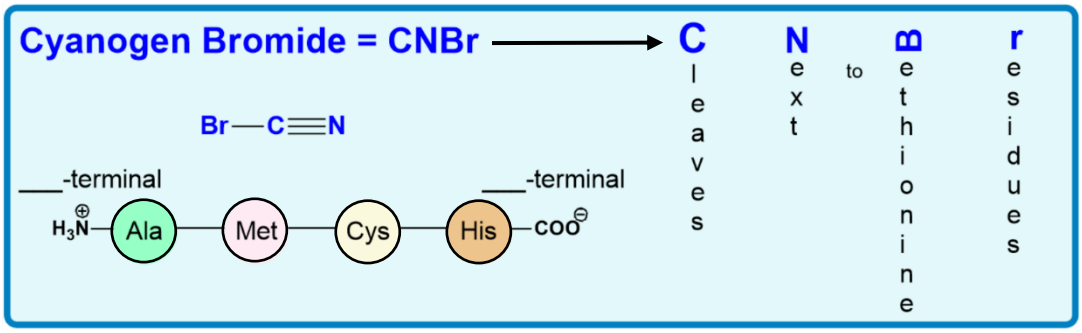
CONCEPT: CHEMICAL CLEAVAGE OF BONDS

●Many different chemicals are used to cleave bonds within proteins; we will cover the more *common* ones.

●*Cyanogen Bromide* (_____) cleaves peptide bonds on the _____ side of *Met* amino acid residues.

EXAMPLE: Where will CNBr cleave the peptide?



PRACTICE: What fragments result from treating the following peptide with CNBr?

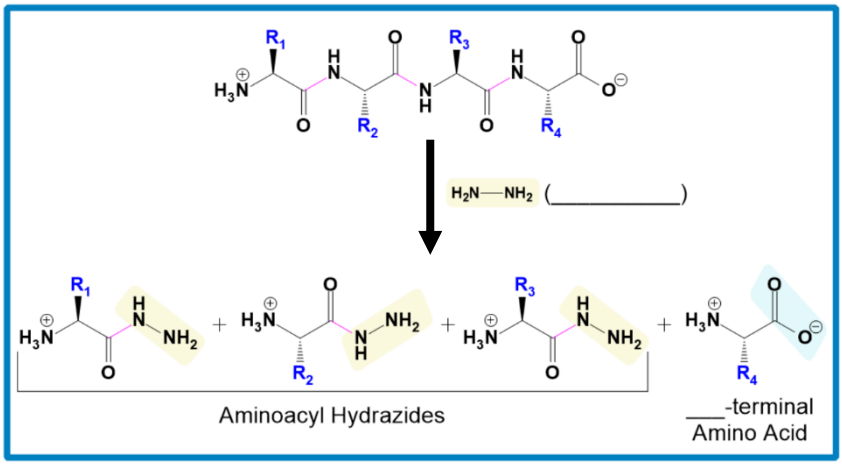
Asp-Met-Leu-Phe-Met-Arg-Ala-Tyr-Gly-Asn $\xrightarrow{\text{CNBr}}$

Hydrazinolysis for C-terminal Residues

●*Hydrazinolysis*: uses the chemical agent *hydrazine* to identify the ___-terminal amino acid residue of a peptide.

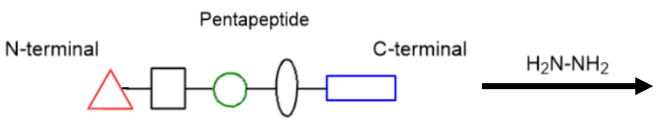
- □ Hydrazine: forms aminoacyl hydrazides with every amino acid residue _____ for the C-terminal residue.
- □ Free C-terminal amino acid can be distinguished from other residues & identified.

EXAMPLE: Hydrazinolysis.



"**Z**" is the *last* letter of the alphabet & hydra**Z**ine identifies the _____ residue (C-terminal residue) of a peptide.

PRACTICE: Sketch the approximate result of the following reaction.

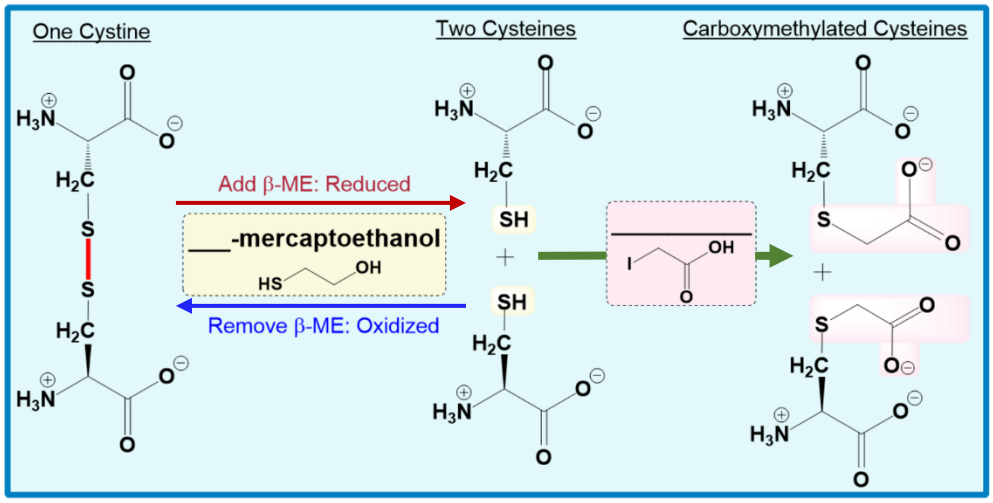


CONCEPT: CHEMICAL CLEAVAGE OF BONDS

β-mercaptoethanol + Iodoacetate

- *Disulfide Bonds* _____ with the sequencing procedure and must be broken *prior* to Edman Degradation Seq.
  - □ *β-mercaptoethanol + iodoacetate* permanently break the _____ bonds of cystine amino acid residues.
  - □ Iodoacetate: *carboxymethylates* cysteine sulfhydryl groups, which _____ reformation of disulfides.

EXAMPLE:



PRACTICE: Which of the following statements about cystine is correct?

a) Cystine is formed through a peptide linkage between two cysteines.

b) Cystine forms when the $-CH_2-SH$ group is oxidized to form a $-CH_2-S-S-CH_2-$ disulfide bridge between 2 cysteines.

c) Cystine is formed by the oxidation of the carboxylic acid groups on two cysteines.

d) Two cystines are released when a $-CH_2-S-S-CH_2-$ disulfide bridge is reduced to $-CH_2-SH$.

Recap of Chemical Bond Cleavage

EXAMPLE: Fill-in the blanks to recap the effects of the chemicals on proteins.

Chemical Agent	Result of Chemical Agent on Protein
1-fluoro-2,4-dinitrobenzene (FDNB) _____ Reagent	Covalently *labels* the free ___-terminal residues of *all* polypeptide chains.
6M Hydrochloric Acid (HCl)	Cleaves _____ Peptide Bonds & Releases *Free* Amino Acids.
Cyanogen Bromide (CNBr)	Cleaves Peptide Bonds on ___-terminal Side of _____
Hydrazine (NH_2NH_2)	Initiates Hydrazinolysis to Identify ___-terminal *Amino Acids*.
β-mercaptoethanol + Iodoacetate	Breaks _____ bonds of Cystine Residues & Prevents their Re-formation.

CONCEPT: CHEMICAL CLEAVAGE OF BONDS

PRACTICE: Given the amino acid sequence DAHGHFLKAGMT, what reagent would cleave the last residue, T, from the peptide? That is, one of the products after treatment will be the free amino acid: T.

a) Trypsin.
b) Phenyl isothiocyanate.
c) Cyanogen bromide.
d) Iodoacetate.
e) Chymotrypsin.

PRACTICE: One method to prevent disulfide bond interference with protein sequencing procedures is:

a) Cleaving proteins with proteases that specifically recognize disulfide bonds.
b) Protecting the disulfide bridge against spontaneous reduction to cysteinyl sulfhydryl groups.
c) Reducing disulfide bridges and preventing their reformation by further modifying the -SH groups.
d) Removing cystines by proteolytic cleavage.
e) Sequencing proteins that do not contain cysteine residues.