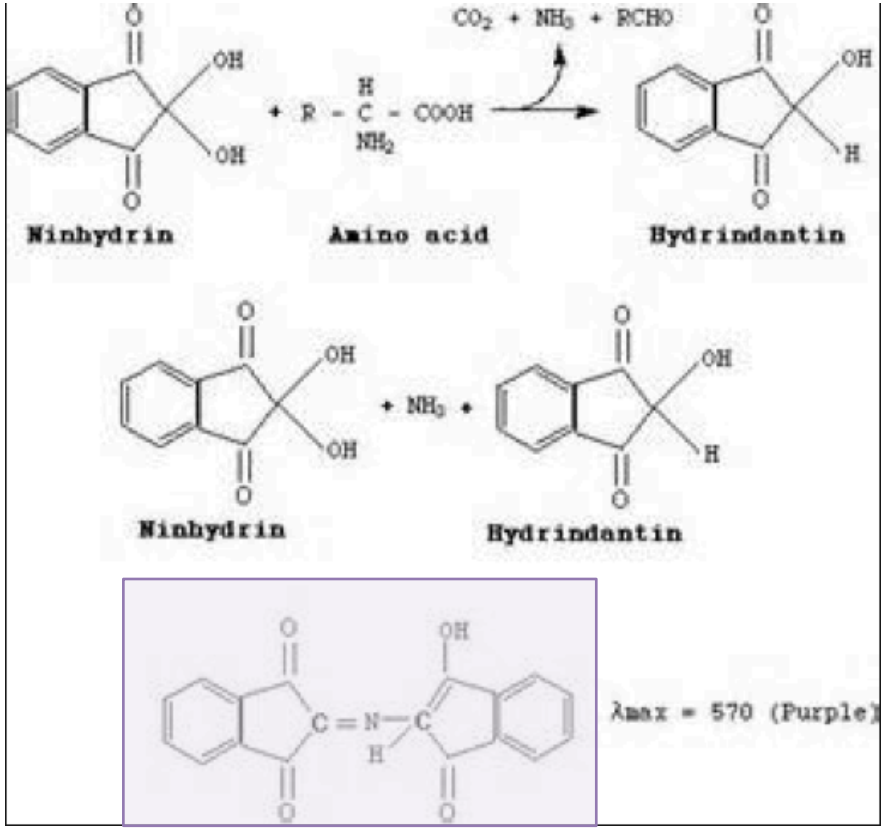
Reagents for Protein Sequence Determination

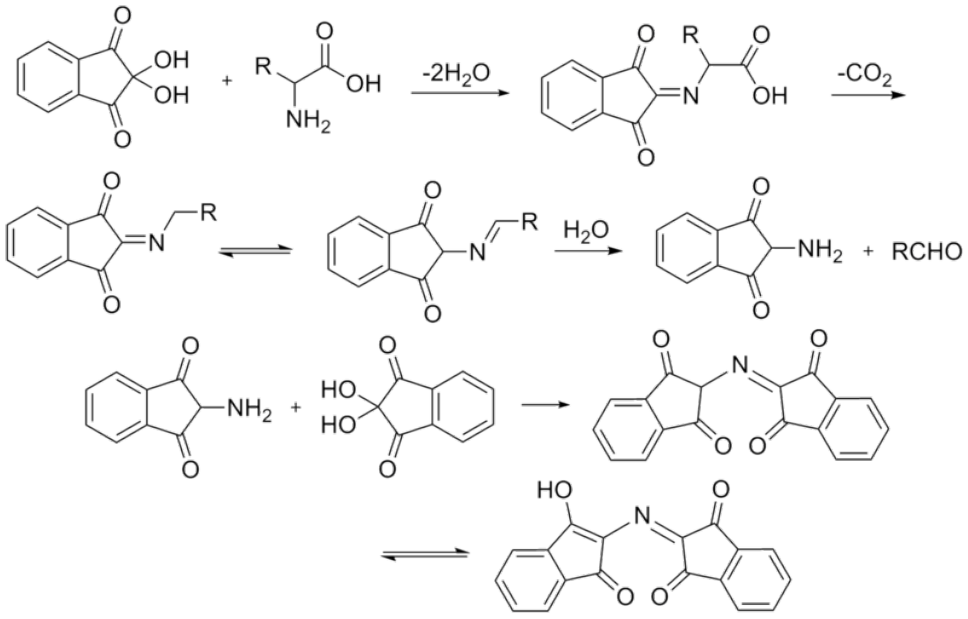
1. Ninhydrin: for quantitative reaction with amino groups of any amino acid
2. Edman Degradation: for reaction with amino terminus of proteins/ peptides and reduction of peptide by one residue
3. N-terminal analysis: for reaction with amino terminus of proteins/ peptides, but complete hydrolysis of peptide required
   a. FDNB
   b. Dansyl chloride
   c. Fluorescamine
   d. *o*-phthalaldehyde

Ninhydrin

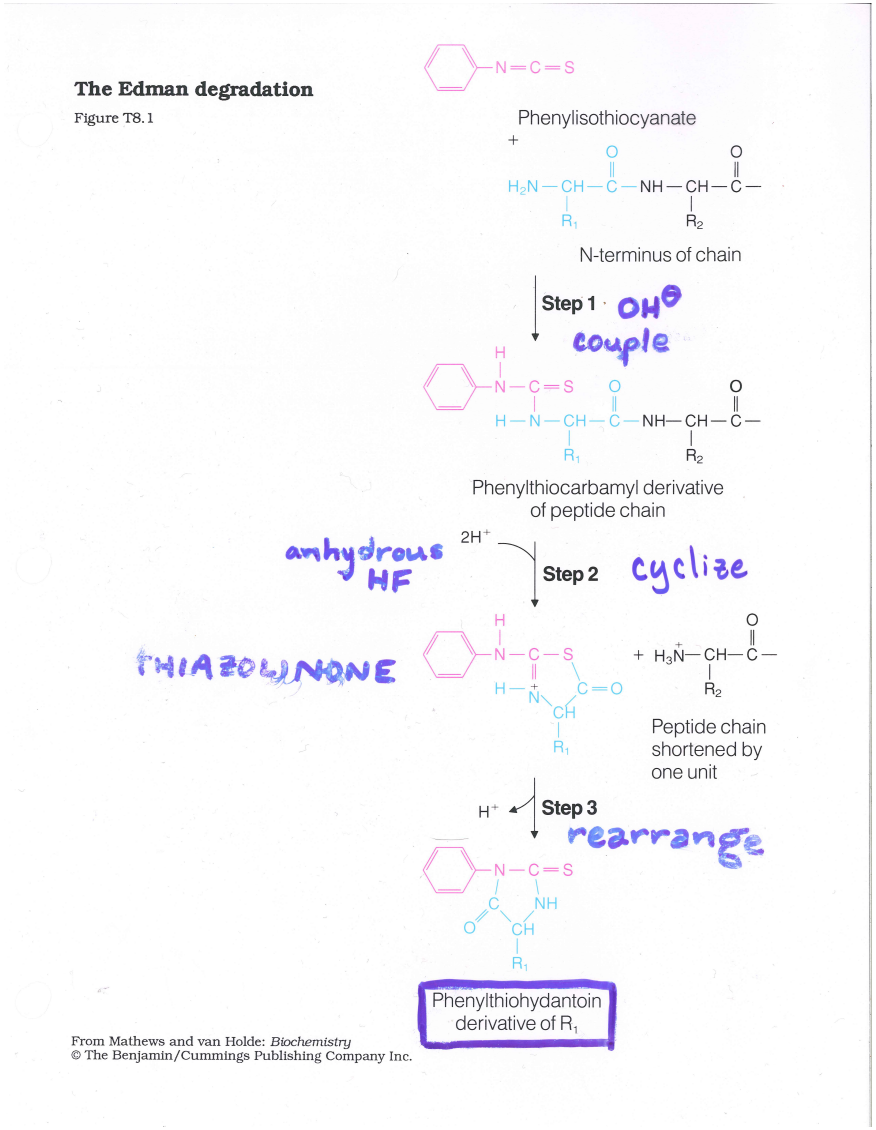


The ninhydrin reaction quantitatively reacts with amino acids in a complex reaction that produces ammonia from the amine, carbon dioxide from the carboxylate, and the Ca becomes an aldehyde of the R-group. The key is the reaction of the intermediate hydrindantin with another mole of ninhydrin and the ammonia to produce the large molecule, called Ruhemann's purple, which is a deep purple and easily quantified by absorbance at 570 nm.

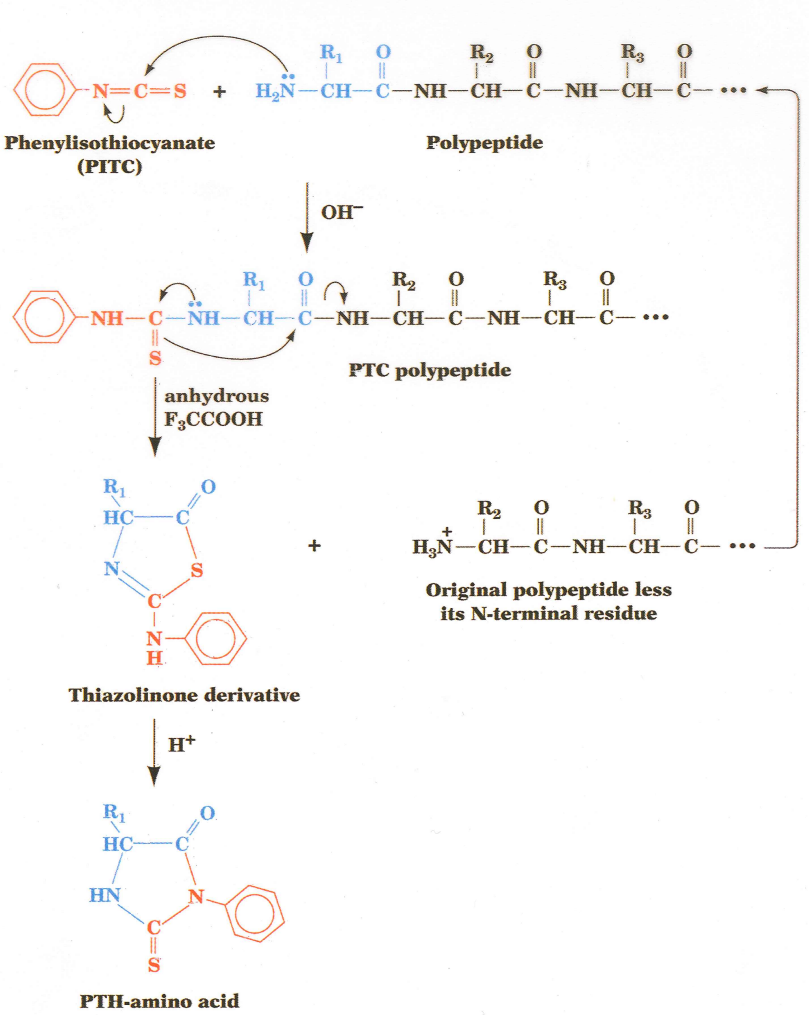
Mechanism:



Edman Degradation

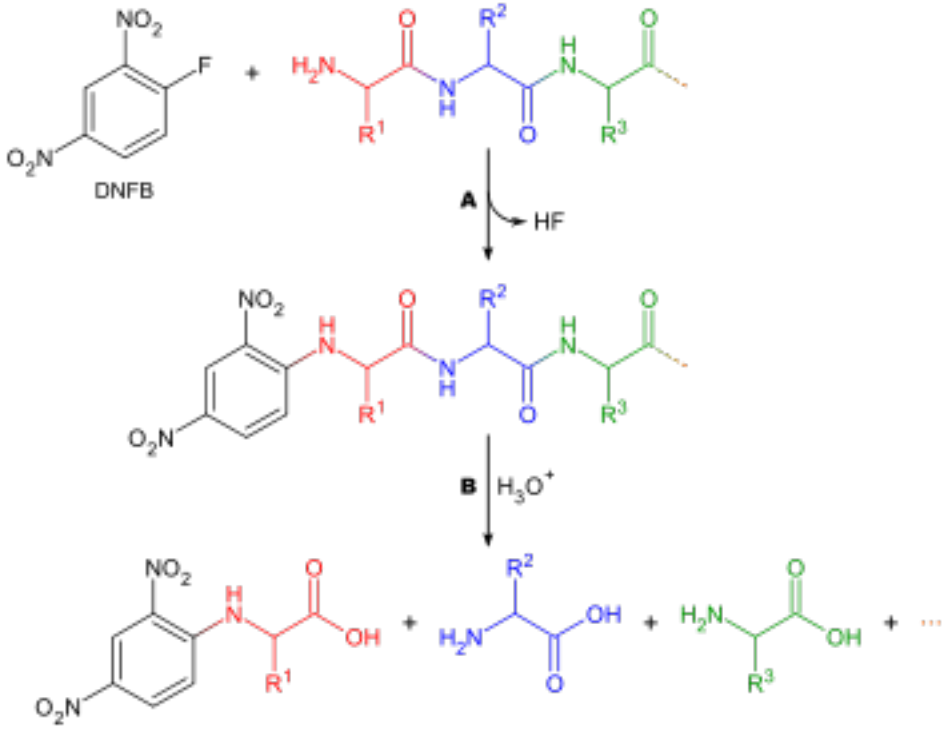


OR

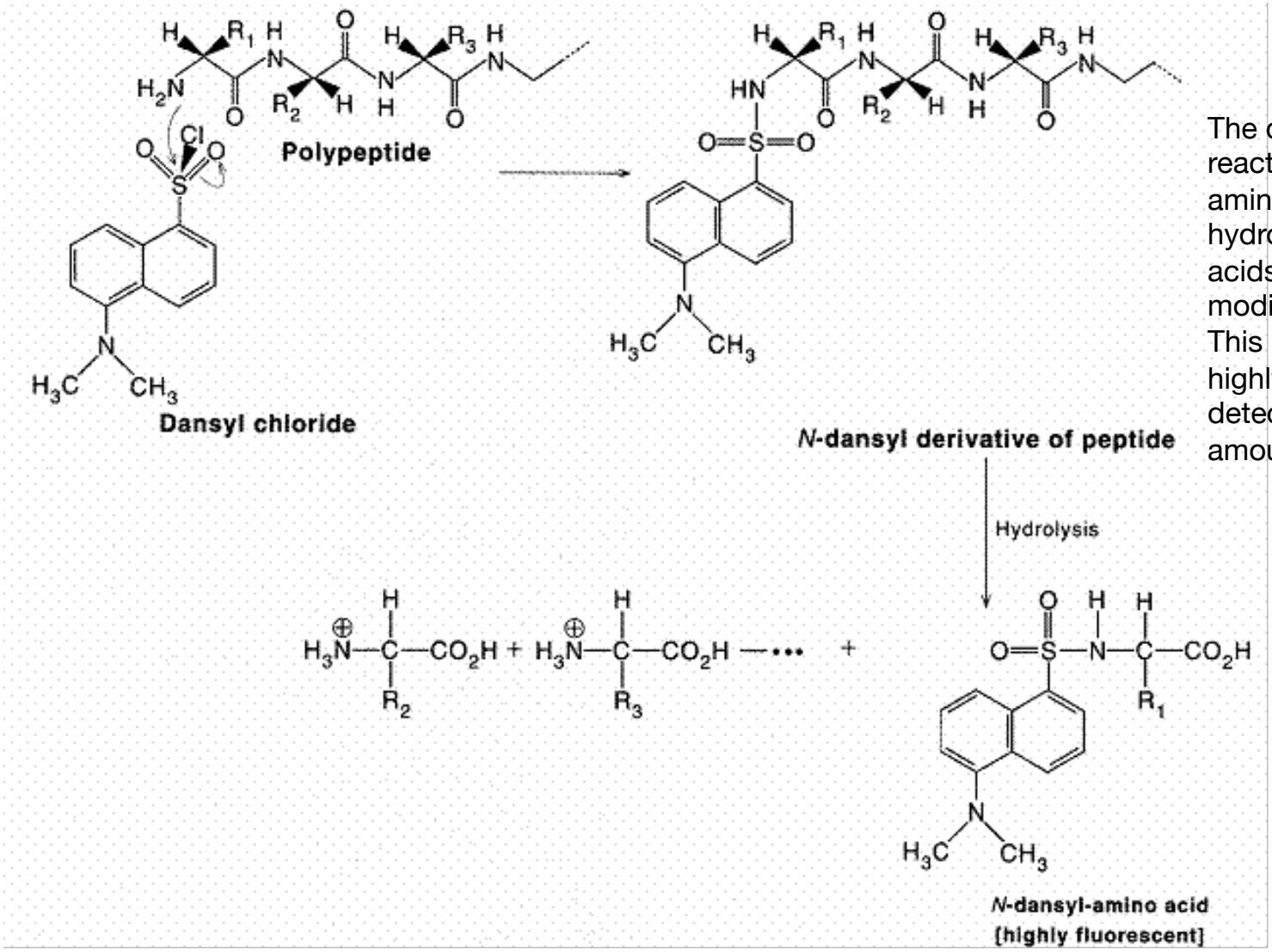


Biochemical Reactions for N-terminal analysis

Sanger Reagent
(fluorodinitrobenzene-FDNB; aka dinitrofluorbenzene-DNFB)



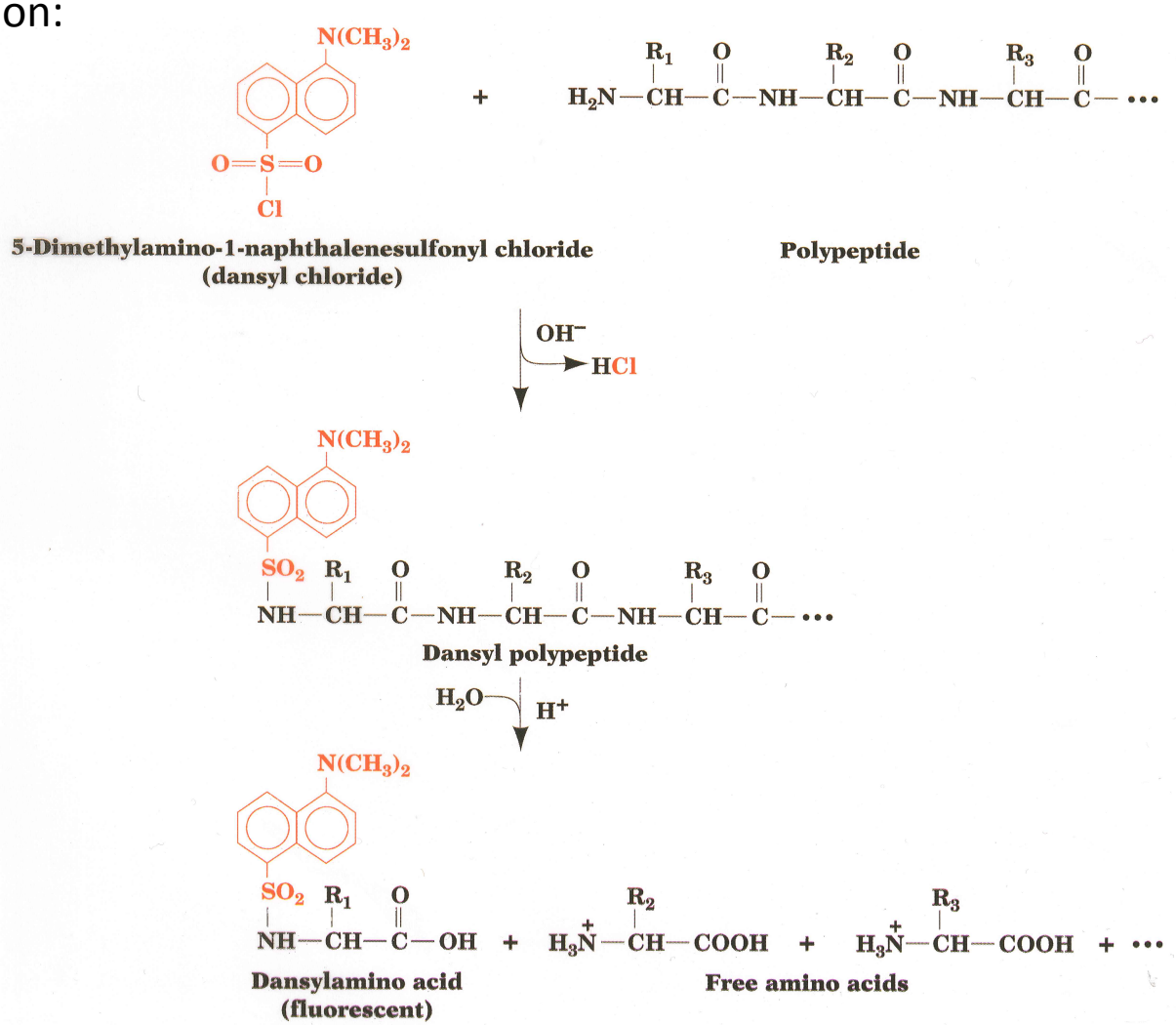
Dansyl chloride



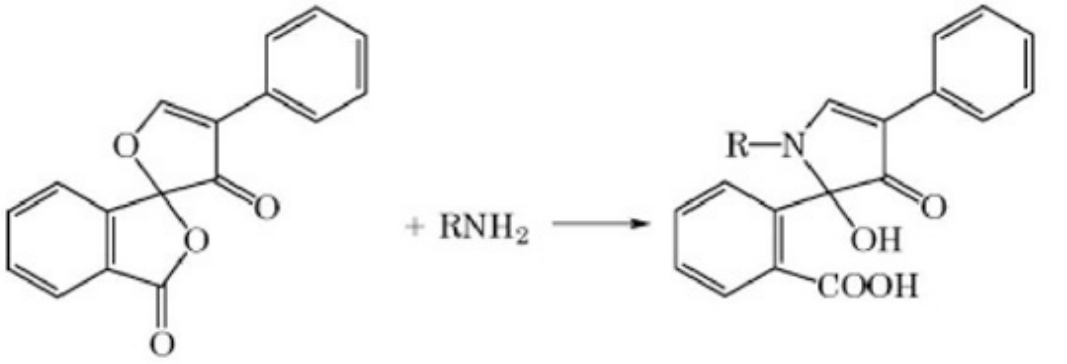
The dansyl choride reagent reacts with amino terminal amino acid. Subsequence acid hydrolysis yields all the amino acids plus the N-terminal one modified by the dansyl group. This modified amino acid is highly fluorescent and allows detection from very small amounts of protein

Dansyl chloride

Another depiction:



Fluorescamine & *o*-Phthalaldehyde

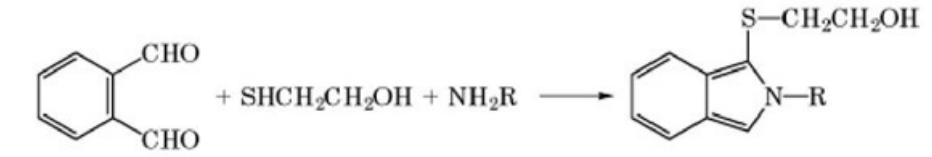


[I] Fluorescamine

[III] Fluorescent compound

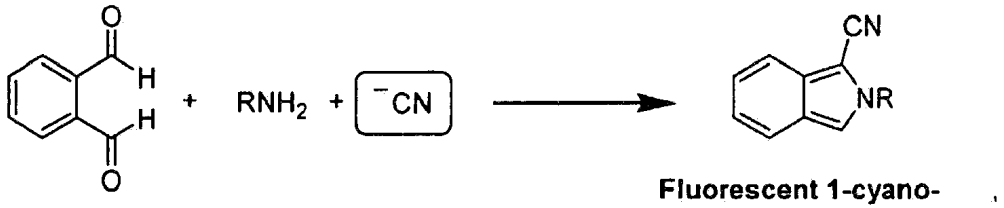
Fluorescamine [I] reacts readily with primary amino groups to form highly fluorescent compounds [II]. Even though fluorescamine itself is nonfluorescent. The fluorescent products have an excitation maximum at 390 nm and an emission maximum at 475 nm. These properties make fluorescamine ideal for detecting amino groups, especially in proteins, peptides, and amino acids. It is 10 to 100 times more sensitive in detecting primary amino groups than the ninhydrin reaction.

Because fluorescamine has low solubility and stability in water, o-phthalaldehyde [III] (OPA), which reacts similarly with primary amino groups and gives highly fluorescent products [IV]. OPA is sometimes substituted for fluorescamine:



[III] o-Phthalaldehyde

[IV] Fluorescent product



OPA

Fluorescent 1-cyano-2-substituted benzoisoindole

EX λ 320 nm, EMλ 380 nm

These reactions need to include either a thiol (β-mercaptoethanol depicted) or cyanide anion.

[what-when-how]

Example of primary-structure determination