Chem 135: First Midterm

September 28th, 2007

Please provide all answers in the space provided. Extra paper is available if needed. You may not use calculators for this exam, but you are free to use (previously unassembled!) molecular model kits. Including the title page, there should be 7 pages in this exam booklet.

Good Luck!

Name: Key

(1) _____ (17 points)

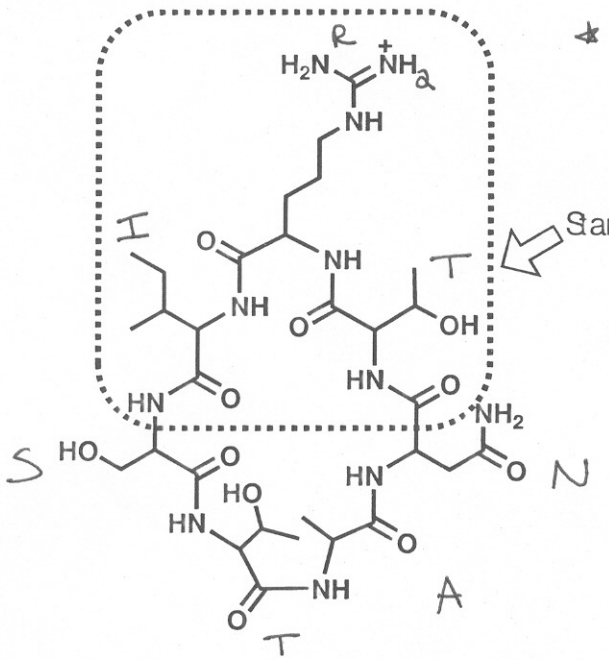
(2) _____ (12 points)

(3) _____ (32 points)

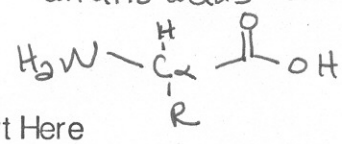
(4) _____ (39 points)

TOTAL _____ (100 points)

1. Your Chem 135 GSIs have recently isolated a new pain killer from Peet's coffee beans (yet another reason not to drink Starbucks!). As shown below, this compound exists as a ring, where the N- and C-termini of a polypeptide have joined to form a new amide bond. The stereochemistry of this structure has been omitted intentionally. The dotted outline is only for use in part b.



* Remember correct order for amino acids (N → C)



* move counterclockwise

a) Starting from the site that is labeled with the arrow, provide the one letter abbreviations for the amino acids in this polypeptide. Write your sequence in the conventional order (7 points).

7

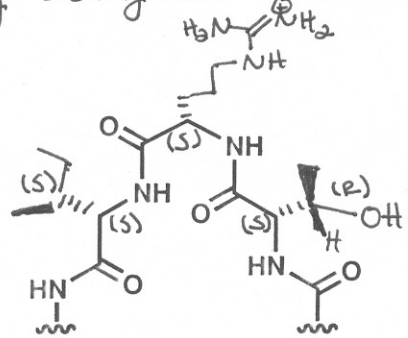
TRISTAN

1 pt for correct 1 letter code for each a.a.

b) Assume this cyclic peptide was synthesized from naturally occurring amino acids. Considering only the outlined portion of the structure shown above, redraw the side chains on the backbone segment below, clearly indicating the correct configuration of all stereocenters (10 points).

2 pts for correctly ID'ing each stereocenter

10



/17

2. Draw the dipeptide Trp-His with the amide bond in the ***cis*** conformation, as defined in class. Adjust the protonation state of the side chains to be what you would expect at pH 2 and indicate the pK_a of ALL groups that are ionizable under normal biological conditions. Use our "official" Chem 135 values for your answer (12 points).

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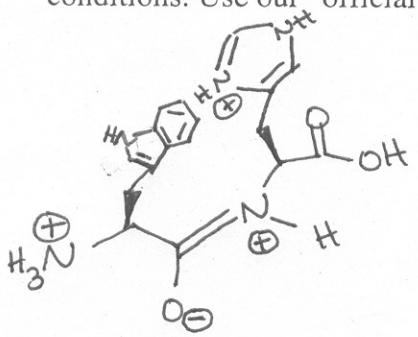


correct backbone +1
correct a.a. +1 each
cis conformation +1
correct C_α stereochem +1 each
correct ionization state of C-term, N-term, His +0.5 each

pKas - 0.5 each
N term ~ 8
C-term ~ 4
His ~ 6.5

What is the net charge of this dipeptide at:

pH = 2?

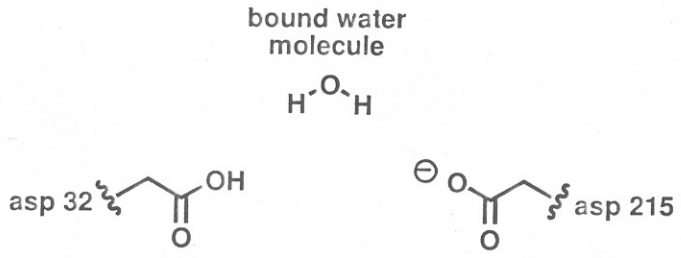
pH = 7?

pH = 10?

3

N term	C-term	His	Net charge	
+	0	+	+2	pH 2
+	−	0	0	pH 7
0	−	0	−1	pH 10

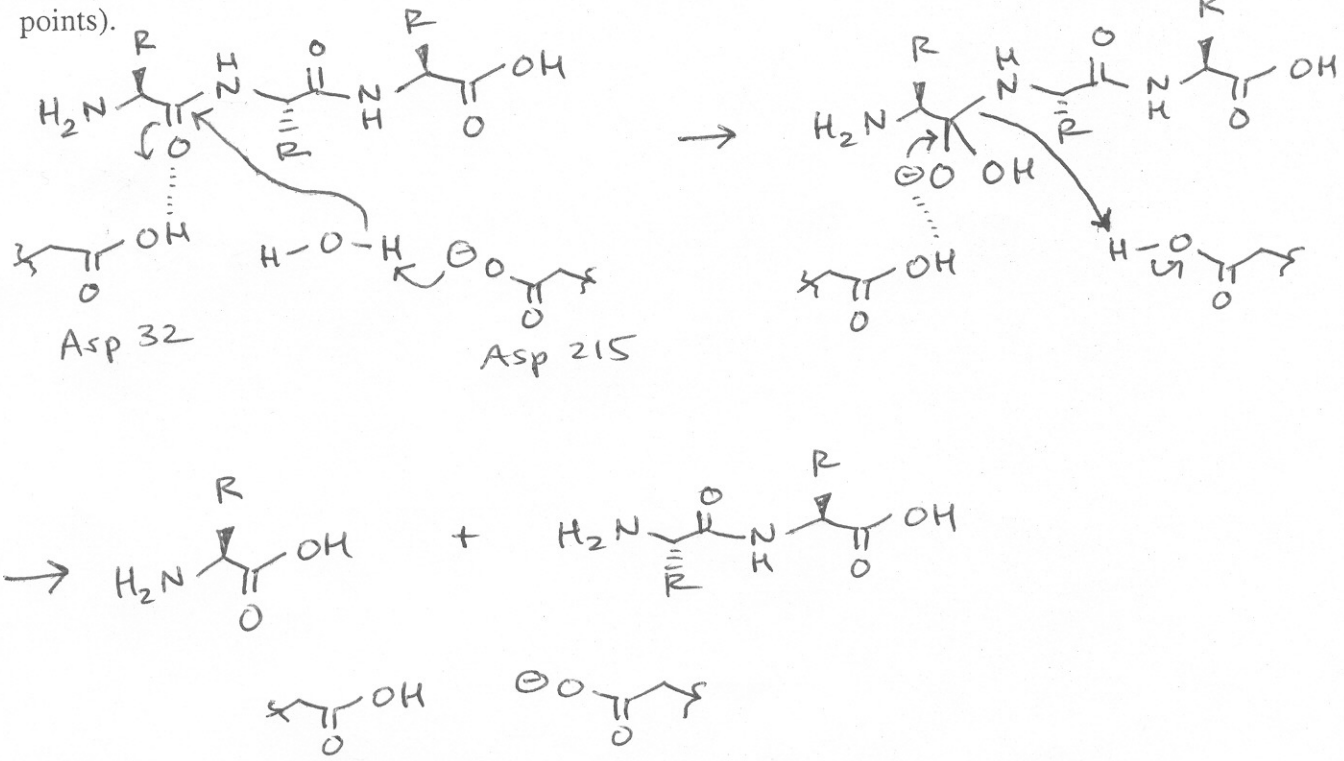
3. One of the most important proteases in your body is the enzyme pepsin, which is used by your stomach to break down the proteins that you eat. Pepsin belongs to the aspartic acid protease family because the active site relies on two aspartic acid residues to carry out its catalytic function. The active site also contains a bound water molecule that is essential for peptide bond hydrolysis. The diagram below depicts how the two asp residues and the bound water are positioned in the pepsin active site:



pepsin active site

(question continued on next page)

a.) Based on the structure of the active site, draw a generic tripeptide (you can abbreviate the side chains as "R" groups) and provide a detailed arrow-pushing mechanism that clearly indicates the role of the asp residues in the hydrolysis of a peptide bond. (12 points).



b.) What 3 requirements must the enzyme fulfill to promote peptide bond cleavage? Briefly explain how your mechanism accounts for all 3 (6 points).

1.) Activate the carbonyl toward electrophilic attack – H-bonding by Asp 32 polarizes C=O bond.

2.) Activate the nucleophile – deprotonation of H_2O by Asp 215 makes it more nucleophilic

3.) Stabilization of leaving group – N of scissile bond stabilized by protonation by Asp 215

c.) Pepsin cleaves only after the amino acids W, Y, and F. What do these amino acids have in common? Given that pepsin does *not* cleave after amino acids like D, Q, or K, speculate as to the nature of the binding pocket that confers this cleavage specificity (4 points). W, Y, and F are nonpolar, aromatic amino acids. Since pepsin does not cleave after polar or charged amino acids (D, Q, K), the binding pocket most likely also contains aromatic, nonpolar amino acids.

d.) Pepsin works optimally in your stomach at pH 3, but at pH 7 it has very little activity. Based on your mechanism, explain why pH 3 is more ideal than pH 7 for this protease (4 points). The mechanism requires Asp in both protonated and deprotonated form. At pH 3, both forms exist in reasonable amounts ($pK_a \sim 4$). At pH 7, all of the Asp residues are deprotonated and thus cannot activate the C=O or the N leaving group.

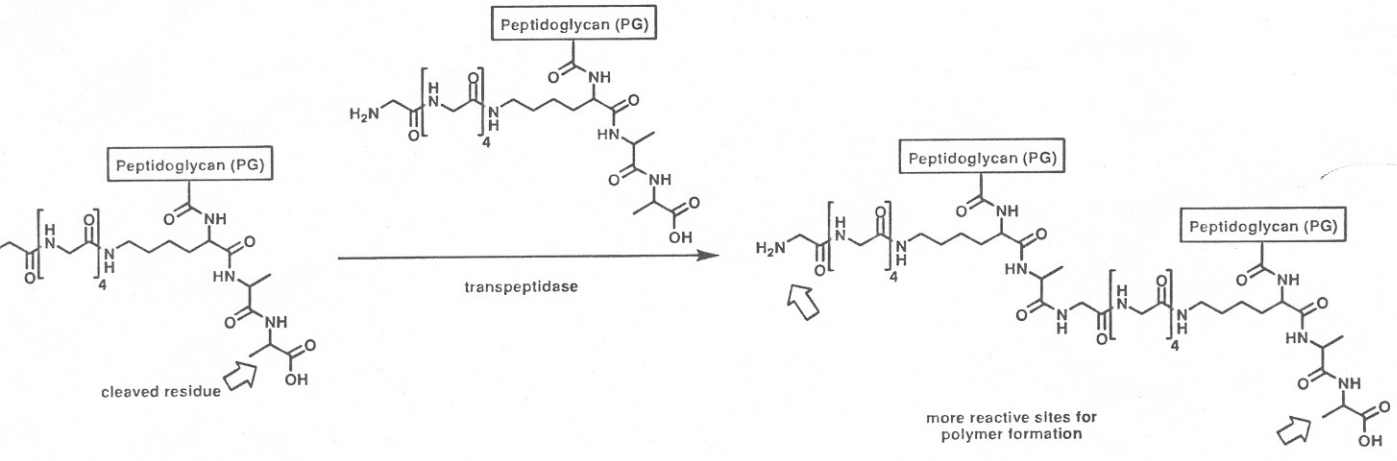
e.) If you changed one of the aspartic acid residues to an asparigine, what effect would you expect the mutation to have on K_M for the enzyme? What effect would you expect for k_{cat}? Briefly explain your answers (6 points).

Effect of K_M: Little to none. The mutation does not affect the aromatic binding pocket. (We also accepted $K_M \uparrow$ if you stated that Asn is a worse H-bonder than Asp)

Effect of k_{cat}:

K_{cat} will decrease by a lot. Asn cannot shuttle protons like Asp. (or stabilize the C=O w/H-bonding) so the catalytic ability of the enzyme will be severely compromised.

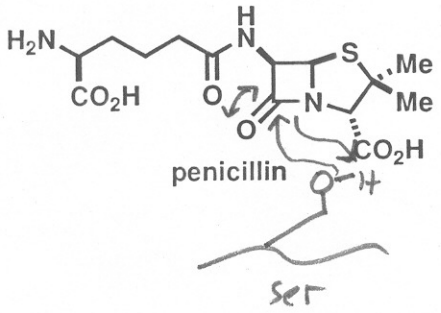
4. As bacteria grow, they must expand the size of the peptidoglycan (PG) cell wall that surrounds them. This is achieved by transamidases, which are a special class of protease enzymes. Most of them contain the same catalytic triad that you would expect for a serine protease, and they use it to cleave a peptide bond at the end of a PG building block (marked with the arrow below). However, these enzymes differ from normal proteases in that they do not use water to complete the hydrolysis reaction. Instead, the role of the water molecule is played by an amino group on an adjacent PG chain. This links the two segments together, forming a dimer. As this reaction occurs repeatedly, a dense polymeric net is produced to provide structure to the bacterial cell:



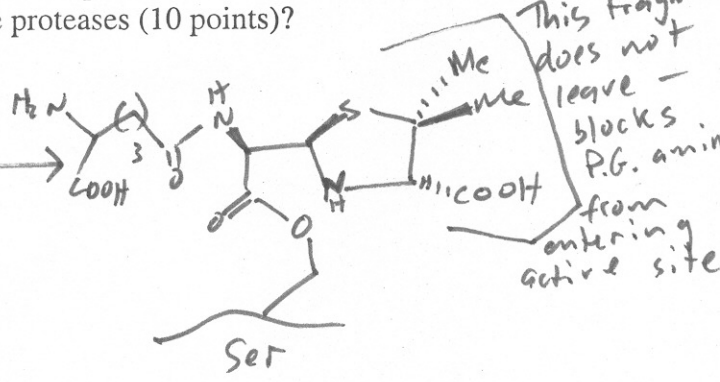
(question continued on next page)

a) Penicillin is a cyclic antibiotic that reacts irreversibly with the transamidase enzyme. Following this reaction, incoming PG units are blocked from entering the active site. This effectively halts cell wall biosynthesis, and ultimately kills the bacterial cell. By examining the structure of this drug and thinking about the mechanism of the transamidase, draw the product that this reaction would produce. What structural feature makes penicillin especially reactive toward serine proteases (10 points)?

(1)



(2)



This fragment does not leave — blocks P.G. amin from entering active site

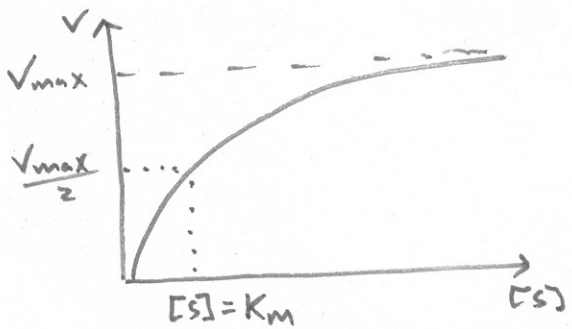
(2) The strained β-lactam ring makes penicillin especially reactive toward serine proteases.

b) Not surprisingly, bacteria have countered the use of penicillin by evolving a new enzyme called beta-lactamase, which serves to destroy this drug. The gene for this enzyme is not part of the bacterial genome, but is instead carried on a plasmid. Provide an accurate sketch of an *E. coli* bacterium and label the key structures that were discussed in class. Indicate the location and structure of a plasmid that would contain the beta-lactamase gene (10 points).

10 free points !!

c) Beta-lactamase has been shown to exhibit Michaelis-Menten kinetics. Provide the Michaelis-Menten equation and a graph showing how the rate of this enzyme depends on substrate concentration. Also indicate how you would use this diagram to determine the values of k_{cat} and K_M (10 points).

① MM eq: rate $= V = \frac{k_{cat}[S][E]_T}{K_m + [S]}$

② 

③ $K_m = [S]$ at $\frac{V_{max}}{2}$

and

$V_{max} = k_{cat}[E]_T$

$\Rightarrow k_{cat} = \frac{V_{max}}{[E]_T}$

d) Suppose a particular beta-lactamase has the parameters listed below. By examining these values, do you think this enzyme has evolved to function at high (>1 mM) or low (<0.1 micromolar) concentrations of penicillin? Briefly explain your answer. Starting with the Michaelis-Menten equation, provide a simplified rate law that you would obtain under the conditions that you chose and provide **an expression and a value** for the effective rate constant (6 points).

For beta-lactamase: $k_{cat} = 1 \times 10^2\ s^{-1}$ $K_M = 1 \times 10^{-6}$ M

① This enzyme has evolved to function at low [penicillin] – we have a micromolar K_m & enzymes frequently have $K_m \approx [S]$ (remember K_m can be thought of as the dissociation const. for E·S complex).

② MM: $V = \frac{k_{cat}[S][E]_T}{K_m + [S]} \xrightarrow[(K_m \gg [S])]{@\ low\ [S]} V = \frac{k_{cat}[S][E]_T}{K_m}$

③ $k_{eff} = \frac{k_{cat}}{K_m} = \frac{10^2\ s^{-1}}{10^{-6}\ M} = 10^8\ s^{-1}M^{-1}$

e) What is significance of the effective rate constant value that you calculated in part d (3 points)?

– Rate constants of $\sim 10^8\ M^{-1}s^{-1}$ are close to the diffusion limit. This enzyme is approaching "catalytic perfection"!