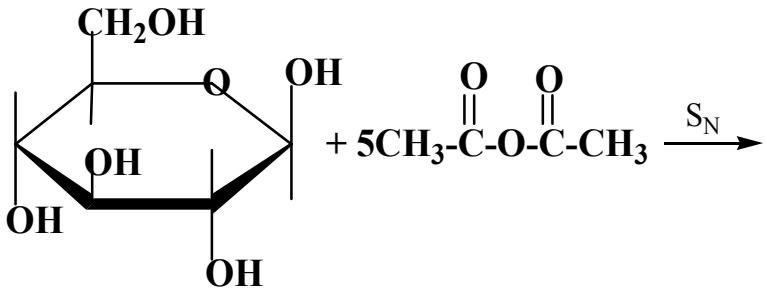
(b) the reactions typical for alcohols

• Formation of ethers

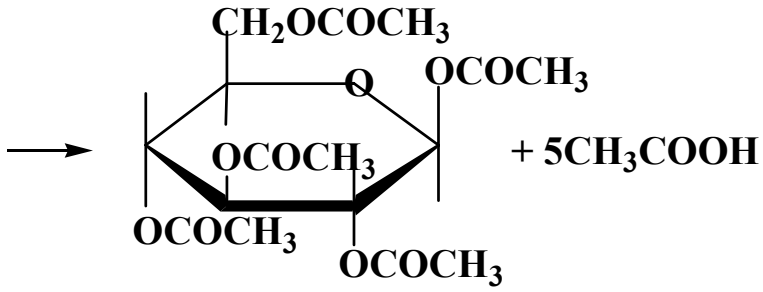


O-metyl-2,3,4,6-tetrametyl-β-D-glycopyranoside

• Formation of esters



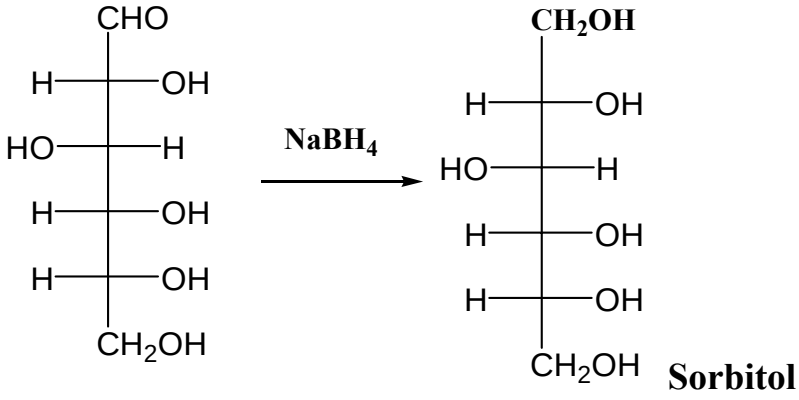
acetic anhydride



β-D-glycopyranoside pentaacetate

(c) the reactions typical for aldehydes and ketones

• Reduction of monosaccharides



Sorbitol

• Oxidation of monosaccharides

The Tollen's silver mirror test:

Glucose + $[Ag(NH_3)]OH \rightarrow Ag \downarrow$ + products of oxidation

Tollen's reagent

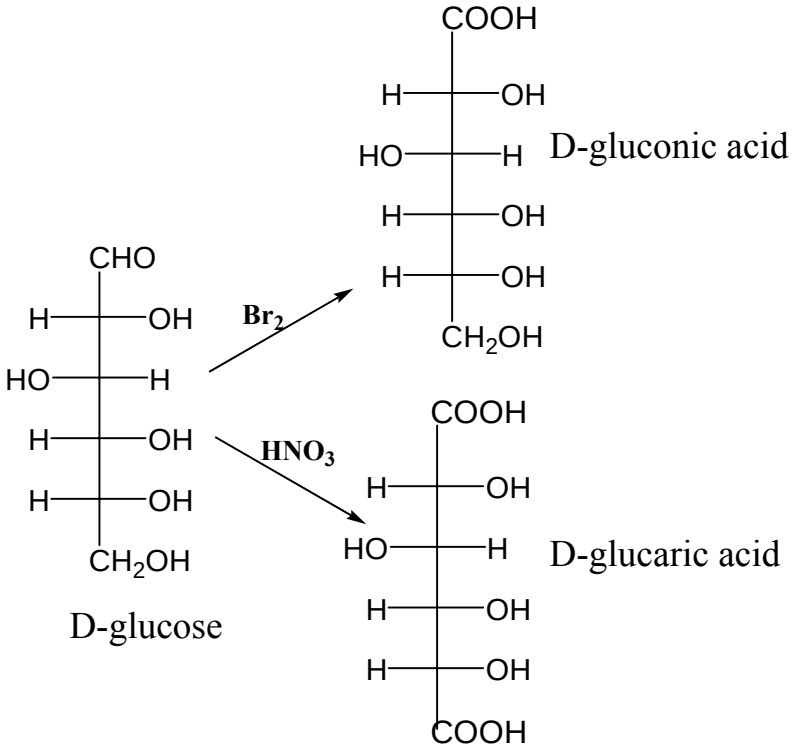
The Fehling's and Benedict's tests

Glucose + Cu^{2+} comp $\rightarrow Cu_2O \downarrow$ + products of oxidation

brick-red
precipitate

The Fehling's reagent is Cu^{2+} complexed with tartrate ion.
The Benedict's reagent is Cu^{2+} complexed with citrate ion.
The oxidation in neutral and acid media runs with the formation of different acids.

The oxidation of aldoses with mild oxiditizing agents (Br_2 water) gives so-called **aldonic acids**. Strong oxiditizing agents such as nitric acid attack the aldehyde group and the primary alcohol group, producing dicarboxylic acids called **aldoric acids**.



2.4. Laboratory work

MONOSACCHARIDES

Experiment №1 The evidence of several hydroxyl groups in the D-glucose molecule

Pour 5 drops of 2% copper (II) sulfate solution into a test tube and treat it by 5 drops of 10% sodium hydroxide solution. It will results in a blue copper (II) hydroxide precipitate formation. Dissolve this precipitate in glycose. Mark the color of an obtained solution. This reaction is a test on alcohols containing more than one hydroxyl groups (polyhydric alcohols). Write the equations of fulfilled reactions.

Experiment № 2. The Tollens' silver mirror test

Pour 1drop of 5% silver nitrate $AgNO_3$ solution into a test tube and treat it by 2 drops of 10% sodium hydroxide NaOH solution. Dissolve the prepared dark-brown precipitate in the excess of aqueous ammonia. A prepared transparent solution is used as a reagent to detect glucose and other aldoses in their solutions. It is known as the Tollens' reagent. Treat the Tollens' reagent with 2 drop of 0.5% glucose solution and heat it in the fire of a spirit lamp. If the test tube is thoroughly clean, the silver deposits as mirror on the glass surface. Write the equations for fulfilled reactions.

Experiment № 3. The Trommer's test (a reaction of monosaccharides with copper (II) hydroxide)

Pour 1-2 mL of glucose solution into test tube and treat it with 1 mL of 10% sodium hydroxide NaOH solution and same drops of 1% copper (II) sulfate solution. Heat a prepared mixture up to the boiling point. What can you see? Write the equations for fulfilled reactions

Experiment № 4. The Fehling's test (a reaction of monosaccharides with Fehling's reagent)

Pour 0.5 mL of 2 M copper (II) sulfate solution with 0.5 mL of 2 M sodium hydroxide solution. Dissolve the prepared precipitate with 3% sodium potassium tartrate solution. A prepared solution is used as a reagent to detect monosaccharides in their solutions (the Fehling's reagent). Treat 1-2 mL of glucose solution with Fehling's reagent. Heat the mixture up to the boiling point. What can you see? Write an equation for the fulfilled reaction. Compare the results of Trommer's and Fehling's tests. What test is more useful? Why?

2.5. Exercises for the self-control

Draw the Haworth projection for

- β-D-fructofuranose
- α-D –ribosefuranose
- α-D – galactopyronose
- β-D-mannopyronose

2.6. Classification Oligo- and Polysaccharides

Oligosaccharides are condensation products of two to ten monosaccharide units.

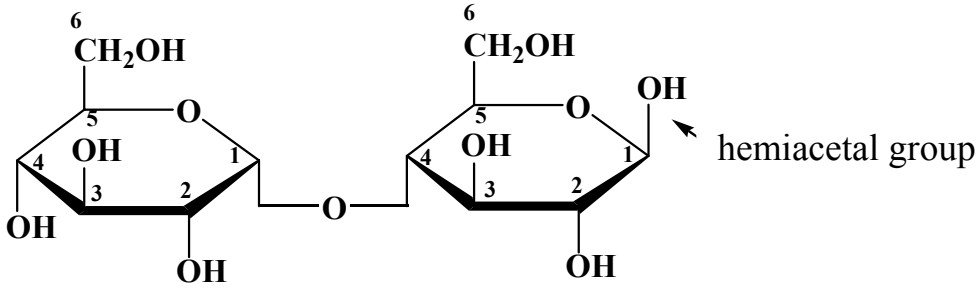
Polysaccharides are condensation products of more that ten monosaccharide units. They are:

- Homopolysaccharides – consist of the identical monosaccharide units (starch, cellulose)
- Heteropolysaccharides – consist of the different monosaccharides units (hyaluronic acid, chondroitin sulfate, heparin)

2.7. Disaccharides

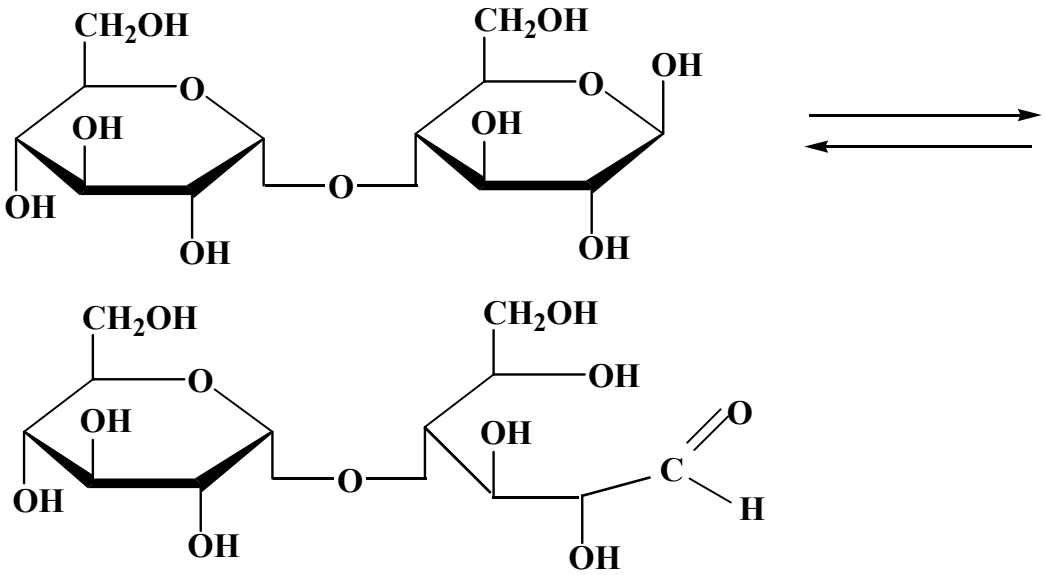
Disaccharides are condensation products of two monosaccharide units. (maltose, lactose, sucrose). They may be classified as reducing and nonreducing sugars. The reducing sugars contain hemiacetal groups in their molecules and exhibit reducing properties ; their solutions mutarotate. Nonreducing sugars do not contain hemiacetal groups; their solutions do not mutarotate.

➢ **Maltose** or malt sugar is the reducing disaccharide. It does not occur widely in nature and is obtained by partial hydrolysis of starch.

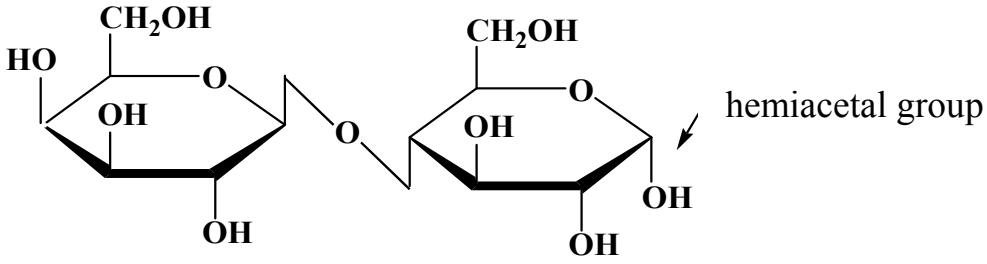


α-D-glucopyranosyl-(1→4)-β-D-glucopyranose

Maltose solutions mutarotate:

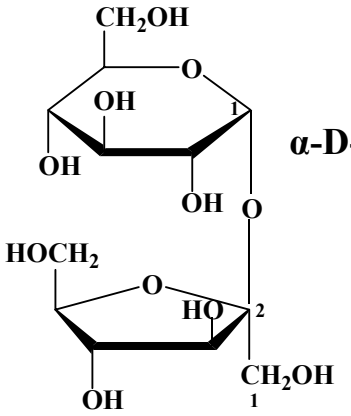


Lactose is the reducing disaccharide. It is the major sugar in human and cow's milk (4-8%). It plays an essential role in forming nonpathogenic micro flora in digestive tract of infants



β-D-galactopyranosyl-(1→4)-α-D-glucopyranose

➢ **Sucrose** is the nonreducing disaccharide. It occurs in all photosynthetic plants where it functions as an energy source. Its solutions do not mutarotate.

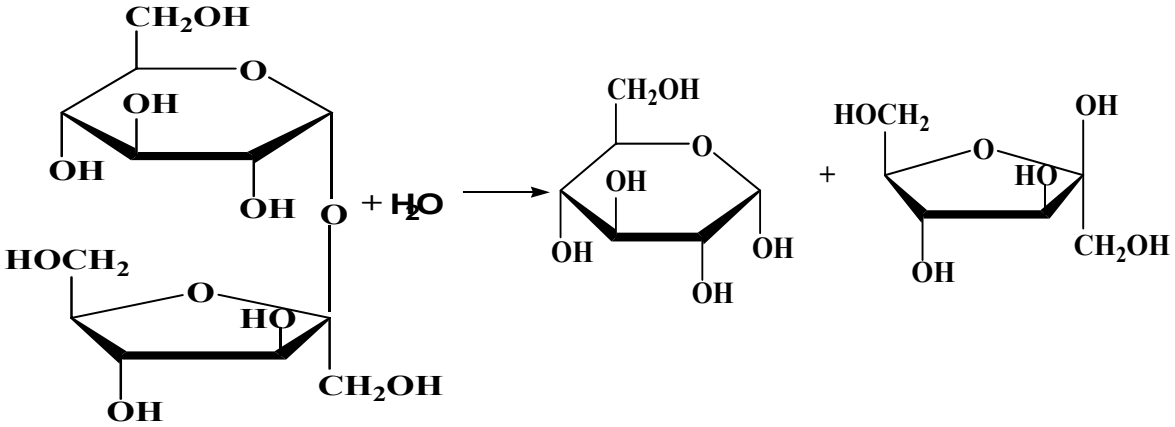


α-D-glucopyranosyl-(1→2)-β-D-fructofuranoside

2.8. Chemical properties of disaccharides

➢ **They undergo hydrolysis in acidic medium**

Glycosidic bond can be hydrolyzed in acidic media with the formation of monosaccharides.



Sucrose **α-D- glucopyranose** **β-D-fructofuranose**
(Invert sugar)

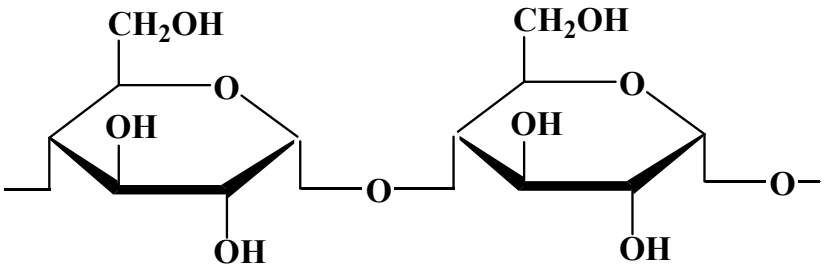
➢ Reducing disaccharides can be oxidized by different oxidizing agents; they give positive Tollens', Fehling' and Benedict's tests.

➢ Nonreducing disaccharides cannot be oxidized by different oxidizing agents; they give negative Tollens', Fehling' and Benedict's tests.

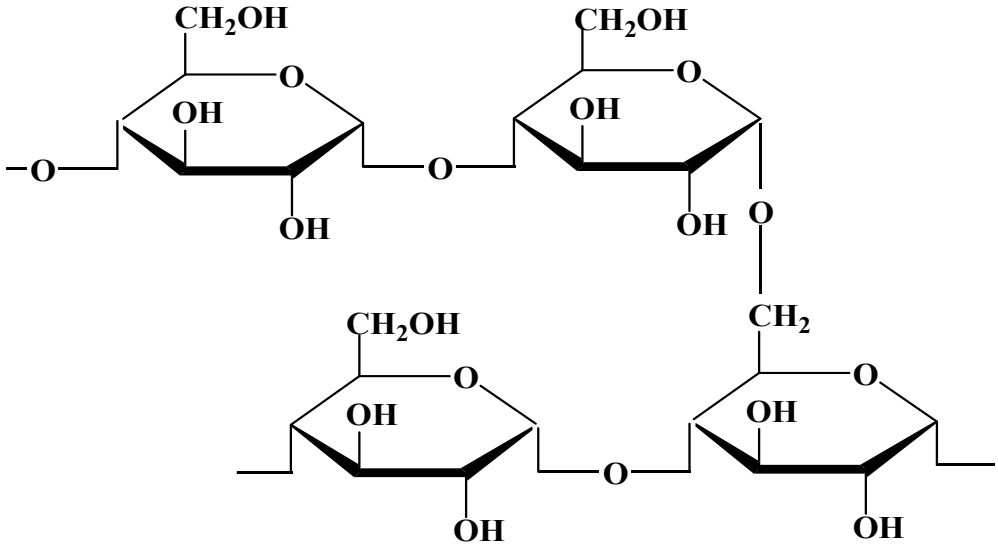
2.9. Homopolysaccharides

➢ **Starch** is the energy-storing carbohydrate of plants. It is a major component of cereals, potatoes, corn and rice. It is the form in which glucose is stored by plants for later use. Starch can be separated by various techniques into two fractions: amylase (10–20%) and amylopectin (80–90%). Its macromolecules ate built from α-glucose which are linked by α (1-4) and α (1-6) glycosidic bonds.

Amylase's structure. Its unbranched molecules are coiled in helix.



Amylpectin's structure. Its molecules are highly branched. Glycogen is the storage polysaccharide in animals, which is a structural analog of amylopectin, but is more highly branched (with the branch every 8 to 12 glucose units).



Starch undergoes acid catalyzed hydrolysis:

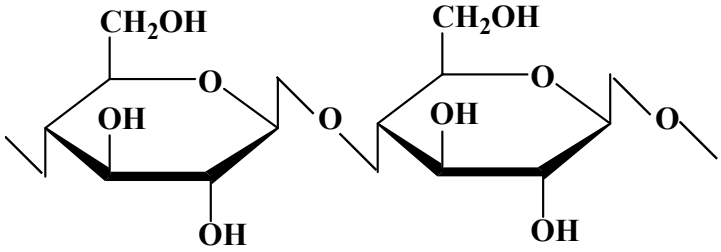
$(C_6H_{10}O_5)_n + n\ H_2O \rightarrow (C_6H_{10}O_5)_m + m\ H_2O \rightarrow (C_6H_{10}O_5)_x + x\ H_2O \rightarrow$

Starch — Soluble starch — Dextrin

$\rightarrow n/2\ C_{12}H_{22}O_{11} + n/2\ H_2O \rightarrow n\ C_6H_{12}O_6$

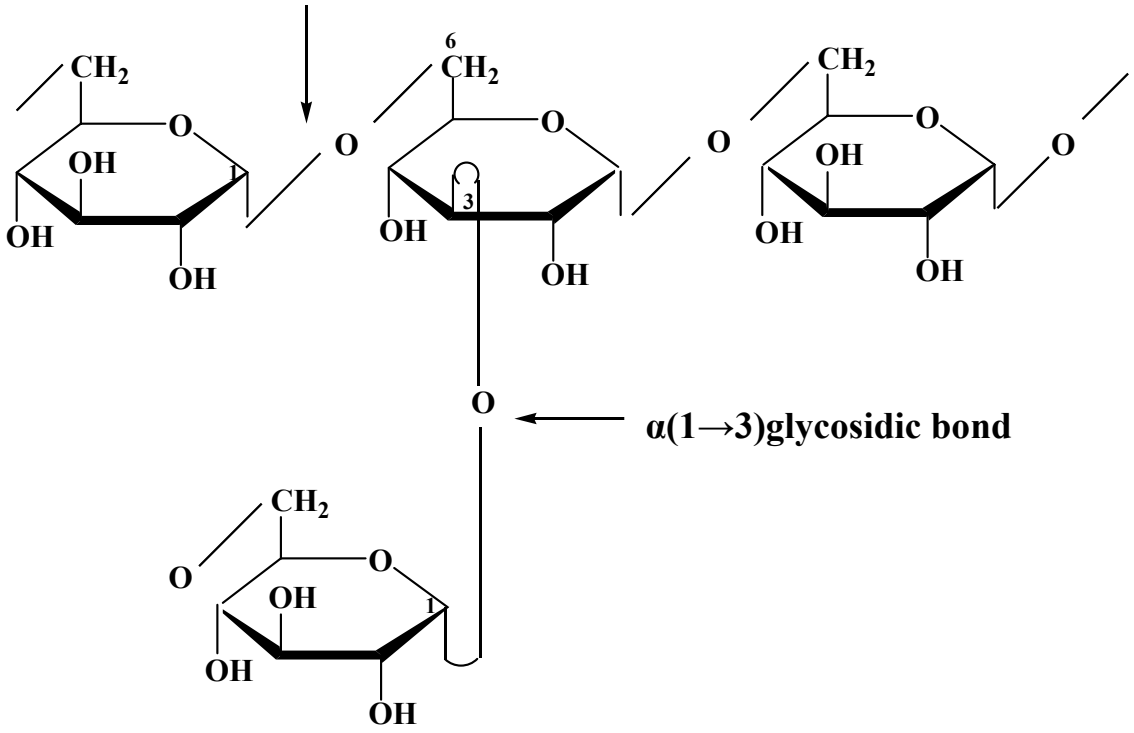
Maltose — α-glucose

➢ **Cellulose** is the most abundant homopolysaccharide. It is the chief constituent of the framework of plants. It is formed from β-glucose linked by β (1-4) glycosidic bonds.



➢ **Dextrans** are the homopolysaccharides produced by special bacteria in sucrose solutions. It is used in pharmacology to produce plasma substitutent "polyglukin". It is formed from α-glucose linked by α (1-4) glycosidic bonds in main chain and by α (1-2), α (1-3) and α (1-4) glycosidic bonds at branch points.

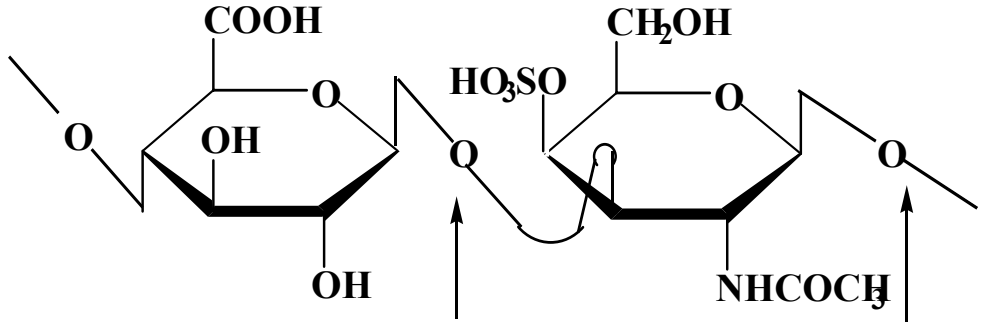
α(1→6)glycosidic bond



2.10. Heteropolysaccharides

A big group of heteropolysaccharides is known as mucopolysaccharides. They provide the ground of packing substance in connective tissues. They are: Chondroitin sulfate is one of the most important heteropolysaccharide of connective tissue. Its unbranched chains are built up from the disaccharide unit linked by β(1→4)glycosidic bond. Each unit is built up from D-glucuronic acid and D-galactoseamine joined by β(1→3) glycosidic bonds. Amino group is acylated by acetic acid and OH-group attached to C-4 or C-6 is acylated by sulfuric acid.

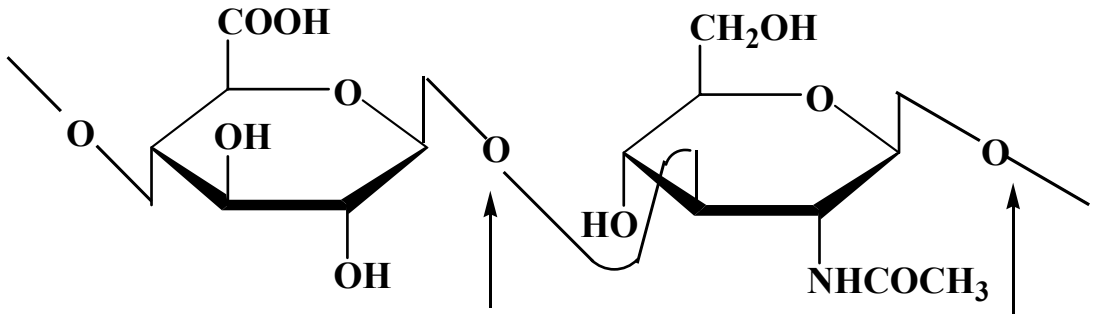
Chondroitin sulfate's disugar fragment.



β(1→3)glycosidic bond **β(1→4)glycosidic bond**

➢ In Hyaluronic acid each disaccharide unit consists from D-Glucuronic acid and ad-gluccoseamine linked by β(1→3) glycosidic bond. It protects tissues from pathogenic bacteria.

Hyaluronic acid's disugar fragment



β(1→3)-glycosidic bond **β(1→4) glycosidic bond**

2.11. Laboratory work

DI-AND POLYSACCHARIDES

Experiment №1 Nonreducing properties of sucrose

Pour 1 drop of 1% sucrose solution into a test tube and treat it with 6 drops of 10% sodium hydroxide solution. Dilute the prepared solution with 5-6 drops of water and add 1 drop of 2% $CuSO_4$ solution into it. It results in the formation of blue transparent solution of copper (II) complex salt with sucrose.

Heat a prepared solution in the fire of spirit lamp up to the boiling point. Can you see any changes in a solution color? Compare the obtained result with the result of the same experiment with glucose. Make a conclusion about reducing property of sucrose.

Experiment № 2. Reducing properties of lactose

Put 1 drop of 1% lactose solution into a test tube and treat it with 4 drops of 10% sodium hydroxide solution. Add 1 drop of 2% copper (II) sulfate solution into it. Dissolve a prepared copper (II) hydroxide precipitate in lactose solution under stirring. The dissolving results in the formation of blue copper (II) complex salt with lactose solution.

Heat a prepared solution in the fire of spirit lamp up to the boiling point. Can you see any changes in a solution color? Compare the obtained result with the result of the same experiment with glucose. Make a conclusion about reducing property of lactose. Write out the equation for lactose interaction with copper (II) hydroxide.

Experiment № 3. Iodine-starch test

Pour 5 drops of 0.5% starch colloidal solution into a test tube and treat it with 1 drop of dilute iodine solution. A solution turns blue. Heat a solution. Can you see any changes in its color? Explain why the blue color of iodine-starch complex disappears under heating and appears again under cooling?

Experiment № 4. Acid catalyzed starch hydrolysis

Pour 8-10 drops of starch solution into a test tube and add treat it with 2 drops of concentrated sulfuric acid. Heat a prepared mixture during 20-25 minuets in a glass with boiling water.

Take 1 ml of a solution after hydrolysis with a pipette and treat it with 1 drop of dilute iodine solution. If iodine-starch test is positive treat a solution in a test tube with 8 drops of 10% NaOH solution and add 1 drop of 2% copper (II) sulfate solution. . What can you see? Is Trommer's test positive or not? Write the equations of fulfilled reactions.

Experiment № 5. Cellulose dissolving in the Schweitzer reagent

The Schweitzer reagent is a copper (II) carbonate ammonia solution. Prepare this solution and put a small piece of cotton into it. A cotton will be dissolved in the Schweitzer reagent under stirring with a glass stick.

Pour approximately 1 ml of a transparent cellulose solution into another test tube and add 4-5 ml of distilled water into it. Pour the mixture into a glass with 10-12 ml of dilute hydrochloric acid. A solution turns colorless and free cellulose releases as white gel looking precipitate.

Does cellulose dissolved in the Schweitzer reagent undergo hydrolysis?

2.12. Exercises for the self-control

1. Draw the Haworth projection for maltose. Name it. Write out an equation for maltose hydrolysis. Explain why maltose is a reducing sugar.

2. Draw the structure of amylopectin. Name the type of a glycosidic bond which link glucose units in a amylopectin macromolecule.

3. From what monosaccharide units a cellulose macromolecule is built from? Write a formula for the trios' fragment of its molecule. Name the type of glycosidic bond in cellulose.

CHAPTER 3

AMINO ACIDS

After reading this chapter, you should be able to:

- **Define α-amino acids and their classification and nomenclature;**
- **Describe biosynthesis of nonessential α-amino acids;**
- **Discuss chemical properties of α-amino acids which lay at the bottom of their functioning in vivo.**

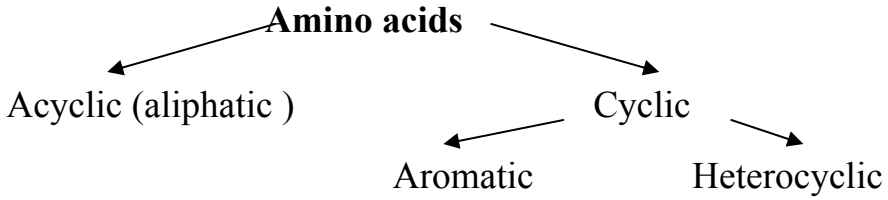
3.1. Classification and nomenclature of α-amino acids

General formula for amino acids, which are building blocks of proteins, is

$$H_2N - CH(R) - COOH$$

There are different types for amino acids classification

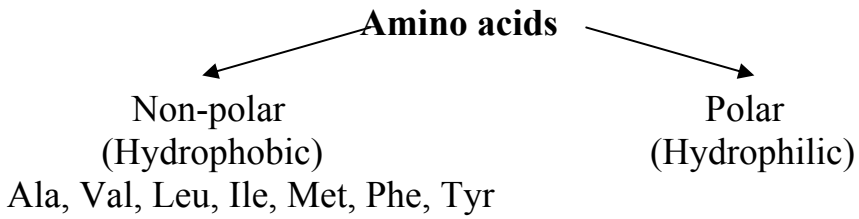
(a) Classification of amino acids according to molecular framework



(b) Classification of amino acids according to the number of *AMINO*- and *CARBOXYLIC* groups

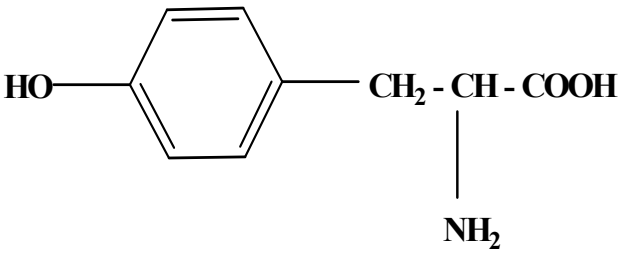
- Mono amino monocarboxylic acids (NEUTRAL)
- Mono amino dicarboxylic acids (ACIDIC)
- Diamino monocarboxylic acids (BASIC)

(c) Classification of amino acids according to the structure of their radicals

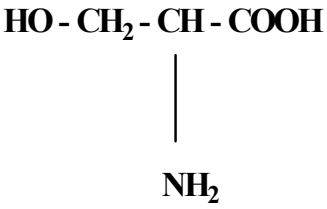


POLAR AMINO ACIDS

Containing ionisable groups which undergo ionization in vivo	**Withought ionisable groups**



Tyrosine



Serine

(d) Amino acids may be classified as:

- Essential, which cannot be synthesized in a human body and must be included in the diet in the form of proteins.

They are: **Val, Leu, Ile, Thr, Met, Lys, Phe and Trp**

- Nonessential, which can be synthesized in a human body

3.2. Names and formulas of the common amino acids

I. ALIPHATIC AMINO ACIDS

1. MONOAMINO MONOCARBOXYLIX ACIDS

$H_2N - CH_2 - COOH$

Amino acetic acid**,**

2-amino ethanoic acid**, Glycine (Gly)**

$CH_3 - CH - COOH$

NH_2

α – aminopropionic acid,2-aminopropanoic acid, **Alanine (Ala)**

$CH_2 - CH - COOH$

OH NH_2

α-amino-β-hydroxy propionic acid,

2-amino-3-hydroxypropanoic acid, **serine, (Ser)**

$CH_2 - CH - COOH$

SH NH_2

α-amino-β-thio propionic acid,

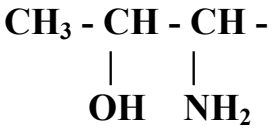
2- amino-3-mercaptopropanoic acid, **Cystein (Cys)**

$CH_3 - CH - CH - COOH$

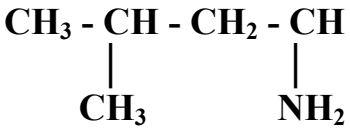
CH_3 NH_2

α-aminoisovaleric acid,

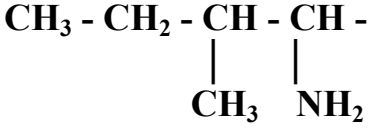
2-amino-3-methylbutanoic acid, **Valine (Val)**



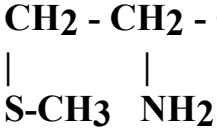
α-amino-β-hydroxybutyric acid,
2-amino-3-hydroxybutanoic acid, **Threonine (Thr)**



α-amino isocaproic acid, 2-amino-4-methylpentanoic acid, **Leucine (Leu)**

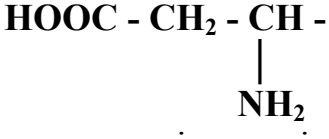


α-amino-β-methylvaleric acid,
2-amino-3-methylpentanoic acid, **Isoleucine (ILe)**

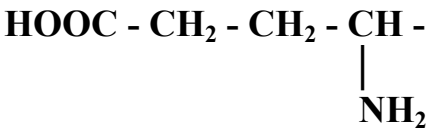


α-amino-γ-methylthiobutyric,
2-amino -4-methylthiobutanoic acid, **methyonine (MET)**

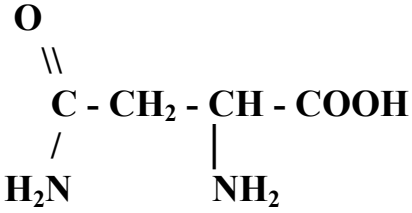
2. MONOAMINO DICARBOXYLIC ACIDS



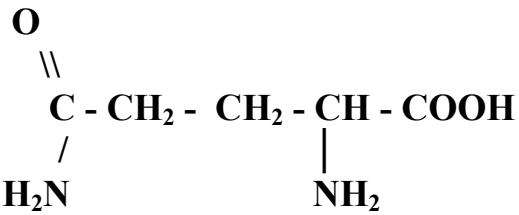
α-amino succinic acid,
2-amino butanedioic acid, **Aspartic acid (Asp)**



α-aminoglutaric acid,
2-aminopentanedioic acid, **Glutamic acid (Glu)**

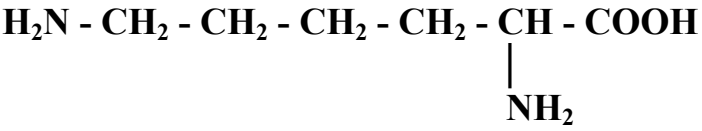


Aspartic acid amide, **Asparagine (Asn)**

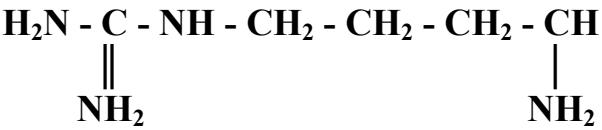


Glutamic acid amide, **Glutamine (Gln)**

3. DIAMINO MONOCARBOXYLIC ACIDS

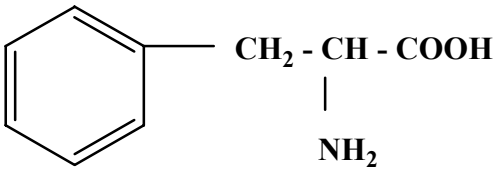


α,ε –diamino caproic acid, 2,6-diaminohexanoic acid, **Lysine (Lys)**

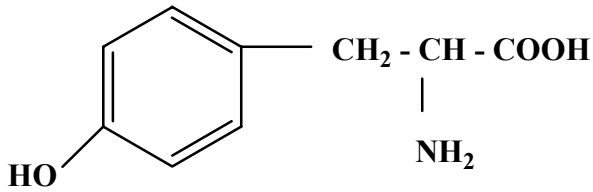


α-amino-Δ –guanidyl valeric acid,
2-amino-5-guanidylpentanoic acid, **Arginine (Arg)**

II. AROMATIC α-AMINO ACIDS

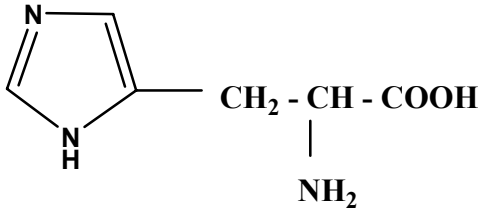


α-amino-β-phenyl propionic acid,
2-amino-3-phenyl propanoic acid,
Phenylalanine (Phe)

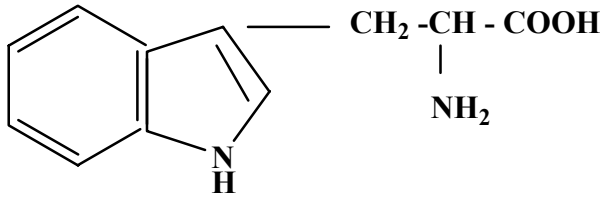


2-amino-3-(4- hydroxy phenyl)-propanoic acid, **Tyrosine (Tyr)**

III. HETEROCYCLIC α-AMINO ACIDS

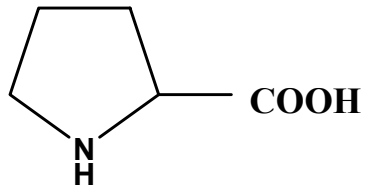


α-amino-β- imidazolyl propionic acid,
2-amino-3-imidazolyl propanoic acid
Histidine (His)

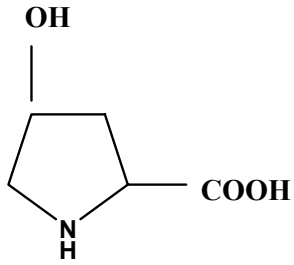


α- amino-β- indolyl propionic acid
2-amino-3-indolyl propanoic acid
Tryptophan (Trp)

IV. IMINO ACIDS



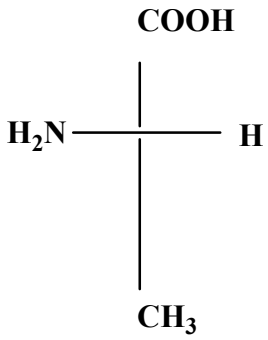
Pyrrolidine-2-carboxylic acid,
Proline (Pro)



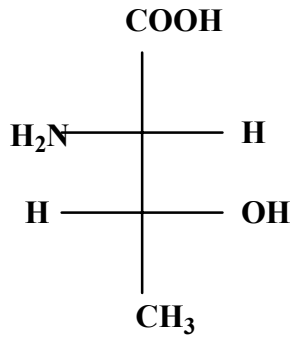
4-hydroxy pyrrolidine-2-carboxylic acid,
Hydroxy proline (HO-Pro)

3.3. Stereoisomerism of α-Amino Acids

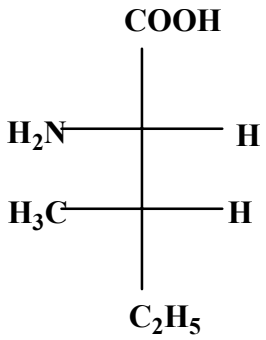
All α-amino acids are chiral, except Glycine. They contain stereogenic centers and form optical isomers. It was proved that only L-amino acids take part in proteins' biosynthesis:



L-Alanine



L-Threonine

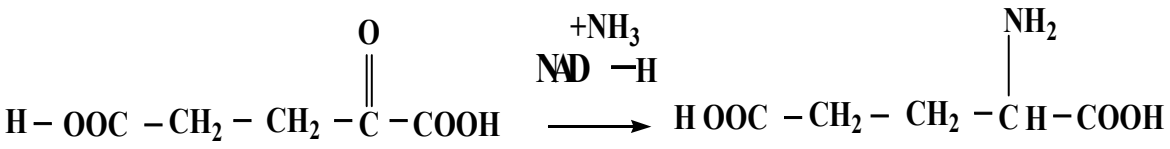


L-Isoleucine

3.4. Biosynthesis of α-Amino Acids

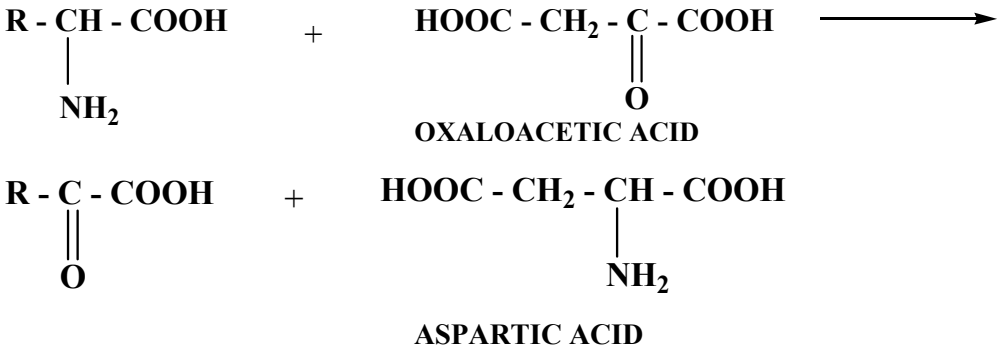
The body can manufacture a number of α-amino acids from intermediates that appear in the catabolism of nonprotein substances. The precursors of α-amino acids in a body are α-keto acids. There are two pathways for the biosynthesis of nonessential amino acids in vivo.

1) Reductive Amination



α-Ketoglutaric acid Glutamic acid

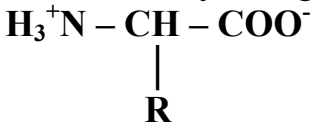
2) Transamination



3.5. Chemical properties of α-amino acids

(a) Amphoteric properties

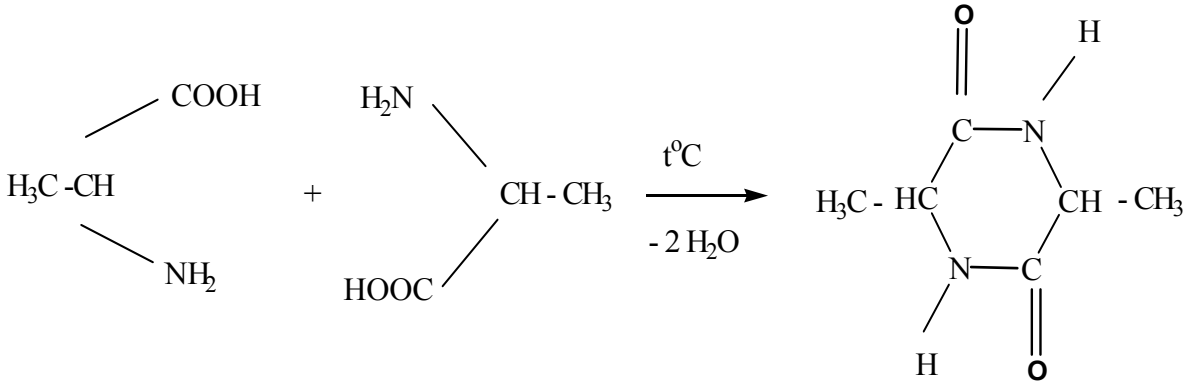
The simultaneous presence of amino- and carboxylic groups is responsible for the amphoteric character of α- amino acids. This results in the dipolar structure of acids and their salt like properties (high melting and boiling points, high solubility in water and relatively low solubility in organic solvents):



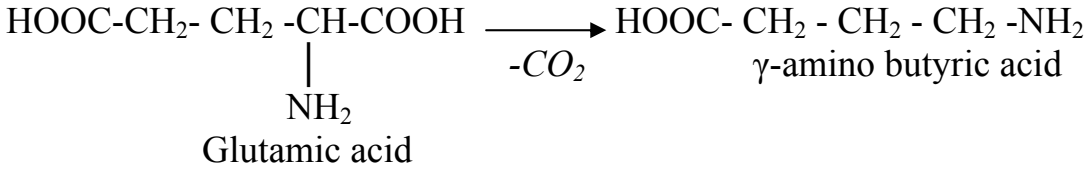
Dipolar ion

(b) α-location of amino group is responsible for intermolecular cyclization

(c) Decarboxylation of α-Amino Acids (removal of carbon dioxide) is a main pathway for bio amines formation in a body.

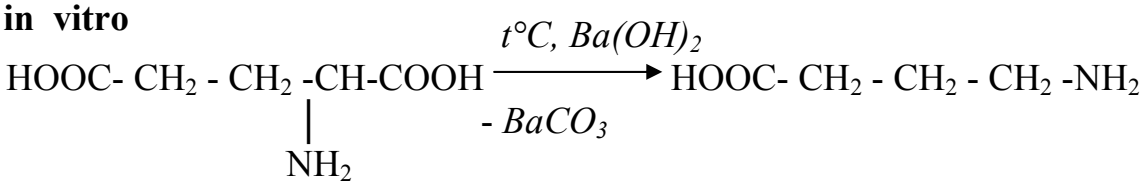


in vivo



Glutamic acid γ-amino butyric acid

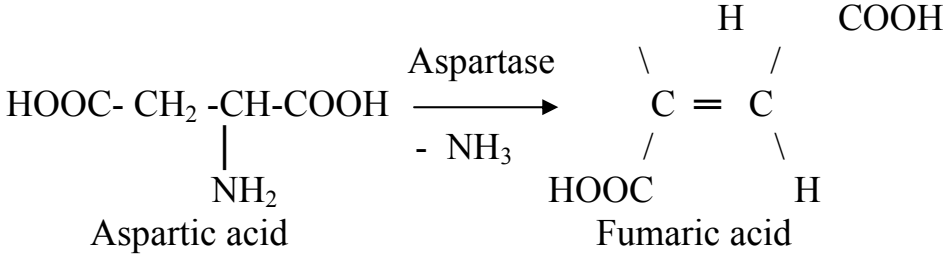
in vitro



(d**) Deamination of α-Amino acids** (removal of amino groups) may be direct and oxidative.

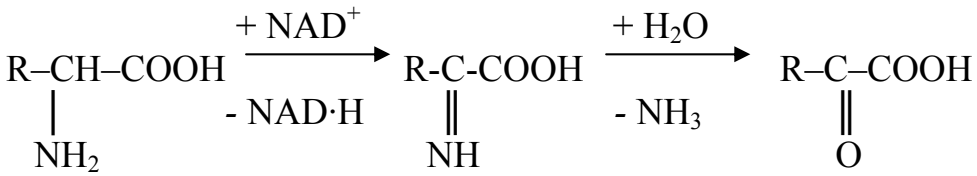
In vivo

➢ Direct Deamination:



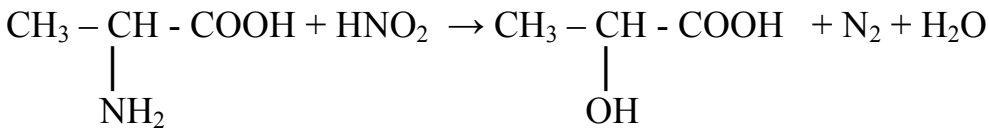
Aspartic acid Fumaric acid

➢ Oxidative Deamination:



In vitro

➢Oxidative Deamination **(Van-Slake Method)**



Alanine α-hydroxypropionic acid

This reaction is used in volumetric analysis to detect amino acids' content in analyzed solutions according to the volume of liberated nitrogen gas.

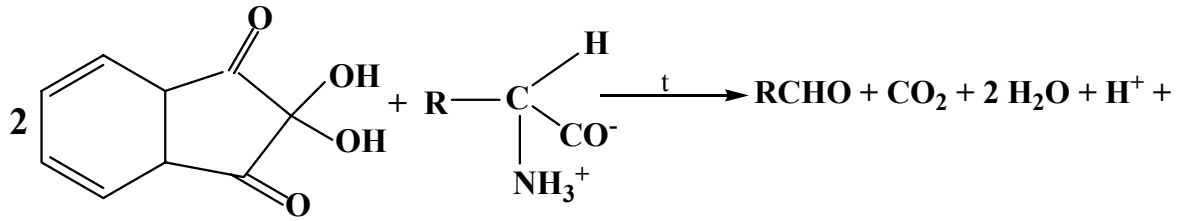
3.6. Laboratory work

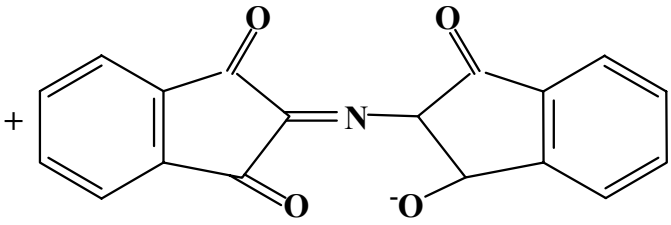
α-AMINO ACIDS

Experiment 1. The Ninhydrin reaction

Ninhydrin is a useful reagent for detecting amino acids and determining the concentration of their solutions. It is the hydrate of a cyclic triketone, and when

it reacts with an amino acid, a violet dye is produced. The overall reaction, whose mechanism is complex, is as follows:





Violet anion

Only the nitrogen atom of the violet dye comes from the amino acid; the rest of the amino acid is converted to an aldehyde and carbon dioxide. Therefore, *the same violet dye is produced from all a-amino acids with a primary amino group,* and the intensity of its color is directly proportional to the concentration of the amino acid present. Only proline, which has a secondary amino group, reacts differently to give a yellow dye, but this, too, can be used for analysis.

Pour 4 drops of 1% glycine solution and treat it with 2 drops of 0.1 % ninhydrin solution. Heat the mixture up to the boiling point. What can you see?

Experiment 2. Oxidative Deamination of α-Amino Acids with nitrous acid **(**Van-Slake Method**)**

Pour 5 drops of 1% glycine solution into a test tube and treat it with equal volume of 5% of sodium nitrite solution. Then add 2 drops of concentrated sulfuric acid and stir a prepared solution. What can you see? Write an equation for the reaction of glycine with nitrous acid.

Experiment 3. The formation of copper (II) complex salt with glycine

Pour 1 ml of 1% glycine solution into a test tube and treat it with some crystals of dry copper (II) carbonate salt. Heat the mixture up to the boiling point. What can you see? Write an equation for the fulfilled reaction.

3.7. Exercises for the self-control

1. Draw the Fisher projections for leucine enantiomers. Name them. Is leucine essential or nonessential amino acid?

2. Write an equation for the serine reaction with formaldehyde. For what purpose this reaction is used in a biochemical analyses?

3. Write an equation for the reaction of phenylalanine transamination with α-ketoglutaric acid. Name the products. What enzyme and coenzyme are involved in this process?

4. Write the equation for the reductive amination that produces glutamic acid. What coenzyme is involved in this process?

5. Write out the scheme for tryptophan decarboxylation. Under what conditions it may occurs in vivo and in vitro?

6. Illustrate the amphoteric nature of methionine by writing equations for its reactions with one equivalent of

(a) hydrochloric acid,

(b) sodium hydroxide

CHAPTER 4

PEPTIDES AND PROTEINS

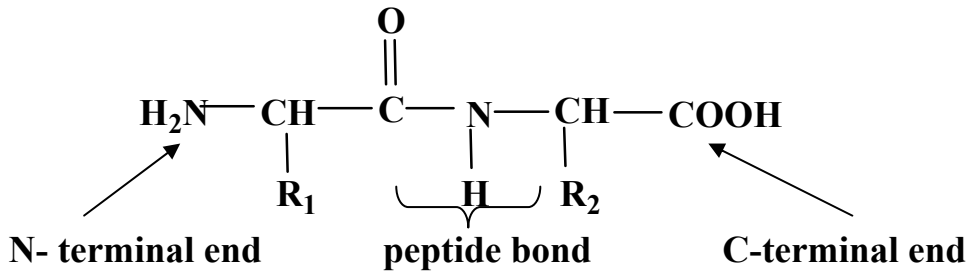
After reading this chapter, you should be able to:

- **Define structure and nomenclature of polypeptides chains;**
- **Describe electron structure of a peptide bond;**
- **Discuss primary structure of proteins determination;**
- **Explain strategy of peptide synthesis;**
- **Summarize chemical properties of peptides and proteins**

4.1. Electron structure of peptides bond

Peptides are natural or synthetic substances built up of α-amino acids residues which are linked by amide (peptide) bond. An amide bond arises between the carboxyl group of one amino acid and the α-amino group of another amino acid.

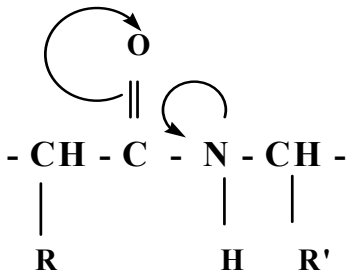
General formula for tripeptides

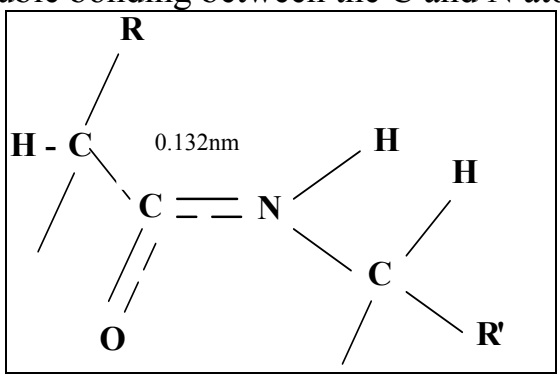


This amide bond was called a **peptide bond** by Emil Fisher, who first proposed the primary structure for proteins.

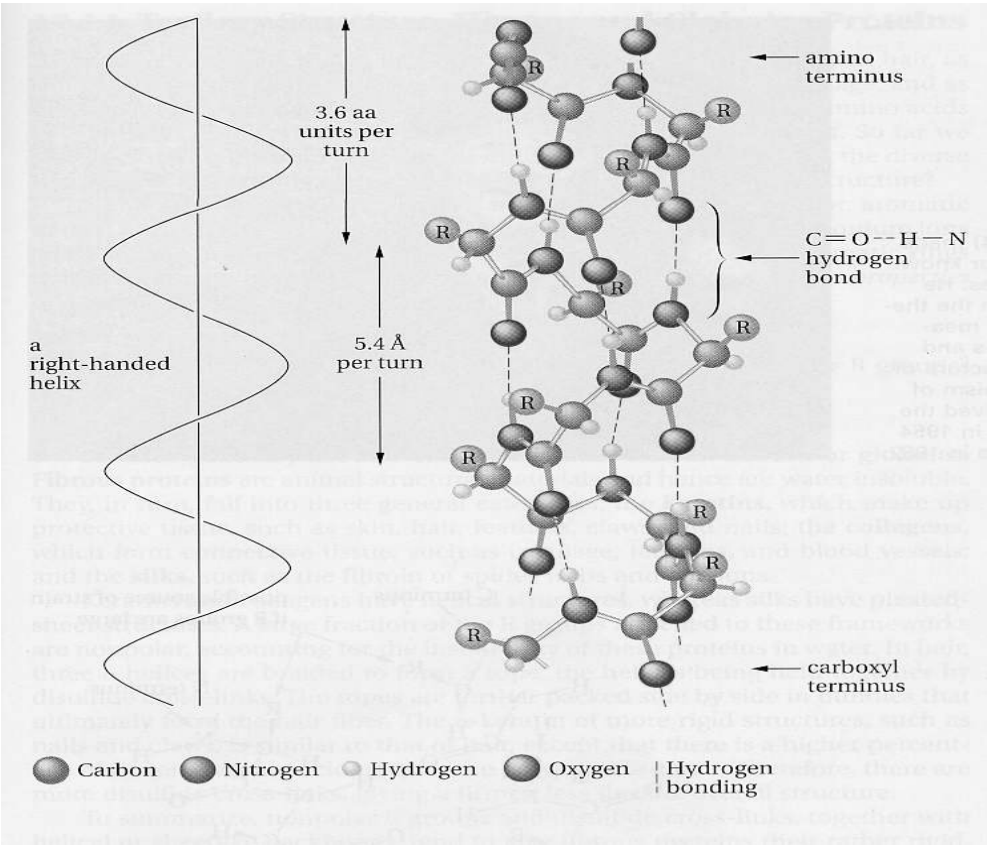
Biological functions of peptides and proteins depend upon a peptide group (unit) structure. Carbonyl carbon is sp^2-hybridized due to which all its bonds lay in one plane with angles between them 120º. As the result the peptide group is a rigid planar unit with hydrogen of the substituted amino group nearly always trans (opposite) to the oxygen of carbonyl group.

p,π-conjugation of a nitrogen lone electron pair and a carbonyl group π-bond is responsible for a partial double bonding between the C and N atoms:





The partial double bonding decreases the freedom of rotation about the peptide bond (it is rigid), but doesn't prevent rotation about the α-carbons and a peptide bond. Theoretically these rotations must give rise to the huge number of polypeptide chain conformations, but practically only two of them were proved to be stable. They are α-helix and β-strand, known as the secondary structure of proteins.

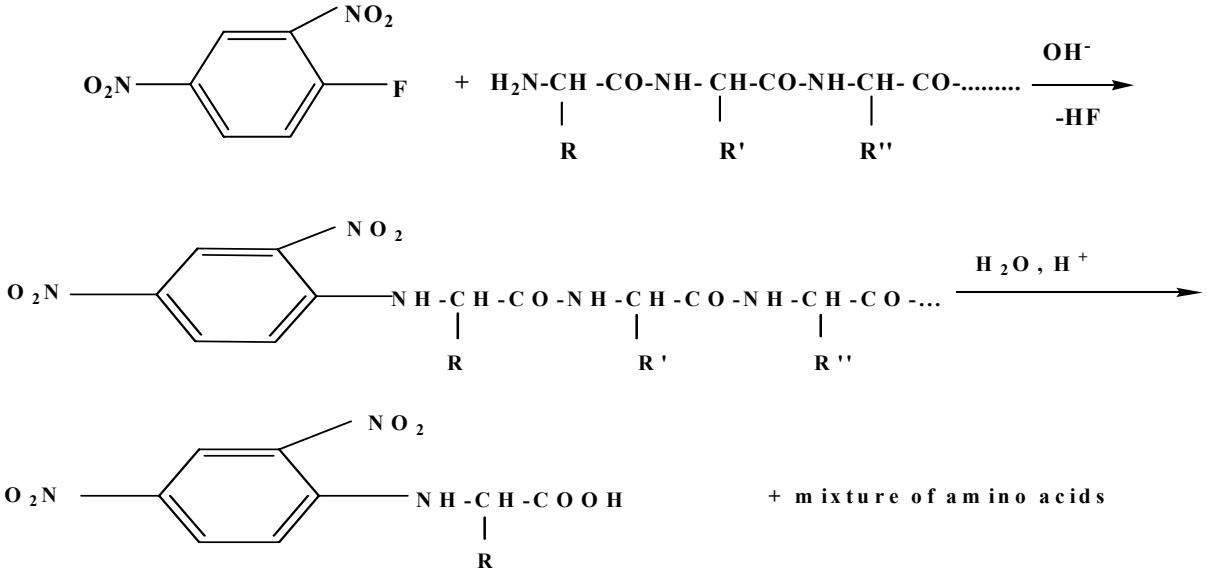


4.2. Primary structure of proteins determination

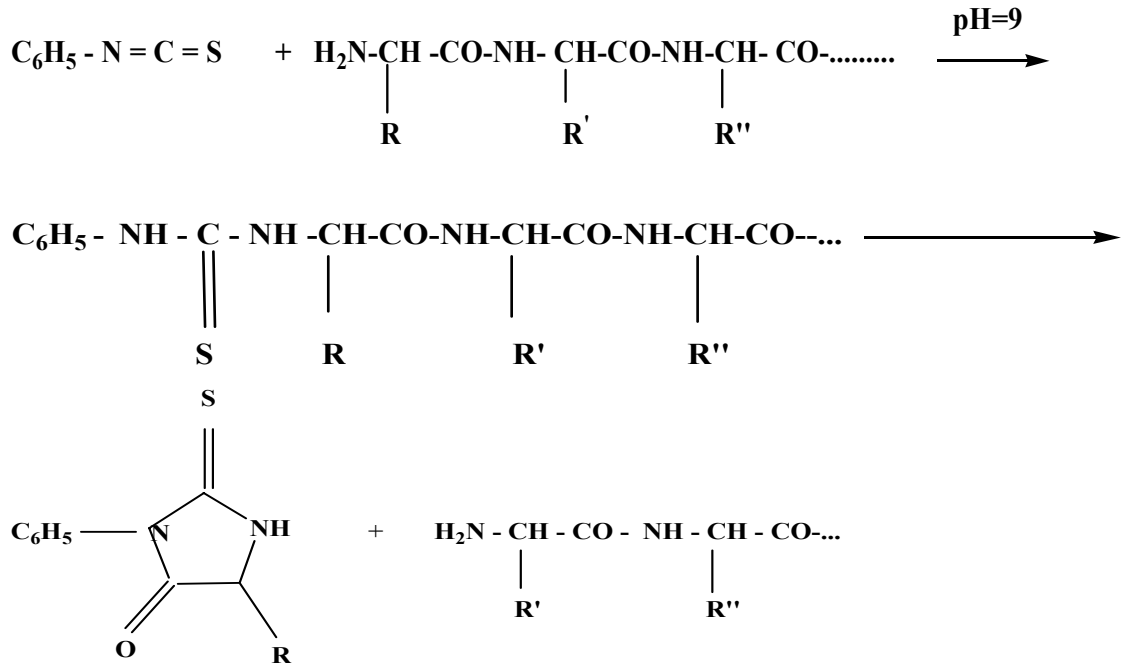
The primary structure of proteins is the sequence of amino acids residues in a completely assembled polypeptide chain.

The primary structure of peptides and proteins may be determined by sequence elimination of N-terminal amino acids with their later identification. Now days there are a lot of such methods.

(a) **The Sanger method for determining the N-terminal residue of a peptide.** 2, 4-Dinitrofluorobenzene (**DNFB**) is used to label N-terminal amino acids prior to hydrolysis and identification.



(b) **The Edman degradation method.** The Edman's reagent is phenyl isothiocyanate. The steps in selectively labeling and releasing the N-terminal amino acid are shown in the following scheme:



It's the ideal method for sequencing peptides or proteins, which have been automated. So currently amino acid 'sequinators' can easily determine, in a day, the sequence of the first 50 or so amino acids in a peptide, starting at the N-terminal end.

4.3. Strategy of peptide synthesis

Many methods have been developed to link amino acids in a controlled manner. Most of them involve the protection of the amino group in the first and the carboxylic group of the second amino acid. For example, synthesis the dipeptide - Gly-Leu involves the following steps:

The first step – protection of amino group in Gly molecule

$$(H_3C)_3C–O–CO\text{-}Cl + HOOC – CH_2 – NH_2 \rightarrow$$

butoxycarbonulchloride (BOC) — Gly

$$HOOC\text{-}CH_2\text{-}NH\text{-}CO\text{-}O\text{-}C(CH_3)_3 + HCl$$

or in short **HOOC-CH$_2$-NH-*BOC***

The second step – the carboxylic group activation in Gly molecule

$$BOC\text{–}NH–CH_2–COOH + PCl_5 \rightarrow BOC\text{–}NH–CH_2–COCl + POCl_3 + HCl$$

The third step – the carboxylic group protection in Leu molecule

$$(CH_3)_2CH\text{-}CH_2\text{-}\underset{\displaystyle NH_2}{\underset{|}{CH}}\text{-}COOH + C_2H_5OH \xrightarrow[-H_2O]{H^+} (CH_3)_2CH\text{-}CH_2\text{-}\underset{\displaystyle NH_2}{\underset{|}{CH}}\text{-}\overset{\displaystyle O}{\overset{//}{C}}\backslash OC_2H_5$$

The fourth step – the formation of a peptide bond

$$BOC\text{–}NH–CH_2–\overset{\displaystyle O}{\overset{//}{C}}\backslash Cl + H_2N – \underset{\displaystyle CH_2CH(CH_3)_2}{\underset{|}{CH}} – COOC_2H_5 \rightarrow$$

$$BOC\text{–}NH–CH_2– CO – NH – \underset{\displaystyle CH_2CH(CH_3)_2}{\underset{|}{CH}} – COOC_2H_5$$

The fifth step – deprotection of amino- and carboxylic groups in Gly-Leu

$$(CH_3)_3C – O\text{-}CO – NH – CH_2 – CO – NH – \underset{\displaystyle CH_2CH(CH_3)_2}{\underset{|}{CH}} – COOC_2H_5$$

$$\downarrow HBr, CH_3COOH \qquad \downarrow H_2O, OH^-$$

$$(CH_3)_2C = CH_2 + CO_2 \qquad C_2H_5OH$$

isobutene

$$H_2N – CH_2 – CO – NH – \underset{\displaystyle CH_2CH(CH_3)_2}{\underset{|}{CH}} – COOH$$

Gly-Leu

4.4. Chemical properties of peptides and proteins

Proteins and peptides are polyelectrolytes which contain ionisable groups

- Acidic (-COOH, -SH and other);
- Basic ($-NH_2$)

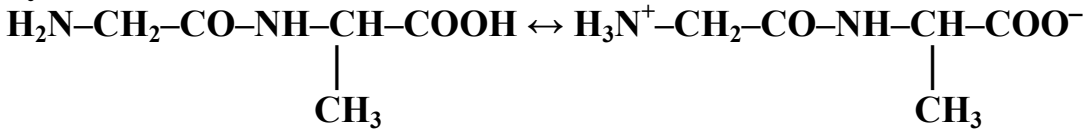
Polyelectrolytes containing both acidic and basic ionisable groups are known as amphoteric electrolytes. **Proteins exhibit their specific properties being in the isoelectric state, which is characterized by the zero net electric charge on macromolecules:**

$$NH_3^+ – R –COO^-$$

Isoelectric point (pI) is the pH at which the net charge on a macromolecule is zero. pI is an important characteristic for all peptides and proteins. The approximate pI values of peptides can be determined according to their chemical structure.

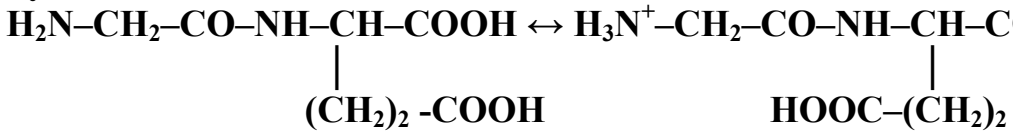
For example:

(a) Gly - Ala



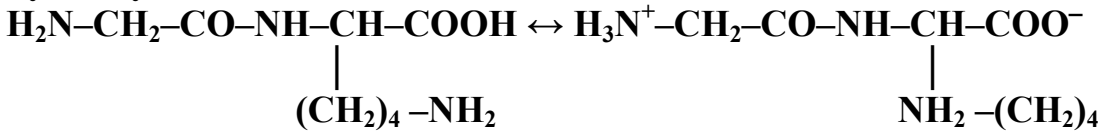
pI≈7

(b) Gly - Glu



pI<7, because a side carboxylic group ionization is suppressed in acidic medium

(d) Gly - Lys



pI>7, because a side amino group ionization is suppressed in basic medium.

Tests on proteins

The Biuretic Test on peptide bonds – proteins solutions turn violet when treated with a base and copper (II) sulfate solutions.

The Ninhydrin Reaction – proteins and α-amino acids solutions turn violet when treated with ninhydrin under heating.

The Xanthoprotenic Reaction – proteins solutions turn yellow when treated with concentrated nitric acid under heating. This reaction occurs due to tyrosine and tryptophan presence in proteins.

The Melon reaction – proteins solutions turn brick red when treated with a reagent consist from mercury (II) nitrate and mercury (II) nitrite. This reaction occurs due to tyrosine presence in proteins.

The Adamkevitch Reaction – a dark-violet ring in the interface appears when protein is treated with a mixture of concentrated acetic and glyoxilic acids. This reaction occurs due to and tryptophan presence in proteins.

The Phol Reaction – black colored lead salt precipitates from protein solution when it is treated with $(CH_3COO)_2Pb$ in basic medium. This reaction occurs due to sulfur containing amino acids contained in proteins.

4.5. Laboratory work

PEPTIDES AND PROTEINS

Experiment 1: The Biuretic Test on peptide bonds

Pour 5-6 drops of egg protein solution into a test tube and treat it with equal volume of 10% sodium hydroxide solution. After that add 1-2 drops of copper (II) sulfate solution to the prepared mixture. What can you see? Write an equation for the fulfilled reaction.

Experiment 2: The Xanthoprotenic Reaction

Pour 10 drops of egg protein solution into a test tube and treat it with 2 drops of concentrated nitric acid. Heat a prepared mixture. What can you see? Write an equation for the fulfilled reaction.

Experiment 3: The Phol Reaction

This reaction occurs due to sulfur containing amino acids contained in proteins. Pour 10 drops of egg protein solution into a test tube and treat it with 20 drops of 10% sodium hydroxide solution. Heat a prepared mixture up to the boiling point. Treat the solution with 5 drops of 10% lead (II) acetate solution. What can you see?

4.6. Exercises for the self-control

1. Write the structure for glycylglycine, and show the resonance contributors to the peptide bond. At which bond is rotation restricted?

2. Write out the complete structural formula for Phe – Tyr – Thr. Mark peptide bonds and point out N- and C-terminal ends. At approximately what pH will the isoelectric point come, and what is the structure of this tripeptide dipolar ion?

3. Write out an equation for alanine amino group protection by t-butoxycarbonyl chloride. Name the mechanism for this reaction.

4. Name the test on a peptide bond. Write out an equation for it. What is the color of an obtained salutation?

5. Write an equation for the hydrolysis of

(a) leucylserine,

(b) valyltyrosylmethionine.

CHAPTER 5

NUCLEIC ACIDS

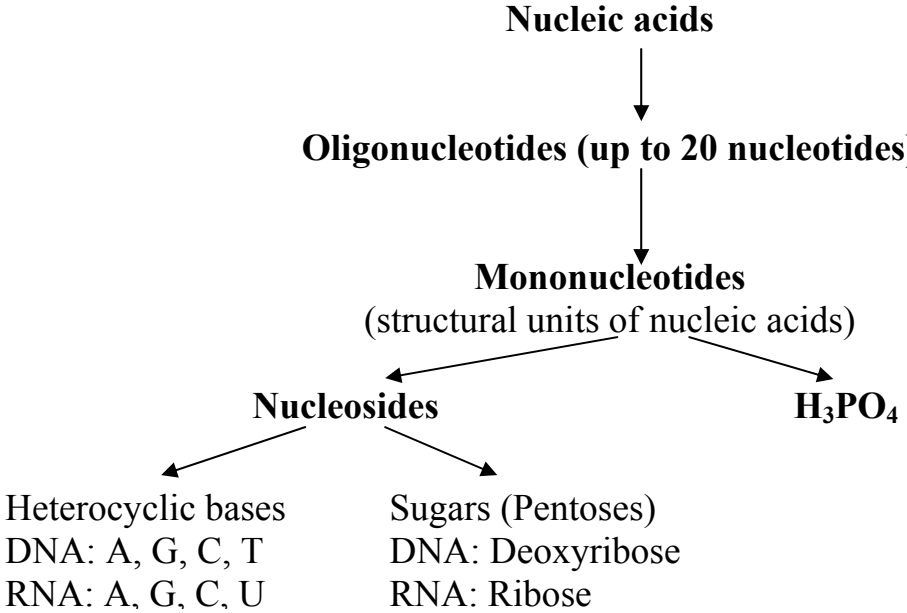
After reading this chapter, you should be able to:

- **Define general structure of nucleic acids;**
- **Describe structure and tautomerism of the pyrimidines and the purines;**
- **Discuss structure, nomenclature and properties of nucleosides and nucleotides;**
- **Explain the primary and secondary structure of DNA;**
- **Summarize structure, chemical properties and biological functions of ATP.**

5.1. General structure of nucleic acids.

Nucleic acids are biopolymers, which are responsible for storing and transmitting genetic information from one generation to another.

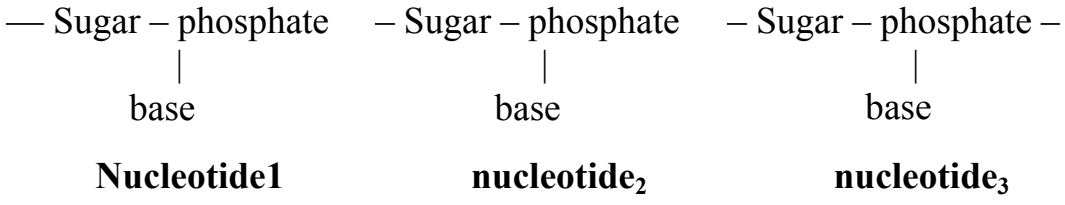
Hydrolysis of nucleic acids gives a complete description of their primary structure and structural units.



DNA and RNA differ in sugars and heterocyclic bases.

According to the obtained data **Nucleic acids** are defined as macromolecules built up from mononucleotides linked to form giant molecules.

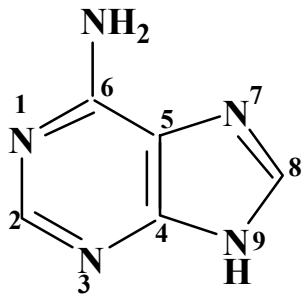
Schematic structure of nucleic acids may be represented by the following scheme:



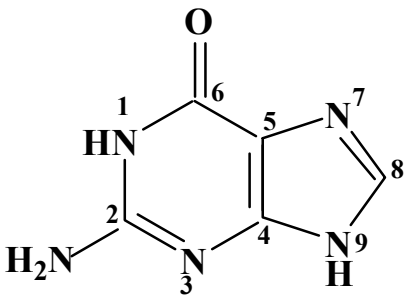
The heterocyclic bases fall into two categories:

- The purines (adenine – A; guanine – G).
- The pyrimidines (uracil – U; thymine – T; cytosine – C).

THE PURINES BASES

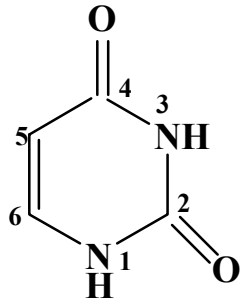


Adenine – A
6-aminopurine

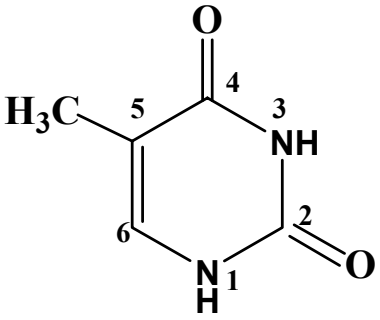


Guanine – G
2-amino -6-oxopurine

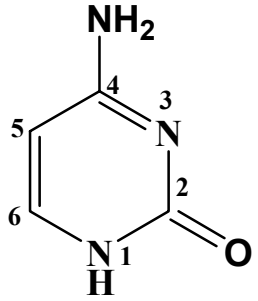
THE PYRIMIDINES BASES



Uracil – U
2,4-dioxopirimidine



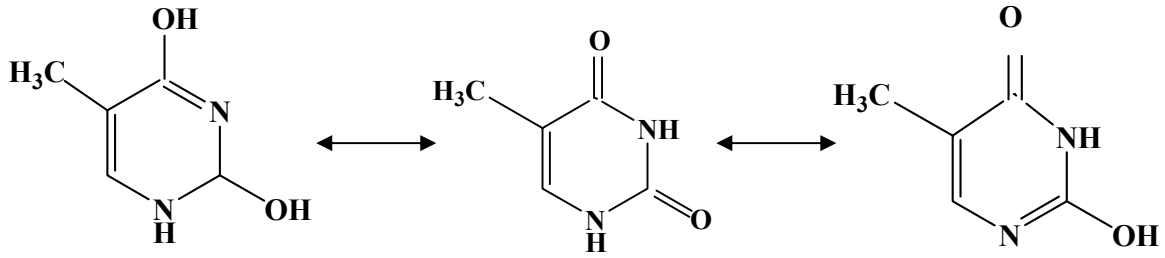
Thymine – T
5-methyl -2,4-dioxo-pirimidine



Cytosine – C
4-amino - - 2-oxopirimidine

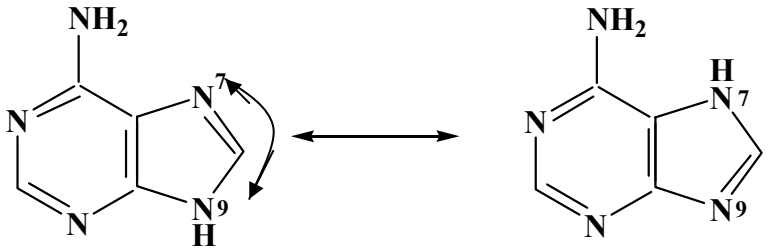
They all are aromatic systems, which can exist in lactam and lactime forms simultaneously (the phenomenon of lactam-lactime tautomerism). But only their lactam forms present in nucleic acids due to their higher stability.

LACTAM –LACTIME TAUTOMERISM

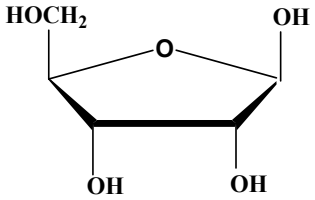


The purines exhibit prototropic tautomerism (the reversible proton migration from N-9 to N-7). But still NH -9 isomers are components of nucleic acids.

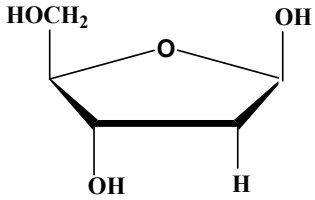
PROTOTROPIC TAUTOMERISM



Pentoses are involved in nucleic acids in their cyclic forms with β-configuration of anomeric carbon atom:



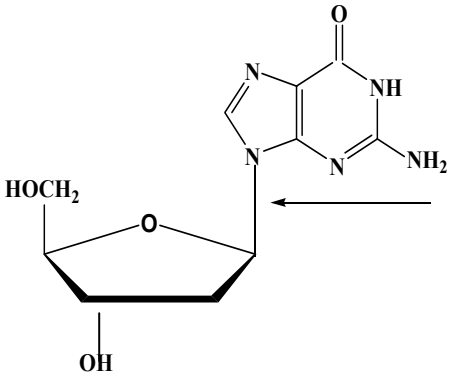
β-D-ribofuranose



β-D-dioxoribofuranose

5.2. Nucleosides

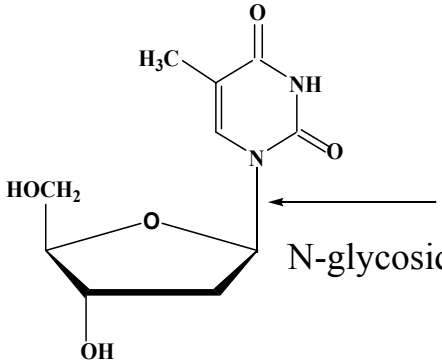
Nucleosides are N-glycosides, in which the pyrinidine or purine base is connected to the anomeric carbon (C'-1) of the sugar. The pyrimidines are connected at N-1 and the purines at N-9 N-glycosides have structures similar to those of O-glycosides. For example:



DEOXYGUANOSINE
9-β-D-DEOXYRIBOFURANOSYL GUANINE

N-glycosidic bond

Ending *osine* is typical for purine nucleosides.



THYMIDINE
1-β-D-DEOXYRIBOFURANOSYL THYMINE

N-glycosidic bond

Ending *idine* is typical for pyrinidine nucleosides.

Prefix deoxy is used for nucleosides, containing deoxoribose.

Nucleosides fall into two categories:

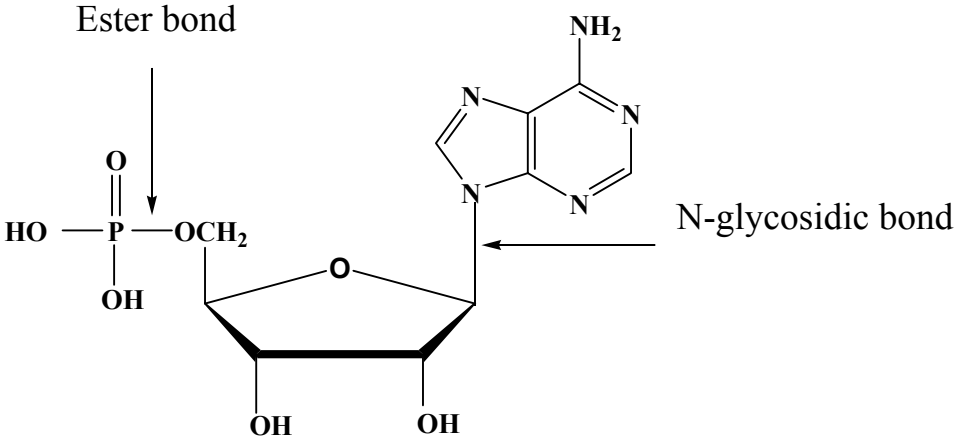
- purines;
- pyrimidines.

5.3. Mononucleotides

Nucleotides are phosphate esters of nucleosides. A hydroxyl group in the sugar part of a nucleoside is esterified with phosphoric acid either at 5' or the 3'-positions. Nucleotides are found primary as the monomeric units of nucleic acids, however, they also are required for numerous other important functions within the cell.

Nucleotides fall into two categories:

- ribonucleotides;
- deoxyribonucleotides.



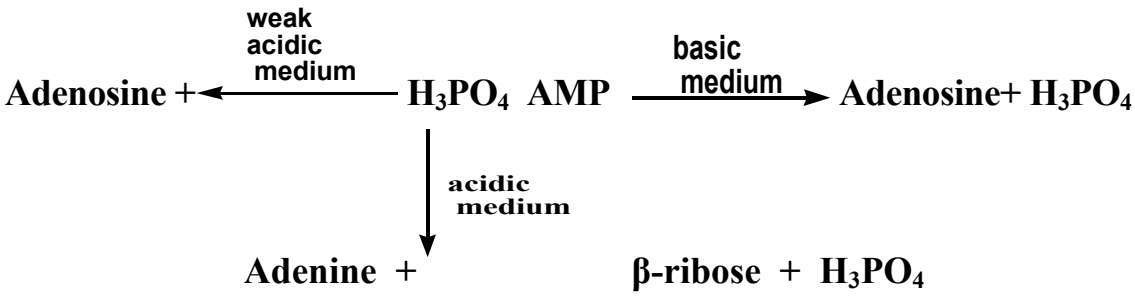
ADENOSINE -5′-MONOPHOSPHATE

AMP

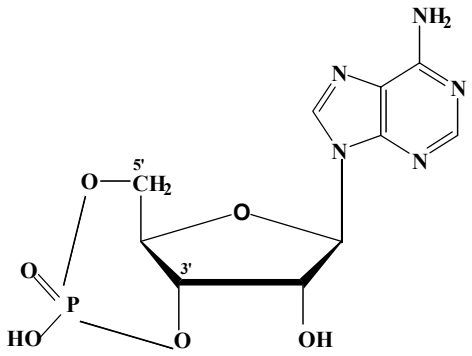
Mononucleotides are rather strong acids and exist as anions at physiological pH values (pH = 7.35).

Mononucleotides undergo partial hydrolysis in basic and weak acidic media and complete hydrolysis in strong acidic medium (pH = 1). They also may undergo enzymatic hydrolysis.

A SCHEME OF AMP HYDROLYSIS



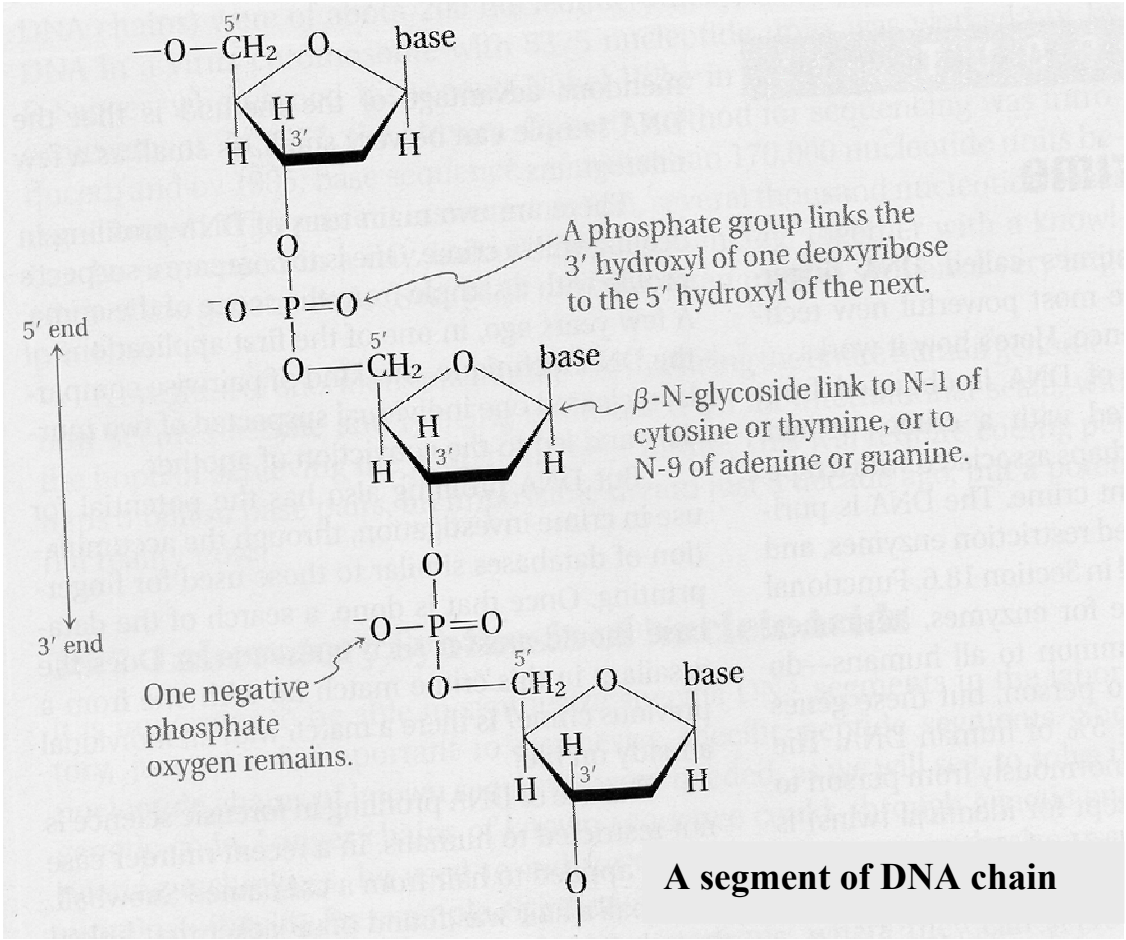
In cyclic mononucleotides both 5’ and 3’ hydroxyl groups are acylated with phosphoric acid. Such compounds serve as mediators of numerous important cellular processes such as second messengers in signal transduction events. The predominant second messenger is cyclic AMP (cAMP).



Adenosine -3‘-5‘-cyclic monophosphate -(c-AMP)

5.4. Primary and secondary structure of nucleic acids

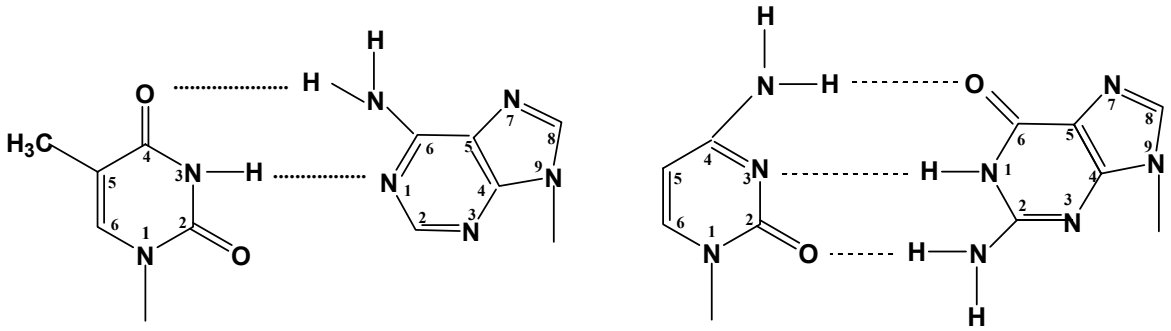
The primary structure of nucleic acids is the sequence of mononucleotide units linked through phosphodiester bonds. A phosphate group links the 3’ hydroxyl of the one pentose to the 5’ hydroxyl of the next.



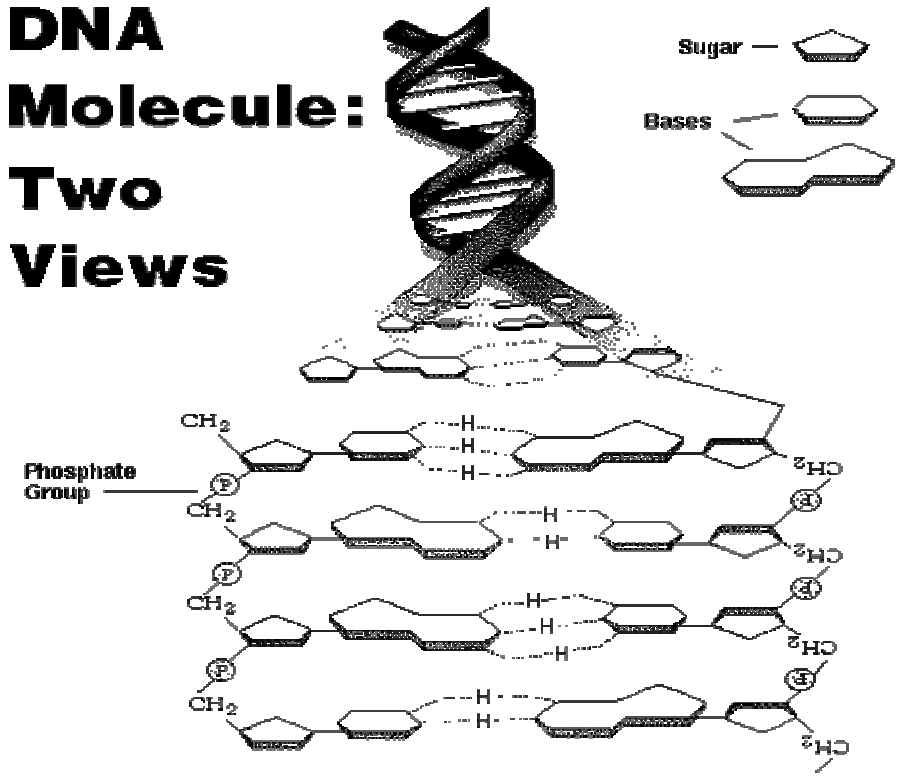
A segment of DNA chain

Watson and Crick proposed the model of secondary DNA structure in 1953. In the Watson-Crick model, the bases are in the interior of the helix aligned at a nearly 90 degree angle relative to the axis of the helix. Purine bases form hydrogen bonds with pyrimidines, in the crucial phenomenon of base pairing. This model predicted that DNA would exist as a helix of two complementary antiparallel strands, wound around each other in a rightward direction and stabilized by H-bonding between bases in adjacent strands.

The two chains are held together by purine-pyrimidine base pairs connected by hydrogen bonds. Adenine is always paired with thymine, and guanine is always pared with cytosine. The key feature if the structure is the complementarity of the base pairing.



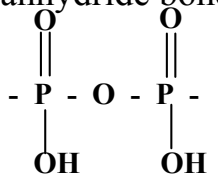
➢The diameter of the helix is 2 nm.



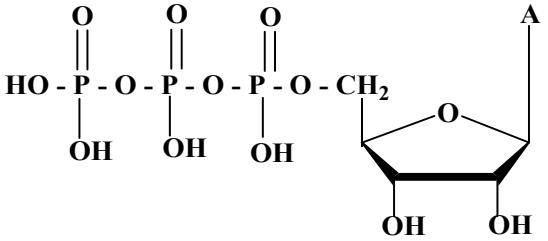
For their outstanding work in discovering the double helical structure of DNA, Watson and Crick were awarded the 1962 Nobel Prize for Physiology and Medicine.

5.5. ATP

Adenosine triphosphate (ATP) is a high – energy triphophate ester used in living systems to provide chemical energy for metabolic needs. ATP accumulates energy, which is released in the process of carbohydrates and lipids oxidation. It contains two macroergertic anhydride bonds:

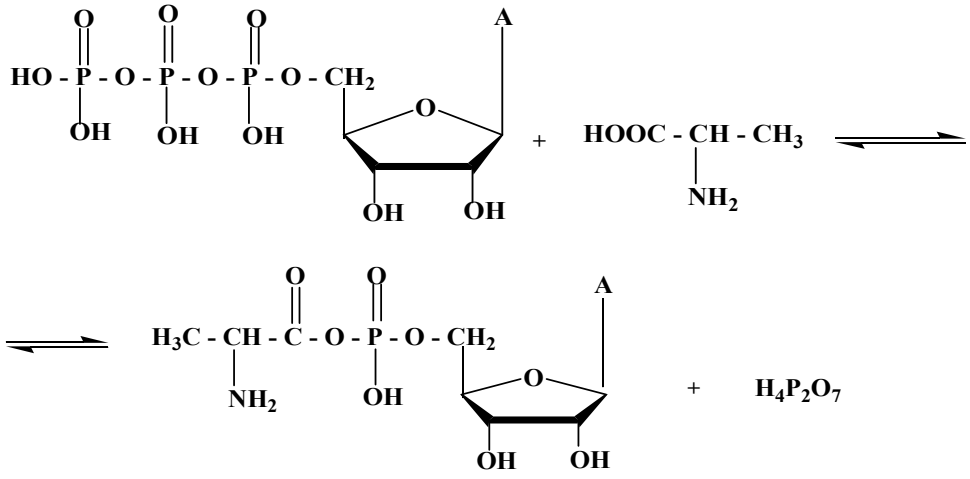


ATP accumulate energy which is released at the result of carbohydrates and lipids oxidation in vivo.



ATP contains two phosphoric anhydride bonds and considerable energy is released when ATP is hydrolyzed to ADP and further to AMP (32 kJ/mol).

ATP activates α-amino acids, which is prior to proteins' biosynthesis. This activation is the result of ATP interaction with amino acids.



Alanyladenilate **Diphosphoric acid**

5.6. Exercises for the self-control

1. Describe lactam-lactime and prototropic tautomerism of guanine.
2. Draw the structure of thymidine-5'-mononucleotide and write an equation for its hydrolysis in basic medium. Name the products.
3. Draw the structure for c-CMP.
4. Draw the full structure of the RNA trinucleotide A-U-G (written from 5' to 3'-end).
5. Write out an equation for ATP reaction with

(a) Serine, (b) methionine.

GLOSSARY

Amino group. The functional group — NH_2.

Aromaticity. The unusual stability of certain fully conjugated cyclic systems.

Bronsted-Lowry acid. A proton donor.

Bronsted-Lowry base. A proton acceptor.

Carbocyclic compounds. Compounds that contain rings of carbon atoms.

Carbohydrates. Originally, compounds such as aldoses and ketoses, having the stoichiometric formula $C_n(H_2O)_n$, hence "hydrates of carbon". The generic term carbohydrate includes monosaccharides, oligosaccharides and polysaccharides as well as substances derived from monosaccharides by reduction of the carbonyl group (alditols), by oxidation of one or more terminal groups to carboxylic acids, or by replacement of one or more hydroxy group(s) by a hydrogen atom, an amino group, thiol group or similar groups. It also includes derivatives of these compounds.

Chair conformation(s). The most stable conformation of a six-membered ring in which all bonds are staggered; the chair conformation of cyclohexane.

Conjugation. Multiple bonds that are separated by one single bond (C = C— C = C or C = C—C=O).

Epimers Diastereomers that differ in 1 configuration at only one stereogenic center.

Ester(s) Carboxylic acid derivatives in which the hydroxyl group is reply by an alkoxy (OR) group.

Fatty acids. Long-chain carboxylic acids obtained from saponification of fats and oils.

Fatty acids. Long-chain carboxylic acids obtained from saponification of fats and oils. **Stereogenic carbon atom** (stereogenic center) A carbon atom to which four different groups are attached.

Functional group is an atom or small group of atoms in a molecule that gives the molecule characteristic chemical properties.

Heterocyclic compounds. Cyclic compounds in which one or several carbon atoms are replaced by heteroatoms (O, S or N atoms).

Lipids (from *greek* ”lipos” – fat) are constituents of plants and animals that are characterized by their solubility properties.

Monosaccharides are polyhydroxyl aldehydes (aldoses) and polyhydroxy ketones (ketoses). According to the number of carbon atoms present they are: triose, tetrose, pentose, hexose. In nature pentoses and hexoses are most abundance.

Nucleic acids are defined as macromolecules built up from mononucleotides linked to form giant molecules.

Nucleosides are N-glycosides, in which the pyrinidine or purine base is connected to the anomeric carbon (C'-1) of the sugar.

Nucleotides are phosphate esters of nucleosides. A hydroxyl group in the sugar part of a nucleoside is esterified with phosphoric acid either at 5' or the 3'-positions.

Nucleotides are phosphate esters of nucleosides. A hydroxyl group in the sugar part of a nucleoside is esterified with phosphoric acid either at 5' or the 3'-positions. The primary structure of nucleic acids is the sequence of mononucleotide units linked through phosphodiester bonds.

Oligosaccharides are condensation products of two to ten monosaccharide units. **Disaccharides** are condensation products of two monosaccharide units. (maltose, lactose, sucrose)

Oxidation reaction(s). A reaction that increases the oxidation state of atoms in a molecule or ion. In organic chemistry this frequently involves reactions in which C—H bonds are replaced by C — O bonds. It is a net decrease in the number of bonds to hydrogen or electropositive elements, or a net increase in the number of bonds to electronegative elements. A net loss of electrons.

Peptides are natural or synthetic substances built up of α-amino acids residues which are linked by amide (peptide) bond. **The primary structure of proteins** is the sequence of amino acids residues in a completely assembled polypeptide chain.

Polyelectrolytes containing both acidic and basic ionisable groups are known as amphoteric electrolytes.

Polysaccharides are condensation products of more that ten monosaccharide units.

Racemic mixture. A 50:50 mixture of enantiomers.

Reduction. It is a net increase in the number of bonds to hydrogen or electropositive elements, or a net decrease in the number of bonds to electronegative elements. A net gain of electrons.

Saponification of lipids is hydrolysis, which occurs in basic media. and gives a mixture of salts of fatty acids and glycerol.

Soaps. The salts (usually sodium) of long-chain fatty acids.

Stereoisomers Isomers with the same attachment of atoms but different arrangements of atoms in space— for example, *cis-trans* isomers of alkenes or cycloalkanes, conforma-tional isomers of alkanes, a pair of enantiomers, or a pair of diastereomers.

Tautomerism The process of inter-conversion of tautomers, such as keto and enol forms of a carbonyl compound.

Tautomers Structural isomers that differ in the location of a proton and a double bond.

The Adamkevitch Reaction – a dark-violet ring in the interface appears when protein is treated with a mixture of concentrated acetic and glyoxilic acids. This reaction occurs due to and tryptophan presence in proteins.

The Biuretic Test on peptide bonds – proteins solutions turn violet when treated with a base and copper (II) sulfate solutions.

The Melon reaction – proteins solutions turn brick red when treated with a reagent consist from mercury (II) nitrate and mercury (II) nitrite. This reaction occurs due to tyrosine presence in proteins.

The Ninhydrin Reaction – proteins and α-amino acids solutions turn violet when treated with ninhydrin under heating.

The Phol Reaction – black colored lead salt precipitates from protein solution when it is treated with $(CH_3COO)_2Pb$ in basic medium. This reaction occurs due to sulfur containing amino acids contained in proteins.

The Xanthoprotenic Reaction – proteins solutions turn yellow when treated with concentrated nitric acid under heating. This reaction occurs due to tyrosine and tryptophan presence in proteins.

CONTENT