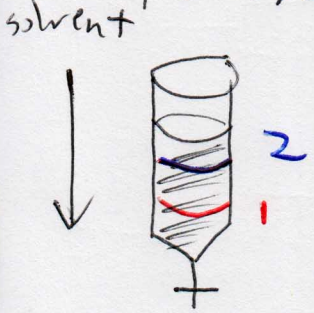
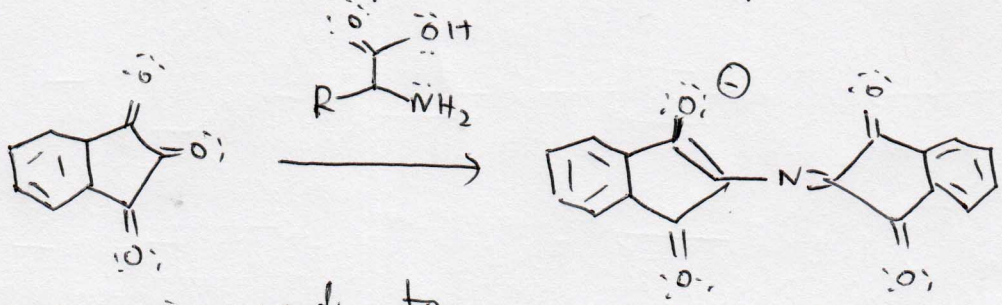
Chromatography can be used to determine the types and quantities of amino acids in a polypeptide, but cannot be used to determine a sequence since to prepare for chromatography a polypeptide is first hydrolyzed.





Ninhydrin is used to visualize amino acids, but (purple) not identify them (product of ninhydrin has no R group)

← fractions - different portions of the solvent eluting (coming out) from the column that may contain one or (non-ideally) more solutes.

Beer's law - $A = \varepsilon \cdot c \cdot l$

l ← length that light travels through a sample

c ← concentration (molarity) of sol'n

ε ← unique property of an individual compound

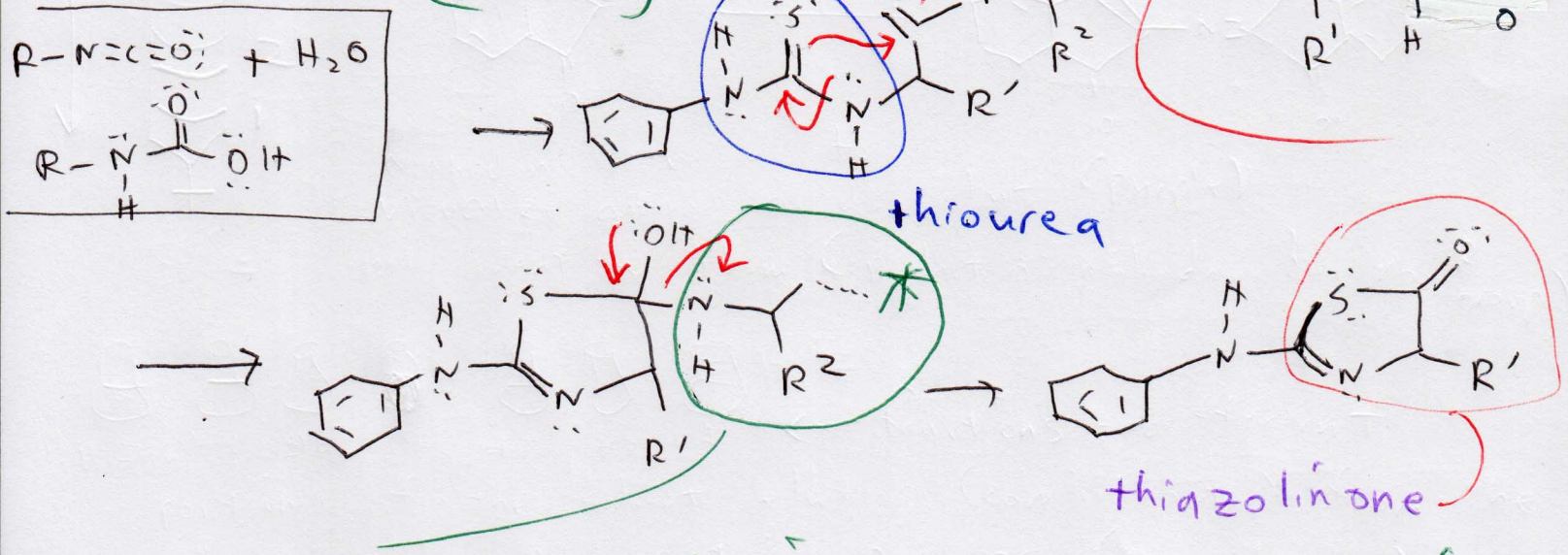
Since the absorbance and volume of a fraction can be measured, and since the path length used to measure a sol'n would be known, the concentration (and therefore moles) of an amino acid can be determined.

—Remember! In chromatography, the identity of an amino acid is determined by retention time.

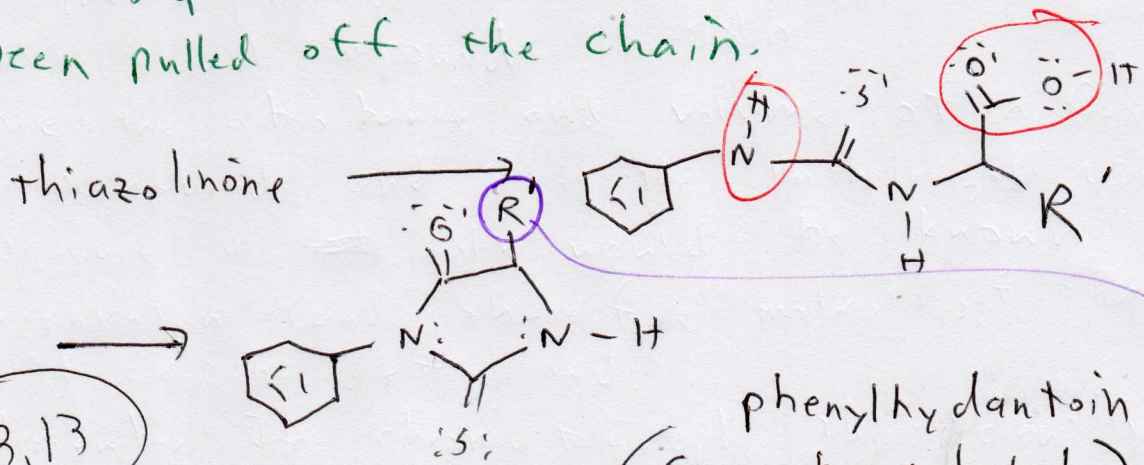
Sequencing

Edman degradation – Allows individual amino acids to be cleared from a polypeptide chain in order, allowing sequencing of that chain.

Reagent: Phenyl isothiocyanate (PITC)



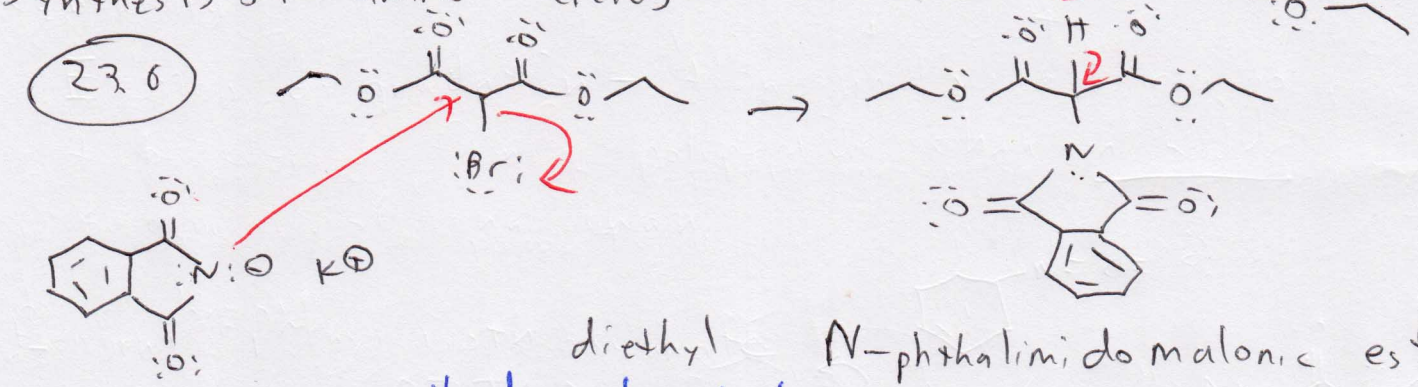
At this point of the mechanism, the remainder of the polypeptide is ejected from the PITC derivative. The PITC derivative can therefore be used to identify the amino acid that had been pulled off the chain.



23.13

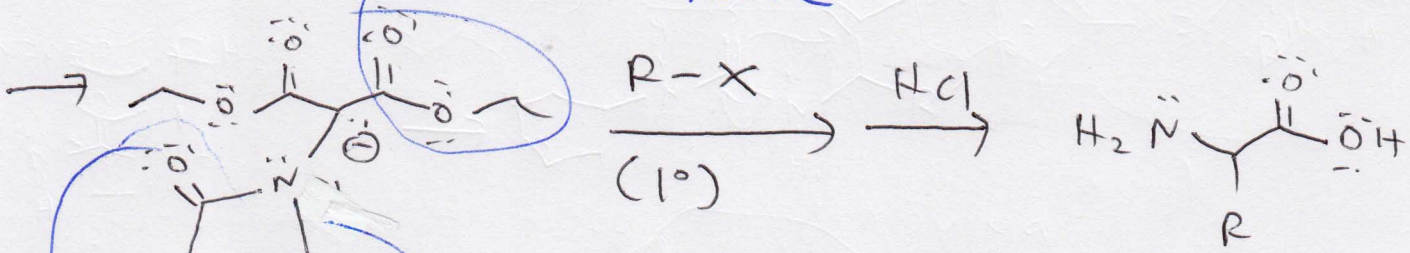
Synthesis of amino acids

(23.6)



diethyl N-phthalimidomalonic ester

will decarboxylate



will be hydrolyzed