NMR-ASSISTED BIOMETAL CHELATION STUDY OF NEW L-CARNOSINE MIMICS: PROMISING NEUROPROTECTIVE AGENTS[1]

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Alzheimer's Disease (AD) is a **neurodegenerative** syndrome that slowly destroys memory and thinking skills, resulting from complex changes in the brain that begin years before symptoms appear and lead to the loss of brain cells and their connections.[2] As a neurodegenerative syndrome, it has a multifactorial etiology stemming from: 1) a decrease in the activity of the Ubiquitin-Proteasome System (UPS), which entails the accumulation of altered proteins; 2) the loss of function of molecular chaperones, resulting in protein misfolding; 3) the deposition of aggregated proteins; 4) an **imbalance of metal ions** that promotes aggregation and neuroinflammation.
In this context, NPs with multi-target pharmacological activities appear to be a fundamental mine for the design of new drugs for treating diseases with multi-pathogenic factors.
L-Car is a dipeptide (*Figure 1*) widely distributed in mammalian tissues and serves as a potential drug candidate[3] for neurodegenerative syndromes due to its radical scavenger, anti-inflammatory, antiaggregant, antiglycation, and **well-known zinc-chelating activities**.[4]

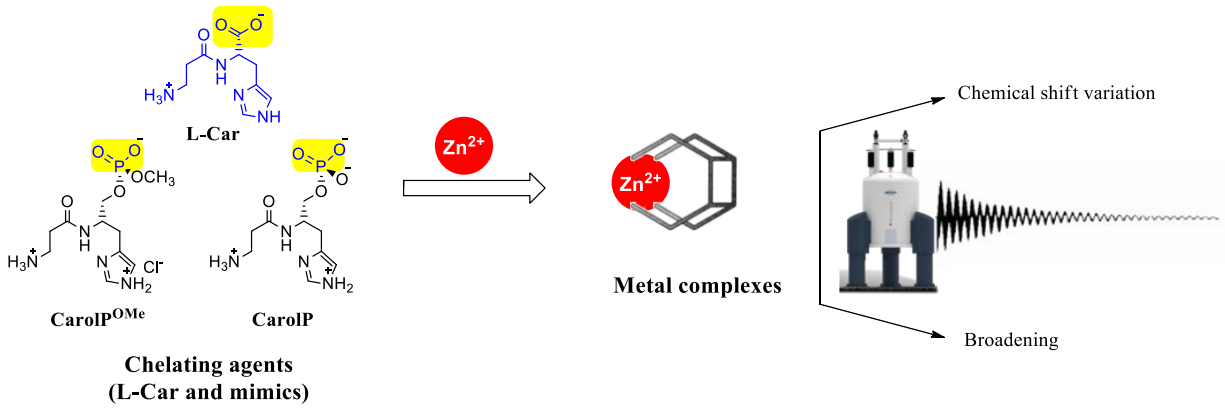

***Figure 1**: L-Car and mimics Zn(II) titration via NMR strategy.*

Unfortunately, the therapeutic potential of L-Car is limited by its low bioavailability, as it is rapidly degraded by serum carnosinase (CN1), a specific zinc dipeptidase that hydrolyses L-Car into β-alanine and L-histidine by recognizing the carboxyl group. Different studies on both the mechanism of CNs digestion as well as the affinity for the hPEPT1 transporter, involved in the intestinal absorption of L-Car, have inspired useful modifications to the dipeptide skeleton, with the aim of improving its stability, thus enhancing the protective functions.[5] Many modifications have involved both the C-terminal group[6,7] and the amino group at the N-terminal[8] as well as the introduction of side chains into β-alanine.[9]

In this frame, here, we synthesized new **L-Car mimics** characterized by phosphate isosteres of the carboxylic group starting from the L-Carnosinol (L-Carol) synthon, where the carboxylic group is reduced to OH group (*Figure 1*). The strategic synthesis of a tailored L-Carol unit significantly broadens the synthetic toolbox for the rational design of structurally diverse L-Car mimics, paving the way for the development of next-generation compounds with enhanced therapeutic potential.
The stability of the newly settled mimics to serum carnosinase CN1 will also be discussed.
Therefore, here we illustrate the capability of the novel mimics Carolp and Carolp^{OMe} (*Figure 1*) to chelate the Zn(II) ion through **titration experiments** using the **NMR technique** and to compare it with that of L-Car.
The titration experiments were designed to ensure the reproducibility of the results. Notably, in the presence of the titrating salt, the mimics exhibited high chelating power at pH 7, unlike the precursor L-Car. The NMR analyses were performed at pH 7 and T 310 K to simulate physiological conditions, revealing two main consistent events in the spectra: a variation in chemical shift, common to both L-Car and the mimics, and broadening until complete flattening of the signals, occurring only for Carolp. The shifts were indeed larger for the mimics compared to their precursor. Based on the regions of shift and/or broadening, we anticipated the structures of the complexes (*Figure 2*), which will be confirmed by further experiments. Replacing the carboxylic group with the phosphate group enhances interaction with Zn(II), and this finding provides a basis for further experiments involving co-titration and diffusion and for designing and synthesizing novel isosteres with improved chelating profiles.

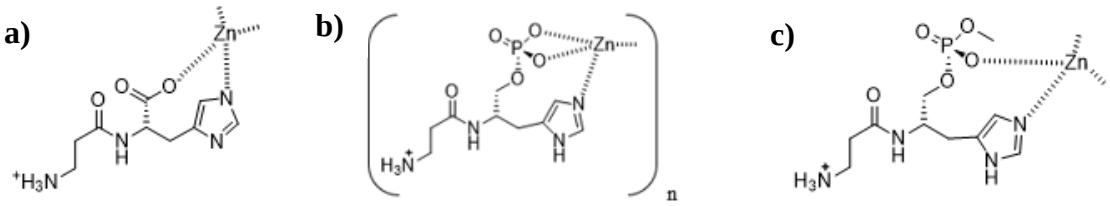

Figure 2*: Prediction of Zn(II) complex via NMR strategy of a) L-Car, b) Carolp, c) Carolp^{OMe}.*

Acknowledgements**:** *Gruppo Italiano Discussione Risonanze Magnetiche (GIDRM), C/O Prof. Daniel Oscar Cicero, Università degli studi di Roma "Tor Vergata", Via della Ricerca Scientifica 1, 00133 Rome, Italy*

[1] These studies were supported by AIPRAS Onlus (http://www.aipras.org/)
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