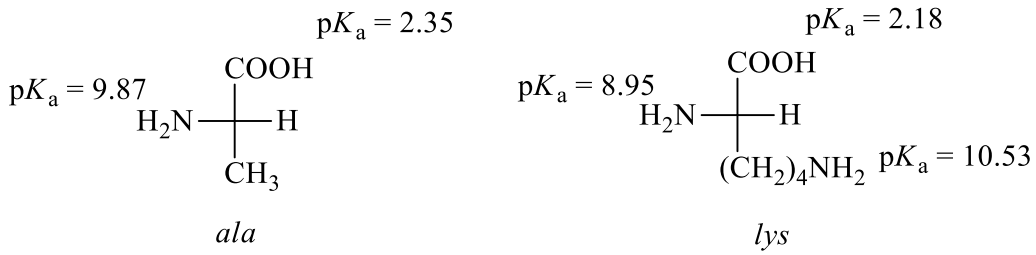
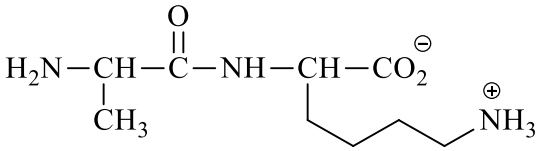
Marks 5

- Alanine (*ala*) and lysine (*lys*) are two amino acids with the structures given below as Fischer projections. The pK_a values of the conjugate acid forms of the different functional groups are indicated.



ala *lys*

Draw the structure of the dipeptide *ala-lys* in its zwitterionic form.



Note that the amine group on the side chain is more basic so it is the one that is protonated.

Would you expect the dipeptide to be acidic, neutral or basic? Give a brief reason for your choice.

Basic. The side chain in lysine is basic whilst that in alanine is neutral.

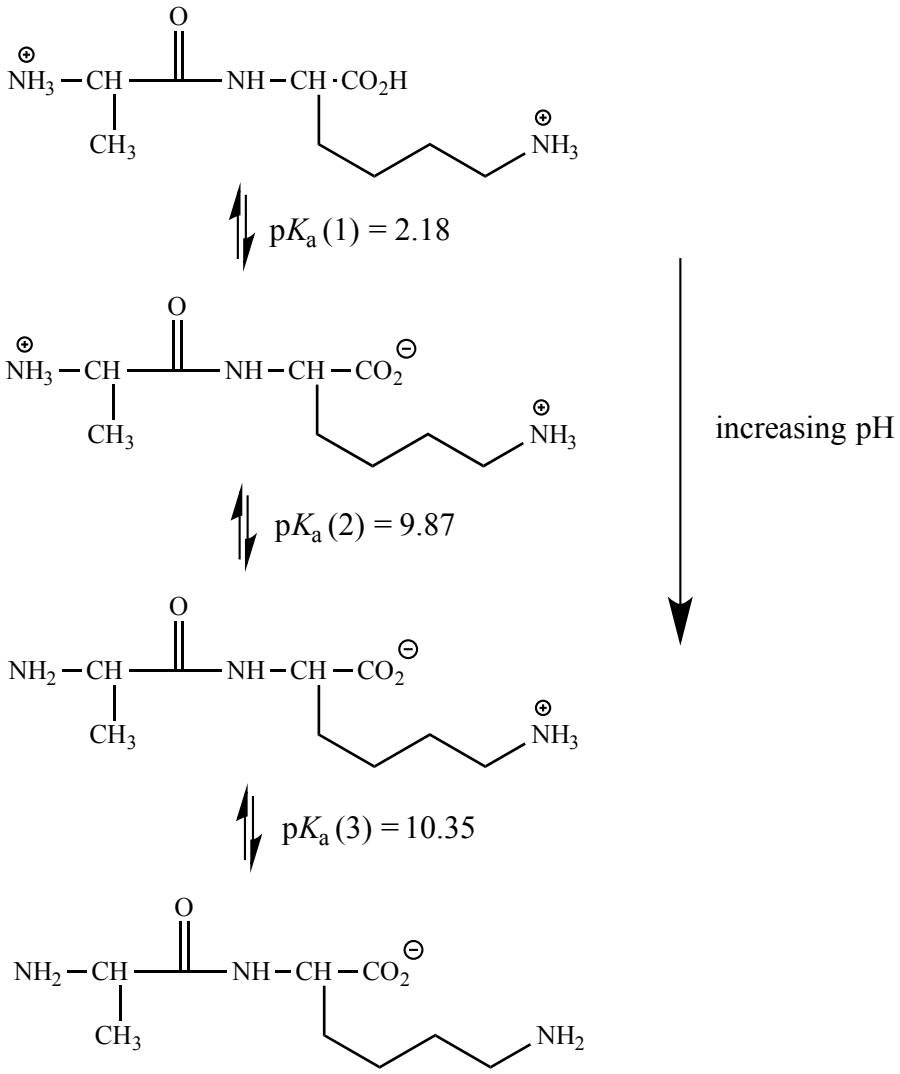
Estimate the isoelectric point of the dipeptide.

The isoelectric point occurs when the peptide has no overall charge. The dipeptide has 2 amine and one carboxylic acid group left after the formation of the amide bonds.

The zwitterionic form is the third form drawn overleaf, occurring between pH = 9.87 and 10.35.

pI is half way between these values: pI = ½ (9.87 + 10.35) = 10.20.

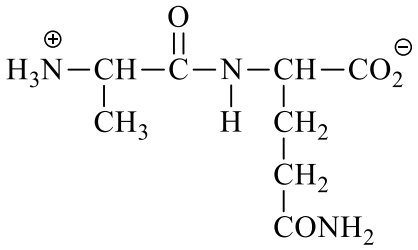
ANSWER CONTINUES ON THE NEXT PAGE

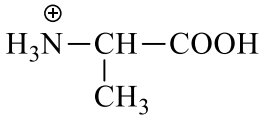


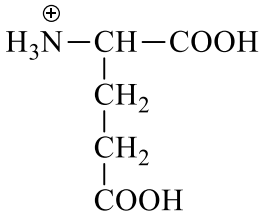
Answer: **10.20**

Marks 3

- Draw all products from the acid hydrolysis of the following dipeptide, indicating the correct charge state under these conditions.



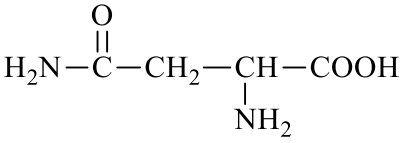




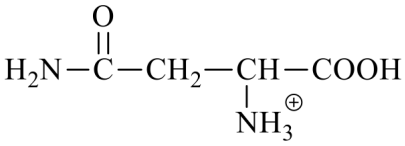
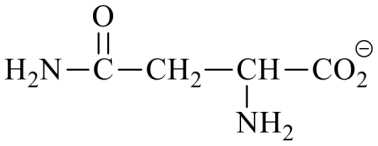
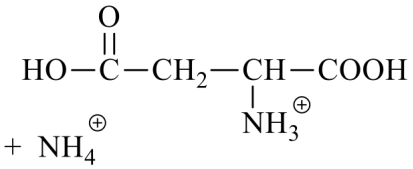
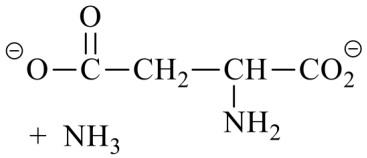
THE REMAINDER OF THIS PAGE IS FOR ROUGH WORKING ONLY.

Marks 6

- The amino acid, asparagine, was isolated from asparagus juice in 1806. The uncharged form, **Y**, is given below.



Draw the constitutional formula of the product(s) formed in the reaction of **Y** with the following reagents.

Cold, dilute hydrochloric acid 	Cold, dilute sodium hydroxide 
Hot, 6 M hydrochloric acid 	Hot, 6 M sodium hydroxide 

THE REMAINDER OF THIS PAGE IS FOR ROUGH WORKING ONLY.

Marks 6

- Alanine (R = CH_3) and lysine (R = $CH_2CH_2CH_2CH_2NH_2$) are two common amino acids. Using *ala* and *lys* to represent the two amino acids, represent all constitutional isomers of the tripeptide formed from one *ala* and two *lys* units.

ala-lys-lys lys-ala-lys lys-lys-ala

Comment, giving your reason(s), on whether the tripeptide(s) will be acidic, neutral or basic in character.

The alanine side chain is neutral whilst the lysine side chain is basic. The tripeptide will therefore be basic.

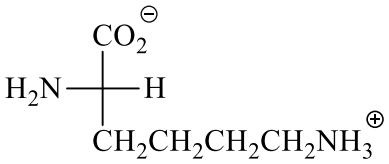
The pK_a values of lysine are 1.82 (α-COOH), 8.95 (α-$NH_3^{\oplus}$) and 10.53 (side chain). What is the value of the isoelectric point of lysine?

Fully protonated, lysine has a –COOH group and two NH_3^+ groups. It has a +2 charge. To get a neutral form, both =NH_3^+ groups must be deprotonated. The isoelectric point is thus the mean of the pK_a values for these two groups:

pI = ½ (8.95 + 10.53) = 9.74

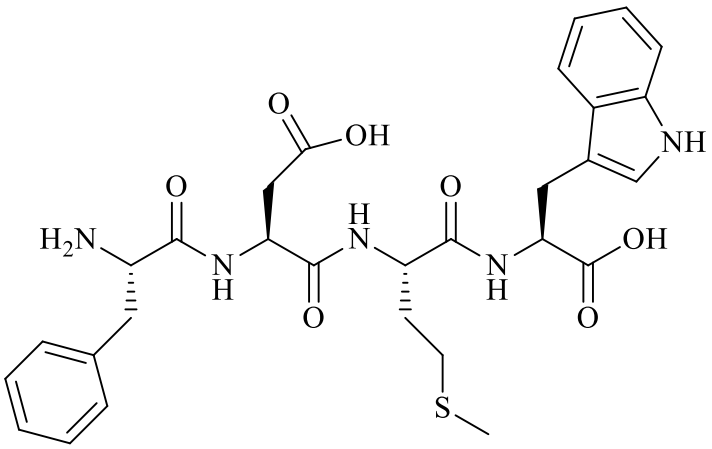
pI = **9.74**

Draw the Fischer projection of the zwitterionic form of lysine.



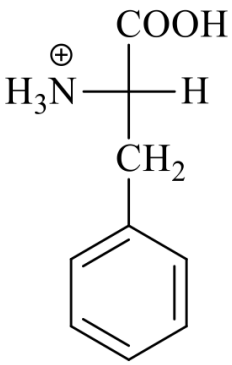
Marks 6

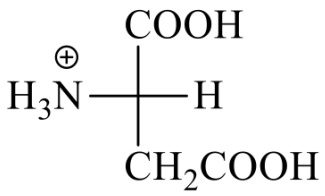
- Cholecystokinin tetrapeptide (CCK-4), (Phe-Asp-Met-Trp) is a peptide fragment derived from the larger peptide hormone cholecystokinin. Unlike cholecystokinin, which has a variety of roles in the gastrointestinal and central nervous systems, CCK-4 acts primarily in the brain as an anxiogenic.

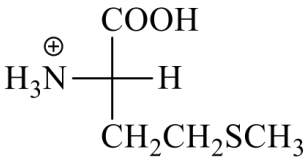


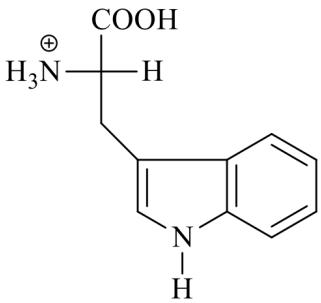
CCK-4

Draw the Fischer projections of the four L-amino acids that result from the acid hydrolysis of CCK-4.







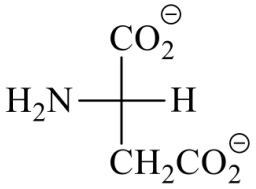


THIS QUESTION CONTINUES ON THE NEXT PAGE.

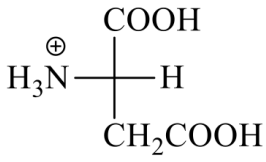
What is the major species present when aspartic acid (Asp) is dissolved in water at pH 12 and pH 1? The pK_a values of aspartic acid are 1.88 (α-COOH), 9.60 (α-$NH_3^{\oplus}$) and 3.65 (side chain).

Marks 4

pH 12

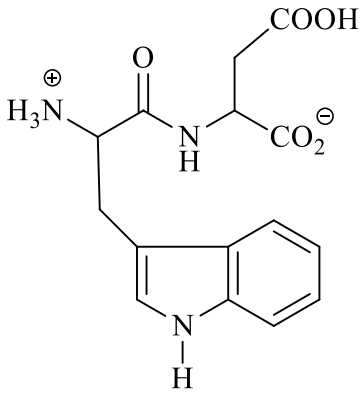


pH 1

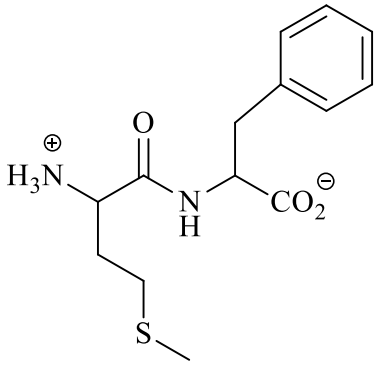


Give the constitutional formulas for the following dipeptides in their zwitterionic states.

Trp-Asp



Met-Phe

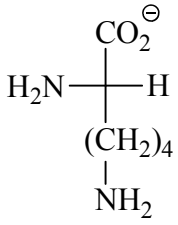


THE REMAINDER OF THIS PAGE IS FOR ROUGH WORKING ONLY.

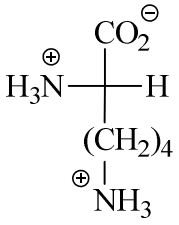
Marks 4

What is the major species present when lysine (Lys) is dissolved in water at pH 12 and pH 5.6. The pK_a values of lysine are 1.82 (α-COOH), 8.95 (α-$NH_3^{\oplus}$) and 10.53 (side chain).

pH 12

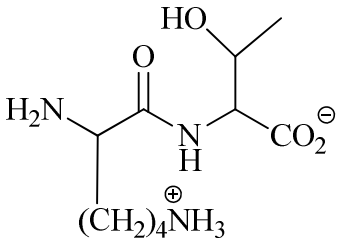


pH 5.6

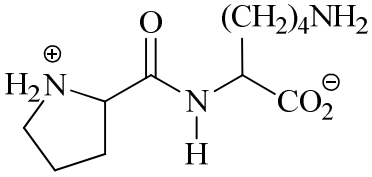


Give the constitutional formulas for the following dipeptides in their zwitterionic states. The pK_a values of proline (Pro) are 1.95 and 10.64.

Lys-Thr

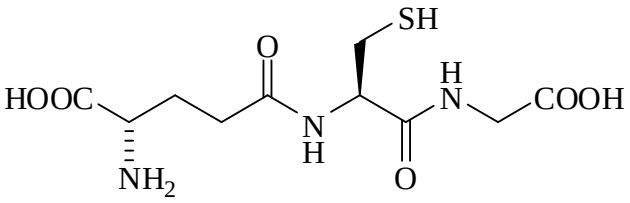


Pro-Lys



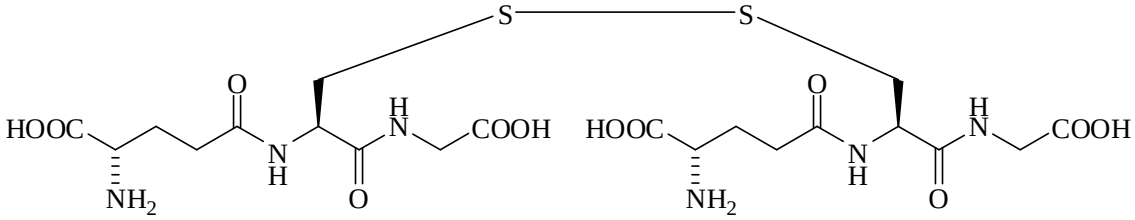
Marks 10

- Glutathione is an important tripeptide (Glu-Cys-Gly) which acts as an antioxidant, protecting cells from toxins such as free radicals. It is an unusual peptide in that the peptidic linkage with glutamic acid (Glu) involves the carboxylic acid group in the side chain.

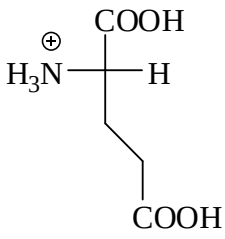


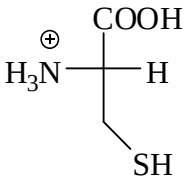
glutathione

Give the product when glutathione undergoes oxidation.



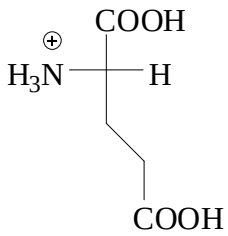
Draw the Fischer projections of the three amino acids (in their natural absolute configurations, where applicable) that result from the vigorous acid hydrolysis (with 6 M HCl) of glutathione.



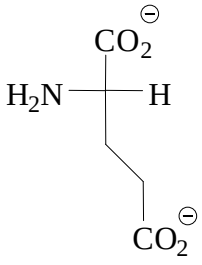


Draw the major species present when glutamic acid (Glu) is dissolved in water at pH 1 and pH 12. The pK_a values of glutamic acid are 2.1 (α-COOH), 9.5 (α-$NH_3^{\oplus}$) and 4.0 (side chain).

pH 1

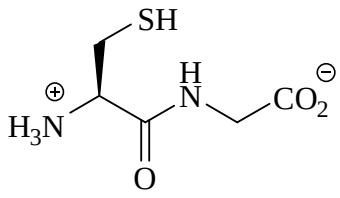


pH 12



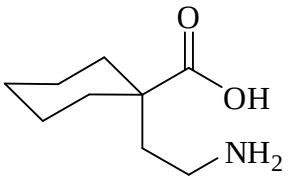
ANSWER CONTINUES ON THE NEXT PAGE

Give the constitutional formula for the dipeptide Cys-Gly in its zwitterionic state.



Marks 4

- Neurontin® is a pharmaceutical now widely used for the treatment of nerve pain. The structure of the active ingredient in Neurontin, gabapentin, is shown below. The pK_a value for the carboxyl group is 3.68, whilst the pK_b value for the amine group is 3.30.



gabapentin

Explain whether gabapentin can reasonably be described as an amino acid.

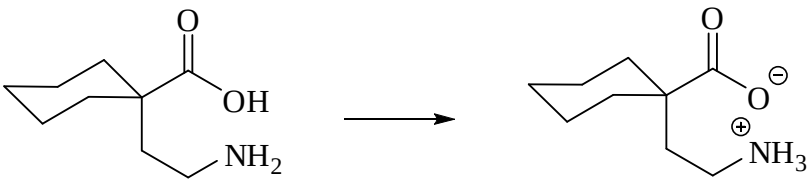
Gabapentin has both an amine and a carboxylic acid functional group, so it is an amino acid.

It is not an α-amino acid (like those found in proteins) as the amino group is not attached to the carbon next to the COOH group.

Orally-delivered pharmaceutical agents that contain amine functional groups are often prepared as hydrochloride salts, rather than as free amines. Suggest a reason why gabapentin is not delivered as a hydrochloride salt, illustrating your answer with a suitable diagram.

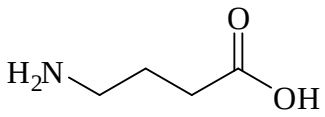
Salt formulations are mainly used to prevent oxidation of the free amine group. The amine group is converted to a quaternary ammonium salt which is more stable.

However, gabapentin already exists in a zwitterionic form at normal pH, with the amine group protonated to form the more stable quaternary ammonium ion.



ANSWER CONTINUES ON THE NEXT PAGE

Gabapentin was originally synthesised as it was anticipated that it would bind to the same receptors as the neurotransmitter GABA (4-aminobutanoic acid). Draw the structure of GABA. Suggest a reason why it might have been anticipated that gabapentin would interact with GABA receptors, and what form such interactions might take.



GABA (4-aminobutanoic acid).

Both GABA and gabapentin have the same basic features - a four carbon chain with a terminal NH_2 and a terminal COOH group. These functional groups are likely to be involved in receptor binding through interactions such as H-bonding.

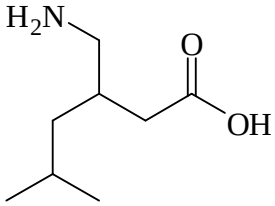
ANSWER CONTINUES ON THE NEXT PAGE

Marks 4

This expectation has proven to be incorrect, as gabapentin does not interact well with GABA receptors. Suggest a reason why this might be the case.

The bulky cyclohexyl group interferes with the binding of gabapentin at the GABA receptor site. This could be due to either steric reasons (the group is too large to fit into the receptor site) or its hydrophobic nature is a poor match for the equivalent part of the receptor.

Pregabalin (marketed under the trade name Lyrica) has been developed as a successor to gabapentin as it is more potent. Its structure is shown below.



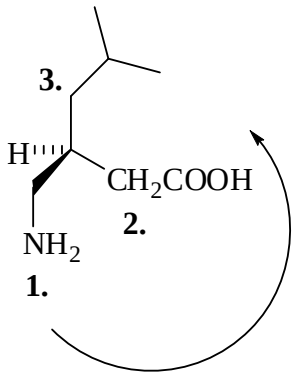
pregabalin

The pharmaceutical formulation contains only the (*S*) enantiomer of pregabalin. Rank the substituents around the stereocentre in decreasing order of priority.

highest priority lowest priority

$-CH_2NH_2$	**$-CH_2COOH$**	**$-CH_2CH(CH_3)_2$**	**–H**

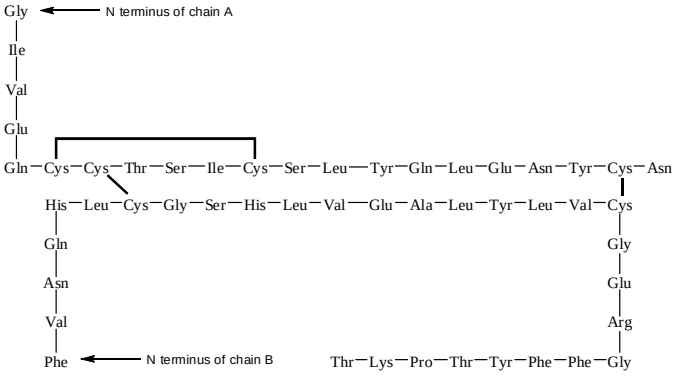
Draw the (*S*) enantiomer of pregabalin.

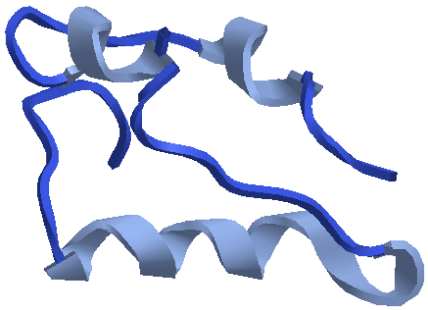


1 → 2 → 3 is anticlockwise with 4 at the back: (*S*)

Marks 4

- Insulin is an important hormone involved in the regulation of glucose availability in the body. It consists of two peptide chains, one consisting of 21 amino acids (the "A" chain) and one of 30 amino acids (the "B" chain). Below are two representations of insulin, one showing the amino acid sequence and the other a stylised ribbon diagram.





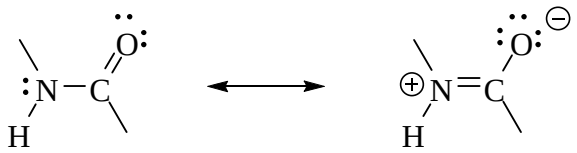
Define the terms *primary structure*, *secondary structure* and *tertiary structure* in relation to proteins. Illustrate your answer with appropriate diagram(s) and by making reference to the representations shown above.

ANSWER CONTINUES ON THE NEXT PAGE.

The peptide links in a protein chain are said to be *resonance stabilised*. Use a diagram to explain what is meant by this term, and indicate one important consequence relating to protein structure and one important consequence relating to the chemistry of proteins.

Marks 4

Resonance occurs when two or more Lewis structures can be drawn for the same compound. In such cases, the true structure is none of those drawn, but rather a weighted average of all of them.



The amide functional group has two major resonance contributors as shown. As a consequence of resonance, the peptide bond is rigid, planar and strong.

Consequence for structure: This rigidity and the charge on the oxygen are ideal for the formation of α-helices and β-pleated sheets via H-bonding.

Consequence for chemistry: The involvement of the N lone pair in resonance, means that the N is unavailable for protonation and is non-basic. The peptide bond is therefore relatively inert.

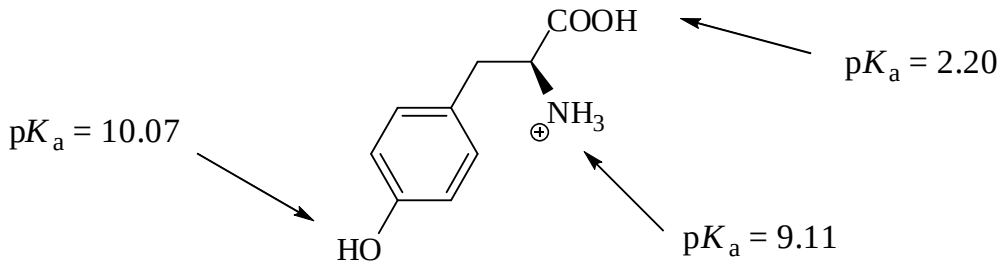
Modern medicine now uses insulin analogues (where one or more of the amino acid residues has been changed) in the treatment of diabetes. In one such analogue, glargine insulin, the changes have increased the isoelectric point of the enzyme from 5.4 to 6.7, thereby reducing its solubility at physiological pH. Explain how changes in the primary amino acid sequence can alter the pI and solubility of the analogue without altering its interaction with blood-glucose.

Changing surface amino acids (for example by changing charged groups to uncharged or polar groups to non-polar) alters the pI and hence the solubility of the protein.

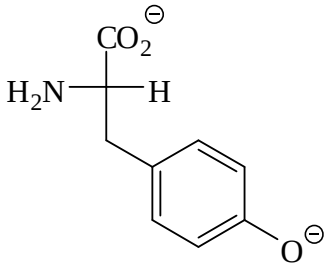
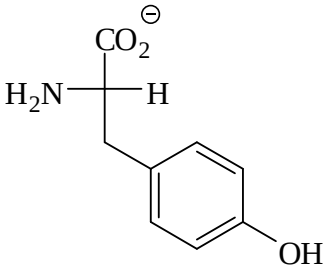
As long as the residues changed are not near the active site and do not change its shape, the mode of action of the enzyme is not affected.

Marks 6

- The structure of L-tyrosine in 1 M HCl is drawn below. The pK_a for each acidic group is indicated on the diagram.



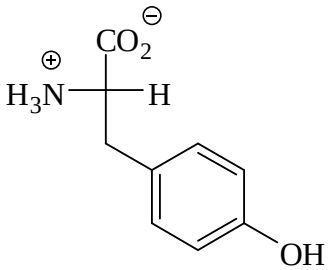
Draw Fischer projections of the predominant species present in a solution of tyrosine at pH 11.0 and pH 9.6. Indicate the overall charge of these species.

Fischer projection of tyrosine at pH 11.0	Fischer projection of tyrosine at pH 9.6
	
Overall charge: **-2**	Overall charge: **-1**

What is the isoelectric point (pI) of tyrosine? **½ (9.11 + 2.20) = 5.66**

Draw the predominant species of tyrosine at the isoelectric point.

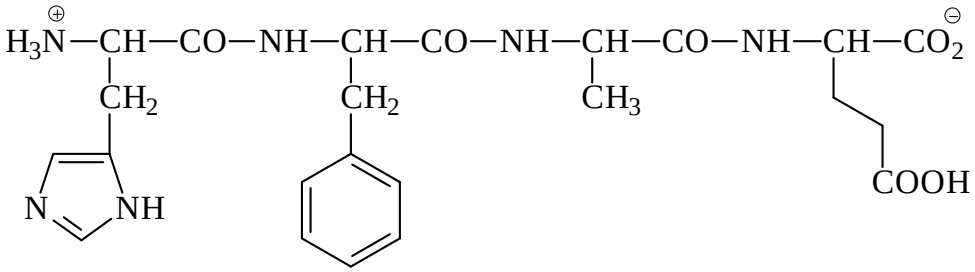
Fischer projection of tyrosine at its isoelectric point.



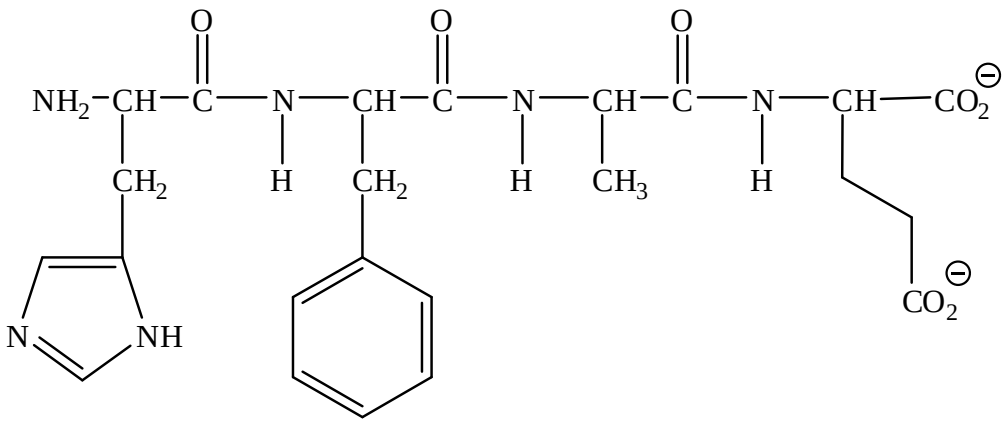
Marks 10

- The structure of the naturally occurring tetrapeptide His-Phe-Ala-Glu, **A**, is shown below as the zwitterion.

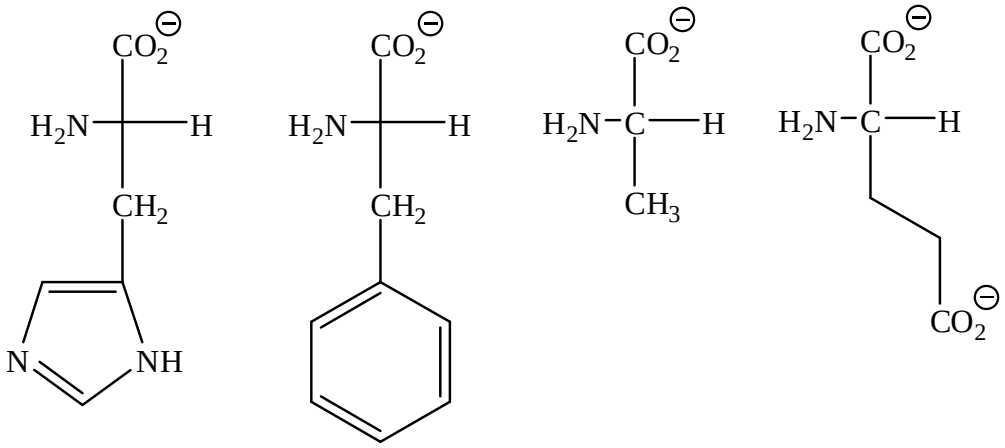
A



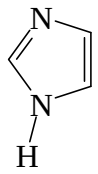
Give the product(s) obtained when **A** is treated with cold 1 M NaOH solution.

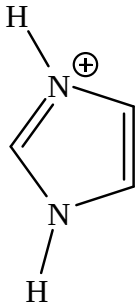


Give the Fischer projections of the four L-amino acids in their correct ionic states obtained from the vigorous basic hydrolysis (6 M KOH) of **A**.



The heterocycle present in the sidechain of histidine is imidazole, whose structure is shown on the right. Give the structure of the product formed when imidazole is treated with HCl. State, giving reasons, whether the product is aromatic.

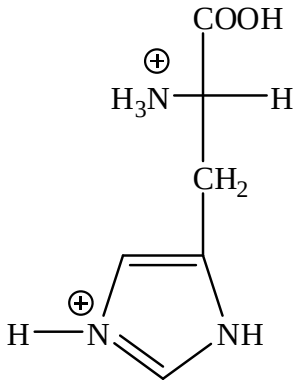




The product is aromatic as all ring atoms are sp^2 hybridised and there are 6 π electrons (4 in π bonds and 2 from the neutral N atom).

This agrees with the Hückel rule, which requires (4n+2) electrons in the π system for aromaticity.

What is the major species present when histidine is dissolved in water at pH 1. The pK_a values of histidine are 1.82 (-COOH), 9.17 (-$NH_3^{\oplus}$) and 6.04 (sidechain).



Given that the pK_a of the carboxylic acid group of leucine is 2.32 and the pK_b of the amine group is 4.24, do you expect the classical or the zwitterionic form to predominate when leucine is dissolved in water? In other words, does the following equilibrium lie to the right or left? Show your reasoning.

$$H_2N{-}CH(CH_2CH(CH_3)_2){-}COOH \rightleftharpoons H_3\overset{\oplus}{N}{-}CH(CH_2CH(CH_3)_2){-}\overset{\ominus}{C}O_2$$

The equilibrium for the K_a of the acid group is:

$$\mathbf{H_2N\text{-}CHR\text{-}COOH \rightleftharpoons H_2N\text{-}CHR\text{-}COO^- + H^+}$$

for which:

$$\mathbf{K_{a(COOH)} = \frac{[H^+][H_2NCHR-COO^-]}{[H_2NCHR-COOH]} = 10^{-2.32}}$$

The equilibrium for protonation of the amine group is:

$$\mathbf{H_2N\text{-}CHR\text{-}COO^- + H^+ \rightleftharpoons H_3N^+\text{-}CHR\text{-}COO^-}$$

for which:

$$\mathbf{K = \frac{[H_3N^+-CHR-COO^-]}{[H_2NCHR-COO^-][H^+]} = \frac{1}{K_{a(NH_3^+)}} = \frac{1}{10^{-9.76}} = 10^{+9.76}}$$

in which pK_a + pK_b = 14 has been used.

The equilibrium for formation of the zwitterionic form in the question is:

$$\mathbf{H_2N\text{-}CHR\text{-}COOH \rightleftharpoons H_2N^+\text{-}CHR\text{-}COO^- + H^+}$$

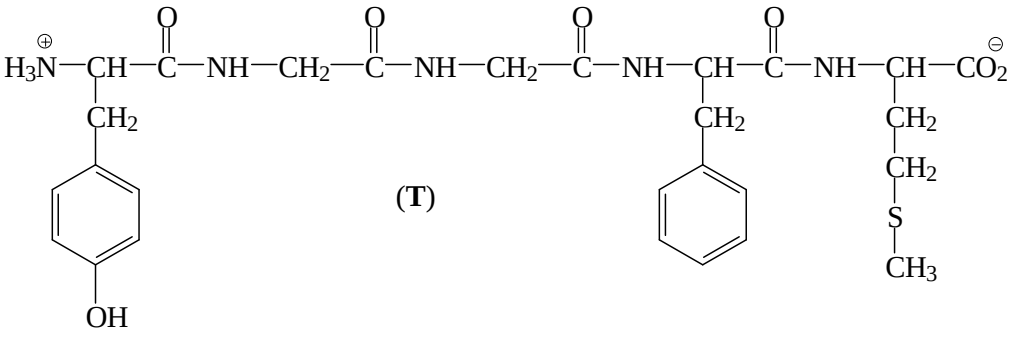
for which:

$$\mathbf{K' = \frac{[H_3N^+-CHR-COO^-][H^+]}{[H_2NCHR-COOH]} = \frac{K_{a(COOH)}}{K_{a(NH_3^+)}} = 10^{-2.32} \times 10^{+9.76} = 10^{7.44} >> 1}$$

As the equilibrium constant >> 1, the equilibrium lies far to the right and so the zwitterionic form dominates.

Marks 7

- The neurohormone Tyr-Gly-Gly-Phe-Met (**T**) known as methionine enkephalin is a naturally occurring peptide which controls pain perception in vertebrates.



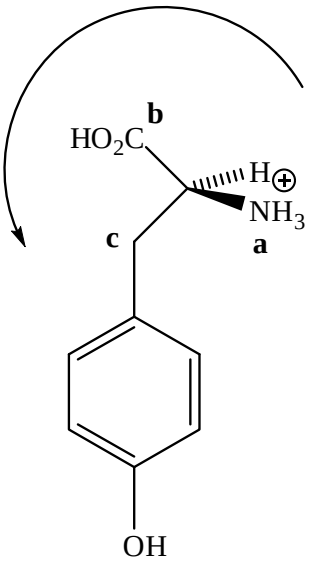
(**T**)

Name the functional groups in (**T**).

tertiary ammonium ion, phenol, amide, arene, carboxylate ion, thioether

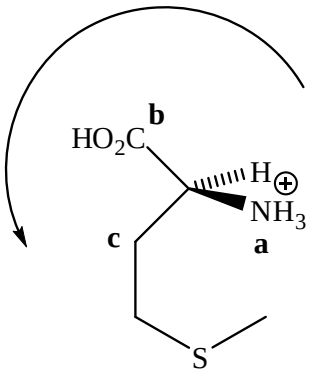
Four amino acids (tyrosine, glycine, phenylalanine and methionine) are obtained on complete acid hydrolysis of (**T**). Draw the stereoformulas of L-tyrosine and L-methionine in the boxes below. Indicate their absolute configurations using the (*R*)- and (*S*)- convention.

L-tyrosine



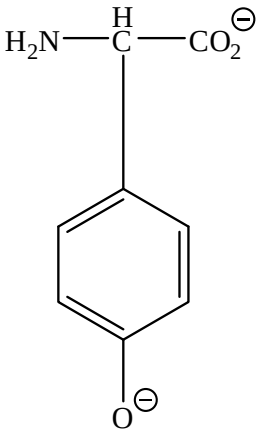
Absolute configuration **S**

L-methionine



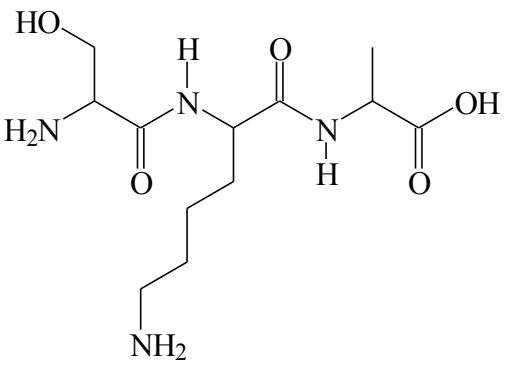
Absolute configuration **S**

Give the constitutional formula for the product obtained when tyrosine, the *N*-terminal amino acid in peptide (**T**), is dissolved in 1 M NaOH solution.


H
H2N
C
CO2
O

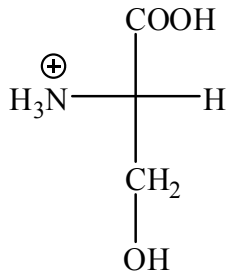
Marks 11

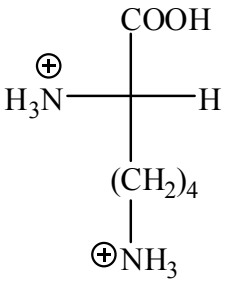
- The constitutional formula of the tripeptide seryllysylalanine (Ser-Lys-Ala), **M**, is shown below.

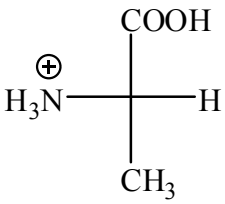


(**M**)

Draw the Fischer projections for the L-configurations of the amino acids formed when compound **M** is hydrolysed with hot 6 M hydrochloric acid.

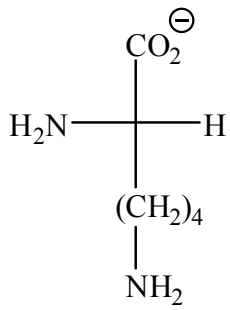




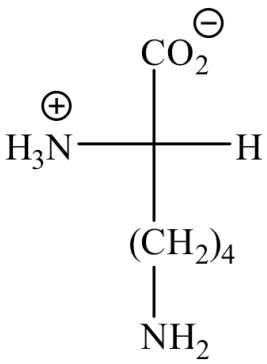


The pK_a values of lysine are pK_{a1} = 1.82 (α-COOH), pK_{a2} = 8.95 (α-$NH_3^{\oplus}$) and pK_{a3} = 10.53 (-$(CH_2)_4NH_3^{\oplus}$). Give the structures of the predominant species present in a solution of lysine at pH 12 and at pH 5.6.

pH 12.0



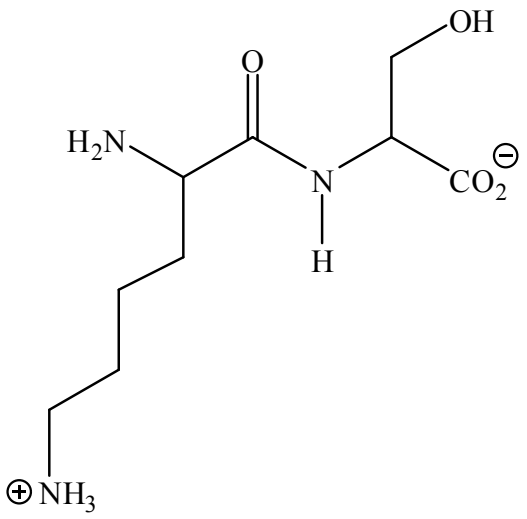
pH 5.6



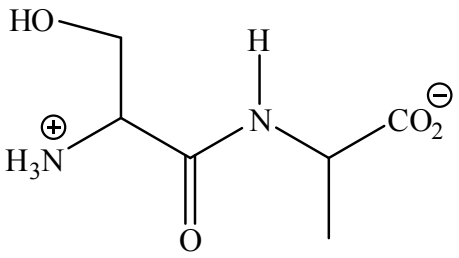
ANSWER CONTINUES ON THE NEXT PAGE

Give the constitutional formulas for the following dipeptides in their zwitterionic states.

Lys-Ser



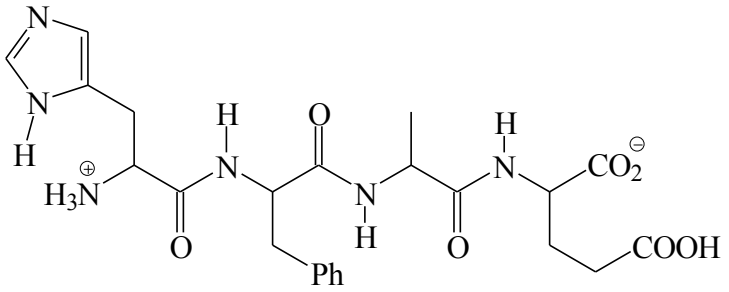
Ser-Ala



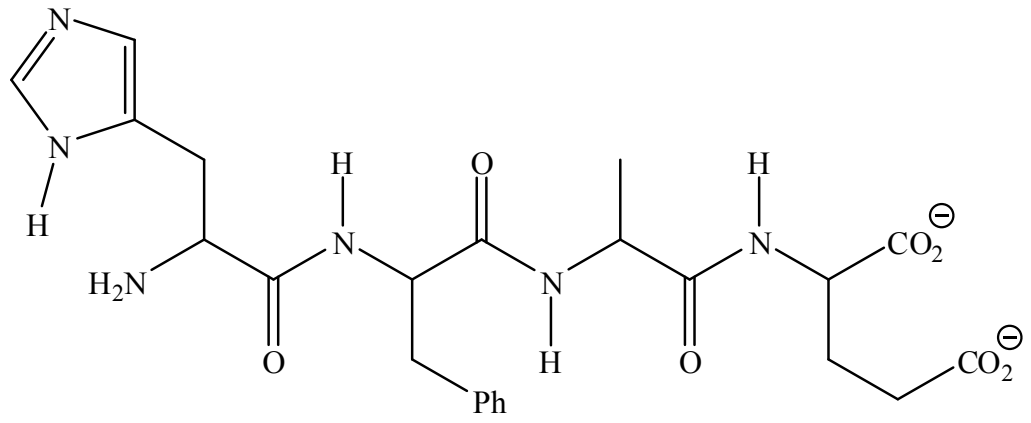
Marks 9

- The structure of the naturally occurring tetrapeptide His-Phe-Ala-Glu, **A**, is shown below as the zwitterion.

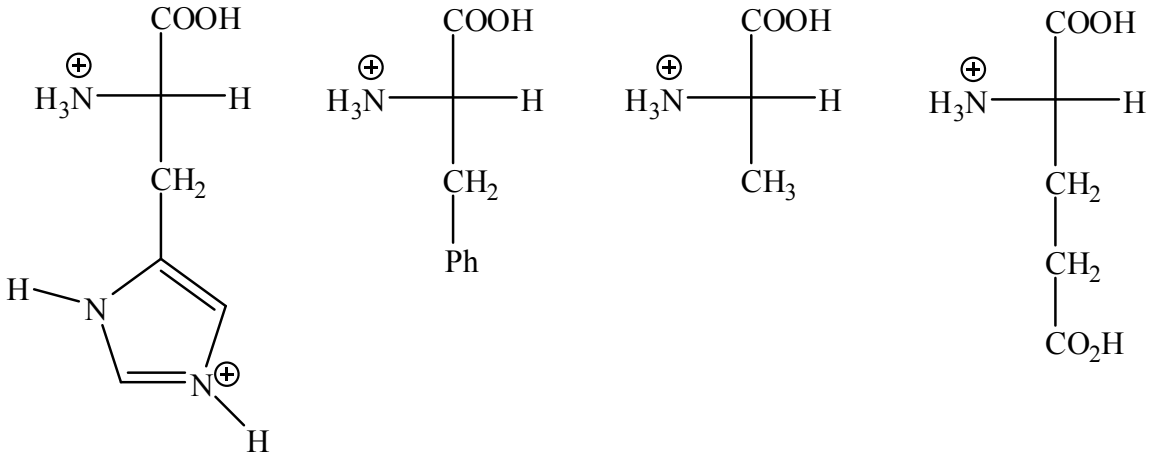
A



Give the product(s) obtained when **A** is treated with cold 1 M NaOH.

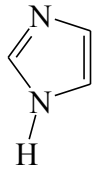


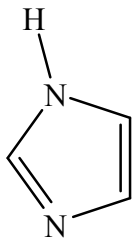
Vigorous acid hydrolysis of **A** gives four products. Give the structures of these four products in their correct ionic states as Fischer projections.



ANSWER CONTINUES ON THE NEXT PAGE

The heterocycle present in the sidechain of histidine is imidazole, whose structure is shown on the right. Give the structure of a tautomer of imidazole and state, giving reasons, whether your tautomer is aromatic.





Imidazole is aromatic: there are 6 π electrons, the ring is planar and each ring atom is sp^2 hybridized. The C=C and C=N bonds both contributes 2 π electrons. The N-H nitrogen atom has two electrons in a p-orbital which also contribute to the π system.

The lone pair on the second nitrogen are housed in a sp^2 hybrid direct away from the molecule and do not contribute to the π electrons.

What is the major species present when histidine is dissolved in water at pH 12. The pK_a values of histidine are 1.82 (-COOH), 9.17 ($-NH_3^{\oplus}$) and 6.04 (sidechain).

$$\mathbf{pI} = \frac{\mathbf{6.04 + 9.17}}{\mathbf{2}} = \mathbf{7.61}$$

At pH = 12: