

\[
\begin{align*}
    & \overset{\text{NH}_2}{R} \overset{\text{CO}_2\text{H}}{\text{R}} = \text{sidechain} \\
\end{align*}
\]

\text{\(\alpha\)-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
Dipolar Structure

Zwitterion:

\[
\begin{align*}
  &\text{H}_2\text{N} - \text{CO}_2\text{H} \\
  &\text{H}_3\text{N} - \text{CO}_2^- \\
  &\text{H}_2\text{N} -> \text{CO}_2^-
\end{align*}
\]

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

Amino acids are classified according to their sidechains

1. Hydrophobic:

   \[
   \begin{align*}
   &\text{(S)-(+)-Alanine (Ala, A)} \\
   &\text{(S)-(+)-Valine (Val, V)} \\
   &\text{(S)-(-)-Leucine (Leu, L)} \\
   &\text{(S)-(-)-Metionine (Met, M)} \\
   &\text{(S)-(-)-Phenylalanine (Phe, F)} \\
   &\text{(S)-(-)-Tryptophan (Trp, W)}
   \end{align*}
   \]

2. Uncharged polar groups

   \[
   \begin{align*}
   &\text{(R)-(-)-Cysteine (Cys, C)} \\ &\text{(S)-(-)-Asparagine (Asn, N)} \\
   &\text{(S)-(-)-Glutamine (Gln, Q)} \\
   \end{align*}
   \]

\( \text{pKa} ~ 13 \)
\( \text{pKa} ~ 13 \)
\( \text{pKa} ~ 10.5 \)
\( \text{pKa} ~ 8.3 \)
3. Acidic

\[\text{(S)-(+) Aspartic Acid (Asp, D) \quad pK_a \approx 3.9}\]
\[\text{(S)-(+) Glutamic Acid (Glu, E) \quad pK_a \approx 4.1}\]

4. Basic

\[\text{(S)-(+) Lysine (Lys, K) \quad pK_a \approx 10.5}\]
\[\text{(S)-(+) Histidine (His, H) \quad pK_a \approx 6.0}\]
\[\text{(S)-(+) Arginine (Arg, R) \quad pK_a \approx 12.5}\]

**Amino Acid Synthesis:**
New unnatural amino acids with altered properties
new therapeutics (lead compounds)
mechanistic probes

\[\text{L-DOPA}\]
Traditional Amino Acid Syntheses:

Strecker Synthesis

Amidomalonate Synthesis

Reductive Amination
Azlactone Synthesis

These are racemic syntheses!!

Resolution: separation of enantiomers
Asymmetric Synthesis of Amino Acids

\[ \text{pro-chiral} \]

\[ \text{pro S-face} \quad \text{pro R-face} \]

\[ \text{H}_2, \text{Pd/C - heterogeneous catalysis} \]

\[ \text{H}_2, (\text{Ph}_3\text{P})_3\text{RhCl (Wilkinson’s catalyst)} - \text{homogeneous catalysis} \]

\[ \text{R} \quad \text{C} \quad \text{O} \quad \text{2} \quad \text{H} \]

\[ \text{B} \quad \text{r} \quad \text{2}, \text{PBr}_3 \]

\[ \text{RCH} \quad \text{Br} \]

\[ \text{O} \]

\[ \text{Br} \quad \text{Br} \]

\[ \text{R} \quad \text{Br} \]

\[ \text{H} \quad \text{H} \]

\[ \text{H} \quad \text{CO}_2\text{H} \]

\[ \text{R} \quad \text{Br} \]

\[ \text{H} \quad \text{H} \]

\[ \text{H} \quad \text{CO}_2\text{H} \]

\[ \text{R} \quad \text{Br} \]

\[ \text{S} \quad \text{S} \]

\[ \text{R} \quad \text{C} \quad \text{O} \quad \text{2} \quad \text{H} \]

\[ \text{B} \quad \text{r} \quad \text{B} \quad \text{r} \quad \text{R} \quad \text{C} \]

\[ \text{O} \]

\[ \text{Br} \quad \text{Br} \]

\[ \text{R} \quad \text{C} \]

\[ \text{O} \quad \text{2} \quad \text{H} \]

\[ \text{B} \quad \text{r} \quad \text{B} \quad \text{r} \quad \text{R} \quad \text{C} \]

\[ \text{O} \]

\[ \text{Br} \quad \text{Br} \]

\[ \text{R} \quad \text{C} \]

\[ \text{O} \quad \text{2} \quad \text{H} \]
Chiral Auxiliaries

- **Acid Chloride** + Ph

- **NBS**

- **N₂**

- **LiOH**

- **H₂, Pd/C**

- **LDA**

- **THF**

- **SO₂N₂**

- **LDA, THF**

- **D- amino acids**
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.

Peptide Coupling: need for protecting groups

selectively remove $P_n$ peptide synthesis

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

- benzyl (Bn)
- benzhydryl
- t-butyl

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)

Solid-Phase Peptide Synthesis (SPPS)

(Lloyd-Williams et al., Chapter 2, pp. 19-92)

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

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
Newer coupling reagents:
(Lloyd-Williams et al., p. 53-55)

- Phosphonium salts
- Uronium salts:
  - (salts of urea, not uranium)

![Phosphonium salts and Uronium salts](image)

Why not N to C peptide synthesis?
(Lloyd-Williams et al. pp. 116-119)

![Peptide synthesis](image)

VERY acidic easily racemized (scrambled)
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

Standard $\alpha$-amino protecting group is FMOC
removed (deprotected) with base (piperidine)

Orthogonal Protection Strategy: if the $\alpha$-amino group has a base-labile protecting group, then the C-terminus and the side chains require base-stable protecting groups
**Sidechain Protecting Groups:** (Lloyd-Williams et al., pp. 23-39) for nitrogen sidechain functional groups

- **NH₂ of lysine** (Lloyd-Williams et al., pp. 24-26)

  \[
  \text{Cu}^{2+} \quad 1) (\text{tBuOCO})_2\text{O} \quad 2) \text{H}_2\text{S} \quad \text{BOC group removed with acid}
  \]

  - cBu: removed w/ H+ or w/ hydrogenolysis
  - Alloc: selectively removed w/ Pd(0)

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

  \[
  \text{H}_2\text{O} \quad \text{stable to mild base, removed with acid}
  \]

  - Trityl (Tr)
  - Boc
  - Tosyl (Ts)

- **Indole nitrogen of tryptophan** (often not protected)

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

  \[
  \text{stable to mild base, removed with acid}
  \]
• Amides of asparagine and glutamine (Lloyd-Williams et al., pp. 32-33) usually not protected—could dehydrate to -C≡N

• Guanidine group of arginine—often not protected (Lloyd-Williams et al., pp. 26-28)

for oxygen sidechain functional groups
• carboxylate groups of aspartate and glutamate (Lloyd-Williams et al. pp. 33-35)

• alcohols of serine, threonine and tyrosine (Lloyd-Williams et al. pp. 35-35)
Sulfur of Cysteine (Lloyd-Williams et al. pp. 39-40)

Disulfides of cysteine (cystine) redox active amino acid side chain
(Lloyd-Williams et al., Chapter 5, pp. 209-236)

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.
Amide-linked resins

![Diagram of Amide-linked resins]

Ester-linked Resins

![Diagram of Ester-linked resins]

Other Resins:

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

- Wang Resin
  (Lloyd-Williams et al., pp. 143-144)
Rink (amide) resin (Lloyd-Williams et al., pp. 45-46)

Tanta gel

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

Deprotection of the peptide (Lloyd-Williams et al., pp. 71-75)
sidechain protecting groups
cleavage from the solid support

Acid hydrolysis: $\text{CF}_3\text{CO}_2\text{H}, \text{HF}$
anisole or p-cresol is added as an alkylation scavenger

Purification
High performance liquid chromatography (HPLC)
electrophoresis

Analysis
mass spectrometry
Ribonuclease A - 124 amino acids catalyzes the hydrolysis of RNA
Solid-phase synthesis of RNase A:

Synthetic RNase A: 78 % activity
0.4 mg was synthesized
2.9 % overall yield
average yield ~ 97% per coupling

```
LYS GLU THR 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
```

pdb code: 1AFL

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)
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

Couple at cysteine (Kent, Tam)
Native peptide ligation

(Lloyd-Williams et al., pp. 190-195)

Thioester: a less reactive
activated acid
More general peptide ligation strategy

Staudinger reaction

\[
\text{Staudinger Ligation:} \\
\text{R-} \text{N}_2 \text{N} + \text{PPh}_3 \xrightarrow{\Delta} \text{R-N-PPh}_3 + \text{N}_2 \xrightarrow{\text{H}_2\text{O}} \text{R-NH}_2 + \text{O=PPh}_3
\]
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
Intramolecular Native Peptide Ligation Strategy

Solves the stereochemical scrambling problem but not the dimer formation issue

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

44
Attached the first amino acid to the solid support via the α-amino group

On-support cyclization