Synthesis and biological activity of ultra-short antimicrobial peptides bearing a novel non-coded amino acid

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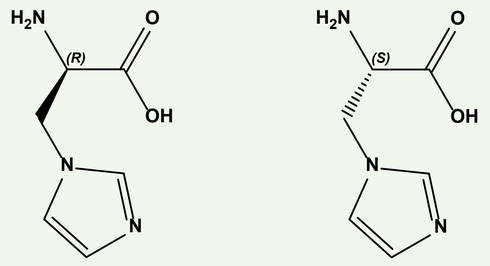
Aim

Antimicriobial resistance is a major concern for human health worldwide. Antimicrobial peptides and lipopeptides represent a promising alternative to conventional drugs in the fight against bacterial infections because of their ability to destroy cell membranes, causing damage difficult to fix, with a mechanism different from that of conventional antibiotics that act on specific targets. The major drawback in using peptides as drugs is their easy degradability by the enzymatic system of the human organism. The introduction of non-coded amino acids (NCAAs) should impart resistance to proteolysis.

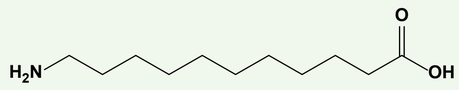
We focused on 2-amino-3-(1H-imidazol-1-yl)propanoic acid as the NCAA for our investigation, synthesizing both enantiomers in high yield and optical purity. Starting from known ultrashort antimicrobial peptides bearing a lipid moiety at the N-terminus (1), we designed and synthesized different analogs in which we inserted a new NCAA at different positions. We also investigated whether the insertion of the lipid chain at the C-terminus impacts the biologic activity of these compounds.



(R)- and (S)-2-amino-3-(1H-imidazol-1-yl)propanoic acid
(D- and L-**His***)

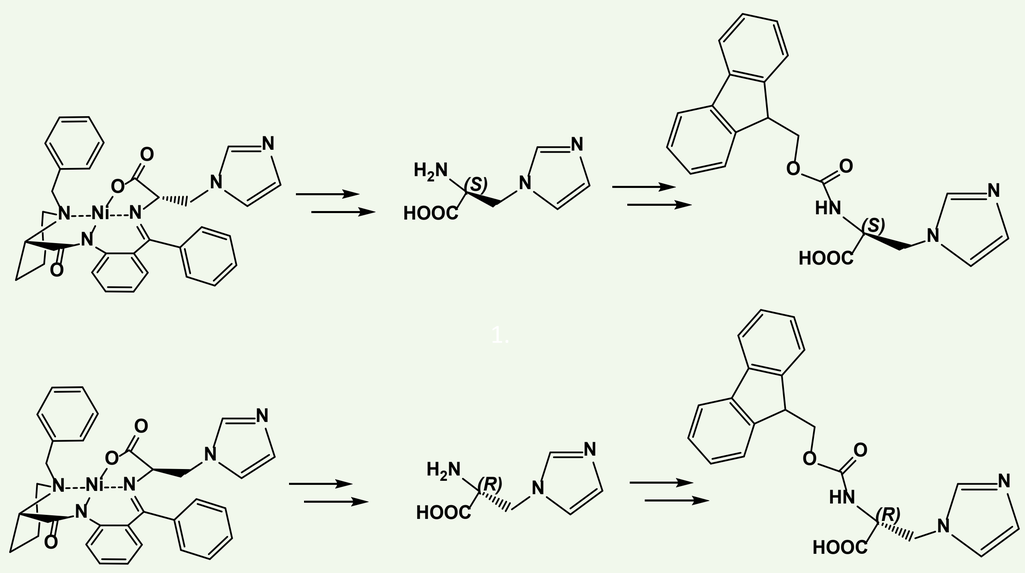
Peptide Sequences	
P10	Palm-His-Ala-D-Ala-His-NH_2
P11	Palm-L-His*-Ala-D-Ala-L-His*-NH_2
P12	Palm-D-His*-Ala-D-Ala-D-His*-NH_2
P13	Ac-His-Ala-D-Ala-His-Aun-NH_2
P14	Ac-L-His*-Ala-D-Ala-L-His*-Aun-NH_2
P15	Ac-L-His*-Ala-D-Ala-L-His*-Aun-NH_2
P16	Laur-His-Ala-D-Ala-His-NH_2
P17	Laur-L-His*-Ala-D-Ala-L-His*-NH_2
P18	Laur-D-His*-Ala-D-Ala-D-His*-NH_2

Palm=$C_{15}H_{31}$-CO- ; **Laur**= $C_{11}H_{23}$-CO-



11-aminoundecanoic acid (**Aun**)

Amino Acid Synthesis



Belokon', Y. N., Sagyan, A. S., Djamgaryan, S. M., Bakhmutov, V. I., & Belikov, V. M. (1988). Asymmetric synthesis of β-substituted α-amino acids via a chiral Ni(II) complex of dehydroalanine. Tetrahedron, 44(17), 5507–5514.

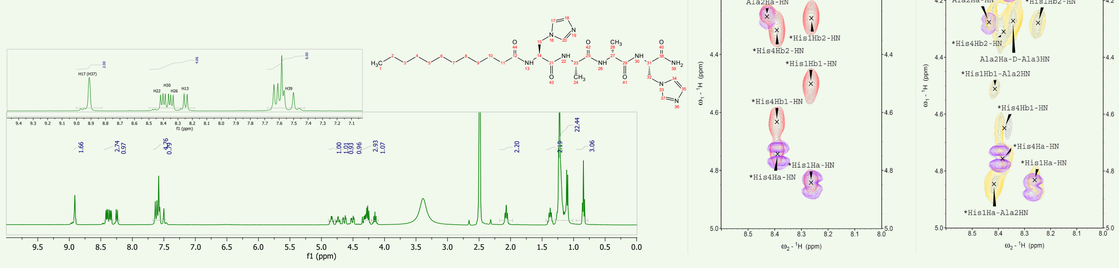
SPPS & Peptides Characterization

SPPS
- Fmoc/tBu
- Activation: DIC/Oxyme
- Cleavage: TFA/TIS/H_2O (95:2.5:2.5)

Sample	R_t(min)*	Purity (%)	MW_{calc}	MW_{exp}
P10	20,0	97	671,9	671,4
P11	19,9	95	671,9	671,4
P12	19,8	95	671,9	671,4
P13	10,6	99	658,8	658,4
P14	10,6	99	658,8	658,3
P15	10,9	99	658,8	658,4
P16	16,0	96	615,8	615,3
P17	16,2	96	615,8	615,3
P18	16,3	95	615,8	615,3

*column Phenomenex Kinetex, 0.46x10cm, 100Å, 3.5µ; 5-95%B over 30 min

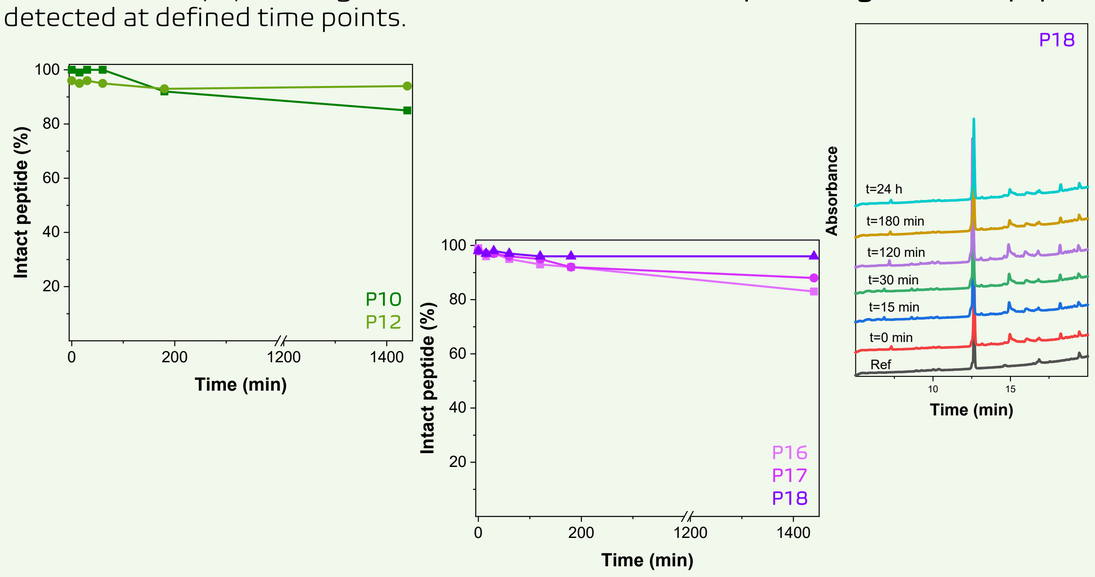
NMR characterization of P17



Superposition of 2D-NMR spectra of **P17**
(**A**: TOCSY-COSY; **B**: ROESY-COSY)

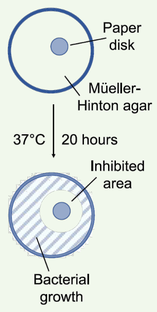
In serum stability

The stability of the peptides in the presence of human serum was monitored by HPLC–MS. The rate of peptide degradation was calculated as the **percentage of intact peptide** detected at defined time points.



The introduction of the NCAA 2-amino-3-(1H-imidazol-1-yl)propanoic acid does not affect significantly the resistance to degradation *in serum* of the ultrashort peptides under investigation, regardless of the acyl chain at N-terminus.

Antimicrobial activity



The antibacterial activity was tested on different Gram-positive and Gram-negative bacterial strains, according to the standardized disk diffusion Kirby–Bauer method. (2) Peptide activity was determined by measuring the diameters (in mm) of bacteria growth inhibition around the paper disks.

	Gram-negative bacteria				
Sample	Klebsiella aerogenes ATCC 13048	Salmonella enterica ATCC 13076	Escherichia coli ATCC 25922	Pseudomonas aeruginosa ATCC 27853	Acinetobacter baumannii ATCC 19606
P10	-	10	-	-	-
P11	-	-	-	-	-
P12	-	8	-	-	-
P13	-	-	-	-	-
P14	-	-	-	-	-
P15	-	-	-	-	-
P16	-	9	-	-	-
P17	10	8	9	8	-
P18	9	-	7	8	-

	Gram-positive bacteria									Fungi (yeast)
Sample	Bacillus Subtilis ATCC 6633	Staphylococcus aureus ATCC 25923	Enterococcus faecalis ATCC 29212	Streptococcus pyogenes ATCC 19615	Streptococcus mitis (clinical isolate)	Streptococcus oralis (clinical isolate)	Streptococcus pyogenes Group A (clinical isolate)	Streptococcus Group G (clinical isolate)	Enterococcus faecalis ATCC 51299	Saccharomyces cerevisiae ATCC 9763
P10	-	-	-	-	-	-	-	-	-	-
P11	-	-	-	-	-	-	-	-	-	-
P12	-	-	-	10	8	8	8	-	-	-
P13	-	-	-	-	-	-	-	-	-	-
P14	-	-	-	-	-	-	-	-	-	-
P15	-	-	-	-	-	-	-	-	-	-
P16	-	-	-	-	-	-	-	-	-	-
P17	11	9	12	-	-	-	-	-	-	12
P18	12	12	9	17	10	-	12	-	-	14

P17 and P18, containing the NCAA and the lauryl moiety at the N-terminus, resulted as the most promising candidates with a broad spectrum of activity against both Gram-positive and Gram-negative bacteria and yeast. Cytotoxicity tests are in progress.

References

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