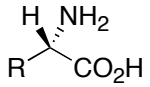


R = sidechain

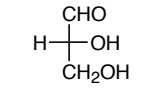
α- Amino Acid

20 common amino acids
- 19 are 1°-amines, 1 (proline) is a 2°-amine

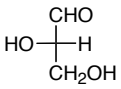
19 amino acids are "chiral"
- 1 (glycine) is achiral (R=H)

The configuration of the "natural" amino acids is L

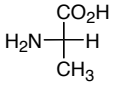
1



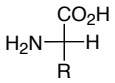
D-glyceraldehyde

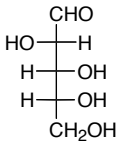


L-glyceraldehyde

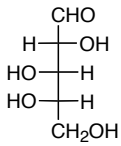


L-alanine

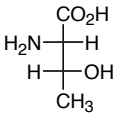




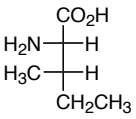
D-arabinose



L-arabinose



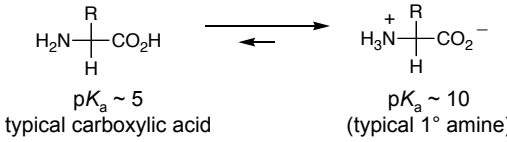
L-theronine
(2S,3R)



L-isoleucine
(2S,3S)

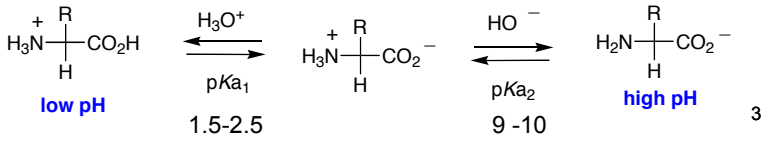
2

Amino acids exist as a zwitterion: a dipolar ion having both a formal positive and formal negative charge (overall charge neutral).



Amino acids are *amphoteric*: they can react as either an acid or a base. Ammonium ion acts as an acid, the carboxylate as a base.

Isoelectric point (pI): The pH at which the amino acid exists largely in a neutral, zwitterionic form (influenced by the nature of the sidechain)



3

Bronsted Acid: proton donor (H^+)

weak acids (and bases) do not fully dissociate

$$H\text{-}A \rightleftharpoons H^+ + A^-$$

$$K_a = \frac{[H^+]\,[A^-]}{[H\text{-}A]} \quad \textit{acid dissociation constant}$$

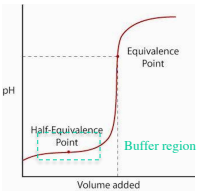
$$pK_a = -\log K_a$$

$$pH = -\log [H^+]$$

Henderson-Hasselbalch Equation: Relates pK_a with pH

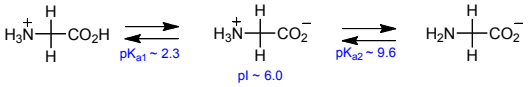
$$pH = pK_a + \log \frac{[A^-]}{[H\text{-}A]}$$

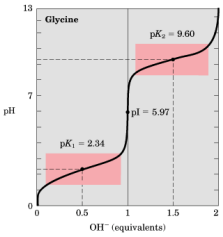
when $[A^-] = [H\text{-}A]$, the pH = pK_a

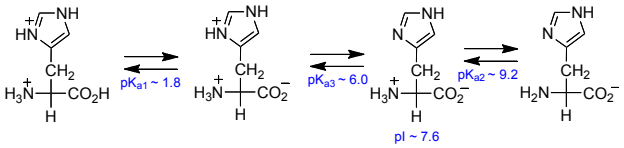


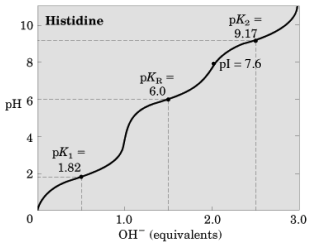
For a review see: http://themedicalbiochemistrypage.org/

4







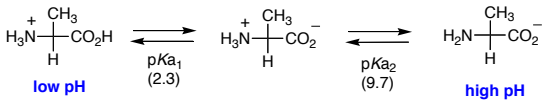


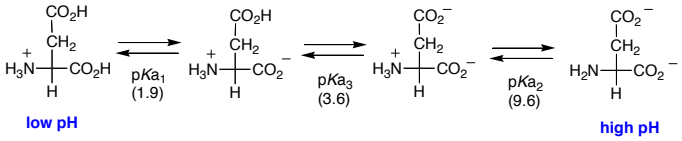
http://cti.itc.virginia.edu/~cmg/Demo/titr.html

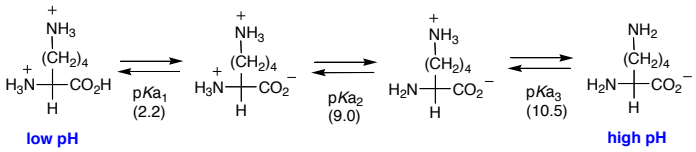
5

Isoelectric point (pI): The pH at which the amino acid exists largely in a neutral, zwitterionic form

$$pI = \frac{pKa_x + pKa_y}{2}$$



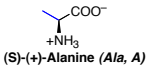


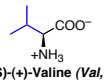


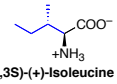
6

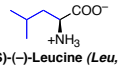
Amino acids are classified according to their sidechains

1. Hydrophobic:

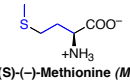

(S)-(+)-Alanine *(Ala, A)*

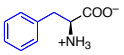

(S)-(+)-Valine *(Val, V)*

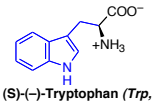

(2S,3S)-(+)-Isoleucine *(Ile, I)*


(S)-(−)-Leucine *(Leu, L)*


(S)-(−)-Proline *(Pro, P)*

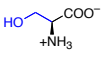

(S)-(−)-Methionine *(Met, M)*


(S)-(−)-Phenylalanine *(Phe, F)*

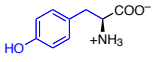

(S)-(−)-Tryptophan *(Trp, W)*

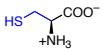
2. Uncharged polar groups

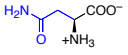

Glycine *(Gly, G)*

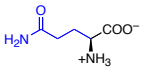

(S)-(−)-Serine *(Ser, S)*
pKa ~ 13


(2S,3R)-(−)-Threonine *(Thr, T)*
pKa ~ 13

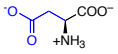

(S)-(−)-Tyrosine *(Tyr, Y)*
pKa ~ 10.5

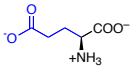

(R)-(−)-Cysteine *(Cys, C)*
pKa ~ 8.3


(S)-(−)-Asparagine *(Asn, N)*

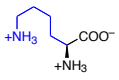

(S)-(+)-Glutamine *(Gln, Q)*

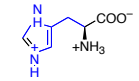
3. Acidic

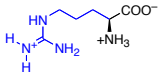

(S)-(+)-Aspartic Acid *(Asp, D)*
pKa ~ 3.9


(S)-(+)-Glutamic Acid *(Glu, E)*
pKa ~ 4.1

4. Basic


(S)-(+)-Lysine *(Lys, K)*
pKa ~ 10.5


(S)-(−)-Histidine *(His, H)*
pKa ~ 6.0

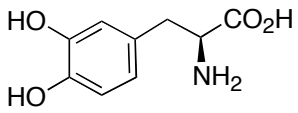

(S)-(+)-Arginine *(Arg, R)*
pKa ~ 12.5

Amino Acid Synthesis:

New unnatural amino acids with altered properties

new therapeutics (lead compounds)

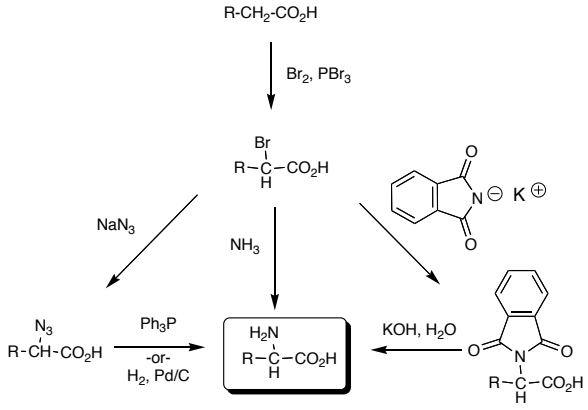
mechanistic probes



L-DOPA

9

Traditional Amino Acid Syntheses:



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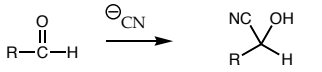
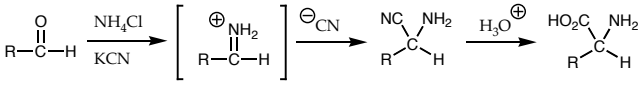
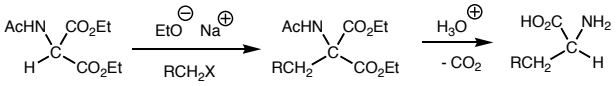
Strecker Synthesis



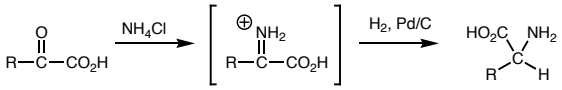
cyanohydrin



Amidomalonate Synthesis



Reductive Amination



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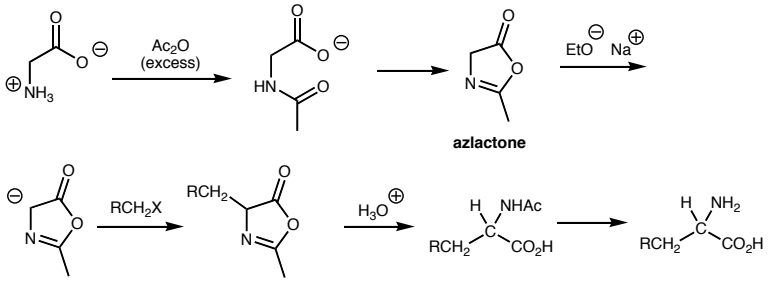
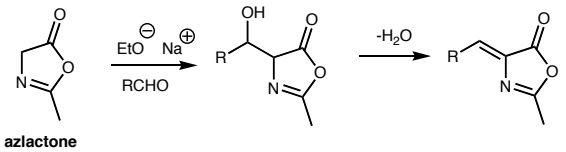
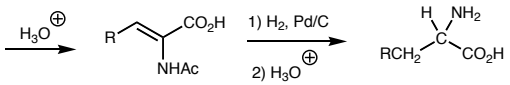
Azlactone Synthesis



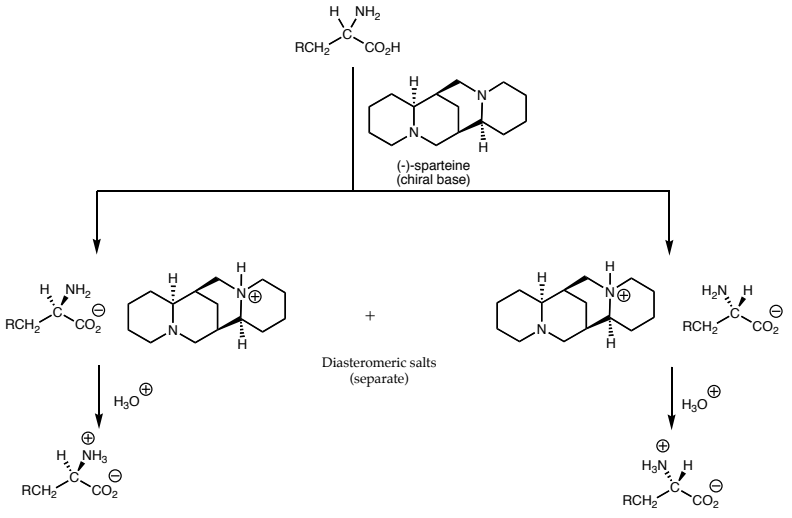




12

These are racemic syntheses!!

Resolution: separation of enantiomers



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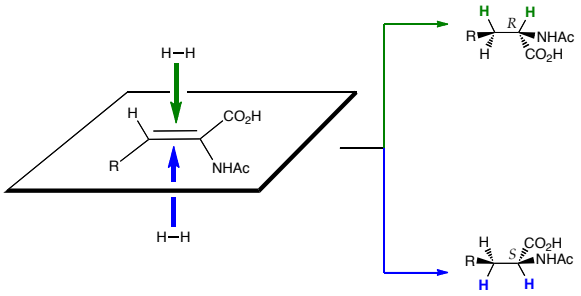
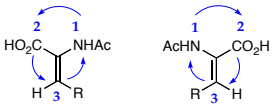
Asymmetric Synthesis of Amino Acids



pro-chiral

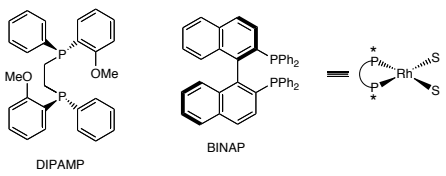


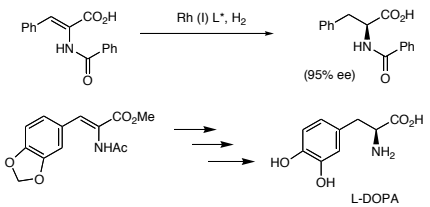
pro S-face (si)

pro R-face (re)

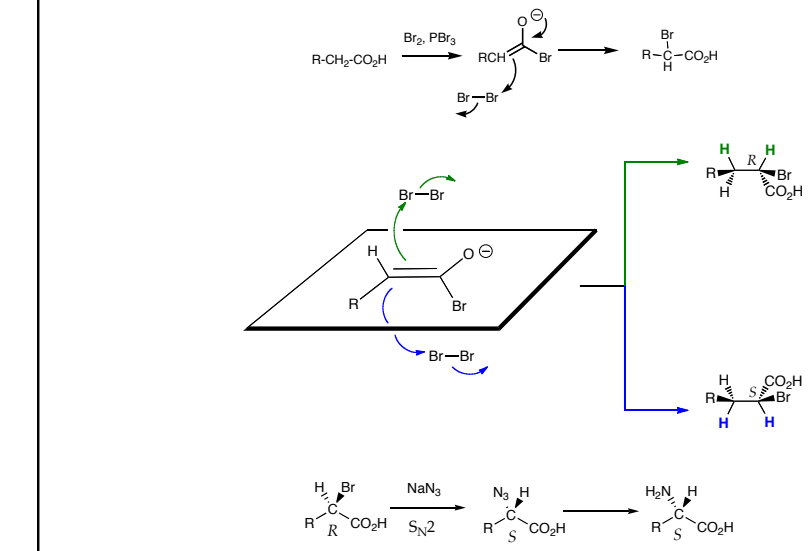
H_2, Pd/C - heterogeneous catalysis

H_2, $(Ph_3P)_3RhCl$ (Wilkinson's catalyst) - homogeneous catalysis





14



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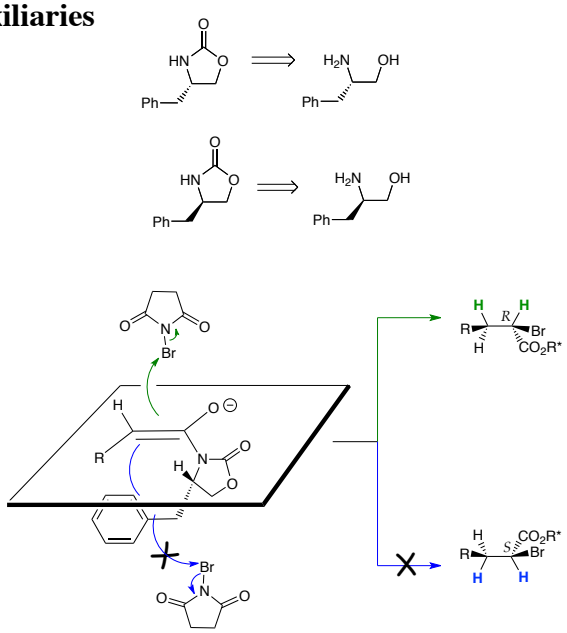
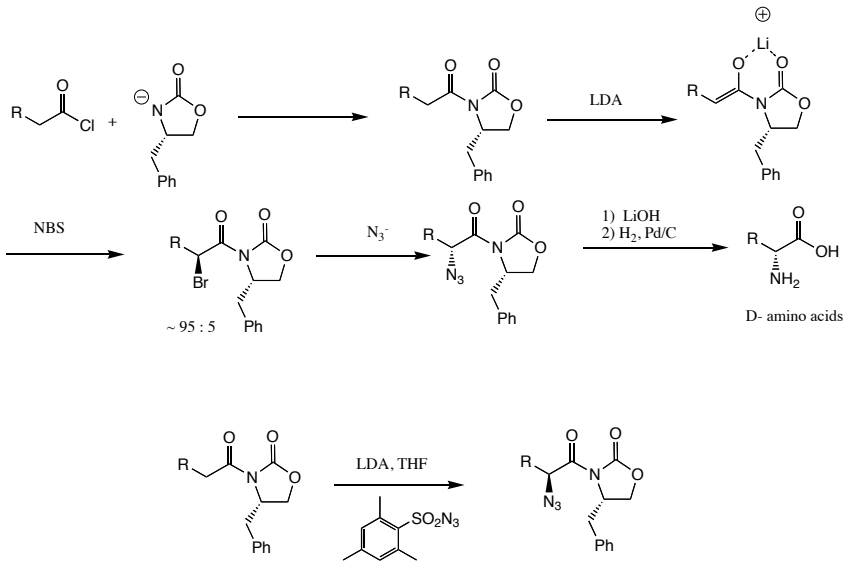
Chiral Auxiliaries

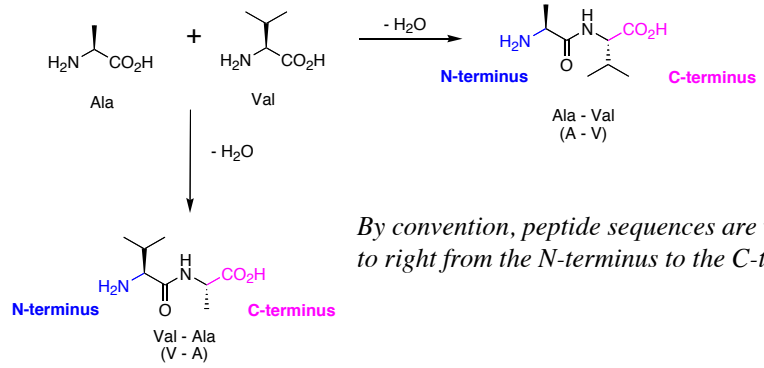


16

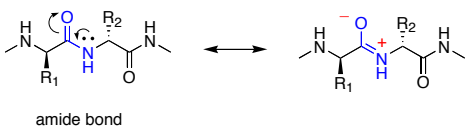


17

Peptides



By convention, peptide sequences are written left to right from the N-terminus to the C-terminus

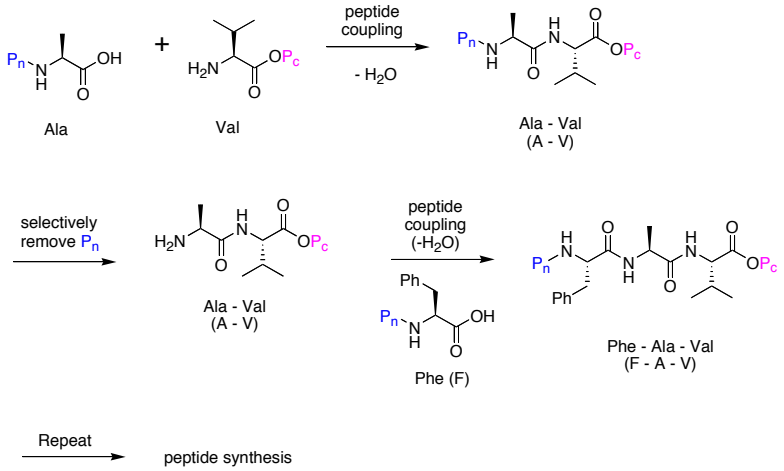


C=N double bond character due to this resonance structure

restricts rotations
resistant to hydrolysis

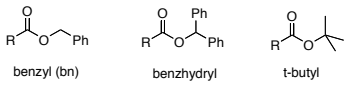
18

Peptide Coupling: need for protecting groups

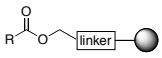


19

C-protecting groups (Lloyd-Williams et al., p. 11)

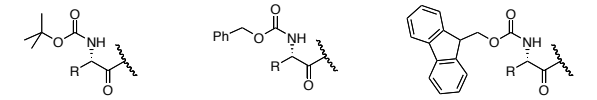


removed with mild acid



insoluble solid support (resin)

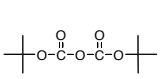
N-protecting groups (Lloyd-Williams et al., p. 10)



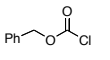
tert-butylcarbamoyl (t-BOC)

benzyloxycarbamoyl (cBz)

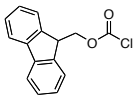
fluorenylmethylcarbamoyl (FMOC)



removed with mild acid



removed with mild acid or by hydrogenolysis



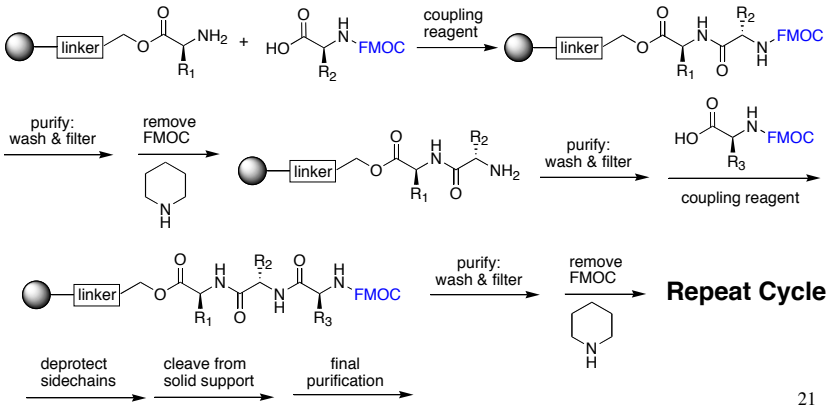
removed with mild base (piperidine)

20

Solid-Phase Peptide Synthesis (SPPS)

(Lloyd-Williams et al., Chapter 2, pp. 19-82)

- peptides up to ~ 100 amino acids can be synthesized in a laboratory
- laboratory synthesis is from the C-terminus to the N-terminus
- nature synthesizes peptides from N to C.



21

Mechanism of Peptide Coupling (Lloyd-Williams et al., p. 48)

22

Mechanism of stereochemical scrambling

Additives can suppress the scrambling (Lloyd-Williams et al., pp. 120-121)

Peptide coupling reagent (one-pot):

N-protected carboxylic acid, C-protected amine

DCC, HOBT

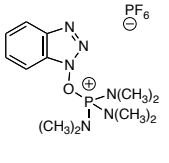
23

Newer coupling reagents:

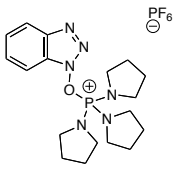
(Lloyd-Williams et al., p. 53-55)

phosphonium salts

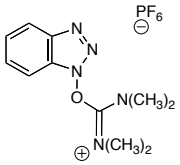
uronium salts:
(salts of urea, not uranium)



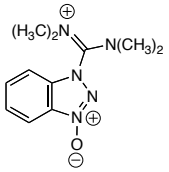
BOP



PyBOP



HATU

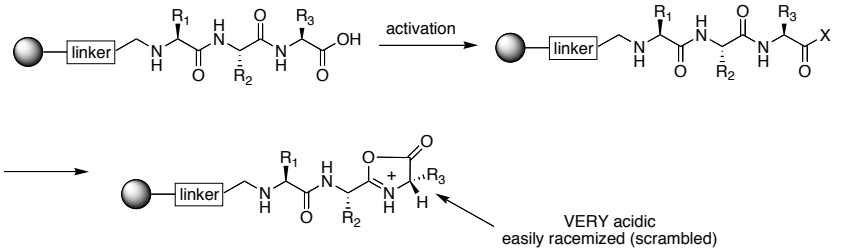


actual structure

24

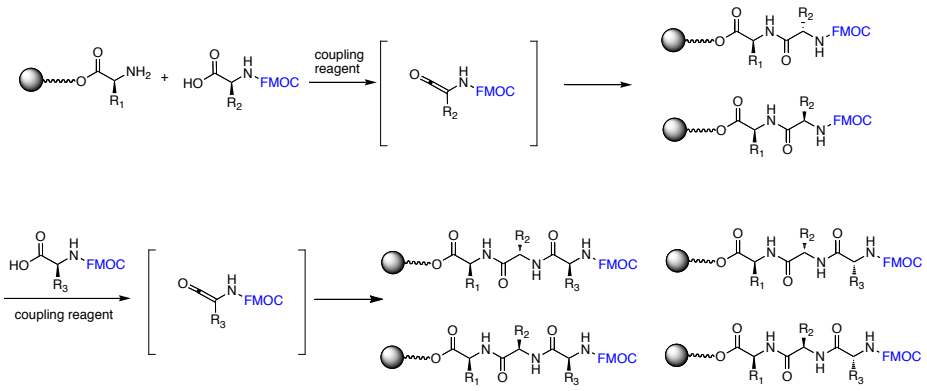
Why not N to C peptide synthesis?

(Lloyd-Williams et al. pp. 116-119)



25

Importance of maintaining stereochemical integrity during the coupling step:

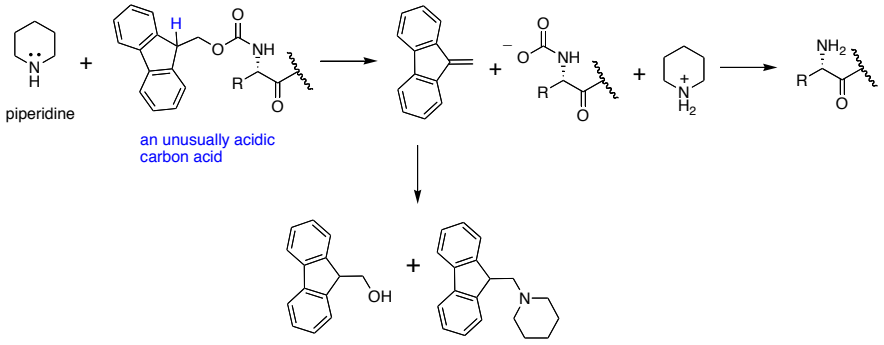


Number of possible stereoisomers = 2^n where n= # of chiral centers

A peptide w/ 10 AA residues has 2^{10} possible stereoisomers

26

Standard α-amino protecting group is FMOC
removed (deprotected) with base (piperidine)

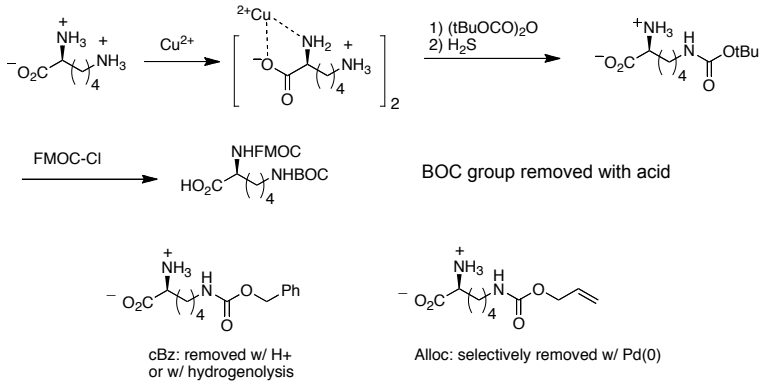


Orthogonal Protection Strategy: if the α-amino group has a base-labile protecting group, then the C-terminus and the side chains require base-stable protecting groups

27

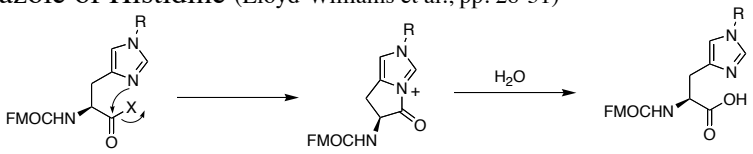
Sidechain Protecting Groups: (Lloyd-Williams et al., pp. 23-39)
for nitrogen sidechain functional groups

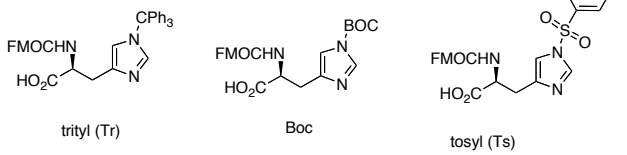
• NH_2 of lysine (Lloyd-Williams et al., pp. 24-26)



28

• Imidazole of Histidine (Lloyd-Williams et al., pp. 28-31)



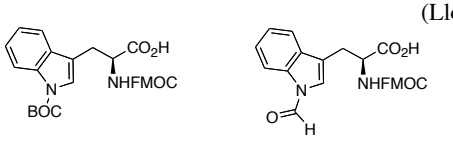


trityl (Tr) Boc tosyl (Ts)

stable to mild base, removed with acid

• Indole nitrogen of tryptophan (often not protected)

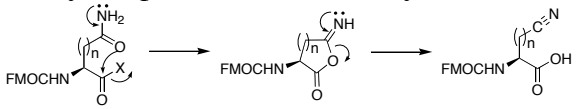
(Lloyd-Williams et al., p. 31)



stable to mild base
removed with acid

29

• Amides of asparagine and glutamine (Lloyd-Williams et al., pp. 32-33)
usually not protected- could dehydrate to -C≡N

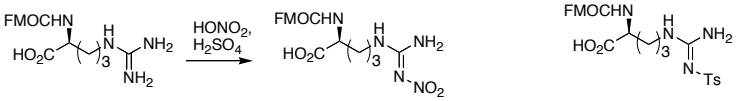


Benzyl (Bn) Trityl (Tr)

stable to mild base,
removed with acid

• Guanidine group of arginine- often not protected

(Lloyd-Williams et al., pp. 26-28)



removed with hydrogenolysis
or with Zn(0) in acetic acid

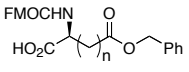
removed with HF

30

for oxygen sidechain functional groups

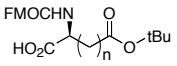
• carboxylate groups of aspartate and glutamate

(Lloyd-Williams et al. pp. 33-35)



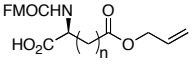
Benzyl (Bn)

removed with acid or hydrogenolysis



t-butyl

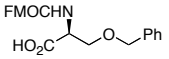
removed with acid



allyl

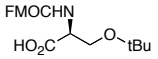
removed with Pd(0)

• alcohols of serine, threonine and tyrosine (Lloyd-Williams et al. pp. 35-35)



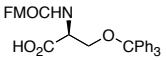
Benzyl (Bn)

removed with acid or hydrogenolysis



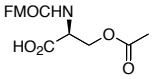
t-butyl (tBu)

removed with acid



trityl (Tr)

removed with acid

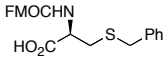


acetate (Ac)

removed with acid

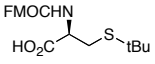
31

Sulfur of Cysteine (Lloyd-Williams et al. pp. 39-40)



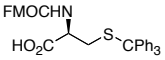
Benzyl (Bn)

removed with acid



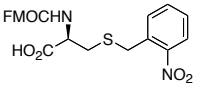
t-butyl (tBu)

removed with acid

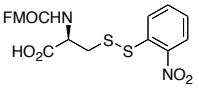


trityl (Tr)

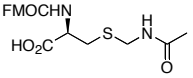
removed with acid



o-nitrobenzyl removed photochemically



removed with Ph_3P or with $HOCH_2CH_2SH$

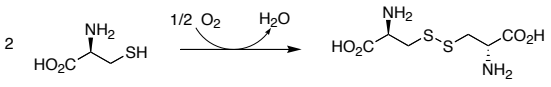


stable to acid

removed with HO^- or Hg(II)

Disulfides of cysteine (cystine) redox active amino acid side chain

(Lloyd-Williams et al., Chapter 5, pp. 209-236)



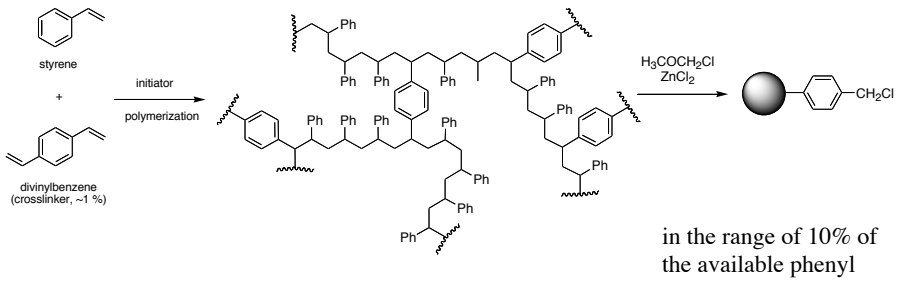
32

Solid-Phase Peptide Synthesis: The solid support (resin, bead, etc.)

(Lloyd-Williams et al., pp. 19-21, 41-46)

Merrifield Resin: R. Bruce Merrifield, Rockefeller University, 1984 Nobel Prize in Chemistry:

for his development of methodology for chemical synthesis on a solid matrix.



in the range of 10% of the available phenyl groups are functionalized

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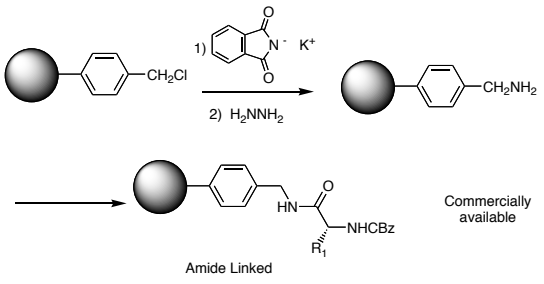
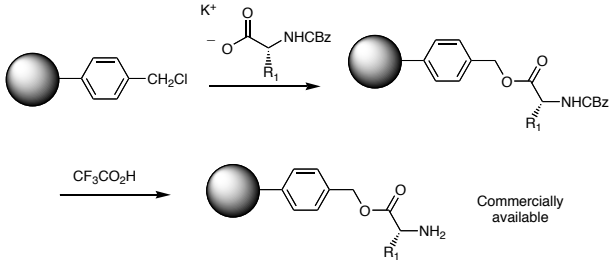
Amide-linked resins

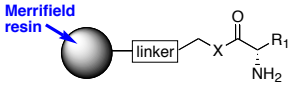


Ester-linked Resins



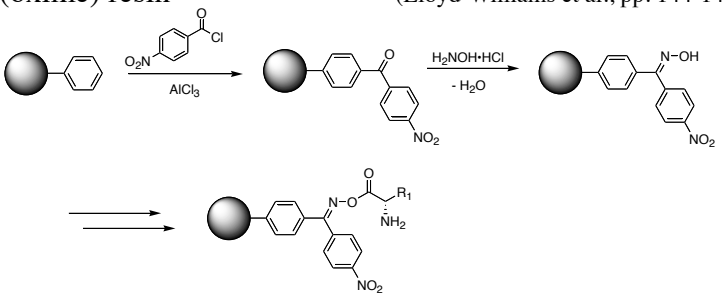
34

Other Resins:



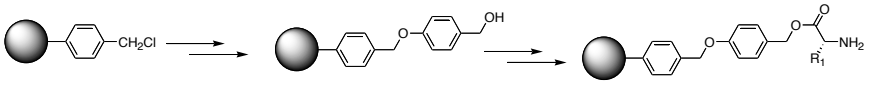
Kaiser (oxime) resin

(Lloyd-Williams et al., pp. 144-145)



Wang Resin

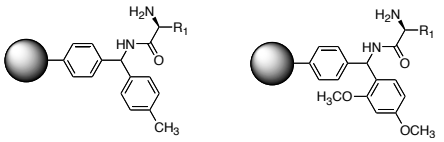
(Lloyd-Williams et al., pp. 143-144)



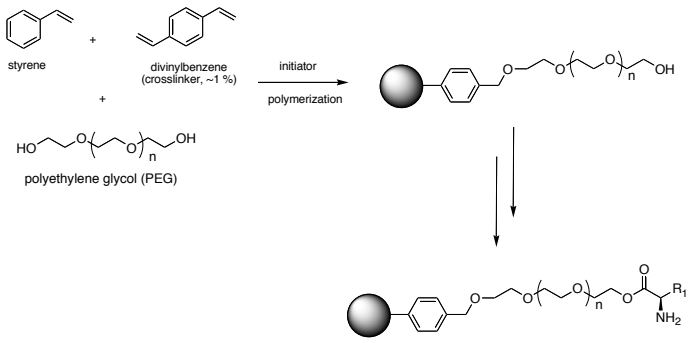
35

Rink (amide) resin

(Lloyd-Williams et al., pp. 45-46)



Tenta gel



Solublizes the synthetic peptide
Particularly good for the synthesis of long peptides

36

Deprotection of the peptide (Lloyd-Williams et al., pp. 71-75)

sidechain protecting groups

cleavage from the solid support

Acid hydrolysis: CF_3CO_2H, HF

anisole or p-cresol is added as an alkylation scavenger

Purification

High performance liquid chromatography (HPLC)

electrophoresis

Analysis

mass spectrometry

37

Ribonuclease A- 124 amino acids

catalyzes the hydrolysis of RNA

Solid-phase synthesis of RNase A:

B. Gutte & R. B. Merrifield, *J. Am. Chem. Soc.* **1969**, *91*, 501-2.

Synthetic RNase A: 78 % activity

0.4 mg was synthesized

2.9 % overall yield

average yield ~ 97% per coupling

```
LYS GLU THR ALA ALA ALA LYS PHE GLU ARG
GLN HIS MET ASP SER SER THR SER ALA ALA
SER SER SER ASN TYR CYS ASN GLN MET MET
LYS SER ARG ASN LEU THR LYS ASP ARG CYS
LYS PRO VAL ASN THR PHE VAL HIS GLU SER
LEU ALA ASP VAL GLN ALA VAL CYS SER GLN
LYS ASN VAL ALA CYS LYS ASN GLY GLN THR
ASN CYS TYR GLN SER TYR SER THR MET SER
ILE THR ASP CYS ARG GLU THR GLY SER SER
LYS TYR PRO ASN CYS ALA TYR LYS THR THR
GLN ALA ASN LYS HIS ILE ILE VAL ALA CYS
GLU GLY ASN PRO TYR VAL PRO VAL HIS PHE
ASP ALA SER VAL
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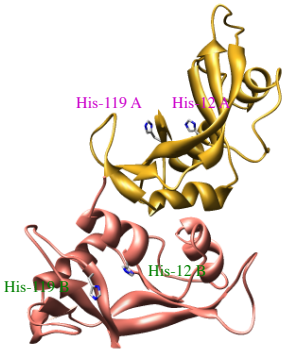

pdb code: 1AFL

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Linear vs. Convergent Synthesis

SPPS- linear synthesis of peptides, many steps, low overall yield, inefficient for long peptides and proteins

Convergent Synthesis (segmental coupling strategy)- make short peptides by SPPS then couple the short peptides, in solution, to give longer ones. Less linear steps and higher overall yield if the segmental coupling is efficient.

(Lloyd-Williams et al., Chapter 3, pp. 95-137, Chapter 4, pp.139-207)

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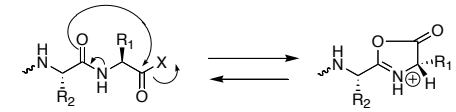
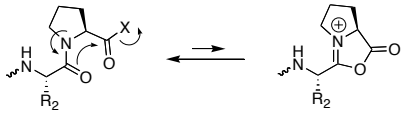
Must activate the C-terminus of a peptide segment
recall there are problems with the N to C peptide synthesis

(Lloyd-Williams et al., pp. 116-120)



Scrambling of stereochemistry

couple at glycine,
R_1=H, no stereochemistry



couple at proline- unstable azlactone, little scrambling of stereochemistry

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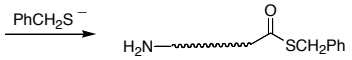
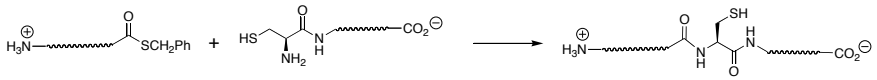
Couple at cysteine (Kent, Tam) (Lloyd-Williams et al., pp. 190-195)
Native peptide ligation



Thioester: a less reactive activated acid



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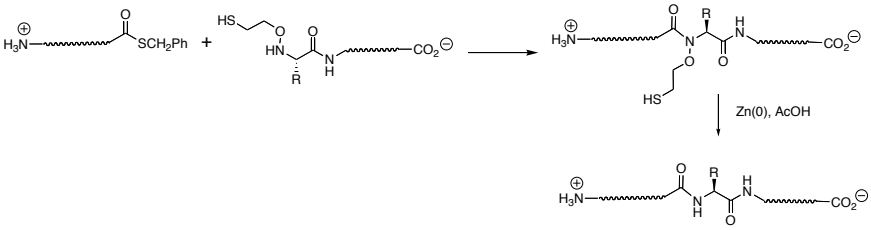
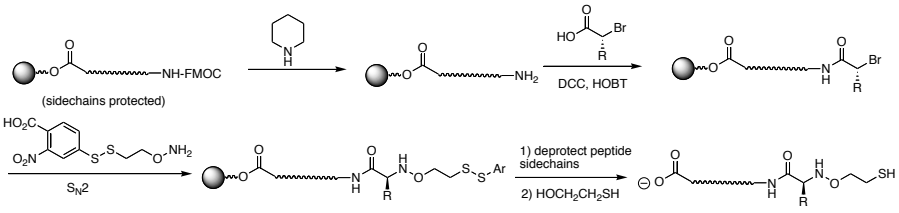
More general peptide ligation strategy





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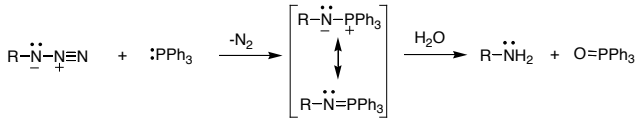
Staudinger reaction



Staudinger Ligation:

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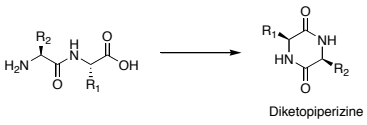
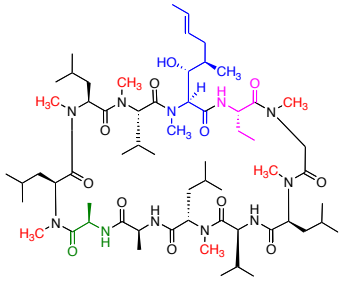
Cyclic Peptides





Cyclosporin A

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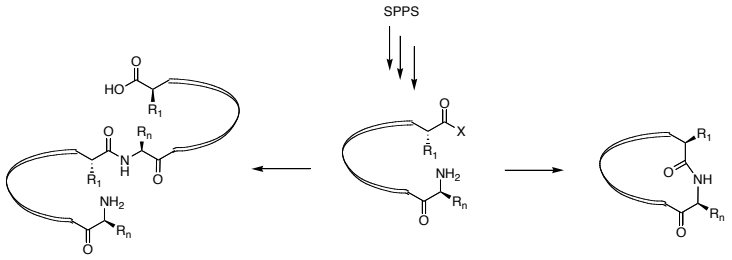
Cyclic Peptide Synthesis

Problems with the solution-phase cyclization reaction

- stereochemical scrambling
  - use acyl azide, acyl thioester, HATU or PyBop in the coupling reaction
- dimerization
  - high dilution conditions favors cyclization



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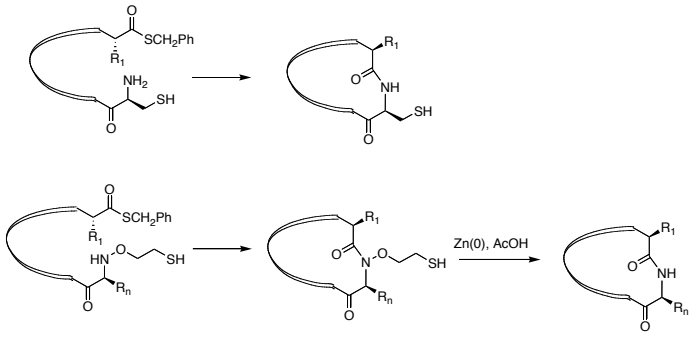
Intramolecular Native Peptide Ligation Strategy



Solves the stereochemical scrambling problem but not the dimer formation issue

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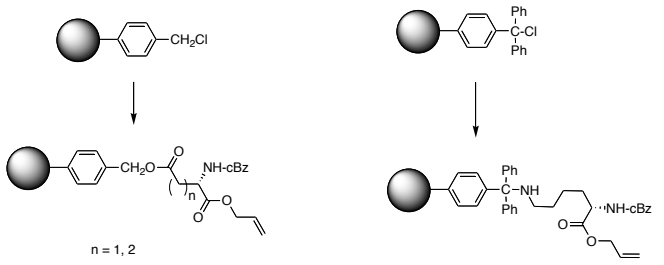
Cyclization on the solid support will solve the dimerization problem

Attached the first amino acid through the side chain

applicable for Asp, Glu and Lys

Requires a carboxylate protecting group that is removed under conditions other than acid or base → Allyl



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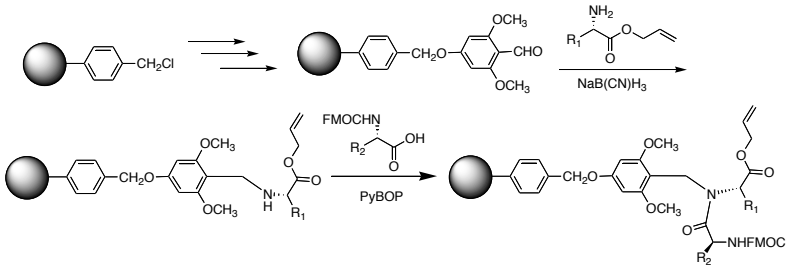
Attached the first amino acid to the solid support via the α-amino group



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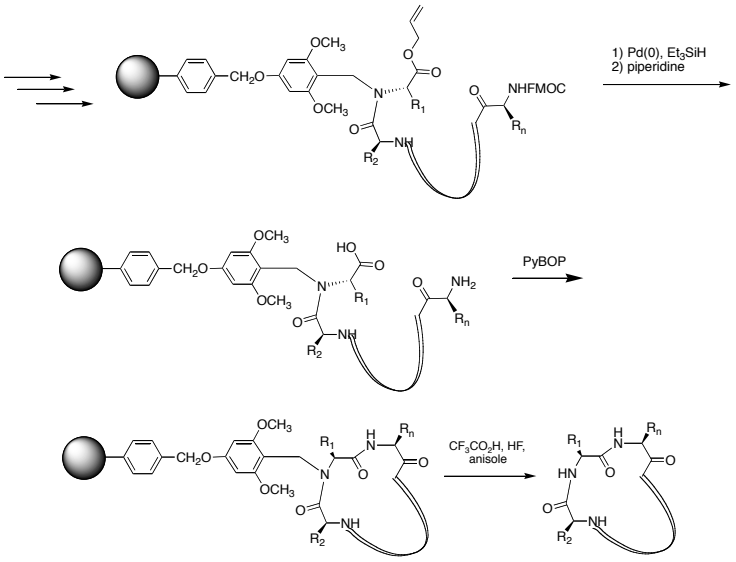
On-suppport cyclization



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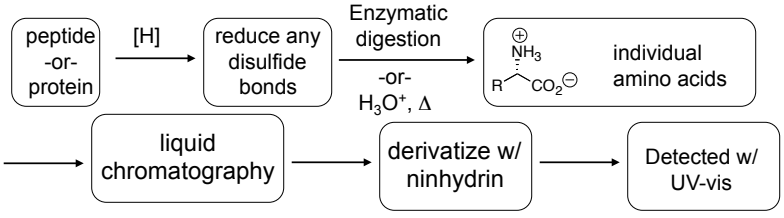
Peptide and Protein Analysis

Primary (1°) structure of a peptide or protein is the amino acid sequence

Amino acid analyzer- automated instrument to determine the amino acid content of a peptide or protein. Individual amino acids are separated by hplc, then detected by post-column derivatization



Different amino acids have different chromatographic mobilities (retention times)

1972 Nobel Prize in Chemistry
William Stein
Stanford Moore

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