KAHA Ligation Generates Diverse β-Peptides for Drug Discovery

β-Amino Acids and β-Peptides

• **Structure:** Elongated one carbon atom
  – Additional positions for substitution.
• **Present in some natural products and pharmaceuticals**
• **Tunable secondary, tertiary, and quaternary structures**
• **Enhanced structural and metabolic stability relative to α-peptides**

![α-peptide structure](image1)

![β-peptide structure](image2)

![Jasplakinolide (NP)](image3)

![Sitaliptin (antidiabetic)](image4)
Synthesis of β-Amino Acids

• Requires a chiral starting material or asymmetric synthesis.
  – Arndt-Eistert homologation of α-amino acids.
  – Chiral auxiliary methods

• General approach to β-amino acid synthesis (Bode 2010)

KAHA (Ketoacid-hydroxylamine) Peptide Synthesis

\[
\text{R}^1\text{CO}_2\text{H} + \text{R}^2\text{HN}⁻\text{O}\text{Me}\text{O}\text{Me}\text{O}\text{Me} \rightarrow \text{R}^1\text{NH}⁻\text{R}^2\text{CO}_2\text{Me} \text{Me}
\]

1:1 tBuOH/H₂O (0.5 M) 1 hr, 40 C 93% Yield

Production of Non-Ribosomal Peptides (NRPs)

- Non-ribosomal peptides (NRPs)
  - Produced by enzyme assemblies (NRPS).
  - Three kinds of building blocks.
  - Examples: vancomycin, cyclosporin, and surfactin.

- Increasing NRP diversity:
  - Manipulation of cell machinery
  - KAHA ligation with diverse building blocks ("Synthetic Fermentation")
Synthetic Fermentation

Products are oligomers of varying length and sequence.

Product distribution controlled by monomer quantity.
Monomer Addition Order

Initiation | First elongation | Second elongation | Termination
--- | --- | --- | ---
5:1 BuOH/buffer pH 7.0 (0.10 M) | 45 °C, 2 h | 45 °C, 2 h | 45 °C, 2 h
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Retention time (min)
Target: HCV Protease NS3/4A

- Hepatitis C Virus
  - Infects 140 million people worldwide
  - Leads to chronic liver disease
  - No vaccination and limited treatment

- Protease NS3/4A
  - Has vital role in HCV replication
  - Shallow and substrate-exposed binding region (tough target)
  - Inhibitor binding energy derived from weak lipophilic and electrostatic interactions.

Culture 1

- $\alpha$-ketoamide forming terminators to target protease serine.
- Hydrophobic side chains
Culture 2

• Same model as culture 1 with different building blocks.
Culture 3 (Focused)

- $M^2$, $M^4$, and $M^5$ were incubated with each of combination of $I$ and $T$.
- $I^3$ and $T^4$ were active even at low concentrations.
- In well C2, approximately 40 different combinations of $M^2$, $M^4$, and $M^5$. 

![Diagram of molecules and grid showing concentration and dilution]
Culture 4 (Addition Biased)

- Only two monomers in each culture.
- Control product distribution by varying concentrations of $\mathbf{M}$ and addition order.
  - A1-A3: 1 eq. of one $\mathbf{M}$
  - A4-B2: 2 eq. of one $\mathbf{M}$
  - B3-C1: Sequential addition of two $\mathbf{M}$s
  - C2-C4: Simultaneous addition of two $\mathbf{M}$s
- Five possible oligopeptides:
  - $\mathbf{I}^3\mathbf{M}^5\mathbf{M}^5\mathbf{T}^4$, $\mathbf{I}^3\mathbf{M}^5\mathbf{M}^2\mathbf{T}^4$, $\mathbf{I}^3\mathbf{M}^5\mathbf{M}^5\mathbf{M}^5\mathbf{T}^4$, $\mathbf{I}^3\mathbf{M}^5\mathbf{M}^2\mathbf{M}^2\mathbf{T}^4$, $\mathbf{I}^3\mathbf{M}^5\mathbf{M}^5\mathbf{M}^5\mathbf{M}^2\mathbf{T}^4$
• With just $M^5$, more than 1 eq. gave higher activity.
• Higher ratios of $M^5$ to $M^2$ resulted in higher activity.
• Possible active compounds: $I^3-M^5-M^5-M^5-T^4$ and $I^3-M^5-M^5-M^2-T^4$. 
Lead Confirmation

- Incubating $I^3$ with 3.6 eq of $M^5$ gave lead compound in 24% isolated yield.
- $IC_{50} = 1.0 \mu M$
- Boceprevir ($IC_{50} = 0.35 \mu M$)
  - Approved by FDA in 2011.
  - Also targets protease NS3/4A.

“Synthetic Fermentation”

- Uses KAHA ligation
  - Highly chemoselective
  - Mild conditions
  - Nearly all unprotected functional groups tolerated
- Analogous to non-ribosomal peptides with I, M, and T building blocks.
- Can generate diverse product mixtures with small numbers of building blocks.
- May facilitate drug discovery by non-specialists or in remote locations.