Biochemistry

Unit 1 · Foundations: Water, pH and Carbohydrates

Every molecule of life follows ordinary chemistry: a few elements, a small set of functional groups, and water, which shapes everything through weak bonds and pH. This unit lays that ground and then climbs through the carbohydrates, from single sugars to the sugar chains on your own cells. After it you can classify any carbohydrate, draw and name its structures, prepare a buffer, explain the lab tests for sugars and plan how a glycosidic bond is made.

Taught at 8 of 10 | Usually Sem 3 | 9 chapters

About this unit

The unit starts with the chemistry every later unit uses. Chapter 1.1 shows what biomolecules are made of and how small building blocks join into large molecules. Chapter 1.2 explains water, the weak interactions it allows, and how buffers hold pH steady. Chapters 1.3 to 1.6 then climb the carbohydrate ladder: how carbohydrates are classified, the structure and isomers of single sugars, pairs of sugars joined by the glycosidic bond, and long polysaccharides. Chapter 1.7 shows sugars working with proteins and lipids (glycosaminoglycans, proteoglycans and glycoconjugates), Chapter 1.8 collects the reactions and lab tests of sugars, and Chapter 1.9 shows how cells and chemists build glycosidic bonds on purpose.

Chapters

1. Biomolecules and the chemical foundations of biochemistry
2. Water, weak interactions, pH and biological buffers
3. Carbohydrates: classification and functions
4. Monosaccharides: structure, isomerism and mutarotation
5. Disaccharides and the glycosidic bond
6. Polysaccharides: starch, glycogen, cellulose and chitin
7. Glycosaminoglycans, proteoglycans and glycoconjugates
8. Properties and reactions of sugars; reducing sugars
9. Glycosylation and oligosaccharide synthesis

How to use this book

- Read the lead paragraph and the figure of each chapter first; together they carry the one idea.

- Cover the solution of each worked example, do it yourself with a pencil and calculator, then compare step by step.
- Answer every "Check yourself" question on paper before you open it; the marks show how long the exam answer should be.

1.1 Biomolecules and the chemical foundations of biochemistry

Biochemistry explains life in the language of chemistry. A living cell is built from a few elements, arranged into a small set of building blocks (sugars, amino acids, nucleotides, fatty acids) that join into large molecules. The rules that govern a reaction in a test tube (covalent bonds, functional groups, shape and energy) also govern a reaction in a liver cell. Learn these rules once and every later unit becomes a set of examples.

What biochemistry studies, and why

Biochemistry has four aims: to describe the structures of the molecules of life; to explain how they recognise and bind one another; to trace how cells make and break them (metabolism, Units 5 and 6); and to find out how all of this is controlled and inherited.

The scope is wide because almost every question about living things is, at bottom, a chemical one. In **medicine**, a blood-glucose test, a lipid profile and a liver-enzyme test are biochemical measurements. In **food and agriculture**, the ripening of a mango, the setting of curd and the rising of idli batter are enzyme and carbohydrate chemistry. In **industry**, detergent enzymes, recombinant insulin, vaccines and biofuels all start from knowing which molecule does what.

Elements and building blocks

Of the roughly 90 natural elements, only about 30 are essential to living things. Four of them, carbon, hydrogen, oxygen and nitrogen, make up more than 99% of the mass of most cells. Phosphorus and sulfur come next. Ions such as Na^+, K^+, Mg^{2+}, Ca^{2+} and Cl^- are needed in bulk, and trace elements such as Fe, Zn, Cu, Se and I in small amounts, mostly inside enzymes.

Carbon is the backbone because each carbon atom forms four stable covalent bonds, single or double, to other carbons or to H, O and N. Carbon can therefore build chains, branches and rings of almost any size, and its tetrahedral bonds give these skeletons a definite three-dimensional shape. Water, the medium for all of this, makes up about 70% of the mass of most cells (Chapter 1.2).

Most biomolecules belong to four classes. Three are true polymers, made by joining many similar units through one kind of bond (Figure 1.1.1). Lipids are the exception: they are

assembled from fatty acids and other parts but do not form long repeating chains (Unit 3). Cells also hold thousands of small molecules, the metabolites such as pyruvate, citrate and ATP, that are the intermediates of metabolism.

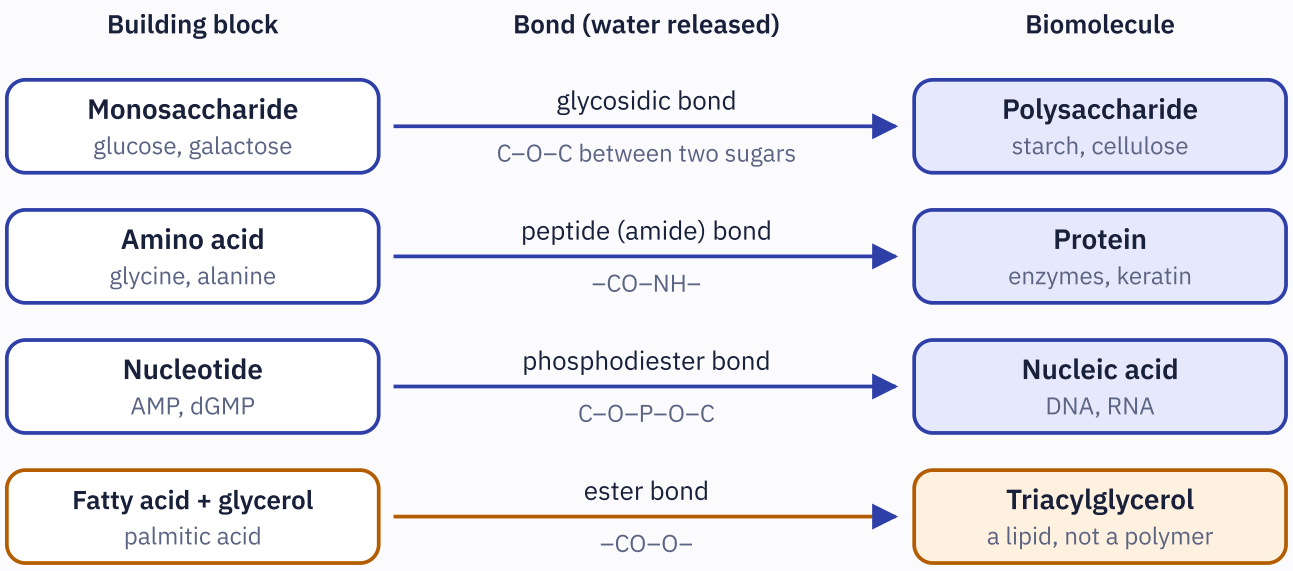


Figure 1.1.1 The three polymer classes and the lipids. Every bond shown forms when water is removed (condensation) and breaks when water is added back (hydrolysis). Notice that a triacylglycerol is made by the same kind of chemistry but is not a long chain of repeating units.

Joining two building blocks removes a molecule of water: an –OH from one partner and an –H from the other. This is a **condensation** (dehydration) reaction. The reverse, splitting the bond by adding water back, is **hydrolysis**. Digestion is hydrolysis; building a protein or a starch granule is condensation, paid for with energy from ATP or an activated donor (Chapter 1.9 and Unit 5).

Polymers also have direction. A polysaccharide has a reducing end and a non-reducing end (Chapter 1.6), a protein an amino end and a carboxyl end, and a nucleic acid a 5′ end and a 3′ end. The order of units along the chain carries information: twenty kinds of amino acid in a chain of 100 can be arranged in 20^{100} ways, far more than the number of atoms in the observable universe.

Functional groups

A functional group is a set of atoms that reacts in a characteristic way wherever it sits. Most biomolecules carry several, and their solubility, charge and reactivity follow from them. The charges shown are those at pH 7, explained in Chapter 1.2.

GROUP	STRUCTURE	WHERE YOU MEET IT
Hydroxyl	–OH	sugars, alcohols, serine

GROUP	STRUCTURE	WHERE YOU MEET IT
Aldehyde (carbonyl)	–CHO	open-chain aldoses such as glucose
Ketone (carbonyl)	>C=O	ketoses such as fructose; pyruvate
Carboxyl	–COOH (–COO^-)	amino acids, fatty acids, uronic acids
Amino	–NH_2 (–NH_3^+)	amino acids, amino sugars
Amide	–CO–NH–	peptide bond, N-acetylglucosamine
Ester	–CO–O–R	fats and oils, acetylated sugars
Ether or acetal	C–O–C	glycosidic bonds
Phosphoryl	–O–PO_3^{2-}	sugar phosphates, nucleotides, ATP
Thiol (sulfhydryl)	–SH	cysteine, coenzyme A
Sulfate ester	–O–SO_3^-	glycosaminoglycans (Chapter 1.7)

Shape: configuration, conformation and chirality

Two molecules with the same atoms and bonds can still differ in shape, and in biology shape decides function. The **configuration** of a molecule is the fixed arrangement of its atoms in space; changing it means breaking a covalent bond. Cis and trans double bonds, and the two mirror-image forms of a chiral molecule, differ in configuration. A **conformation** is any shape the molecule can take by rotating about single bonds, without breaking any. A sugar ring flexing between chair forms, or a protein chain folding, changes conformation only.

A carbon atom with four different groups attached is a **chiral centre**. It can exist as two non-superimposable mirror images (enantiomers), like a left and a right hand. Living systems are strongly one-handed: proteins are built from L-amino acids and most natural sugars are D-sugars (Chapter 1.4). Enzymes and receptors are themselves chiral, so they usually accept only one enantiomer. Even our noses tell the two forms of carvone apart: one smells of spearmint, the other of caraway seeds.

Shape is held in place by weak interactions (Chapter 1.2), and energy decides which reactions run; how cells capture and spend energy is bioenergetics (Unit 5).

WORKED EXAMPLE

Question (6 marks). (a) Ten glucose molecules ($C_6H_{12}O_6$, molar mass 180.16 g/mol) join in a straight chain. How many water molecules are released, and what is the molar mass of the chain? (b) Estimate the molar mass of a protein of 300 amino acid residues. (c) Name the bond formed in each case.

1. A straight chain of n units has n − 1 links, and each link releases one H_2O (18.02 g/mol). For n = 10: **9 water molecules**.
2. Molar mass of the chain = 10 × 180.16 − 9 × 18.02 = 1801.6 − 162.2 = **1639.4 g/mol**.
3. Check another way: each glucose inside a chain is a residue $C_6H_{10}O_5$ (162.14 g/mol), and one water's worth of atoms stays at the two ends: 10 × 162.14 + 18.02 = 1639.4 g/mol. The answers agree.
4. For proteins, the average mass of an amino acid residue (water already removed) is about 110 Da, where 1 Da corresponds to 1 g/mol. So 300 × 110 ≈ 33,000 Da, that is **about 33 kDa**.
5. The glucose chain is held by glycosidic bonds; the protein by peptide (amide) bonds.

Rule: a polymer's molar mass is never just n times the monomer's; subtract (n − 1) waters.

EXAM TRAPS

- "Biomolecule" is not the same as "macromolecule". Lipids and small metabolites such as pyruvate are biomolecules, but they are not polymers.
- Condensation and hydrolysis are often swapped. Condensation releases water and makes the bond; hydrolysis uses water and breaks it.
- Configuration or conformation? Rotation about a single bond changes conformation; a change of configuration needs a bond to break. The α to β change of glucose is a change of configuration, because the ring must open (Chapter 1.4).
- The letters D and L describe configuration, not the direction in which a sugar rotates light (Chapter 1.4).
- Structures are often drawn with –COOH and $–NH_2$, but at pH 7 these groups exist as $–COO^-$ and $–NH_3^+$. Give the charged forms when the question is about a cell.

CHECK YOURSELF

– REMEMBER **Which four elements make up more than 99% of the mass of most cells?** 2 marks

Carbon, hydrogen, oxygen and nitrogen.

– UNDERSTAND **Why are lipids called biomolecules but not polymers?** 2 marks

They are assembled from a few different parts (glycerol, fatty acids, phosphate, bases) joined mainly by ester bonds, and they do not form long chains of one repeating unit. Large lipid structures such as membranes are held by weak forces, not by a covalent chain.

– APPLY **Calculate the molar mass of a straight chain of 25 glucose residues.** 3 marks

25 × 162.14 + 18.02 = 4053.5 + 18.0 = **4071.5 g/mol** (or 25 × 180.16 − 24 × 18.02, which gives the same).

– APPLY **Name the functional groups in pyruvate ($CH_3–CO–COO^-$) and in glucose 6-phosphate.** 4 marks

Pyruvate: a ketone (carbonyl) group and a carboxylate group. Glucose 6-phosphate: several hydroxyl groups, the aldehyde (hemiacetal in the ring form) at C1, and a phosphoryl group as a phosphate ester at C6.

– ANALYZE **Twenty amino acids are enough to build millions of different proteins. Show why with a calculation.** 4 marks

Each position can hold any of 20 amino acids, so a chain of n residues has 20^n possible sequences. Even a 10-residue peptide has $20^{10} \approx 1.0 \times 10^{13}$ sequences. The order of units carries the information, just as 26 letters make every book.

– ANALYZE **Why does an enzyme usually act on only one enantiomer of its substrate?** 3 marks

The active site is built from L-amino acids and is itself chiral. The substrate must touch it at three or more points at once; its mirror image can match at most two of them, like a left hand in a right glove.

BEYOND THE SYLLABUS

Modern biochemistry measures thousands of molecules at once. Metabolomics uses mass spectrometry to profile the small molecules of a cell, and cryo-electron microscopy and AI prediction (AlphaFold) show the shapes of the large ones. Why life uses only one hand of each chiral molecule, called homochirality, is still an open question in origin-of-life chemistry. Search next for "metabolomics", "Human Metabolome Database" and "homochirality". None of this is for the exam.

1.2 Water, weak interactions, pH and biological buffers

Water is the medium of life, a reactant in every hydrolysis and a sculptor of biomolecules. Its bent, polar molecules hydrogen bond to one another and to polar groups, push nonpolar groups together, and split very slightly into H^+ and OH^-. That last property gives the pH scale, and buffers (a weak acid with its conjugate base) keep pH steady in blood, cells and the lab.

Water and the hydrogen bond

Oxygen is more electronegative than hydrogen, so in a water molecule it pulls the shared electrons towards itself: the O carries a partial negative charge (δ−) and each H a partial positive charge (δ+). The molecule is bent, with an H–O–H angle of 104.5°, so the charges do not cancel and water is a dipole. A **hydrogen bond** forms when a δ+ hydrogen, already bonded to an O or N, is attracted to a lone pair on another O or N. Each water molecule can make up to four: two through its hydrogens and two through its oxygen (Figure 1.2.1, left).

A hydrogen bond in liquid water has a dissociation energy of about 23 kJ/mol, against about 470 kJ/mol for the covalent O–H bond. Each lasts only picoseconds, but at any moment most molecules are bonded, so liquid water is a constantly reshuffling network. This explains water's high boiling point, specific heat and heat of vaporisation (sweat cools you because breaking the bonds takes heat). In ice every molecule is locked into four bonds in an open lattice, so ice is less dense than water and floats.

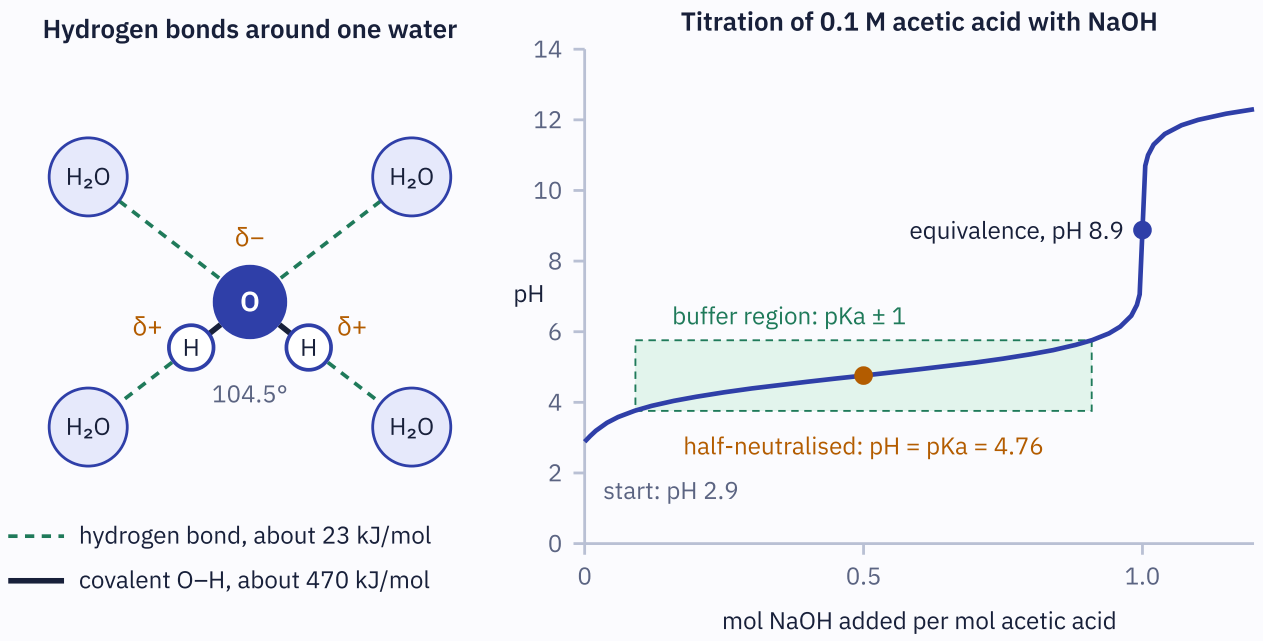


Figure 1.2.1 Left: one water molecule can donate two hydrogen bonds and accept two. Right: titration of 0.1 M acetic acid, computed point by point (volume change ignored). Notice how flat the curve is around pH 4.76, where acid and conjugate base are equal: that is the buffer region.

Weak interactions and the hydrophobic effect

Covalent bonds hold the atoms of a biomolecule together; weak (non-covalent) interactions hold its shape and let molecules recognise each other. Each is weak and breaks easily, but hundreds together are strong and specific.

INTERACTION	WHAT ATTRACTS WHAT	EXAMPLE
Hydrogen bond	δ+ H on O or N to a lone pair on O or N	DNA base pairs, protein helices, sugar–water
Ionic (electrostatic)	opposite charges, such as $–COO^-$ and $–NH_3^+$	salt bridges in proteins, Mg^{2+} with ATP
Hydrophobic effect	nonpolar groups clustering away from water	oil droplets, membranes, protein cores
van der Waals	short-lived dipoles of atoms in close contact, a few kJ/mol each	tightly packed protein interiors, enzyme–substrate fit

Water weakens ionic attractions. By Coulomb's law the force between charges q_1 and q_2 at distance r is $F = q_1 q_2/(4\pi\varepsilon_0\varepsilon r^2)$, where ε_0 is the permittivity of free space and ε the dielectric constant of the medium. For water ε is about 78.5 at 25 °C, so opposite charges attract with only about 1/80 of their force in a vacuum. That is why salts dissolve: water surrounds and screens

each ion (hydration). Polar and charged molecules such as sugars are hydrophilic; nonpolar ones such as oils are hydrophobic.

The **hydrophobic effect** is not a true attraction between nonpolar groups. A nonpolar molecule cannot hydrogen bond, so the water around it is forced into an ordered cage, which lowers entropy. When nonpolar groups cluster, caged water is set free and its entropy rises. Amphipathic molecules (a polar head and a nonpolar tail, as in soaps and phospholipids) therefore form micelles and bilayers, tails inside (Unit 3).

Ionisation of water, pH and pKa

Water ionises slightly: $H_2O \rightleftharpoons H^+ + OH^-$ (the H^+ is really carried as H_3O^+). The ion product of water is $K_w = [H^+][OH^-] = 1.0 \times 10^{-14}$ M² at 25 °C, where square brackets mean molar concentration; in pure water $[H^+] = [OH^-] = 10^{-7}$ M. The pH is defined as $pH = -\log_{10}[H^+]$, so neutral water at 25 °C has pH 7. The scale is logarithmic: one pH unit is a tenfold change in $[H^+]$.

A weak acid HA dissociates only partly: $HA \rightleftharpoons H^+ + A^-$, where A^- is its conjugate base. Its acid dissociation constant is $K_a = [H^+][A^-]/[HA]$, and $pK_a = -\log K_a$; the lower the pKa, the stronger the acid. Taking logarithms of Ka gives the Henderson–Hasselbalch equation:

$$K_a = [H^+][A^-] / [HA]$$
$$\log K_a = \log[H^+] + \log([A^-]/[HA])$$
$$-\log[H^+] = -\log K_a + \log([A^-]/[HA])$$
$$pH = pK_a + \log([A^-]/[HA])$$

When $[A^-] = [HA]$, the log term is zero and pH = pKa: the acid is exactly half dissociated. That is the flat middle of the titration curve in Figure 1.2.1.

Biological buffers and how to prepare one

A **buffer** is a mixture of a weak acid and its conjugate base that resists a change in pH when a little acid or base is added. Added H^+ is taken up by A^- (forming HA); added OH^- takes H^+ from HA (forming A^- and water). The ratio $[A^-]/[HA]$, and so the pH, changes only a little. A buffer works best at pH = pKa, usefully within pKa ± 1, and its capacity grows with concentration.

Blood plasma (pH 7.35 to 7.45) relies on the bicarbonate system, $CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$, with an apparent pKa of 6.1. It works despite that pKa because it is open: the lungs remove CO_2 and the kidneys adjust HCO_3^-. Inside cells the phosphate pair $H_2PO_4^- \rightleftharpoons H^+ + HPO_4^{2-}$ (pKa 7.2 in dilute solution, about 6.9 at cellular ionic strength) does most of the work, helped by histidine side chains of proteins (pKa about 6). Blood pH below 7.35 is acidosis (as when ketone bodies pile up in uncontrolled diabetes, Unit 6); above 7.45 is alkalosis (as after hyperventilation).

Common lab buffers are phosphate, acetate (pKa 4.76), Tris (pKa about 8.1) and HEPES (pKa about 7.5). The procedure:

1. Choose a buffer with pKa within 1 unit of the pH you need, that does not interfere with the experiment.
2. Find the ratio $[A^-]/[HA] = 10^{(pH - pKa)}$.
3. From concentration and volume, work out moles, then grams (a hydrate has a different molar mass).
4. Dissolve in about 80% of the final volume, check with a calibrated pH meter at the working temperature (Tris shifts about 0.03 pH unit per °C), and adjust with dilute acid or base.
5. Make up to volume in a volumetric flask.

WORKED EXAMPLE

Question (6 marks). Prepare 1.0 L of 0.10 M sodium phosphate buffer, pH 7.4, from anhydrous NaH_2PO_4 (119.98 g/mol) and Na_2HPO_4 (141.96 g/mol). Take pKa = 7.20.

1. Ratio: $[HPO_4^{2-}]/[H_2PO_4^-] = 10^{(7.4 - 7.2)} = 10^{0.2} = 1.585$.
2. Let $[H_2PO_4^-] = x$. Then $x + 1.585x = 0.10$ M, so $x = 0.10/2.585 = 0.0387$ M, and $[HPO_4^{2-}] = 0.0613$ M. Check: 0.0387 + 0.0613 = 0.100 M.
3. In 1.0 L that is 0.0387 mol and 0.0613 mol.
4. Masses: NaH_2PO_4 = 0.0387 × 119.98 = **4.64 g**; Na_2HPO_4 = 0.0613 × 141.96 = **8.70 g**.
5. Dissolve in about 800 mL of water, check with the pH meter, adjust and make up to 1.0 L. The reading is usually a little below 7.4, because ionic strength lowers the effective pKa.

EXAM TRAPS

- pH is logarithmic: pH 6 has ten times the $[H^+]$ of pH 7, not "a little more".
- In Henderson–Hasselbalch the ratio is base over acid, $[A^-]/[HA]$. Writing it upside down flips the sign of the log term.
- A buffer has a range, pKa ± 1. Acetate cannot buffer at pH 7.4, however concentrated it is.
- Neutral pH is 7.0 only at 25 °C, because Kw changes with temperature.
- The hydrophobic effect is driven by the entropy of water, not by an attraction between nonpolar molecules; "hydrophobic bond" is a misleading name.

CHECK YOURSELF

– REMEMBER **Write the Henderson–Hasselbalch equation and define each term.** 2 marks

pH = pKa + log([A⁻]/[HA]); pKa = –log Ka of the weak acid, [A⁻] is the conjugate base concentration and [HA] the undissociated acid concentration.

– UNDERSTAND **Why does oil not mix with water?** 3 marks

Oil cannot hydrogen bond, so water forms ordered cages around it. Droplets merging frees caged water, and that gain in the water's entropy drives the separation (the hydrophobic effect).

– APPLY **A 1 L buffer has 0.10 M acetic acid and 0.10 M sodium acetate (pKa 4.76). Find the pH after adding 0.010 mol HCl, and compare with pure water.** 4 marks

H^+ converts acetate to acetic acid: [HA] = 0.11 M, [A^-] = 0.09 M. pH = 4.76 + log(0.09/0.11) = 4.76 – 0.09 = **4.67**, a drop of only 0.09. The same HCl in 1 L of pure water gives [H^+] = 0.01 M, so pH falls from 7 to **2**.

– APPLY **Blood has [HCO_3^-] = 24 mM and pCO_2 = 40 mmHg (dissolved CO_2 = 0.03 mM per mmHg; pKa 6.1). Find the pH, and the pH if pCO_2 rises to 60 mmHg.** 4 marks

CO_2 = 1.2 mM: pH = 6.1 + log(24/1.2) = 6.1 + 1.30 = **7.40**. At 60 mmHg, CO_2 = 1.8 mM: pH = 6.1 + log 13.3 = **7.22**, a respiratory acidosis (breathing too little).

– ANALYZE **For an enzyme assay at pH 7.2, choose between acetate (pKa 4.76), phosphate (7.2) and Tris (8.1). Justify.** 3 marks

Phosphate: its pKa equals the target pH, so capacity is greatest. Tris is at the edge of its range and drifts with temperature; acetate is outside its range. If phosphate inhibits the enzyme or precipitates Ca^{2+}, use HEPES.

– ANALYZE **The bicarbonate pKa (6.1) is far from 7.4, yet it is the main buffer of blood. Explain.** 3 marks

It is an open system: the lungs adjust CO_2 within minutes and the kidneys adjust HCO_3^- over hours to days, so the ratio [HCO_3^-]/[CO_2] stays near 20.

BEYOND THE SYLLABUS

Good's buffers (HEPES, MES, MOPS and others) were designed in the 1960s to suit biology: pKa near 7, little binding of metal ions, and no passage through membranes. In research, weak interactions are now known to drive liquid–liquid phase separation, in which proteins and RNA condense into membraneless droplets inside cells. Search next for "Good's buffers" and "biomolecular condensates". None of this is for the exam.

1.3 Carbohydrates: classification and functions

Carbohydrates are polyhydroxy aldehydes or ketones, or substances that give these on hydrolysis. They are the most abundant biomolecules on Earth: every year photosynthesis turns more than 100 billion tonnes of CO_2 and water into cellulose and other plant products. In living things they are fuel, fuel stores, building materials and identity tags on cell surfaces. Every university's syllabus opens carbohydrates with this classification, and it is one of the most common long-answer questions.

You need: functional groups and the glycosidic bond as a condensation (Chapter 1.1).

What counts as a carbohydrate

Many simple carbohydrates have the empirical formula $(CH_2O)_n$, which is where the old name "hydrates of carbon" comes from. The formula is a poor definition. Deoxyribose ($C_5H_{10}O_4$) is a carbohydrate that does not fit it, while formaldehyde (CH_2O), acetic acid ($C_2H_4O_2$) and lactic acid ($C_3H_6O_3$) fit it but are not carbohydrates. Some carbohydrates also contain nitrogen (amino sugars), phosphorus (sugar phosphates) or sulfur (sulfated glycosaminoglycans). Define carbohydrates by structure instead: several –OH groups plus an aldehyde or ketone group, or a polymer built from such units.

Classification by size

The main classification counts sugar units (Figure 1.3.1).

- **Monosaccharides** are simple sugars that cannot be hydrolysed into smaller carbohydrates. Each gets two descriptions: by its carbonyl group (an **aldose** has an aldehyde at C1; a **ketose** has a ketone, usually at C2) and by its number of carbons (triose 3, tetrose 4, pentose 5, hexose 6, heptose 7). Combined, glucose is an aldohexose, fructose a ketohexose, ribose an

aldopentose, glyceraldehyde an aldotriose and dihydroxyacetone a ketotriose. Their structures and isomers are Chapter 1.4.

- **Oligosaccharides** contain 2 to about 10 units joined by glycosidic bonds. Disaccharides (maltose, lactose, sucrose) are the most important (Chapter 1.5). Raffinose, a trisaccharide of galactose, glucose and fructose, is found in rajma, chana and soybean; our small intestine cannot digest it, so gut bacteria ferment it, and the gas they make is the familiar side effect of a pulse-rich meal. Many oligosaccharides in cells are attached to proteins and lipids (Chapter 1.7).
- **Polysaccharides** (glycans) contain many units, often hundreds to thousands. A **homopolysaccharide** has one kind of unit (starch, glycogen, cellulose, chitin, inulin); a **heteropolysaccharide** has two or more kinds (hyaluronic acid, heparin, chondroitin sulfate, the peptidoglycan of bacterial cell walls). Unlike a protein, a polysaccharide is made without a template, so its chain length varies from molecule to molecule and it has no single molecular mass (Chapter 1.6).

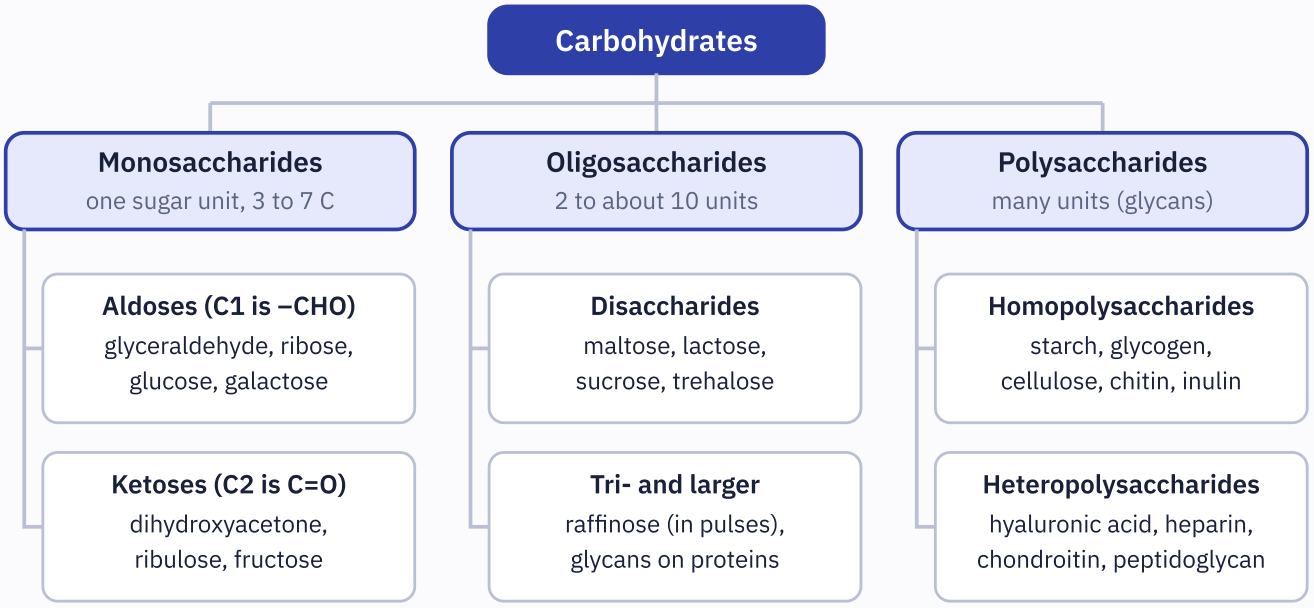


Figure 1.3.1 Classification of carbohydrates by the number of sugar units, with the second level each class is split into. Notice that "homo" and "hetero" refer to the kinds of unit, not to branching: branched glycogen is still a homopolysaccharide.

Chemically modified monosaccharides are called **derived sugars**: deoxy sugars (2-deoxyribose in DNA), amino sugars (glucosamine and galactosamine, often N-acetylated), sugar acids (gluconic acid, glucuronic acid, sialic acids), sugar alcohols (sorbitol, mannitol, xylitol) and sugar phosphates (glucose 6-phosphate). The reactions that make them are in Chapter 1.8.

Functions of carbohydrates

1. **Fuel.** Digestible carbohydrate supplies about 17 kJ (4 kcal) per gram. Glucose is the main fuel of the brain and the only fuel of red blood cells, which have no mitochondria.
2. **Fuel storage.** Plants store starch, animals and fungi store glycogen, and some plants such as chicory store inulin, a fructose polymer.
3. **Structure.** Cellulose builds plant cell walls and cotton; chitin builds the shells of insects and crabs and the walls of fungi; peptidoglycan builds bacterial cell walls (the target of penicillin); glycosaminoglycans give cartilage its spring.
4. **Parts of other biomolecules.** Ribose is part of RNA, ATP, NAD^+, FAD and coenzyme A; deoxyribose is part of DNA (Unit 4).
5. **Recognition and signalling.** Sugar chains on proteins and lipids act as tags read by other proteins: the ABO blood groups, influenza virus attaching to sialic acid on our cells, and white blood cells rolling to a site of infection all depend on them (Chapters 1.7 and 1.9).
6. **Protection and lubrication.** Hyaluronic acid lubricates joints; mucins in mucus protect the gut and airways.

WORKED EXAMPLE

Question (8 marks). Classify each of the following, with a reason and one source or role: glyceraldehyde, dihydroxyacetone, ribose, fructose, sucrose, raffinose, glycogen, hyaluronic acid. Then find the energy in a meal with 60 g of digestible carbohydrate.

CARBOHYDRATE	CLASS	REASON	SOURCE OR ROLE
Glyceraldehyde	Aldotriose	3 C, aldehyde at C1	its phosphate is a glycolysis intermediate
Dihydroxyacetone	Ketotriose	3 C, ketone at C2, no chiral carbon	its phosphate is a glycolysis intermediate
Ribose	Aldopentose	5 C, aldehyde	RNA, ATP
Fructose	Ketohexose	6 C, ketone at C2	fruits, honey
Sucrose	Disaccharide	glucose + fructose	sugarcane, table sugar
Raffinose	Trisaccharide	galactose + glucose + fructose	pulses
Glycogen	Homopolysaccharide	only glucose, branched	energy store in liver and muscle
Hyaluronic acid	Heteropolysaccharide	two kinds of unit (glucuronic acid, N-acetylglucosamine)	lubricates joints

1. Energy = mass × energy per gram = 60 g × 17 kJ/g = **1020 kJ**, about 1.0 MJ.
2. In kilocalories: 60 g × 4 kcal/g = **240 kcal**.

Examiners give marks for the reason column: the class alone earns half.

EXAM TRAPS

- "Carbohydrate = $(CH_2O)_n$" is not a definition: deoxyribose breaks it, and lactic acid fits it without being a carbohydrate.
- Not every carbohydrate is sweet. "Sugar" usually means mono- and disaccharides; starch and cellulose are tasteless.
- Dihydroxyacetone has no chiral carbon, so it has no D and L forms; glyceraldehyde is the smallest chiral sugar.
- Homo- and heteropolysaccharide refer to the kinds of unit, not to branching or bond type.
- Glycogen is often called "animal starch", but it is more branched than amylopectin (Chapter 1.6).

CHECK YOURSELF

– REMEMBER **Define a monosaccharide and give one aldotriose and one ketotriose.** 2 marks

A monosaccharide is a simple sugar that cannot be hydrolysed into smaller carbohydrates. Aldotriose: glyceraldehyde. Ketotriose: dihydroxyacetone.

– REMEMBER **Name two storage and two structural polysaccharides.** 2 marks

Storage: starch, glycogen (also inulin). Structural: cellulose, chitin (also peptidoglycan).

– UNDERSTAND **Why do pulses such as rajma cause gas when rice does not?** 2 marks

Pulses contain raffinose and related oligosaccharides that our small intestine cannot digest. They reach the colon, where bacteria ferment them and release gases. Rice starch is digested and absorbed before it gets there.

– APPLY **Classify sedoheptulose (7 carbons, ketone at C2) and 2-deoxyribose.** 2 marks

Sedoheptulose is a ketoheptose. 2-Deoxyribose is a derived sugar: an aldopentose lacking the –OH at C2.

– APPLY **A glass of sweet lassi has 30 g of sugar. How much energy is that in kJ and kcal?** 2 marks

30 × 17 = **510 kJ**; 30 × 4 = **120 kcal**.

– ANALYZE **Why do red blood cells depend entirely on glucose, while muscle can also burn fat?** 3 marks

Red blood cells have no mitochondria, so they cannot oxidise fatty acids or run the citric acid cycle. Glycolysis, which needs glucose, is their only source of ATP. Muscle has mitochondria and can oxidise fatty acids.

BEYOND THE SYLLABUS

Glycobiology studies the full set of sugars a cell makes, its "glycome", using mass spectrometry and glycan arrays. The CAZy database classifies the enzymes that make and break carbohydrates, and biorefineries use such enzymes to turn crop waste into fuels and chemicals (see the Environmental Biotechnology and Bioenergy subject). Dietary fibre research now focuses on how gut bacteria ferment polysaccharides into short-chain fatty acids. Search next for "glycome", "CAZy" and "dietary fibre gut microbiome". None of this is for the exam.

1.4 Monosaccharides: structure, isomerism and mutarotation

A monosaccharide is a chain of 3 to 7 carbons carrying one aldehyde or ketone group and several –OH groups. Most of its carbons are chiral, so each sugar has many stereoisomers, and in water the chain closes into a ring. Ring closure creates one more chiral centre, the anomeric carbon, whose two forms (α and β) slowly interconvert. This chapter teaches you to draw a sugar three ways (Fischer, Haworth, chair) and to name every kind of isomer an examiner asks about.

You need: chirality, configuration and conformation (Chapter 1.1); aldoses and ketoses (Chapter 1.3).

Fischer projections and the D/L system

In a Fischer projection the carbon chain is drawn vertically with C1, the most oxidised end, at the top. Horizontal bonds point towards you and vertical bonds away. The reference is glyceraldehyde, the simplest chiral sugar: in D-glyceraldehyde the –OH on the chiral carbon is on the right; in L-glyceraldehyde it is on the left.

For longer sugars, D or L is set by the chiral carbon farthest from the carbonyl group (C5 in glucose and fructose, C4 in ribose): –OH on the right means D. Almost all natural sugars are D.

The label says nothing about the direction of optical rotation, which is measured and written (+) for dextrorotatory or (–) for levorotatory. D-glucose is (+), but D-fructose is (–).

Kinds of isomers

Glucose, fructose, galactose and mannose all have the formula $C_6H_{12}O_6$, so they are isomers. The relationships to know are:.

TYPE	MEANING	EXAMPLE
Structural (functional) isomers	same formula, different carbonyl group	glucose (aldose) and fructose (ketose)
Enantiomers	mirror images; every chiral centre inverted	D-glucose and L-glucose
Diastereomers	stereoisomers that are not mirror images	D-glucose and D-galactose
Epimers	diastereomers differing at only one chiral centre	glucose and mannose (C2); glucose and galactose (C4)
Anomers	epimers at the new centre made by ring closure	α- and β-D-glucopyranose

A molecule with n chiral centres has at most 2^n stereoisomers. An open-chain aldohexose has four (C2 to C5), so there are $2^4 = 16$ aldohexoses, 8 D and 8 L. A ketohexose has three (C3 to C5), giving $2^3 = 8$. Mannose and galactose differ at two centres (C2 and C4), so they are diastereomers but not epimers.

Ring formation: pyranoses, furanoses and anomers

An aldehyde reacts with an alcohol to give a hemiacetal; a ketone gives a hemiketal. Inside a sugar the alcohol is the molecule's own –OH. In glucose the C5–OH attacks the C1 aldehyde, closing a six-membered ring of five carbons and one oxygen. Six-membered sugar rings are **pyranoses** (after pyran) and five-membered ones are **furanoses** (after furan). Fructose forms a furanose when its C5–OH attacks the C2 ketone, the form found in sucrose (Chapter 1.5).

Ring closure turns the carbonyl carbon (C1 of glucose) into a new chiral centre, the **anomeric carbon**. In the Haworth projection, a flat hexagon seen edge-on with the ring oxygen at the back right, every group on the right in the Fischer projection points down, and the CH_2OH (C6) of a D-sugar points up. The anomer with the C1–OH down, opposite the CH_2OH, is **α**; with C1–OH up, on the same side, it is **β** (Figure 1.4.1).

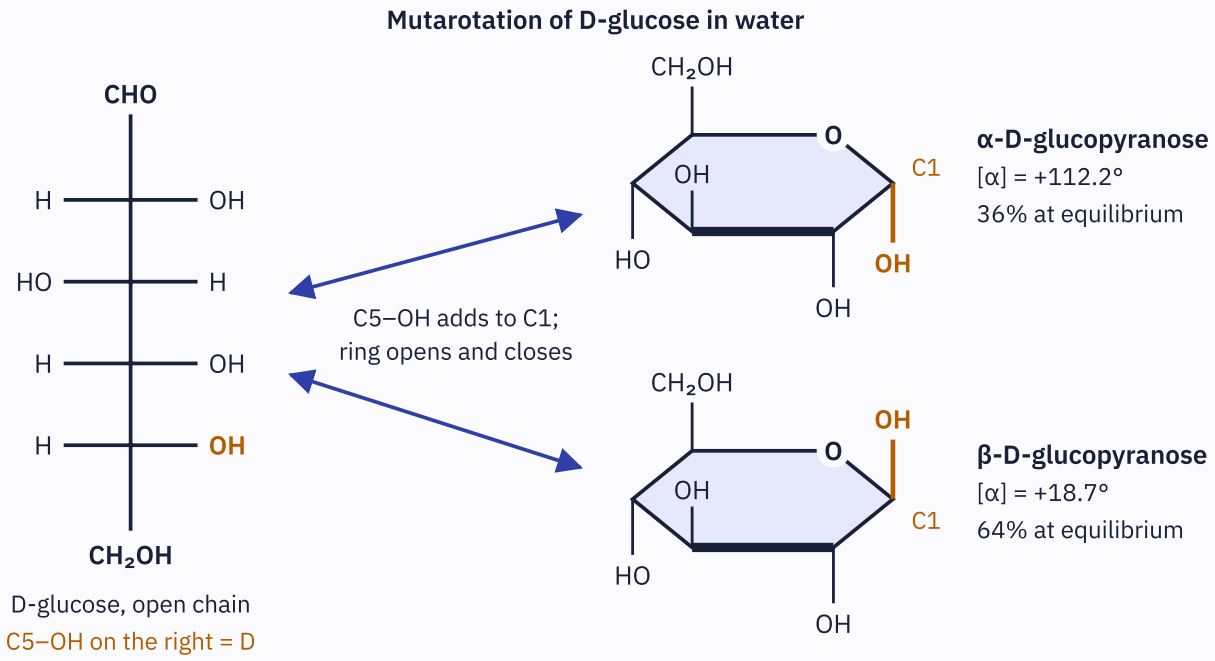


Figure 1.4.1 The open chain of D-glucose closes into two rings that differ only at C1, the anomeric carbon (highlighted). Groups on the right in the Fischer projection point down in the Haworth ring; ring hydrogens are left out for clarity. Notice that α has the C1–OH on the opposite side to CH_2OH and β on the same side; in water they interconvert through the open chain until the mixture reaches +52.7°.

Mutarotation and conformation

Crystals of pure α-D-glucose dissolved in water have a specific rotation of +112.2°; pure β-D-glucose gives +18.7°. With time both solutions drift to the same value, +52.7°. This change in optical rotation is **mutarotation**. It happens because the ring opens to the open-chain aldehyde and closes again, either way, until the anomers reach equilibrium. The open chain is only a tiny fraction (well under 1%). Acid and base speed mutarotation up, and cells have an enzyme for it, mutarotase. Only sugars with a free anomeric carbon mutarotate; in a glycoside or in sucrose the anomeric carbon is locked (Chapter 1.5).

Haworth rings are flat for convenience, but a real pyranose ring is puckered into a **chair**. In the chair each ring carbon has one axial bond (parallel to the ring axis) and one equatorial bond (pointing outwards), and bulky groups are more stable equatorial. In β-D-glucopyranose every bulky group (four –OH and the CH_2OH) is equatorial, one reason β is the main form at equilibrium. In α the C1–OH is axial; a stereo-electronic effect called the anomeric effect partly stabilises an axial group at C1, which is why α still makes up about a third. Flipping between chairs changes conformation, not configuration.

WORKED EXAMPLE

Question (5 marks). Fresh solutions of α-D-glucose ([α] = +112.2°) and β-D-glucose ([α] = +18.7°) both change to +52.7°. (a) Name and explain the change. (b) Calculate the percentage of each anomer at equilibrium, ignoring the open chain. (c) What does the answer say about stability?

1. (a) Mutarotation: each anomer opens to the aldehyde form and recloses as either anomer until equilibrium.
2. (b) Let x be the fraction of α. The observed rotation is the weighted average: 112.2x + 18.7(1 − x) = 52.7.
3. Rearrange: 112.2x − 18.7x = 52.7 − 18.7, so 93.5x = 34.0 and x = 0.364.
4. So α = **36.4%** and β = **63.6%**. In general, fraction α = ([α]eq − [α]β) / ([α]α − [α]β).
5. (c) β is favoured, because in its chair all bulky groups are equatorial.

EXAM TRAPS

- D does not mean dextrorotatory: D-fructose is levorotatory. D/L is read from the highest-numbered chiral carbon (C5 in hexoses), not from C2.
- Mannose and galactose are not epimers: they differ at two centres.
- Mutarotation is not racemisation. D-glucose stays D-glucose; only C1 changes.
- Ring closure adds a chiral centre: the open-chain aldohexose has 4, the pyranose has 5 (2^5 = 32 stereoisomers).
- For D-sugars in Haworth, α means the anomeric –OH is down, trans to the CH_2OH; β means up.

CHECK YOURSELF

– REMEMBER **Define epimers and anomers, with one example each.** 2 marks

Epimers differ in configuration at only one chiral carbon (D-glucose and D-galactose at C4). Anomers are epimers at the anomeric carbon created by ring closure (α- and β-D-glucopyranose).

– UNDERSTAND **In which direction do the –OH groups at C2, C3 and C4 of D-glucose point in the Haworth ring?** 2 marks

Right in Fischer becomes down in Haworth: C2–OH down, C3–OH up (it was on the left), C4–OH down; the CH_2OH at C5 points up.

– UNDERSTAND **Why does the rotation of a fresh β-D-glucose solution rise from +18.7° to +52.7°?** 2 marks

Some β opens to the aldehyde and recloses as α, which rotates light far more (+112.2°). The rotation rises until the α:β ratio reaches equilibrium (about 36:64).

– APPLY **How many stereoisomers are possible for (a) an aldopentose, (b) a ketopentose, (c) a glucopyranose?** 3 marks

(a) 3 chiral centres: 2^3 = **8**. (b) 2 chiral centres: 2^2 = **4**. (c) 5 chiral centres: 2^5 = **32**.

– APPLY **A hexose has [α] = +150.7° for its α anomer and +52.8° for β; its equilibrium value is +80.2°. Find the percentage of α.** 3 marks

Fraction α = (80.2 − 52.8)/(150.7 − 52.8) = 27.4/97.9 = 0.28, so **28% α** and 72% β.

– ANALYZE **Why is the anomeric carbon C1 in glucose but C2 in fructose?** 2 marks

The anomeric carbon is the former carbonyl carbon, which becomes the hemiacetal or hemiketal carbon on ring closure. Glucose's aldehyde is at C1; fructose's ketone is at C2.

– ANALYZE **β-D-glucopyranose is the most abundant form of glucose in water. Explain with conformation.** 3 marks

In the $^4C_1$ chair of β-D-glucopyranose all five bulky substituents are equatorial, so there are no crowded axial groups. In α the C1–OH is axial, which costs some stability, only partly offset by the anomeric effect.

BEYOND THE SYLLABUS

Chemists assign α and β by NMR: the coupling between H1 and H2 is larger in β, where both are axial, than in α. Rare sugars are now made by enzymes that change one centre at a time; D-allulose, the C3 epimer of D-fructose, is produced this way as a low-calorie sweetener. The anomeric effect remains a lively topic in physical organic chemistry. Search next for "anomeric effect", "rare sugars Izumoring" and "carbohydrate NMR". None of this is for the exam.

1.5 Disaccharides and the glycosidic bond

Two monosaccharides join when the anomeric –OH of one condenses with an –OH of the other, releasing water. The new link, the glycosidic bond, is an acetal. Which carbons it joins, and whether it is α or β, decide everything else about the disaccharide: its name, whether our enzymes can cut it, and whether it is a reducing sugar.

You need: the anomeric carbon, α and β (Chapter 1.4).

Making and naming the glycosidic bond

A sugar ring is a hemiacetal: its anomeric carbon carries both the ring oxygen and an –OH. When that anomeric –OH reacts with an –OH of another molecule, water is lost and the anomeric carbon now carries two –OR groups. It has become an acetal, and the new link is an **O-glycosidic bond**; the product is a **glycoside**. When the partner is another sugar, the product is a disaccharide. When the nitrogen of a base takes the place of the –OH, the bond is an N-glycosidic bond, as in nucleosides (Unit 4). How cells and chemists actually make the bond is Chapter 1.9.

Two consequences follow. The anomeric carbon in the bond is locked: it can no longer open to the aldehyde, so that unit cannot mutarotate or reduce Cu^{2+}. And its configuration is fixed as α or β, which enzymes recognise exactly. To name a disaccharide:

1. Start with the unit whose anomeric carbon is in the bond (the non-reducing end), written on the left.
2. Give its configuration at the anomeric carbon (α or β) and, in full names, its ring size (pyranose or furanose).
3. Give the two carbons joined, the arrow pointing from the anomeric carbon: (1→4).
4. Name the second unit. If both anomeric carbons are in the bond, use a double arrow (↔) and give both configurations.

Maltose is therefore Glc(α1→4)Glc, in full α-D-glucopyranosyl-(1→4)-D-glucopyranose. Sucrose is Glc(α1↔2β)Fru, or β-D-fructofuranosyl α-D-glucopyranoside.

The disaccharides to know

DISACCHARIDE	UNITS AND LINK	REDUCING?	SOURCE OR USE
Maltose	Glc(α1→4)Glc	yes	starch digestion; malt from germinating barley

DISACCHARIDE	UNITS AND LINK	REDUCING?	SOURCE OR USE
Isomaltose	Glc(α1→6)Glc	yes	branch points of starch and glycogen
Cellobiose	Glc(β1→4)Glc	yes	breakdown of cellulose
Lactose	Gal(β1→4)Glc	yes	milk sugar
Sucrose	Glc(α1↔2β)Fru	no	sugarcane and beet; table sugar
Trehalose	Glc(α1↔1α)Glc	no	insects and fungi; protects cells from drying
Lactulose	Gal(β1→4)Fru	yes	forms when milk is heated; a laxative

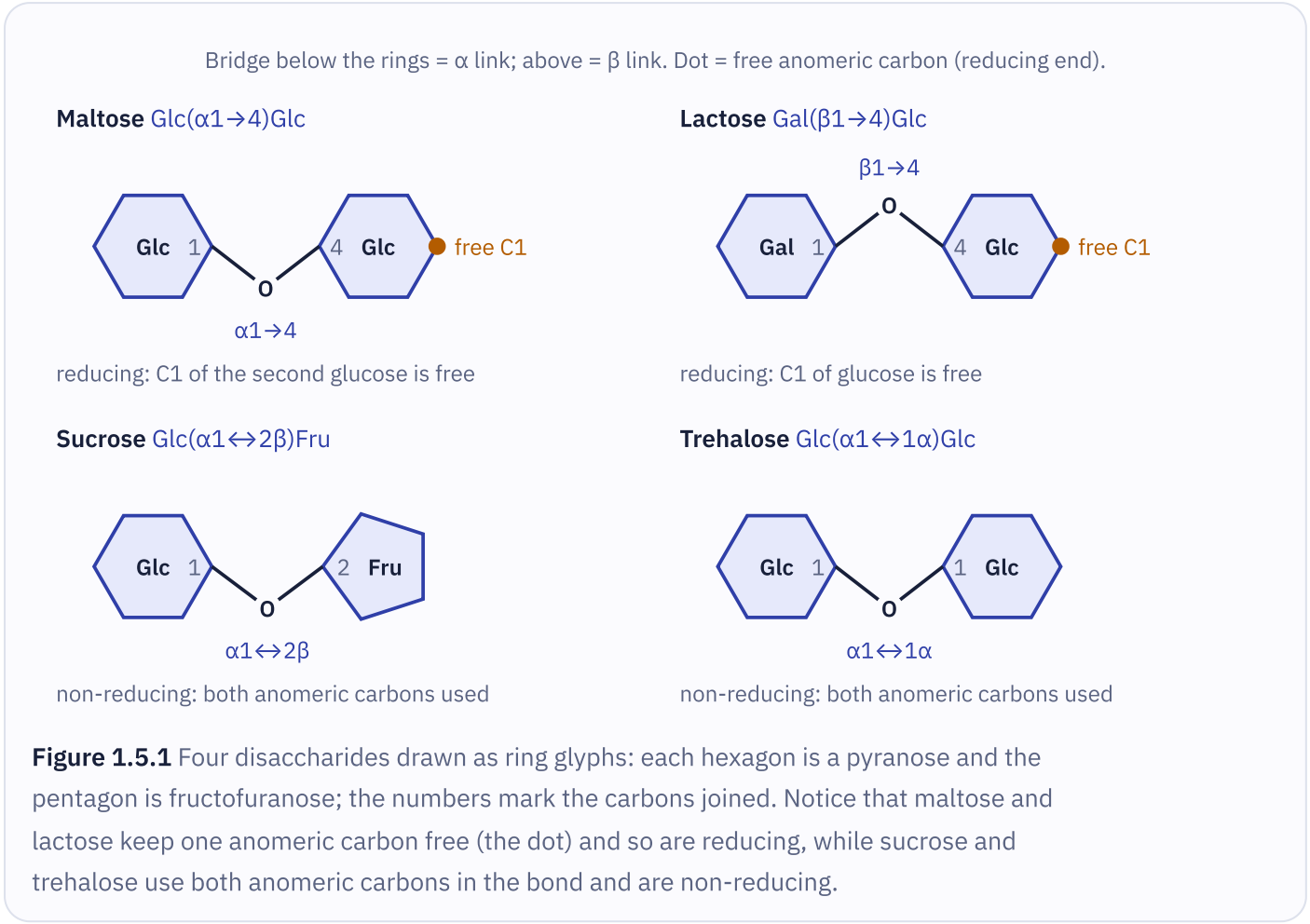


Figure 1.5.1 Four disaccharides drawn as ring glyphs: each hexagon is a pyranose and the pentagon is fructofuranose; the numbers mark the carbons joined. Notice that maltose and lactose keep one anomeric carbon free (the dot) and so are reducing, while sucrose and trehalose use both anomeric carbons in the bond and are non-reducing.

Hydrolysis: cutting the bond

Glycosidic bonds are stable at neutral pH but are hydrolysed by hot dilute acid or by enzymes called glycosidases, each strictly specific for α or β. In the small intestine maltase cuts maltose, sucrase cuts sucrose and lactase (a β-galactosidase) cuts lactose. We have no enzyme for the β1→4 link between glucoses, which is why cellulose passes through us as fibre (Chapter 1.6).

Many adults make much less lactase than they did as children. Undigested lactose then reaches the colon, where bacteria ferment it and cause gas and bloating: this is lactose intolerance. Curd

and yogurt are easier on such people because the bacteria that set them have already used part of the lactose, and lactose-free milk is made by adding the lactase enzyme.

Hydrolysis of sucrose gives an equal mixture of glucose and fructose called **invert sugar**, because the sign of optical rotation inverts from + to −. The enzyme that does it is invertase (sucrase). Honey is largely invert sugar, made by the bees' own invertase; it is sweeter than sucrose and resists crystallising, so confectioners use it.

Artificial sweeteners

Some are sugar derivatives and some are not. **Sucralose** is sucrose with three –OH groups replaced by chlorine atoms; our sucrase cannot cut it, so it passes through with almost no calories. **Steviol glycosides** from the leaves of *Stevia rebaudiana* are glycosides in the strict sense: sugar units attached by glycosidic bonds to a non-sugar part. **Aspartame** is not a sugar but a dipeptide methyl ester (aspartyl-phenylalanine); because it releases phenylalanine, its labels warn people with phenylketonuria. Saccharin and acesulfame K are unrelated synthetic compounds. The US FDA gives their sweetness relative to sucrose as about 200 times for aspartame and acesulfame K, 200 to 400 for steviol glycosides, 200 to 700 for saccharin, and 600 for sucralose.

WORKED EXAMPLE

Question (6 marks). 34.2 g of sucrose (342.3 g/mol) is dissolved to 100 mL and completely hydrolysed by invertase. Specific rotations: sucrose +66.5°, glucose +52.7°, fructose −92.4°. (a) Write the reaction and find the mass of products. (b) Find the observed rotation in a 1 dm tube before and after. (c) Explain the name "invert sugar".

1. Sucrose + H_2O → glucose + fructose. Moles of sucrose = 34.2/342.3 = 0.100 mol, giving 0.100 mol of glucose (18.0 g) and 0.100 mol of fructose (18.0 g). The products weigh 36.0 g; the extra 1.8 g is the water used (0.100 × 18.02).
2. Observed rotation $\alpha = [\alpha] \times l \times c$, where $[\alpha]$ is the specific rotation, l the tube length in dm and c the concentration in g/mL.
3. Before: c = 34.2 g/100 mL = 0.342 g/mL, so $\alpha = +66.5 \times 1 \times 0.342 =$ **+22.7°**.
4. After: glucose at 0.180 g/mL gives +52.7 × 0.180 = +9.49°; fructose at 0.180 g/mL gives −92.4 × 0.180 = −16.63°. Total α = **−7.14°**.
5. The sign changed from + to −, so the mixture is "inverted". Its specific rotation is (52.7 − 92.4)/2 = −19.9°.

EXAM TRAPS

- Sucrose is non-reducing because both anomeric carbons are in the bond. Lactose is reducing even though its link is β1→4: the C1 of its glucose is free.
- Maltose, isomaltose, cellobiose and trehalose are all two glucoses; only the link differs. Learn them by the link.
- A glycoside is any sugar acetal (methyl glucoside, steviol glycosides), not only a disaccharide.
- "Inversion" in invert sugar means the sign of optical rotation changes, not a change of configuration at a carbon.
- Aspartame is a dipeptide, not a carbohydrate.

CHECK YOURSELF

– REMEMBER **Give the units and the linkage of lactose and of sucrose.** 2 marks

Lactose: galactose and glucose, Gal(β1→4)Glc. Sucrose: glucose and fructose, Glc(α1↔2β)Fru.

– UNDERSTAND **Why does maltose show mutarotation but sucrose does not?** 2 marks

Maltose has one free anomeric carbon (on the second glucose) whose ring can open and reclose as α or β. In sucrose both anomeric carbons are locked in the glycosidic bond, so no ring can open.

– APPLY **Name Glc(β1→4)Glc and say whether it is reducing.** 2 marks

Cellobiose, the repeating unit released from cellulose. It is reducing, because the C1 of the second glucose is free.

– APPLY **Which of maltose, sucrose, trehalose, lactose and cellobiose reduce Benedict's reagent?** 2 marks

Maltose, lactose and cellobiose (one free anomeric carbon each). Sucrose and trehalose do not.

– ANALYZE **Starch and cellulose both give glucose on acid hydrolysis, yet we digest only starch. Explain at the level of the bond.** 3 marks

Starch has α1→4 (and α1→6) links, which our amylases and maltase recognise. Cellulose has β1→4 links; enzymes are specific for the anomeric configuration and humans make no β1→4 glucosidase (cellulase). Hot acid is not specific, so it cuts both.

– ANALYZE **Why can many lactose-intolerant people eat curd without trouble?** 2 marks

The lactic acid bacteria that set curd ferment part of the lactose before it is eaten, so less reaches the colon; the live bacteria also carry β-galactosidase that keeps acting in the gut.

BEYOND THE SYLLABUS

Glycosidases are grouped into families by sequence in the CAZy database, and engineered ones now run in reverse to make new sugars. Food companies use β-galactosidase to turn lactose into galacto-oligosaccharides, prebiotics that feed beneficial gut bacteria, and human milk oligosaccharides such as 2′-fucosyllactose are now made by fermentation for infant formula. Search next for "galacto-oligosaccharides", "human milk oligosaccharides" and "glycoside hydrolase families". None of this is for the exam.

1.6 Polysaccharides: starch, glycogen, cellulose and chitin

Polysaccharides are long chains of sugar units, and a small change in the glycosidic bond changes everything about them. α1→4 links make chains that coil and pack into compact, digestible fuel stores (starch and glycogen); β1→4 links make straight chains that stack into tough, insoluble fibres (cellulose and chitin).

You need: α and β glycosidic links and reducing ends (Chapter 1.5).

Starch: amylose and amylopectin

Starch is the fuel store of plants, packed into granules in the seeds, tubers and roots we eat (rice, wheat, potato, tapioca). It is a mixture of two glucose polymers. **Amylose** is unbranched: D-glucose units joined only by α1→4 links, from a few hundred to several thousand per chain. The α link puts a bend at every unit, so the chain coils into a helix with about six glucose units per turn; iodine slips inside the helix and gives the deep blue-black colour of the starch test. **Amylopectin** has the same α1→4 chains plus α1→6 branch points about every 24 to 30 units. It is much larger than amylose and gives a red-violet colour with iodine. Most starches are roughly 20 to 30% amylose; "waxy" types such as sticky rice are almost all amylopectin.

Digestion starts in the mouth with salivary α-amylase and continues with pancreatic α-amylase. Both cut internal α1→4 links, giving maltose, maltotriose and branched fragments called limit dextrins; enzymes on the intestinal lining finish the job, including the α1→6 branches. Cooking

matters. Heating starch in water makes the granules swell and lose their order (gelatinisation), which makes them easy to digest. On cooling, amylose chains slowly pair up again (retrogradation), and part of the starch in cooled rice or bread then resists digestion.

Glycogen

Glycogen is the glucose store of animals, fungi and bacteria. Like amylopectin it is an α1→4 chain with α1→6 branches, but it branches more often, about every 8 to 12 units, and is more compact. It lies as granules in the cytosol, mainly of liver and skeletal muscle, and each molecule grows on a primer protein, glycogenin. Liver glycogen keeps blood glucose up between meals; muscle glycogen fuels the muscle itself.

Branching is a design for speed. A branched polymer has one reducing end but many non-reducing ends, one more than its number of branch points (Figure 1.6.1), and the enzyme glycogen phosphorylase removes glucose from the non-reducing ends. More ends mean more places to work at once, so glucose is released fast in a sprint or when a meal is late. Branching also makes glycogen more soluble. Storing glucose as one giant molecule instead of millions of free ones keeps the osmotic pressure of the cell low, as the worked example shows.

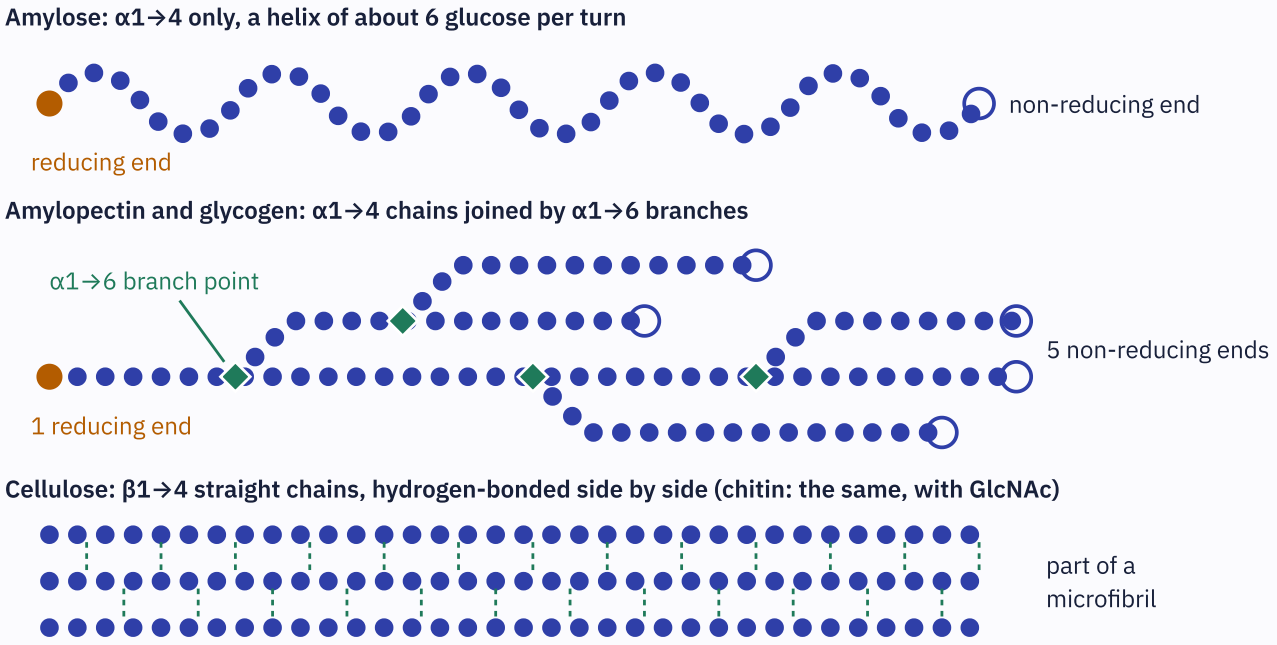


Figure 1.6.1 Each dot is one glucose unit. Amylose (top) is a single coiled chain. Amylopectin and glycogen (middle) branch through α1→6 links (diamonds); notice that four branch points give five non-reducing ends (rings) but still only one reducing end. Cellulose chains (bottom) are straight and hydrogen-bonded (dashes) into microfibrils.

Cellulose

Cellulose is the most abundant organic polymer on Earth: the main material of plant cell walls, almost all of a cotton fibre, and a large part of wood (with lignin and hemicellulose). It is an unbranched chain of 10,000 to 15,000 D-glucose units joined by β1→4 links. The β link turns each glucose 180° relative to its neighbour, so the chain is straight and flat rather than helical. Hydrogen bonds within each chain and between neighbouring chains lock parallel chains into crystalline microfibrils with great tensile strength, and water cannot get in, so cellulose is insoluble.

Humans make no enzyme that cuts the β1→4 link (cellulase). Cellulose therefore passes through us as dietary fibre, which adds bulk and feeds gut bacteria. Cows, goats and termites digest it only because microbes in their gut make cellulases. Industry uses cellulose for paper, cotton and viscose textiles, and derivatives such as carboxymethylcellulose, a thickener in food and toothpaste.

Chitin

Chitin is cellulose's counterpart in animals and fungi: unbranched β1→4 chains of N-acetyl-D-glucosamine (GlcNAc), a glucose whose C2–OH is replaced by an acetamido group (–NH–CO–CH_3). The chains pack like cellulose into hydrogen-bonded sheets that build the hard exoskeletons of insects, crabs and prawns and the cell walls of fungi. After cellulose, it is the most abundant polysaccharide in nature. Hot alkali removes most acetyl groups to give chitosan, whose free amino groups become positively charged in dilute acid, so it dissolves there. Chitosan, made from prawn and crab shell waste, is used in wound dressings, water treatment and edible coatings.

POLYMER	UNIT	LINKS	BRANCHING	IODINE COLOUR	ROLE
Amylose	glucose	α1→4	none	blue-black	plant fuel store
Amylopectin	glucose	α1→4, α1→6	every 24 to 30	red-violet	plant fuel store
Glycogen	glucose	α1→4, α1→6	every 8 to 12	red-brown	animal fuel store
Cellulose	glucose	β1→4	none	none	plant cell wall
Chitin	GlcNAc	β1→4	none	none	exoskeleton, fungal wall

WORKED EXAMPLE

Question (6 marks). A liver cell stores glycogen equivalent to 0.40 M glucose, while the glycogen molecules themselves are about 0.01 μM. (a) Using the van 't Hoff equation, find the osmotic pressure at 37 °C due to (i) 0.40 M free glucose and (ii) 0.01 μM glycogen. (b) A glycogen molecule of 6,000 glucose units branches every 10 units. How many non-reducing ends does it have, and why does that matter?

1. The van 't Hoff equation is $\Pi = icRT$, where Π is osmotic pressure, i the number of particles per molecule (1 for glucose), c the molar concentration, R = 0.08314 L·bar/(mol·K) and T the absolute temperature (37 °C = 310 K).
2. (i) $\Pi = 0.40 \times 0.08314 \times 310 =$ **10.3 bar**, about ten times atmospheric pressure: enough to pull in water and burst the cell.
3. (ii) 0.01 μM = 1.0×10^{-8} M, so $\Pi = 1.0 \times 10^{-8} \times 0.08314 \times 310 =$ **2.6×10^{-7} bar**, negligible. The osmotic load falls $0.40/10^{-8} = 4 \times 10^{7}$-fold.
4. (b) Branch points ≈ 6,000/10 = 600, so non-reducing ends = 600 + 1 = **601**, with a single reducing end.
5. Glycogen phosphorylase works at non-reducing ends, so about 600 ends can release glucose at the same time: fast mobilisation in exercise or fasting.

EXAM TRAPS

- Amylose is unbranched. Amylopectin branches every 24 to 30 units and glycogen every 8 to 12; do not swap the numbers.
- Starch and cellulose are both glucose polymers. The difference is the α or β link, not the monomer.
- Chitin's unit is N-acetylglucosamine, not glucose.
- However large, a polysaccharide has only one reducing end, so it gives a weak or negative reducing-sugar test.
- Iodine colours: amylose blue-black, amylopectin red-violet, glycogen red-brown.

CHECK YOURSELF

– REMEMBER **Give the glycosidic links in amylose, amylopectin and cellulose.** 3 marks

Amylose: α1→4 only. Amylopectin: α1→4 chains with α1→6 branches. Cellulose: β1→4 only.

– UNDERSTAND **Why is a cellulose chain straight while amylose coils into a helix?** 2 marks

In the β1→4 link each glucose is turned 180° to its neighbour, so the chain runs straight and can hydrogen bond to parallel chains. The α1→4 link puts the same bend at every unit, so the chain curls into a helix.

– UNDERSTAND **Why does chitosan dissolve in dilute acetic acid while chitin does not?** 2 marks

Chitosan has free $-NH_2$ groups that take up H^+ to become $-NH_3^+$; the charged chains repel one another and hydrate. Chitin's acetylated amides do not ionise, and its chains stay locked in hydrogen-bonded sheets.

– APPLY **An amylopectin molecule of 12,000 units branches every 25 units. Find its branch points, non-reducing ends and reducing ends.** 3 marks

Branch points = 12,000/25 = **480**; non-reducing ends = 480 + 1 = **481**; reducing ends = **1**.

– APPLY **Predict the iodine colour for potato starch, liver glycogen and filter paper.** 2 marks

Potato starch: blue-black (from its amylose). Glycogen: red-brown (short branched chains form no long helix). Filter paper is cellulose: no colour.

– ANALYZE **Glycogen branches more often than amylopectin. Relate this to the needs of animals.** 3 marks

Animals must release glucose fast (running, fasting). More branches mean more non-reducing ends for phosphorylase to act on at once, and a more soluble, compact granule. A plant seed releases its starch slowly during germination, so fewer branches suffice.

BEYOND THE SYLLABUS

Food scientists breed and process grains for more "resistant starch", which raises blood glucose more slowly. Cellulose broken into nanometre-wide fibres (nanocellulose) makes strong, transparent films, and cellulase enzymes turn crop residues such as rice straw into second-generation ethanol (see the bioenergy subject). Inherited defects in the enzymes that make or break glycogen cause the glycogen storage diseases (Unit 6). Search next for "resistant starch", "nanocellulose" and "glycogen storage disease". None of this is for the exam.

1.7 Glycosaminoglycans, proteoglycans and glycoconjugates

Glycosaminoglycans (GAGs) are long, unbranched heteropolysaccharides built from one repeating disaccharide of an amino sugar and, usually, a uronic acid, most of them carrying sulfate groups. Their dense negative charge makes them stiff, extended and water-loving, which is why cartilage is springy and joint fluid is slippery. Attached to a core protein, GAGs form proteoglycans; together with glycoproteins and glycolipids these make up the glycoconjugates, the sugar-bearing molecules that coat every cell.

You need: heteropolysaccharides (Chapter 1.3); weak interactions and osmosis (Chapter 1.2).

What a glycosaminoglycan is

Each GAG repeats one disaccharide hundreds of times. One partner is an amino sugar: N-acetylglucosamine (GlcNAc), N-acetylgalactosamine (GalNAc) or, in heparin, N-sulfated glucosamine (GlcNS). The other is usually a uronic acid: D-glucuronic acid (GlcA), which is glucose with C6 oxidised to –COOH, or its C5 epimer L-iduronic acid (IdoA). Keratan sulfate is the exception, with galactose in place of the uronic acid. Sulfate esters ($–OSO_3^-$) on many sugars, plus the carboxylates ($–COO^-$), make GAGs the most negatively charged molecules in the body. Their old name, mucopolysaccharides, comes from the slimy feel of their solutions.

Charge explains function. Neighbouring negative groups repel, so each chain stretches out and sweeps a huge volume. The charges attract cations such as Na^+, and water follows by osmosis (Chapter 1.2), so GAGs form a hydrated gel. Squeeze the gel and water is pushed out; release it and water flows back in. That is the cushioning of cartilage. Hyaluronic acid adds slipperiness: its solutions are viscous at rest but thin out when sheared, ideal for a moving joint.

GAG	REPEATING DISACCHARIDE	SULFATED?	FOUND IN	ROLE
Hyaluronic acid	GlcA(β1→3)GlcNAc, repeats β1→4	no	joint fluid, vitreous humour, cartilage, skin	lubricant, shock absorber
Chondroitin sulfate	GlcA(β1→3)GalNAc-4S or -6S	yes	cartilage, tendon, bone	resists compression
Dermatan sulfate	IdoA(α1→3)GalNAc-4S	yes	skin, blood vessels, heart valves	flexibility; binds collagen
Keratan sulfate	Gal(β1→4)GlcNAc-6S, no uronic acid	yes	cornea, cartilage	corneal transparency

GAG	REPEATING DISACCHARIDE	SULFATED?	FOUND IN	ROLE
Heparin	IdoA-2S(α1→4)GlcNS-6S (main unit)	most of all	granules of mast cells	anticoagulant
Heparan sulfate	like heparin, fewer sulfates, more GlcA	yes	cell surfaces, basement membranes	binds growth factors

Two of these stand apart. **Hyaluronic acid** (hyaluronan) is the only GAG that is not sulfated and not bound covalently to a protein. It is also the largest, reaching millions of daltons, and is made at the plasma membrane rather than in the Golgi. **Heparin** is the most heavily sulfated. Mast cells store it in granules, and as a drug it prevents clotting by helping the plasma protein antithrombin switch off clotting enzymes. Dermatan sulfate is simply chondroitin sulfate in which some GlcA has been turned into IdoA. Its list misprints keratan sulfate as "keratin sulphate", and keratin is a protein.

Proteoglycans

A **proteoglycan** is a core protein carrying one or more covalently attached GAG chains; by mass it is mostly carbohydrate. Most chains join a serine of the core through a fixed linker, the tetrasaccharide GlcA–Gal–Gal–Xyl–Ser, with xylose bonded to the serine.

In cartilage the main proteoglycan is **aggrecan**. Each aggrecan core carries many chondroitin sulfate chains and shorter keratan sulfate chains, and many aggrecans bind along one hyaluronic acid chain, held there by a small link protein. The result is a huge bottle-brush aggregate (Figure 1.7.1) trapped in a mesh of collagen fibres: collagen resists stretching, the aggregate resists compression. Loss of aggrecan is an early event in osteoarthritis. Other proteoglycans sit on cell surfaces (syndecans span the membrane) or in basement membranes (perlecan), where their heparan sulfate chains bind growth factors and guide signalling.

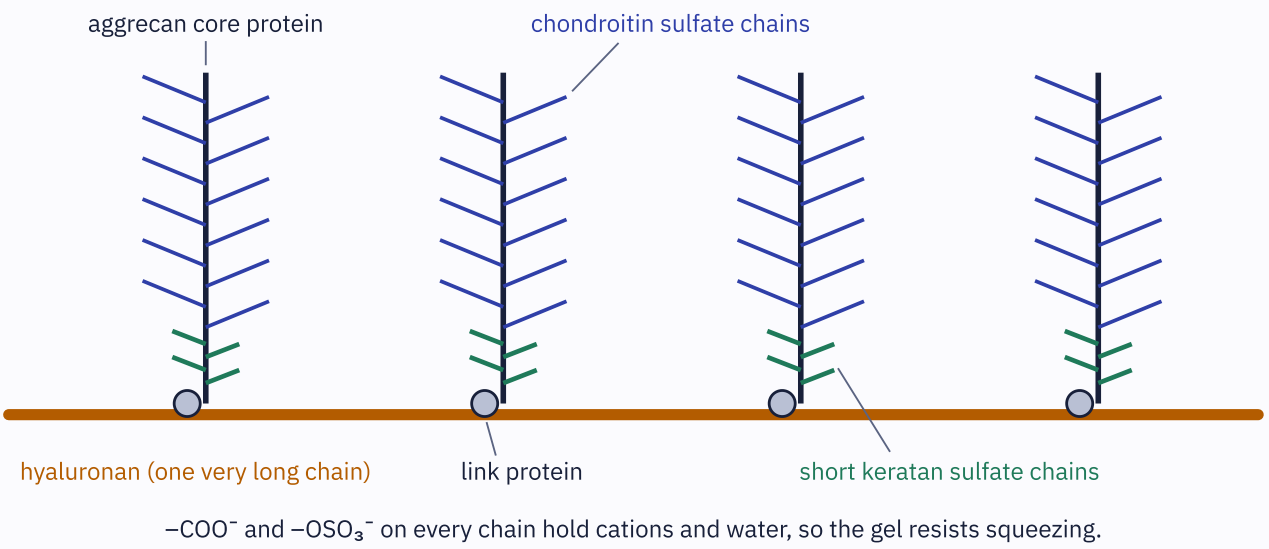


Figure 1.7.1 The proteoglycan aggregate of cartilage (not to scale, and with far fewer chains than the real thing). Aggrecan cores carry long chondroitin sulfate and short keratan sulfate chains and bind, through link proteins, along a single hyaluronan chain. Notice that the GAG chains, not the proteins, fill most of the space: that space is held open by charge and water.

Glycoconjugates: glycoproteins and glycolipids

Glycoconjugate is the umbrella term for any protein or lipid with sugars covalently attached. Proteoglycans are one kind. **Glycoproteins** are proteins carrying one or more short, often branched oligosaccharides (glycans). Most secreted and cell-surface proteins are glycoproteins, including antibodies, many hormones and the mucins of mucus, which carry so many sugars that they form a gel. Their glycans attach either to the amide nitrogen of asparagine (N-linked) or to the –OH of serine or threonine (O-linked); how cells add them is Chapter 1.9. **Glycolipids** are lipids with a sugar head: in animals mostly glycosphingolipids such as cerebrosides and gangliosides (Unit 3), and in Gram-negative bacteria the lipopolysaccharide of the outer membrane, which acts as endotoxin.

Do not mix up the two protein conjugates. A proteoglycan is mostly carbohydrate, with long, unbranched, repeating, sulfated GAG chains. A glycoprotein is mostly protein, with short, branched, non-repeating glycans. Glycans act as identity tags read by sugar-binding proteins called lectins: the ABO blood groups differ only in the last sugar of a glycan, influenza virus binds sialic acid on our airway cells, and selectins on blood-vessel walls catch the glycans of white blood cells to slow them at a site of infection. GAGs are broken down step by step in lysosomes, and an inherited lack of one of these enzymes makes them pile up in tissues, as in the mucopolysaccharidoses such as Hurler syndrome (Unit 6).

WORKED EXAMPLE

Question (6 marks). At pH 7 each carboxyl and each sulfate group carries one negative charge; N-acetyl groups are uncharged. (a) Find the charge per repeating disaccharide of hyaluronate, chondroitin 4-sulfate, keratan sulfate (one sulfate) and the main heparin unit (IdoA-2-sulfate with N-sulfated, 6-sulfated glucosamine). (b) Find the charge of a heparin chain of 20 such units. (c) Explain why protamine, a small arginine-rich protein, reverses heparin after heart surgery.

1. Hyaluronate: GlcA (−1, carboxylate) + GlcNAc (0) = **−1**.
2. Chondroitin 4-sulfate: GlcA (−1) + GalNAc-4-sulfate (−1) = **−2**.
3. Keratan sulfate: Gal (0) + GlcNAc-6-sulfate (−1) = **−1**; there is no uronic acid.
4. Heparin: IdoA-2-sulfate (−1 carboxylate, −1 sulfate) + GlcNS-6-sulfate (−1 N-sulfate, −1 O-sulfate) = **−4**.
5. (b) 20 units × (−4) = **−80** charges on one chain.
6. (c) Arginine side chains are positive at pH 7. Protamine binds heparin by ionic attraction, neutralises its charge and stops it from helping antithrombin.

Ranking by charge per unit: heparin, then chondroitin and dermatan sulfate, then hyaluronate and keratan sulfate.

EXAM TRAPS

- Keratan sulfate, not "keratin sulfate": keratin is a protein of hair and nails.
- Hyaluronic acid is the only GAG that is unsulfated and not covalently bound to a core protein.
- Heparin and heparan sulfate are different: heparin is more sulfated and stored in mast cells; heparan sulfate sits on cell surfaces.
- Proteoglycan is not glycoprotein: mostly sugar with long repeating chains, against mostly protein with short branched glycans.
- Dermatan sulfate contains L-iduronic acid, the C5 epimer of D-glucuronic acid.

CHECK YOURSELF

– REMEMBER **Name the two sugars of the hyaluronic acid repeat, and the one GAG that has no uronic acid.** 2 marks

D-glucuronic acid and N-acetyl-D-glucosamine. Keratan sulfate has galactose instead of a uronic acid.

– UNDERSTAND **Why are GAG solutions viscous and slippery?** 2 marks

The chains are long and stiffened by repelling negative charges, so they occupy a large volume and trap water, giving a viscous gel. Under shear the chains line up, so the fluid thins and lubricates.

– UNDERSTAND **Give three differences between a proteoglycan and a glycoprotein.** 3 marks

Proteoglycan: mostly carbohydrate; long unbranched GAG chains of repeating disaccharides; usually sulfated. Glycoprotein: mostly protein; short branched glycans with no repeating unit; sulfate rare.

– APPLY **What is the charge of a chondroitin 4-sulfate chain of 50 disaccharide units at pH 7?** 2 marks

Each unit is −2 (one carboxylate, one sulfate), so 50 × (−2) = **−100**.

– ANALYZE **Explain why cartilage resists compression and then recovers its shape.** 3 marks

Aggrecan aggregates pack fixed negative charges into the collagen mesh. These hold cations and water, creating a swelling pressure. Compression squeezes water out against this pressure; when the load is removed, the charges draw water back and the tissue re-swells.

– ANALYZE **Many invasive bacteria secrete hyaluronidase, called a "spreading factor". Explain the name.** 2 marks

The enzyme cuts the hyaluronan that keeps connective tissue a thick gel. As the gel thins, bacteria and their toxins move through the tissue more easily.

BEYOND THE SYLLABUS

Heparin for medicine is still extracted from pig intestine. In 2008, batches contaminated with an oversulfated chondroitin sulfate harmed patients, and regulators then added tests such as NMR to tell real heparin from look-alikes. Fondaparinux, a fully synthetic copy of the five-sugar heparin sequence that binds antithrombin, shows that chemical synthesis (Chapter 1.9) can replace an animal product. Hyaluronic acid is used in knee injections, eye surgery and cosmetic fillers. Search next for "heparin contamination 2008", "fondaparinux" and "glycocalyx". None of this is for the exam.

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.

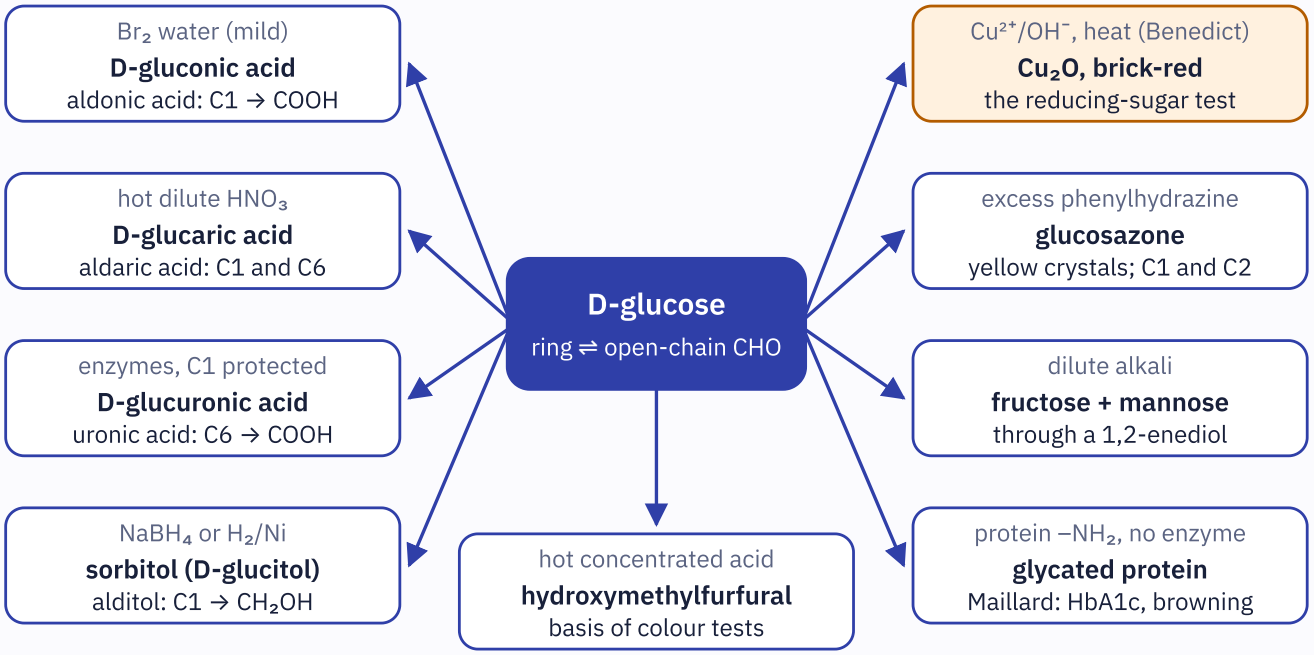


Figure 1.8.1 The main reactions of glucose: oxidations and the reduction on the left, reactions of the open-chain carbonyl on the right, dehydration below. The top line of each box is the reagent. Notice that most of these reactions act on the carbonyl group, which is free only when the anomeric carbon 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)
Bial	orcinol, HCl, $FeCl_3$; heat	blue-green	pentose
Iodine	I_2 in KI	blue-black; red-brown	starch; glycogen

TEST	REAGENT	POSITIVE RESULT	SHOWS
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.

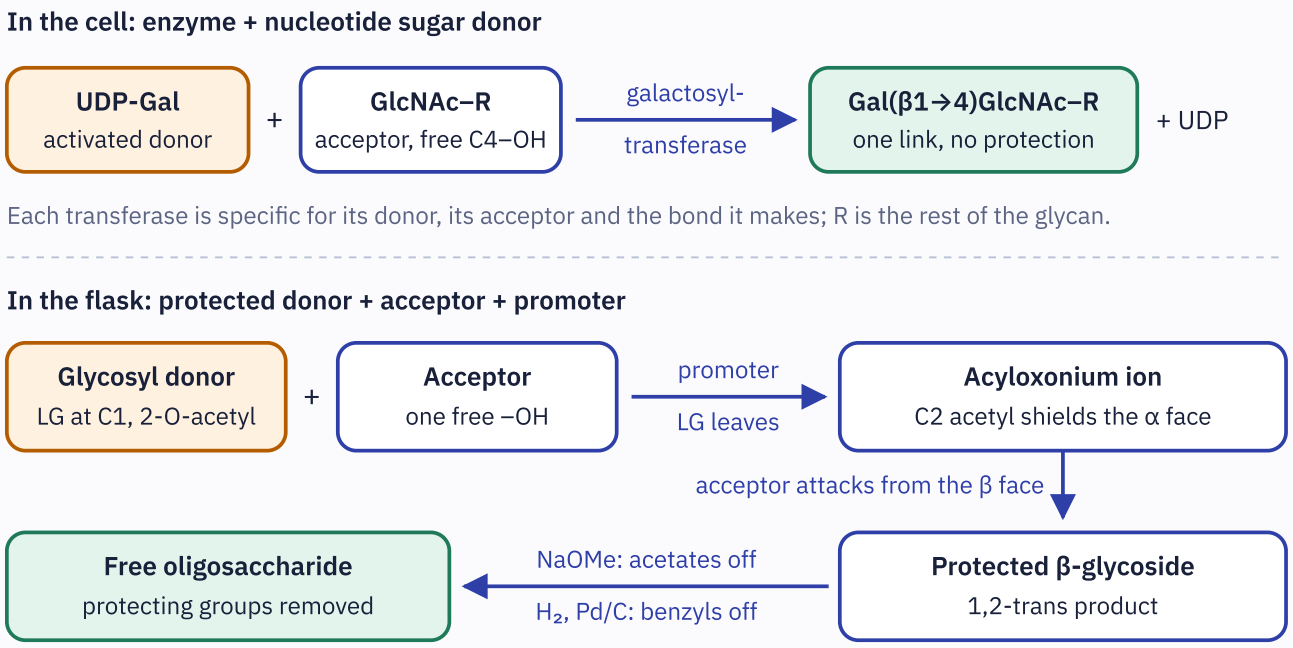


Figure 1.9.1 Two ways to make the same kind of bond. Top: a cell uses an enzyme and a nucleotide sugar, so no protecting groups are needed. Bottom: a chemist masks every –OH but one, activates the donor with a promoter and uses a participating 2-O-acetyl group to force the β (1,2-trans) product, then 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

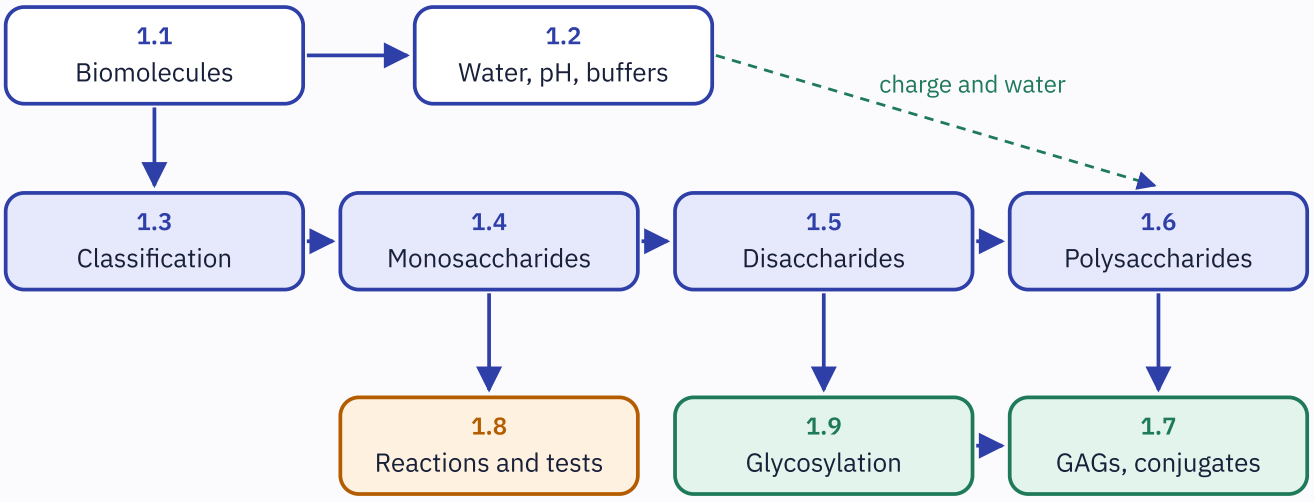
Conjugate vaccines join a bacterial capsule polysaccharide to a carrier protein so that infants' immune systems respond to it; India's Universal Immunisation Programme uses such vaccines against Haemophilus influenzae type b (in the pentavalent vaccine) and pneumococcus. In 2004 Cuba introduced a Hib 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.

Unit summary

KEY TERMS AND FORMULAS

TERM	MEANING OR FORMULA	CHAPTER
Condensation, hydrolysis	joining two units releases H_2O; hydrolysis adds it back and breaks the bond	1.1
Polymer molar mass	n × M(monomer) − (n − 1) × 18.02 g/mol	1.1
Hydrogen bond	δ+ H on O or N attracted to a lone pair on O or N; about 23 kJ/mol in water	1.2
Hydrophobic effect	nonpolar groups cluster because freeing ordered water raises its entropy	1.2
Ion product, pH	$K_w = [H^+][OH^-] = 1.0 \times 10^{-14}$ M² at 25 °C; pH = −log[H^+]	1.2
Henderson–Hasselbalch	pH = pKa + log([A^-]/[HA]); a buffer works within pKa ± 1	1.2
Aldose, ketose	aldehyde at C1; ketone, usually at C2	1.3
Stereoisomers	at most 2^n for n chiral centres (16 aldohexoses)	1.4
Epimer, anomer	differ at one chiral centre; anomers differ at the anomeric carbon (α or β)	1.4
Mutarotation	fraction α = ([α]eq − [α]β)/([α]α − [α]β); glucose about 36% α, 64% β	1.4
Glycosidic bond	acetal made from an anomeric –OH, written for example Glc(α1→4)Glc	1.5
Observed rotation	α = [α] × l × c, with l in dm and c in g/mL	1.5
Branching rule	non-reducing ends = branch points + 1; always one reducing end	1.6
Osmotic pressure	Π = icRT (van 't Hoff)	1.6
Glycosaminoglycan	repeating disaccharide of an amino sugar and a uronic acid, mostly sulfated	1.7
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

TERM	MEANING OR FORMULA	CHAPTER
1,2-trans rule	a participating C2 ester gives the 1,2-trans glycoside (β for glucose, α for mannose)	1.9



Leads on to Unit 2 (glycoprotein backbones), Unit 3 (glycolipids) and Unit 5 (burning and storing glucose).

Figure 1.U.1 How the chapters connect. The two foundation chapters feed the carbohydrate ladder (1.3 to 1.6), which branches into reactions (1.8), bond-making (1.9) and glycoconjugates (1.7). Notice the dashed link: the water chemistry of Chapter 1.2 (hydrogen bonds, osmotic pressure, charge) explains why cellulose is insoluble, why glucose is stored as glycogen and, through 1.6, why GAGs hold so much water.

Takeaways

1. Life runs on ordinary chemistry: a few elements, a few functional groups, and bonds made by condensation and broken by hydrolysis.
2. Water's polarity and hydrogen bonds explain solubility and the hydrophobic effect; weak interactions, many at once, hold biomolecules in shape.
3. pH = pKa + log([A^-]/[HA]). A buffer works within pKa ± 1, and blood is held near pH 7.4 by the open bicarbonate system.
4. Carbohydrates are classified by size (mono-, oligo-, polysaccharides); monosaccharides also by carbonyl group and carbon number.
5. A ring sugar has an anomeric carbon; α and β interconvert through the open chain (mutarotation), about 36% α and 64% β for glucose.
6. The glycosidic bond locks an anomeric carbon. Its position and its α or β configuration decide the name, the reducing nature and which enzymes can cut it.
7. α1→4 links give coiled fuel stores (starch, glycogen); β1→4 links give straight fibres (cellulose, chitin). Branching gives many non-reducing ends for fast release.

8. GAGs are long, negatively charged, water-holding chains; on core proteins they form proteoglycans, and with glycoproteins and glycolipids they make up the glycoconjugates.
9. Reducing sugars have a free anomeric carbon; their carbonyl chemistry underlies the Benedict, osazone and DNS tests and the HbA1c test of diabetes.
10. Every glycosylation needs an activated donor: nucleotide sugars and transferases in cells, protected donors, promoters and participating groups in the flask.

Exam question bank

Remember and understand

– REMEMBER **What is biochemistry? Describe its scope and the chemical basis of life: the main elements, functional groups, building blocks and the bonds that join them.** 8 marks

Outline: definition (the chemistry of living things, molecules and reactions); scope with one example each in medicine (blood glucose, lipid profile), food and agriculture (ripening, curd) and industry (detergent enzymes, recombinant insulin, biofuels); about 30 of the roughly 90 natural elements are essential, and C, H, O and N make up more than 99% of the mass of most cells; functional groups (hydroxyl, carbonyl, carboxyl, amino, phosphate, sulfhydryl) with where each occurs; four building blocks and three true polymers (polysaccharides, proteins, nucleic acids), lipids as non-polymers; condensation and hydrolysis; weak interactions and chirality give shape. A labelled chart of monomer, bond and polymer earns marks.

– REMEMBER **Define carbohydrates and classify them with examples. Give the main functions of each class.** 10 marks

Outline: polyhydroxy aldehydes or ketones, or substances that give them on hydrolysis; why $(CH_2O)_n$ fails (deoxyribose breaks it, lactic acid fits it); monosaccharides by carbonyl group (aldose, ketose) and carbon number (glyceraldehyde and dihydroxyacetone, ribose, glucose, fructose, galactose) and derived sugars; oligosaccharides (maltose, lactose, sucrose; raffinose); homopolysaccharides (starch, glycogen, cellulose, chitin) and heteropolysaccharides (hyaluronic acid, chondroitin sulfate, heparin); functions: fuel (about 17 kJ or 4 kcal per gram), storage, structure, parts of nucleotides and coenzymes, recognition, lubrication. A classification tree is expected.

– UNDERSTAND **Explain why water is called the solvent of life. Describe hydrogen bonds, ionic and van der Waals interactions and the hydrophobic effect, with one biological example of each.** 10 marks

Outline: bent molecule (104.5°), partial charges, dipole; the hydrogen bond (δ+ H on O or N to a lone pair on O or N), about 23 kJ/mol against 470 kJ/mol for O–H; up to four per molecule, the ice lattice floats; high boiling point, specific heat and heat of vaporisation; salts dissolve in hydration shells, sugars by hydrogen bonding; ionic attraction between charged groups (salt bridges in proteins); van der Waals contact (stacked DNA bases); hydrophobic effect driven by the entropy of released water (protein cores, micelles, bilayers); many weak bonds give stable but reversible structures (base pairing, enzyme–substrate binding).

– UNDERSTAND **Draw the Fischer and Haworth structures of D-glucose. Explain anomers, epimers and mutarotation with the specific rotation values. Why is β-D-glucopyranose the major form in water?** 12 marks

Outline: Fischer projection with the C5–OH on the right (D); four chiral centres, $2^4 = 16$ aldohexoses; the C5–OH adds to the C1 aldehyde to give a hemiacetal pyranose with a new chiral centre at C1 (the anomeric carbon); α has the C1–OH on the opposite side to CH_2OH, β on the same side; epimers differ at one centre (glucose and mannose at C2, glucose and galactose at C4); mutarotation: α (+112.2°) and β (+18.7°) both change to +52.7° through the open chain; about 36% α and 64% β; in the chair of β every bulky group is equatorial.

– UNDERSTAND **Describe the structure, sources and properties of maltose, lactose and sucrose. Explain why sucrose is non-reducing and what "invert sugar" means. Name two artificial sweeteners and how much sweeter they are than sucrose.** 10 marks

Outline: maltose Glc(α1→4)Glc, from starch digestion, reducing; lactose Gal(β1→4)Glc, in milk, reducing, cut by lactase (lactose intolerance); sucrose Glc(α1↔2β)Fru, from cane and beet, both anomeric carbons in the bond, so no free carbonyl: non-reducing, no mutarotation, no osazone; trehalose for comparison; acid or invertase hydrolysis turns the rotation from +66.5° to negative, because fructose (−92.4°) outweighs glucose (+52.7°); structures with linkages drawn; sweeteners as the US FDA gives them (aspartame about 200 times, sucralose 600 times).

– UNDERSTAND **Compare starch, glycogen, cellulose and chitin in monomer, linkage, branching, shape, location and function.** 12 marks

Outline: a comparison table; amylose α1→4, unbranched, a helix of about six units per turn, blue-black with iodine; amylopectin α1→4 with α1→6 branches every 24 to 30 units, red-violet; glycogen branches every 8 to 12 units, compact granules in liver and muscle, red-brown; cellulose β1→4 glucose, 10,000 to 15,000 units, straight hydrogen-bonded chains in microfibrils of plant cell walls; chitin β1→4 N-acetylglucosamine in insect and crab shells and fungal walls; storage polymers have many non-reducing ends for quick release; humans digest only the α-linked ones.

– UNDERSTAND **Write notes on glycosaminoglycans: hyaluronic acid, chondroitin sulfate, dermatan sulfate, keratan sulfate and heparin, giving the repeating unit, location and function of each.** 12 marks

Outline: definition (unbranched repeating disaccharides of an amino sugar and usually a uronic acid, mostly sulfated, highly negative, hydrated and extended); hyaluronic acid GlcA(β1→3)GlcNAc, not sulfated and not bound to protein, joint fluid and vitreous humour, lubricant; chondroitin sulfate GlcA with GalNAc-4S or -6S, cartilage, resists compression; dermatan sulfate with IdoA, skin and blood vessels; keratan sulfate Gal with GlcNAc-6S and no uronic acid, cornea; heparin IdoA-2S with GlcNS-6S, mast cells, anticoagulant through antithrombin; heparan sulfate on cell surfaces; proteoglycans and the aggrecan–hyaluronate aggregate.

Apply

– APPLY **Prepare 1.0 L of 0.10 M acetate buffer, pH 5.00, from 1.0 M acetic acid and 1.0 M sodium acetate (pKa 4.76). Find the pH after adding 1.0 mL of 1.0 M HCl, and compare with adding the same acid to 1.0 L of water.** 10 marks

Outline: $\log([A^-]/[HA]) = 5.00 - 4.76 = 0.24$, so the ratio is 1.74; $[A^-] = 0.10 \times 1.74/2.74 =$ **0.0635 M** and [HA] = **0.0365 M**; mix 63.5 mL of sodium acetate and 36.5 mL of acetic acid, dilute to about 950 mL, check with a calibrated pH meter, adjust, make up to 1.0 L. The 1.0 mmol of H^+ turns acetate into acetic acid: 62.5 and 37.5 mmol, pH = 4.76 + log(62.5/37.5) = **4.98**, a fall of only 0.02. In water, $[H^+] = 1.0 \times 10^{-3}$ M and the pH falls from 7.0 to **3.0**. Useful range 3.76 to 5.76; capacity is greatest at pH = pKa and rises with concentration.

– APPLY **A glucose solution that has stood overnight reads +5.27° in a 2 dm polarimeter tube. (a) Find its concentration. (b) What does a fresh solution of pure α-D-glucose of the same concentration read at once, and after some hours? (c) Explain the change, and give the first reading for fresh β-D-glucose.** 8 marks

Outline: α = [α] × l × c, so c = 5.27/(52.7 × 2) = **0.0500 g/mL** (5.00 g per 100 mL); (b) at once 112.2 × 2 × 0.0500 = **+11.22°**, falling to +5.27°; (c) mutarotation: the ring opens and closes until about 36% α and 64% β; fresh β reads 18.7 × 2 × 0.0500 = **+1.87°** and rises to the same +5.27°, because the equilibrium does not depend on which anomer was dissolved.

– APPLY **An amylopectin fragment has 600 glucose residues, with a branch point on average every 25 residues. Find (a) the number of α1→6 and α1→4 bonds, (b) the non-reducing and reducing ends, (c) the molar mass, (d) the water used in complete hydrolysis. (e) Why is glucose stored as a branched polymer?** 10 marks

Outline: 600 residues have 599 glycosidic bonds; 600/25 = 24 branch points, so **24 α1→6** and 599 − 24 = **575 α1→4**; non-reducing ends = 24 + 1 = **25**, with **one** reducing end; molar mass = 600 × 180.16 − 599 × 18.02 = **97,302 g/mol** (Chapter 1.1); complete hydrolysis uses **599 H_2O** and gives 600 glucose; branching gives many ends where enzymes release glucose at the same time (Unit 5), and one large molecule adds almost nothing to osmotic pressure (Chapter 1.6).

– APPLY **Five unlabelled tubes contain starch, maltose, sucrose, ribose and galactose. Using the Molisch, iodine, Benedict, Barfoed, Bial and mucic acid tests, plan a scheme that identifies each, with the expected result in every tube.** 10 marks

Outline: Molisch gives a violet ring in all five (all carbohydrates); iodine is blue-black only with **starch**; Benedict gives brick red with maltose, ribose and galactose, none with **sucrose** (no free anomeric carbon) and practically none with starch (one reducing end per very long chain); Barfoed gives a red precipitate within minutes with ribose and galactose (monosaccharides) but not with **maltose**; Bial gives blue-green only with **ribose** (a pentose); mucic acid gives insoluble crystals only with **galactose**; confirm sucrose by boiling with dilute HCl, neutralising, and repeating Benedict, which now turns positive. A results table earns marks.

– APPLY **A secreted human protein contains the stretches Asn–Gly–Ser, Asn–Pro–Thr, Asn–Ala–Ala and Asn–Val–Thr, and a Ser/Thr-rich region. (a) Which asparagines can carry N-glycans? (b) Where can O-glycans go, and how do they start? (c) Predict its glycans when made in E. coli and in CHO cells.** 8 marks

Outline: (a) the sequon is Asn–X–Ser/Thr with X not proline: Asn–Gly–Ser **yes**; Asn–Pro–Thr **no** (X is proline); Asn–Ala–Ala **no** (no Ser or Thr two places on); Asn–Val–Thr **yes**; a sequon is needed, but not every sequon is used; (b) O-glycans go on the –OH of Ser or Thr, with no consensus sequence, mostly in the Golgi, and mucin-type chains start with GalNAc; (c) E. coli makes the protein bare, because it lacks this machinery; CHO cells add the dolichol-built $Glc_3Man_9GlcNAc_2$ block in the ER, trimmed and extended in the Golgi into human-like complex glycans, plus O-glycans.

Analyze and evaluate

– ANALYZE **Glycogen and hyaluronate are both large polysaccharides in contact with water, yet one is a compact fuel store and the other a space-filling gel. Analyse this using osmotic pressure, charge and hydrogen bonding.** 12 marks

Outline (spans Chapters 1.2, 1.6 and 1.7): Π = icRT counts particles: 0.40 M free glucose at 37 °C would give about 10 atm (0.40 × 0.08206 × 310), while the same glucose as glycogen gives almost none, so storing a polymer protects the cell; glycogen is uncharged and highly branched, so it packs into dense granules; hyaluronate and the sulfated GAGs carry negative charges on every disaccharide, which repel, keep chains extended and hold counter-ions whose osmotic pull draws in water, so a small mass fills a large volume as a viscous gel; in cartilage, aggrecan on hyaluronate holds this water, compression squeezes it out and charge repulsion takes it back; conclusion: neutral and compact suits storage, charged and extended suits space-filling and shock absorption.

– ANALYZE **"A sugar is reducing when its anomeric carbon is free." Use this rule to explain why (a) fructose is reducing though it is a ketose, (b) sucrose and trehalose are non-reducing, (c) methyl α-D-glucoside neither mutarotates nor reduces Benedict's reagent, (d) starch gives almost no Benedict's test, (e) HbA1c rises in diabetes.** 12 marks

Outline (spans Chapters 1.4, 1.5 and 1.8): a free anomeric carbon is a hemiacetal or hemiketal, so the ring opens to a carbonyl that reduces Cu^{2+} to Cu_2O; (a) the C2 of fructose is free, and in hot alkali it isomerises through an enediol to aldoses; (b) sucrose, Glc(α1↔2β)Fru, and trehalose, Glc(α1↔1α)Glc, use both anomeric carbons, so no open form exists; (c) a methyl glycoside is an acetal, locked shut; (d) starch has one reducing end per thousands of units, too few to see; (e) open-chain glucose condenses with a free $–NH_2$ of haemoglobin to a Schiff base and then a stable Amadori product, without enzymes; the percentage follows average glucose over a red cell's life of about 120 days.

– ANALYZE **Starch and cellulose are both polymers of D-glucose, yet we digest only one, and one forms soft granules while the other forms strong fibres. Analyse how a single difference at the anomeric carbon causes all of this.** 10 marks

Outline (spans Chapters 1.5 and 1.6): α1→4 against β1→4; in α each unit sits at an angle to the next, so the chain coils into a helix of about six units per turn, packs loosely and holds iodine; in β each unit is turned 180°, so the chain is straight and hydrogen bonds within and between chains lock parallel chains into insoluble crystalline microfibrils of great tensile strength; enzymes are stereospecific: salivary and pancreatic α-amylase and maltase cut only α links, humans make no cellulase, while cows, goats and termites rely on gut microbes; chitin repeats the β pattern with GlcNAc; conclusion: one stereochemical change at C1 sets shape, packing, solubility and digestibility.

– EVALUATE **Compare how a cell attaches sugars with how a chemist makes an oligosaccharide in the flask. Then evaluate the better route for (i) a therapeutic antibody and (ii) a defined sugar antigen for a vaccine.** 12 marks

Outline (spans Chapters 1.7 and 1.9): shared principle: an activated donor, an acceptor –OH and a new bond at the anomeric carbon; in cells: nucleotide sugar donors (UDP-galactose, GDP-mannose, CMP-sialic acid), glycosyltransferases specific for donor, acceptor and linkage, no protecting groups, ER and Golgi, products a mixture of glycoforms because no template is copied; in the flask: protecting groups on every –OH but the acceptor's, a donor with a leaving group (bromide, trichloroacetimidate or thioglycoside) and a promoter, a participating C2 ester for 1,2-trans links, one pure product but many steps; (i) antibody: CHO cells, because the protein must fold and carry a human-like N-glycan that affects immune activity and lifetime in blood, and E. coli adds none; (ii) antigen: chemical or chemoenzymatic synthesis for one pure, repeatable structure, as in Cuba's synthetic Hib vaccine (2004).

Glossary

Aldose, ketose — A monosaccharide whose carbonyl group is an aldehyde at C1 (glucose), or a ketone, usually at C2 (fructose).

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.

Buffer — A solution of a weak acid and its conjugate base that resists a change in pH; it works within pKa ± 1.

Condensation, hydrolysis — Condensation joins two molecules with loss of water; hydrolysis adds water to break the bond again.

D and L — The configuration of the chiral carbon farthest from the carbonyl: –OH on the right in a Fischer projection is D.

Enantiomers — Non-superimposable mirror images, with every chiral centre inverted, such as D- and L-glucose.

Epimers — Sugars that differ at only one chiral centre, such as glucose and galactose (C4).

Fischer projection	A drawing of the open chain with carbons in a vertical line, C1 at the top, and groups to the left or right.
Glycation	Attachment of a reducing sugar to a free amino group of a protein without an enzyme, as in HbA1c.
Glycoconjugate	A protein or lipid with sugars covalently attached: glycoproteins, proteoglycans and glycolipids.
Glycosaminoglycan (GAG)	An unbranched chain of repeating disaccharides of an amino sugar and usually a uronic acid, mostly sulfated and negatively charged.
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.
Haworth projection	A flat drawing of a sugar ring, with each group shown above or below the ring.
Hemiacetal	The product of an alcohol adding to an aldehyde; the ring form of an aldose is one, and a ketose ring is a hemiketal.
Homopolysaccharide, heteropolysaccharide	A polysaccharide of one kind of unit (starch, cellulose), or of two or more kinds (hyaluronic acid).
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.
Hydrophobic effect	The clustering of nonpolar groups in water, driven by the entropy gained by the water molecules released.
Invert sugar	The equimolar glucose and fructose mixture from hydrolysis of sucrose; its optical rotation is negative.
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.
pKa	−log Ka; the pH at which an acid is half dissociated.

Protecting group	A temporary group that blocks an –OH so that a chemical reaction happens only at the chosen position.
Proteoglycan	A core protein carrying one or more covalently attached GAG chains; mostly carbohydrate by mass.
Pyranose, furanose	A sugar in a six-membered ring (five carbons and one oxygen), or in a five-membered ring (four carbons and one oxygen).
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.
Specific rotation, [α]	The observed rotation divided by tube length (dm) and concentration (g/mL); a constant of each compound at a stated temperature and wavelength.

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.