Strategies for Peptide Synthesis: An Overview

Han, S., Kim, Y. *Tetrahedron*, 2004, 60, 2447-2467
Albericio, F. *Current Opinion in Chemical Biology*, 2004, 8, 211-221
Humphrey, J., Chamberlin, R. *Chem. Rev.*, 1997, 97, 2243-2266
Outline

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2) Coupling reagents
   - Carbodiimides
   - Uronium Reagents
   - Phosphonium Reagents
   - Organophosphorous Reagents
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   - Racemization Pathways
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   - $\alpha,\alpha$-disubstituted Amino Acids
   - Peptide Macrocyclizations
   - N-methyl Amino Acids
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4) Applications
   - Process Scale Solid Phase Peptide Synthesis
Introduction

- Amide Bonds are Ubiquitous in Nature

- A large number of Natural products are based upon a peptide framework and exhibit a spectrum of biological activity

- Currently there are many peptide therapeutics in development

- The current pursuit of non-natural amino acid mimics makes coupling chemistry paramount for drug discovery and scientific advancement

- There is no single strategy for amide bond formation that is a magic bullet

- Advances in coupling chemistry have made formation of the most difficult amide bonds possible
General Strategy For Peptide Bond Formation

\[ \text{Peptide Bond Forming Reagent} \rightarrow \text{X = Activating Group} \]

\[ R'HN\rightarrow COOH \rightarrow R'HN\rightarrow COX \rightarrow R'HN\rightarrow CO\rightarrow \text{NR}''\rightarrow CO\rightarrow R''' \]
Carbodiimides: Representative Examples/Comparisons

**Water Soluble by-product is easily removed in aqueous work-up**

**Urea formed is partially soluble in many solvents and hard to purify via column chromatography**

**Urea formed is soluble in most organics. This is advantageous in solid phase synthesis.**

$\text{EDC}$ $\text{DCC}$ $\text{DIC}$

$\text{BEC}$ $\text{CIC}$ $\text{BMC}$ $\text{BDDC}$

$N,N$-dicyclopentylcarbodiimide
**Carbodiimides: Basic Structure and Mechanism**

**N-acyl Urea Formation**

**Common Activators: Accelerate Reaction and Suppress Byproduct Formation**
Lou Carpino: Peptide Giant
UMass, Amherst

- Developed benzotriazole based aminium reagent, HATU, and elucidated the active form of the coupling agent
- Introduced HOAt as an efficient additive for coupling reactions
- Introduced the widely used Fmoc protecting group
- Pioneered the use of amino acid fluorides as coupling agents
Uronium reagents: Basic Structure and Reactive Species

- Originally the uronium isomer was thought to be the active species

- Upon solving of the x-ray crystal structure, it was found that the guanidinium species was predominate

- However, the uronium could be prepared and was found to be more reactive than the guanidinium salt

- Original attempts to classify the reactive species were misguided based on known thermodynamic stabilities

- The two forms are readily distinguished by a shift in the IR absorption from ~1710 cm⁻¹ (Uronium) to ~1670 cm⁻¹ (guanidinium)

**Uronium reagents: Overview/Cost Analysis**

-HATU is the most reactive uronium reagent, however it can be cost prohibitive on large scales and is often used only as a last resort.

-HBTU is the more cost effective alternative and is acceptable for most coupling applications, however lower yielding couplings can become problematic on industrial scales and with long peptides.

- HCTU has been developed as an effective alternative to HATU on industrial scales, the higher reactivity of this species is attributed to the more reactive CI-HOBt intermediate.

-TSTU and TNTU are useful alternatives under aqueous reaction conditions.
Uronium reagents: Mechanism and Side Reactions

Intramolecular general base catalysis enhances reactivity

Guanidine Formation

This side reaction highlights the importance of stoichiometry and pre-activation of the acid component. This can generally be avoided with the proper precautions.
Uronium reagents: Efficiency Comparison

\[
\text{CH}_3\text{CH}_3\ \text{OH} + \text{H}_2\text{N} \rightarrow \text{Fmoc-Deg-Phe-OFm}
\]

<table>
<thead>
<tr>
<th>Coupling Agent</th>
<th>HPLC Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>HATU</td>
<td>94</td>
</tr>
<tr>
<td>HBTU</td>
<td>85</td>
</tr>
<tr>
<td>HAPyU</td>
<td>92</td>
</tr>
<tr>
<td>HAMDU</td>
<td>57</td>
</tr>
<tr>
<td>HDTU</td>
<td>64</td>
</tr>
</tbody>
</table>

*J. Org. Chem. 1998, 63, 9678-9683*
**Phosphonium Reagents: The Basics**

**Common Reagents**

- Reagents bearing the dimethyl amine moiety produce HMPA as a toxic byproduct, and thus their pyrrolidine based analogues are preferred.

- Halogenophosphonium reagents have been shown to be more efficient coupling reagents in the coupling of N-methylated amino acids.

"Difficult" because of steric hindrance

\[
\text{Boc-Pro-MeVal-OMe} \quad \text{PyBroP} = 79\% \\
\text{PyClO} = 85\% \\
\text{PyBOP} = 26\%
\]

*J. Org. Chem. 1994, 59, 2437-2446*
**Phosphonium Reagents: Thioamide Formation**

\[ \text{ROSH} + \text{H}_2\text{NR}^{'-} \xrightarrow{\text{PyBOP}} \text{R'}\text{SNR'} + \text{R'CO} \]

2.5 : 1

(68% overall yield of both products)

- Thioamides are useful probes in peptide structure and function, particularly for the elucidation of the contribution of backbone hydrogen bonding

- These peptide analogues are not readily accessed through thioacylation in an analogous fashion to standard peptides

- This example uses the oxophilicity of the phosphonium coupling reagent to direct thioamide formation preferentially over amide formation

- Interestingly, this selectivity is reversed (1:24) with the use of PyBrOP

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Note: Thioamides are also readily prepared using Lawesson's reagent

\[ \text{RCONHR} + \text{S-P-S} \xrightarrow{\text{H}_3\text{CO}} \text{RNSNR} \]

Organophosphorous Reagents

- Originally developed as an alternative to the mixed anhydride method
- The use of organophosphorous reagents gives enhanced regioselectivity toward the carbonyl of the phosphoric-carboxylic mixed anhydride

Common Organophosphorous Reagents

- DPPA: Oil, difficult to handle
- MPTA: Crystalline, stable
- DECP: Useful for nucleophilic amines
- BOP-Cl: Especially good for N-alkyl amino acids
Organophosphorous Reagents: Practical Considerations

Boger's Total Synthesis of Sandramycin

- Macrocyclization was accomplished under mild conditions using NaHCO₃ as a base
- Use of stronger bases led to decomposition

**Acid Halogenation**

- Acid halides are typically used when steric congestion prohibits the use of standard coupling reagents
- Currently there are a number of commercially available Fmoc amino acid fluorides, however Boc and Cbz groups present problems in the coupling of acid halides
- For difficult couplings the more reactive and less expensive acid chloride would be preferred, however until recently an appropriate protecting group was not available

**Carboxyanhydride Formation: An Unwanted Side reaction**

This side product can be significantly reduced with careful selection of protecting groups for the amine functionality. Boc protected amines form the carboxyanhydride byproduct much more readily than the corresponding Fmoc or Cbz amino acids.

*J. Am. Chem. Soc. 1996, 118, 9796-9797*
Acid Chlorides

- Acid Chlorides were first introduced for peptide coupling in 1903 by Emil Fischer

- However, when the amine functionality is protected as a carbamate, the oxazolinone is readily accessed, leading to racemization and undesirable side products

- These problems can be avoided by the use of a sulfonyl protecting group, but deprotection conditions may be too harsh for many peptides

- This problem was solved by Vedejs and coworkers

**Common reagents to Make Acid Chlorides**

- Cyanuric Chloride
- CDMT
- Oxalyl Chloride
- BTC
- Thionyl Chloride
- Phosphorous oxychloride (POCl₃)
**Acid Chlorides: New Sulfonyl Protecting Groups**

- Both sulfonyl chlorides are readily accessed from the commercially available mercapto derivatives
- The corresponding sulfonamides are synthesized in high yield for a number of amino acid derivatives, including zwitterionic amino acids
- Both groups can be selectively removed in the presence of other sulfonamides under reducing conditions including: Zn/HOAc, Al-Hg/ether, and H₃PO₄

\[ \text{ThsCl} \quad \text{"thisyl chloride"} \]

\[ \text{BtsCl} \quad \text{"betsyl Chloride"} \]

\[ \begin{align*}
\text{1) } & \text{SOCl}_2 \\
\text{2) } & \text{Na}_2\text{CO}_3
\end{align*} \]

\[ \text{95\% Yield} \]

\[ \text{99.75 \text{ de (crude)}} \]

*J. Am. Chem. Soc. 1996, 118, 9796-9797*
**Acid Fluorides**

**Common Acid Fluorinating Reagents**

![Cyanuric Fluoride](image)

DAST can be used in the absence of base to promote acid fluoride formation. Particularly useful in preparing Fmoc amino acid fluorides.

**Advantages/Disadvantages of the Approach**

- Promotes the formation of amide bonds at sterically hindered sites
- Most carbamate protected amino acids are stable compounds that do not form the N-carboxyanhydride byproduct
- More water stable than the corresponding acid chlorides
- Arg and His are not stable and need to be generated in situ
- The amino acid fluorides show similar reactivity to activated esters and may not be appropriate for difficult couplings at unreactive sites

*J. Am. Chem. Soc. 1995, 117, 5401-5402*

Acid Fluorides Applied

- Danishefsky and co-workers applied an acid fluoride mediated coupling in route to 5-N-acetylrafideemin when standard coupling reagents failed to produce a diastereomerically pure compound

J. Am. Chem. Soc. 1999, 121, 11953-11963
Microwave Assisted Synthesis

Solid Phase Synthesis of the Acyl Carrier Peptide

1) Piperidine, DMF, µw
   1min
2) Asn-Fmoc, PyBOP
   HOBT, DIEA, DMF, µw
   2 min

GLY-NHFOc

GLY-ASN-NHFOc

VQAAIDYING
>95%

- Researchers at CEM Corporation were able to rapidly and efficiently assemble a decameric peptide in >95% overall yield with microwave assisted synthesis.

- The reactions were enhanced through microwave radiation allowing for higher resin substitution, less reagent excess and higher coupling yields due to decreased intramolecular aggregation.

*Biopolymers, 2003, 71, 361*

<table>
<thead>
<tr>
<th>Reagent</th>
<th>PyBOP</th>
<th>HATU</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reaction Time(min)</td>
<td>20</td>
<td>1.5</td>
</tr>
<tr>
<td>Temperature(°C)</td>
<td>110</td>
<td>110</td>
</tr>
<tr>
<td>Solvent</td>
<td>DMF</td>
<td>DMF</td>
</tr>
</tbody>
</table>

*Synthesis, 2002, 11, 1592-1596*
Racemization Pathways

Oxazolone Formation

Epimerization can be controlled with the appropriate rate enhancing, racemization suppressants.
Racemization Suppressants

HOBT

Most commonly used racemization suppressant. Often used in combination with carbodiimide chemistry.

HOAt

HOAt is more effective than HOBT due to intramolecular general base catalysis. Caution should be used on large scales, as HOAt is slightly explosive.

HODhbt

HODhbt has yet to find widespread use due to a ring opening side reaction.

HOCT

When used with DIC, HOCT suppressed all racemization except with histidine.

PTF

When PTF is used in combination with HBTU, the coupling efficiency is equivalent to that of HATU. Unsuitable for phosphorous reagents because of strong P-F bond.

Org. Lett. 2003, 5, 975-977

- Racemization suppression agents also work to enhance the reactivity of intermediates in the coupling reaction

- Also, Cu(II) salts have been found to act as racemization suppressants in the presence of standard coupling agents (CuCl₂, Cu(OBT)₂, Cu(OAT)₂), J. Pept. Sci. 2001, 7, 115-120
**Difficult Couplings: α,α dialkyl**

- Disubstituted amino acids are particularly challenging because of the propensity for racemization of the penultimate residue, and diketopiperazine formation

- Steric congestion also makes these residues less reactive to standard peptide coupling techniques

- Both of these are a result of the geminal dialkyl effect

- Dialkyl substituents force the peptide from its preferred straight chain, anti-periplanar conformation into a staggered conformation, decreasing the bond angle in the chain and facilitating ring formation

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**Diketopiperazine Formation**

Diketopiperazine formation is especially problematic in the synthesis of peptides containing N-methylated amino acids. This is in part due to N-alkyl peptide exhibiting a greater preference for the Z-amide.
\( \alpha, \alpha \) dialkyl : Solutions

**Barton PTOC Ester**

\[
\begin{align*}
\text{ZHN} & \quad \text{O} \quad \text{N} \\
\text{O} \quad \text{S} \quad \text{Ph} & \quad \text{R} \quad \text{R}^\prime \quad \text{N} \\
\text{CO}_2 \text{O} & \quad \text{R}^\prime \quad \text{R}^\prime \quad \text{R} & \quad \text{H}_3 \text{C} \quad \text{CH}_3 \\
\end{align*}
\]

\[
\begin{align*}
\text{PhS} & \quad \text{N} \\
\text{CO}_2 \text{Et} & \quad \text{R}^\prime \quad \text{R}^\prime \quad \text{N} \\
\end{align*}
\]

\[
\begin{align*}
\text{ZHN} & \quad \text{O} \quad \text{N} \\
\text{H}_3 \text{C} & \quad \text{CH}_3 \\
\end{align*}
\]

95%

**Proposed Mechanism**

- PTOC esters can be generated in situ and coupled, but for higher yields isolation is recommended
- Coupling proceeds under base free conditions, eliminating racemization
- Free amines can be used in place of sulfeneamides but yields decrease

*Tetrahedron, 1996, 52, 9367-9386*
α,α dialkyl : Solutions

Azirine Method for Solution Phase Coupling

-Z-Val-Aib
  95%

-Z-Val-Aib-Aib-NMePh
  99%

-Azirine Method was used in the formation of hindered bonds in the natural product peptide, Alamethicin

-This gave high yields and negligible racemization for the following "difficult" peptides

Carbodiimides: Macrocyclization

Cyclotheonamide A
(A serine protease inhibitor, isolated from a marine sponge)

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>BOP-Cl, DMAP</td>
<td>25</td>
</tr>
<tr>
<td>BOP, DMAP</td>
<td>38</td>
</tr>
<tr>
<td>EDC, HOBr</td>
<td>24</td>
</tr>
<tr>
<td>DCC, HOBr</td>
<td>41</td>
</tr>
<tr>
<td>DPPA, NaHCO₃</td>
<td>25</td>
</tr>
<tr>
<td>BBC, DIEA</td>
<td>36</td>
</tr>
</tbody>
</table>

Macrocyclizations

Silverman's Traceless Linker

1) n-BuLi allylchlorodimethyl silane 82%
2) t-BuLi DMF 82%

1) (S,S)-Et-DuPHOS-Rh, H₂
2) (Boc)₂O, DMAP
3) Hydrazine, MeOH 93%

J. Am. Chem. Soc. 1999, 121, 8407-8408
Org. Lett. 2000, 2, 3743-3746

Orthogonal protecting groups allow for chain extension in either direction.
Perfect for macrocyclizations
Macrocyclizations

Silverman's On resin Cyclization With a Traceless Linker

- Silverman applied his side chain attachment methodology to the synthesis of a peptide based natural product, sansalcamide A

- The ten step synthesis was carried out in 67% overall yield and >95% purity

- Allowed for on-resin cyclizations with peptides not bearing polar sidechains

- Solid phase methodology eliminates need for high dilution, and reduces dimerization and oligomerization

- Especially important because many biologically active peptides are exclusively hydrophobic

*Org. Lett.* **2000**, *2*, 3743-3746
Macrocyclizations: Tentoxin

Novel Incorporation of Difficult Residues

- The N-methyl dehydrophenylalanine could not be installed through standard peptide couplings
- The dehydro amino acid was installed by an elimination reaction of the unprotected precursor
- N-methylation was also carried out regioselectively on solid support due to the enhanced acidity of the amide proton of the dehydro-residue

Org. Lett. 2003, 5, 2115-2118
Difficult Couplings: N-methyl Amino Acids

Acid Catalyzed Cleavage of Imino Acid Sequences

![Chemical Structure]

Proceeds through Z-amide conformer. Minor side reaction however and is generally too slow to cause significant problems.

- N-alkyl amino acids are more prone to cleavage and diketopiperazine formation because the Z-amide is more populated than in standard amino acids, making cyclizations favorable.

- Furthermore, racemization is more problematic because the alpha proton is the most acidic proton whereas in natural amino acids the amide proton would be deprotonated first.

- The steric bulk of N-alkyl AAs reduces the nucleophilicity of the amine, slowing the reaction rate and leading to undesirable byproducts.

- It is important to note that in the case of N-methyl AAs, HOBT suppresses the reaction rate and benzotriazole based reagents should be avoided in most cases.

**N-methyl Amino Acids: Solutions**

*BOP-Cl as an Effective Coupling Agent of Me-AAs*

- Intramolecular base catalysis is proposed to allow for efficient coupling with BOP-Cl where other phosphorous based reagents fail

- BOP-Cl allows for one-pot couplings of N-MeAAs because of selective reaction with the carboxylate

- Primary amines react with BOP-Cl yielding undesired side products

- It is critical to employ high purity BOP-Cl and is best to prepare it from ethanol, diethylcarbonate and PCl₅, *Bull. Soc. Chim. Belg.* 1986, 95, 203

- SPPS does not proceed efficiently with BOP-Cl. HOAt based reagents are the preferred reagents for SPPS of N-methyl AAs and do not suffer the low reactivity of HOBt esters
- For large peptides, often times solid phase synthesis is not an operative pathway

-Peptides/proteins must be made by convergent methods in which smaller pieces are brought together to form the whole oligomer

-There are two ways to accomplish this, solution phase couplings using standard peptide bond forming reagents or native chemical ligation

-Solution phase couplings are often made chemoselective by a protein's propensity for folding, this however requires additional protection strategies if multiple segments are to be coupled
Segment Condensations

Native Chemical Ligation (Kent Ligation)

\[
\text{H_2C} \quad \text{C} \quad \text{COSR} + \text{Cys COS'Na}^+ \quad \text{H}_2\text{O, pH 7}
\]

- Solid phase chemical ligation avoids the need to protect internal residues within the peptide segments
- Also, tedious purification of intermediates is no longer an issue
- Assembly of the peptide components can proceed in either the C to N or N to C directions, the C to N direction however requires an additional transient protecting group on the N-terminal cysteine
- Racemization is not a problem because peptides are coupled under neutral conditions
- Coupling using a C-terminal thio acid eliminates cyclization products derived from self-condensation

Segment Condensations: Mechanism

Native Chemical Ligation (Kent Ligation)

\[
\text{Peptide}_1 \text{SR} + \text{+H}_3\text{N} - \text{Peptide}_2 \xrightarrow{\text{H}_2\text{O, pH 7}} \text{Chemoselective Reaction}
\]

\[
\begin{align*}
\text{Peptide}_1 & \xrightarrow{\text{Spontaneous Rearrangement}} \\
\text{Peptide}_1 \text{NH} & \xrightarrow{\text{Peptide}_2} \text{Peptide}_1 \text{SH}
\end{align*}
\]

\[J. \text{Am. Chem. Soc., 1999, 121, 8720-8727}\]
Segment Condensations: Applied

Solid Phase Protein Synthesis

Solid Phase Chemical Ligation (SPCL)

"Accelerated" SPCL

- GV-PLA2, a 118 residue phospholipase was synthesized using SPCI in four segments with three ligation reactions

- The molecule contains six disulfide bonds which could potentially compete with the ligation reaction

- The major peak was collected, found to correspond to the mass of the full length peptide, and shown to possess full biological activity

- An accelerated method was used in which concentrated solutions of each peptide component were used and ligation reactions proceeded for 1 hour, instead of the usual overnight reaction time

Segment Condensations: Improved

Chemical Ligation of Non-cysteine Containing Peptides

![Chemical Ligation Diagram]

**Table 2.** Desulfurization Conditions for PGB1

<table>
<thead>
<tr>
<th>entry</th>
<th>metal reagent</th>
<th>reaction medium</th>
<th>yield (%)</th>
<th>potential problem</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pd/Al₂O₃</td>
<td>0.1 M phosphate, pH 5.8, 6 M guanidine</td>
<td>80</td>
<td>hydrogenation of tryptophan</td>
</tr>
<tr>
<td>2</td>
<td>Raney nickel</td>
<td>0.1 M phosphate, pH 5.8, 6 M guanidine</td>
<td>82</td>
<td>demethylthiolization of methionine</td>
</tr>
</tbody>
</table>

- Native Chemical Ligation sequence can be carried out followed by a desulfurization step to afford a ligated product linked at an alanine residue

- Desulfurization readily occurred in the presence of Raney Ni or with Pd/Al₂O₃ under hydrogen atmosphere

- The products of both reactions could be directly lyophilized and for the most part underwent minimal side reactions

*J. Am. Chem. Soc.* **2001**, *123*, 526-533


**Peptide Synthesis: Process Considerations**

<table>
<thead>
<tr>
<th>Peptide pharmaceuticals manufactured by chemical synthesis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Peptide</strong></td>
</tr>
<tr>
<td>Adrenocorticotropic hormone (1-24)</td>
</tr>
<tr>
<td>Bombesin</td>
</tr>
<tr>
<td>Growth/hormone releasing factor (1-28)</td>
</tr>
<tr>
<td>Integrin</td>
</tr>
<tr>
<td>Oxytocin</td>
</tr>
<tr>
<td>Aliskiren (angiotensin II receptor antagonist)</td>
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<tr>
<td>Thrombin (TPA)</td>
</tr>
<tr>
<td>Thrombin α-1</td>
</tr>
<tr>
<td>Thrombin releasing hormone</td>
</tr>
<tr>
<td>Vasopressin analogues</td>
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<tr>
<td>Desmopressin</td>
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<td>Felypressin</td>
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<tr>
<td>Glypressin</td>
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<td>Oxine</td>
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<tr>
<td>Angiotensin-converting enzyme inhibitors</td>
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<tr>
<td>Enalapril, Lisinopril</td>
</tr>
<tr>
<td>Somatostatin and analogues</td>
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<td>Somatostatin and analogues</td>
</tr>
<tr>
<td>Octreotide</td>
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<tr>
<td>Lanreotide</td>
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<tr>
<td>Luteinizing-hormone releasing hormone</td>
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<tr>
<td>LH/Hgonalised and antagonists</td>
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<td>Leuprolide</td>
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<td>Goserelin</td>
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<td>Human</td>
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<tr>
<td>Salmon</td>
</tr>
<tr>
<td>Eot</td>
</tr>
<tr>
<td>Dichotomel (calcitonin)</td>
</tr>
</tbody>
</table>

C, classical solution phase chemical synthesis; SP, solid phase peptidic synthesis.

- There are more than 40 chemically synthesized peptide therapeutics on the market today (a four-fold increase from 1990)

- All examples at left are potent hormones or hormone analogues that are required in relatively small quantities (<50 kg/ year)

- These peptides represent research undertaken at least 10 years ago and hence represent the inefficient, expensive methodology of peptide synthesis in the early 90s

- It was once thought that peptide therapeutics could be expected to cost $75-100 per gram per amino acid residue to produce

- With current advancements in chemistry and economies of scale, peptides could be produced at <$1 per gram on a multi tonne scale

- The economic advancements led to the development of enfuvirtide (a.k.a. Fuzeon or T-20)

*Nat. Rev. Drug Disc., 2003, 2, 587-593*
Peptide Synthesis: Process Considerations

Key Problems in Process Scale Peptide Synthesis

1) Contaminants and Impurities
   a) Racemization: Difficult to detect in large peptides
      - Use proper racemization suppressants, low dielectric constant solvents, and short activation times
   b) Reaction By-Products:
      - Ureas, phosphonium salts, and scavengers are the most common byproducts
   c) Oxidized peptides: Peptides rich in cysteine are easily oxidized and form dimers and higher order oligomers in solution
   d) Peptide Sequence Failures
      - Deletions can be minimized by using multiple methods for detection of primary amines (Ninhydrin, Trinitrobenzene Sulphonic Acid, and NF-31)

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Nat. Rev. Drug Disc., 2003, 2, 587-593
Chimica Oggi. June 2003, 6-11

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Single-Site Deletion

Single-Site Double Hit
Peptide Synthesis: Process Considerations

Key Problems in Process Scale Peptide Synthesis

2) Toxic and Hazardous Reagents Used in Synthesis
   a) Storage and Bulk Use of Reagents
      - Triazole based reagents such as HBTU can have stability problems
      - This is especially pronounced with HOAt which is sensitive to friction and spark, leading to burning and explosion
      - The use of such reagents may require an expensive safety upgrade

   b) Hazardous Reagents
      - Carbodiimides and benzotriazole reagents are known to cause skin irritation and contact dermatitis with prolonged use
      - They also cause sensitization of the respiratory tract over time

3) Yield
   - Ramifications of low yielding reactions are obvious, however the extent of the problem is magnified in a stepwise synthesis with few purification steps

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*Nat. Rev. Drug Disc.,* 2003, 2, 587-593
*Chimica Oggi. June 2003,* 6-11
**Peptide Synthesis: Enfuvirtide**

- Enfuvirtide is a 36 residue peptide that selectively inhibits HIV-1 membrane fusion
- It has been approved for treatment of HIV patients in the US
- Projected requirements are 3 tonnes per year, where patients will receive 180 mg per day or 80g per year
- The drug was developed at Trimeris and a streamlined synthesis was developed by Roche on tonne scales
- This involved a hybrid solid/solution phase synthesis where small segments were first made by SPPS and then coupled in solution

**Ac-Tyr-Thr-Ser-Leu-Ile-His-Ser-Leu-Ile-Glu-Glu-Ser-Gln-Asn-Gln-Gln-Glu-Lys-Asn-Glu-Gln-Glu-Leu-Glu-Leu-Asp-Lys-Trp-Ala-Ser-Leu-Trp-Asn-Trp-Phe-NH₂**

Route 1: Linear Solid Phase Peptide Synthesis
- Fmoc SPPS conducted for the 36 residue sequence
- Greater than 2 equivalents of Fmoc-AA were used per coupling
- Furthermore, upon cleavage from the resin, the peptide was only ~30-40% pure
- This required difficult, low throughput chromatographic separation
- Overall yield was 6-8%
- This was an expensive and inefficient initial synthesis, but allowed access to enough material for clinical trials

*Nat. Rev. Drug Disc., 2003, 2, 587-593*
**Peptide Synthesis: Enfuvirtide**

Ac-Tyr-Thr-Ser-Leu-Ile-His-Ser-Leu-Ile-Glu-Glu-Ser-Gln-Asn-Gln-Gln-Glu-Lys-Asn-Glu-Gin-Glu-Leu-Glu-Leu-Asp-Lys-Trp-Ala-Ser-Leu-Trp-Asn-Trp-Phe-NH₂

Route 2: Combination of SPPS and fragment condensations

- Three side chain protected fragments are constructed using a super acid sensitive resin, 2-chlorotrityl resin

- Resin is not patent protected and can be easily recycled, also attachment is racemization-free

- The three fragments were synthesized using HBTU/HOBt and 1.5 eq of Fmoc protected amino acids, no re-couple cycles were necessary

- Each fragment is isolated in >85% yield and >90% purity

- Each fragment can be synthesized in one week and in 300-500 kg scales

- To make the process efficient solvent recycling must occur, while yields are >99% per coupling, the cost is 75L of solvent per kilogram resin

- Five solution phase reactions complete the peptide which is then isolated in 30% overall yield

- The segment condensations were optimized to show less than 1% racemization

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Conclusions

- Amide Bonds are ubiquitous in nature and elsewhere
- Effective methods to make these bonds are critical to extending pharmaceutical and bio-organic research
- There however is no ONE method that is effective in every situation
- Unfortunately the process of selecting the proper tool is somewhat empirical, however the tool box is well stocked and most difficulties can be overcome