

Chemistry 434/534
Exam #1
February 24, 1998

Instructions: Answer the questions using your own paper. Read the question carefully to make sure you understand what I'm asking for. Useful information is found at the end of the exam.

1. (15 points)

- Which of the following groups will be nucleophilic at pH = 7? Consider the side chain ONLY in your answer.
Ala, Arg, Met, Cys, Ser, Lys, Asn
- Why is Phe less nucleophilic than Ser at pH = 7? Consider the side chain ONLY in your answer.
- Why is the side chain of Cys more acidic than the side chain Ser?

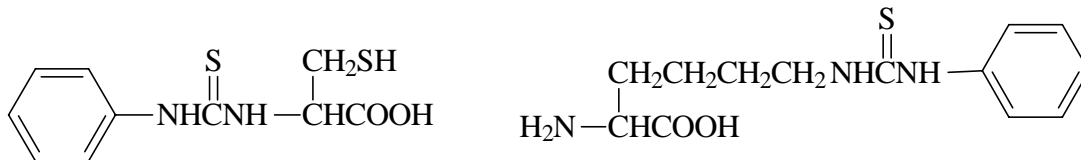
2. (25 points)

You have isolated a 14 amino acid residue peptide from carrots and wish to determine its sequence. Below are the data you obtained from various experiments.

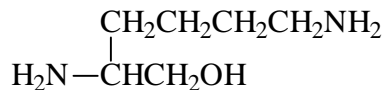
Total acid hydrolysis of the reduced and carboxymethylated peptide yielded the following amino acid composition:



Reaction of the peptide with phenyl isothiocyanate followed by total acid hydrolysis yielded two modified amino acids:



Methylation/reduction followed by total acid hydrolysis yielded one amino acid with an altered α -carboxylic acid:



The reduced and carboxymethylated peptide was then subjected to a number of cleavage reactions. The isolated fragments were analyzed for amino acid content by *total acid hydrolysis*. The results are tabulated below:

Enzyme cleavage sites:

Trypsin: C terminus of Lys and Arg
 Chymotrypsin: C terminus of Phe, Tyr and Trp

S. aureus: C terminus of Asp and Glu

Elastase: C terminus of Ala and Gly

Ala ₂ ArgAspCys ₂ Glu ₂ LysMetPheProThrTyr

(AA Analysis – repeated from p. 1)

Chymotrypsin:

CT-1 CysTyr

CT-2 Ala₂AspGluMetPhe

CT-3 ArgCysGluLysProThr

Trypsin

T-1 LysPro

T-2 Ala₂ArgAspCys₂Glu₂MetPheThrTyr

CNBr

CB-1 ArgCysGluLysPheProThr

CB-2 Ala₂AspCysGluMetTyr

In this case *only*, the peptide was first reduced and then reacted with ethyleneimine to aminoethylate the Cys residues. It was then subjected to digestion with trypsin followed by total acid hydrolysis of the fragments.

Trypsin #2:

TR-1 AECys

TR-2 ArgThr

TR-3 LysPro

TR-4 Ala₂AspAECysGlu₂MetPheTyr

Elastase

E-1 AspAlaGlu

E-2 AlaCysTyr

E-3 ArgCysGluLysMetPheProThr

S. aureus protease

S-1 AlaGluMetPhe

S-2 ArgCysLysProThr

S-3 AlaAspCysGluTyr

- Provide the amino acid sequence of the peptide. Use the three-letter abbreviation for each amino acid.
- If the peptide was *not* reduced and carboxymethylated, treatment with CNBr yielded a 14 amino acid residue peptide (i.e., no *net* cleavage occurred). What does this result tell you about the structure of the peptide?

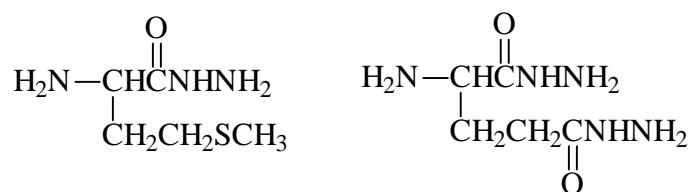
3. (15 points)

The carrot extract also yielded a tripeptide that showed interesting biological activity. Total acid hydrolysis of this peptide, termed substance T, revealed three amino acids:

AlaGluMet

Unexpected difficulties were encountered in attempts to sequence the tripeptide. Substance T did *not* react with Sanger's reagent or with phenyl isothiocyanate. It also did *not* react with *S. aureus* protease. Cyanogen bromide treatment of substance T produced a free amino acid, Ala, and a dipeptide.

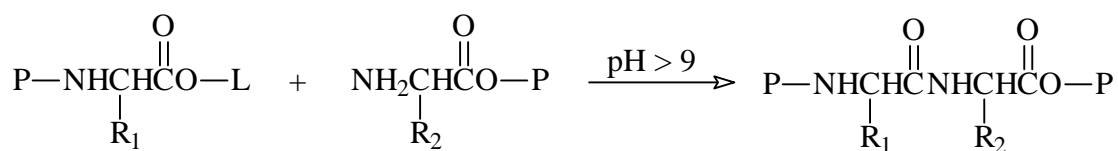
Treatment of substance T with hydrazine yielded two modified amino acids and Ala. The structures of the modified amino acids were determined to be:



- Provide the *structure* of substance T. (Draw the chemical structure *not* the three letter codes for the sequence.)
- Explain why substance T did not react with Sanger's reagent or with *S. aureus* protease.

4. (35 points)

Peptide bond formation between two amino acids involves activation of the α -COOH of one amino acid and protection of the α -COOH of the other amino acid, i.e.,

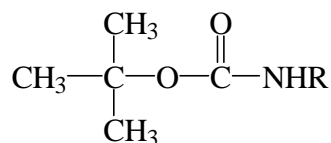


P = protecting group; L = activating group

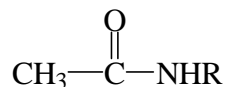
Answer the following questions pertaining to the coupling chemistry

- Why must the α -COOH of the N-terminal amino acid be activated? (That is, why can't you just couple using the free carboxylic acid?)
- Why must the reaction be run at basic pH?
- The α -NH₂ of the N terminal amino acid is prevented from reacting by the use of a protecting group. Two possible choices for an amine protecting group could be BOC and acetamide (product formed by reaction of the amino acid with acetic anhydride). First, draw the structures of an amine protected with BOC and protected as an

acetamide. Show, using these structures, that either protecting group would prevent the amine from reacting in the peptide bond forming reaction. Then explain why acetamide could *not* be used as a protecting group in peptide synthesis.

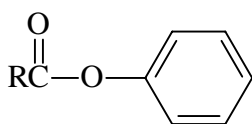


BOC protected amine

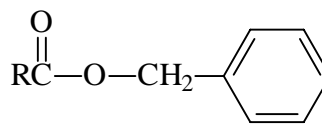


acetamide

- D. Esters can be used in peptide synthesis as either activating agents or as protecting groups depending on the structure of the ester. In the two structures shown below, structure I is an activated ester, while structure II is a protected carboxylic acid:



I



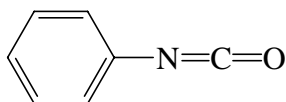
II

- Explain *why* I is an activated ester while II is a protected carboxylic acid. (Your answer should include the mechanism of peptide bond formation at basic pH.)
- Suppose structure I was modified to include a nitro group at the *para*- position. Would this modification make the structure a better activating group or a worse activating group? Justify your answer using structures.
- Suppose structure II was modified to include a nitro group at the *para*- position. Would this modification make the structure a better protecting group or a worse protecting group? Justify your answer using structures.

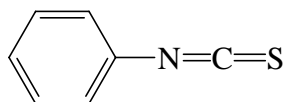
5. (10 points)

Consider the mechanism of the Edman degradation to answer the following questions.

- The Edman degradation consists of three steps: coupling, cleavage and conversion. Why is it best to perform the conversion reaction after the cleavage step (i.e., in the *absence* of the rest of the peptide)?
- Do you suppose that phenylisocyanate would work as well as phenylisothiocyanate in this series of reactions? Briefly explain your reasoning.



phenyl isocyanate



phenyl isothiocyanate