27.14
The Strategy of Peptide Synthesis
General Considerations

Making peptide bonds between amino acids is not difficult.

The challenge is connecting amino acids in the correct sequence.

Random peptide bond formation in a mixture of phenylalanine and glycine, for example, will give four dipeptides.

Phe—Phe  Gly—Gly  Phe—Gly  Gly—Phe
General Strategy

1. Limit the number of possibilities by "protecting" the nitrogen of one amino acid and the carboxyl group of the other.

N-Protected phenylalanine

C-Protected glycine
2. Couple the two protected amino acids.
3. Deprotect the amino group at the N-terminus and the carboxyl group at the C-terminus.

\[
\begin{align*}
&\text{X--NHCHC--NHCH}_2\text{C--Y} \\
&\text{CH}_2\text{C}_6\text{H}_5 \\
&\downarrow \\
&\text{H}_3\text{NCHC--NHCH}_2\text{CO} \\
&\text{CH}_2\text{C}_6\text{H}_5 \\
&\text{Phe-Gly}
\end{align*}
\]
27.15
Amino Group Protection
Amino groups are normally protected by converting them to amides.

Benzylloxycarbonyl (C₆H₅CH₂O—) is a common protecting group. It is abbreviated as Z.

Z-protection is carried out by treating an amino acid with benzylloxycarbonyl chloride.
Protect Amino Groups as Amides

CH₂OCCl + H₃N⁺CHCO⁻

1. NaOH, H₂O
2. H⁺

CH₂OC—NHCHCOH

(82-87%)
Protect Amino Groups as Amides

is abbreviated as: ZNHCHCOH or Z-Phe

CH₂C₆H₅
An advantage of the benzyloxy carbonyl protecting group is that it is easily removed by:

a) hydrogenolysis

b) cleavage with HBr in acetic acid
Hydrogenolysis of Z-Protecting Group

\[ \text{Reactions}
\]

- **Before Hydrogenolysis:**
  - Initial structure with protecting group and functional groups.

- **After Hydrogenolysis:**
  - Functional groups are reduced, revealing the underlying structure.

**Equation:**

\[ \text{Product} \quad \% \]

\[ \text{H}_2, \text{Pd} \]

**Diagram:**

- Initial structure with protecting groups.
- Functional groups are indicated with specific bonds and atoms.
- Bond formation and breaking indicated by arrows.
- Reaction conditions noted.
HBr Cleavage of Z-Protecting Group

\[
\text{HBr} + \text{NHCHCHCNHCH}_2\text{CO}_2\text{CH}_2\text{CH}_3 \rightarrow \text{CH}_2\text{Br} + \text{CO}_2 + \text{H}_3\text{N}\text{CHCNHCH}_2\text{CO}_2\text{CH}_2\text{CH}_3 \text{Br}^- \quad (82\%) 
\]
The tert-Butoxycarbonyl Protecting Group

is abbreviated as: BocNHCHCOH or Boc-Phe
**HBr Cleavage of Boc-Protecting Group**

\[
\text{O} \quad \text{(CH}_3\text{)}_3\text{CO} \quad \text{-NHCHCNHCH}_2\text{CO}_2\text{CH}_2\text{CH}_3 \\
\text{CH}_2\text{C}_6\text{H}_5 \\
\downarrow \text{HBr} \\
\text{H}_3\text{C} \quad \text{C} \equiv \text{CH}_2 \quad \text{CO}_2 \quad \text{H}_3\text{NCHCNHCH}_2\text{CO}_2\text{CH}_2\text{CH}_3 \\
\text{CH}_2\text{C}_6\text{H}_5 \quad \text{Br}^- \quad (86\%) 
\]
27.16
Carboxyl Group Protection
Carboxyl groups are normally protected as esters.

Deprotection of methyl and ethyl esters is by hydrolysis in base.

Benzyl esters can be cleaved by hydrogenolysis.
Hydrogenolysis of Benzyl Esters

\[
\text{C}_6\text{H}_5\text{CH}_2\text{OC} \text{NHCHCNHNHCH}_2\text{COCH}_2\text{C}_6\text{H}_5 \\
\text{CH}_2\text{C}_6\text{H}_5 \\
\downarrow \text{H}_2, \text{Pd} \\
\text{C}_6\text{H}_5\text{CH}_3 \quad \text{CO}_2 \quad \text{H}_3\text{NCHCNHNHCH}_2\text{CO}^- \quad \text{CH}_3\text{C}_6\text{H}_5 \\
\text{CH}_2\text{C}_6\text{H}_5 \quad \text{(87%)}
\]
27.17
Peptide Bond Formation
The two major methods are:

1. coupling of suitably protected amino acids using $N,N'$-dicyclohexylcarbodiimide (DCCI)
2. via an active ester of the N-terminal amino acid.
DCCI-Promoted Coupling

\[
\begin{align*}
\text{O} & \quad \text{O} \\
\text{ZNHCH\text{C}_6\text{H}_5} \quad + \quad \text{H}_2\text{NCH}_2\text{COCH}_2\text{CH}_3 \\
\text{ZNHCHC} \quad \text{NHCH}_2\text{COCH}_2\text{CH}_3 \\
\text{CH}_2\text{C}_6\text{H}_5 & \quad \text{CH}_2\text{C}_6\text{H}_5 \\
\end{align*}
\]

DCCI, chloroform

(83%)
Mechanism of DCCI-Promoted Coupling

\[
\text{ZNHCHCOH} + \text{C}_6\text{H}_{11}\text{N} = \text{C} = \text{NC}_6\text{H}_{11} \rightarrow \text{C}_6\text{H}_{11}\text{N} - \text{C} - \text{OCCHNHZ} \]

\[
\text{C}_6\text{H}_{11}\text{N} \quad \text{CH}_2\text{C}_6\text{H}_{15}
\]
Mechanism of DCCI-Promoted Coupling

The species formed by addition of the Z-protected amino acid to DCCI is similar in structure to an acid anhydride and acts as an acylating agent.

Attack by the amine function of the carboxyl-protected amino acid on the carbonyl group leads to nucleophilic acyl substitution.

\[
\begin{align*}
\text{H} & \quad \text{O} \\
\text{C}_6\text{H}_{11}\text{N} & \quad \text{OCCHNHZ} \\
\text{C}_6\text{H}_{11}\text{N} & \quad \text{CH}_2\text{C}_6\text{H}_5
\end{align*}
\]
Mechanism of DCCI-Promoted Coupling

\[
\begin{align*}
\text{H} & \quad \text{O} \\
\text{C}_6\text{H}_{11}\text{N} & \quad \text{ZNHCH} \quad \text{NHCH}_2\text{COCH}_2\text{CH}_3 \\
\text{C}_6\text{H}_{11}\text{NH} & \quad \text{CH}_2\text{C}_6\text{H}_5 \\
\text{H} & \quad \text{O} \\
\text{C}_6\text{H}_{11}\text{N} & \quad \text{OCCH} \quad \text{NH}_2 \\
\text{C}_6\text{H}_{11}\text{N} & \quad \text{CH}_2\text{C}_6\text{H}_5
\end{align*}
\]
A \textit{p}-nitrophenyl ester is an example of an "active ester."

\textit{p}-Nitrophenyl is a better leaving group than methyl or ethyl, and \textit{p}-nitrophenyl esters are more reactive in nucleophilic acyl substitution.
The Active Ester Method

\[
\text{ZNHCHCO} - \text{NO}_2 + \text{H}_2\text{NCH}_2\text{COCH}_2\text{CH}_3
\]
The Active Ester Method

\[
\text{ZNHCHCO} - \text{CH}_2\text{C}_6\text{H}_5 + \text{H}_2\text{NCH}_2\text{COCH}_2\text{CH}_3 \xrightarrow{\text{chloroform}} \text{ZNHCHC} - \text{NHCH}_2\text{COCH}_2\text{CH}_3 + \text{HO} - \text{CH}_2\text{C}_6\text{H}_5
\]

(78%)
27.18
Solid-Phase Peptide Synthesis:
The Merrifield Method
Solid-Phase Peptide Synthesis

In solid-phase synthesis, the starting material is bonded to an inert solid support.

Reactants are added in solution.

Reaction occurs at the interface between the solid and the solution. Because the starting material is bonded to the solid, any product from the starting material remains bonded as well.

Purification involves simply washing the byproducts from the solid support.
The solid support is a copolymer of styrene and divinylbenzene. It is represented above as if it were polystyrene. Cross-linking with divinylbenzene simply provides a more rigid polymer.
Treating the polymeric support with chloromethyl methyl ether (ClCH$_2$OCH$_3$) and SnCl$_4$ places CICH$_2$ side chains on some of the benzene rings.
The side chain chloromethyl group is a benzylic halide, reactive toward nucleophilic substitution ($S_{N2}$).
The chloromethylated resin is treated with the Boc-protected C-terminal amino acid. Nucleophilic substitution occurs, and the Boc-protected amino acid is bound to the resin as an ester.
The Merrifield Procedure

\[
\text{CH}_2 - \text{CH} - \text{CH}_2 - \text{CH}_2 - \text{CH} - \text{CH}_2 - \text{CH}_2 - \text{CH}
\]

\[
\text{BocNHCH}_2\text{CO}^\text{–}
\]

\[
\text{CH}_2\text{Cl}
\]
The Merrifield Procedure

Next, the Boc protecting group is removed with HCl.
DCCI-promoted coupling adds the second amino acid
The Merrifield Procedure

Remove the Boc protecting group.
The Merrifield Procedure

Add the next amino acid and repeat.
The Merrifield Procedure

Remove the peptide from the resin with HBr in CF$_3$CO$_2$H

H$_3$N$^+$ peptide - C$\equiv$NH$\equiv$CHC$\equiv$NH$\equiv$CHCO$\equiv$CH$_2$

Remove the peptide from the resin with HBr in CF$_3$CO$_2$H
The Merrifield Procedure

\[
\begin{align*}
\text{peptide} & \quad C - NHCHC - NHCHCO^- \\
& \quad R' \quad R
\end{align*}
\]
Merrifield also automated his solid-phase method.

Synthesized a nonapeptide (bradykinin) in 1962 in 8 days in 68% yield.

Synthesized ribonuclease (124 amino acids) in 1969.

369 reactions; 11,391 steps

Nobel Prize in chemistry: 1984