Name: _____WHITE_____ Student Number: _____

Answer the following questions on the computer scoring sheet. 1 mark each

1. Which of the following amino acids would have the highest **relative mobility R_f** in normal thin layer chromatography?

A) Lys **B) Leu** C) Asn D) Thr E) Asp 1) A **B** C D E

least polar

2. Which of the following amino acids is most likely to be found **on the outer surface** of a properly folded protein?

A) Ile B) Phe **C) Asp** D) Val E) Met 2) A B **C** D E

most polar

3. Which of the following amino acids prefers β-sheet **secondary structure**?

A) Ile B) Pro C) Ala D) Gly E) Glu 3) **A** B C D E

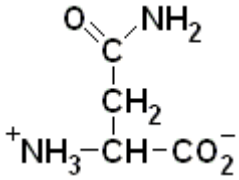
branched on β-carbon

4. Which of the following amino acids **can donate H-bonds from its side chain**?

A) Phe **B) Gln** C) Ala D) Leu E) Gly 4) A **B** C D E

O=C-NH_2 on side chain

5. **Identify the amino acid** drawn on the right:



A) Asparagine B) Arginine C) Threonine

D) Glutamine E) Lysine

5) **A** B C D E

6. Which of the following statements correctly describes why **Ninhydrin** is used in amino acid and peptide analysis?

A) to detect amino acids by a color reaction

B) to identify the N-terminal amino acid

C) as the product of the cyclization step in Edman's reaction

D) to reduce disulphide bonds

E) to cut the peptide chain at methionine 6) **A** B C D E

7. By what factor does a typical enzyme **speed up reaction rate** compared to uncatalyzed reaction?

A) 10 fold B) 100 fold C) 1000 fold

D) 10000 fold **E) 1000000000 fold** 7) A B C D **E**

8. Which of the following represent the **catalytic triad** of chymotrypsin?

A) Phe, Tyr and Trp B) His, Lys and Arg **C) Asp, His and Ser**

D) Gly, Ser and Phe E) Ala, Gly and Ser

8) A B **C** D E

9. In the structure of the α-helix, **which of the following pairs of groups is linked together by a hydrogen bond**?

A) α-NH of amino acid 1 to α-CO of amino acid 3
B) α-NH of amino acid 1 to α-CO of amino acid 4
C) α-NH of amino acid 1 to α-CO of amino acid 5
D) α-CO of amino acid 1 to α-NH of amino acid 4

E) α-CO of amino acid 1 to α-NH of amino acid 5 9) A B C D **E**

10. What **initial reaction velocity** v_o is observed if substrate concentration in an enzyme reaction is $0.5 \times K_M$ and V_{max} is 2.4×10^{-6} mol L^{-1} min^{-1}?

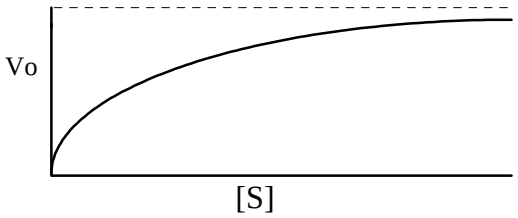
A) 1.2×10^{-6} mol L^{-1} min^{-1}.
B) 6.0×10^{-7} mol L^{-1} min^{-1}.
C) 2.4×10^{-6} mol L^{-1} min^{-1}.
D) 8.0×10^{-7} mol L^{-1} min^{-1}.
E) 1.6×10^{-6} mol L^{-1} min^{-1}.

$1/3\ V_{max}$ 10)A B C **D** E

11. What **substrate concentration**, expressed as a multiple of K_M, must be present when an enzyme reaction is observed to have an initial rate $v_o = 0.75\ V_{max}$.?

A) [S] = $0.25 \times K_M$ B) [S] = $0.75 \times K_M$ C) [S] = $1.33 \times K_M$
D) [S] = $3.0 \times K_M$ E) [S] = $4.0 \times K_M$ 11)A B C **D** E

12. The plot at right represents **initial rate** of an enzyme catalyzed reaction as a **function of [S].**



Why does the curve level off towards the right?

A) Substrate is running out.
B) Affinity for substrate is low and it's not binding to the enzyme
C) Product is accumulating and causing the reverse reaction to occur
D) All the available enzyme is fully occupied with substrate
E) An inhibitor must be present and is slowing down the reaction 12)A B C **D** E

13. What is the **net charge** of the following peptide at pH 5.0?

-0.5 +1 +1
+Ala-Cys-Leu-Ser-Glu-Lys-Met-Tyr-His-Val-Thr –

A) + 0.5 B) +1 **C) +1.5** D) +2 E) +2.5 13)A B **C** D E

14. Which of the following represents the **major energy contribution** that causes a typical protein to fold in its usual tertiary structure?

A) Disulfide bonds B) Hydrogen bonds
C) Ion pairs D) Salt bridges
E) Hydrophobic and non-polar interactions 14)A B C D **E**

15) Which of the following best describes the role of the **oxyanion hole** in chymotrypsin?

A) Its negative charge helps His 57 capture H^+ and become positive

B) It binds the substrate C=O group in a position that helps form the transition state

C) It makes a better nucleophile for the reaction

D) It is responsible for recognizing aromatic amino acids as the targets for hydrolysis

E) It is responsible for recognizing positively charged amino acids for hydrolysis

15)A **B** C D E

16) How are **K_M and V_{max}** deduced from a **Woolf-Hanes** plot?

A) K_M is the slope and V_{max} is the y intercept

B) K_M is the y intercept and V_{max} is the slope

C) $-K_M$ is the x intercept and $1/V_{max}$ is the slope

D) $-1/K_M$ is the x intercept and $1/V_{max}$ is the slope

E) $-1/K_M$ is the x intercept and $1/V_{max}$ is the y intercept

16)A B **C** D E

17) What are the characteristics effects of a **competitive inhibitor** on enzyme kinetics?

A) K_M decreases and V_{max} is unchanged

B) K_M increases and V_{max} is unchanged

C) K_M is unchanged and V_{max} is decreased

D) K_M is unchanged and V_{max} increases

E) K_M decreases and V_{max} is increased

17)A **B** C D E

18) Which of the following statements about a **noncompetitive inhibitor** is **NOT true**?

A) Substrate can bind to the EI complex

B) Inhibitor can bind to the ES complex

C) K_i is the concentration of inhibitor causing rate of catalysis to decrease by 50%

D) Very high [S] can overcome the effect of the inhibitor

E) The inhibitor and the substrate do not share the same binding site

18)A B C **D** E

19) The **extinction coefficient** of adenosine monophosphate is **1.51×10^4 L $mol^{-1}cm^{-1}$** at 260 nm. If a sample 1.00 cm thick has absorbance **0.604 at 260 nm**, what is the **concentration** of adenosine monophosphate?

A) 4.00×10^{-5} mol/L

B) 4.00×10^{-4} mol/L

C) 1.04×10^{-3} mol/L

D) 2.5×10^{-3} mol/L

E) 9.6×10^{-6} mol/L

19)**A** B C D E

20) Which of the following definitions describes **turnover number** of an enzyme?

A) moles per liter of substrate converted to product per second

B) moles of substrate converted per second

C) moles of substrate converted per mole of enzyme per second

D) molecules of substrate converted per molecule of enzyme per second

E) both C) and D) are correct

20)A B C D **E**

Name _____**white**_____

21 a). How does chymotrypsin **bind and recognize** aromatic amino acids Phe, Tyr and Trp, but not smaller non polar amino acids such as Ala or Val? (no need to discuss catalytic mechanism) 2 marks

½ mark each point

Selected side chain fits in a nonpolar pocket in the chymotrypsin
Pocket is large, to fit aromatic amino acids
benzene ring makes good contact with pocket
either Ala or Val are small, and don't make good van der Waals contact with pocket
or small Ala or Val may allow H_2O into non polar pocket

Note: the binding pocket is **not the oxyanion hole**, which binds the C=O group of the substrate and has **nothing to do with selecting a specific amino acid**, but is part of the catalytic centre.

b) How does the enzyme trypsin **bind and recognize** peptide chains and target the **positively charged** amino acids Lys and Arg, but not His? (no need to discuss catalytic mechanism) 2 marks

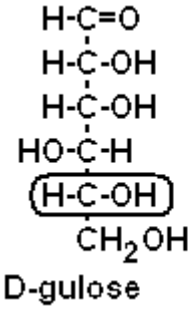
½ mark each point

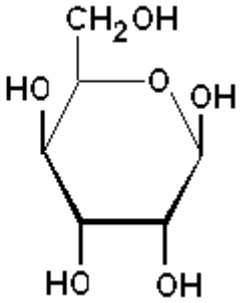
H-bonding groups of trypsin peptide backbone to enzyme
Selected side chain fits in a long narrow pocket in trypsin
Negative charge at bottom of pocket attracts positive Lys or Arg
either His ring is excluded by narrow shape of pocket
or His ring is too large to fit in the pocket

Note: marks were lost for **describing the properties of the target amino acids instead of describing how the enzyme recognizes them** (this applies to part a also).
Read the question carefully!

c) The hexose **D-gulose** has the Fischer straight chain structure shown on the left; use this to complete the Haworth structure on the right with **appropriate orientation of ring -OH groups**.

D-gulose





β-D-gulopyranose

Note: in Haworth form, -OH groups on the ring are shown **up** or **down**, not sideways or at an angle

2 marks

½ mark each

Indicate which -OH group of the straight chain becomes the ring -O- in the pyranose form.

circled in figure above or O-5 or on carbon 5 1 mark

How is D-altrose related to D-glucose?

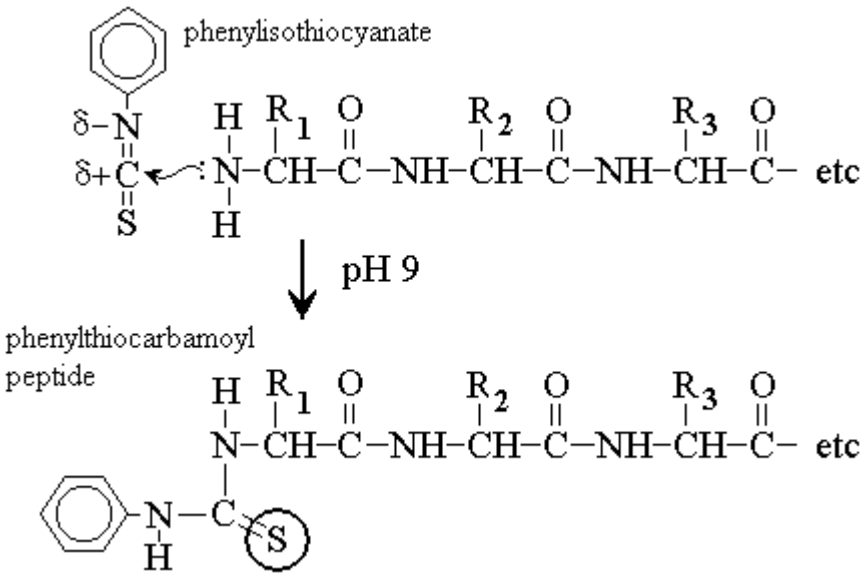
Epimer at C-2 or configuration **inverted at C-2** ½ mark
Epimer at C-3 or configuration **inverted at C-3** ½ mark 1 mark
or **Epimer** (without specifying location) ½ mark max

The question describes D-altrose as a **hexose**; since glucose is also a hexose, we are looking for **relationship within that family**, not something trivial like having 6 C atoms or having D-stereochemistry

total 8

Name _____**white**_____

22. The diagram shows the coupling step of the Edman reaction



a) Why are mildly basic conditions (pH 9) needed for the coupling reaction?

pK_a = 8 for N-terminal (see data page), so pH > pKa

At pH 9, the N terminal is deprotonated ½ mark

and **can act as a nucleophile** to couple to phenylisothiocyanate ½ mark

b) What **change in conditions** causes the cyclization reaction to proceed? What prevents coupling and cyclization from proceeding **at the same time**, and why does this make Edman's method so effective?

Change to **acid** conditions ½ mark

coupling and cyclization **can't occur simultaneously or are mutually exclusive** ½ mark

either each reaction step can **go to completion without prematurely starting the next** ½ mark

or the two step process keeps the reaction cycle in phase so **only one amino acid is released per cycle** ½ mark

max 1½ mark

c) During the cyclization step, which atom in the phenylthiocarbamoyl group **acts as nucleophile** to attack the first peptide bond? **Circle the atom concerned in the diagram above.** ½ mark

d) Why is **only the first peptide bond** attacked in the cyclization step?

The reactive **sulfur/nucleophilic sulfur can only reach the first peptide bond** 1 mark

or **only one S available to react** ½ mark (does not fully explain why only nearest reacts)

e) Why is **water excluded** during the cyclization reaction?

Why is the Edman method a significant improvement over Sanger's method?

exclusion of H_2O **does not permit** or **prevents hydrolysis** of the **remaining peptide bonds** ½ mark ½ mark

Edman's method leaves the rest of the chain intact so that the **reactions can be repeated** ½ mark

Sanger's method used hydrolysis to release the first amino acid, which **destroys the rest of the chain** ½ mark

total 6

Name _____**white**_____

23 a) A polypeptide was analyzed by breaking it into short oligopeptides and determining the amino acid sequence of each oligopeptide. One sample of the polypeptide was digested with **chymotrypsin**, and a second sample of the same polypeptide was digested with **trypsin**. The results of each sequence analysis are listed below:

oligopeptides generated by trypsin	**oligopeptides generated by chymotrypsin**
Gly-Ile-Lys	Asp-Ala-Gly
Met-Val-Arg	Leu-Ile-Trp
Met-Lys-Pro-Arg	Met-Val-Arg-Ala-Gln-Phe
Cys-Glu-Phe-Asp-Ala-Gly	Asp-Ser-Lys-Gly-Ile-Lys-Met-Lys-Pro-Arg-Cys-Glu-Phe
Ala-Gln-Phe-Leu-Ile-Trp-Asp-Ser-Lys	

Use this information to write out the complete sequence of the original polypeptide. 3 marks
(use data page for rough work)

Met-Val-Arg | Ala-Gln-Phe-Leu-Ile-Trp-Asp-Ser-Lys | Gly-Ile-Lys | Met-Lys-Pro-Arg | Cys-Glu-Phe-Asp-Ala-Gly

Met-Val-Arg-Ala-Gln-Phe | Leu-Ile-Trp | Asp-Ser-Lys-Gly-Ile-Lys-Met-Lys-Pro-Arg-Cys-Glu-Phe | Asp-Ala-Gly

3 marks for the **complete sequence** (not necessary to show both)
½ for one pair correctly assembled
1 mark for 3 in a row correctly assembled
lose 1 mark if sequence is correct but with one extra peptide is tacked on at either end
lose 1 mark if sequence is correct except one peptide is missing

b) You need to separate a mixture of peptides of varied size. Briefly explain how **gel filtration chromatography separates molecules of different size**. 3 marks

Score for the following points in the description

A gel is a porous water-filled polymer network ½ mark

Pore size is comparable to size of peptides being separated ½ mark
or **gel has pores up to a maximum size of molecule or exclusion limit**

Gel is formed into beads so that buffer or **solvent can flow around outside** ½ mark

Large (peptides or **molecules) (can't enter pores** or **are excluded from the gel)** ½ mark
and remain in the (flowing buffer or **mobile phase) so pass through quickly** ½ mark

Smaller (peptides or **molecules) enter pores of gel so their passage is delayed** ½ mark

Mobility is proportional to log molar mass ½ mark

3 marks max

total 6