“Peptides: Synthesis and Biological Interest”
**Therapeutic Agents**

![Pie chart showing the distribution of drugs approved by the US FDA by chemical species.](image)

*Figure 1. Distribution of the drugs approved by the US FDA by chemical species.*
# Therapeutic peptides approved by the FDA (2009-2011)

Table 1. Peptides approved by the US FDA during the period 2009–2011.

<table>
<thead>
<tr>
<th>Generic name (trade name)</th>
<th>Disease/target</th>
<th>Properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ecallantide (Kalbitor®)</td>
<td>Hereditary angioedema</td>
<td>Plasma kallikrein inhibitor</td>
</tr>
<tr>
<td>Telavancin (Vibativ®)</td>
<td>Skin infection</td>
<td>Antibacterial agent</td>
</tr>
<tr>
<td>Romidepsin (Istodax®)</td>
<td>Cutaneous T-cell lymphoma</td>
<td>HDAC inhibitor</td>
</tr>
<tr>
<td>Liraglutide (Victoza®)</td>
<td>Type 2 diabetes</td>
<td>GLP-1 receptor agonist</td>
</tr>
<tr>
<td>Boceprevir (Victrelis™)</td>
<td>Hepatitis C Virus genotype 1</td>
<td>NS3/4A protease inhibitor</td>
</tr>
<tr>
<td>Telaprevir (Incivek®)</td>
<td>Hepatitis C Virus genotype 1</td>
<td>NS3/4A protease inhibitor</td>
</tr>
<tr>
<td>Brentuximab vedotin (Adcetris™)</td>
<td>Hodgkin’s lymphoma</td>
<td>CD30 directed</td>
</tr>
<tr>
<td>Icatibant (Firazyr®)</td>
<td>Hereditary angioedema</td>
<td>Bradykinin B2 receptor antagonist</td>
</tr>
</tbody>
</table>
Proteins

- Biopolymers of $\alpha$-amino acids.
- Amino acids are joined by peptide bond.
- They serve a variety of functions:
  - Structure
  - Enzymes
  - Transport
  - Protection
  - Hormones
Structure of Amino Acids

α carbon atom
α-amino group
side chain
an α-amino acid

H₂N—CH—C—OH

α carbon atom
α-amino group
side chain
an α-amino acid

H₂N—CH—C—OH

α carbon atom
α-amino group
side chain
an α-amino acid

H₂N—CH—C—OH

α carbon atom
α-amino group
side chain
an α-amino acid

H₂N—CH—C—OH

α carbon atom
α-amino group
side chain
an α-amino acid

H₂N—CH—C—OH

α carbon atom
α-amino group
side chain
an α-amino acid

several individual amino acids

peptide bonds

a short section of a protein

Copyright © 2010 Pearson Prentice Hall, Inc.
Amino Acids

- $\text{NH}_2$ on the carbon next to $\text{COOH}$. 
- Glycine, $\text{NH}_2\text{CH}_2\text{COOH}$, is simplest.
- With $\text{R}$ side chain, molecule is chiral.
- Most natural amino acids are $\text{L}$-amino acids, related to $\text{L}$-(-)-glyceraldehyde.
- Direction of optical rotation, (+) or (-), must be determined experimentally.
Standard Amino Acids

- Twenty standard $\alpha$-amino acids.
- Differ in side-chain characteristics:
  - $\sim$H or alkyl
  - Contains an $\sim$OH
  - Contains sulfur
  - Contains a nonbasic nitrogen
  - Has $\sim$COOH
  - Has a basic nitrogen
<table>
<thead>
<tr>
<th>Name</th>
<th>Symbol</th>
<th>Abbreviation</th>
<th>Structure</th>
<th>Functional Group in Side Chain</th>
<th>Isoelectric Point</th>
</tr>
</thead>
<tbody>
<tr>
<td>side chain is nonpolar, H or alkyl</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>glycine</td>
<td>G</td>
<td>Gly</td>
<td>(\text{H}_2\text{N}--\text{CH}--\text{COOH})</td>
<td>none</td>
<td>6.0</td>
</tr>
<tr>
<td>alanine</td>
<td>A</td>
<td>Ala</td>
<td>(\text{H}_2\text{N}--\text{CH}--\text{COOH})</td>
<td>alkyl group</td>
<td>6.0</td>
</tr>
<tr>
<td>*valine</td>
<td>V</td>
<td>Val</td>
<td>(\text{H}_2\text{N}--\text{CH}--\text{COOH})</td>
<td>alkyl group</td>
<td>6.0</td>
</tr>
<tr>
<td>*leucine</td>
<td>L</td>
<td>Leu</td>
<td>(\text{H}_2\text{N}--\text{CH}--\text{COOH})</td>
<td>alkyl group</td>
<td>6.0</td>
</tr>
<tr>
<td>*isoleucine</td>
<td>I</td>
<td>Ile</td>
<td>(\text{H}_2\text{N}--\text{CH}--\text{COOH})</td>
<td>alkyl group</td>
<td>6.0</td>
</tr>
<tr>
<td>*phenylalanine</td>
<td>F</td>
<td>Phe</td>
<td>(\text{H}_2\text{N}--\text{CH}--\text{COOH})</td>
<td>aromatic group</td>
<td>5.5</td>
</tr>
<tr>
<td>proline</td>
<td>P</td>
<td>Pro</td>
<td>(\text{H}_2\text{N}--\text{CH}--\text{COOH})</td>
<td>rigid cyclic structure</td>
<td>6.3</td>
</tr>
<tr>
<td>side chain contains an (-\text{OH})</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>serine</td>
<td>S</td>
<td>Ser</td>
<td>(\text{H}_2\text{N}--\text{CH}--\text{COOH})</td>
<td>hydroxyl group</td>
<td>5.7</td>
</tr>
<tr>
<td>*threonine</td>
<td>T</td>
<td>Thr</td>
<td>(\text{H}_2\text{N}--\text{CH}--\text{COOH})</td>
<td>hydroxyl group</td>
<td>5.6</td>
</tr>
<tr>
<td>Name</td>
<td>Symbol</td>
<td>Abbreviation</td>
<td>Structure</td>
<td>Functional Group in Side Chain</td>
<td>Isoelectric Point</td>
</tr>
<tr>
<td>-------------------</td>
<td>--------</td>
<td>--------------</td>
<td>-----------------</td>
<td>-------------------------------</td>
<td>-------------------</td>
</tr>
<tr>
<td>tyrosine</td>
<td>Y</td>
<td>Tyr</td>
<td>H₂N—CH—COOH</td>
<td>phenolic—OH group</td>
<td>5.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><img src="image" alt="tyrosineStructure" /></td>
<td></td>
<td></td>
</tr>
<tr>
<td>side chain contains sulfur</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cysteine</td>
<td>C</td>
<td>Cys</td>
<td>H₂N—CH—COOH</td>
<td>thiol</td>
<td>5.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><img src="image" alt="cysteineStructure" /></td>
<td></td>
<td></td>
</tr>
<tr>
<td>*methionine</td>
<td>M</td>
<td>Met</td>
<td>H₂N—CH—COOH</td>
<td>sulfide</td>
<td>5.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><img src="image" alt="methionineStructure" /></td>
<td></td>
<td></td>
</tr>
<tr>
<td>side chain contains nonbasic nitrogen</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>asparagine</td>
<td>N</td>
<td>Asn</td>
<td>H₂N—CH—COOH</td>
<td>amide</td>
<td>5.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><img src="image" alt="asparagineStructure" /></td>
<td></td>
<td></td>
</tr>
<tr>
<td>glutamine</td>
<td>Q</td>
<td>Gln</td>
<td>H₂N—CH—COOH</td>
<td>amide</td>
<td>5.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><img src="image" alt="glutamineStructure" /></td>
<td></td>
<td></td>
</tr>
<tr>
<td>*tryptophan</td>
<td>W</td>
<td>Trp</td>
<td>H₂N—CH—COOH</td>
<td>indole</td>
<td>5.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><img src="image" alt="tryptophanStructure" /></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Name</td>
<td>Symbol</td>
<td>Abbreviation</td>
<td>Structure</td>
<td>Functional Group in Side Chain</td>
<td>Isoelectric Point</td>
</tr>
<tr>
<td>---------------</td>
<td>--------</td>
<td>--------------</td>
<td>-----------</td>
<td>--------------------------------</td>
<td>-------------------</td>
</tr>
<tr>
<td>side chain is acidic</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>aspartic acid</td>
<td>D</td>
<td>Asp</td>
<td>H₂N—CH—COOH</td>
<td>carboxylic acid</td>
<td>2.8</td>
</tr>
<tr>
<td>glutamic acid</td>
<td>E</td>
<td>Glu</td>
<td>H₂N—CH—COOH</td>
<td>carboxylic acid</td>
<td>3.2</td>
</tr>
<tr>
<td>side chain is basic</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>*lysine</td>
<td>K</td>
<td>Lys</td>
<td>H₂N—CH—COOH</td>
<td>amino group</td>
<td>9.7</td>
</tr>
<tr>
<td>*arginine</td>
<td>R</td>
<td>Arg</td>
<td>H₂N—CH—COOH</td>
<td>guanidino group</td>
<td>10.8</td>
</tr>
<tr>
<td>*histidine</td>
<td>H</td>
<td>His</td>
<td>H₂N—CH—COOH</td>
<td>imidazole ring</td>
<td>7.6</td>
</tr>
</tbody>
</table>

*essential amino acid

Copyright © 2010 Pearson Prentice Hall, Inc.
Properties of Amino Acids

- High melting points, over 200°C.
- More soluble in water than in ether.
- Larger dipole moments than simple acids or simple amines.
- Less acidic than most carboxylic acids; less basic than most amines.

\[
\begin{align*}
\text{pK}_a &= 10 \\
\text{pK}_b &= 12 \\
\end{align*}
\]
Resonance Stabilization

- The peptide bond is an amide bond.
- Amides are very stable and neutral.
An oligopeptide is made out of four to ten amino acids.

Peptide structures are drawn with the N-terminal end at the left.

Peptides are named from left to right: arginylprolylprolyl……arginine.
Peptide Bond Formation

- The amino group of one molecule condenses with the acid group of another.
- Polypeptides usually have molecular weight less than 5,000.
- Protein molecular weight is 6,000–40,000,000.
Solution Phase Peptide Synthesis

- First, protect the amino group at the N terminus with benzyl chloroformate.
- Activate the carboxyl group with ethyl chloroformate to form anhydride of carbonic acid.
- Couple the next amino acid.
- Repeat activation and coupling until all amino acids needed have been added.
- Remove the protecting group.
Peptide Synthesis: Solid Phase

X : Cl, Br, SH, OH, NH₂

1) Deprot.
2) Coupling

Cleavage
Peptide Synthesis: Solid Phase
Peptide Synthesis: Solid Phase

Carbodiimides

DIP (DIPCDI, 8)  EDC (EDAC,WSC, 9)  DCC (DCCI, 10)

R-N=C=N-R

R-N=C=N-R

R-N=C=N-R

R-N=C=N-R

R-N=C=N-R

R-N=C=N-R

R-N=C=N-R

R-N=C=N-R

R-N=C=N-R

R-N=C=N-R

R-N=C=N-R

R-N=C=N-R

R-N=C=N-R

R-N=C=N-R

R-N=C=N-R

R-N=C=N-R

R-N=C=N-R
Peptide Synthesis: Solid Phase

Aminium Salts

- HBTU (11) X = CH, Y = H, Z = PF₆
- TBTU (12) X = CH, Y = H, Z = BF₄
- HCTU (13) X = CH, Y = Cl, Z = PF₆
- TCTU (14) X = CH, Y = Cl, Z = BF₄
- HATU (15) X = N, Y = H, Z = PF₆

Phosphonium Salts

- PyBOP (16) X = CH
- PyAOP (17) X = N
Peptide Synthesis: Reaction Monitoring

General Considerations

Constant monitoring of the reaction to facilitate optimization of the yield and minimize side reactions is necessary.

On-Bead Methods:
- Fast, reliable and sensitive
- Use of common analytical techniques (Colorimetric / Fluorescent detection)

Destructive Methods

Off-Bead Methods:
Cleavage of resin bound materials and analysis by traditional Org Chem methods

Advantage:
Highly Accurate

Drawbacks:
- Resin beads are lost (lost of material and difficult to quantify)
- Analysis requires time (hours)

Peptide Synthesis: Reaction Monitoring

Colorimetric tests for: OH, CO$_2$H, CHO and more...

Ninhydrin

Kaiser Test

Blue beads (+)
Free amines

Yellow beads (-)
Complete Coupling

Complete coupling ?
Reaction with Ninhydrin

- Used to visualize spots or bands of amino acids separated by chromatography or electrophoresis.
- Deep purple color formed with traces of any amino acid.

Chemical reaction:

\[
\text{H}_2\text{N} - \text{CH} - \text{COOH} + 2 \text{ninyhydrin} \xrightarrow{\text{pyridine}} \text{Ruhemann's purple} + \text{CO}_2 \uparrow + \text{R} \rightarrow \text{CHO}
\]

Copyright © 2010 Pearson Prentice Hall, Inc.
General Considerations

Magic Angle Spinning (MAS-NMR), derives from the observation that spinning a heterogeneous NMR sample at the magic angle (54.736°) reduces the line-broadening of solid/swollen polymer samples. Using a high-resolution NMR probe of reduced volume (Nanoprobe) that holds all the sample in the active region of the receiver coil, allows acquisition of SP MAS-1H NMR spectra with line widths as small as 1Hz using a 500 MHz spectrometer.

- The quality of the MAS-1H NMR spectrum in primarily influenced by the nature of the polymer matrix
- Special techniques can be employed with PEG resins (high mobility and flexibility) that will provide spectra of comparable quality to their solution-phase counterpart.
- Hydrophobic PS resins with short tether (e.g., wang linker) give poor quality spectra
- 2D MAS techniques such as 2D-COSY AND TOCSY has also been developed

MS AND IR are also very useful tools
Advantages of Solid Phase Synthesis

- Growing chain, built from C to N terminus, is attached to polystyrene beads.
- Intermediates do not have to be purified.
- Excess reagents are washed away with a solvent rinse.
- Process can be automated.
- Larger peptides can be constructed.
We know how to synthesize peptides on solid phase, but how could we synthesize several peptides at a time?
Parallel Synthesis of 27 tripeptides requires $3 + 9 + 27 = 39$ Coupling Reactions

What can we do if we want to synthesize 1 million of peptides?
Peptide Synthesis: Mixture Libraries

Parallel Synthesis of 27 tripeptides requires 3 Reactions !!!!!

Problems:
- Different monomers will have different reactivities
- Different reactivities of certain dimeric sequences
- Complex mixtures on resin beads precludes the on-resin assays
- Complex deconvolution process for in-solution assays
Peptide Synthesis: Split and Mix
Split and Mix Libraries: Screening

Fluorescently Labeled Protein

Structure of the biologically active peptide?

Peptide sequencing by Edman Degradation
Sequencing from the N Terminus

- **Edman degradation**: The reaction with phenyl isothiocyanate followed by hydrolysis removes the N terminus amino acid.
- The phenylthiohydantoin derivative is identified by chromatography.
- Use for peptides with < 30 amino acids.
Edman Degradation

Step 1: Nucleophilic attack by the free amino group on phenyl isothiocyanate, followed by a proton transfer, gives a phenylthiourea.

\[
\begin{align*}
\text{Ph}-\text{N}=\text{C} \rightleftarrows \text{Ph}-\text{N}^{+} \rightleftarrows \text{HN}^{-} \rightleftarrows \\
\text{H}_{2}\text{N} \rightleftarrows \text{CH} \rightleftarrows \text{C} \rightleftarrows \text{NH} \rightleftarrows \\
\text{peptide} \rightleftarrows \text{peptide} \rightleftarrows \text{peptide} \rightleftarrows \\
\text{R}^{1} \rightleftarrows \text{R}^{1} \rightleftarrows \text{R}^{1} \rightleftarrows \\
\text{O} \rightleftarrows \text{O} \rightleftarrows \text{O} \rightleftarrows \\
\end{align*}
\]

Copyright © 2010 Pearson Prentice Hall, Inc.

Step 2: Treatment with HCl induces cyclization to a thiazolinone and expulsion of the shortened peptide chain.

\[
\begin{align*}
\text{NHPh} \rightleftarrows \text{N} \rightleftarrows \text{HN}^{-} \rightleftarrows \\
\text{H} \rightleftarrows \text{C} \rightleftarrows \text{C} \rightleftarrows \text{NH}_{2} \rightleftarrows \\
\text{peptide} \rightleftarrows \text{peptide} \rightleftarrows \text{peptide} \rightleftarrows \\
\text{R}^{1} \rightleftarrows \text{R}^{1} \rightleftarrows \text{R}^{1} \rightleftarrows \\
\text{O} \rightleftarrows \text{O} \rightleftarrows \text{O} \rightleftarrows \\
\text{protonated phenylthiourea} \rightleftarrows \text{a thiazolinone} \rightleftarrows \text{peptide} \rightleftarrows \text{peptide} \rightleftarrows \text{peptide} \rightleftarrows \\
\text{H}_{2}\text{O}^{-} \rightleftarrows \text{H}_{3}\text{O}^{+} \rightleftarrows \\
\end{align*}
\]

Copyright © 2010 Pearson Prentice Hall, Inc.
In the final step (step 3) the thiazoline isomerizes to the more stable phenylthiohydantoin.
Edman Degradation (Continued)