CONCEPT: PEPTIDE SEQUENCING: EDMAN DEGRADATION

Phenyl isothiocyanate (PITC)

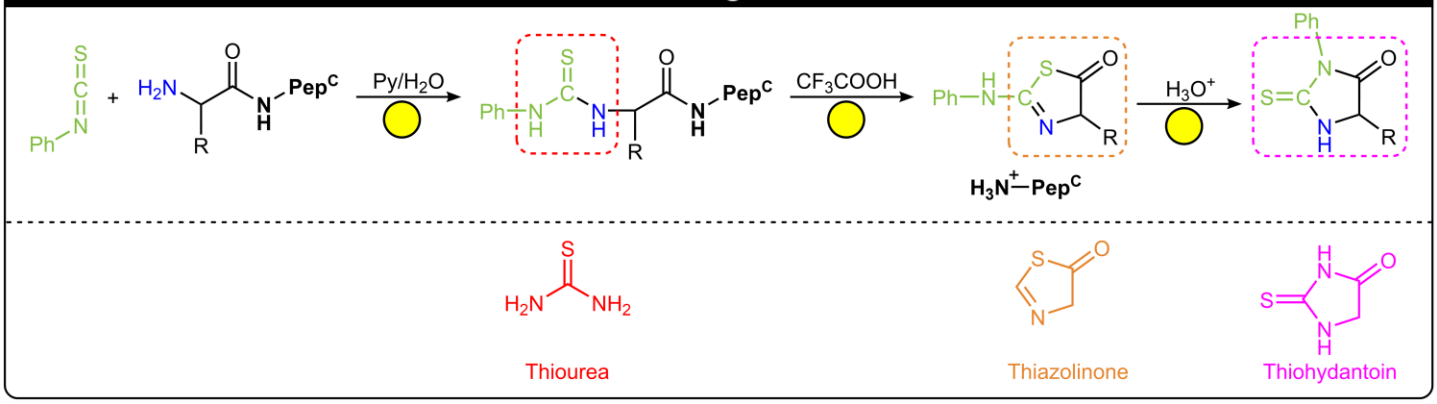
Ph–N=C=S

_____ reagent

Intro to Edman Degradation

- Edman degradation is a _____ method to sequence a peptide from the __-terminal.
  - □ Uses phenyl isothiocyanate (_____) as the main reagent.
- Each degradation cycle (__ reactions) cleaves __ amino acid residue from the peptide chain.
  1. **Nucleophilic Addition:** PITC reacts with N-terminal ____ group to produce a _____ derivative.
  2. **Cyclization & Cleavage:** Thiourea derivative cyclizes into a _____ derivative.
     - N-terminal amino acid is cleaved from the peptide chain (_____).
  3. **Rearrangement:** Thioazolinone derivative rearranges to a more stable phenylthiohydantoin (_____) derivative.

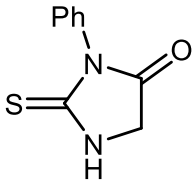
Edman Degradation



- N-terminal amino acid is identified via _____ characterization of the _____ derivative.

EXAMPLE: Complete the structure of the PTH derivative formed by Edman degradation of the following tetrapeptide:

Tyr-Pro-Trp-Phe



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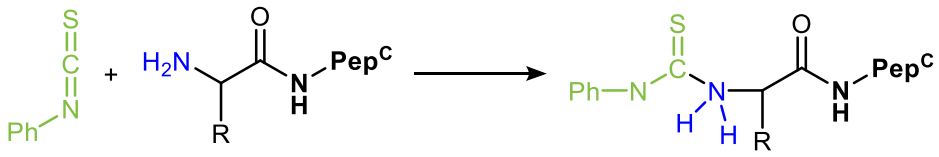
1 Nucleophilic Addition

- Reaction of the $-NH_2$ with PITC is analogous to nucleophilic _____ to a carbonyl group.

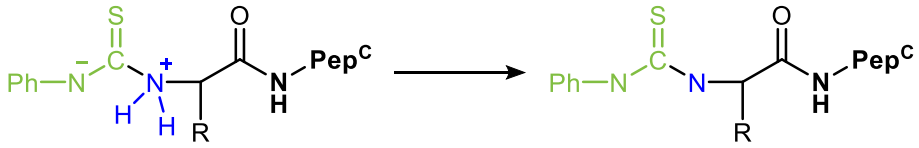
Step 1
Nucleophilic Attack

Step 2
Proton Transfer

STEP 1: $-NH_2$ attacks the ___ atom of PITC.



STEP 2: A H^+ is transferred from $-NH_2$ to the PITC ___ atom.



EXAMPLE: Which of the following is an unlikely step in the nucleophilic addition of a peptide to PITC?

a) Amino group of N-terminal amino acid attacks the C atom of PITC.

b) Formation of a resonance-stabilized ion after nucleophilic attack.

c) Proton transfer leading to the formation of a thiourea derivative.

d) Nucleophilic attack of the S atom of PITC on the carbonyl group of the N-terminal amino acid.

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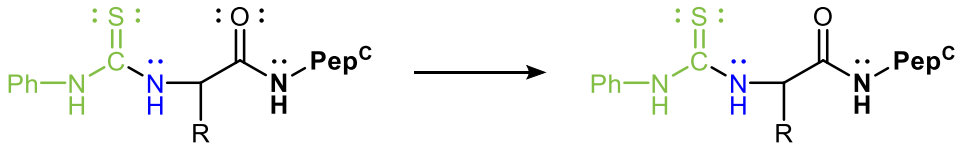
(2) Cyclization & Cleavage

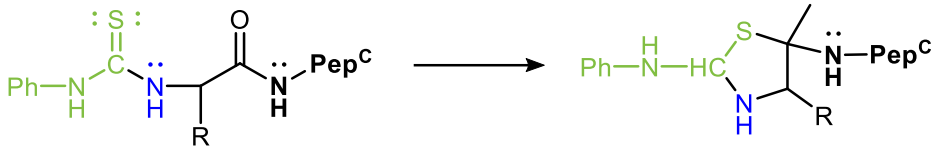
- Formation of the thioazolinone derivative takes place via nucleophilic acyl _____ mechanism.

Step 1	Step 2	Step 3	Step 4	Step 5
Protonation	Nucleophilic Attack	Proton Transfer	Leaving Group	Deprotonation

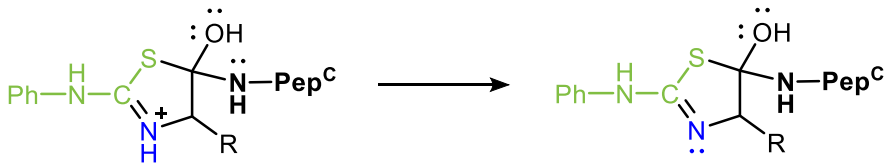
STEP 1: The carbonyl ___ of the N-terminal amino acid is protonated.



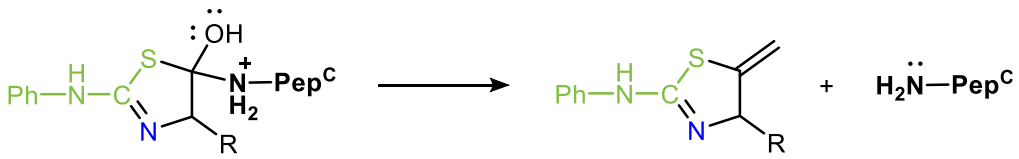
STEP 2: An _____molecular nucleophilic attack forms a cyclic tetrahedral intermediate.



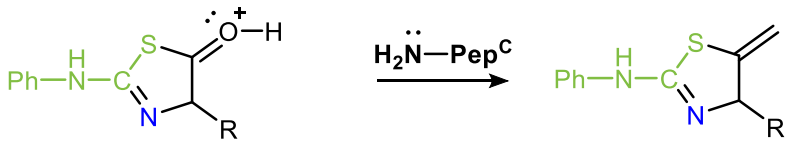
STEP 3: A H^+ transfer makes the peptide chain a better _____ group.



STEP 4: The –OH group pushes its electrons to reform the carbonyl and kick out the _____ chain.



STEP 5: Carbonyl ___ is deprotonated to give a thiozolinone derivative.



EXAMPLE: Which of the following statements about thioazolinone formation in Edman degradation is incorrect?

a) Thiazolinone forms through an intramolecular nucleophilic attack.

b) The tetrahedral intermediate cannot convert into thiazolinone without a proton transfer step.

c) The N-terminal amino acid is cleaved from the peptide chain before ring formation takes place.

d) The S atom from PITC, instead of the N atom, attacks the carbonyl group because it is more nucleophilic.

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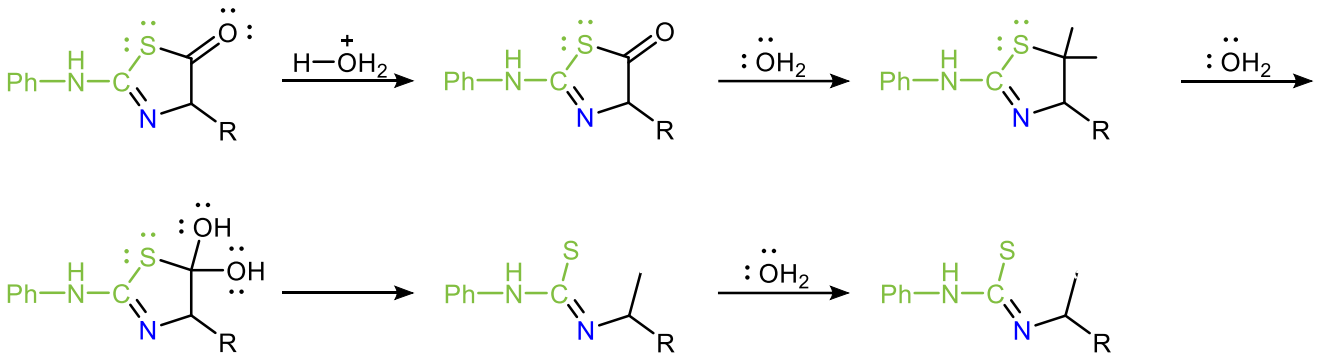
3 Rearrangement

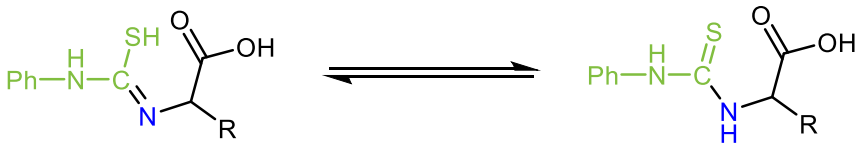
- Rearrangement of thiazolinone to thiohydantoin takes place in ___ steps.

Step 1	Step 2	Step 3	Step 4
Ring Opening	Tautomerization	Rotation	Ring Closure

STEP 1: The thioazolinone ring opens via an acid-catalyzed NAS _____ reaction.

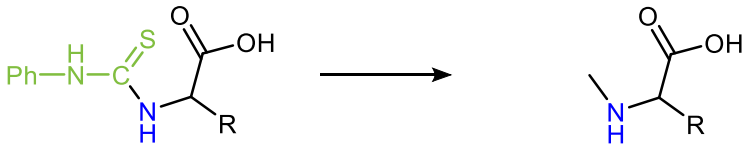


STEP 2: Tautomerization of the hydrolysis product takes place.

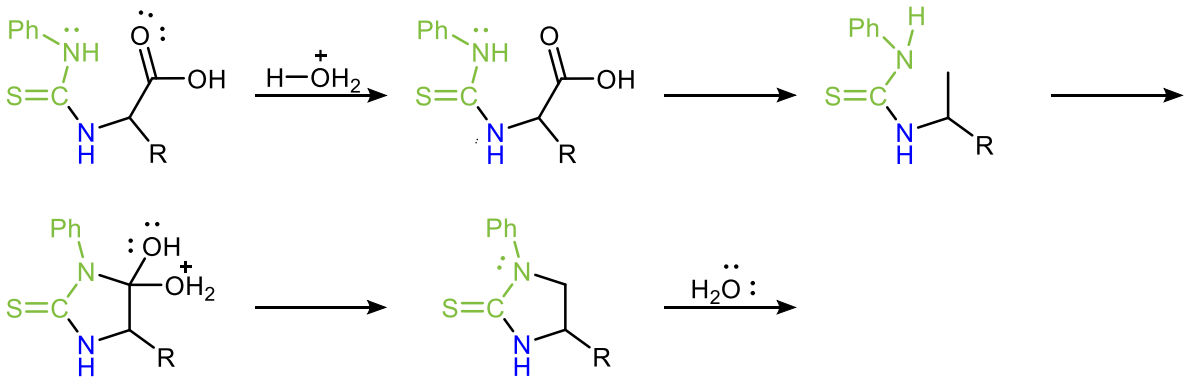


_____ form _____ form

STEP 3: Rotation takes place along the C–N bond.



STEP 4: Ring closure takes place through an _____-catalyzed intramolecular _____ reaction.



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EXAMPLE: Acetylation at the N-terminal is a commonly seen posttranslational modification. Explain why the following peptide cannot be sequenced using Edman degradation. (Hint: draw the thiazolinone structure.)

PRACTICE: Bradykinin is an important nonapeptide with many biological functions. A student performed a series of experiments to determine its structure and obtained the following results:

1. Complete hydrolysis of the peptide yielded 2 Arg, 2 Phe, 3 Pro, Gly, and Ser.
2. Edman degradation produced PTH-Arg.
3. Treatment with carboxypeptidase A did not produce any fragments.
4. Treatment with chymotrypsin produced Arg, a pentapeptide, and a tripeptide with composition Pro, Ser, Phe.
5. A tripeptide produced during partial acidic hydrolysis was confirmed to be Pro-Gly-Phe.

____-____-____-____-____-____-____-____-____-____

Edman: PTH-Arg

____-____-____-____-____-____-____-____-____-____

Carboxypeptidase A = No fragments

____-____-____-____-____-____-____-____-____-____

Chymotrypsin = pentapeptide, tripeptide (Pro, Ser, and Phe), Arg

____-____-____-____-____-____-____-____-____-____

____-____-____-____-____-____-____-____-____-____

Tripeptide Pro-Gly-Phe

____-____-____-____-____-____-____-____-____-____

CONCEPT: PEPTIDE SEQUENCING: EDMAN DEGRADATION

PRACTICE: Use the following information to determine the structure of a synthetic heptapeptide:

1. Complete hydrolysis of the peptide yields 2 Thr, Cys, Met, Trp, Lys, and Phe.
2. Edman degradation produces PTH-Phe.
3. Treatment with carboxypeptidase A cleaves off Thr.
4. Cleavage with cyanogen bromide produces a dipeptide and a pentapeptide
5. Treatment with trypsin produces a tetrapeptide and a tripeptide Thr-Cys-Thr.

____-____-____-____-____-____-____

Edman: PTH-Phe

____-____-____-____-____-____-____

Carboxypeptidase A = Thr

____-____-____-____-____-____-____

Cyanogen bromide = dipeptide and a tripeptide

____-____-____-____-____-____-____ or ____-____-____-____-____-____-____

Trypsin = tetrapeptide and tripeptide (Thr-Cys-Thr)

____-____-____-____-____-____-____

____-____-____-____-____-____-____