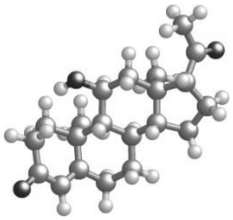

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Class: ……

Date: …… **/** …… **/** ……

The Structure and Biological Activity of Penicillin

Requirements:

- Molecular modelling kit.
- Molecular modelling software (for optional integration with information technology).

Learning Objectives:

In this activity you will make molecular models of a simple β-lactam ring and 6-aminopenicillanic acid (the nucleus of the penicillin molecule). Construction of these models will allow you to investigate penicillin's *stereochemistry* and mode of action as an antibacterial drug. To extend the investigation further, you will construct molecular models of several different penicillins. This will allow you to study how the different structures of the various side-chains allow penicillins to be effective against specific bacterial infections.

Prior Knowledge:

Biology	Chemistry
Bacteria – structure and function	Identification of organic functional groups
Enzyme – structure and function	Typical reactions of organic functional groups
	Optical isomerism in organic molecules
	Valence shell electron pair repulsion theory (Advanced Questions)
	Organic reaction mechanisms (Advanced Questions)

Introduction

Penicillin is an antibiotic discovered by Alexander Fleming at St. Mary's Hospital in London in 1928. Antibiotics are a class of drug used in medicine to treat diseases that are caused by *bacterial* infections. It is a common misconception that antibiotics can be used to treat diseases that are caused by *viral* infections.

Instructions:

Work in small groups of 2 – 3 students for this activity. This will allow you to construct the models more rapidly, and will also allow you to discuss the answers to the following questions while studying the models.

Part One: At Introduction to Bacteria Structure and Function

Question One:

How does a bacterial infection cause disease within the body of an animal? Consider what processes take place during the life-cycle of the bacteria and how this may affect the host organism.

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Question Two:

a) Name three different diseases that can be successfully treated using penicillin:

- ……
- ……
- ……

b) Name three different diseases that will *not* respond to treatment using penicillin:

- ……
- ……
- ……

Question Three:

Bacteria are single celled organisms or *prokaryotes*. Similar to plant cells, each bacterial cell is surrounded by a cell wall.

a) What possible functions are performed by the bacteria's cell wall?

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b) Penicillin works by inhibiting an enzyme called *transpeptidase*. This enzyme's activity is essential in biosynthesis of the bacterial cell wall. At which point(s) of the bacteria's life-cycle do you expect transpeptidase to be most active?

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<u>Part Two:</u> Modelling Penicillins

Question Four:

Construct a model of a simple β-lactam ring as shown in **Figure 1**.

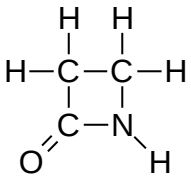

Figure 1. The β-lactam ring.

a) Name the functional group that is present in the β-lactam ring.

……

b) Suggest why the β-lactam ring is susceptible to attack by acids and alkalis.

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Question Five:

Now convert the model of the β-lactam ring into a model of 6-aminopenicillanic acid as shown in **Figure 2**. This is the *penicillin nucleus*, common to all penicillins. Study the graphical formula of 6-aminopenicillanic acid very carefully. The molecule has a very precise *stereochemistry* and so it is important that your molecular model has the correct geometry.

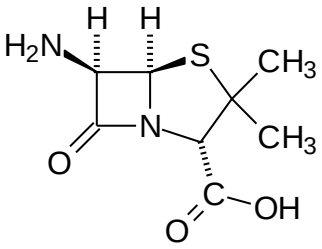
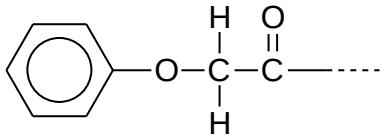


Figure 2. 6-aminopenicillanic acid.

Identify all of the *chiral* carbon atoms present in 6-aminopenicillanic acid. Mark these on the graphical formula shown in **Figure 2** using asterisks (∗).

Question Six:

If your molecular modelling kit contains sufficient atoms and bonds, you could construct a side-chain and convert your model of 6-aminopenicillanic acid into a model of a penicillin. For example, by substituting a hydrogen atom (H) on the amine group (–NH_2) with the following structure:



Converts 6-aminopenicillanic acid into penicillin V, shown in **Figure 3**.

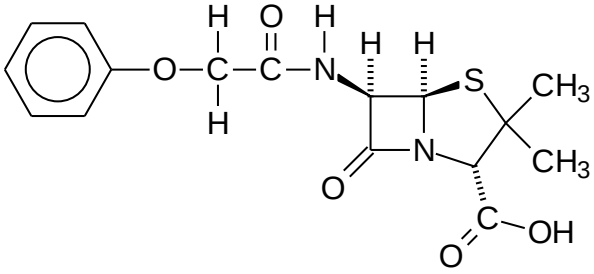


Figure 3. Penicillin V.

a) The other optical isomers of penicillin V demonstrate far less biological activity against bacteria. With reference to your knowledge of enzymes, explain why the correct stereochemistry is essential to penicillin's biological activity.

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b) The structure of penicillin V was worked out during the 1940s, but it was not synthesised commercially until 15 years later. Give reasons why totally synthetic penicillins have never been produced commercially on a large scale.

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<u>Part Three:</u> Examining the side-chain (**)**

Table 1 shows the R groups in the side-chains of some natural and some semi-synthetic penicillins:

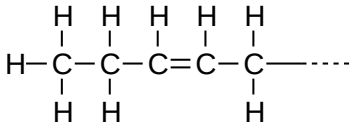
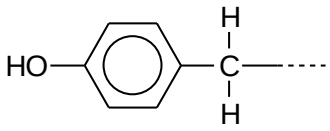
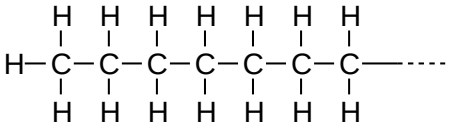
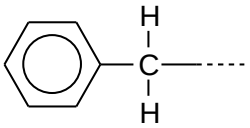
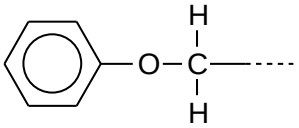
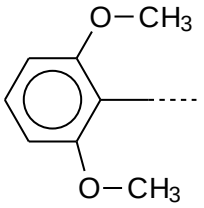
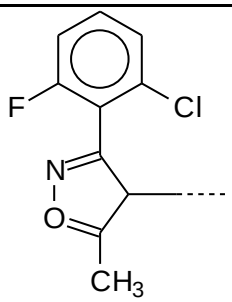
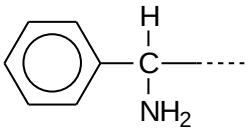
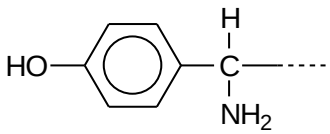
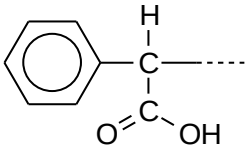
Name:	R Group in the Side-chain:	Natural or Semi-synthetic:	Properties:
Penicillin F		Natural	Not used commercially
Penicillin X		Natural	Not used commercially
Penicillin K		Natural	Not used commercially
Penicillin G		Natural	General infections, gonorrhoea and syphilis
Penicillin V		Natural and Semi-synthetic	General infections, ear, nose and throat
Methicillin		Semi-synthetic	Controlling resistant *Staphylococcus*
Flucloxacillin		Semi-synthetic	Controlling resistant *Staphylococcus*
Ampicillin		Semi-synthetic	Lung and wound infections
Amoxycillin		Semi-synthetic	Lung and urinary tract infections
Carbenicillin		Semi-synthetic	Pneumonia and burns

Table 1. Penicillin Side-chains.

Question Seven:

a) Clearly explain the differences between *natural*, *semi-synthetic* and *totally synthetic* penicillins. State an advantage and a disadvantage for each one.

Natural: ……

……

Semi-synthetic: ……

……

Synthetic: ……

……

b) β-Lactamase is an enzyme, produced by bacteria, which destroys the β-lactam ring of penicillin rendering it ineffective as an antibiotic. Consider the R group in the side-chains of methicillin and flucloxacillin. What structural features of these penicillins appear to be important in resisting attack by the β-lactamase enzyme? (**Hint:** look at the groups attached to the first carbon atom in the side-chain. Remember that large groups affect the size and shape of a molecule).

……

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c) Suggest how penicillins such as methicillin and flucloxacillin are able to resist attack by the β-lactamase enzyme.

……

……

d) Penicillins act by inhibiting a bacterial enzyme known as *transpeptidase*. This enzyme's activity is essential in construction of the bacterial cell wall. How might the active site of the transpeptidase enzyme differ from the active site of the β-lactamase enzyme?

……

……

<u>Part Four:</u> Advanced Questions

Question Eight:

6-aminopenicillanic acid, shown in **Figure 2**, does not demonstrate any significant antibacterial activity until a side-chain, shown in **Table 1** is attached. Using this information, deduce the structure of the penicillin *pharmacophore*:

Graphical formula of the penicillin pharmacophore:

Question Nine:

Refer to the information provided in **Table 2**. Use your knowledge of valence shell electron pair repulsion theory to suggest why the β-lactam ring readily reacts to form *open-chain* (as opposed to cyclic) compounds.

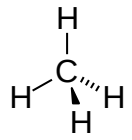
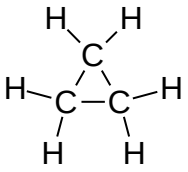
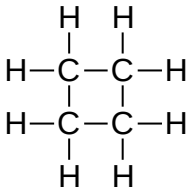
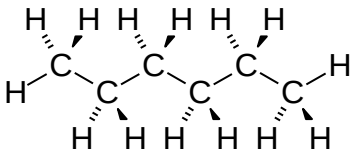
Methane (*Reference*)	**Three C Ring**	**Four C Ring**	**Open-chain**
			
Bond Angle = 109.5°	Bond Angle = 60.0°	Bond Angle = 90.0°	Bond Angle = 109.5°

Table 2. A comparison of bond angles in cyclic and open-chain molecules.

……

……

Question Ten:

The electronegativity values (Pauling Scale) of the chemical elements present in Penicillin V are given:

H = 2.1 **C** = 2.5 **N** = 3.0 **O** = 3.5 **S** = 2.5

a) Use these electronegativity values to assign bond polarity at the active site of the transpeptidase enzyme and within the β-lactam ring of Penicillin V shown in **Figure 4**.

b) Using curved arrows to represent the movement of a pair of electrons, show how the active site of the transpeptidase enzyme attacks the β-lactam ring of Penicillin V.

c) Complete **Figure** 4. to show the open-chain penicillin structure covalently bonded to the active site of the transpeptidase enzyme, *i.e.* show how transpeptidase is inhibited by Penicillin V.

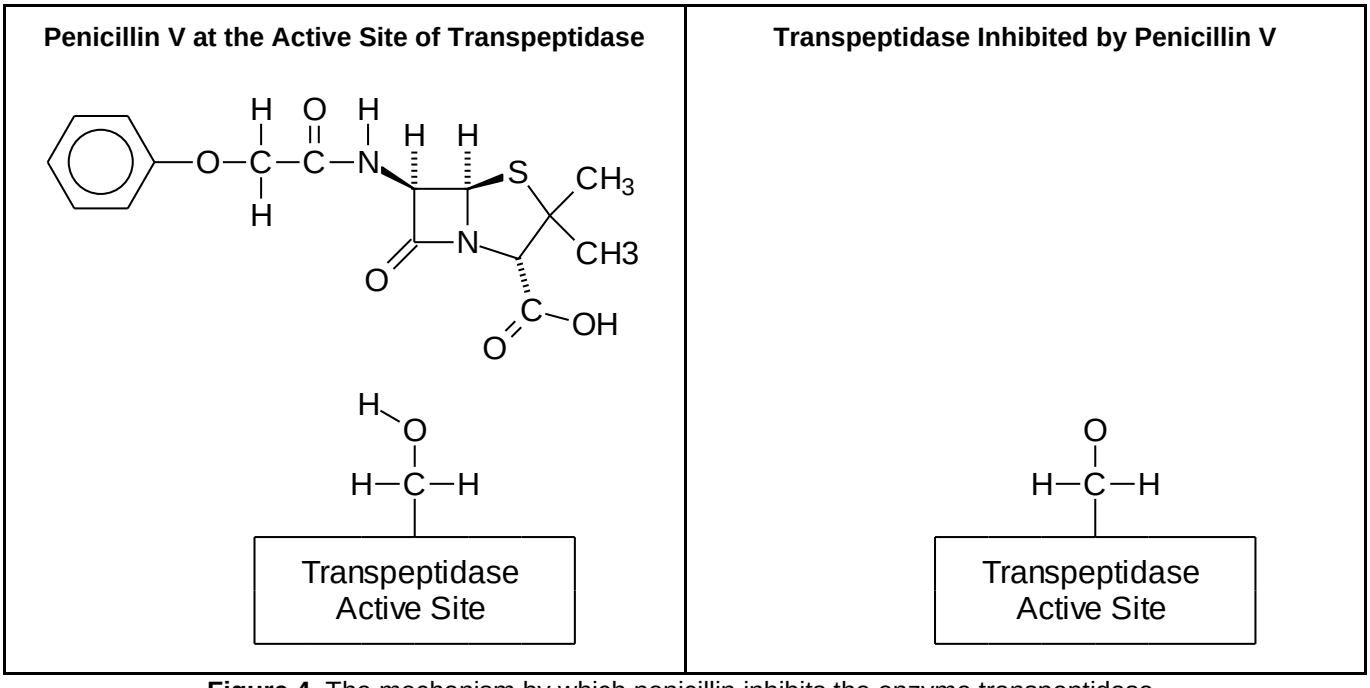


Figure 4. The mechanism by which penicillin inhibits the enzyme transpeptidase.

Adapted from:

Burton, G., Holman, J., Lazonby, J., Pilling, G., & Waddington, D. (2000). *Salters advanced chemistry activities and assessment pack* (2nd ed.). Oxford: Heinemann.

Answers

Question One:

The bacteria produces metabolites, or waste products, that are toxic to the host organism.

Question Two:

a) Any bacterial disease, *e.g.* anthrax, typhoid, diphtheria, gonorrhoea, syphilis, bacterial meningitis.

b) Any viral disease, *e.g.* influenza, smallpox, chickenpox, bird flu, AIDS, Ebola.

Question Three:

a) The bacterial cell wall will prevent the cell from expanding and rupturing when it is place in pure, or nearly pure, water.

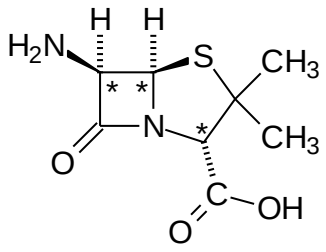
b) The enzyme transpeptidase will be most active during bacterial cell division and growth.

Question Four:

a) Amide group.

b) The carbonyl group is polar: $\delta+$ C=O $\delta-$ hence the $\delta+$ C can undergo attack by hydroxide ions (OH^-) while the $\delta-$ O can undergo attack by hydrogen ions (H^+). The amide undergoes acid or base catalysed hydrolysis, in a similar way that esters are hydrolysed.

Question Five:



Question Six:

a) Changes to the stereochemistry around the chiral carbon atoms changes the shape of the penicillin molecule, so that it no longer fits well into the active site of the transpeptidase enzyme.

b) The synthesis is complex and yields are low. It is difficult to achieve the correct stereochemistry at each chiral carbon atom. The process is uneconomical compared to using 6-aminopenicillanic acid from moulds.

Question Seven:

a)

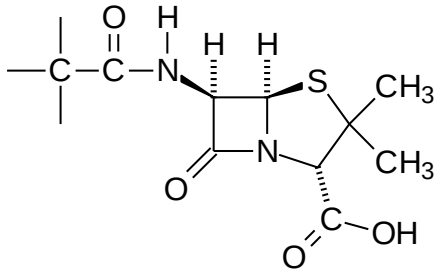
Natural:	These penicillins are created entirely by the mould with the appropriate side-chains attached.
Semi-synthetic:	These penicillins are synthesised from 6-aminopenicillanic acid, which is obtained by enzyme hydrolysis of naturally produced penicillin.
Synthetic:	These penicillins are prepared in the laboratory by artificial means from simple organic compounds.

b) Methicillin and flucloxacillin are both resistant to attack by the β-lactamase enzyme. Both penicillins have very large / bulky side-chains. In each case, the carbon atom bonded directly to C=O in the side-chain is completely substituted.

c) Large / bulky side-chains make a profound difference to the overall size and shape of the penicillin molecule. This prevents a good fit between the penicillin molecule and the active site of the β-lactamase enzyme.

d) Methicillin and flucloxacillin fit well into the active site of the transpeptidase enzyme, but not into that of β-lactamase. The active site of the β-lactamase enzyme may therefore be smaller than that of the transpeptidase.

Question Eight:



Question Nine:

The tetrahedral bond angle around a saturated carbon atom is 109.5°. The bond angles in the β-lactam ring are 90°. Repulsion between the bonding and non-bonding pairs of electrons in the valence shells of carbon and nitrogen will cause the 4-membered β-lactam ring to be very strained. The β-lactam ring therefore reacts readily to produce a stable, strain-free open-chain structure in which bonding and non-bonding pairs of electrons in the valence shells of carbon and nitrogen are much further apart.

Question Ten:

a) In the β-lactam ring: δ+ C=O δ–.
At the transpeptidase active site: δ+ H–O δ–.

b) The mechanism should show nucleophilic attack by δ+ H–O δ– on δ+ C=O δ–.

c)