МІНІСТЕРСТВО ОХОРОНИ ЗДОРОВ'Я УКРАЇНИ ХАРКІВСЬКИЙ НАЦІОНАЛЬНИЙ МЕДИЧНИЙ УНІВЕРСИТЕТ

CARBOHYDRATES: MONOSACCHARIDES. OLIGO- AND POLYSACCHARIDES

Methodical instructions for 1st year students' self-work in Biological and Bioorganic Chemistry (module 1)

ВУГЛЕВОДИ: МОНОСАХАРИДИ. ОЛІГО- ТА ПОЛІСАХАРИДИ

Методичні вказівки для самостійної роботи студентів 1-го курсу з біологічної та біоорганічної хімії (модуль 1)

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Subject I. MONOSACCHARIDES

Motivational characteristic of subject

Monosaccharides are structural components of glycogen, heparin, and other important biopolymers. They are formed in the metabolism and participate in biological oxidation processes.

Objective

To study the stereochemical structure, tautomeric equilibrium and to be able to make qualitative reactions for the important monosaccharides.

Training questions

1. To know the classification of monosaccharides according to the number of carbon atoms and functional composition.

2. To know the classification of monosaccharides according to the cycle dimension and location of hemiacetal hydroxyl group.

3. To study the most stable conformations of hexoses.

4. To learn the properties associated with the presence of hemiacetal (glycoside) hydroxyl group in monosaccharide molecules.

5. To be able to illustrate properties of monosaccharides associated with the presence of carbonyl and hydroxyl groups (oxidation, reduction, formation of ethers, esters, hexoses epimerization in alkaline medium).

Methodological questions

1. Heterofunctionality is one of the most important features of monosaccharides which are involved in life processes.

2. The interaction of part and whole in the molecules of monosaccharides as heterofunctional compounds.

Initial level

1. Chemical properties of the hydroxyl and aldehyde groups. The mechanism of formation of hemiacetals and acetals.

2. The conformation of cyclohexane.

3. The concept of tautomerism.

Practical skills

1. To be able to predict the properties of important monosaccharides.

2. To be able to carry out the reactions for monosaccharides.

Test questions

1. What biologically important products can be obtained by the oxidation of glucose under different conditions?

2. Write the reaction of β -D-galactopyranose (Haworth formula) with ethanol. Name the product.

3. Write the schemes and explain the mechanism of formation of cyclic forms of ribose and fructose.

Teaching tasks

Task №1. What isomers are characteristic for monosaccharides? What is the difference between optical isomers?

Solution. Pentoses and hexoses are the most common in nature. Usually they have unbranched chains, so quantity of the isomers is expected to be quite small. However, monosaccharides contain several asymmetric carbon atoms and therefore they show optical isomerism. Thus, aldohexoses have four asymmetric carbon atoms and 16 optical antipodes. They are divided into eight pairs of D-and L-hexoses which differ in the arrangement of substituents around asymmetric carbon atoms and have the same properties, except for sign of rotation.



Isomers which have different arrangement of hydroxyl groups at some chiral centers have not only different optical activity but also differ in some physical and chemical properties and named differently (glucose, galactose, mannose, etc.).

The aldehyde and keto-formulas clearly explain the reactions of these substances. However, there are some properties which can not be explained by the structure of these formulas. Aldoses do not give some characteristic aldehyde reactions. Glucose, e.g., under normal conditions does not produce coloration with Schiff's reagent. At the same time, some reactions (formation of glycosides) can not be explained by oxo forms.

Task №2. What is the essence of tautomeric transformations of monosaccharides, what is their influence on the chemical properties?

Solution. Aldehyde or keto structure is one of tautomeric forms of monosaccharides and most of saccharides exist as cyclic tautomeric forms which are internal hemiacetals of polyhydric aldehydes and ketones.

Tautomeric transition of aldehyde or ketone form into cyclic one is the formation of internal hemiacetal due to displacement of hydrogen atom from hydroxyl group at the fifth or fourth carbon atom to a carbonyl group. As a result the carbon atoms become connected through oxygen with the formation of six- or five-membered ring:



The formation of the cyclic form is accompanied by formation of new hydroxyl group which is absent in the open-chain form. It is called hemiacetal hydroxyl group. Its properties differ from the other hydroxyl groups because of two reasons. First of all, it is in equilibrium with the carbonyl group and second of all, it is bound with carbon connected to oxygen of the cycle which attracts electronic density. Carbon atom which is linked to a hemiacetal hydroxyl group is asymmetric, thus the existence of two cyclic isomers is possible. They are named as $-\alpha$ and $-\beta$ anomers - the forms in which the location of hemiacetal hydroxyl group is the same as the location of hydroxyl at the asymmetric carbon atom which defines belonging of the saccharide to D- and L-series.

English chemist Haworth offered to represent cyclic forms of monosaccharides in the form of regular polygons that lie perpendicular to the plane of the figure:



I Cyclic monosaccharides can exist in the same conformations as other cycles, which consist of tetragonal carbon atoms, e.g., chair conformation.



Task №3. What biologically important products can be obtained by oxidation and reduction of glucose?

Solution. The presence of different functional groups in monosaccharides, as well as their ability to tautomeric transformations results in high reactivity and variety of chemical properties of monosaccharides. They easily enter oxidation reactions. Products depend on the strength of the oxidizing agents and the pH.

Glucose on reaction with bromine water in neutral medium is oxidized into hydroxy acid with same number of carbon atoms:



Stronger oxidizing agents (e.g., concentrated nitric acid) oxidize not only aldehyde group but also the primary alcohol group into carboxyl groups. The products are dibasic hydroxy acids:



The most pronounced transformations of monosaccharides take place in alkaline medium. Interaction with the ammoniacal silver nitrate or copper(II) hydroxide results in breaking of the carbon chain with formation of mixture of different hydroxy acids.

Reduction with hydrogen in the presence of metallic nickel converts monosaccharides into polyhydric alcohols:



Glucose is reduced into hexatomic alcohol sorbitol, which is used as a sugar substitute for diabetics.

Task №4. Write the reaction of monosaccharides with phosphoric acid.

Solution. The alcoholic hydroxyls of glucose participate in the formation of ethers with alcohols and esters with acids. Phosphoric acid esters of monosaccharides are of great biological importance.



Phosphate formation is the first step of the biochemical transformations of monosaccharides.

Task №5. What transformations of monosaccharides occur in alkaline medium?

Solution. In alkaline medium monosaccharides undergo isomerization via the formation of the enol form:



D-glucose Endiol form of D-fructose

Isomerization of monosaccharides and their derivatives takes place in important biochemical transformations.

Task №6. What transformations of monosaccharides take place under the action of microorganisms?

Solution. Conversion of monosaccharides by the action of microorganisms is called fermentation. Depending on the process conditions and the type of microorganisms there are several types of fermentation:

$$C_{6}H_{12}O_{6} \xrightarrow{\bullet} 2CH_{3}-CHOH-COOH \qquad \text{lactic} \\ CH_{3} - (CH_{2})_{2} - COOH+2CO_{2}+2H_{2} \qquad \text{butyric}$$

The highest reactivity of the hemiacetal hydroxyl group as compared to other hydroxyl groups allows, under certain conditions (boiling of glucose in alcohol solution containing 2% of dry hydrochloric acid), to obtain acetals of saccharides – glycosides.



Some natural glycosides serve as medicines, e.g., cardiac glycosides.

Task №7. What is the biological role of monosaccharides?

Solution. Ribose and deoxyribose are of great biological importance. They are the components of ribonucleic and deoxyribonucleic acids.

The most common aldohexose is glucose. It is also known as grape sugar, as it is contained in the grape juice. Glucose is also contained in other sweet fruits. There are about 0.1% of glucose in blood.

Fructose is the representative of ketohexoses. This one is the sweetest of all carbohydrates. In large quantity it is found in honey.

Task №8. Give the structure of the amino sugars. Characterize their properties.

Solution. Derivatives of monosaccharides obtained by substitution of alcoholic hydroxyl with amino group are called amino saccharides. They possess strong reducing properties. Residues of amino saccharides enter the composition of certain polysaccharides.





2-deoxy-2-amino-α-D-galactopyranose (galactosamine) 2-deoxy-2-amino-α-D-glucopyranose (glucosamine)

Revision exercises

№ 1

1. Write the formula of α -D-glucopyranose (Haworth formula). Specify atom which determines its belonging to the D-series.

2. Write the reaction of ethylamine with β -D-deoxyribofuranose. What is the type of glycosides? Name it. Write the hydrolysis reaction.

3. Write the reduction reaction of alkaline copper(II) hydroxide solution with glucose. What is the use of this reaction?

№ 2

1. Write the formula of β -D-glucopyranose and its 6-phosphate (Haworth formulas). Specify atom which determines its belonging to the D-series.

2. Write the hydrolysis reaction of methyl- β -D-2,3,4,6-tetramethylgalactopyranoside. Name the product. What is the pH for hydrolysis?

3. What property of glucose is the basis of its interaction with Fehling's reagent? Specify the composition of the reagent.

<u>№</u> 3

1. Write the formula of α -D-galactopyranose (Haworth formula). Specify atom which determines its belonging to the D-series.

2. Write the reaction of β -D-ribofuranose (Haworth formula) with methanol. Specify the conditions of the reaction. Name the compound obtained.

3. Write the reaction of glucaric acid formation from D-glucose. Specify the conditions.

<u>№</u> 4

1. Write the formula of β -D-galactopyranose (Haworth formula). Specify atom which determines its belonging to the D-series.

2. Write the hydrolysis reaction of methyl- β -D-1,3,4,6-tetramethylfructofuranoside (Haworth formula). Name the compound obtained.

3. Which properties of glucose are shown in "silver mirror" test?

<u>№</u> 5

1. Write open-chain formulae of D-mannose and D-xylose. Specify atom which determines its belonging to the D-series.

2. Write the reaction of β -D-fructofuranose (Haworth formula) with ethanol. Name the compound obtained. Write its hydrolysis.

3. Which reaction can prove the existence of several hydroxyl groups in hexose?

<u>№</u> 6

1. Show phenomenon of oxo-oxy tautomerism on example of D-ribose (furanose cycle). Which biopolymers contain D-ribose?

2. Write the hydrolysis reaction of completely methylated sucrose (Haworth formula). Does the product have reducing properties?

3. Which polysaccharides are called homopolysaccharides? Name the monosaccharide units of dextran and specify the nature of bonds between them. Give structure of the fragment of the main chain.

<u>№</u> 7

1. Write the formula of β -D-ribofuranose (Haworth formula). Specify atom which determines its belonging to the D-series.

2. Write the reaction of β -D-glucopyranose (Haworth formula) with excess of acetic acid anhydride. Name the compound obtained. Write its hydrolysis.

3. Write the reaction of D-glucuronic acid decarboxylation. Name the product obtained.

<u>№</u> 8

1. Write the formula of β -D-deoxyribofuranose (Haworth formula). Specify atom which determines its belonging to the D-series.

2. Give the reaction equation of α -D-glucopyranose (Haworth formula) with excess of dimethylsulfate. Name the compound obtained. Write its hydrolysis.

3. Write the reaction of oxidation of D-galactose into D-galactonic acid. Specify the conditions.

<u>№</u> 9

1. Write the formula of β -D-deoxyribofuranose (Haworth formula). Specify atom which determines its belonging to the D-series.

2. Write the reaction of β -D-galactopyranose (Haworth formula) with ethanol in the presence of HCl. Name the compound obtained. Write its hydrolysis.

3. Give an equation for the formation of D-gluconic acid from D-glucose. What is the oxidizing agent?

№ 10

1. Write open-chain formulae of D-ribulose and D-xylulose. Specify atom which determines its belonging to the D-series.

2. Write the reaction of β -D-ribofuranose with ethylamine. Define the type of glycosides. Write its hydrolysis.

3. What property of glucose is shown in Trommer reaction?

№ 11

1. Write the formula of β -D-fructofuranose (Haworth formula). Specify atom which determines its belonging to the D-series.

2. Write the reaction equation of β -D-glucopyranose (Haworth formula) with methyl iodide. Name the product obtained. Write its hydrolysis.

3. Write the reaction of glucose reduction with ammoniacal silver oxide solution. What is the use of this reaction?

№ 12

1. Write the formula of α -D-fructofuranose (Haworth formula). Specify atom which determines its belonging to the D-series.

2. Give reaction equation for the formation of β -D-glucopyranoside (Haworth formula). Specify the conditions. Write the hydrolysis of the compound.

3. Write reaction equation of glucose with Fehling's reagent.

№ 13

1. Write the formula of 1,6-diphosphate- β -D-fructofuranose in the open-chain form and in Haworth formula. Specify atom which determines its belonging to the D-series.

2. Give the equation of the reaction of β -D-glucopyranose with ethanol in dry HCl. Name the product. Write the hydrolysis of reaction.

3. How to obtain pentoses from hexuronic acids?

№ 14

1. Write 2- α -D and 2- β -D-glucosamine in pyranose ring as Haworth formulas. Specify atom which determines its belonging to the D-series.

2. Write the hydrolysis reaction of penta-acetyl- β -D-galactopyranose. Name the product.

3. What reaction can show reducing properties of glucose?

№ 15

1. Write 2- α -D and 2- β -D-galactosamine in pyranose ring as Haworth formulas. Specify atom which determines its belonging to the D-series.

2. Write the reaction of β -D-glucopyranose (Haworth formula) with methanol. Name the product.

3. What reaction can prove the presence of some hydroxyl groups in glucose?

Subject II. OLIGO- and POLYSACCHARIDES

Motivational characteristic of subject

Polysaccharides are important biopolymers. They are found in many organs, tissues and systems of the organism.

Objective

To study the principles of structure and main chemical reactions of essential homoand heteropolysaccharides in relation to their biological functions.

Training questions

1. To know the monosaccharide composition, structure, and conformation of the most important disaccharides (maltose, lactose, cellobiose, sucrose).

2. To study the fractional composition of starch, linear and spatial structure of amylose and amylopectin, and glycogen.

3. To study the main chemical properties of starch: hydrolysis, inclusion compounds.

4. To know the composition and structure of the main heteropolysaccharides: hyaluronic acid, heparin, chondroitin sulfate.

Methodological questions

Structure and properties of polysaccharides illustrate the fundamental laws of dialectics.

Initial level

1. Tautomerism of monosaccharides. Glycosides.

2. Conformations of aldohexoses.

3. Reducing properties of aldohexoses.

Practical skills

- 1. To be able to predict the properties of the polysaccharides.
- 2. To be able to carry out qualitative reactions to polysaccharides.

Test questions

1. Illustrate the phenomenon oxo-oxy tautomerism on example of disaccharide lactose (Haworth formula).

2. Write the fragments of amylose and cellulose, which consist of D-glucose residues and explain the differences between their structures.

3. What are the monosaccharides in glycogen? Write the fragment structure of the glycogen molecule. Specify the type of linkage between the monosaccharide units.

Teaching tasks

Task № 1. What are oligosaccharides? Give examples. Characterize bonds between the monosaccharide units and chemical properties.

Solution. All polysaccharides can be regarded as anhydrides of simple sugars which are obtained by removal of one or more molecules of water from two and more monosaccharides molecules. Polysaccharides are divided into two subgroups: oligosaccharides - relatively low-molecular weight carbohydrates, which give a small number of molecules of monosaccharides on hydrolysis (name is derived from the Greek "oligos" - few) and high-molecular weight polysaccharides which are composed of hundreds and thousands of monosaccharide residues.

Oligosaccharides exhibit a number of properties which are similar to those of simple carbohydrates: they are highly soluble in water and have a sweet taste.

Disaccharides are the most important. They all are glycosides, that is, a molecule of water is released from two molecules of monosaccharides with obligatory participation of hemiacetal (glycosidic) hydroxyl group. Hydroxyl group of the second molecule which participates in the formation of a glycosidic bond may be either alcoholic or hemiacetal one. In the first case the disaccharide molecule has one hemiacetal hydroxyl, and therefore

such compounds have similar properties with monosaccharides, i.e. they can be easily oxidized, so they are known as reducing disaccharides.

If the disaccharide molecule is formed involving hemiacetal hydroxyls of both monosaccharides they are known as non-reducing disaccharides.

The reducing disaccharides are able to tautomerism due to the presence of hemiacetal hydroxyl group in the molecule. Therefore, they enter chemical reactions that are characteristic for monosaccharides (oxidation with silver oxide, osazones formation, etc.). The most important reducing disaccharides are maltose (malt sugar) and lactose (milk sugar).



 α -maltose

 α -D-glucopyranose α -D-glucopyranose

In maltose molecule two residues of α -D-glucose are linked by 1,4-glycosidic bond; lactose consists of residues of D-galactose and D-glucose in β -form.

Sucrose is an example of non-reducing sugars. It consists of residues of D-glucose and D-fructose. Sucrose has no free glycosidic hydroxyl, so it can not be transformed into the open carbonyl form and therefore does not reduce silver oxide and copper(II) hydroxide. Like all polysaccharides, sucrose is capable of hydrolyzing and exhibits the properties of polyols.

Task № 2. Which compounds are called homopolysaccharides?

Solution. Polysaccharides are high-molecular weight substances, which are composed of hundreds or thousands of monosaccharide residues. These residues may be identical (homopolysaccharides) or different (heteropolysaccharides). Starch and cellulose are the main homopolysaccharides. These polysaccharides are hydrolyzed into glucose.

The composition of both compounds is expressed as $(C_6H_{10}O_5)n$. Glucose residues are joined by glycosidic linkages in the long chains, wherein one molecule provides its hemiacetal hydroxyl and second molecule provides its alcoholic hydroxyl group:



Cellulose and starch are practically deprived of reducing properties, as they have only one hemiacetal hydroxyl group per several hundreds or even thousands of glucose residues.

Task № 3. What are the products of partial and complete hydrolysis of starch?

Solution. At rapid heating starch breaks into simpler molecules which are called dextrins with the same molecular formula as that of the starch, but with a smaller number of units. Further refluxing with mineral acids gives subsequent cleavage with formation of maltose initially and then glucose. Thus, a simplified starch hydrolysis can be represented as follows:

 $(C_6H_{10}O_5)_n \rightarrow (C_6H_{10}O_5)_x \rightarrow (C_{12}H_{22}O_{11}) \rightarrow C_6H_{12}O_6$ Starch dextrins maltose glucose

Task № 4. Give the structures of the vegetable and animal starch.

Solution. Analysis of the structure of starch showed that it is not a unified substance, but it is a mixture of the two fractions - amylose and amylopectin. In most plants starch is comprised of 15-20% of amylose and 80-85% of amylopectin. Amylose molecule is constructed from α -D-glucose units linked by 1,4-glycosidic bonds.

Amylose molecule contains from 200 to 1000 glucose units and has a spatial structure, forming a spiral coil which consists of six glucose residues. This spatial

structure is a consequence of the axial location of hemiacetal hydroxyl group in glucose. Inside spiral amylose molecule there is a channel with a diameter of about 5 nm. This channel can include molecules of suitable size, forming a special kind of complexes, the so-called inclusion compounds. Complex of amylose with iodine can serve as example of such compounds. It is blue in color, and its formation is used for the qualitative determination of starch.

Amylopectin is branched polysaccharide with a molecular weight of over one million (polymerization degree is 6000). Amylopectin, as well as amylose, consists of α -D-glucose, but its molecule has more than 30 branches due to 1,6-glycosidic linkages. Due to branched structure amylopectin has spherical shape.



amylopectin

Animal starch - glycogen is contained in all animal organisms. Its molecules are very similar to those of amylopectin, but glycogen molecules are much more branched. Glycogen is well soluble even in cold water. Glycogen serves as storage carbohydrate in the organism. Its content in liver is about 20% and about 4% in muscles. All the processes of life, primarily the work of the muscles, are accompanied by the breakdown of glycogen, this process leads to release of energy.

Task № 5. Give the composition and structure of cellulose.

Solution. Cellulose is the most abundant naturally occurring polysaccharide. It forms walls of the plant cells, gives the mechanical strength and elasticity to the plant tissues. The average molecular weight of the cellulose of plant origin is in the range of 100,000 to 1,000,000. Cellulose consists of residues of β -D-glucose linked by 1,4-glycosidic bonds and has a linear structure:



Task № 6. What are heteropolysaccharides? What are their constituent parts?

Solution. Heteropolysaccharides are polysaccharides built from the residues of different monosaccharides. They are widely distributed in nature in the form of complexes with proteins. Hyaluronic acid is contained in connective tissue, vitreous body of the eye, synovial fluid. On hydrolysis it produces glucosamine, glucuronic acid and acetic acid. The structural components of hyaluronic acid are glucuronic acid and N-acetylglucosamine linked by 1,3-glycosidic bonds:



β-1,3-glycosidic bond

Heteropolysaccharide - heparin is in the organs and tissues of animals and humans. Especially there are a lot of heparine in the liver, lungs, heart, and skeletal muscles. It was isolated in a crystalline state, and is widely used as substance for preventing blood coagulation (anticoagulant). Heparin molecule includes glucuronic acid, L-iduronic acid, and partially sulfated glucosamine:



heparin

Revision exercises

<u>№</u> 1

1. Give the mechanism of the reaction of β -D-galactopyranose (Haworth formula) with ethanol. Name the product.

2. What are the monosaccharide units in cellulose? Write a fragment of cellulose structure and specify the linkage between the monosaccharides.

<u>№</u> 2

1. Write formulae of enantiomers of fructose. How many chiral centers are in this molecule?

2. Write the hydrolysis of sucrose. Name the product. Specify the glycosidic bond in sucrose molecule.

<u>№</u> 3

1. Show phenomenon of oxo-oxy tautomerism on example of D-galactose. Which disaccharide contains galactose?

2. What are mucopolysaccharides? Write the structure of the components of chondroitin-6-sulfate and specify the nature of the linkage between them.

1. Write reaction of methyl- β -glucopyranoside formation. Write its hydrolysis reaction equation.

2. Show the structure of chondroitin-4-sulfate. Name monomers.

<u>№</u> 5

1. What happens to α -D-ribose in an alkaline medium? Write the reaction.

2. Write the reaction equation of glycoside formation from disaccharide maltose and ethanol.

<u>№</u> 6

1. Write the reaction of β -D-glucopyranose (Haworth formula) with methyl iodide. Name the product. Give the reaction of hydrolysis.

2. Name fractions of vegetable starch. How do they differ in structure?

№ 7

1. What monosaccharides are called epimers? Write the epimerization of D-glucose.

2. Write formula of disaccharide consisting of D-glucuronic acid and N-acetylglucosamine linked by 1,3-glycosidic bond. Which biopolymer is composed of such disaccharides?

<u>No</u> 8

1. Which reaction can prove the presence of multiple hydroxyl groups in glucose molecule? Write equation for this reaction.

2. Give the structure of amylopectin. Which products are obtained by its hydrolysis?

<u>№</u> 9

1. Give the mechanism of the reaction of β -D-fructofuranose with ethanol. What is the reaction product?

2. Give the structure of sucrose and explain the reason for the absence of reducing properties.

№ 10

1. Write the reaction of formation of 1,6-diphosphate of β -D-fructose from the corresponding monosaccharide and phosphoric acid.

2. What are the monosaccharide units of glycogen? Write the structure of the glycogen molecule and show the chain branching.

№ 11

1. Show phenomenon of oxo-oxy tautomerism on example of fructose. Which disaccharide contains fructose?

2. Write the hydrolysis of lactose (milk sugar). Name the products and bond in the molecule of lactose.

№ 12

1. Write the Haworth formula of α -D-galactopyranose and its reaction with methanol. Give the mechanism of the reaction.

2. Write the hydrolysis of sucrose. Name the resulting monosaccharides. Does sucrose possess reducing properties?

№ 13

1. Show phenomenon of oxo-oxy tautomerism on example of galactose. Which disaccharide contains galactose?

2. Write the hydrolysis of maltose (malt sugar). Name the products and bond in the molecule of maltose.

№ 14

1. Write formulae of enantiomers of galactose. How many chiral centers are in this molecule?

2. Write the hydrolysis of cellobiose. Name the products and bond in this molecule.

№ 15

1. What monosaccharides are called epimers? Write the epimerization of D-glucose.

2. Write formula of disaccharide consisting of D-glucuronic acid and N-acetylglucosamine linked by 1,3-glycosidic bond. Which biopolymer is composed of such disaccharides?

SUGGESTED READINGS

1. Biologically important classes of bioorganic compounds. Biopolymers and their structural components: Theoretical course of biological and bioorganic chemistry, Module 1 / A.O. Syrovaya, E.R. Grabovetskaya, N.M. Tkachuk et al. – Kharkov: KhNMU. – 2013.– 183 p.

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Навчальне видання

Вуглеводи: Моносахариди. Оліго- та полісахариди

Методичні вказівки для самостійної роботи студентів 1-го курсу з біологічної та біоорганічної хімії (Модуль 1)

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