# Biochemistry

**Unit 4 · Lesson 1 · Bases, nucleosides and nucleotides**

The notes for Lesson 1 of Unit 4: chapters 4.1 to 4.2 of the unit book, with their figures, worked examples, exam traps and quizzes, in one file. The full unit book, with every chapter, the exam question bank and the glossary, is linked at the end.

Lesson 1 of 4 | 2 chapters | Chapters 4.1 to 4.2

## About these notes

These are the notes for **Lesson 1 · Bases, nucleosides and nucleotides** of the University free n easy video lessons on Biochemistry Unit 4, Nucleic Acids, Vitamins and Minerals. They are chapters 4.1 to 4.2 of the unit book, unchanged, so a page here is the same page in the full book.

## 4.1 Purine and pyrimidine bases

The letters of DNA and RNA are five small, flat molecules called nitrogenous bases. They are rings of carbon and nitrogen atoms, and they come in two families: the purines (adenine and guanine), which have two fused rings, and the pyrimidines (cytosine, thymine and uracil), which have one. The pattern of hydrogen-bond donors and acceptors on each ring decides which base pairs with which, so this chapter is the alphabet for every chapter that follows.

### Two parent rings

A **heterocycle** is a ring that contains at least one atom other than carbon. Both base families are heterocycles with nitrogen in the ring.

- **Pyrimidine**: one six-membered ring with nitrogen at positions 1 and 3 (four carbons, two nitrogens).
- **Purine**: a pyrimidine ring fused to a five-membered imidazole ring, nine ring atoms in all, with nitrogen at positions 1, 3, 7 and 9. The two rings share the C4–C5 bond.

The rings are numbered so that the atom which joins the sugar gets a fixed number: **N1 of a pyrimidine** and **N9 of a purine**. Sugar atoms get primes (1′, 2′, …) so they are never confused with ring atoms.

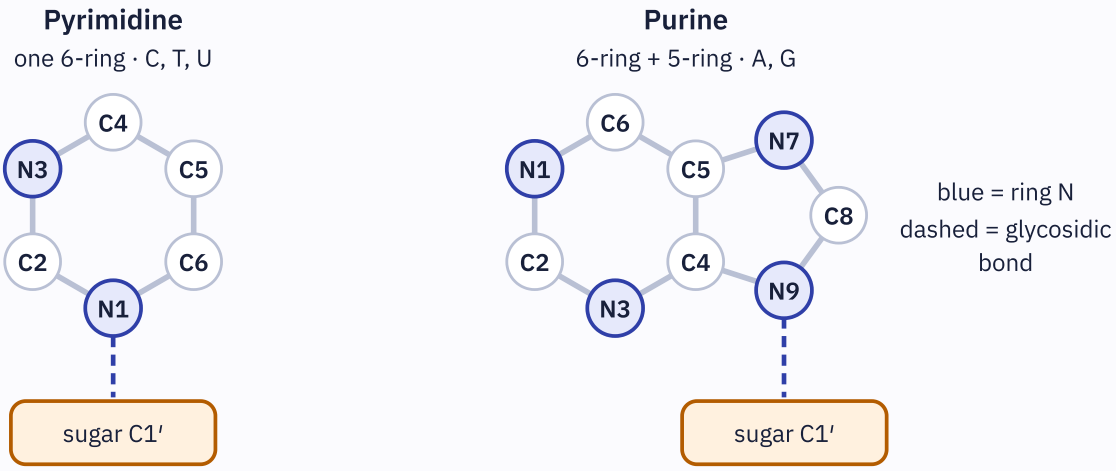



**Figure 4.1.1** The two parent rings with their atom numbers (double bonds omitted for clarity). Notice that the sugar joins a pyrimidine at N1 and a purine at N9, and that the purine is a pyrimidine ring with a five-membered ring fused on at C4–C5.

## The five common bases

Each base is a parent ring carrying amino ($-NH_2$), oxo (C=O) or methyl ($-CH_3$) groups at fixed positions. Learn the positions; examiners ask you to draw them.

| BASE | FAMILY | GROUPS ON THE RING | FORMULA | FOUND IN |
|---|---|---|---|---|
| Adenine (A) | Purine | 6-amino | $C_5H_5N_5$ | DNA and RNA |
| Guanine (G) | Purine | 2-amino, 6-oxo | $C_5H_5N_5O$ | DNA and RNA |
| Cytosine (C) | Pyrimidine | 4-amino, 2-oxo | $C_4H_5N_3O$ | DNA and RNA |
| Thymine (T) | Pyrimidine | 2,4-dioxo, 5-methyl | $C_5H_6N_2O_2$ | DNA |
| Uracil (U) | Pyrimidine | 2,4-dioxo | $C_4H_4N_2O_2$ | RNA |

Thymine is simply 5-methyluracil. The extra methyl group lets DNA repair enzymes tell a real T from a U made when cytosine loses its amino group (deamination of C gives U), which is one accepted reason why DNA uses T.

## Properties that matter

1. **Flat and aromatic.** The rings have conjugated double bonds, so they are planar and stack on top of each other like coins. Stacking is a major force that holds the double helix together (Chapter 4.3).

2. **UV absorption near 260 nm.** The conjugated rings absorb ultraviolet light strongly around 260 nm. Proteins absorb mostly at 280 nm, so the ratio A260/A280 is used to judge DNA purity.
3. **Tautomers.** A tautomer is the same molecule with a hydrogen moved to another atom. Bases can exist in keto or enol forms (–C=O versus –C–OH) and amino or imino forms (–$NH_2$ versus =NH). At pH 7 the **keto and amino forms** dominate, and these forms set the hydrogen-bond pattern that makes A pair with T and G with C. A rare tautomer present at the moment of copying can pair wrongly and cause a point mutation.
4. **Weakly basic, poorly soluble.** Ring nitrogens can accept a proton at low pH. Guanine and the purine end product uric acid are poorly soluble in water; uric acid crystals in joints cause gout (Unit 6).

## Unusual (minor) bases

These are the common bases changed after they are built into a chain, or close relatives of them.

- **In DNA:** 5-methylcytosine (switches genes off in plants and animals.
- **In tRNA:** pseudouridine (ψ, where uracil is joined to ribose through C5 instead of N1), dihydrouridine (D), inosine (its base is hypoxanthine; it sits at the wobble position of some anticodons), ribothymidine and methylated guanosines.
- **In mRNA:** 7-methylguanosine in the 5′ cap of eukaryotic mRNA (Chapter 4.5).
- **Free purine derivatives:** hypoxanthine, xanthine and uric acid (steps of purine breakdown) and the methylxanthines caffeine (1,3,7-trimethylxanthine), theophylline (1,3-dimethyl) and theobromine (3,7-dimethyl) in tea, coffee and cocoa.

## WORKED EXAMPLE

**Question (6 marks).** (a) Calculate the molar mass and percentage of nitrogen by mass in adenine ($C_5H_5N_5$) and cytosine ($C_4H_5N_3O$). Use C = 12.011, H = 1.008, N = 14.007, O = 15.999 g/mol. (b) A base from a hydrolysed nucleic acid is a single-ring compound with oxo groups at C2 and C4 and a methyl group at C5. Name it and say which nucleic acid it came from.

1. Adenine: C = 5 × 12.011 = 60.055; H = 5 × 1.008 = 5.040; N = 5 × 14.007 = 70.035. Molar mass = 135.13 g/mol.
2. %N in adenine = 70.035 ÷ 135.13 × 100 = **51.8%**.
3. Cytosine: C = 4 × 12.011 = 48.044; H = 5.040; N = 3 × 14.007 = 42.021; O = 15.999. Molar mass = 111.10 g/mol.
4. %N in cytosine = 42.021 ÷ 111.10 × 100 = **37.8%**. The purine has more ring nitrogens (four ring N plus one amino N), so it is richer in nitrogen.
5. (b) One ring means a pyrimidine; 2,4-dioxo with 5-methyl is **thymine** (5-methyluracil), so the sample was **DNA**.

## EXAM TRAPS

- Purines are A and G (two rings); pyrimidines are C, T and U (one ring). The bigger name, pyrimidine, is the smaller molecule.
- The sugar bond is at N9 of a purine and N1 of a pyrimidine; writing N1 for adenine loses marks.
- Thymine is in DNA, uracil in RNA. Thymine is not a purine because it has a methyl group.
- Caffeine and uric acid are purines but are not bases of nucleic acids.
- When asked “draw the structure”, show the ring numbering and the keto and amino forms.

## CHECK YOURSELF

– REMEMBER **Name the two purines and the three pyrimidines found in nucleic acids.** 2 marks

Purines: adenine, guanine. Pyrimidines: cytosine, thymine (DNA), uracil (RNA).

– REMEMBER **Which ring atom of a purine forms the glycosidic bond?** 1 mark

N9 (for pyrimidines it is N1).

– UNDERSTAND **Why do the keto and amino tautomers matter for base pairing?** 3 marks

They fix which atoms are hydrogen-bond donors and acceptors. In these forms A presents a pattern that matches T, and G one that matches C. A rare enol or imino form changes the pattern and can pair with the wrong partner, causing a mutation during replication.

– UNDERSTAND **Why is pseudouridine called unusual even though its base is uracil?** 2 marks

Its uracil is linked to ribose through C5, a carbon–carbon glycosidic bond, instead of the usual N1–C1′ bond.

– APPLY **Calculate the molar mass of thymine, $C_5H_6N_2O_2$.** 2 marks

5(12.011) + 6(1.008) + 2(14.007) + 2(15.999) = 60.055 + 6.048 + 28.014 + 31.998 = **126.12 g/mol**.

– APPLY **Caffeine is 1,3,7-trimethylxanthine. Which family does it belong to, and where are its methyl groups?** 2 marks

Purine family (xanthine is 2,6-dioxopurine). Methyl groups on N1, N3 and N7.

– ANALYZE **Cytosine slowly deaminates to uracil. Explain how using thymine instead of uracil helps DNA stay correct.** 4 marks

If DNA normally contained U, a U made from damaged C could not be told apart from a genuine U, and repair could not decide which was wrong. Because DNA uses T (methylated U), any U found in DNA is recognised as damage and removed by uracil-DNA glycosylase, restoring C.

**BEYOND THE SYLLABUS**

Chemists have built extra, synthetic base pairs that are not found in nature and that polymerases can copy, which expands the genetic alphabet beyond four letters. Many drugs are base look-alikes: 5-fluorouracil (cancer) and acyclovir (herpes) work because enzymes mistake them for natural bases or nucleosides. Methylated cytosine is now read genome-wide by bisulfite and nanopore sequencing in epigenetics research. Search “unnatural base pair” and “nucleoside analogue drugs” to go further. This is not for the exam.

## 4.2 Nucleosides and nucleotides

A base on its own carries no information; it has to be fixed to a backbone. Join a base to a five-carbon sugar and you get a **nucleoside**; add one or more phosphate groups to that sugar and you get a **nucleotide**. Nucleotides are the monomers of DNA and RNA, and as free molecules they are also the cell's energy currency (ATP), its signals (cAMP) and parts of its coenzymes ($NAD^+$, FAD, coenzyme A).

You need: the bases and their numbering from Chapter 4.1; pentoses and the glycosidic bond from Unit 1.

### The sugar: ribose or deoxyribose

Both nucleic acids use a pentose in its five-membered (furanose) ring form. RNA uses **β-D-ribose**; DNA uses **2′-deoxy-β-D-ribose**, which has H instead of OH at carbon 2′. That single missing oxygen has large effects. The 2′-OH of RNA can attack the neighbouring phosphate, so RNA is broken down by dilute alkali into 2′,3′-cyclic nucleotides and then 2′- and 3′-monophosphates. DNA has no 2′-OH and survives alkali, which is one reason it is the better long-term store of genes.

### From base to nucleoside to nucleotide

1. **Nucleoside = base + sugar.** The C1′ of the sugar is joined to N9 of a purine or N1 of a pyrimidine by a **β-N-glycosidic bond** (β means the base sits on the same face of the ring as C5′).
2. **Nucleotide = nucleoside + phosphate.** Phosphoric acid forms an ester with a sugar hydroxyl, nearly always the 5′-OH. One phosphate gives a nucleoside monophosphate (AMP), two give a diphosphate (ADP), three a triphosphate (ATP). The phosphates are called α, β and γ counting outward from the sugar.
3. **Polynucleotide.** A phosphate links the 3′-OH of one nucleotide to the 5′-OH of the next. This **3′,5′-phosphodiester bond** repeats to form the sugar–phosphate backbone. The chain has a free 5′-phosphate at one end and a free 3′-OH at the other, and sequences are always written 5′ → 3′.

| BASE | RIBONUCLEOSIDE | DEOXYRIBONUCLEOSIDE | 5′-MONOPHOSPHATE (RNA / DNA) |
|---|---|---|---|
| Adenine | Adenosine | Deoxyadenosine | AMP / dAMP (adenylate) |
| Guanine | Guanosine | Deoxyguanosine | GMP / dGMP (guanylate) |
| Cytosine | Cytidine | Deoxycytidine | CMP / dCMP (cytidylate) |

| BASE | RIBONUCLEOSIDE | DEOXYRIBONUCLEOSIDE | 5′-MONOPHOSPHATE (RNA / DNA) |
|---|---|---|---|
| Uracil | Uridine | (rare) | UMP |
| Thymine | (rare) | Deoxythymidine (thymidine) | dTMP |

A handy pattern: purine nucleosides end in *-osine*, pyrimidine nucleosides in *-idine*.

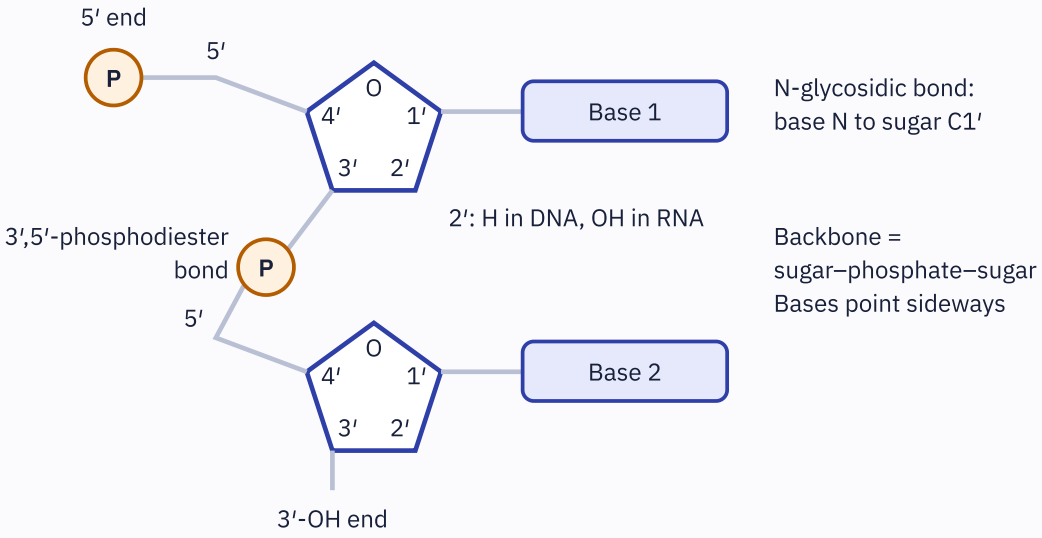



**Figure 4.2.1** Two nucleotides of one strand. Each base hangs from C1′ of its sugar; a phosphate bridges C3′ of the upper sugar and C5′ of the lower one. Notice the direction: the chain starts at a 5′-phosphate and ends at a 3′-OH.

## Bonds in nucleic acids and the jobs of free nucleotides

Inside one strand they are covalent: the N-glycosidic bond (base–sugar), the phosphoester bond (sugar–phosphate) and the 3′,5′-phosphodiester bond (between nucleotides). Between the phosphates of ATP are **phosphoanhydride** bonds, whose hydrolysis releases a lot of free energy (Unit 5, Bioenergetics). Holding two strands together are non-covalent forces: hydrogen bonds between bases and stacking between neighbouring base pairs (Chapter 4.3). Each backbone phosphate carries a negative charge at pH 7, which makes nucleic acids acids and makes them move to the positive electrode in gel electrophoresis.

Free nucleotides do much more than build chains. ATP transfers energy; GTP powers protein synthesis and switches G proteins; cyclic AMP (cAMP, with one phosphate bridging 3′ and 5′ of the same ribose) carries hormone signals inside the cell. $NAD^+$, $NADP^+$, FAD and coenzyme A all contain an adenine nucleotide joined to a vitamin-derived part (Chapter 4.6). UDP-glucose and CDP-choline are activated building blocks for glycogen and phospholipid synthesis, and S-adenosylmethionine is the cell's main methyl donor.

## Estimating DNA: the diphenylamine method

In hot acid (diphenylamine dissolved in glacial acetic acid with concentrated sulfuric acid) the glycosidic bonds between purines and deoxyribose break, and the freed 2-deoxyribose is converted to ω-hydroxylevulinyl aldehyde. This aldehyde reacts with diphenylamine to give a blue colour, read at about 595–600 nm. Ribose does not give this reaction, so RNA does not interfere (RNA is estimated with orcinol instead). The colour is proportional to DNA concentration, so a standard curve of known DNA amounts gives the unknown.

WORKED EXAMPLE

**Question (6 marks).** (a) For the DNA strand 5′-pATGCA-3′, name the nucleotides, count the phosphodiester bonds and estimate the net charge of the backbone phosphates at pH 7. (b) In a diphenylamine assay, a 100 µg/mL DNA standard gives absorbance 0.40 and the blank gives 0.00. The unknown, assayed the same way, gives 0.26. Assuming a straight-line standard curve through zero, find the DNA concentration of the unknown.

1. The nucleotides are dAMP, dTMP, dGMP, dCMP, dAMP (deoxyribonucleotides, because it is DNA and contains T).
2. Five nucleotides are joined by 5 − 1 = **4 phosphodiester bonds**. The “p” at the 5′ end is one extra terminal phosphate.
3. Each internal phosphodiester carries one negative charge (4); the 5′-terminal monophosphate carries between one and two, because its second acidic group has a pKa near 6.5. Net backbone charge is about **−5 to −6** (counting only the phosphates).
4. (b) Slope of the standard line = 0.40 ÷ 100 = 0.0040 per µg/mL.
5. Unknown = 0.26 ÷ 0.0040 = **65 µg/mL DNA**. In a real assay you would use five or six standards and read the value from the fitted line.

EXAM TRAPS

- Nucleoside has no phosphate; nucleotide has at least one. “Adenosine” is a nucleoside; “adenylate” or AMP is a nucleotide.
- A chain of n nucleotides has n − 1 phosphodiester bonds.
- Always write strand direction 5′ → 3′; the 5′ end carries the phosphate, the 3′ end the free OH.
- RNA, not DNA, is hydrolysed by alkali, because of its 2′-OH.
- The diphenylamine test detects deoxyribose, so it measures DNA, not RNA.

## CHECK YOURSELF

– REMEMBER **Define a nucleoside and a nucleotide.** 2 marks

Nucleoside: a base joined to a pentose by a β-N-glycosidic bond. Nucleotide: a nucleoside with one or more phosphate groups esterified to the sugar, usually at 5′.

– REMEMBER **Name the bond that links nucleotides in a strand.** 1 mark

The 3′,5′-phosphodiester bond.

– UNDERSTAND **Why is RNA hydrolysed by 0.1 M NaOH while DNA is not?** 3 marks

RNA's 2′-OH, made more reactive in alkali, attacks the adjacent phosphorus, forming a 2′,3′-cyclic phosphate and cutting the chain. DNA lacks the 2′-OH, so this route is not available.

– UNDERSTAND **Why does the diphenylamine reagent not respond to RNA?** 2 marks

The colour comes from 2-deoxyribose, which forms ω-hydroxylevulinyl aldehyde in hot acid; ribose does not form this product.

– APPLY **Write the full names of dGTP and UMP and say which nucleic acid each is built into.** 2 marks

dGTP: deoxyguanosine 5′-triphosphate, a DNA precursor. UMP: uridine 5′-monophosphate, an RNA unit.

– APPLY **A standard of 50 µg/mL DNA gives A = 0.21 in the diphenylamine assay. An unknown gives 0.63. Find its concentration.** 2 marks

Slope = 0.21/50 = 0.0042. Unknown = 0.63/0.0042 = **150 µg/mL**.

– ANALYZE **$NAD^+$ and FAD are called nucleotide coenzymes. Analyse which part of each molecule is a nucleotide and which part does the chemistry.** 4 marks

Both contain an AMP unit (adenine–ribose–phosphate) that mainly helps enzymes bind them. The reacting part comes from a vitamin: the nicotinamide ring of niacin in $NAD^+$, and the isoalloxazine ring of riboflavin in FAD, which carry electrons and hydrogen.

BEYOND THE SYLLABUS

Short DNA strands (oligonucleotides) for PCR primers are made by machines using phosphoramidite chemistry, which adds one protected nucleotide at a time from 3′ to 5′. Modified nucleotides with a phosphorothioate backbone or a 2′-O-methyl sugar make antisense and siRNA drugs last longer in the body, and the mRNA vaccines used N1-methylpseudouridine in place of uridine. Search “phosphoramidite synthesis” and “modified nucleosides in mRNA vaccines”. This is not for the exam.

## Key terms of this lesson

| TERM | MEANING OR FORMULA | CHAPTER |
|---|---|---|
| Purine / pyrimidine | Two fused rings (A, G) / one ring (C, T, U); sugar at N9 / N1 | 4.1 |
| Nucleoside | Base + pentose (β-N-glycosidic bond at C1′) | 4.2 |
| Nucleotide | Nucleoside + phosphate (usually at 5′) | 4.2 |
| Phosphodiester bonds | n nucleotides in a chain → n − 1 bonds; chain written 5′ → 3′ | 4.2 |

## Glossary (terms used in this lesson)

**Anticodon** Three bases in the tRNA anticodon loop that pair antiparallel with an mRNA codon.

**Codon** A triplet of mRNA bases that specifies one amino acid or a stop signal.

**Coenzyme** A small organic molecule, often made from a vitamin, that an enzyme needs to catalyse its reaction.

**Nucleoside** A nitrogenous base joined to a pentose sugar.

**Nucleotide** A nucleoside with one or more phosphate groups on the sugar.

**Phosphodiester bond** The phosphate bridge between the 3′-OH of one nucleotide and the 5′-OH of the next.

**Tautomer** A form of a molecule with a hydrogen atom moved to another position, such as the keto and enol forms of a base.

## The full unit book

Every chapter of Unit 4, the unit summary, the exam question bank with model answers and the full glossary: Biochemistry · Unit 4 · Nucleic Acids, Vitamins and Minerals (one file, works offline).

## Sources and further reading

### Standard textbooks

- David L. Nelson and Michael M. Cox, *Lehninger Principles of Biochemistry*: the chapters on nucleotides and nucleic acids, and on vitamins as coenzymes (from memory; check the chapter numbers in your edition).
- U. Satyanarayana and U. Chakrapani, *Biochemistry*: the chapters on nucleic acids, vitamins and minerals; widely prescribed in Indian universities (from memory; check).
- Jeremy M. Berg, John L. Tymoczko and Lubert Stryer (later editions with Gregory Gatto), *Biochemistry* (from memory; check).
- Donald Voet and Judith G. Voet, *Biochemistry*: detailed treatment of DNA conformations A, B and Z (from memory; check).

### Open web resources

- Wikipedia, “Nucleic acid double helix”, comparison table of A-, B- and Z-DNA geometry: en.wikipedia.org/wiki/Nucleic_acid_double_helix
- NIH Office of Dietary Supplements, “Vitamin A and Carotenoids: Fact Sheet for Health Professionals”, for the IU and RAE conversion factors: ods.od.nih.gov/factsheets/VitaminA-HealthProfessional

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