B Sc SEM IV Chemistry (Hons): CORE IX
Organic Chemistry

Dr. Kanika Ghosh
Assistant Professor in Chemistry
B C College, Asansol
Kazi Nazrul University

Protein: Synthesis & Analysis

Contact address: ghosh.kanika7@gmail.com
kanika@bccollegeasansol.ac.in

Protein

Proteins are complex organic nitrogenous substances formed in all type of living organisms are composed of C,H,O, N and S in varying contents. In some cases phosphorous and other elements such as Cu, Hg, Fe etc may present.

Proteins are bio-polymer of amino acids. When protein are hydrolysed by acids, alkalis or enzymes, a mixture of amino acids is obtained.

Peptides

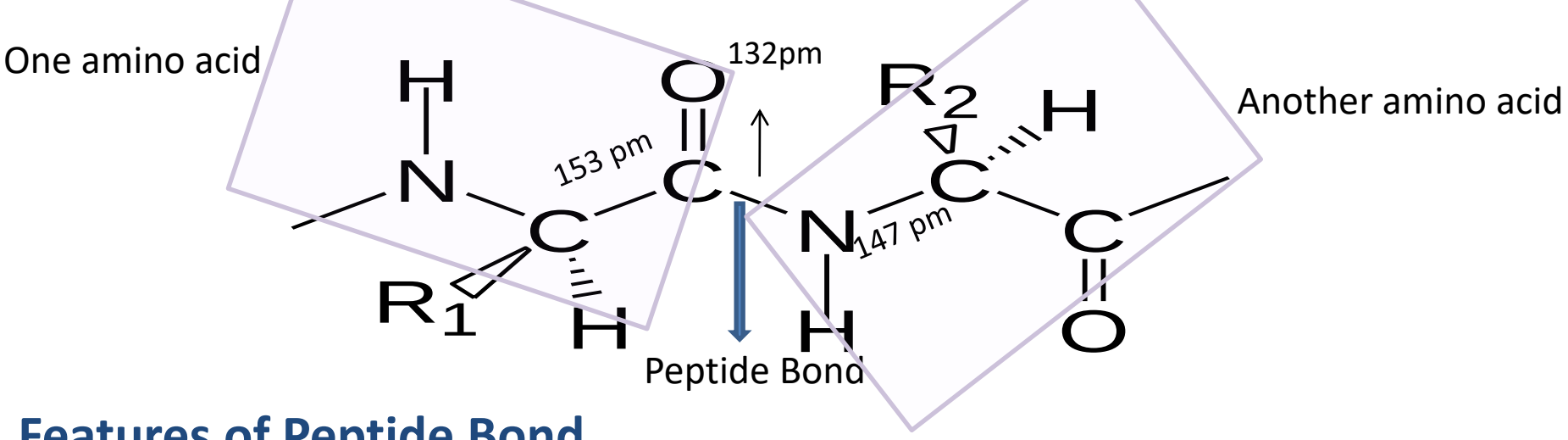
The compound formed by combination of two amino-acids through a peptide bond is called a **dipeptide**. If a third amino-acid joins the dipeptide by forming a peptide bond (new), it is called a **tripeptide**

Oligo-peptides: A peptide containing (3-10) amino acid residues is called an olig- peptide

Polypeptides: A peptide containing more than ten amino acid residues is called an polypeptide

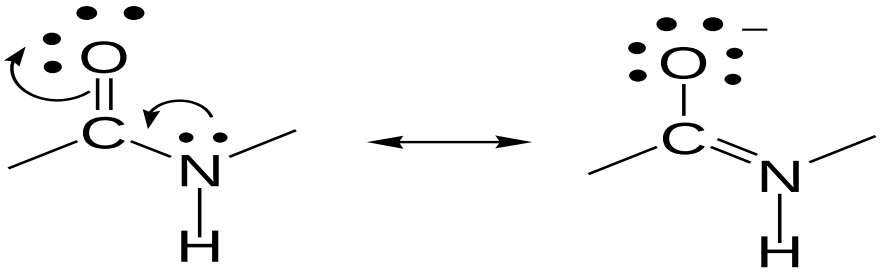
Peptide Bond

Peptide Bond: The amide linkage formed by the reaction of carbonyl group of one amino acid to the amino group of another amino acid is called a peptide bond



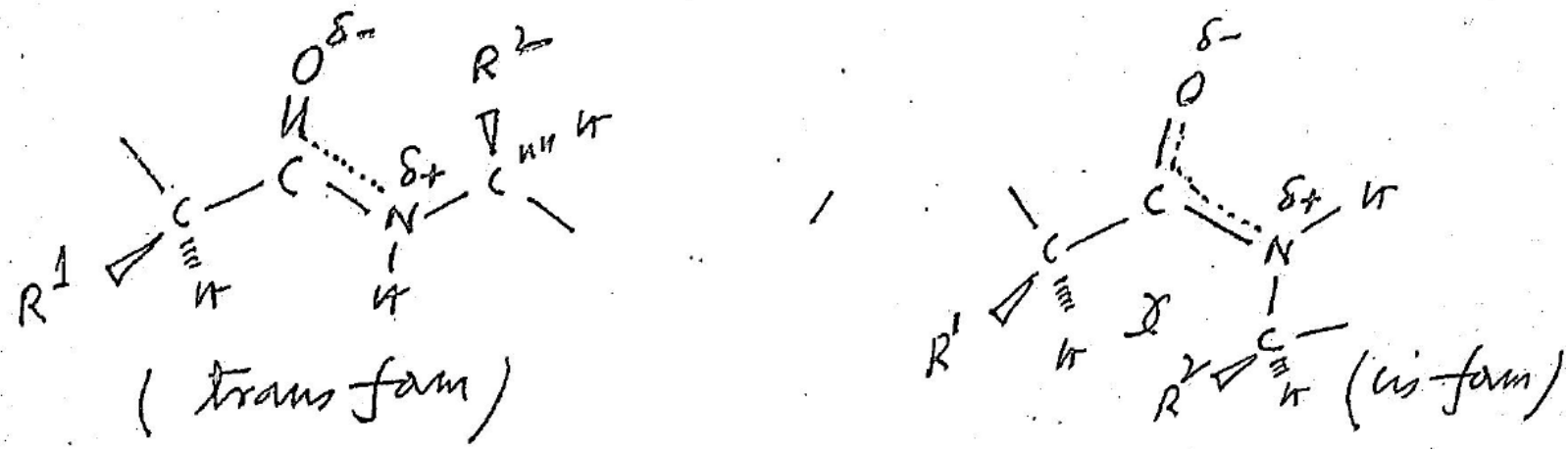
Features of Peptide Bond

1. All the atoms involved in a peptide linkage (-CONH-) are planar. That is 'C' and 'N' atoms are sp^2-hybridised.
2. The carbonyl oxygen atom and the 'H" atom of 'NH' gr are mutually trans.
3. The lone pair electrons on 'N' atom enters in conjugation with adjacent carbonyl (C=O) gr. And thereby the 'C-N' bond gets double bond character.



4. Owing to the double bond character, 'C-N' bond in a peptide is shorter and stronger than normal 'C-N' single bond.
5. Due to the double bond character of 'C-N' bond, free rotation about this bond is restricted And this gives rise to geometrical isomerism.

6.The *trans* form is seen to be about 1000 times more stable than *cis* form.



Denaturing of Proteins

The change in the structure of protein from normal pattern is called denaturing of proteins

Denaturing of proteins can takes place by the following ways:

(1) Heating

(2) Changing in pH

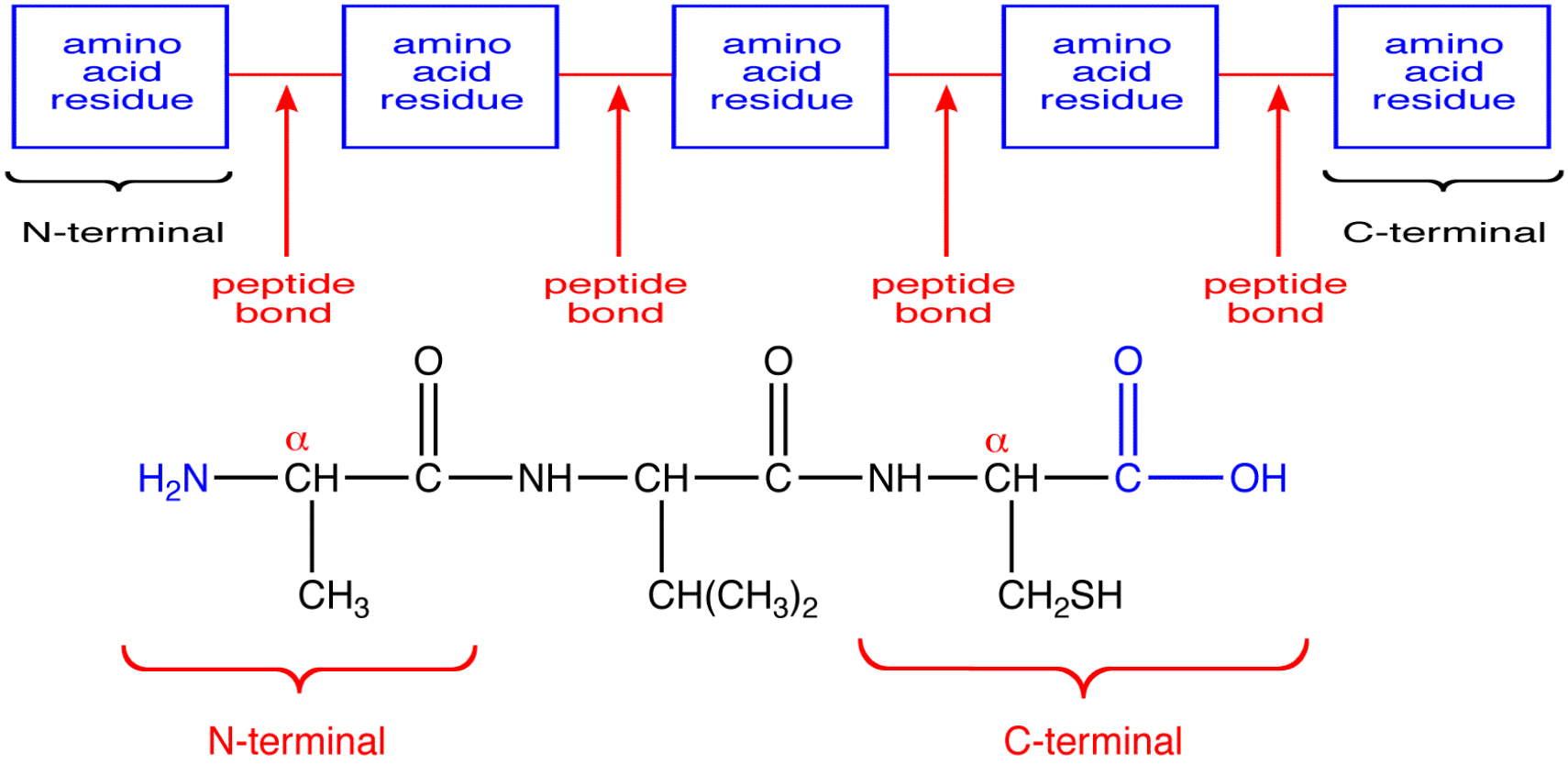
(3) Addition of strong oxidising or reducing agent

(4) By adding detergents

(5) By adding reagents like urea.

In denaturing, the primary structure remain intact but the tertiary structure unfolds from a specific shape to randomly looped chain.

N-Terminal Residue and C-Terminal Amino Acid Residue



In a peptide, the amino acid that contains the free amino group is called N-terminal residue

And the amino acid that contains the free carboxyl group is called C-terminal residue

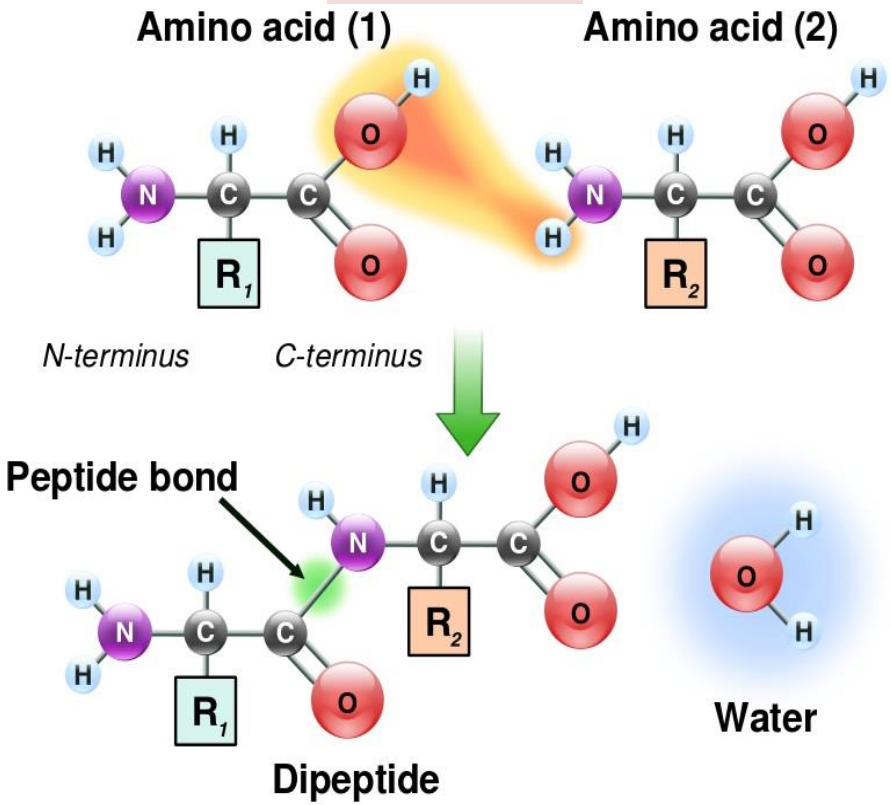
Peptides are always written with the N-terminal residue on the left and the C-terminal residue on the right

Protein Synthesis

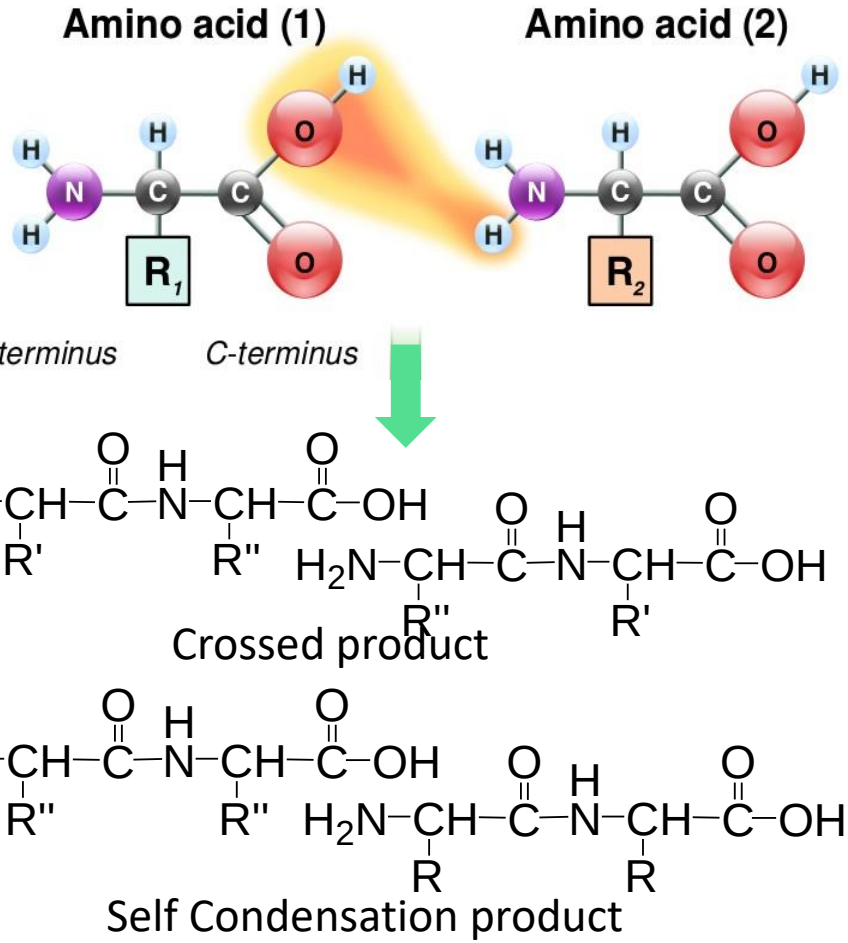
Two amino acid **not** form one dipeptide unit (Having amino end: N-terminal and acid end: C-terminal

In practice two amino acid not for four dipeptide unit

Expected



In Practice



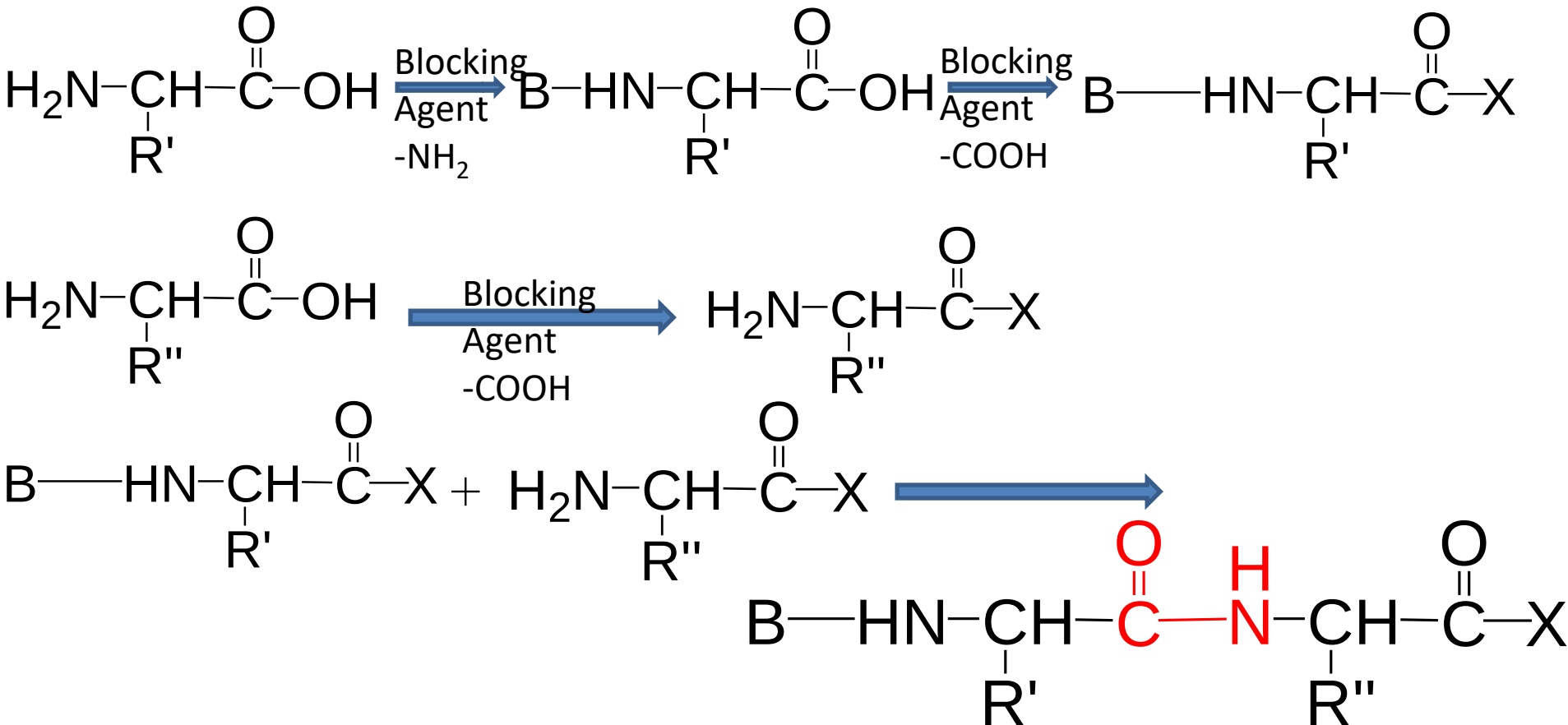
Expected Dipeptide Synthesis

-NH_2 group of **one amino acid** is to be blocked to make **-NH_2 group inactive**

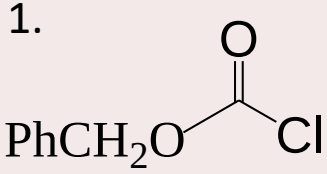
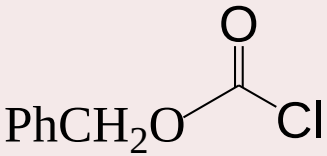
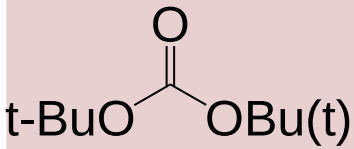
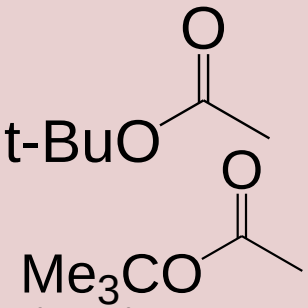
-COOH group of **that amino acid** is to be activated to make **-COOH group active**

-COOH group of **second amino acid** is to be **deactivated by blocking agent**

The N-terminal amino acid unit with blocked amino group and activated acid group is to be treated with second amino acid with blocked –COOH group

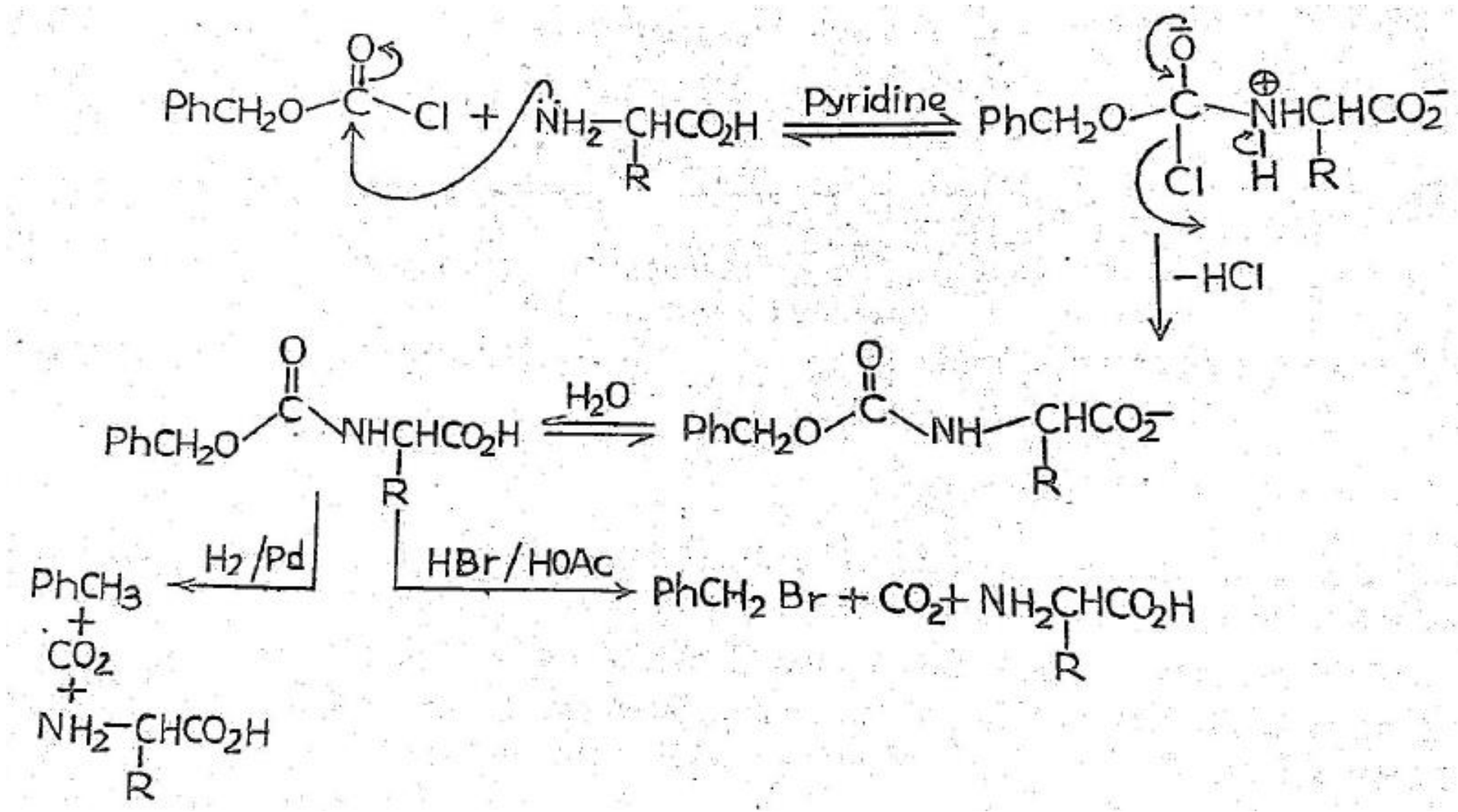


Blocking and Deblocking agents for the $-NH_2$ group of an amino acid unit

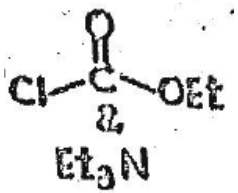
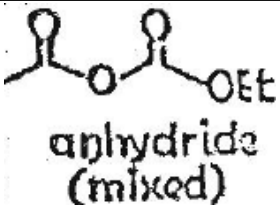
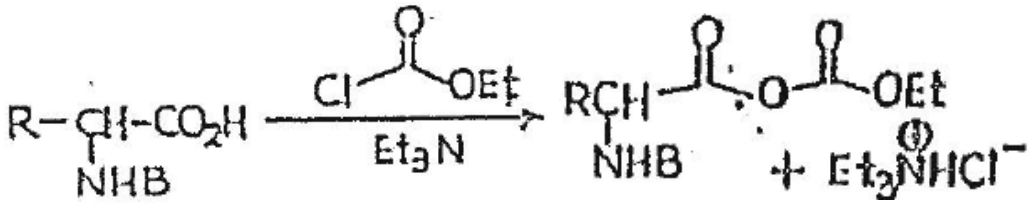
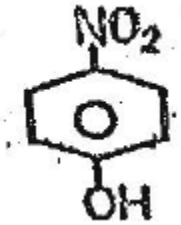
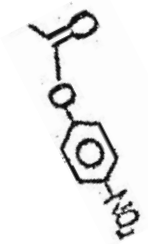
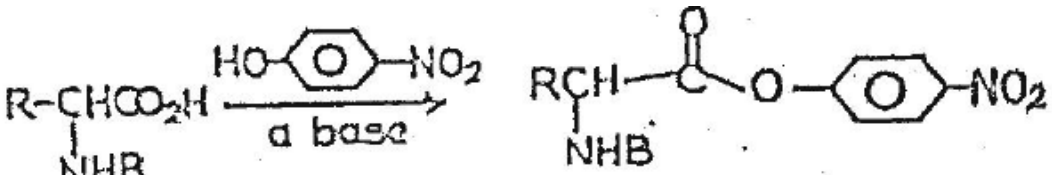
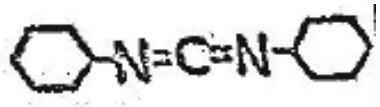
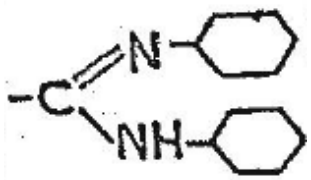
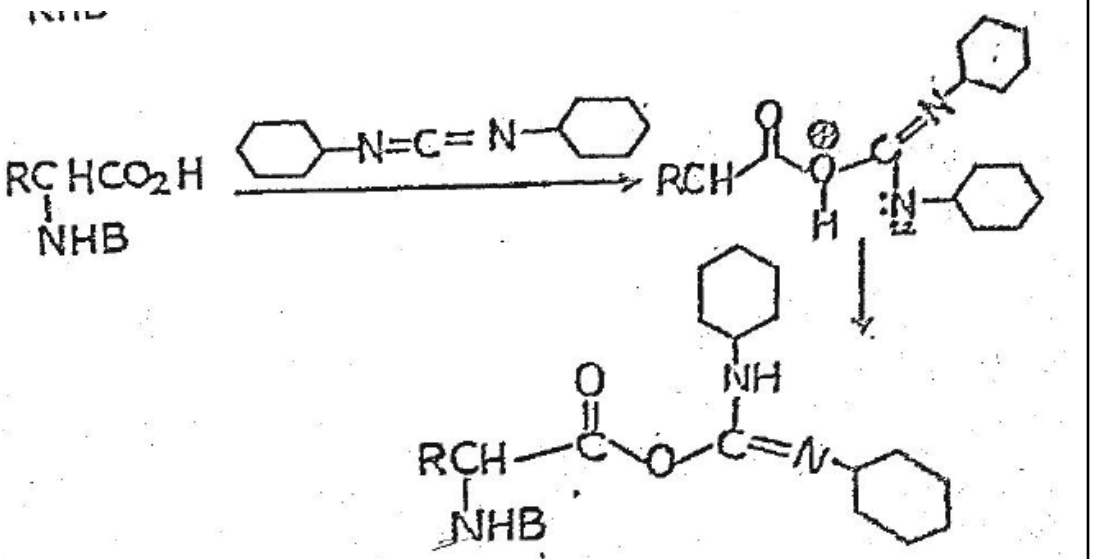
Blocking agent of $-NH_2$ group in the N-terminal amino acid unit of the desired peptide			**Blocking agent of $-NH_2$ group in the peptide synthesis**	
Reagent	Name of reagent	Blocking group and its name	Reagent	Name
1.  and OH- at 25°C	Benzyl chlorformate or benzyloxycarbonyl chloride	 benzyloxycarbonyl chloride group	H_2/Pd or H_2/Pt Or HBr/AcOH cold	$PhCH_3 + CO_2$ + peptide PhCH2Br + CO_2 + peptide
2.  and base at 25°C	D-t-butylcarbonate	 t-butyloxy-carbonyl group (BOC)	HCl Or CF_3COOH In HOAc at 25°C	$(CH_3)_2C=CH_2$ + CO_2 + peptide

Blocking and Deblocking agents for the $-NH_2$ group of an amino acid unit

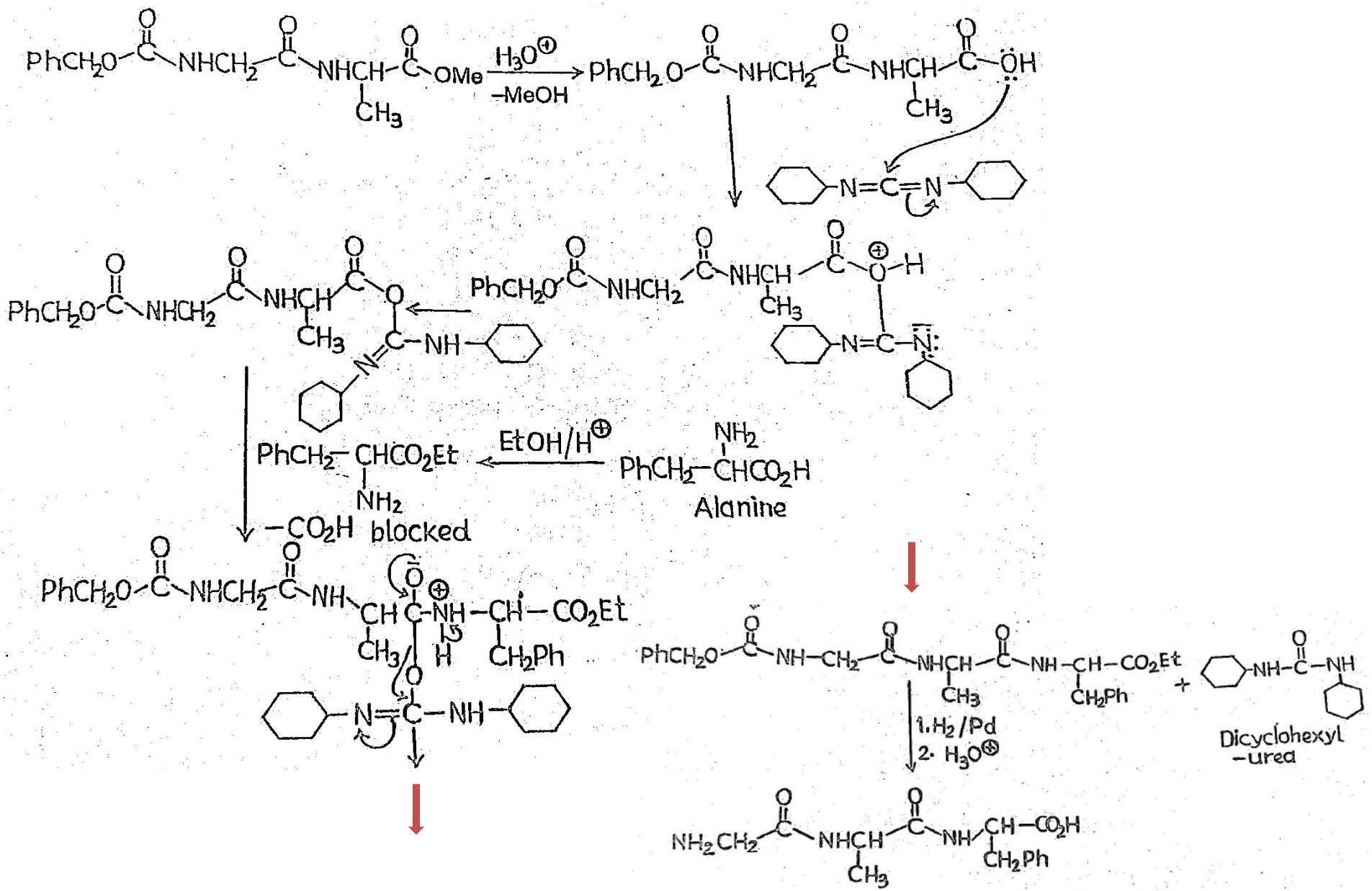
Mechanism of the reaction:



Reagent and reactions for the activation of the carboxylic acid group of –NH2 blocked amino acid unit

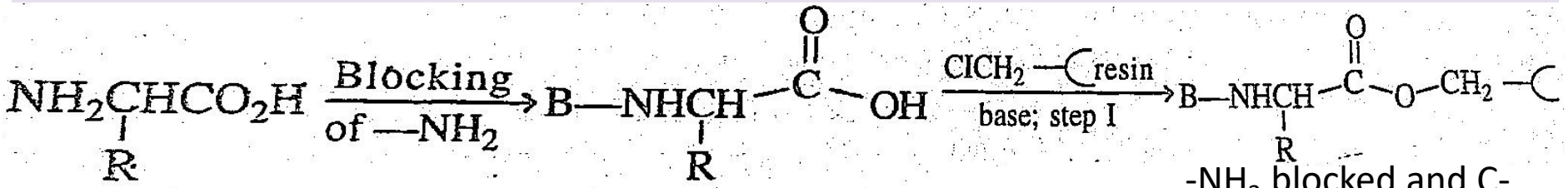
Activating Agents	Activating Group	Reactions
1. 		
2. 		
3. 		

The activation of the carboxylic acid group of $-NH_2$ blocked amino acid unit Mechanism of the reaction:



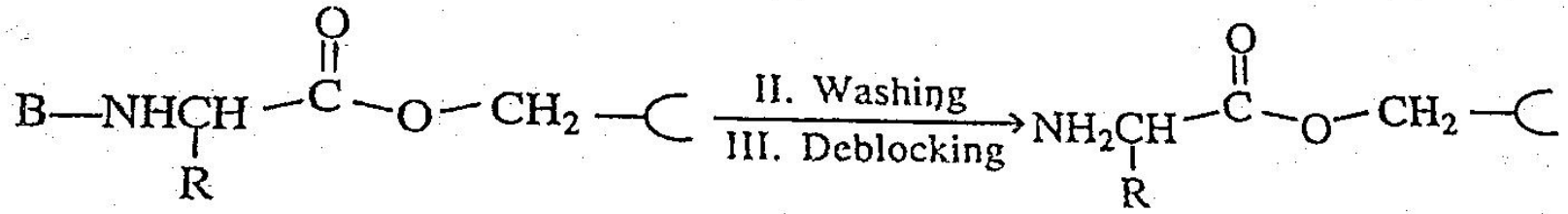
Merrifield Synthesis

Step-I: -NH_2 group is blocked of N-Terminal amino acid of the desired peptide and later it is attached with resin

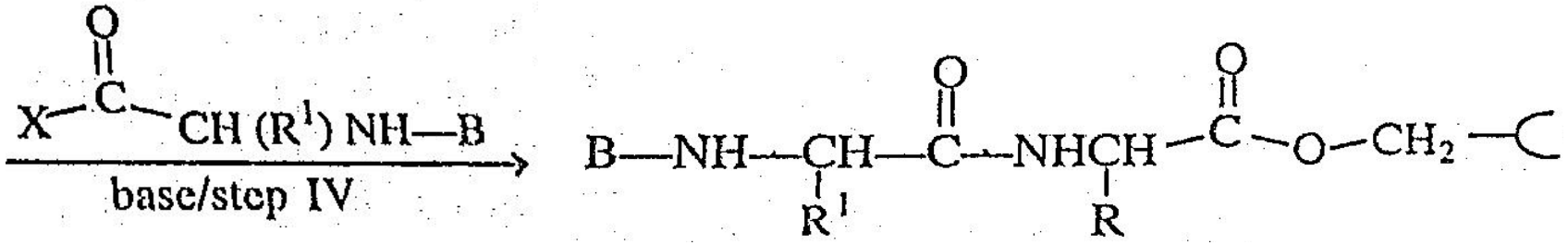


-NH_2 blocked and C-terminal bonded resin

Step-II & III: It is washed and later-NH_2 group is deblocked of N-Terminal amino acid of the desired peptide

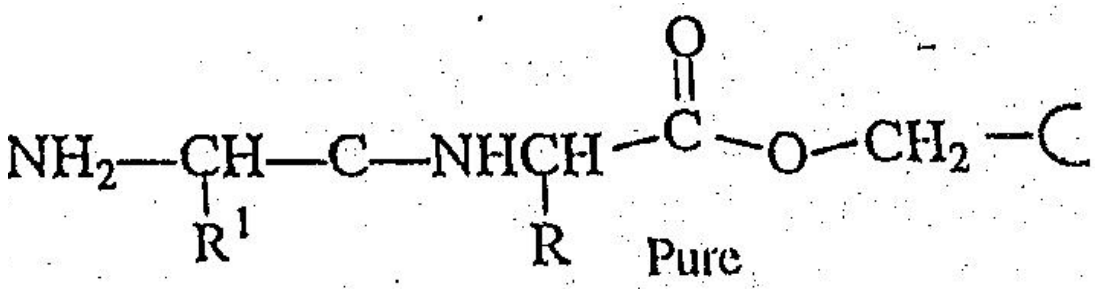


Step-IV: Resin attached amino acid is reacted with blocked –NH_2 and activated –COOH group amino acid



Step-V & VI: Repetition of Step III and IV are performed

Step V & VI
Repetition of Step III & IV



Merrifield Synthesis: Advantages

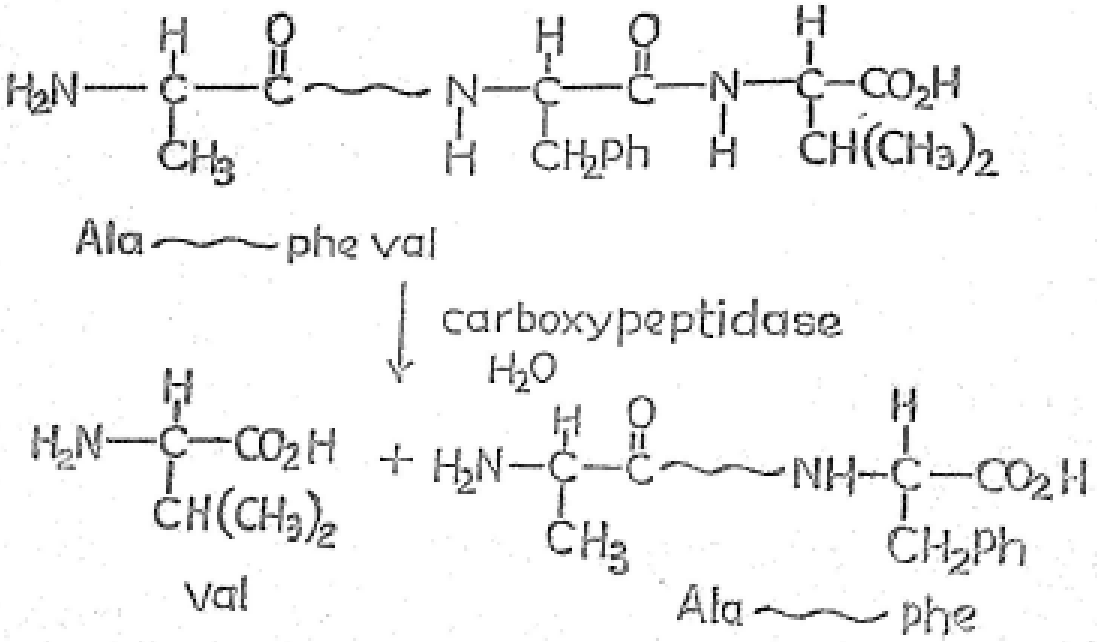
- The impurities are readily removed by washing with a solvent since they are not bonded to resin.

- The separation of intermediates are thus not required.

- The yield is of appreciable amount. So at every step the yield is 99%.

Determination of C terminal amino acid Unit:

The enzyme carboxypeptidase hydrolysis the peptide linkage at the C-terminal which hold an amino acid unit with free –COOH group.

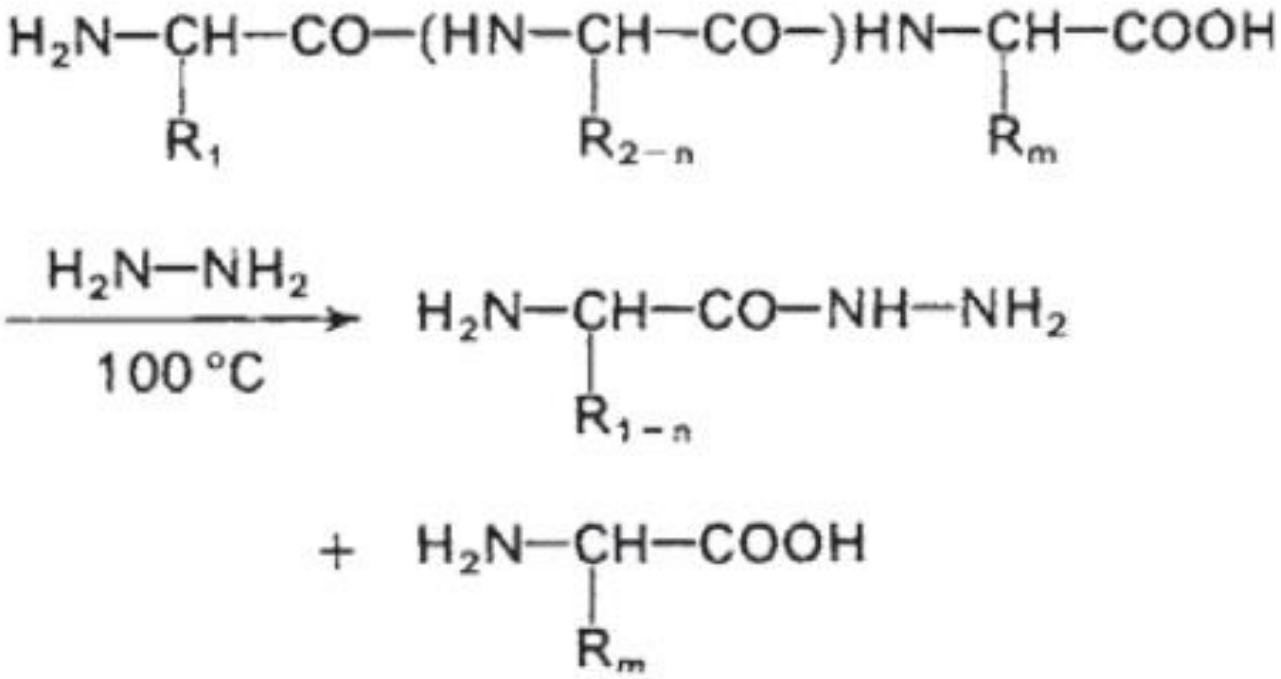


Carboxypeptidase is an exopeptidase.

Carboxypeptidase A cleaves off the C-terminal amino acid as long as it is not Arg or Lys.

Carboxypeptidase B cleaves off the C-terminal amino acid only if it is Arg or Lys.

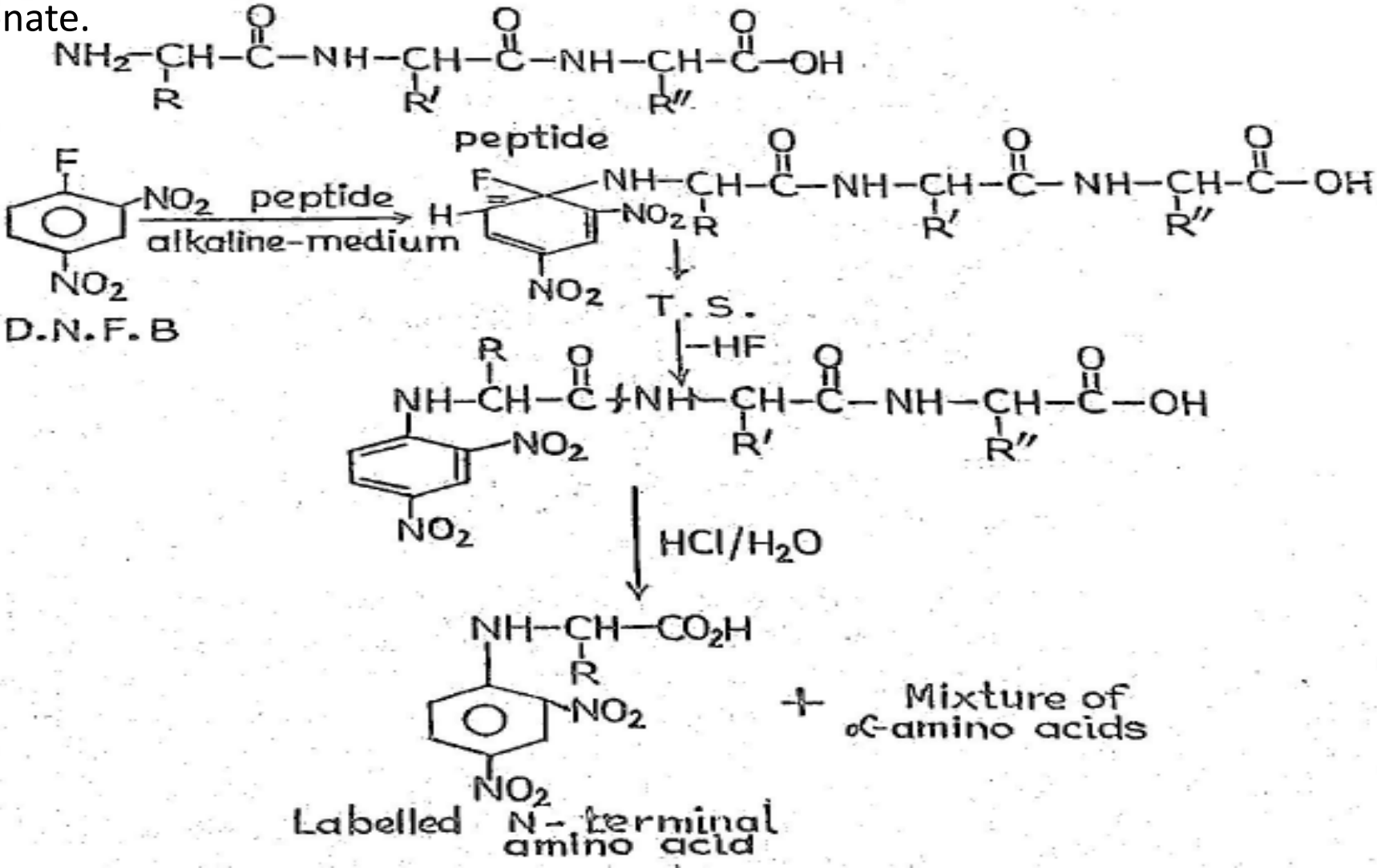
-Determination of C-terminal amino acids is possible *via* the hydrazinolysis procedure recommended by *Akabori:*



-The C-terminal amino acid could be then separated from the amino acid hydrazides by a cation exchange resin.

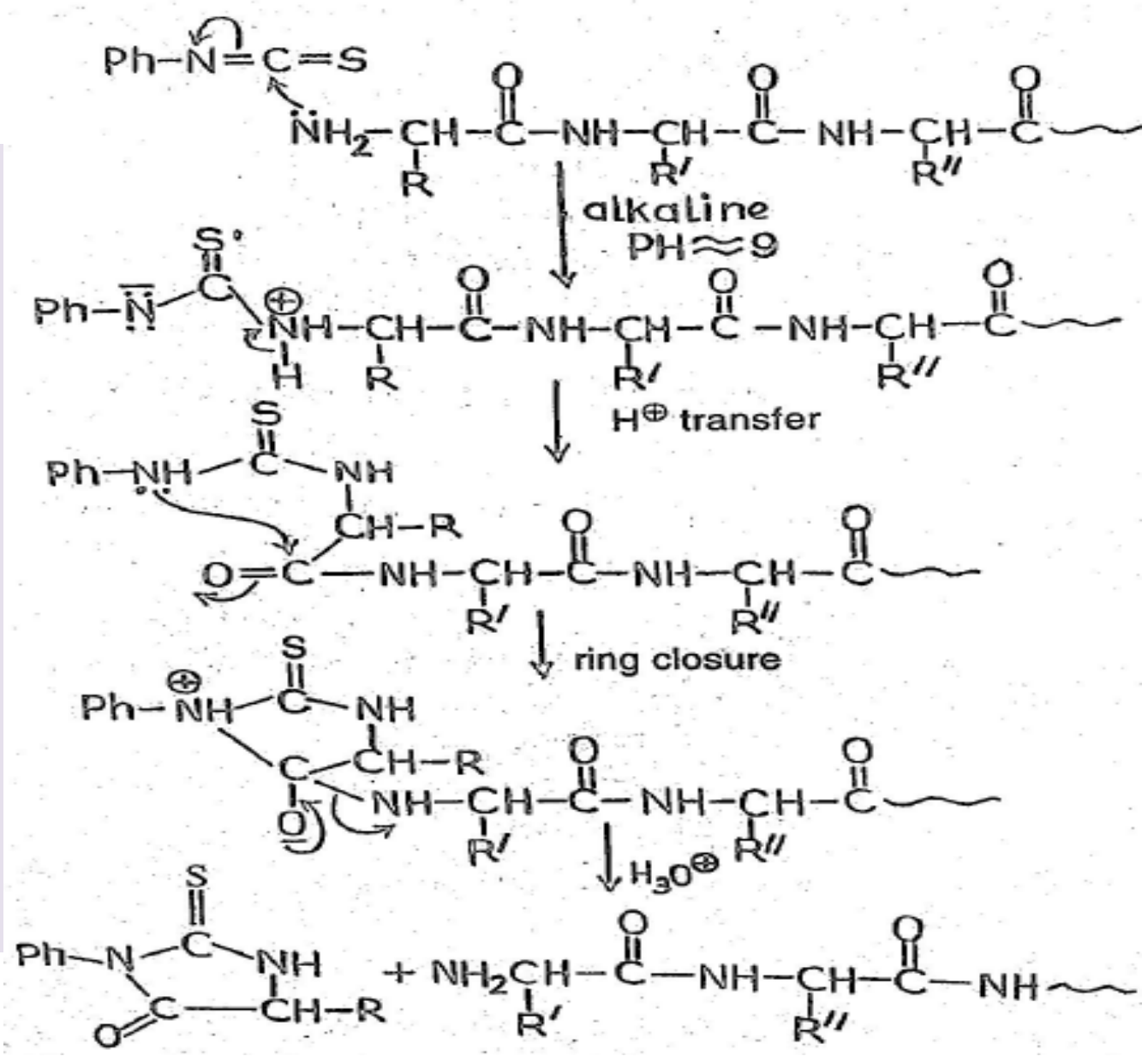
Determination of N terminal amino acid Unit: Sanger's Method:

2,4-Dinitroflurobenzene (DNFB) is the reagent for this method. The reaction between DNFB and Polyamide chain is carried out in a mildly basic solution of aqueous sodium bicarbonate.



Determination of N terminal amino acid Unit: Sanger's Method:

Edman degradation: This involves a nucleophilic addition of the free $-NH_2$ group of the polyamide to C=N of phenyl isocyanate in a mildly basic medium (pH 9.0). The addition product then undergoes a ring closure reaction.



References:

- Organic Chemistry Volume II by I L Finar
- Advanced General Organic Chemistry ; Part 2 by S K Ghos
- Organic Chemistry by R T Morrison, R N Boyd & S K Bhattacharjee
- Organic Chemistry by F A Carey
- Organic Chemistry by Solomons, Fryhle & Snyder
- www.google.com