# **$(RFLR)_3$ retro-inverse peptide containing D-amino acid as antimicrobial agent**

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The emerging bacterial resistance to conventional antibiotics has led to the search for new therapeutic alternatives. Antimicrobial cationic peptides are promising candidates, since they act on bacterial membranes causing their rapid destruction, with low tendency to generate resistance. However, these compounds present low stability against proteases. The aim of this study was to design a retro inverse analog containing D amino acids of oligomer $(RLFR)_3$ (TA4R), in order to evaluate the effect of substitution by D amino acids and inverted sequence on their biological properties and enzymatic stability. For this, RI-dTA4R was synthesized by solid-phase synthesis using Fmoc chemistry. The Minimal inhibitory concentration (MIC) was determined against the following bacterial strains: *Escherichia coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 27853, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25929, *Staphylococcus aureus* Methicillin Resistant SAMR1. Hemolytic activity and enzymatic stability were determined against human erythrocytes and digestive and serum proteases, respectively. DC analyses were performing in aqueous media and membrane mimetic vesicles.

RI-dTA4R showed antimicrobial activity against all bacteria strains tested (MIC=4,6 to 2,3 μM), increasing the inhibitory activity of TA4R. Substitution with D-amino acid allowed obtaining a peptide with improved enzymatic stability against trypsin, chymotrypsin and serum proteases. Nevertheless, this analog was more hemolytic than TA4R, presenting around 50% of hemolysis at 25 μM, but less than 20% of hemolysis at MIC concentration. CD analysis showed that the peptide in DPPG vesicles is strongly structured, adopting a levorotatory helical structure, while in DPPC vesicles it is less structured.

Based on these results, retro-inverse peptides containing all D-amino acid may be considered as potential therapeutic compounds for treatment of infections produced by gram (+) and (-) bacteria with improved enzymatic stability.

Keywords: antimicrobial peptides, D amino acid, enzymatic stability.