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Synthesis of Peptaibolin, an antimicrobial peptide

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Abstract: To tackle one of the biggest global health problems, the resistance of microorganisms to antibiotics, a collective effort in the search for more effective agents against bacteria was required. Peptides with antimicrobial activity have been rasing much attention as a promising alternative for antibiotics. Peptaibols, for instance, are a family of antimicrobial peptides (AMPs) with great biomedical potential, in which the Peptaibolin can be highlighted. Indeed, this peptide has gained relevance due to its small amino acids content, only four, and its acetyl group and a phenylalaninol residue (Phol) at the N-terminal and C-terminal, respectively. Here, we report the synthesis of Peptaibolin through Solid Phase Peptide Synthesis assisted by Microwave heating (MW-SPPS), in a pre-loaded Phe-Wang resin. Starting from a loading of 0.51 mmol/g, two syntheses were made, using two different combinations of coupling reagents. The best option was DIC/Oxima achieving a yield of 50.0%. Proton Nuclear Magnetic Resonance (1H NMR) studies confirmed the peptide structure, while the High Performance Liquid Chromatography (HPLC) technique verified the peptide purity. The peptide solubility was examined against several combinations of solvents. Peptaibolin was not soluble in water, only in organic solvents or in the combination of both. Antimicrobial testing has been conducted using both Gram-positive (Staphylococcus aureus and Staphylococcus epidermidis) and Gram-negative bacteria (Escherichia coli and Pseudomonas aeruginosa). Minimum inhibitory concentration studies demonstrated the resistance of bacteria to the peptide action and the peptide instability in bacterial growth conditions.

Keywords: antimicrobial peptides; peptide synthesis; solid-phase approach; bacteria resistance

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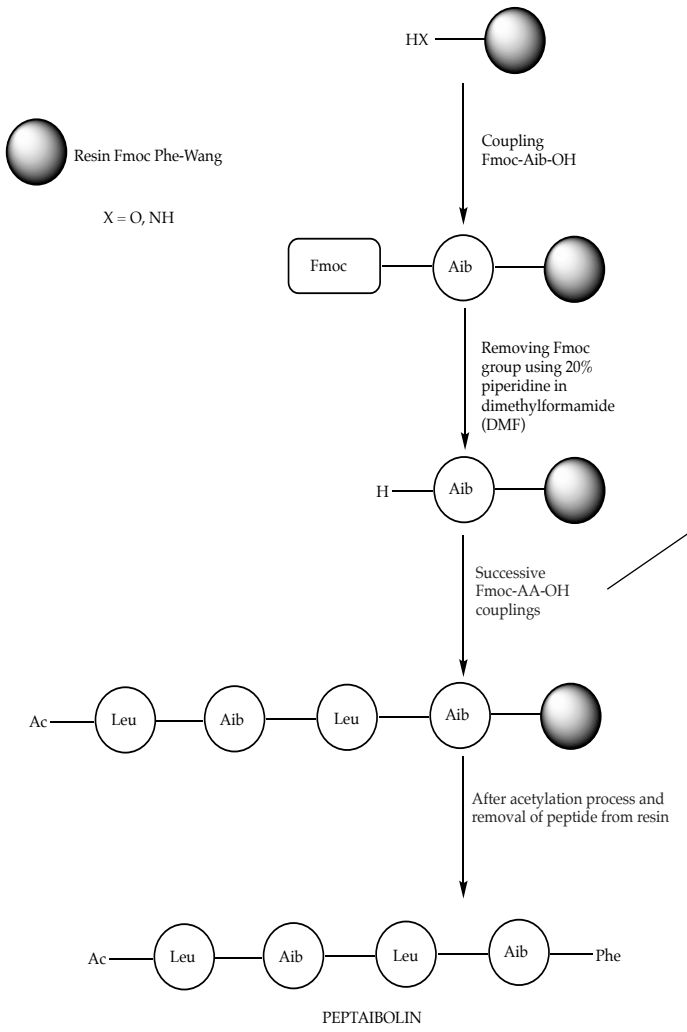
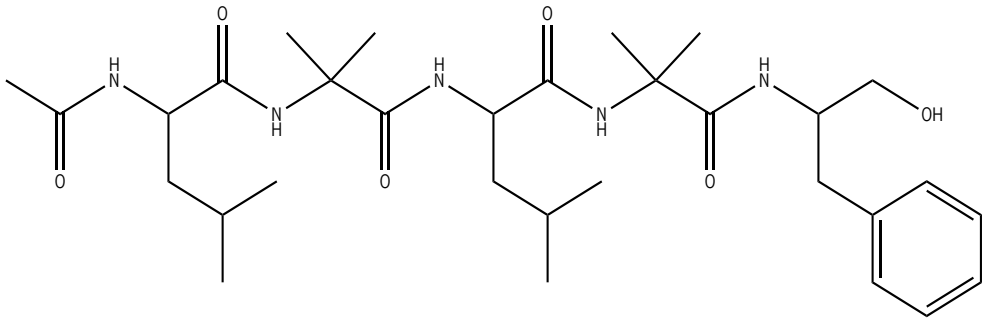
Introduction



Peptaibolin



DIC/HOBt

DIC/Oxime

Study of Properties :

- Yeld according to coupling reagents combination;
- Purity;
- Solubility;
- Antimicrobial activity.

Figure 1- Scheme of the SPPS method using a pre-loaded Fmoc-Phe-Wang resin. All the coupling and deprotecting cycles were performed with assisted heating by microwave.

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Results and Discussion- Synthesis of Peptaibolin by MW-SPPS on Wang resin

- Peptaibolin was synthesized according to the MW-SPPS method where each amino acid is sequentially coupled with the aid of a combination of coupling reagents.
- Two combinations were tested: **DIC/HOBt** (most common on peptide synthesis) and **DIC/Oxime.**
- **Structural confirmation:** Proton Nuclear Magnetic Resonance (^{1}H-NMR) studies;
- **Purity confirmation**: High Performance Liquid Chromatography (HPLC)

Table 1. Comparison between the two coupling reagent combinations in terms of obtained mass, reaction yield and retention time purity.

Coupling Reagents	Mass (g)	Yeld (%)	Retention Time (min)
DIC/HOBt	0,1222	40,6	6,232
DIC/Oxima	0,1520	50,0	6,942

DIC/Oxime coupling reagents had a higher yield as well as a higher purity

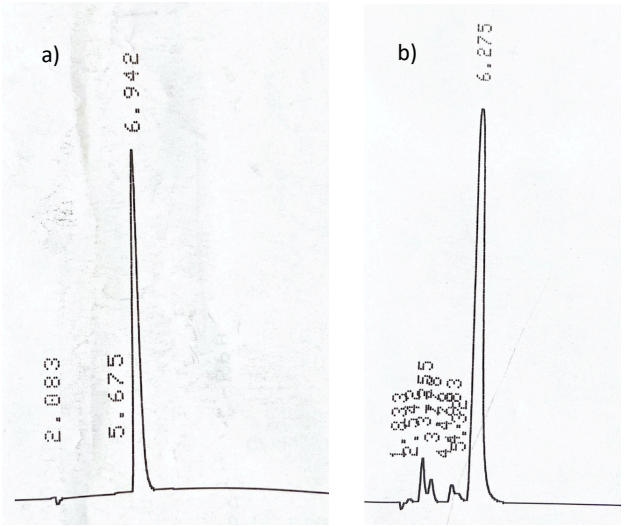

Figure 2- Analytical HPLC of Peptaibolin in a) DIC / Oxima and b) DIC / HOBt.

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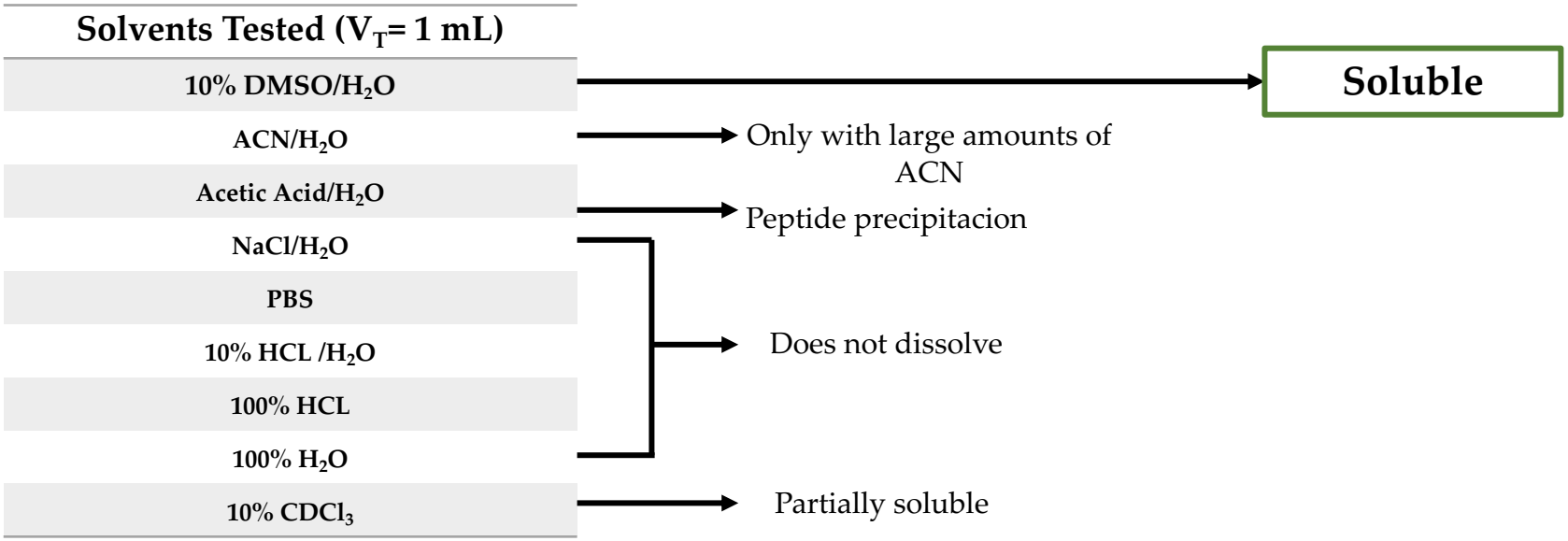
Results and Discussion-Evaluation of Solubility Properties of Peptaibolin

- Performed only on the peptide synthetized with **DIC/Oxime**, mostly because of the highest amount of mass and yield



Peptaibolin was not soluble in water, requiring the addition of other types of organic solvents or even the dissolution of the peptide in a total volume of organic solvents

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Results and Discussion-Antibacterial Assessment

- Peptide dissolved in **10% DMSO/H_2O** for the antimicrobial tests.
- Tests were performed on: *Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli* and *Pseudomonas aeruginosa.*
- After **24 h** of incubation, precipitates were detected in the most Peptaibolin concentrated wells.

Bacterial growth detected in all tested bacteria

MIC studies demonstrated the resistance of bacteria to the peptide action and the peptide instability in bacterial growth conditions

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Conclusions

- DIC/Oxima combination, in addition to maintaining the characteristics of Peptaibolin, provides **better yields** with the **same level of purity** comparing to DIC/HOBt.

- Peptaibolin is **only soluble in organic solvents** or in **organic solvents in combination with H_2O**, making it unsuitable for biomedical applications as AMP.

- Minimum inhibitory concentration studies on *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli* and *Pseudomonas aeruginosa* demonstrated the **resistance of bacteria to the peptide action** and the **peptide instability in bacterial growth conditions**.

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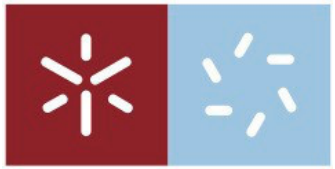

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