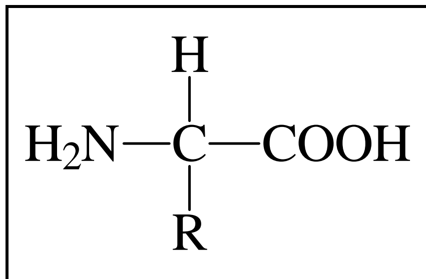
Amino Acids, Polypeptides and Proteins

An α-amino acid is a carboxylic acid which has an amino group attached to the carbon α to the COOH.

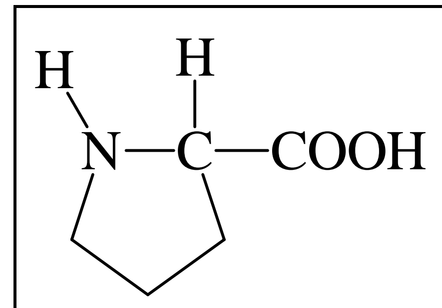
Polypeptides and proteins are composed, either completely or partially, of α-amino acid fragments, called *residues*, which are joined to each other to form chains.

Twenty different amino acids are commonly found in proteins. Nineteen of them are 1° amino acids having this structure:



where the R group is alkyl or substituted alkyl.

The 20th, proline, has a 2° amino group.



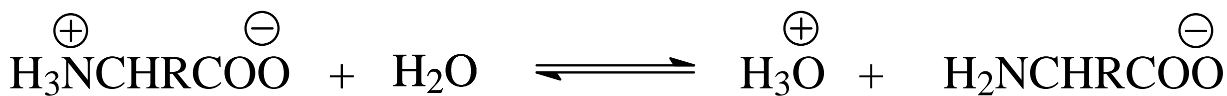
Properties of Amino Acids

- ❋ Non-volatile, high mp. (glycine, 262°C.)
- ❋ Insoluble in non-polar solvents; somewhat soluble in water.
- ❋ Large dipole moments. Glycine: 14D, *cf.* propylamine, 1.4D; propanoic acid, 1.7D
- ❋ K_a and K_b are much smaller than expected — *eg*, for glycine, H_2NCH_2COOH,

  $K_a = 1.6 \times 10^{-10}$, $K_b = 2.5 \times 10^{-12}$

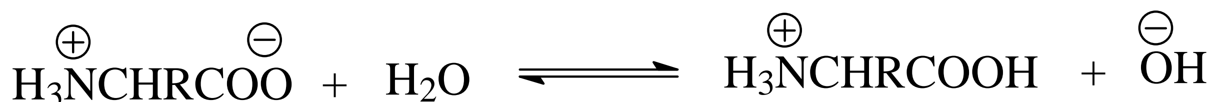
  cf. $K_a \sim 10^{-5}$ for typical COOH,
  $K_b \sim 10^{-4}$ for typical NH_2

All of this suggests the existence of a *zwitterion*, *eg* for glycine: $H_3N^+CH_2COO^-$. In this zwitterionic form the acid is the H_3N^+ (which can donate a proton to become H_2N), not the COOH. Also, the base is the COO^- (which can accept a proton to become COOH), not the NH_2. We cannot directly calculate the K_a for the COOH group and the K_b for the NH_2 group in the non-zwitterionic form of glycine, H_2NCH_2COOH. However, we can calculate the K_a for the COOH group in $H_3N^+CH_2COOH$ and the K_b for the NH_2 group in $H_2NCH_2COO^-$. This is done on the next page where we take advantage of the fact that $K_a \times K_b = 10^{-14}$ in aqueous solution, where K_a and K_b refer to a conjugate acid and base pair.



acid

$$K_{a_{-NH_3^+}} = \frac{\left[H_3O^{\oplus}\right]\left[H_2NCHRCOO^{\ominus}\right]}{\left[H_3\overset{\oplus}{N}CHRCOO^{\ominus}\right]}$$



base

$$K_{b_{-CO_2^-}} = \frac{\left[H_3\overset{\oplus}{N}CHRCOOH\right]\left[OH^{\ominus}\right]}{\left[H_3\overset{\oplus}{N}CHRCOO^{\ominus}\right]}$$

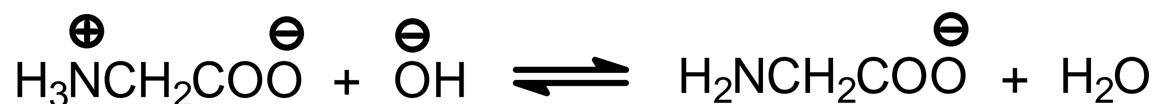
In aqueous solution: $K_a \times K_b = 10^{-14}$
conjugate
acid & base

$K_{a_{-NH_3^+}} \times K_{b_{-NH_2}} = 10^{-14}$; $K_{b_{-NH_2}} = \frac{10^{-14}}{1.6 \times 10^{-10}} = 6.5 \times 10^{-5}$

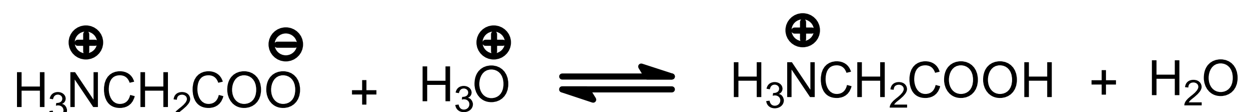
$K_{a_{-COOH}} \times K_{b_{-CO_2^-}} = 10^{-14}$; $K_{a_{-COOH}} = \frac{10^{-14}}{2.5 \times 10^{-12}} = 4 \times 10^{-3}$

These values are reasonable for amines and carboxylic acids.

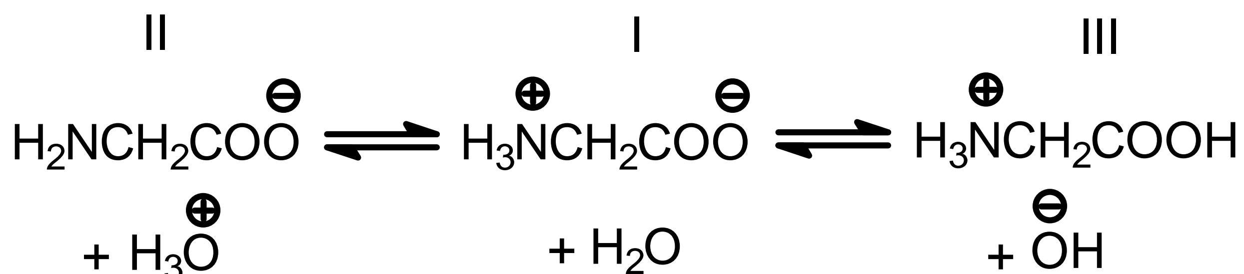
In base:



In acid:



$H_3\overset{\oplus}{N}CH_2CO\overset{\ominus}{O}$ may react as if it were H_2NCH_2COOH because of the equlibria which exist:



In other words, in solution, there are glycine molecules that have a COOH group and others that have an NH_2. [There are even a very few that are completely unionized.]

The hydrogen ion concentration at which [II] = [III] is known as the *isoelectric point* of the amino acid. If the amino acid is placed in an electric field at this pH, it will show no *net* migration toward anode (+) or cathode (-).

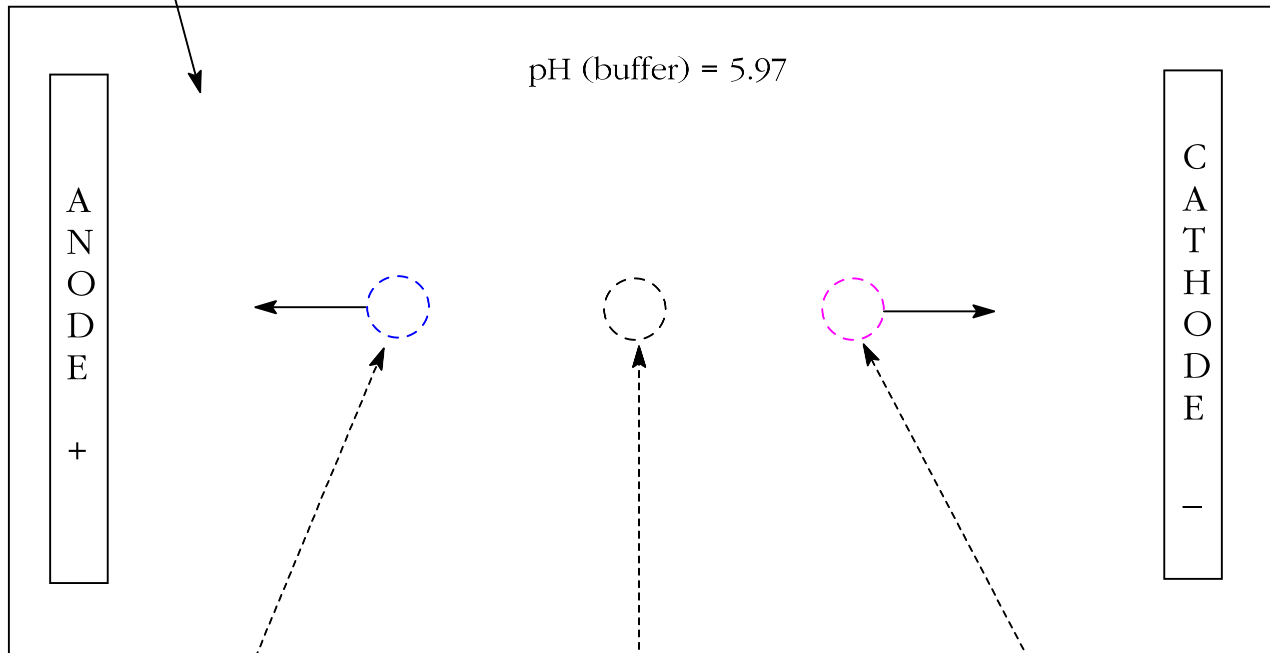
[Remember: Chemical equilibrium is a dynamic process and Lowry-Bronsted acid/base equilibria involving nitrogens and oxygens are usually fast. So, a given amino acid molecule will move toward the anode when it is in anionic (II) form, but a moment later it will be in zwitterionic form (I) and will not move, and a moment later it will be in cationic form (III) and move *back* toward the cathode. So, the molecule does a little dance in place – back and forth – but there is no *net* motion toward either electrode.]

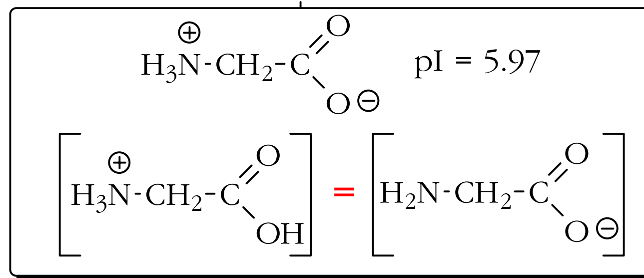
If the pH is increased, [II] > [III] (and a given amino acid molecule spends more time in state II than in state III) so there will be net migration toward the anode.

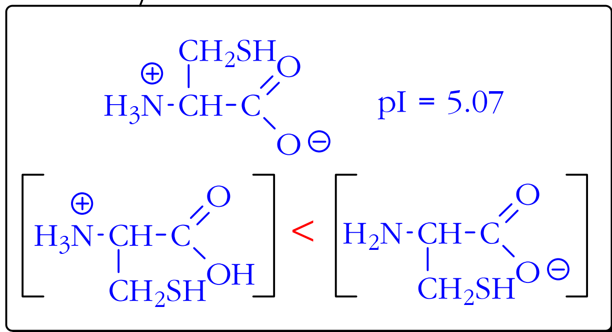
If the pH is decreased, [III] > [II] and there will be net migration toward the cathode.

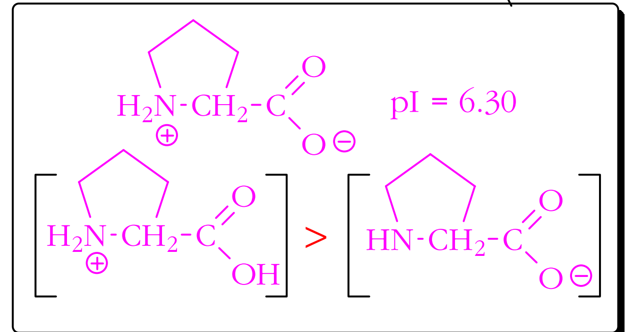
Amino acids with neutral R-groups are more acidic than basic. Therefore, the isoelectric point is acidic, *ie* pI < 7, for these compounds. For amino acids with a basic R, pI > 7. For amino acids with an acidic R, pI << 7.

Different amino acids have different isoelectric points. Consequently, they can be separated from each other by *electrophoresis*:


Gel or paper
pH (buffer) = 5.97
A N O D E
+
C A T H O D E
–

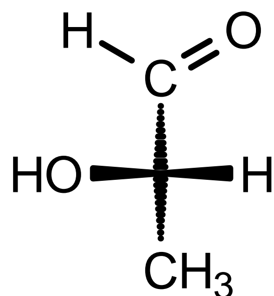

H3N-CH2-C(=O)O⊖
pI = 5.97
[H3N⊕-CH2-C(=O)OH] = [H2N-CH2-C(=O)O⊖]

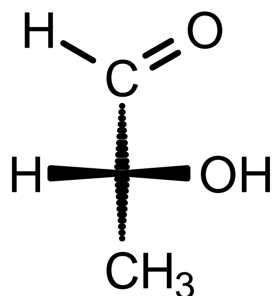

H3N⊕-CH(CH2SH)-C(=O)O⊖
pI = 5.07
[H3N⊕-CH(CH2SH)-C(=O)OH] < [H2N-CH(CH2SH)-C(=O)O⊖]


H2N⊕-CH2-C(=O)O⊖
pI = 6.30
[H2N⊕-CH2-C(=O)OH] > [HN-CH2-C(=O)O⊖]

Amino acids usually are least soluble in water at their isoelectric point.

Configuration of amino acids:

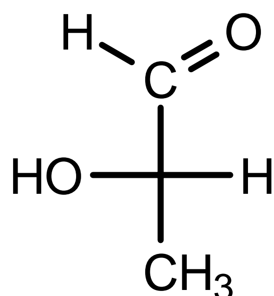


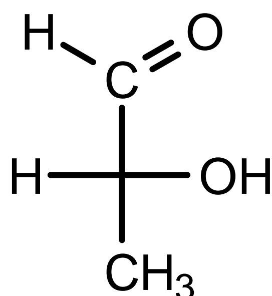


(S)-glyceraldehyde (R)-glyceraldehyde

L-glyceraldehyde D-glyceraldehyde

Fisher Projections:

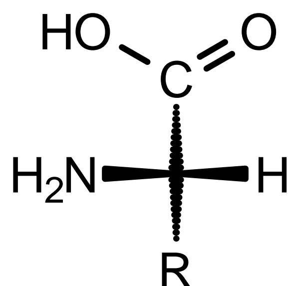


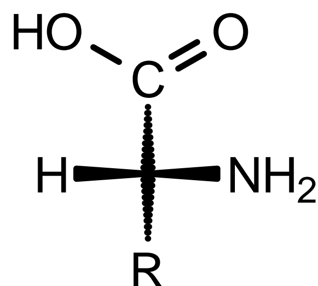


(S)-glyceraldehyde (R)-glyceraldehyde

L-glyceraldehyde D-glyceraldehyde

Amino Acids:





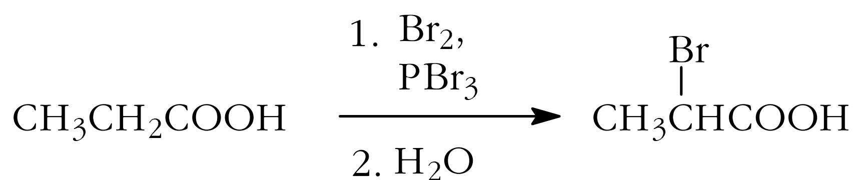
L-amino acid D-amino acid

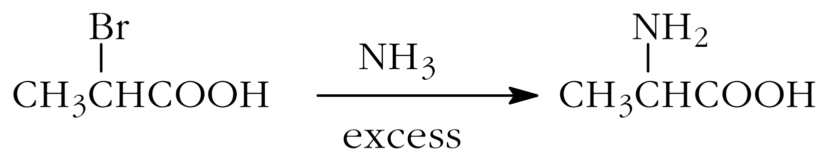
Almost all naturally occurring amino acids, except glycine, have the L-configuration.

Synthesis of Amino Acids in the Laboratory

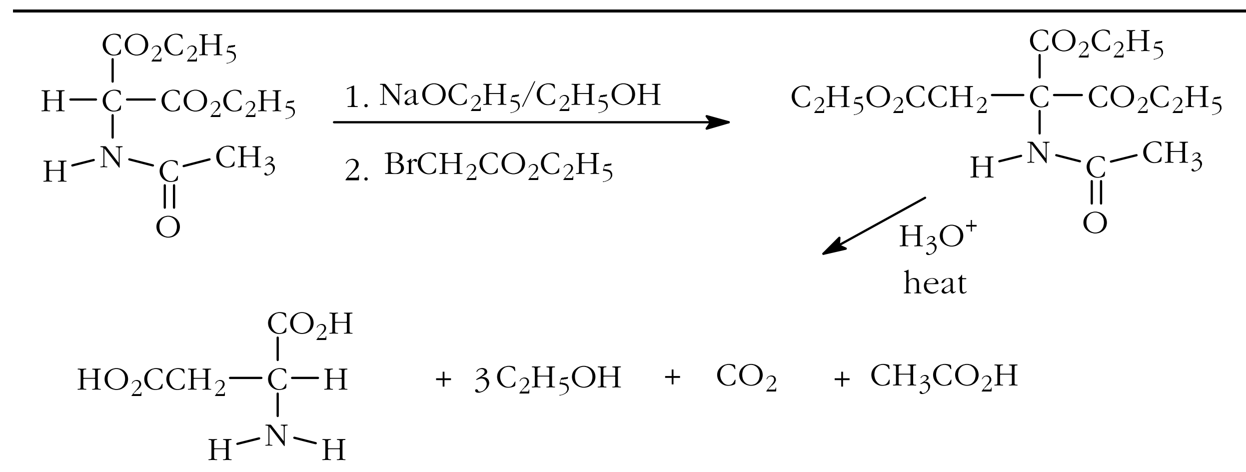
There are several ways to synthesize amino acids. Two of these ways follow. Note that these syntheses will lead to racemic mixtures (except for glycine).

Hell-Volhard-Zelinskii method —



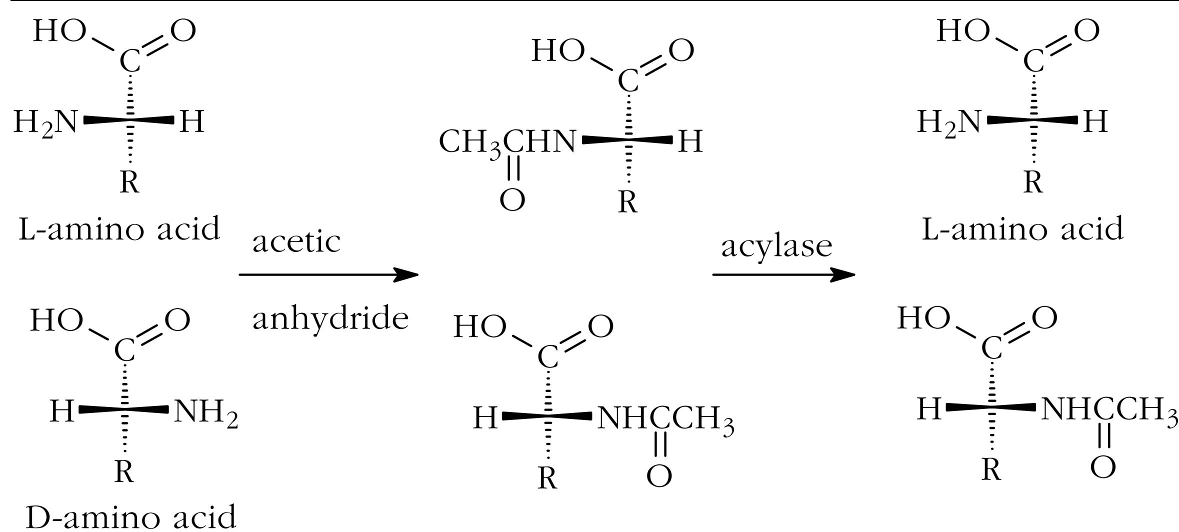


Amidomalonate method —
This method is closely related to the Malonic Ester Synthesis, but instead of using malonic ester diethyl acetamidomalonate is employed.



Since it is usually the L-amino acids that are of interest, the racemic amino acids synthesized above would have to be resolved. The classic method is to form diastereomeric salts. One could form the carboxylate salts of the racemic amino acids using enantiomerically pure alkaloids, eg (-)-brucine or (-)-strychnine, or one could form the ammonium salts using (+)-tartaric acid. In any case the salts are diasteriomeric and can be separated by ordinary means. Once separated, the amino acids can be regenerated from the salts as enantiomerically pure compounds.

An alternative method of resolution is to acetylate the amino group of the racemic mixture using acetic anhydride. The acetylated racemic mixture is then hydrolyzed using an enzyme (carboxypeptidase, hog kidney acylase) that will hydrolyze only one of the enantiomers (actually the L-isomer). [This is OK because the enzyme is chiral and enantiomerically pure.] The hydrolyzed amino acid can then be separated from the unhydrolyzed material by ordinary means. A generic example follows —



The above compounds are easily ;-) separated. Extraction with aqueous acid would likely be successful.