Biochemistry

Unit 1 · Lesson 4 · Sugar reactions and glycosylation

The notes for Lesson 4 of Unit 1: chapters 1.8 to 1.9 of the unit book, with their figures, worked examples, exam traps and quizzes, in one file. The full unit book, with every chapter, the exam question bank and the glossary, is linked at the end.

Lesson 4 of 4 | 2 chapters | Chapters 1.8 to 1.9

About these notes

These are the notes for **Lesson 4 · Sugar reactions and glycosylation** of the University free n easy video lessons on Biochemistry Unit 1, Foundations: Water, pH and Carbohydrates. They are chapters 1.8 to 1.9 of the unit book, unchanged, so a page here is the same page in the full book.

1.8 Properties and reactions of sugars; reducing sugars

Sugars react through two kinds of group: the carbonyl group, available whenever the ring opens, and the many –OH groups. The carbonyl can be oxidised, reduced, moved along the chain or condensed with nitrogen compounds; the –OH groups form esters, ethers and glycosides; hot acid strips water off to give coloured products. A sugar that can reduce a mild oxidising agent such as Cu^{2+} is a reducing sugar, and that one property underlies most lab and clinical sugar tests.

You need: open chain and ring forms (Chapter 1.4); free and locked anomeric carbons (Chapter 1.5).

Physical and biological properties

Mono- and disaccharides are white, crystalline, sweet and very soluble in water, because each –OH hydrogen bonds to water; they do not dissolve in non-polar solvents. Every sugar with a chiral carbon is optically active and, if its anomeric carbon is free, shows mutarotation. Sweetness varies: fructose is the sweetest common sugar and lactose only mildly sweet. Polysaccharides are tasteless and either insoluble (cellulose) or form colloids (starch). The biological roles of sugars were set out in Chapter 1.3; this chapter is about their chemistry.

Reducing sugars

A **reducing sugar** has a free anomeric carbon, so its ring can open to a free aldehyde (or to a ketone that can rearrange into one). In hot alkaline solution the aldehyde reduces Cu^{2+} to Cu^{+}, which precipitates as red Cu_2O, or Ag^{+} to metallic silver, while the sugar is oxidised to a mixture of acids. All monosaccharides are reducing, including the ketose fructose, which in hot alkali isomerises through an enediol into aldoses. Maltose, lactose and cellobiose are reducing; sucrose and trehalose are not. The end of a chain that carries the free anomeric carbon is its reducing end.

Chemical reactions

Figure 1.8.1 maps them around glucose.

- **Oxidation.** A mild oxidant (bromine water) attacks only the aldehyde, giving an **aldonic acid** (glucose → gluconic acid); ketoses are not attacked, so bromine water separates aldoses from ketoses. A strong oxidant (hot dilute nitric acid) oxidises both ends to an **aldaric acid** (glucose → glucaric acid); galactose gives mucic acid, which is insoluble and crystallises out. Enzymes can oxidise C6 alone, giving a **uronic acid**; the liver attaches glucuronic acid to bilirubin and many drugs to make them water-soluble for excretion. Glucose oxidase turns glucose into gluconolactone and H_2O_2, the reaction in most glucometer strips.
- **Reduction.** $NaBH_4$, or H_2 with a nickel catalyst, reduces the carbonyl to an alcohol, giving an **alditol**: glucose → sorbitol, mannose → mannitol, galactose → galactitol, xylose → xylitol. Fructose gives both sorbitol and mannitol, because reduction makes C2 a new chiral centre. In the lens of the eye, excess glucose is reduced to sorbitol, which cannot leave the cells and draws in water, one cause of diabetic cataract; galactitol does the same in galactosaemia.
- **Isomerisation in alkali.** Dilute alkali converts glucose into an equilibrium mixture with fructose and mannose through a shared 1,2-enediol (the Lobry de Bruyn–van Ekenstein transformation).
- **Dehydration.** Hot concentrated acid removes three molecules of water: pentoses give furfural and hexoses give 5-hydroxymethylfurfural (HMF). These condense with phenols to give coloured products, the basis of the Molisch, Seliwanoff and Bial tests.
- **Osazone formation.** Boiled with excess phenylhydrazine (three molecules per sugar), C1 and C2 both react to give a yellow crystalline osazone. Since C2 loses its chirality, glucose, fructose and mannose give the same osazone; crystal shape still tells sugars apart (needle-like sheaves for glucosazone, sunflower-like for maltosazone, powder-puff for lactosazone).
- **Esters.** Phosphate esters such as glucose 6-phosphate start glycolysis (Unit 5); acetic anhydride converts glucose into a pentaacetate, evidence that it has five –OH groups. Glycoside formation is Chapter 1.5.
- **Glycation (Maillard reaction).** Without any enzyme, the open-chain aldehyde of a reducing sugar condenses with a free $–NH_2$ of a protein to form a Schiff base, which rearranges to a stable Amadori product. In cooking, this chemistry browns toast, coffee and fried onions. In

blood, glucose glycates haemoglobin: the percentage of glycated haemoglobin (HbA1c) reflects average blood glucose over the 2 to 3 months a red cell lives, and 6.5% or more is a diagnostic threshold for diabetes.

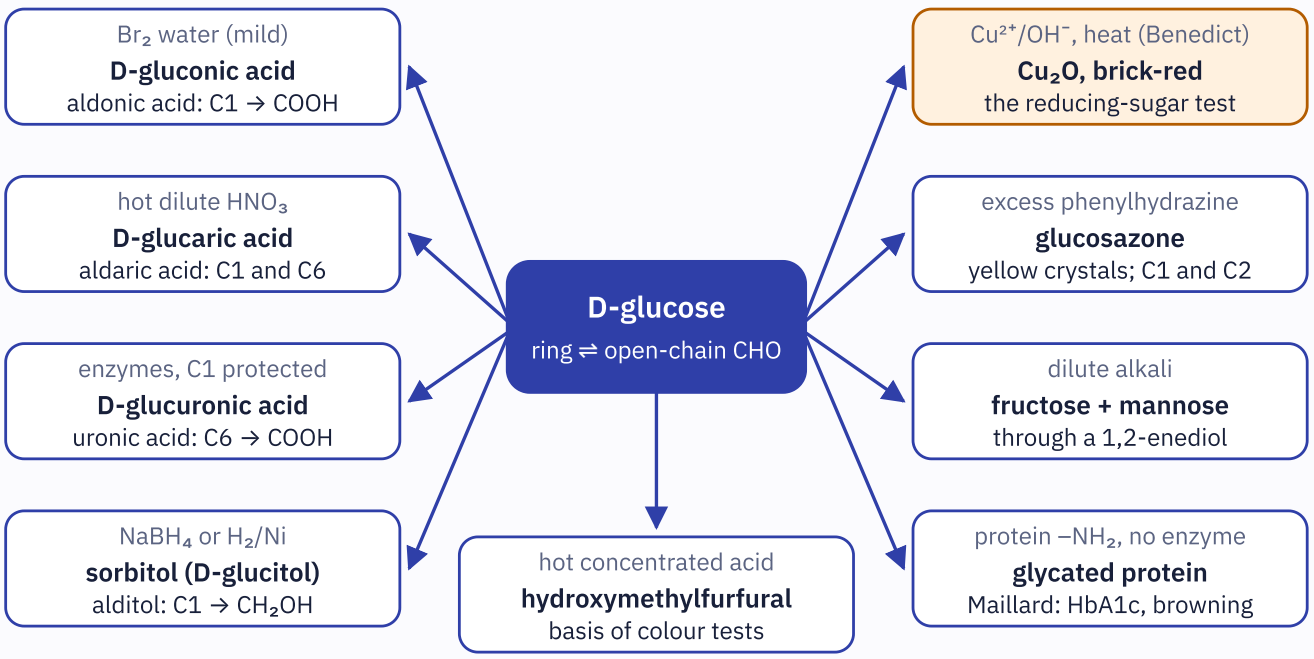


Figure 1.8.1 The main reactions of glucose: oxidations and the reduction on the left, reactions of the open-chain carbonyl on the right, dehydration below. The top line of each box is the reagent. Notice that most of these reactions act on the carbonyl group, which is free only when the anomeric carbon is free; that is why a non-reducing sugar such as sucrose gives them only after hydrolysis.

Lab tests for carbohydrates

TEST	REAGENT	POSITIVE RESULT	SHOWS
Molisch	α-naphthol, then conc. H_2SO_4 down the side	violet ring at the junction	any carbohydrate
Benedict	$CuSO_4$, sodium citrate, Na_2CO_3; boil	green, yellow, then brick-red precipitate	reducing sugar (semi-quantitative)
Fehling	$CuSO_4$ with alkaline sodium potassium tartrate; boil	red precipitate	reducing sugar
Barfoed	copper acetate in dilute acetic acid; heat	red precipitate within a few minutes	reducing monosaccharide (disaccharides react much later)
Seliwanoff	resorcinol in HCl; heat	cherry red, quickly	ketose (sucrose too, after hydrolysis)

TEST	REAGENT	POSITIVE RESULT	SHOWS
Bial	orcinol, HCl, $FeCl_3$; heat	blue-green	pentose
Iodine	I_2 in KI	blue-black; red-brown	starch; glycogen
Osazone	phenylhydrazine, sodium acetate; boil	yellow crystals of typical shape	reducing sugars
Mucic acid	conc. HNO_3; heat, then cool	insoluble crystals	galactose, lactose

For numbers rather than colours, biotech labs use the DNS (3,5-dinitrosalicylic acid) method, which measures total reducing sugar as an orange-brown colour read at 540 nm, for example to assay amylase or cellulase. Clinical labs measure glucose alone with the glucose oxidase–peroxidase (GOD-POD) method.

WORKED EXAMPLE

Question (6 marks). Four unlabelled tubes hold glucose, fructose, sucrose and lactose. Identify each from the results below, and confirm tube C with one more test.

TUBE	MOLISCH	BENEDICT	BARFOED (FAST)	SELIWANOFF	MUCIC ACID
A	+	+	+	–	–
B	+	+	+	+	–
C	+	–	–	+	–
D	+	+	–	–	+

1. All tubes are Molisch-positive, so all hold carbohydrates.
2. C is the only Benedict-negative tube: a non-reducing sugar, so **C is sucrose**. Its positive Seliwanoff fits, because the hot HCl of the reagent hydrolyses sucrose and releases fructose.
3. D is reducing but slow in Barfoed's test, so it is a reducing disaccharide; its positive mucic acid test shows galactose, so **D is lactose**.
4. A and B react fast in Barfoed's test, so both are monosaccharides. B gives a quick cherry red with Seliwanoff, so it is a ketose: **B is fructose** and **A is glucose**.
5. To confirm C, boil it with dilute HCl, neutralise with Na_2CO_3 and add Benedict's reagent. A brick-red precipitate now forms, because hydrolysis has freed glucose and fructose.

EXAM TRAPS

- Fructose is a reducing sugar although it is a ketose: in hot alkali it isomerises through the enediol.
- Sucrose is non-reducing, yet it is Seliwanoff-positive, and Benedict-positive after acid hydrolysis.
- Barfoed's test separates mono- from disaccharides by speed; boiling too long gives a false positive with disaccharides.
- Glucose, fructose and mannose give the same osazone; it cannot tell them apart.
- Glycation is non-enzymatic; glycosylation is enzymatic (Chapter 1.9). HbA1c is glycated, not glycosylated, haemoglobin.

CHECK YOURSELF

– REMEMBER **Name the reagents in Benedict's solution and the precipitate a reducing sugar gives.** 2 marks

Copper(II) sulfate, sodium citrate and sodium carbonate. A reducing sugar gives a brick-red precipitate of copper(I) oxide, Cu_2O.

– UNDERSTAND **Why does fructose give a positive Benedict's test?** 2 marks

Its anomeric carbon is free, and in the hot alkaline reagent the open-chain ketose isomerises through a 1,2-enediol to aldoses (glucose and mannose), whose aldehyde reduces Cu^{2+}.

– APPLY **In a DNS assay, a 1.0 mg/mL glucose standard gives A_{540} = 0.80 (blank-corrected, linear). A sample diluted 1 in 4 gives 0.52. Find its reducing-sugar concentration in mg/mL and in mM (as glucose).** 3 marks

In the assay: 0.52/0.80 × 1.0 = 0.65 mg/mL. Undo the dilution: 0.65 × 4 = **2.6 mg/mL**. As glucose: 2.6 g/L ÷ 180.16 g/mol = **14.4 mM**.

– APPLY **What does $NaBH_4$ give with D-galactose, and why is the product optically inactive?** 2 marks

Galactitol (dulcitol). Turning C1 into CH_2OH makes both ends of the chain identical, and the molecule has an internal mirror plane: it is a meso compound.

– ANALYZE **Suggest two tests to tell glucose from fructose, and the result of each.** 2 marks

Seliwanoff: fructose gives cherry red quickly; glucose only faintly after long heating. Bromine water: glucose (an aldose) decolourises it; fructose does not.

– ANALYZE **Why does HbA1c show blood glucose over months, while a glucometer shows only that moment?** 3 marks

Glycation is slow and practically irreversible, and a red cell lives about 120 days. The glycated fraction of haemoglobin therefore builds up in proportion to the average glucose the cell has met over its life. A glucometer measures free glucose, which changes within minutes after a meal.

BEYOND THE SYLLABUS

HMF made from plant sugars is studied as a platform chemical for bio-based plastics and fuels. The Maillard reaction has a darker side in food: asparagine and reducing sugars in fried or roasted starchy foods form acrylamide, which food regulators try to keep low. Continuous glucose monitors use a glucose oxidase electrode under the skin to read glucose every few minutes. Search next for "HMF biorefinery", "acrylamide in food" and "advanced glycation end products". None of this is for the exam.

1.9 Glycosylation and oligosaccharide synthesis

Glycosylation is the making of a glycosidic bond by moving a sugar from an activated donor onto an acceptor: another sugar, a protein or a lipid. Cells do it with glycosyltransferases and nucleotide sugars, building the glycans of Chapter 1.7; chemists do it with protected sugars and promoters, to make oligosaccharides for drugs and vaccines. Both must solve the same two problems: which –OH reacts, and whether the new bond is α or β.

You need: the glycosidic bond (Chapter 1.5); glycoproteins (Chapter 1.7).

The common principle: an activated donor

Making a glycosidic bond from two free sugars releases water and is uphill: in water, hydrolysis wins (Chapter 1.5). So the sugar to be transferred, the **glycosyl donor**, first gets a good leaving group at its anomeric carbon. When the leaving group departs, the anomeric carbon becomes electron-poor (an oxocarbenium-like state) and is attacked by an –OH or –NH of the **acceptor**. The configuration of the new bond depends on the face from which the acceptor attacks (Figure 1.9.1).

Glycosylation in cells

In cells the leaving group is a nucleotide. The donors are nucleotide sugars such as UDP-glucose, UDP-galactose, UDP-GlcNAc, UDP-glucuronic acid, GDP-mannose, GDP-fucose and CMP-sialic acid, and making them costs a nucleoside triphosphate, which pays for the bond. The enzymes, **glycosyltransferases**, are highly specific: each usually recognises one donor and one acceptor and makes one linkage. That specificity replaces the template that proteins have, so the glycans a cell makes depend on which transferases it expresses. Glycosidases do the reverse and cut glycosidic bonds; in the ER and Golgi they trim glycans as part of normal processing.

N-linked glycosylation, to asparagine, is the main route for secreted and membrane proteins:

1. A 14-sugar block ($Glc_3Man_9GlcNAc_2$) is assembled on the lipid carrier dolichol pyrophosphate in the ER membrane.
2. Oligosaccharyltransferase moves the whole block onto an asparagine of a protein as it enters the ER, but only in the sequence Asn–X–Ser/Thr, where X is any amino acid except proline (the sequon).
3. Glucosidases trim the three glucoses; the chaperones calnexin and calreticulin bind partly trimmed glycans and hold back proteins that have not yet folded.
4. In the Golgi, mannosidases trim further and transferases add GlcNAc, galactose, sialic acid and fucose, giving high-mannose, hybrid or complex glycans that all share the core $Man_3GlcNAc_2$.

O-linked glycosylation adds sugars one at a time to the –OH of serine or threonine, mostly in the Golgi, with no consensus sequence. Mucin-type O-glycans start with GalNAc, and a single O-GlcNAc on nuclear and cytosolic proteins acts as a quick on–off signal. The antibiotic tunicamycin blocks the first step of N-glycosylation and is used in labs to study it.

Blood groups show how one transferase changes a tag. The H antigen is a glycan ending in fucose on galactose. People with the A transferase add GalNAc to it and those with B add galactose; group O people make an inactive enzyme, so their H antigen stays bare. In the rare Bombay phenotype, first described in Bombay (now Mumbai) in 1952, the fucose of the H antigen is never added, so neither A nor B can be built, and such people can receive blood only from other Bombay-phenotype donors.

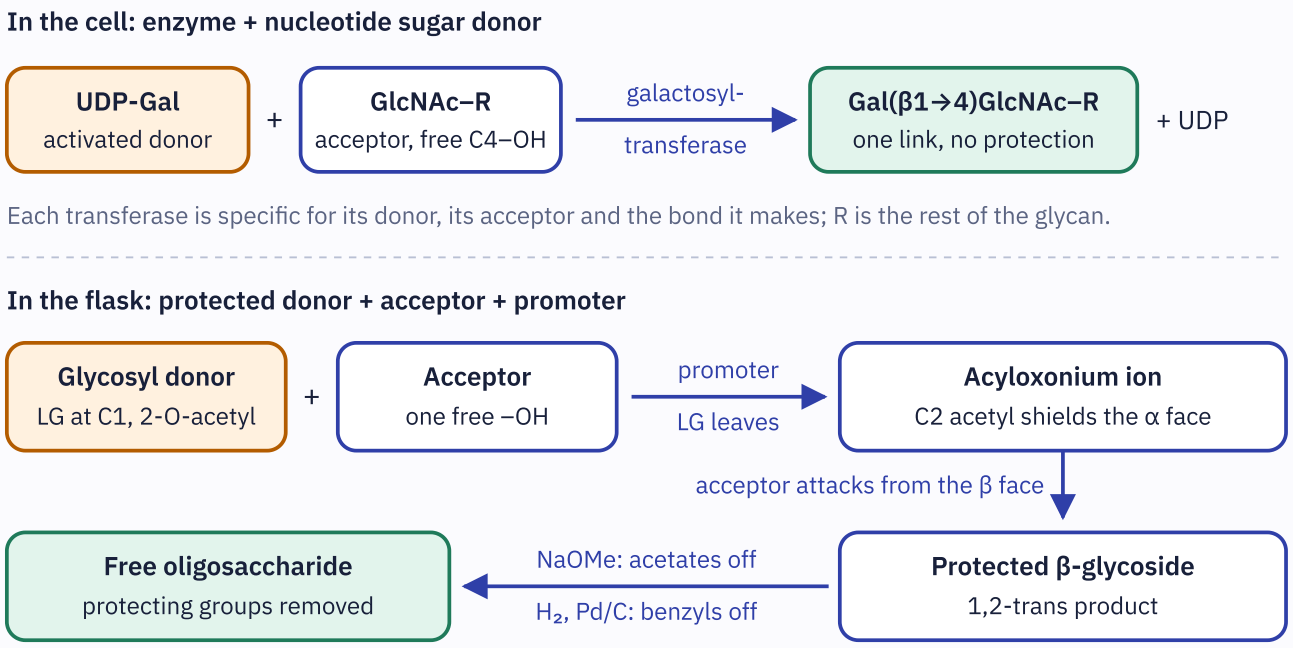


Figure 1.9.1 Two ways to make the same kind of bond. Top: a cell uses an enzyme and a nucleotide sugar, so no protecting groups are needed. Bottom: a chemist masks every –OH but one, activates the donor with a promoter and uses a participating 2-O-acetyl group to force the β (1,2-trans) product, then strips the protecting groups. Notice that in both routes the donor carries a leaving group at its anomeric carbon (LG means leaving group).

Chemical synthesis of oligosaccharides

Glycosylation; general concepts; glycoside bond formation; oligosaccharides". A chemist has no enzyme to choose the –OH or the anomer, so control comes from three tools.

- **Protecting groups.** Every –OH except the one meant to react is masked. Benzyl ethers are "permanent" groups removed at the end by H_2 on palladium; acetyl and benzoyl esters come off with sodium methoxide in methanol (Zemplén conditions); silyl ethers such as TBS come off with fluoride; a 4,6-O-benzylidene acetal masks two –OH at once and comes off with mild acid. A set is **orthogonal** when each group can be removed without touching the others, so one –OH at a time can be unmasked to build a chain or a branch.
- **Donors and promoters.** The donor carries a leaving group at C1: a bromide activated by silver salts (the Koenigs–Knorr method), a trichloroacetimidate activated by a catalytic Lewis acid such as TMSOTf, or a thioglycoside activated by NIS with a strong acid. The promoter pulls off the leaving group to start the reaction.
- **Neighbouring-group participation.** An ester at C2 of the donor swings over to the anomeric carbon as the leaving group departs, forming a ring (an acyloxonium ion) that blocks one face. The acceptor must attack from the other face, giving the 1,2-trans glycoside: β for glucose and galactose, but α for mannose. With a non-participating benzyl ether at C2, mixtures form, often

rich in the α (1,2-cis) product because of the anomeric effect. 1,2-cis links, β-mannosides above all, are the hardest to make.

WORKED EXAMPLE

Question (8 marks). Plan a chemical synthesis of lactose, Gal(β1→4)Glc: name a donor, an acceptor and a promoter, explain how β is ensured, and give the deprotection steps. Compare with how the mammary gland makes lactose.

1. Donor: 2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl trichloroacetimidate. Acetates protect every –OH, and the one at C2 will participate.
2. Acceptor: benzyl 2,3,6-tri-O-benzyl-β-D-glucopyranoside. Benzyl ethers mask C2, C3 and C6 and a benzyl glycoside masks C1, so only the 4-OH is free. Glucose's 4-OH reacts sluggishly, so a reactive donor is chosen.
3. Coupling: catalytic TMSOTf in dry dichloromethane at low temperature. The imidate leaves, the 2-O-acetyl forms an acyloxonium ion over the α face, and the 4-OH attacks from the β face, giving the 1,2-trans β(1→4) bond.
4. Deprotection: NaOMe in methanol removes the four acetates; H_2 over Pd/C removes the four benzyl groups, including the anomeric one, which frees the reducing end. The product is lactose, as an α/β mixture at glucose C1 because it mutarotates.
5. Count: **8 protecting groups** put on and taken off to make **one** bond.
6. In the mammary gland, lactose synthase (a β1,4-galactosyltransferase helped by the protein α-lactalbumin) joins UDP-galactose to glucose in water, in one step, with no protecting groups. Enzymes give perfect selectivity; chemistry gives the freedom to make unnatural links and labelled sugars.

EXAM TRAPS

- Glycosylation is enzymatic and gives defined structures; glycation (Chapter 1.8) is a random, non-enzymatic reaction of sugars with proteins.
- N-linked glycans attach to the amide nitrogen of asparagine; O-linked glycans to the –OH of serine or threonine. The sequon is Asn–X–Ser/Thr with X not proline, and not every sequon is used.
- A participating C2 ester gives the 1,2-trans product: β for glucose and galactose, α for mannose.
- Orthogonal does not mean permanent; it means each group can be removed on its own.
- Glycosyltransferases add sugars; glycosidases remove them.

CHECK YOURSELF

– REMEMBER **Name four nucleotide sugars that cells use as glycosyl donors.** 2 marks

Any four of: UDP-glucose, UDP-galactose, UDP-GlcNAc, UDP-glucuronic acid, GDP-mannose, GDP-fucose, CMP-sialic acid.

– UNDERSTAND **Why must a sugar be activated before it can be transferred?** 2 marks

Joining two free sugars releases water and is thermodynamically uphill; in water the reverse, hydrolysis, is favoured. A good leaving group at C1 (UDP in cells, an imidate or halide in the flask) makes the transfer downhill.

– UNDERSTAND **What makes a set of protecting groups orthogonal? Give an example.** 2 marks

Each group is removed by a reagent that leaves the others intact. Example: acetate (NaOMe), benzyl ether (H_2, Pd/C) and TBS ether (fluoride).

– APPLY **Mark the N-glycosylation sequons in …Asn–Gly–Ser–Leu–Asn–Pro–Thr–Ala–Asn–Ala–Thr–Gln…** 3 marks

Asn–Gly–Ser: a sequon. Asn–Pro–Thr: not a sequon, because X is proline. Asn–Ala–Thr: a sequon. So **two** possible N-glycosylation sites.

– APPLY **A mannosyl donor with a 2-O-acetyl group reacts with an acceptor. Which anomer forms?** 2 marks

The participating acetate gives the 1,2-trans product. Mannose's C2 substituent is axial, so 1,2-trans is the **α-mannoside**.

– ANALYZE **Why are therapeutic antibodies made in mammalian cells such as CHO cells rather than in E. coli?** 3 marks

Antibodies carry an N-glycan that affects how they activate immune cells and how long they last in blood. E. coli has no ER–Golgi pathway and does not add human-type N-glycans; mammalian cells do, and their glycans can be checked and tuned.

BEYOND THE SYLLABUS

Conjugate vaccines join a bacterial capsule polysaccharide to a carrier protein so that infants' immune systems respond to it; India's Universal Immunisation Programme uses such vaccines against Haemophilus influenzae type b (in the pentavalent vaccine) and pneumococcus. In 2004 Cuba introduced a Hib vaccine whose sugar antigen was made entirely by chemical synthesis. Automated glycan assembly now builds oligosaccharides on a solid support, as peptide synthesisers do for peptides, and glycoengineered antibodies lacking core fucose kill target cells more strongly. Search next for "conjugate vaccine", "automated glycan assembly" and "antibody glycoengineering". None of this is for the exam.

Key terms of this lesson

TERM	MEANING OR FORMULA	CHAPTER
Reducing sugar	has a free anomeric carbon; reduces Cu^{2+} to red Cu_2O	1.8
N-glycosylation sequon	Asn–X–Ser/Thr, where X is not proline	1.9
1,2-trans rule	a participating C2 ester gives the 1,2-trans glycoside (β for glucose, α for mannose)	1.9

Glossary (terms used in this lesson)

Anomer Either of the two ring forms, α or β, of a sugar that differ only at the anomeric carbon.

Anomeric carbon The carbonyl carbon that becomes a new chiral centre when the ring closes: C1 of an aldose, C2 of a ketose.

Glycation Attachment of a reducing sugar to a free amino group of a protein without an enzyme, as in HbA1c.

Glycosidic bond The bond from the anomeric carbon of a sugar to an –OH (O-glycosidic) or an –NH (N-glycosidic) of another molecule.

Glycosyltransferase An enzyme that moves a sugar from a nucleotide sugar to an acceptor, making one specific linkage.

Hydrogen bond	The attraction of a δ+ hydrogen on O or N to a lone pair on another O or N; about 23 kJ/mol in water.
Mutarotation	The change in optical rotation of a fresh sugar solution as the α and β anomers interconvert to equilibrium.
Nucleotide sugar	An activated sugar donor, such as UDP-glucose, GDP-mannose or CMP-sialic acid.
Osazone	The yellow crystalline product of a reducing sugar boiled with excess phenylhydrazine.
Protecting group	A temporary group that blocks an –OH so that a chemical reaction happens only at the chosen position.
Reducing sugar	A sugar with a free anomeric carbon, which reduces Cu^{2+} to red Cu_2O in Benedict's test.
Sequon	The sequence Asn–X–Ser/Thr, with X not proline, at which an N-glycan can be attached.

The full unit book

Every chapter of Unit 1, the unit summary, the exam question bank with model answers and the full glossary: Biochemistry · Unit 1 · Foundations: Water, pH and Carbohydrates (one file, works offline).

Sources and further reading

Textbooks

- David L. Nelson and Michael M. Cox, *Lehninger Principles of Biochemistry*, W. H. Freeman; chapters on water and on carbohydrates and glycobiology (from memory; check). Source of the bond energies, the branching frequencies of amylopectin and glycogen and the chain length of cellulose used here.
- Jeremy M. Berg, John L. Tymoczko, Gregory J. Gatto Jr. and Lubert Stryer, *Biochemistry*, W. H. Freeman; chapter on carbohydrates (from memory; check).
- Donald Voet and Judith G. Voet, *Biochemistry*, Wiley (from memory; check).
- U. Satyanarayana and U. Chakrapani, *Biochemistry*, Elsevier India; widely prescribed in Indian universities, with the colour tests for carbohydrates (from memory; check).

- Ajit Varki and others (editors), *Essentials of Glycobiology*, Cold Spring Harbor Laboratory Press, also free on the NCBI Bookshelf; for Chapters 1.7 and 1.9 (from memory; check).

Other standard values in this book, such as the specific rotations of sucrose (+66.5°) and fructose (−92.4°), are textbook values written from memory; check them against your prescribed book.

Open resources opened for this book

- Wikipedia, "Mutarotation": en.wikipedia.org/wiki/Mutarotation. Source of the specific rotations of α- and β-D-glucose (+112.2°, +18.7°), the equilibrium value (+52.7°) and the 36 : 64 ratio of the anomers.
- NPTEL, Module 11 (Carbohydrates), Lecture 30, course 104103071: archive.nptel.ac.in. Confirms the same rotation values and the reducing and non-reducing classes by hemiacetal and acetal form.
- US Food and Drug Administration, "Aspartame and Other Sweeteners in Food": fda.gov. Source of the sweetness of the high-intensity sweeteners relative to sucrose in Chapter 1.5.

Syllabus: the University free n easy universal syllabus, built from top universities' syllabi.

Built for the University free n easy YouTube channel from the universal syllabus of Biochemistry (Oct 2026). Written with top universities' syllabi and standard textbooks; check a formula against your prescribed book before an exam, and report mistakes on the channel.