BioE 11 Midterm 2 – Spring 2019

Number of pages: 11 (including this one!)
Each question is worth 8 points

Last name: Key

First name: _____

Student ID No. (SID): _____

Important note:
This exam will be graded using gradescope. Any answers written outside the designated response areas will therefore not be graded. You can use the backside of the exam as scratch paper.

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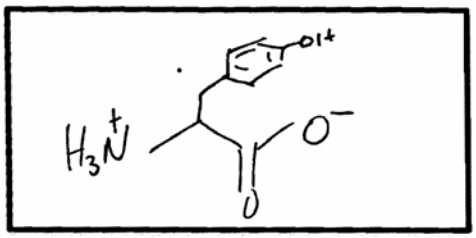
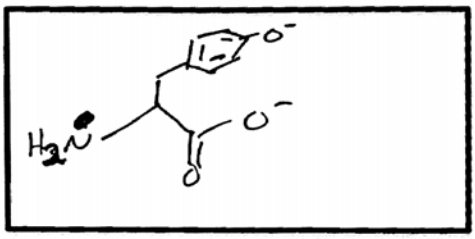
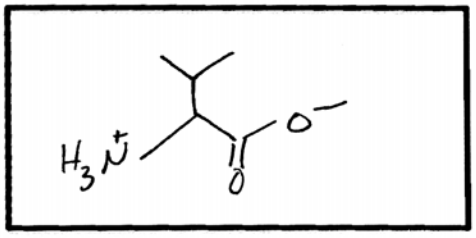
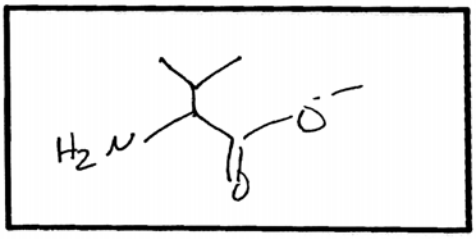
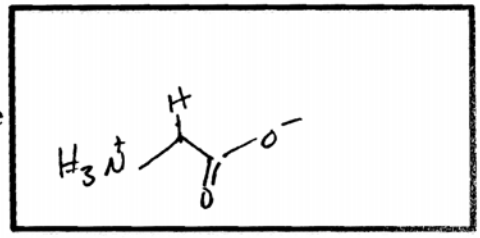
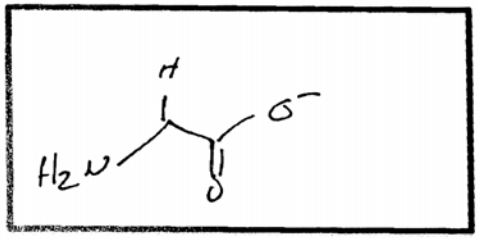
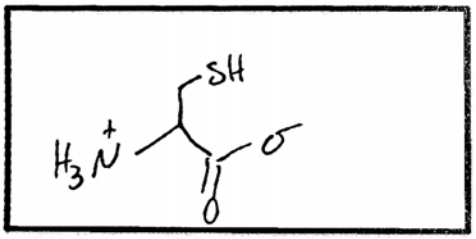
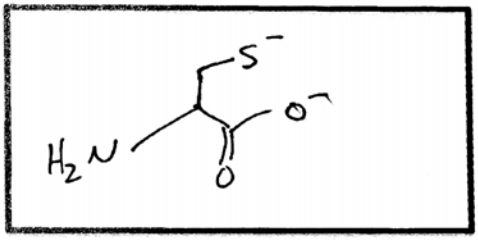
Question 1: Draw the structure of the amino acids listed below. Draw their structures at pH 7.0 and pH 12. The pKa of the tyrosine side chain is 10, and the pKa of the Cysteine side chain is 8.5.

	pH 7.0	**pH 12.0**
Tyrosine		
Valine		
Glycine		
Cysteine		

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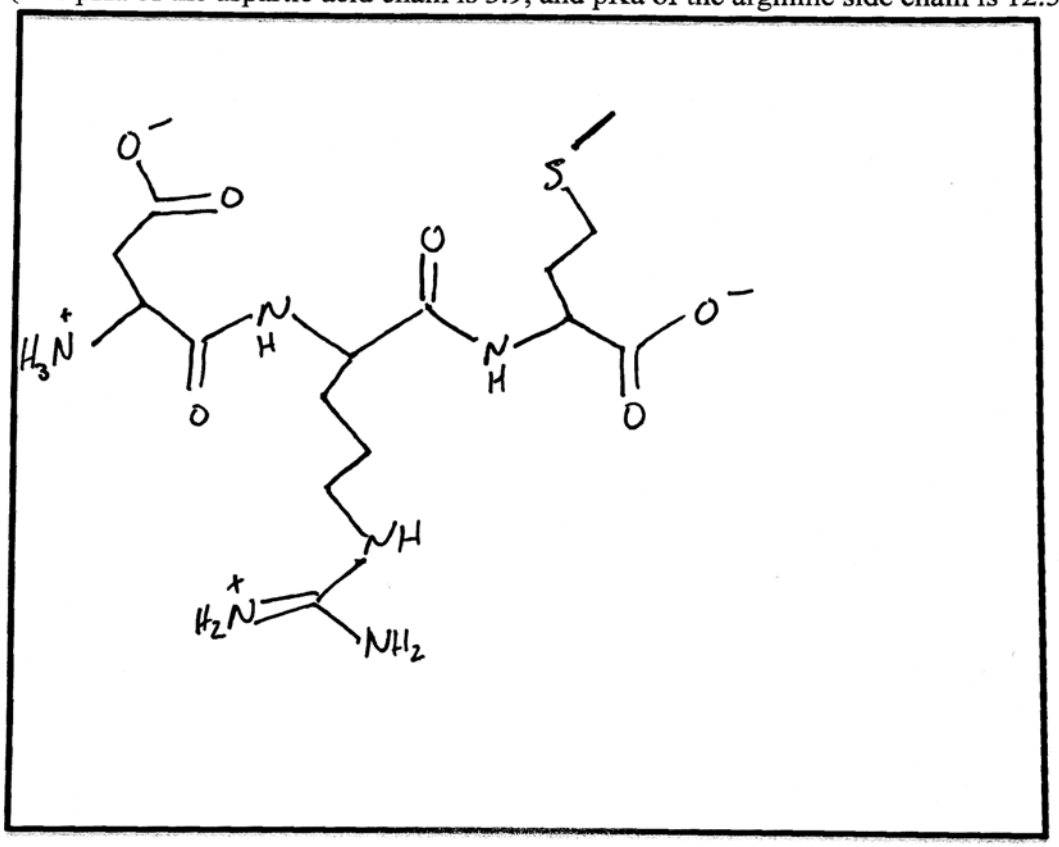
Question 2: Draw the structure of the peptide NH2-Aspartic Acid-Arginine-Methionine-COOH (at pH 7.4).

(The pKa of the aspartic acid chain is 3.9, and pKa of the arginine side chain is 12.5)



What is the net charge of this peptide at pH 7.4?

0

What is the net charge of this peptide at pH 1.0?

+2

C-terminus neutral
Asp. neutral
ARG +1
Net Neutral
N-terminus +1

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Question 3: Poly-glutamic acid is a polymer made of glutamic acids. This polymer forms alpha helices at pH 5.00, but not at pH 7.4. Explain why this is in 2-3 sentences.

The pKa of glutamic acid side chain is 4.25. At a pH of 5, a fraction of the Glu side chains will become protonated, allowing an alpha helix to form. At pH 7.4 all glutamic acids will be negatively charged. This causes repulsion, so the α-helix is unable to form.

Question 4: An antibody binds to an antigen with a Kd of 1X 10^{-8}M. What concentration of free antigen will the fractional binding be 0.1, 0.3, and 0.9. See ligand-binding equation for reference:

$$\theta = \frac{[L]}{[L] + K_d}$$

Show work in space below and put final answers with appropriate units in the small boxes below.

$\theta = \frac{(L)}{(L)+K_d}$

$[(L)+K_d]\theta = (L)$

$(L)\theta + K_d\theta = (L)$

$K_d\theta = (L) - (L)\theta$

$K_d\theta = (L)(1-\theta)$

$(L) = \frac{K_d\theta}{1-\theta}$

Plug & Chug!

$\theta = 0.1, 0.3, 0.9$

0.1: $(L) = \frac{1\times10^{-8}M\,(0.1)}{1-0.1}$

0.1	0.3	0.9
$1.1\times10^{-9}M$	$4.3\times10^{-9}M$	$9\times10^{-8}M$

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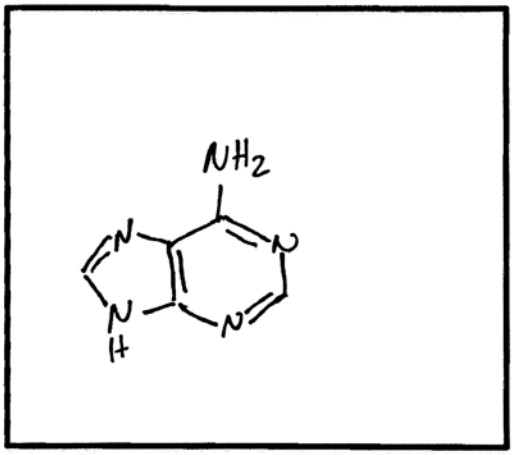
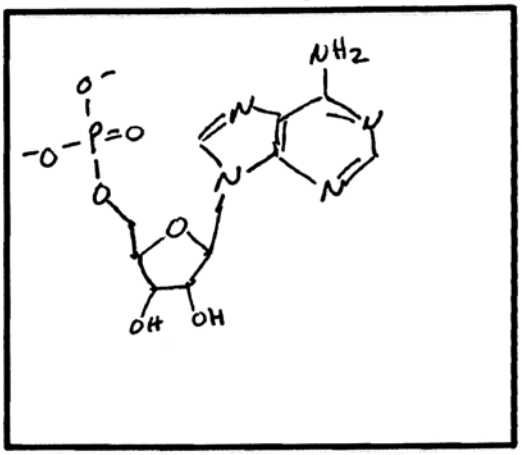
Question 5: Draw the structures of base adenine, adenosine monophosphate, and adenosine triphosphate

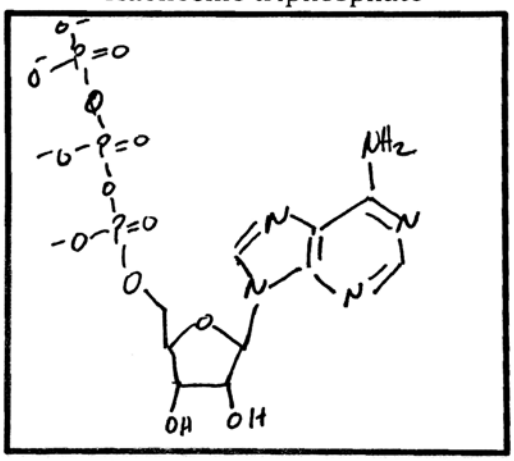
Adenine



Adenosine monophosphate



Adenosine triphosphate



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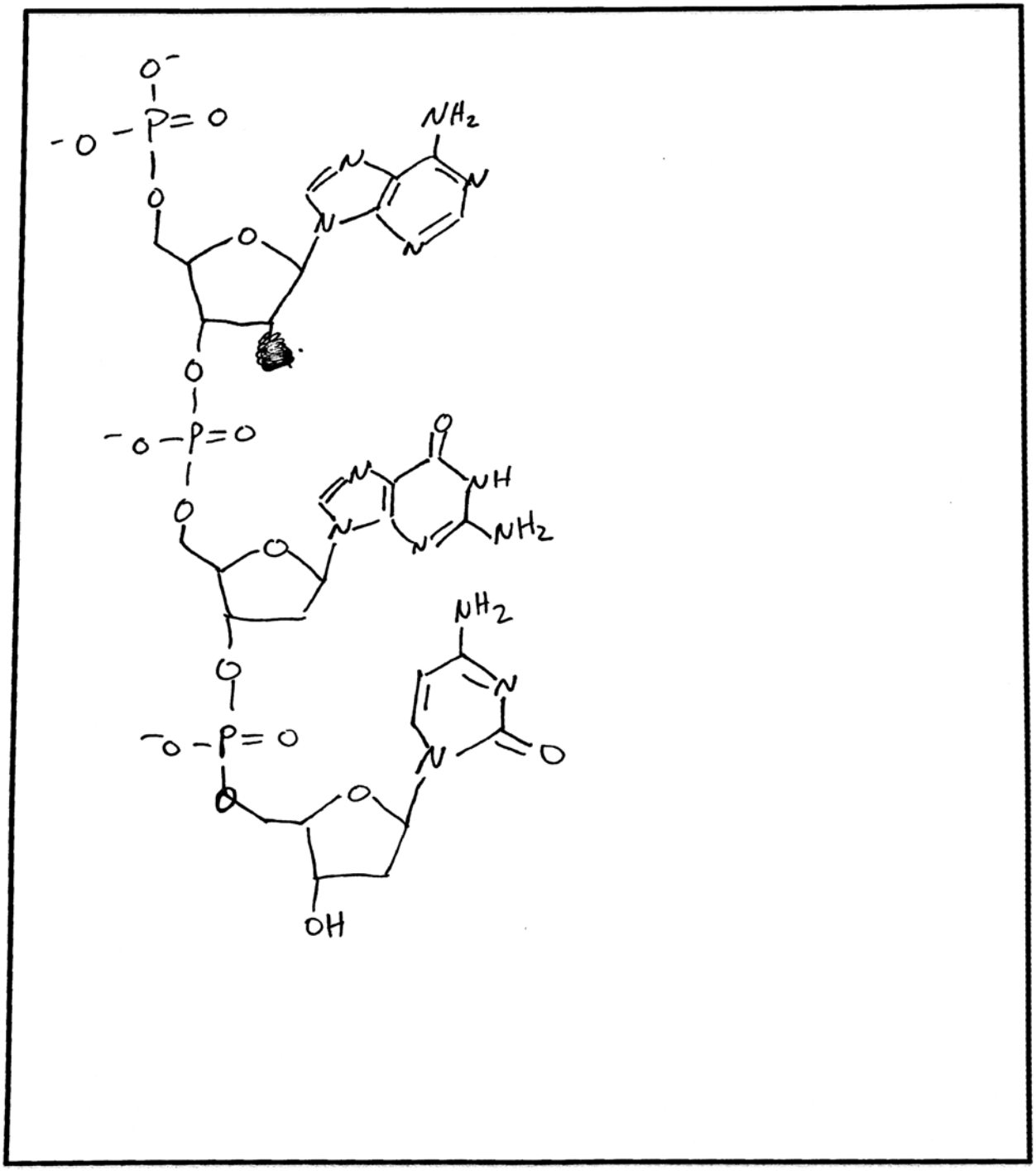
(DNA!)

Question 6: Draw the structure of the 3 base oligonucleotide 5'-AGC-3'. Draw the 5' phosphorylated and the 3' end hydroxylated.



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Question 7:

Primer * * * *
5'——TCAGTCAGTCAGTC
3'——AGTCAGTCAGTCAG-5'

Extension terminates at all Guanines incorporated and at and

The DNA strand above is to be sequenced. This fragment of DNA is placed with the primer and the di-deoxy nucleotide for Guanine, DNA polymerase (Klenow fragment), and the 4 wild type nucleotide triphosphates.

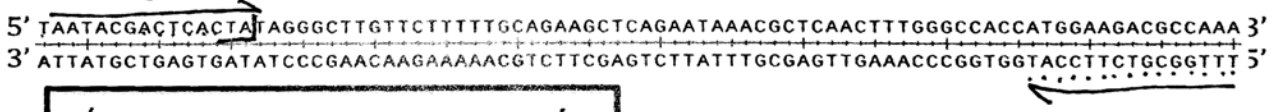
How many product bands would you see if you ran the product on a gel?

4 (We will accept 3)

What are the sequences of these oligonucleotide products?

5'-TCAG-3' 5'-TCAGTCAGTCAG-3'
5'-TCAGTCAG-3' (5'-TCAGTCAGTCAGTC-3')
Complete Band will be synthesized but we will accept as correct if you put the first 3 bands only.

Question 8: The gene of interest shown below is to be amplified by PCR. Design primers that can be used to PCR the entire gene. The primers should be 15 nt long. (Remember DNA sequences are written 5'→3')



5'-TAATACGACTCACTA-3'

5'-TTTGGCGTCCTTCAT-3'

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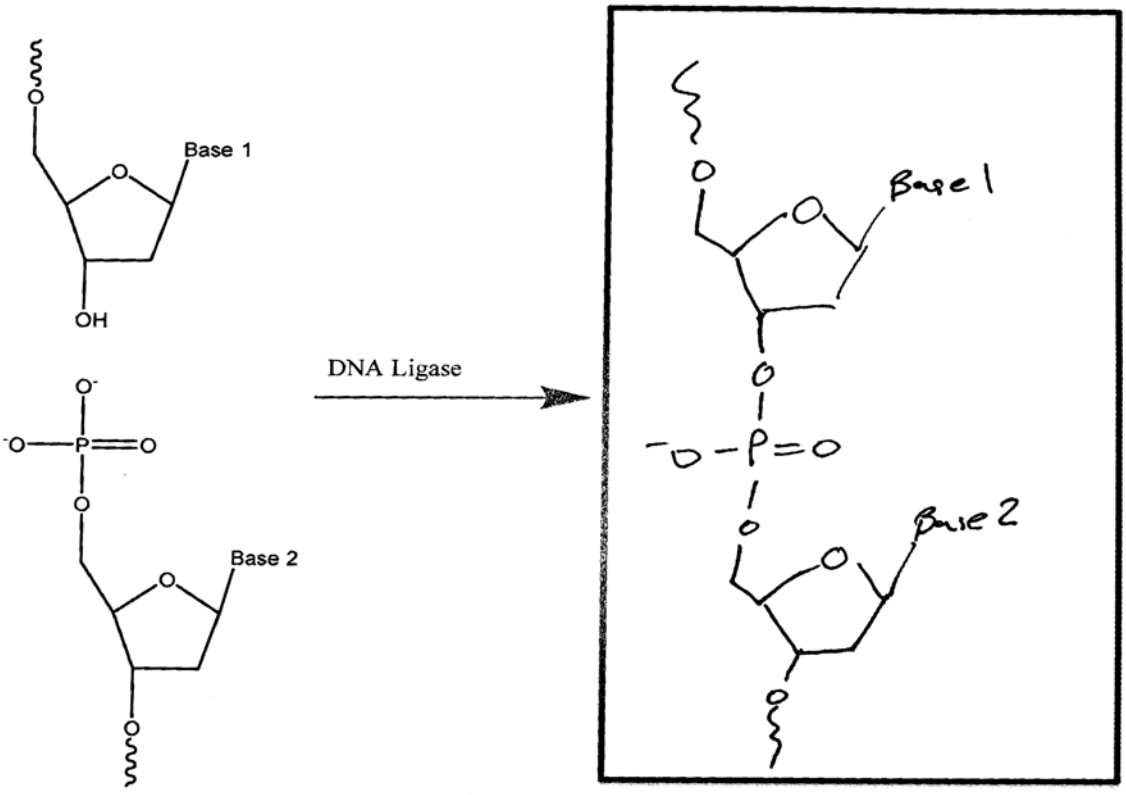
Question 9: An E.coli mutant contains a defective DNA ligase. When these mutants are exposed to ^{3}H-labeled thymine and the DNA produced is run on an agarose gel, two radioactive bands appear, one that is low molecular weight and another that is high molecular weight. Explain what the two bands correspond to, in terms of the DNA replication process.

Without an active DNA ligase, during DNA replication, the Okazaki fragments in the lagging stand will not be connected.
The Low Molecular weight band on the gel corresponds to these unligated fragments. The High MW band is the normally synthesized leading strand, which does not require DNA ligase

Question 10: The enzyme DNA ligase is mixed with the following 2 oligonucleotides. Draw structure of the products.



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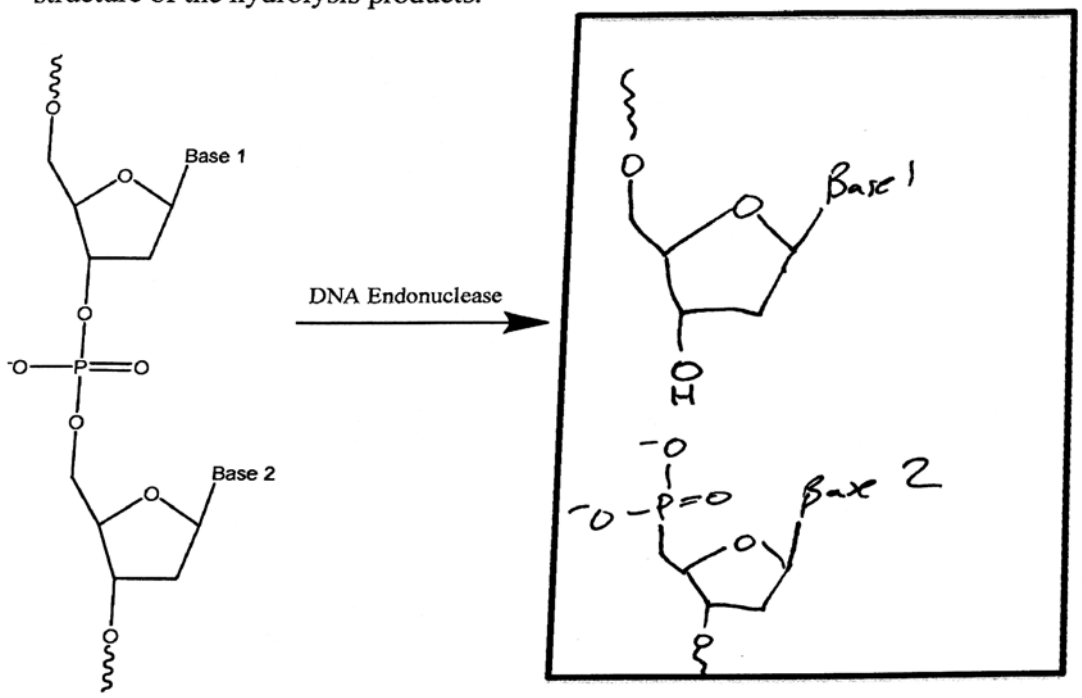
Question 11: An endonuclease is mixed with the following DNA strand. Draw the structure of the hydrolysis products.



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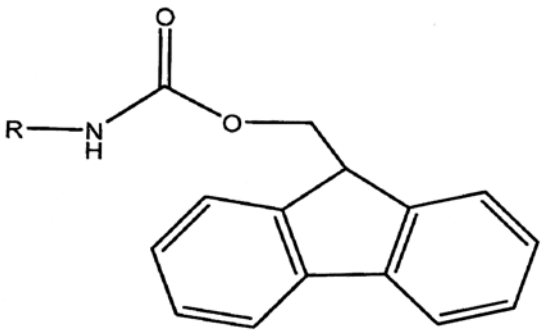
Question 12: Synthesize the peptide NH2-glutamic acid-isoleucine-Lysine-COOH, using solid phase FMOC synthesis. Draw the structures of the protected amino acids you will need to use in the synthesis. List all coupling and deprotection steps.

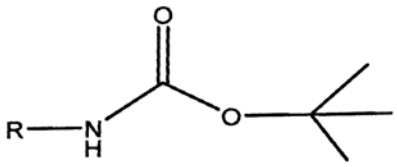
You may use the first amino acid coupled to the bead as a starting reagent.

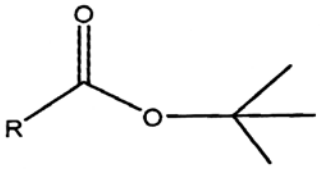
Here are the structures of some protecting groups and reagents for reference.

Fmoc

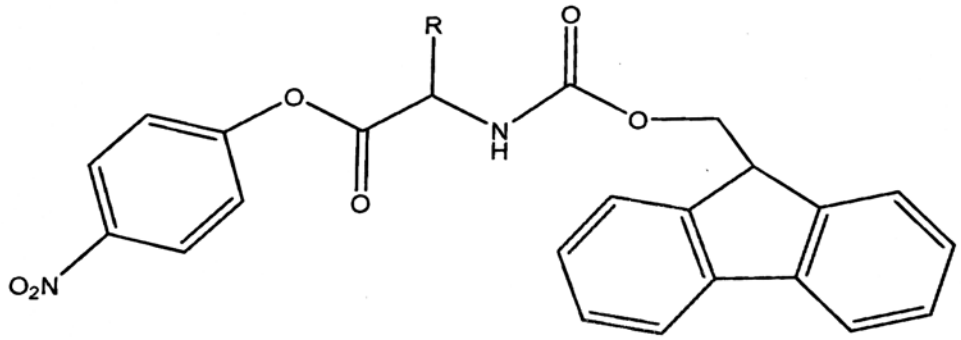


Boc





Activated Fmoc-Protected Amino acid:



Draw the entire synthesis on the following page.

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