Peptide at a Glance

Dr. Matthieu Giraud / GSK, Stevenage, UK / 21-22- October 2009
Levemir®
Insulin detemir
(rDNA origin) Injection
100 units/mL (U-100)
10 mL
For subcutaneous use only.
Important: See insert.
Store at 2°– 8°C (36°– 46°F).
Avoid freezing.

Insulin
Complexity

Increasing Molecular Weight (MW)

Chemical Molecule

Aspirin (180 Da)

Peptide

6mer (804 Da)

Protein

Lysozyme (14,700 Da)

Antibody

Immunoglobulin G (150,000 Da)

Cell / Tissue

Surface of an Animal Cell
Agenda

- Introduction to peptide Chemistry
- Peptide chemistry at University
- The market
- Peptide chemistry in a CMO – case studies
Introduction to Peptide Chemistry
Write from N- to C- terminal
Also Biosynthesis direction

Direction for the synthesis
(C- to N-terminal)
## Options for Peptide Manufacture

<table>
<thead>
<tr>
<th>Chemical Synthesis</th>
<th>Biological Expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solid-phase Peptide Synthesis (SPPS)</td>
<td>Bacterial</td>
</tr>
<tr>
<td>Solution-phase Peptide Synthesis</td>
<td>Yeast</td>
</tr>
<tr>
<td>Hybrid of above-Convergent Peptide Synthesis</td>
<td>Mammalian</td>
</tr>
<tr>
<td></td>
<td>Transgenic</td>
</tr>
</tbody>
</table>
Fermentation
Concatemer Approach

- Peptide multicopy gene → Peptide multicopy protein
- Concatemer optimal design (i.e. for spacer and cutting principle)

Gene synthesis → Lonza Expression System → High Cell Density Fermentation → Concatemer Digest and Spacer Cleavage → Simple Precursor Purification
Angiomax\(^{\text{TM}}\) (bivalirudin)
Bivalent Thrombin Inhibitor

Tetraglycine spacer

D-Phe-Pro-Arg-Pro

Hirudin carboxy-terminal
Pharmacology of Angiomax™

Angiomax(TM) (Bivalirudin) Pharmacology

**Non-Competitive Inhibition**
- Hirulog & Hirudin
- Anion binding site
- Catalytic site

**Competitive Inhibition**
- Hirulog only
- Thrombin

Lonza
Generation of Bivalirudin 18-mer Intermediate

- Removal of medium ions via UF
- Enzymatic digest of concatemer
- Stop enzymatic digest with TFA
- Remove enzymes, host cell impurit. via UF

Trypsin
Leader-R-aa1------aa1-R-aa1------aa1-R-aa1------aa1-R-aa1------
Carboxy-Peptidase B
Peptide Impurity Profile
rec. Bivalirudin-18mer: each peak characterised

18MER = RPGGGNGDFEEIPEEYL
LEADER SEQ. = MSKTTKLFNSLPLDSMEGS

ASP-18MER
VAL-18MER
18MER-(L)
18MER-(YL)
18MER + 72 amu
CYCLIC 18MER
18MER + ARG
L R-18MER
KFNSLPLDMEGS
KFNSLPLDMEGSR

Lonza
Biotec Multi-Purpose Plant
Down stream processing

- Launch plant: broad down stream processing capabilities
SPPS steps
Solid Support: General Features

An optimal solid support should have the following characteristics:

1. Stable to Variation in Temperatures
2. Mobile, Well-Solvated, and Reagent Accessible Sites
3. Acceptable Loadings
4. Good Swelling in Broad Range of Solvents (if applicable)
5. Acceptable Bead Sizes (if applicable)
6. Stable in Acidic, Basic, Reducing, and Oxidizing Conditions
7. Compatible with Radical, Carbene, Carbanion, and Carbenium
8. Mechanically Robust: Batchwise and Flow-Continuous Modes
The Stress of the Bead
Polymeric Support: Gel Type, Polystyrene

Physical Characterization of beads
- **100-200** (75-150 µm) or 200-400 (75-38 µm) mesh
- 1 or 2% crosslinking
- Mechanically robust
- > 99% of the pendant functional groups are located within the matrix

Solvent Compatibility
- DCM
- DMF
- THF
- Toluene
- TFA
Polymeric Support: Gel Type, Polystyrene

Styrene + Divinylbenzene → Polymerization

3-Nov-09
Polymeric Support: PEG-based resins

Aminomethyl-ChemMatrix®

- Highly crosslinked polymer based on PEG 2000.
- Only primary ether bounds (stability towards acidic conditions)
- Loading: circa 0.95 mmol/g
Polymeric Support: Swelling

![Swelling Chart]

- Acetonitrile
- DCM
- DMF
- DMSO
- Methanol
- TFA
- Water

- Polystyrene
- TentaGel
- CLEAR
- Hydroxymethyl-CM
- Aminomethyl-CM HCl
Solid-Phase Peptide Synthesis Strategy

N-Terminal Amine

Side-Chain Functionality

C-Terminal Carboxyl

Backbone Amide

Side-Chain Carboxyl
Stepwise Solid-Phase Peptide Synthesis

AA resin loading + OH → X

Fmoc cleavage

AA coupling + OH → X

Fmoc cleavage

AA coupling + OH → X

Repetitive reaction

Peptide cleavage

H → NH₂
Stepwise SPPS (2) – GD / DSP

Global Deprotection

Purification (HPLC)
Desalting
Lyophilization

* Schema by Prof. F. Albericio, Institut de Recerca Biomèdica, Barcelona, IRB-PCB
Convergent Solid-Phase Peptide Synthesis

Repeat n times

Final deprotection
Analysis Challenges
Control of Reactions

- Colorimetric Methods:
  - ninhydrine
  - chloranil
  - Leclerk
  - bromophenol...

- Spectroscopic Methods:
  - FT-IR
  - Gel-phase
  - MAS-NMR
Peptide in Universities
Tools Box Approach
Some Typical Academic Focus

- Get the targets!
  - using routine elongation procedure (equipments, historical lab know-how)
  - Routine purification (same stationary phase / buffer for any peptides)
  - Isolation using lyophilization
  - Yield?
  - Productivity?
  - Counter ion?
  - Impurities?
  - Analysis (Only ID & HPLC)
Global Pharmaceutical Market

2008 USD 710 Billion

- Small Molecules: 86.4%
- Proteins: 12.3%
- Peptides: 1.3%

USD 9.5 Billion for Peptides
Peptide Pharmaceutical Market in 2008

$9.5 bn and 1400 kg Peptide Market

$9.5 bn
(Peptide Pharmaceutical Market)

$1.3 bn
(Peptide API Market 1400 kg)

$500 m
(Peptide CMO Market)

LONZA

Major Market Developments

- **Growth**: CAGR06-08 10% (revenues)
- 60% in-house vs. 40% CMO, to reach 50% by 2011
- **Technologies**: SPPS, HPPS (LPPS), recombinant technology
- **Pipeline**: Top 3 generics (Leuprorelin, Octreotide, Goserelin) = 50% of market revenues. Peptide products to be launched 2011-2015: 14-38
## Top Peptide-based Drug Product

<table>
<thead>
<tr>
<th>Top Ten</th>
<th>Drug Product</th>
<th>Drug Substance</th>
<th>Amino acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sales &gt;USD 1 Billion</td>
<td>Copaxone</td>
<td>Glatiramer</td>
<td>(4)n</td>
</tr>
<tr>
<td></td>
<td>Lupron</td>
<td>Leuprolide</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Zoladex</td>
<td>Goserelin</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Sandostatin</td>
<td>Octreotide</td>
<td>7</td>
</tr>
<tr>
<td>Sales &lt;USD 1 Billion</td>
<td>Byetta</td>
<td>Exenatide</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td>Decapeptyl</td>
<td>Triptorelin</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Integrerin</td>
<td>Eptifibatide</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Miacalcin</td>
<td>Calcitonin</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>Fuzeon</td>
<td>Enfuvirtide</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>Angiomax</td>
<td>Bivalirudin</td>
<td>20</td>
</tr>
</tbody>
</table>
Growing Market?

After 2010: probably growth by 5 –10% per year
### Some Therapeutic Peptides & their Application

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Sequence</th>
<th>Applications</th>
</tr>
</thead>
</table>
| Desmopressin     | Mpa-Tyr-Phe-Gln-Asn-Cys-Pro-DArg-Gly-NH₂ | - bed wetting  
|                  |                                   | - diabetes insipidus  
|                  |                                   | - mild hemophilia |
| Octreotide       | DPhe-Cys-Tyr-DTrp-Lys-Thr-Cys-Thr-ol | - digestive secreting tumors  
| (Sandostatin)    |                                   | - acromegaly  
|                  |                                   | - pancreatic fistulae |
| Lanreotide       | DNal-Cys-Tyr-DTrp-Lys-Val-Cys-Thr-NH₂ | - same as octreotide  
|                  |                                   | (but longer acting) |
| Leuprolide       | Pyr-His-Trp-Ser-Tyr-Dleu-Leu-Arg-Pro-NHEt | - prostate cancer  
|                  |                                   | - endometriosis  
|                  |                                   | - precocious puberty |
| Calcitonin       | CSNLSTCVLGKLSQELHKLQTPRTNTGSGTP-NH₂ | - osteoporosis  
| (salmon)         |                                   | - Paget’s disease |
| T20 (Fuzeon)     | Ac-YTSLIHSLIESQNNQQEKNEQELLEDKWASLWNWF-NH₂ | - HIV fusion inhibitor |
Fuzeon®

M.W. 4,492

Source: 2004_Trimeris Presentation NP2D event Zermatt
**Fuzeon® inhibition of HIV fusion**

- **Target cell membrane**
- **Viral membrane**
- **Receptor Binding** → **Fusion Intermediate** → **HR2-Zipping** → **Six-Helix Bundle Formation**

**Source:** 2004 Trimeris Presentation NP2D event Zermatt
Fuzeon® inhibition of HIV fusion

Receptor Binding

Fusion Intermediate

HR2-Zipping

Six-Helix Bundle Formation

Target cell membrane

Viral membrane

ENF or T-1249

gp41

HR1

HR2

Fuzeon / T-1249 Inhibition

Source: 2004 Trimeris Presentation NP2D event Zermatt
Fuzeon Route Development

Route 1:
- Linear SPPS
- Rink Amide MBHA Resin

Route 2:
- SPPS of Peptide Fragments
- Solution Fragment Condensation
- 2CTC Resin

Common
- Fmoc-Strategy
- Side-chain protection (Boc, Trityl, and t-butyl)
Fragment Approach

Resin → Fragment 1 (Ac) → Fragment 2 → Fragment 3 → OH

H-Phe-NH₂

NH₂

Fragment 3' → NH₂

Ac

AA(1-12) AA(13-26) AA(27-38)

AA(1-16) AA(17-26) AA(27-35)

T-1249 Fuzeon

Crude peptide → Side Chain Deprotection → Purification → API
Purification & Process Summary

- Single pass RP HPLC purification
- Isolation by precipitation near isoelectric point

- High purity
- High recovery

THANKS to Roche / Trimeris (Fuzeon, T20):

- Leverage a positive attitude from Pharma on Peptide manufacture via SPPS
- Positive effect of the supply chain (Fmoc amino acid + CTC resin)
Fuzeon Process Summary

- Conventional Plant Equipment
- Minimal Isolations
- No Lyophilization
- Reagents and Starting Materials are Subject to Competitive Bidding
- Overall yield, purity, and cycle time meet commercial requirements
Erectile Dysfunction Market

Worldwide

- 150 MM men with ED\textsuperscript{1}
- 18 MM Men Seeking Treatment for ED\textsuperscript{2}

USA

- 30 MM men with ED\textsuperscript{1}
- 3.6 MM Men Seeking Treatment for ED\textsuperscript{2}

618,000 new cases added each year

U.S. Projected Prevalence of Erectile Dysfunction

- Total
- Severe
- Moderate
- Mild

<table>
<thead>
<tr>
<th>Year</th>
<th>Severe</th>
<th>Moderate</th>
<th>Mild</th>
</tr>
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<tbody>
<tr>
<td>2002</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2012</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>2022</td>
<td></td>
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</tbody>
</table>

Sources: \textsuperscript{1}MMAS J Urol, 1994; \textsuperscript{2}Chew et al, Int J Impot Res, 2000
Bremelanotide – Mechanism of Action

Bremelanotide

Visual & Tactile Stimulation

Selective Nerve Signal

Viagra
Levitra
Cialis

GMP
PDE-5

NO
cGMP

Vasodilation & Erection
Bremelanotide – PT141 - Structure
Liquid Phase Strategy & Challenges

- 14 synthesis steps
- Two main Fragments
  - Boc-Asp(OBzl)-His(Dnp)-(D)Phe-OH
  - 2TFA.Arg-Trp(For)-Lys(Z)-OMe
Solid Phase Strategy & Challenges

- Full length elongation
- Cyclization
- Selective side chain protection
- Cost of Goods (Excess of reagents, Solvent, …)
SPPS Route

1) Fmoc-Lys(Alloc)-OH, DIEA, DCM
2) DIEA, MeOH

cycles

1) 20% piperidine in NMP
2) Fmoc-AA-OH

1) Allyl/Alloc cleavage
2) Cyclisation
3) Fmoc-Nle-OH
4) 20% piperidine in NMP
5) Acetylation
6) cleavage
Solution to selective Allyl / Aloc Cleavage

Catalyst
- \( \text{Pd}(\text{Ph}_3)_4 \)
- \( \text{Pd}(\text{OAc})_2 / \text{P(oTol)}_3 \)
- \( \text{PdCl}_2(\text{Ph}_3)_4 \)

Scavengers / proton donor
- HCOOH/DIEA
- Dimedone
- Morpholine
- AcOH/NMM
- Phenylsilane
- \( \text{Me}_2\text{NH}.\text{BH}_3 \) (DMAB)

Design of Experiments
DoE Approach - Screening – Response Surface – Robustness Test

- **Factor Screening DoE**
- **Design Space**
- **Optimization DoE**
Solution to selective Allyl / Aloc Cleavage

Catalyst
- \( \text{Pd(Ph}_3\text{)}_4 \)
- \( \text{Pd(OAc)}_2 / \text{P(oTol)}_3 \)
- \( \text{PdCl}_2(\text{Ph}_3)_4 \)

Scavengers / proton donor
- HCOOH/DIEA
- Dimedone
- Morpholine
- AcOH/NMM
- Phenylsilane
- \( \text{Me}_2\text{NH.BH}_3 \) (DMAB)
Individual Coupling Optimization

Purity 93A%

Prod. + 2

Fmoc-Arg(Pbf)-Trp(Boc)-Lys(Aloc)-OH

SM
β-Ala impurity
Prod.-Boc

MW = 615.76

MW = 616.72
Final Global Deprotection

1) reaction: TFA solution + Scavenger mixture
2) Precipitation in ether

PI-001-P
CN: 35169
Molecular Weight = 1619.96
Exact Mass = 1618
Molecular Formula = C87H106N14O15S

PI-001-roh
CN: 35193
Molecular Weight = 1139.22
Exact Mass = 1138
Molecular Formula = C50H69N14O10.F2O2
Optimization

Reaction optimization (CAD):

- First set of experiments (screening)
  - cocktail composition
- Second set of experiments (optimization)
  - reaction conditions: Temp. / Time…
  - residual solvent from SPPS…

![Diagram showing factors affecting yield](image-url)
Coefficient plot of the model

Investigation: PI-001-roh (screening corr yield) (MLR)
Scaled & Centered Coefficients for Yield-6

N=19         R2=0.946     R2 Adj.=0.893
DF=9         Q2=0.888     RSD=1.9768   Conf. lev.=0.95
Influence of TIS and Phenol
Influence of TFA and Time

Yield

TFA

100 150 200 250 300 350 400

Time

1 2 3 4 5 6

71 72.68 73.4 74.14 74.88 75.62 76.36 77.1 77.84 78.58
Crude Material before DSP
Manufacturing – Different Tools...
Some typical Industrial Focus

- Get the target - on time and quality!
  - Using optimized elongation procedure (equipments, historical know-how, DoE, QbD)
  - Best purification (best stationary phase / buffer)
  - Isolation using precipitation, lyophilization or spray drying
  - High yield & Productivity
  - Meet specification (Mass, Counter ion assay, peptide assay & purity, enantiomeric analysis, residual solvent, heavy metal, bioorganisme…?)
Factors to Success - SPPS Approach

- Be innovative in the choice of protecting groups – Think out of the box
  - Strong sourcing department
  - Back integration of key starting materials (Facility in China)
- State of the art cyclization is on resin
- Use DoE for screening & optimization
- Availability of tank farm for large volume & railway connection
- On site solvent recovery expert and assets
Bright Peptide Future

- Exceptionally versatile molecules
- Can address previously untreatable medical conditions.
- Often very potent, offsetting (high) cost of manufacture.
- Non-toxic: Physiological degradation to amino acids.
- Deleterious side-effects are uncommon: Specificity honed by evolution.
- Scale of manufacture increasing.
- Cost of manufacture decreasing.
- Drug delivery platforms becoming available
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