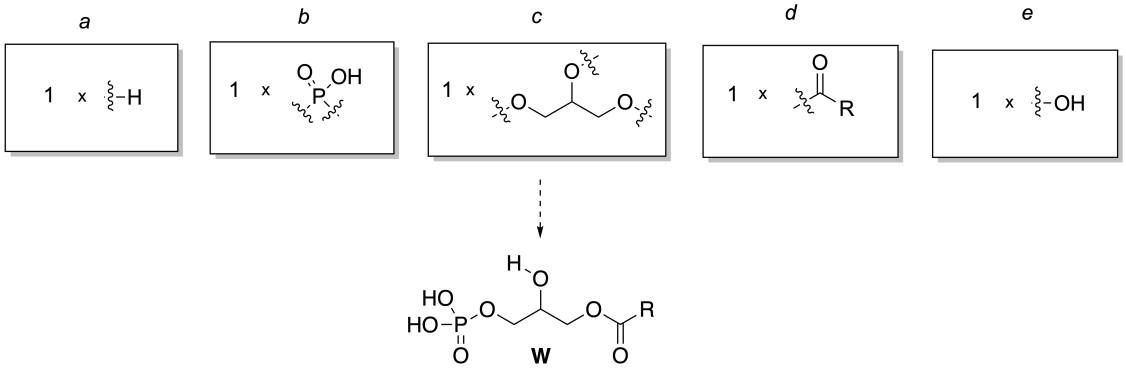
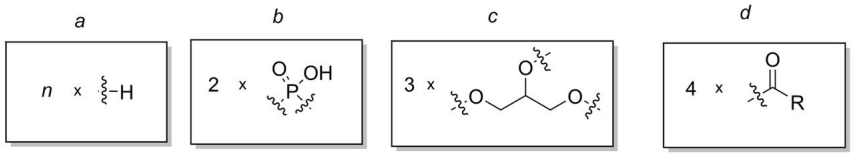
T5. Cardiolipins

6%						
5.1	5.2	5.3	5.4	5.5	5.6	Σ
1	6	4	2	2	3	18

In this problem you are asked to be creative and build molecules from fragments. For instance, if you are required to draw a chiral phospholipid such as **W** using fragments *a-e*, it can be assembled as shown below.



Cardiolipins are a family of acyclic phospholipids differing in fatty acid residues. They are essential for energy production and membrane stability. Defects can lead to certain diseases. **PL1** belongs to this family. Using only the structural elements *a-d* in the quantities stated below, it is possible to assemble the non-ionised form of **PL1**. R is a hydrocarbon substituent in the fatty acid structure.



PL1 does not contain any peroxide bonds.

5.1 **Tick** one correct statement regarding the value of *n* (number of type *a* fragments). 1.0 pt

(a) *n* is an even number,
(b) *n* is an odd number,
(c) *n* can be either an odd or an even number.

SOLUTION:

Each bond involves two atoms, so the total number of atoms with broken bonds (indicated by the wavy line on the fragments of the molecule) must be an even number. For fragments with a known number of repeats in the **PL1** molecule, the total number of atoms with broken bonds is 17 (2·2 + 3·3 + 4). Thus the total number of hydrogen atoms with broken bonds must be an odd number. Therefore, *n* is an odd number.

(a)	(b)	(c)
	x	

Correct answer: 1 point.

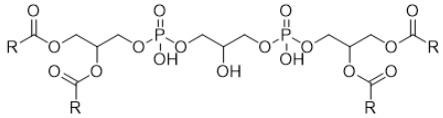
Some cardiolipins are chiral molecules, even if they contain four identical fatty acid residues, as in the case of **PL1**. One enantiomer of **PL1** is found in prokaryotes and eukaryotes, and the other only in archaea. All other diastereomers of **PL1** are achiral molecules, since they have a plane of symmetry. Do not consider the chirality of phosphorus atoms as stereocentres.

PL1 is a diprotic acid with the same acidic groups. Its second deprotonation is less favourable ($pK_{a2} \gg pK_{a1}$) due to the special, stable structure of monoanion **Y**.

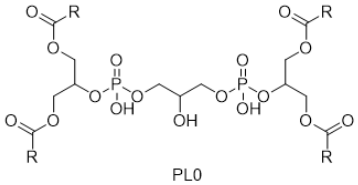
5.2 **Draw** the structure of one enantiomer of **PL1**. **Draw** the structure of **Y** in a way that shows the stabilisation that explains the difference between pK_{a1} and pK_{a2}. Use the abbreviation R for the fatty acid residues. 6.0 pt

SOLUTION:

To obtain the diastereomer of **PL1** with a plane of symmetry, we must construct a symmetric structure from the given fragments. Glycerol backbones can only be linked through phosphate groups since there is no peroxide bond present. The number of glycerol moieties suggests that one glycerol occupies the central position with the remaining fragments arranged around it to achieve an overall plane of symmetry. The number of *a* fragments in **PL1** is *n* = 3·3 – 2·2 – 4 = 1. So, **PL1** has $d_2c-b-c(a)-b-cd_2$ skeleton. Two options, presented below, can have a plane of symmetry:



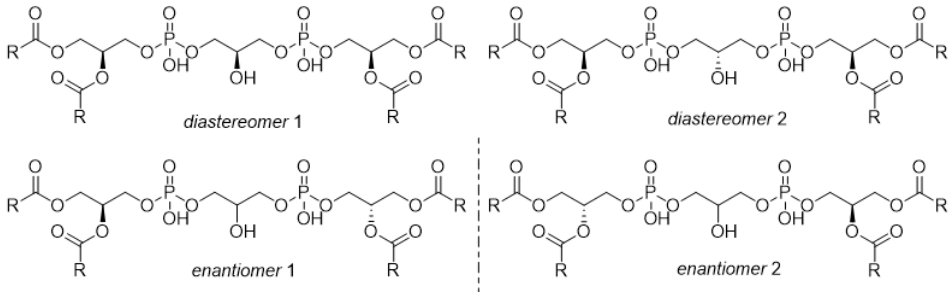
PL1



PL0

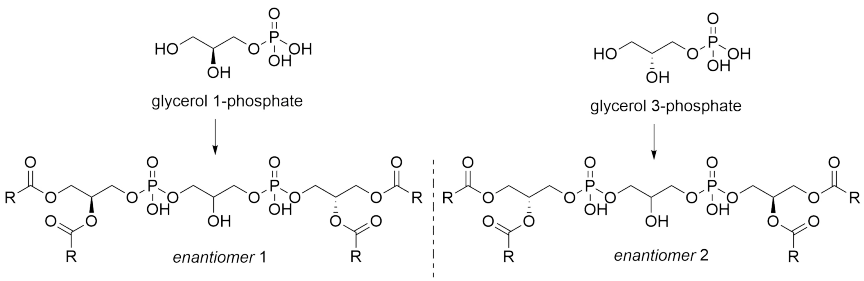
The **PL0** structure is achiral, so it cannot be **PL1**. All possible stereoisomers of **PL1** are as follows:

SOLUTION:

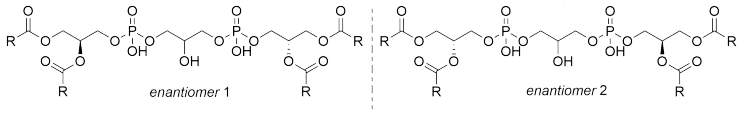


Enantiomer 2 is found in prokaryotes and eukaryotes. These organisms use glycerol 3-phosphate for cardiolipin synthesis, while archaea use glycerol 1-phosphate for this purposes and synthesise *enantiomer 1*:

SOLUTION:

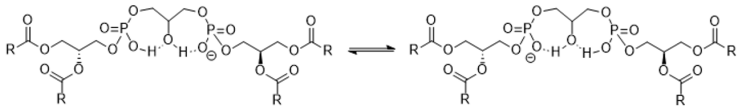


PL1



For drawing of enantiomer 1 or 2 as **PL1**: 4 points.
For drawing the correct structure of **PL1** with wrong or missing stereochemistry: 3 points.
For drawing achiral **PL0** structure: 2 points.
Any other structure that uses exactly 1×*a*, 2×*b*, 3×*c*, 4×*d* and contains no peroxide bond: 1 point.

Intramolecular hydrogen bond between the phosphoric acid residue and the alcohol group significantly reduces the pK_{a2} value.



For any correct tautomer of **Y**: 2 points.
For a tautomer of **Y** (not shown above) with a hydrogen bond between the two phosphate groups: 1 point
Correctly drawn structures with incorrect **PL1** structure are also accepted.
For structures containing hydrogen bond between phosphoric acid residues and esters 0 point, because they are possible for each phosphoric acid residue even in molecular form and don't make difference between pK_{a1} and pK_{a2}.

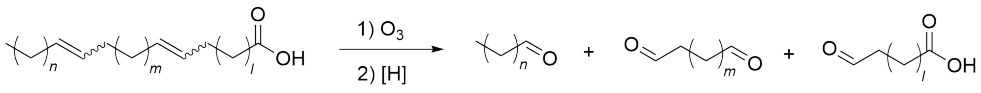
A series of experiments was conducted to identify the fatty acid (RCOOH) in **PL1** found predominantly in mammalian hearts. During reductive ozonolysis, RCOOH forms three different organic products in equimolar amounts.

5.3 **Determine** the molecular formula of the fatty acid (RCOOH), if the non-ionised form of **PL1** contains 255 σ and π bonds between atoms in total. **Support** your answer with calculations. 4.0 pt

If you were unable to find the structural formula of **PL1**, *you can use a-d fragments from* **5.1**.

SOLUTION:

Correct scheme of reductive ozonolysis of RCOOH is as follows:



So, $N_{C=C}$ = 2.
If we take R as C_xH_y, then:
y=(2x+1)-$N_{C=C}$·2=2x-3.(eq.3.1)

Then the formula for calculating the number of bonds in **PL1** can be written as follows:
0.5·1+5·2+11.5·3+4·(3+2x+0.5y)=255,
4x+y=99.(eq.3.2)
Combining eq.3.1 and eq.3.2:
4x+y=4x+2x-3=6x-3=99 $\Longrightarrow$ x=17, y=31.

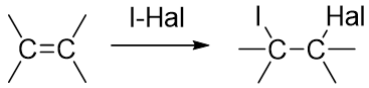
RCOOH – $C_{17}H_{31}COOH$ or $C_{18}H_{32}O_2$
For correct eq.3.2: 2 points.
For correct number of double bonds: 1 point.
For correct formula of $C_{17}H_{31}COOH$: 1 point.

100 g of RCOOH reacts with 181.0 g of iodine. RCOOH also reacts in similar way with **X** and forms an adduct with an iodine mass fraction of 36.57%.

5.4 **Determine** the molecular formula of **X**. **Support** your answer with calculations. 2.0 pt

SOLUTION:

During determination of the iodine number, the following reaction takes place:



The iodine number of RCOOH and the iodine mass fraction in the adduct are described as follows:

$$181.0 = \frac{N_{C=C} \times 253.8}{M_{RCOOH}} \times 100 \Rightarrow \frac{M_{RCOOH}}{N_{C=C}} = 140.2$$

$$0.3657 = \frac{N_{C=C} \times 126.9}{M_{RCOOH} + N_{C=C} \times (126.9 + A_{Hal})} \Rightarrow A_{Hal} = 79.9 \text{ g mol}^{-1}.$$

So, **X** is iodine monobromide, IBr.

If **X** was molecular iodine (I_2), then:

$$\omega_I = \frac{N_{C=C} \times 253.8}{M_{RCOOH} + N_{C=C} \times 253.8} = 0.6441$$

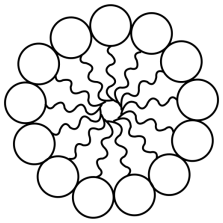
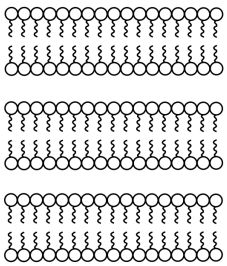
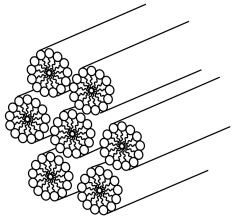
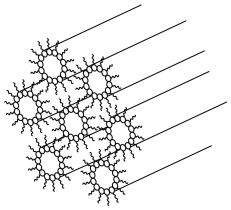
which is incorrect.

For correct formula of **X**: 1 point.

For correct calculations: 1 point.

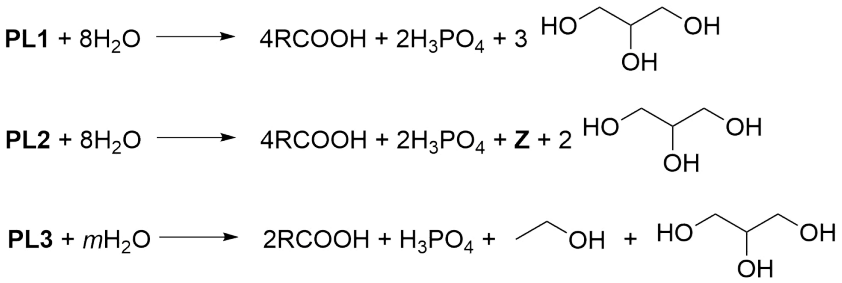
The phase behavior of **PL1** depends on pH. In physiological conditions, **PL1** is a dianion and it forms a lamellar phase.

5.5 Which lipid phase is formed by **PL1** when both acid residues in it are protonated? 2.0 pt
Note that knowledge of **PL1** structure is not required to solve this question.
Tick the correct answer.

			
(a) micellar	(b) lamellar	(c) hexagonal	(d) inverse hexagonal

SOLUTION:
PL1 has 4 acyl chains but only 2 phosphate head groups, so its tail volume is already large relative to the head group — which is why the dianion is lamellar, not micellar/hexagonal. Protonating both acidic groups shrinks the head group further (loss of charge and its water shell), tipping the balance even more toward the tails. Starting from lamellar, this shift leads to the inverse hexagonal phase. Correct answer is (d).
Only for correct answer: 2 point.

The following balanced reaction equations show the hydrolysis of phospholipids **PL1**-**PL3**:



- The non-ionised form of **PL2** contains 254 σ and π bonds between atoms in total. It is a chiral and a more symmetrical molecule than **PL1**,

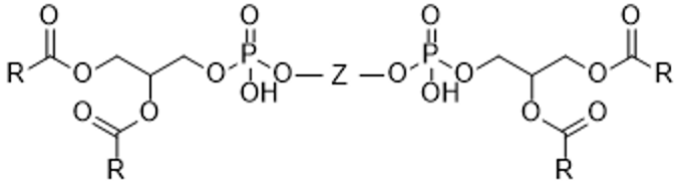
- **Z** contains C, H, and O only, and it doesn't react with H_2 in the presence of a catalyst,

- Phospholipid **PL3** is a charged molecule at physiological pH values, and it exists as pair of enantiomers.

5.6 **Draw** the structures of **PL2** and **PL3** using R to show the fatty acid residues. **Show** stereochemistry where appropriate. 3.0 pt

SOLUTION:

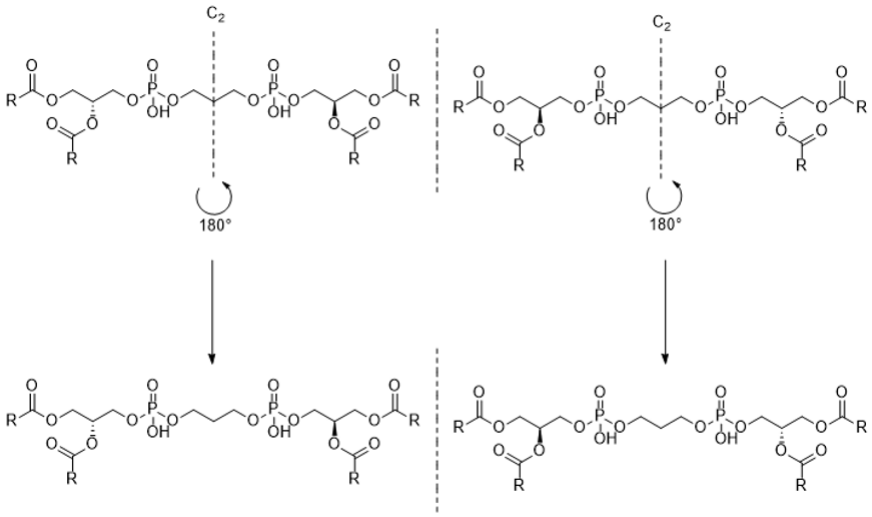
The hydrolysis of **PL1** proceeds through breaking of 8 ester bonds, which requires 8 water molecules. So, **PL2** also contains all these ester bonds and has the same skeleton with **PL1** and the residue of molecule **Z** needs to be located in the centre of **PL2** due to its symmetry:



In the case of **Z** reducing the number of covalent bonds, leads to decreasing numbers of C, H or O atoms:
- removing each C atom decreases the number of covalent bonds by 3;
- removing a pair of H atoms forms a C=C or C=O double bond, which reacts with H_2 in presence of catalyst;
- removing each O atom decreases the number of covalent bonds by 1.

Thus, **Z** is propane-1,3-diol: it contains only C, H, and O; does not react with H_2 in the presence of a catalyst; has one fewer covalent bond; and is more symmetrical than glycerol.

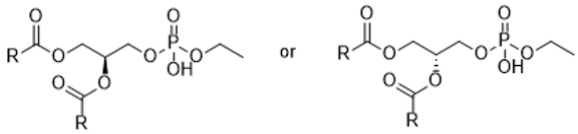
Note that replacing OH with H makes the **PL2** molecule more symmetrical – a C_2 rotation axis appears:



This diastereomer of these enantiomers isn't chiral due to its symmetry plane.

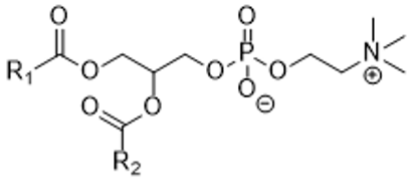
PL3 needs to be synthesised from the same precursors and via the same metabolic pathways as **PL1**. So, it can be one of the following enantiomers:

SOLUTION:



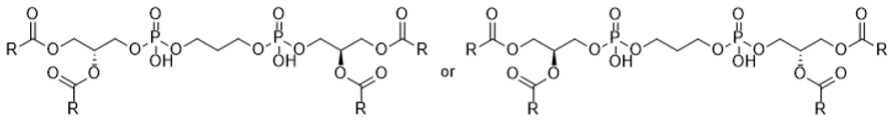
The right structure is typical of prokaryotes and eukaryotes, while the left one is possible for archaea. **PL3** demonstrates the structure of phosphatidic acid derivatives such as phosphatidylcholine.

SOLUTION:

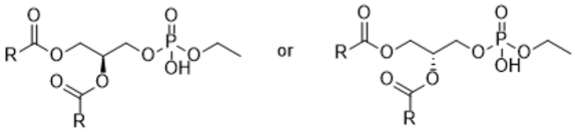


phosphatidylcholine

PL2



PL3



For correct structure of **PL2** with correct stereochemistry: 2 points.

For correct structure of **PL2** with incorrect stereochemistry: 1 point.

For the structure of **PL2** with a keto group in the central position: 1 point.

For correct structure of **PL3** without any stereochemistry: 1 point.

For structure of **PL3** with monoethyl phosphate in central position: 1 point

Correctly drawn structures corresponding to **PL1** in answer to 5.2 are also accepted.