

SUPPORTING INFORMATION

New biotinylated GHK and related copper(II) complex: antioxidant and antiglycant properties against neurodegenerative disorders

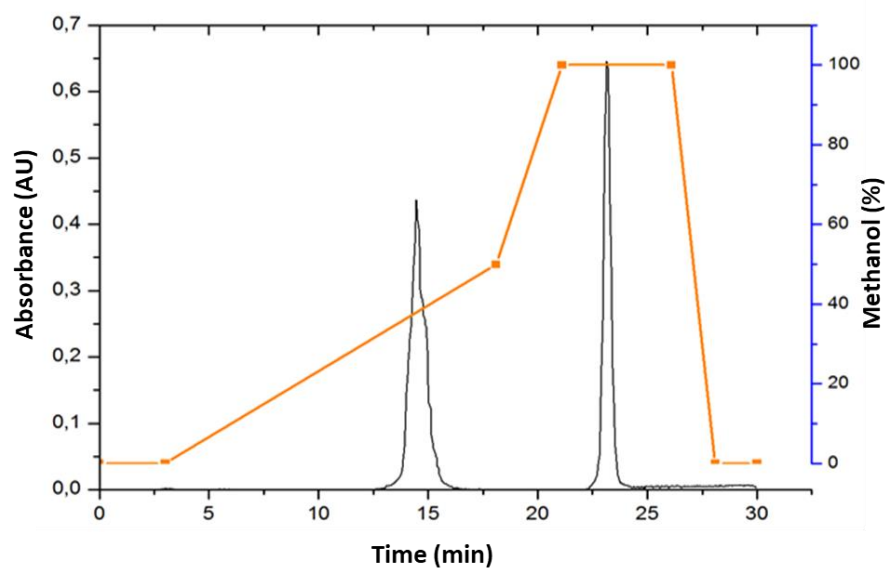
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A



B

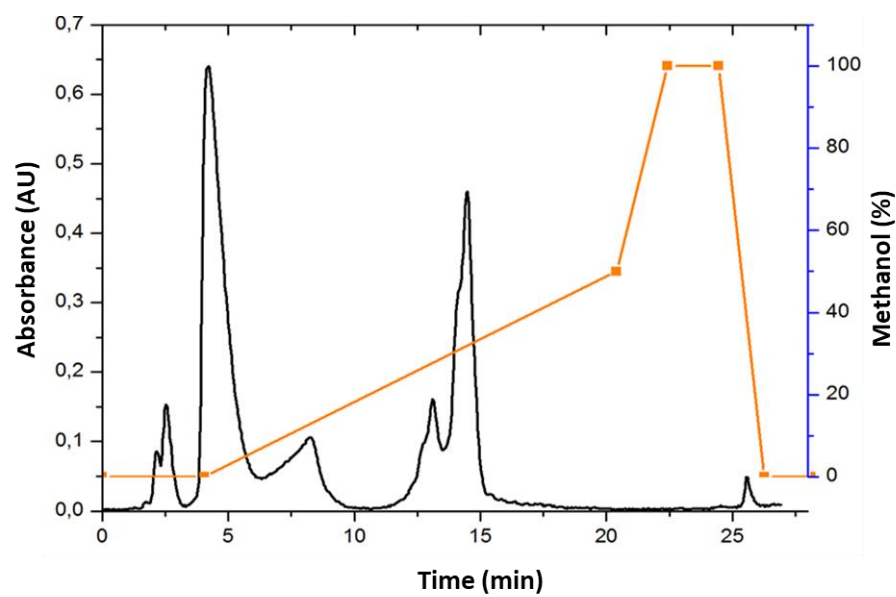


Figure S1. Chromatograms related to purification of (A) GHK-diBOC and (B) BioGHK

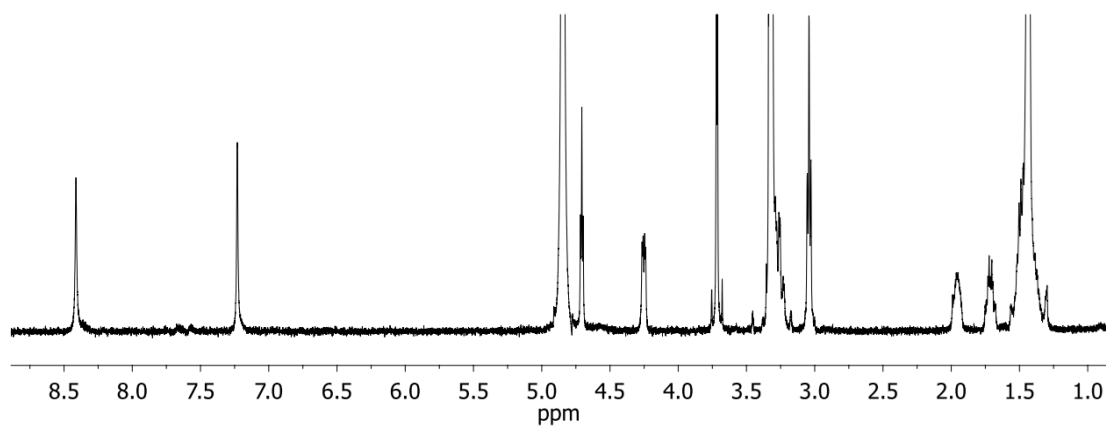
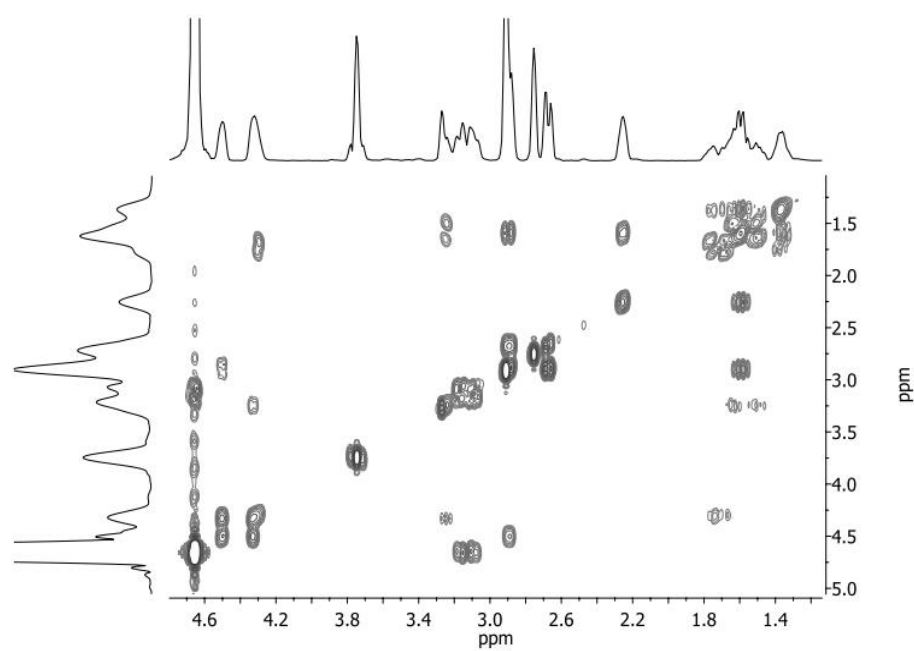


Figure S2. ^1H NMR spectrum of GHK-di-BOC (CDOD, 500 MHz).

A



B

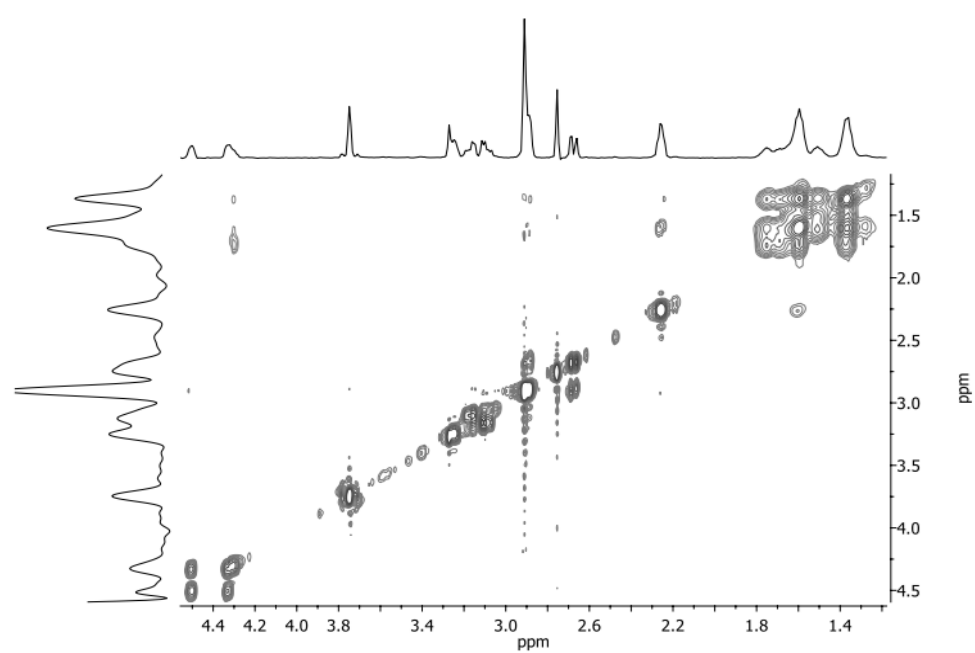


Figure S3. (A) COSY and (B) TOCSY spectra of BioGHK.

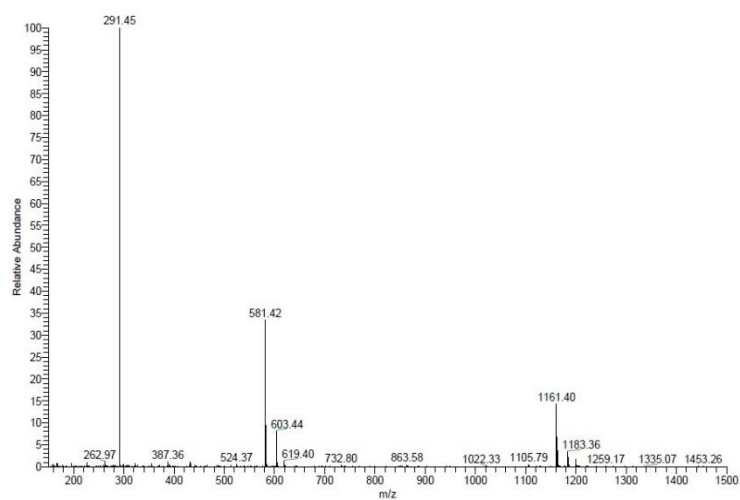


Figure S4. ESI-MS spectrum of BioGHK.

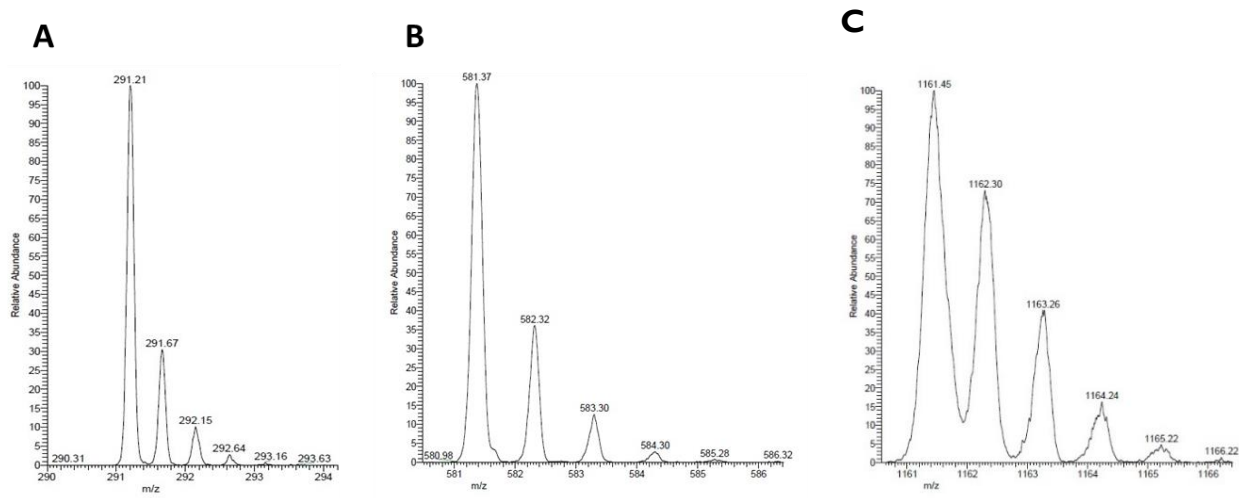


Figure S5. Zoom Scan of the signals relating to the species (A) $[M+2H]^{2+}$, (B) $[M+H]^+$ and (C) $[2M+H]^+$ (M = GHK).

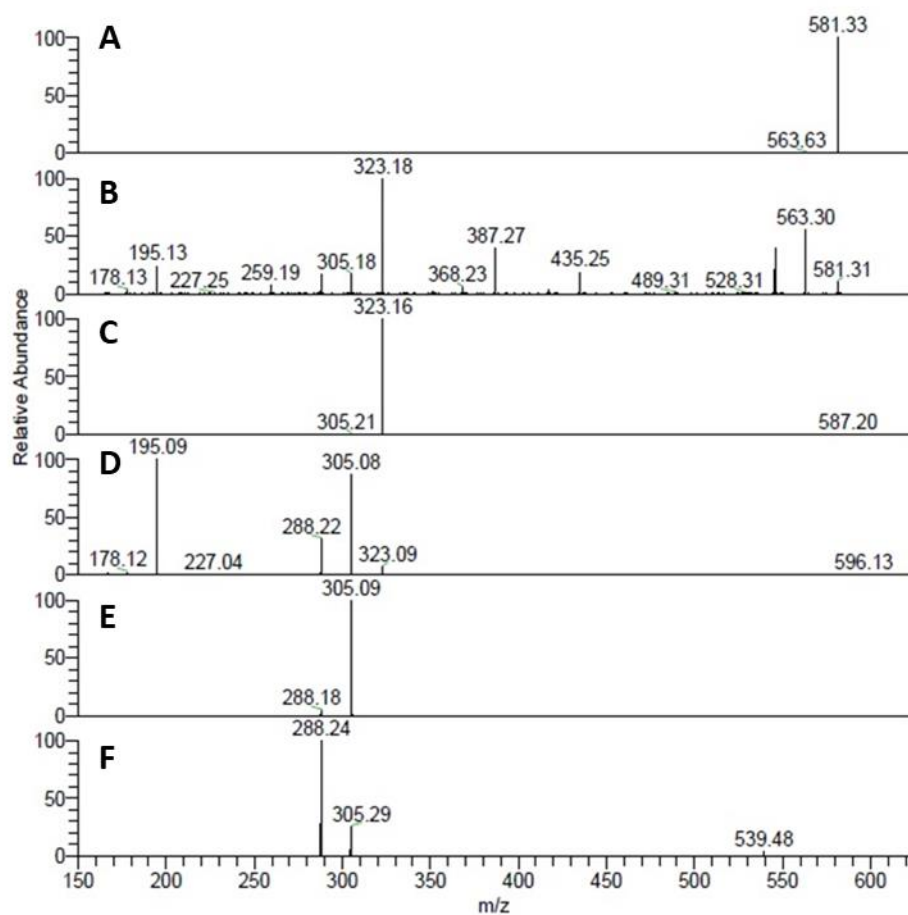


Figure S6. Multidimensional fragmentation of BioGHK, whose the selected mono-charged protonated adduct (A: m/z 581.3, C: m/z 323.2, E: m/z 305.8) were fragmented to produce different signals (B: MS2@581.3, D: MS3@323.2, F: MS4@ 305.8)

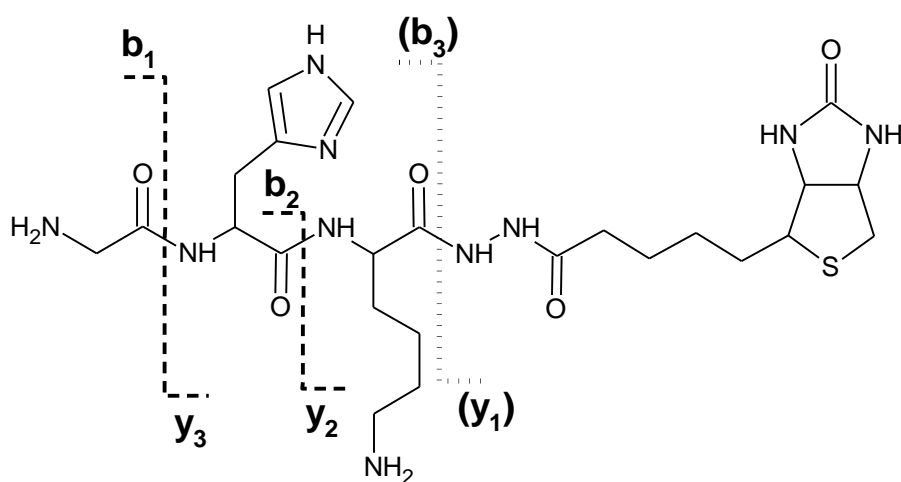


Figure S7. ESI-MS theoretical fragmentation scheme of BioGHK.

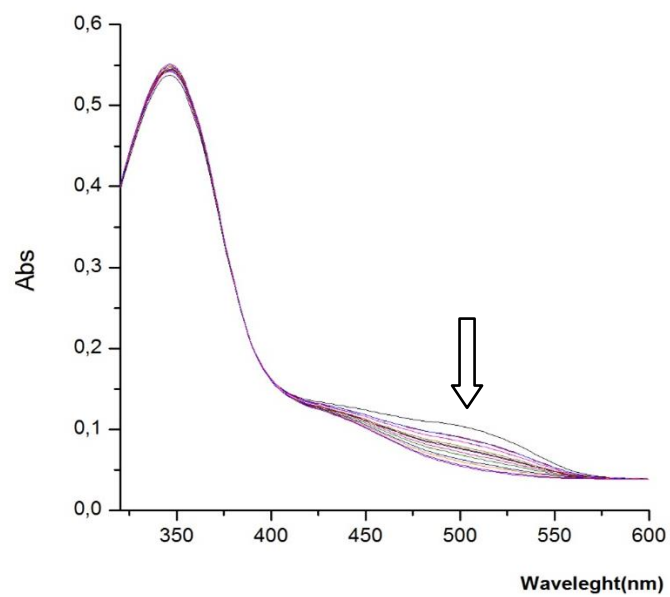


Figure S8. Spectrophotometric titration of Sav-HABA with BioGHK. The arrow shows the decrease trend of the absorption band centered at 500 nm.

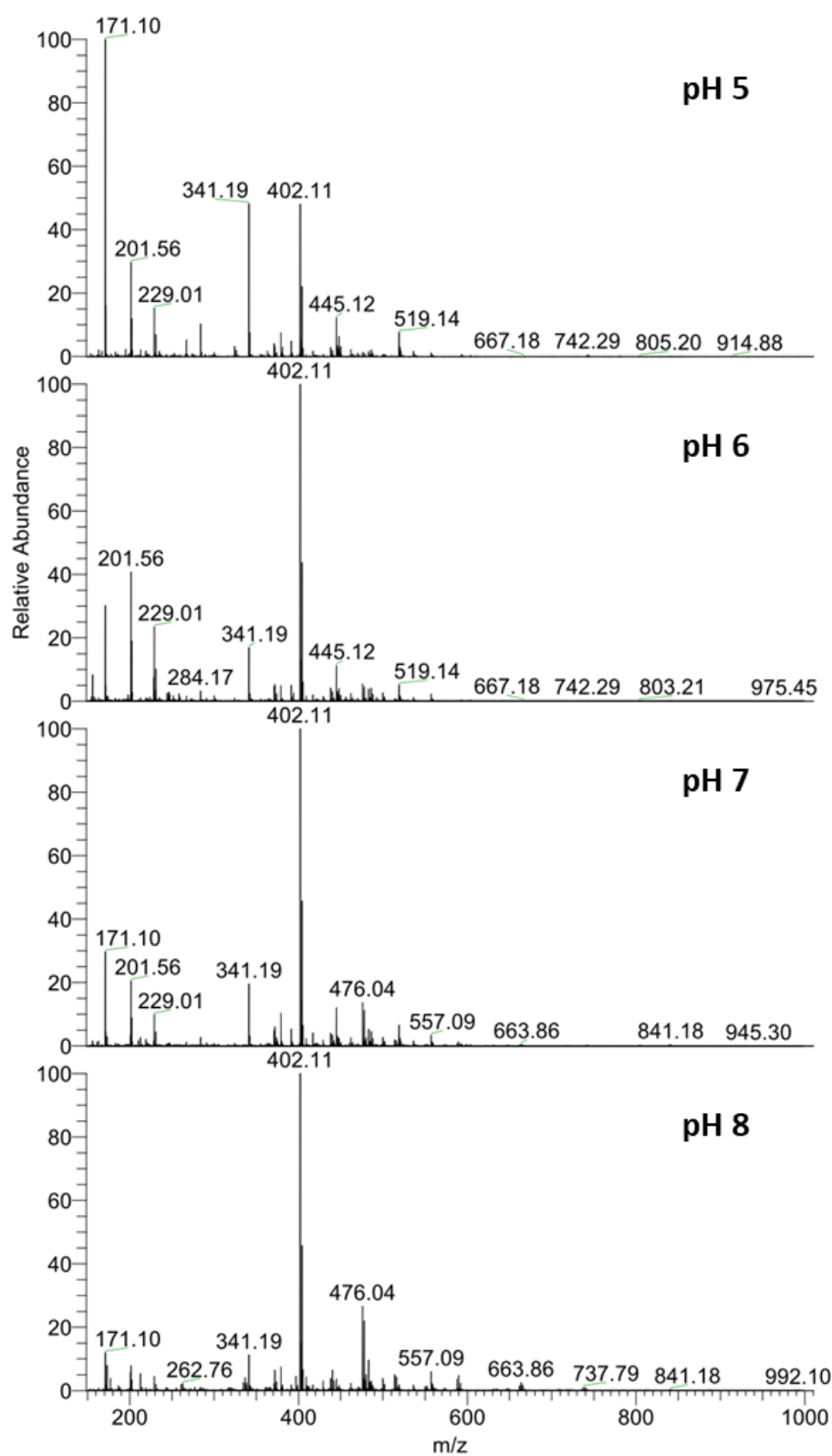


Figure S9. ESI-MS spectra of Cu(II)-GHK complex at various pH values (5-8).

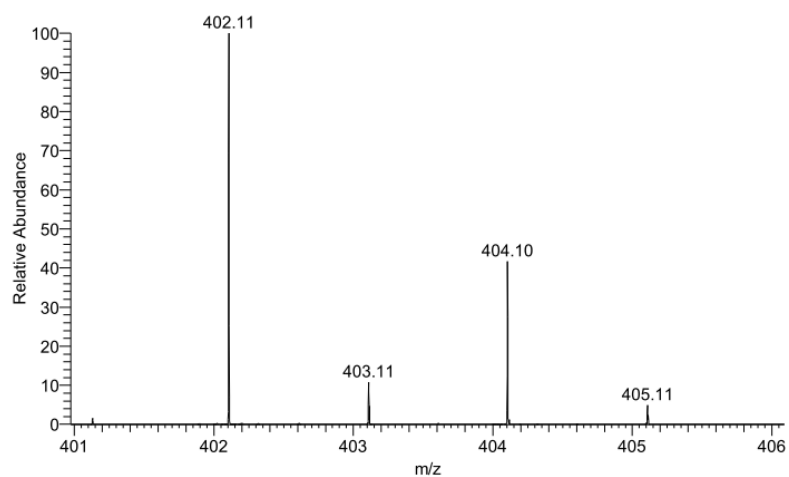


Figure S10. *Zoom scan* of the main complex species of the Cu-GHK system at pH 7.

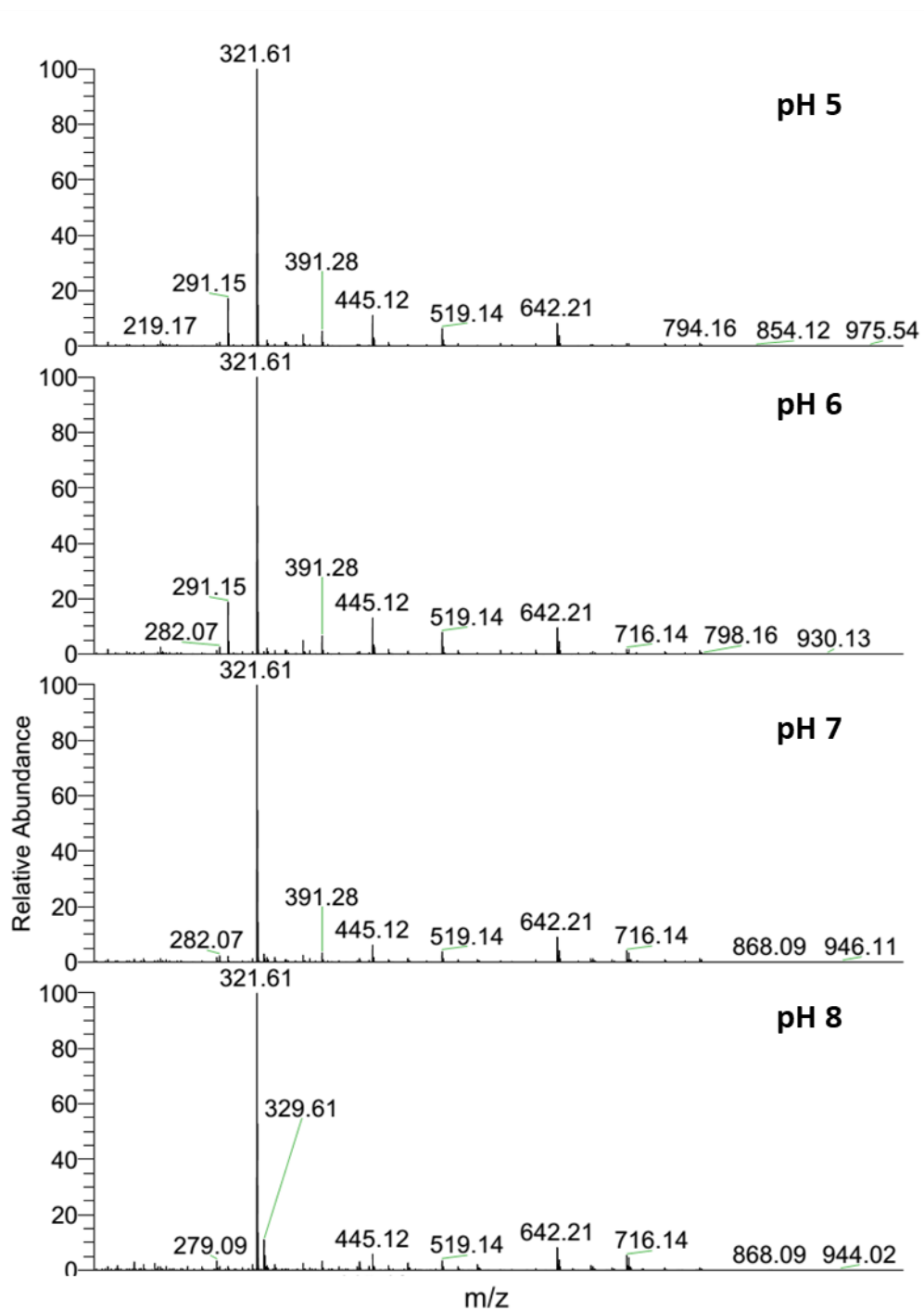
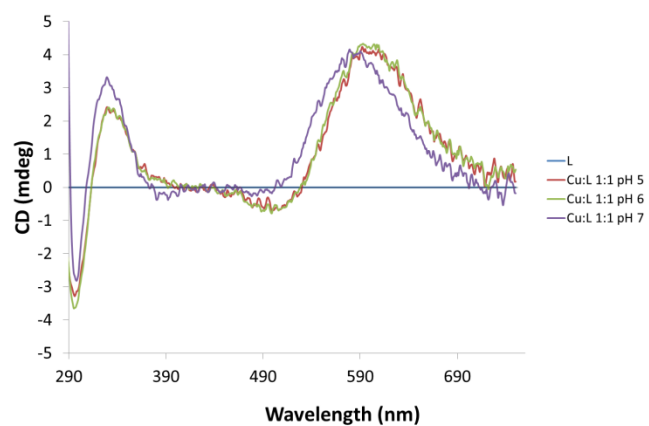
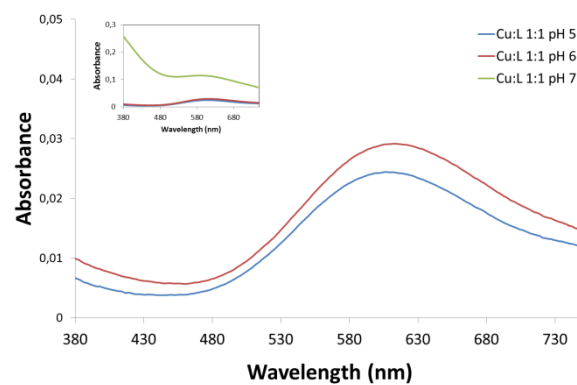


Figure S11. ESI-MS spectra of Cu(II):BioGHK 1:1 at various pH values (5-8).



A



B

Figure S12. A) CD spectra of the Cu(II) complex (L = BioGHK); B) absorbance of the BioGHK/Cu(II) complex in the Vis region.

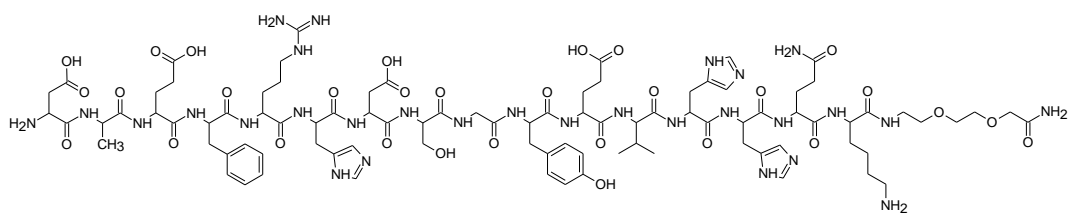


Figure S13. A β_{1-16} amino acid sequence with a PEG residue.

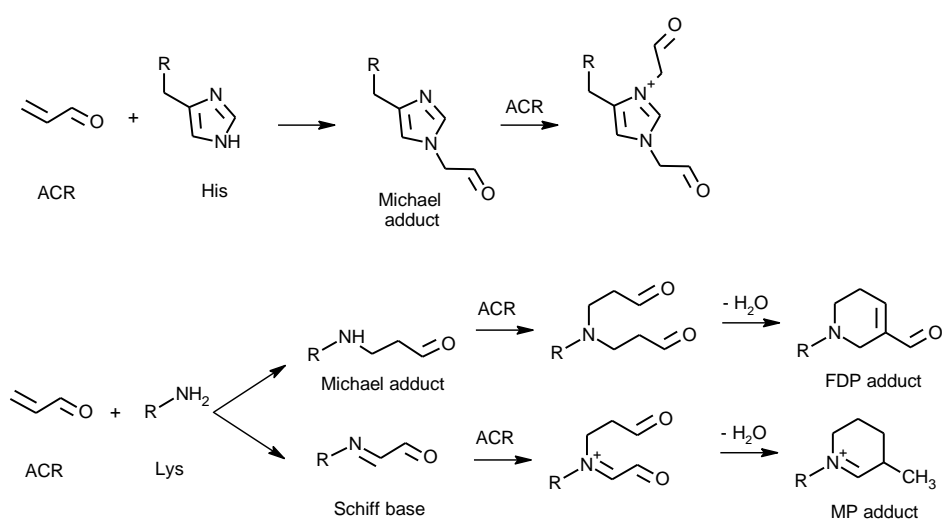


Figure S14. Main adducts between ACR and the histidine and lysine side-chains.

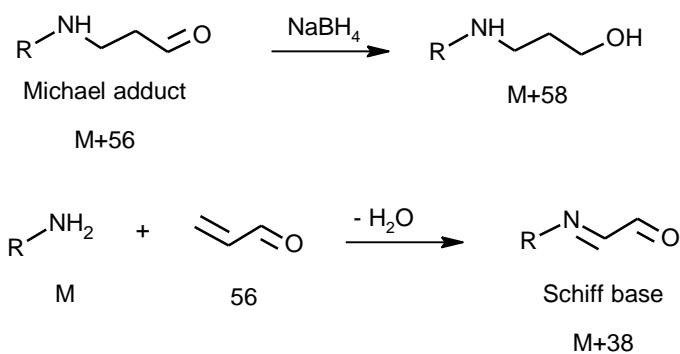


Figure S15. Reduction of the aldehyde group of the Michael adduct by NaBH₄ (upper) and formation of the Schiff base (lower).

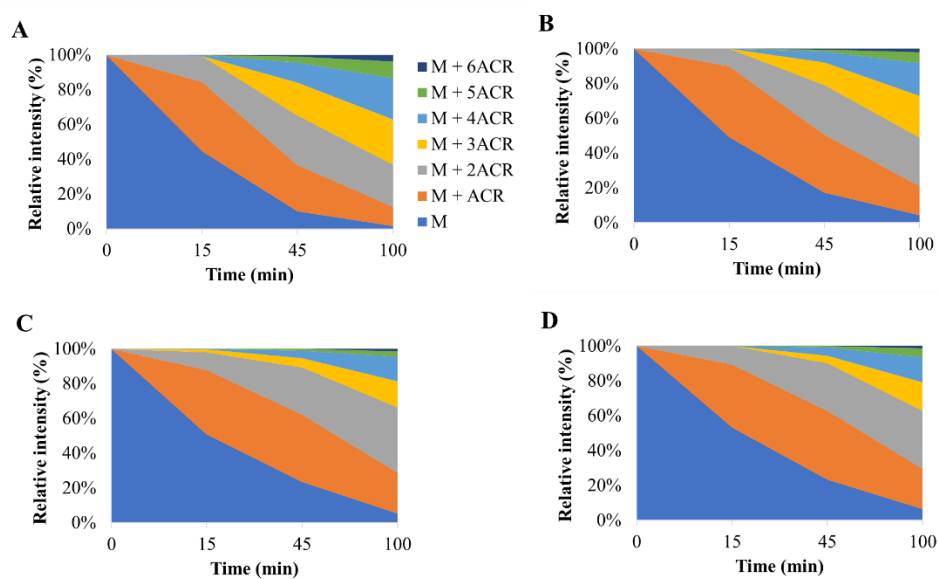


Figure S16. Relative intensity of all species detected by MALDI mass spectrometry following the reaction between A β and ACR in the absence (A) and in the presence of GHK, the A β :GHK molar ratio being 1:1 (B), 1:2 (C) and 1:3 (D)

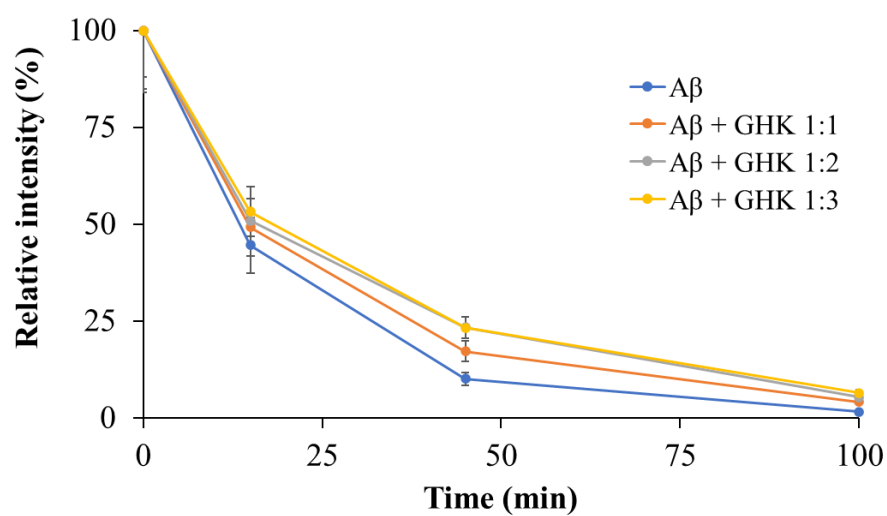
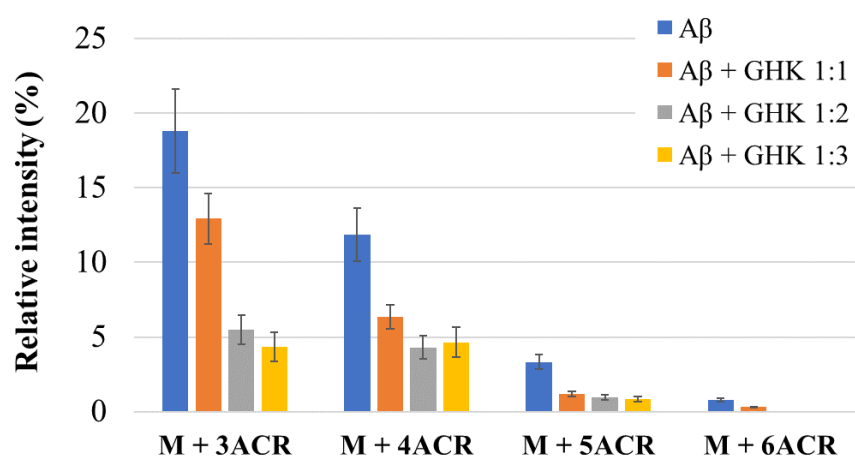
A**B**

Figure S17. (A) Time-dependent amount of non-carbonylated A β in the absence and in the presence of increasing concentrations of GHK. (B) Relative intensity of the carbonylated species of A β at increasing concentrations of GHK, after 45 minutes of reaction.

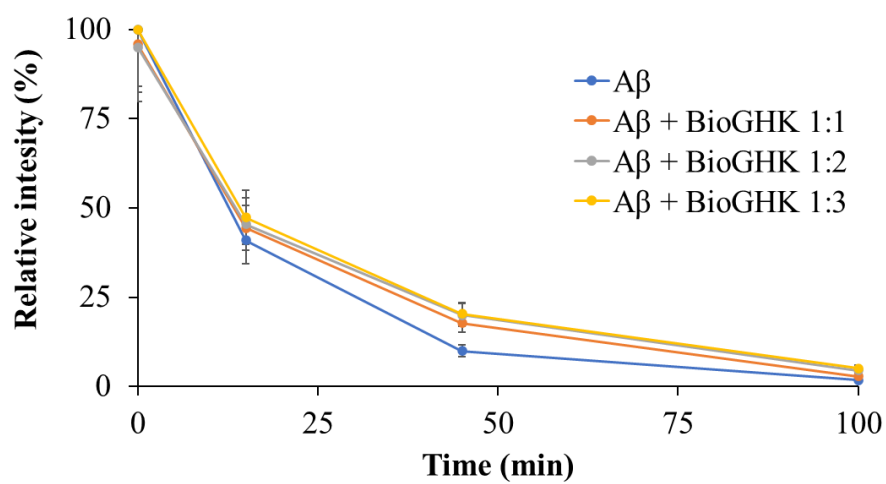


Figure S18: Variation of non-carbonylated A β over time in the absence and in the presence of increasing concentrations of BioGHK.

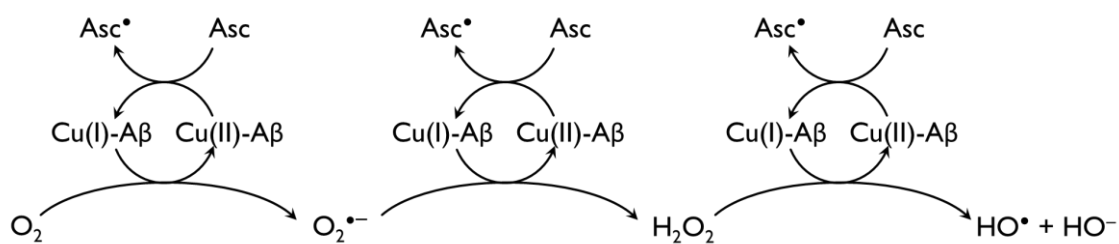
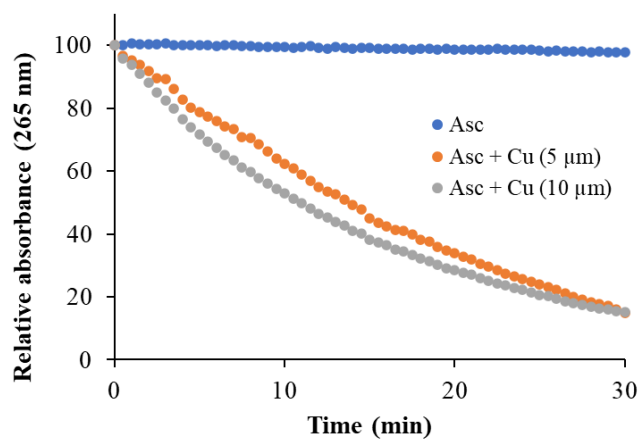


Figure S19. ROS formation determined by the Cu-Aβ complex, in the presence of ascorbate.

A



B

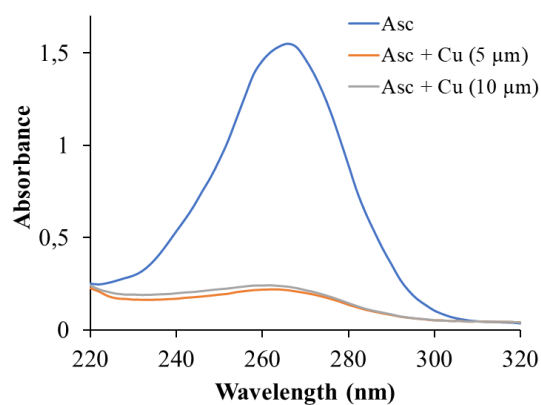


Figure S20. (A) Variation of the absorption of Asc (200 μM) at 265 nm, in the absence and presence of Cu^{2+} (5 and 10 μM). (B) Absorption spectrum of Asc after incubation for 30 min in the absence and in the presence of Cu^{2+} (5 and 10 μM).

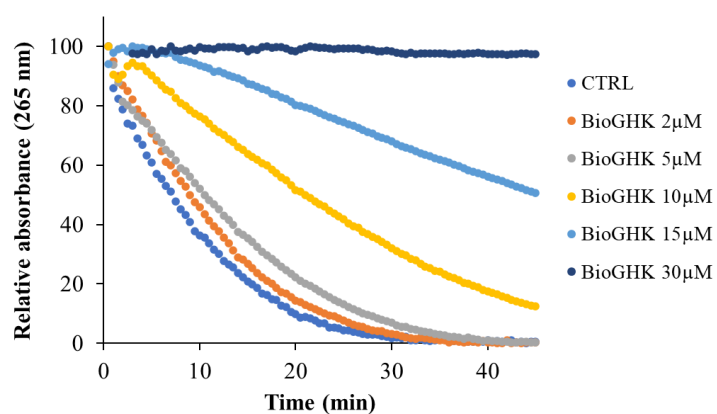
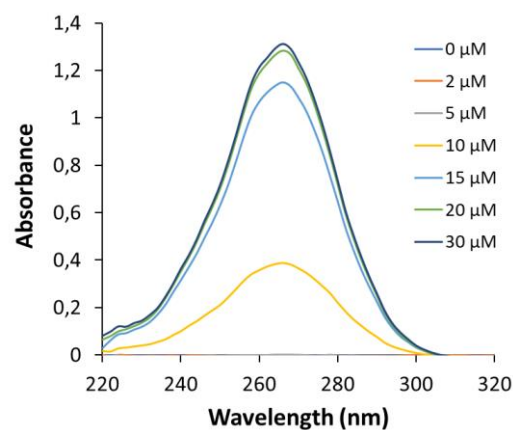
A**B**

Figure S21. (A) Asc oxidation kinetics induced by the Cu^{2+} -A β complex in the absence and in the presence of GHK (0-30 μM). (B) Absorption spectra of ascorbate (200 μM) after incubation for 45 min, in the absence and in the presence of GHK (0-30 μM).

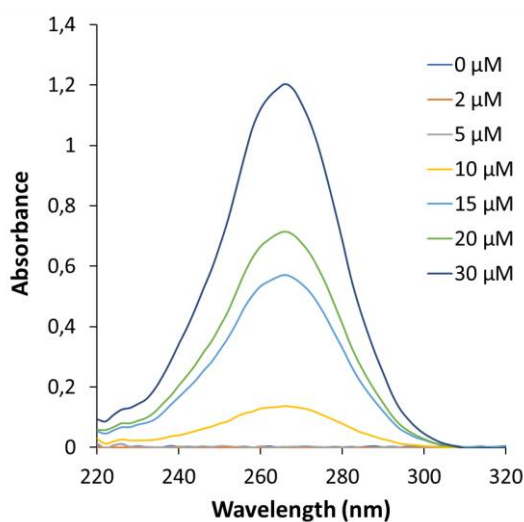


Figure S22. Absorption spectra of ascorbate (200 μM) after incubation for 45 min, in the absence and in the presence of BioGHK (0-30 μM).

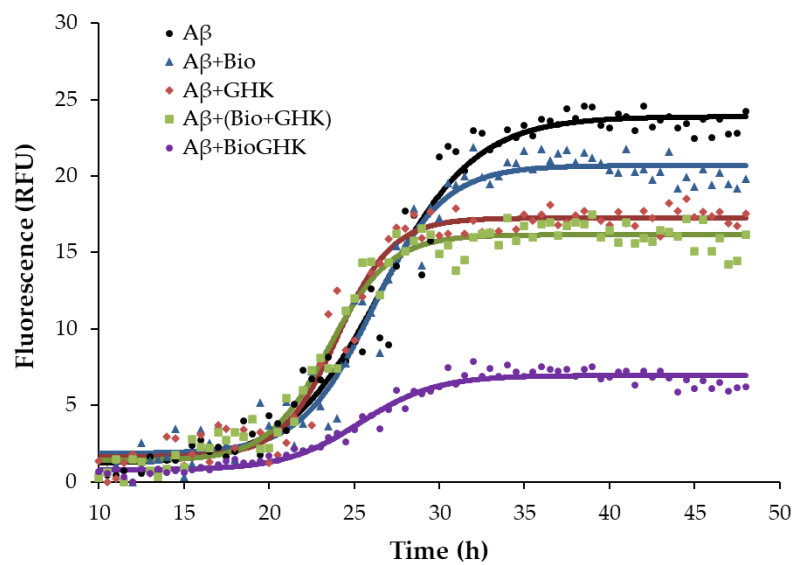
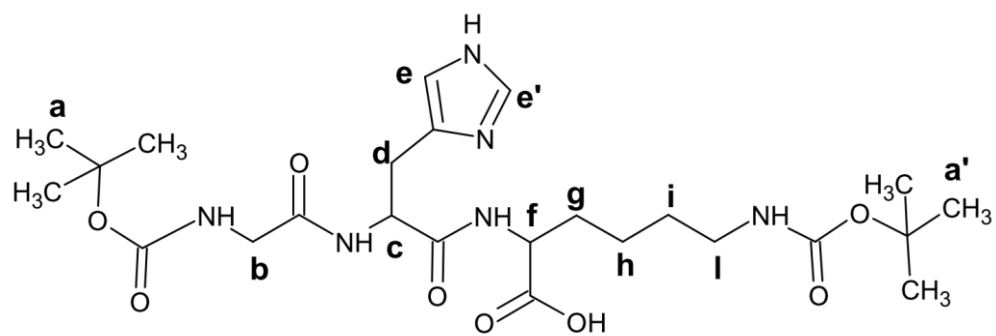


Figure S23. Kinetic profiles of A β_{1-42} aggregation alone (A β , 20 μ M), incubated with biotin (Bio), GHK, an equimolar mixture of Bio and GHK (Bio+GHK), and BioGHK, the A β /compound molar ratio being 1:1.

A



B

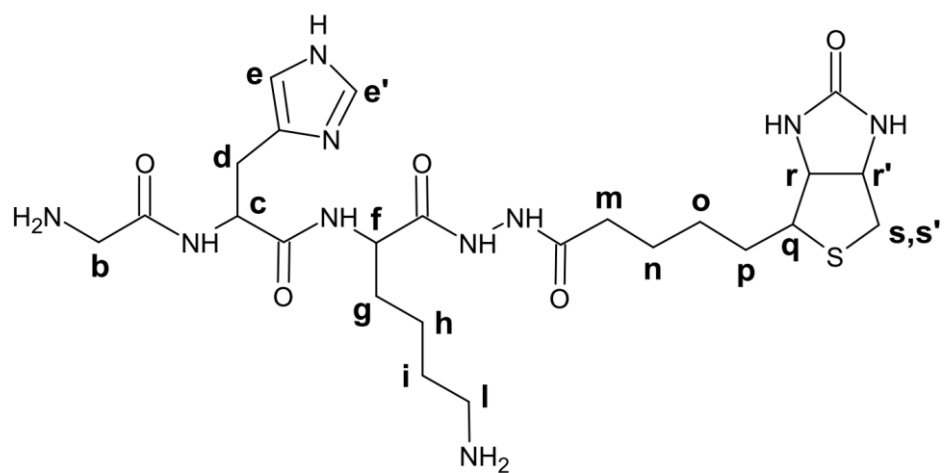


Figure S24. Proton assignment by NMR of GHK-di-BOC (A) and BioGHK (B).