KENYATTA UNIVERSITY

UNIVERSITY EXAMINATIONS 2011/2012

FIRST SEMESTER EXAMINATION FOR THE DEGREE OF MASTER OF SCIENCE

SCH 531: CHEMISTRY OF PROTEINS AND CARBOHYDRATES

DATE: Monday 28th November, 2011 **TIME:** 9.00 a.m. – 12.00 p.m.

INSTRUCTIONS

Answer ALL questions
(A sheet with structures of amino acids and Fischer projections of some monosaccharides is provided)

Q1 (a) The isoelectric point (IEP) of the following amino acids (H_2N-CHR-COOH) varies with the structure of the side chain (R):
(i) 2.98 for glutamic acid; (ii) 6.34 for alanine; (iii) 7.59 for histidine; and (iv) 9.76 lysine.
Account briefly for these differences. [6 marks]

(b) Sketch the acid-base titration curve for diaminopimelic acid, $HOOCCHCH_2CH_2CH_2CHCOOH$ (NH_2 and NH_2), starting from low pH (acidic medium) to higher values resulting from addition of four equivalents of NaOH. On your curve, indicate the pH values corresponding to all pKa values and to IEP of the acid. [6 marks]

(c) The following peptides are subjected to normal electrophoretic analysis at pH 6.0. State whether the peptides will migrate towards the cathode or anode and predict their relative rates of migration.
(i) Gly.Arg, (ii) Phe.Gly.Arg, (iii) Phe.Glu.Glu, and (iv) Phe.Gly.Glu [6 marks]

(d) Three proteins have isoelectric points of 6.0, 7.0 and 8.0. How would they behave across a pH gradient in electrophoresis (Isoelectric Focusing, ISF)? How might you proceed to separate the proteins using ion-exchange chromatography DEAE-cellulose (DEAE = diethylaminoethyl)? [6 marks]

[Total for Q1: 24 marks]

Q2 (a) How many different tetrapeptides can be made with amino acid composition Gly, Ala, Val, Phe? Show the sequences of those with Ala at the N-terminus end. [6 marks]

(b) (i) Drosocin, **A**, is an insect peptide with antimicrobial activity. Use the following information to establish the sequence of amino acids in Drosocin.
Note: To gain full marks your answer must include clear outline of your deductions.

- Total hydrolysis of **A** results in the isolation of the following amino acids: (Arg4, Gly, His, Ile, Lys, Pro6, Ser2, Thr, Tyr, Val)
- Treatment of A with FDNB followed by total acid hydrolysis gave DNP-Gly
- Treatment of A with chymotrypsin gave 2 peptides: **B** (Arg3, His, Ile, Pro4, Ser2, Thr, Val) and **C** (Arg, Gly, Lys, Pro2, Tyr)
- Treatment of A with trypsin gave the following peptides: **D** (Arg, His, Pro2, Ser, Thr), **E** (Arg, Pro2, Ser, Tyr), **F** (Arg, Ile, Pro), **G** (Arg, Pro), **H** (Gly, Lys) and free Val
- Partial acid hydrolysis gave various fragments including the dipeptides, Pro.Thr & Ser.His; and the **only** tripeptides containing arginine were (Arg, Ile, Val) and (Arg, Pro2).

(Amino acid abbreviations separated by commas and given in brackets indicate the peptide *composition*, not the *sequence*.) [16 marks]

(ii) What other experiments could be done to confirm the peptide sequence derived from the above results? [4 marks]

[Total for Q2: 26 marks]

Q3 In peptide synthesis, linking amino acids (or amino acid residues of peptide fragments) need to proceed with (i) protection of functional groups not intended to participate in a linkage; (ii) minimal racemization at carbon adjacent to the activated carboxyl group involved in the formation of the peptide bond.

(a) Carbamates are preferred protective groups for α-amine compared to normal amides because they are more readily removed after peptide formation at the target site. Explain why.
One of the carbamates (flourenylmethoxycarbonyl, FMOC) can be easily removed with organic bases like piperidine, while the others (like benzoyloxycarbonyl and *tert*-butyloxycarbonyl) cannot. Explain. [6 marks]

(b) Explain with mechanistic details why amino acid chlorides enhance the sensitivity of amino acid residues to racemization. Why is proline not similarly affected? [8 marks]

(c) Peptide formation involves free amino group of one amino acid residue and 'active esters' of carboxyl group of the other amino acid residue. Explain how these are made and their electronic characteristics which make them particularly reactive. [8 marks]

[Total for Q3: 22 marks]

Q4 (a) (i) What spectral (e.g. infrared and NMR) evidence is there that shows that aldoses (and ketoses) other than the 4-carbon glyceraldehyde exist predominantly in cyclic rather than linear tautomers? [4 marks]

(ii) D-glucose occurs mainly as an equilibrium mixture of two tautomers, α and β, with axial and equatorial OH, respectively, on C1. The β tautomer is present in larger proportion (64% compared to 34% of the α tautomer). However, equilibration of methyl-D-glucopyranoside tautomers by treatment with HCl in methanol gives the α-form (with hindered axial 1-OMe) and β-

form (with equatorial 1-OMe) in ~ 2:1 proportion. What electronic factors have been attributed to these differences in the relative stabilities of the two sets of tautomers ? [8 marks]

(iii) Unlike D-glucose, similar treatment of D-arabinose gives mainly methyl β-D-arabinopyranoside rather than α-D-arabinopyranoside. Explain. [4 marks]

(b) Put the structures (preferably in chair representations) of the following two disaccharides: α-D-glucopyranosyl-(1↔1)- α-D-glucopyranoside and 4-*O*-β-D-galactopyranosyl-D-glucopyranose. Which, if any, is a reducing sugar? [6 marks]

(c) Biomass (lignocellulose) is considered to have enormous potential for conversion to biofuels and to a series of basic starting materials for chemical industries. Give brief highlights of the current status of R&D in this area. [6 marks]

[Total for Q4: 28 marks]