

Supplementary Information

Transition Metals Enhance Prebiotic Depsipeptide Oligomerization Reactions Involving Histidine

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Materials and Methods

Materials

All reagents were purchased from Sigma-Aldrich and were of analytical grade, unless stated otherwise. The histidine used was in its salt form, L-Histidine monohydrochloride monohydrate (H8125 Sigma). L- β -imidazole lactic acid was purchased from Toronto Research Chemicals (#I355000).

Dry-down reactions

For depsipeptide polymerization experiments, aqueous solutions of 1M glycolic acid (glc) and histidine (His), alanine (Ala), or lysine (Lys) at a 1:1 molar ratio or 5:1 molar ratio (in favor of glc), were allowed to dry at 85 °C for one week. Dry-downs were carried out in the presence or absence of various metals at 1:1 molar ratio (amino acid: M). All metals had chlorine as their anion: ZnCl₂, NaCl, LiCl, KCl, MgCl₂, CoCl₂, CaCl₂, CuCl₂, and NiCl₂. Control reactions contained His alone. For the experiments involving L- β -imidazole lactic acid (his), his solution (200mM) was dried with glycolic acid (glc) at a 1:1 molar ratio, at 85 °C for one week. Dry-downs were carried out in the presence or absence of zinc at 1:1 molar ratio (his: Zn²⁺). Prior to analysis, samples were resuspended in ultrapure water to 100 mM concentration based on original amino acid concentration, vortexed, sonicated and centrifuged at 15,294 x g for 5 min.

Mass spectrometry

All peak assignments correspond to [M-H]⁻ ions. Samples directly infused into mass spectrometer system using the following parameters: Running Solvent: 95% H₂O, 5% Acetonitrile. Flow rate: 0.5mL/min. 5 μ L Injection with H₂O needle wash. UV detection 210nm and 220nm. 0.6cm path length. Scanning ± 65 - ± 2000 m/z. Equipment: ESI-MS – Agilent 6130 single quad MS (Agilent Technologies, Santa Clara, CA) and with UV detector coupled to Agilent 1260 HPLC. Capillary voltage: 3.0kV. Fragmentor voltage: 70V. For direct inject analysis of His-containing depsipeptides, samples were separated via C18-HPLC and fractions were collected and combined from min 2-17 in order to remove any remaining zinc prior to analysis via negative mode ESI-MS.

High Performance Liquid Chromatography

HPLC analyses were conducted using an Agilent 1260 quaternary pump and Agilent 1260 Autosampler with DAD UV-vis detector, with a path length of 1.0cm. Samples were separated using a Phenomenex Kinetex 2.6mmxB-C18100Å, LC column 150x2.1mm. Column temp: 25°C. 10 μ L Injection. Solvents: solvent gradient was as follows: A) 0.1% formic acid in LC-MS grade water, B) LC-MS grade acetonitrile. Flow Rate: 0.3 mL/min. Gradient: 5 min 100% A, 0% B; 20 min ramp to 45% A, 55% B; 10 min 0% A,

100% B; 1 min ramp 100% A, 0% B; 14 min 100% A, 0% B. Wavelengths recorded 210 and 220nm, with entire spectrum 180-400nm detected in 2nm steps.

CD spectroscopy

The CD spectra were collected at 5 °C using a JASCO J-810 CD spectrometer. Scans were from 260 nm to 190 nm with a resolution of 1 nm. The 0.1 mm cuvette contained 0.5 mM solution of His, 2.5mM glc, 50mM Tris buffer (pH=7.2), in the presence or absence of various metals, at varying concentrations (0.05mM – 5mM). For analysis of depsipeptides resulting from dry-down of His and glc, the 0.1 mm cuvette contained 0.5 mM solution of His and 2.5mM glc referring to the starting concentration. The samples also contained 50mM Tris buffer, in the presence or absence of metals.

NMR spectroscopy

Prior to NMR analysis, sample aliquots were lyophilized and dissolved in a phosphate buffer (pH 2.5) in D₂O and NMR spectra were recorded on a Bruker Avance II-500 MHz spectrometer. A long relaxation delay time of 15 seconds was used for dry down mixtures to ensure quantitative integration of the resonances. Data were processed and spectra were plotted with MestReNova software package. For His, the conversion the amino acid into oligomer products was estimated from integration of the free, non-reacted imidazole proton ¹H NMR resonance, with internal integration to its β-protons. For his, the conversion the hydroxy acid into oligomer products was estimated from integration of the free, non-reacted α-proton ¹H NMR resonance, with internal integration to its β-protons.

Supplementary Figures

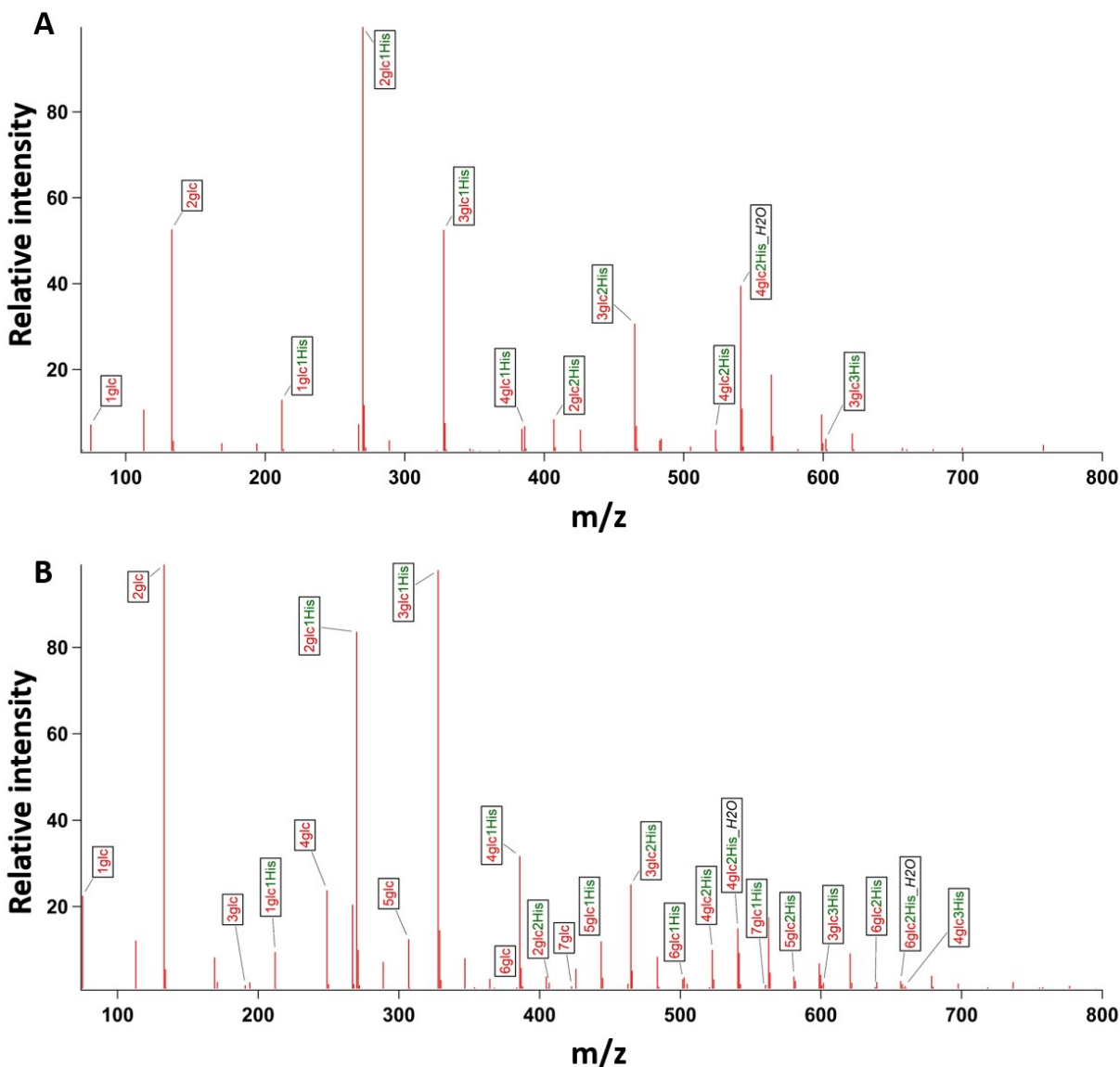


Figure S1. ESI-MS of a dry-down reaction of glc and His verified the increased abundance of longer His-containing depsipeptides following dry-down with zinc. His was dried with glycolic acid (glc) at a 1:1 molar ratio at 85°C for seven days in the absence (A) or presence (B) of zinc, at a 1:1 molar ratio (His:Zn²⁺). For analysis by ESI-MS, samples were separated via C18-HPLC and fractions were collected and combined in order to remove any remaining zinc. Analysis of samples via negative-mode ESI-MS indicated that zinc led to an increased abundance of longer His-containing depsipeptides following dry-down reactions. glc is labeled in red, His is labeled in green. Labeled species correspond to [M-H]⁻ ions.

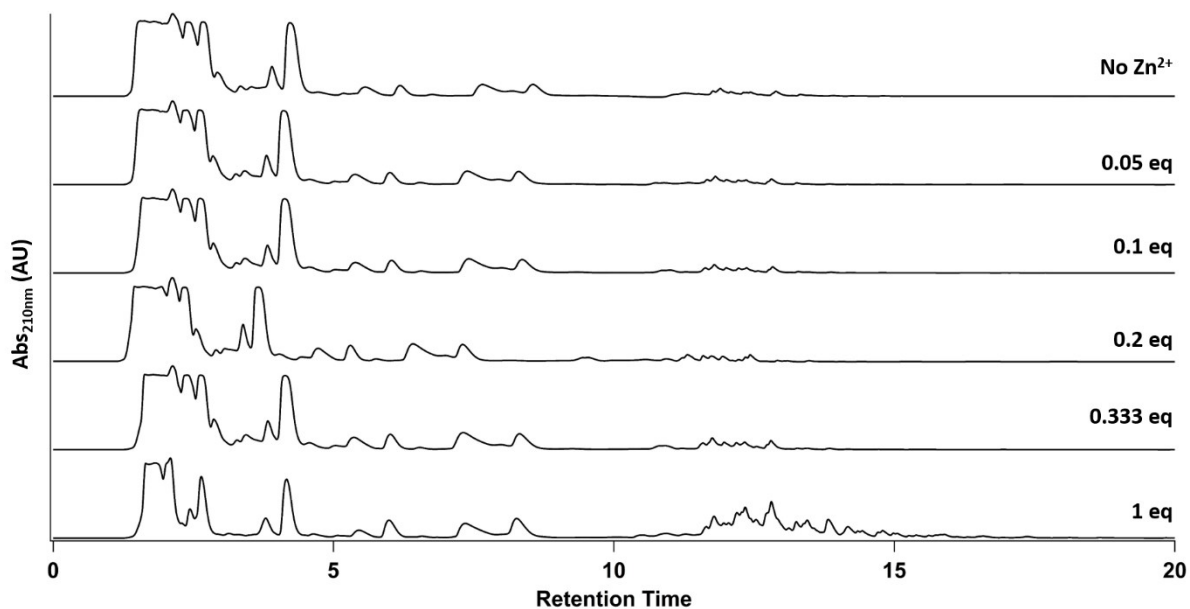


Figure S2. The effect of zinc on polymerization of depsipeptides containing His is dose-dependent. Glycolic acid (glc) and His were dried at 85°C for seven days, at a 1:1 molar ratio, in the presence or absence of zinc at various molar ratios. Number of zinc equivalents added refers to the His monomer (whose amount was held constant). Analysis of product mixtures via C18-HPLC showed that minor effects on polymerization are seen at low added equivalents of zinc, whereas at a 1:1 molar ratio (Zn²⁺:His) the effect is the greatest.

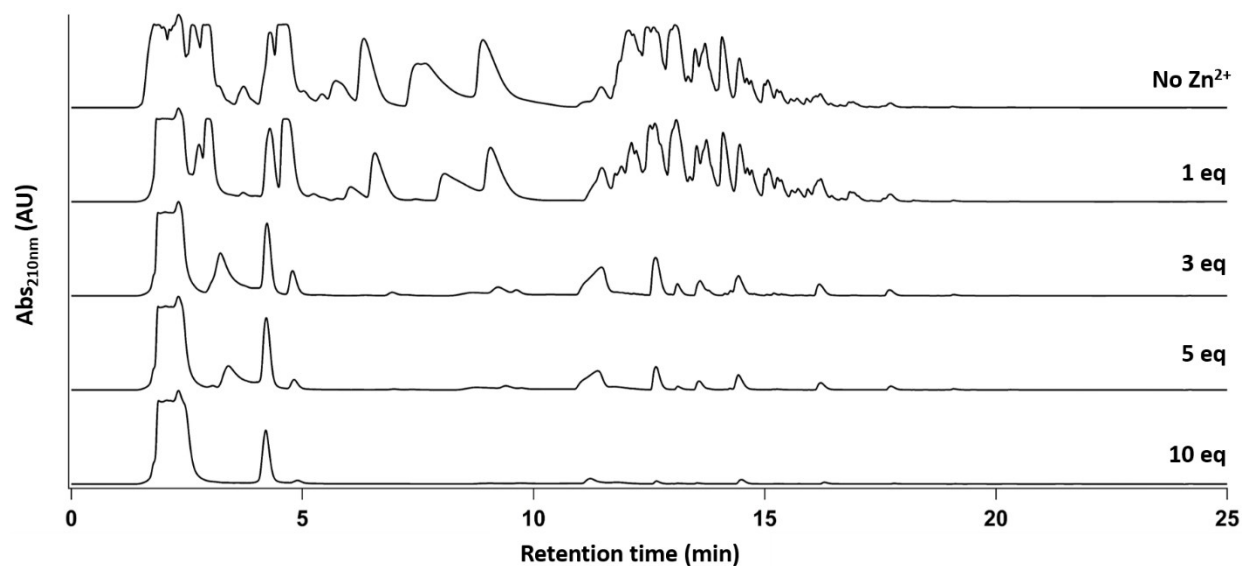


Figure S3. Zinc at high concentrations inhibits polymerization of depsipeptides containing His. Glycolic acid (glc) and His were dried at 85°C for seven days, at a 5:1 molar ratio in favor of glc, in the presence or absence of zinc at various molar ratios. Number of zinc equivalents added refers to the amount His monomer (that was held constant). Analysis of product mixtures via C18-HPLC showed that zinc inhibited polymerization of depsipeptides containing His at high amount of added equivalents.

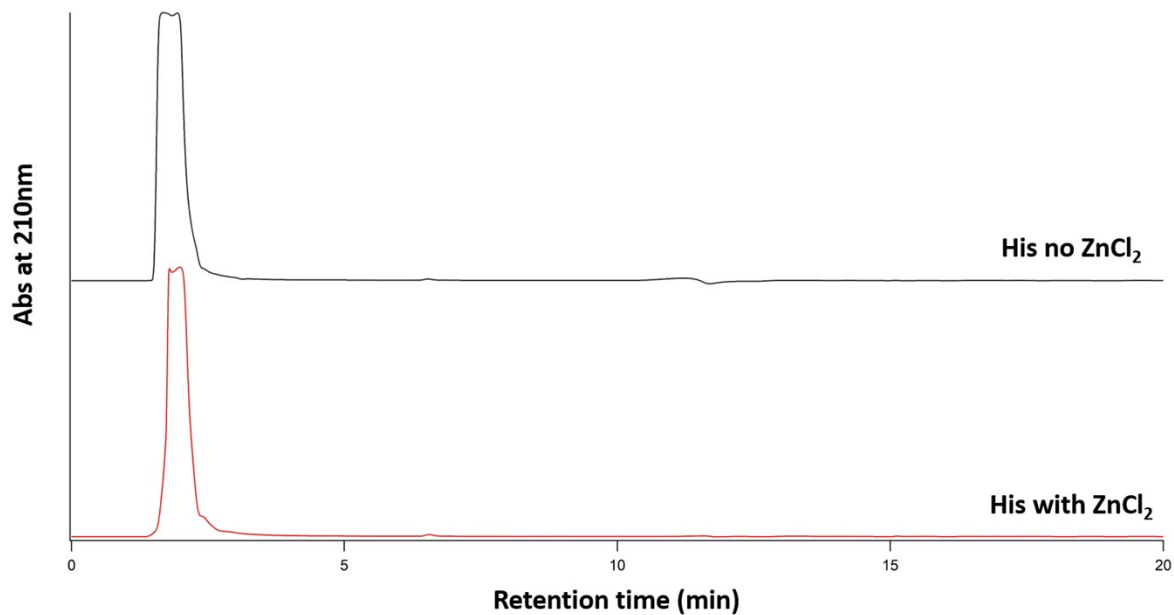


Figure S4. Zinc does not affect control dry-down reactions of His in the absence of glycolic acid. His monomer was dried at 85°C for seven days in the presence or absence of zinc at a 1:1 molar ratio (His:Zn²⁺). Analysis of samples via C18-HPLC verified that no peptides formed upon dry-down reactions of His in the absence of glycolic acid, regardless of the presence of zinc.

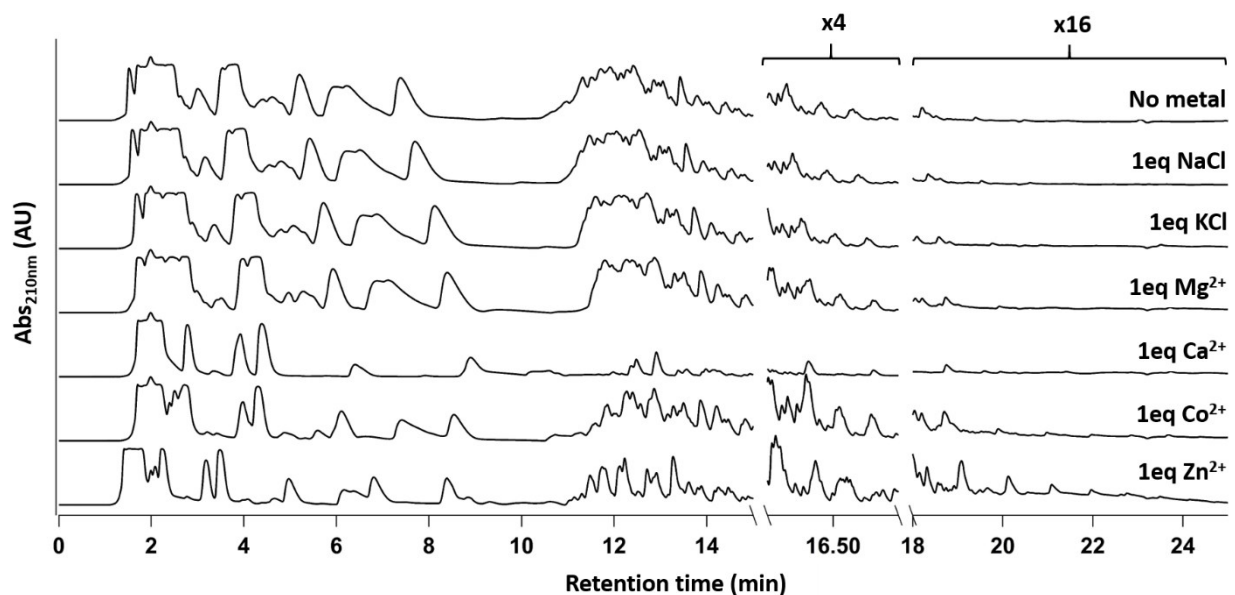


Figure S5. The effect of various metals on oligomerization reactions of His- containing depsipeptides. Glycolic acid (glc) and His were dried at 85°C for seven days, at 5:1 molar ratio in favor of glc, in the presence or absence of various metals. The molar ratio between the metal added to that of His was 1:1. Analysis of product mixtures via C18-HPLC showed that while both Zn²⁺ and Co²⁺ increased the polymerization of various His-containing depsipeptides, Na⁺, K⁺, and Mg²⁺ did not affect it. On the other hand, Ca²⁺ hindered the polymerization of depsipeptides under the dry-down conditions. Notably, the dry-down mixtures containing Zn²⁺ and Co²⁺ had aberrant precipitates, and only the supernatant was analyzed via the HPLC analysis.

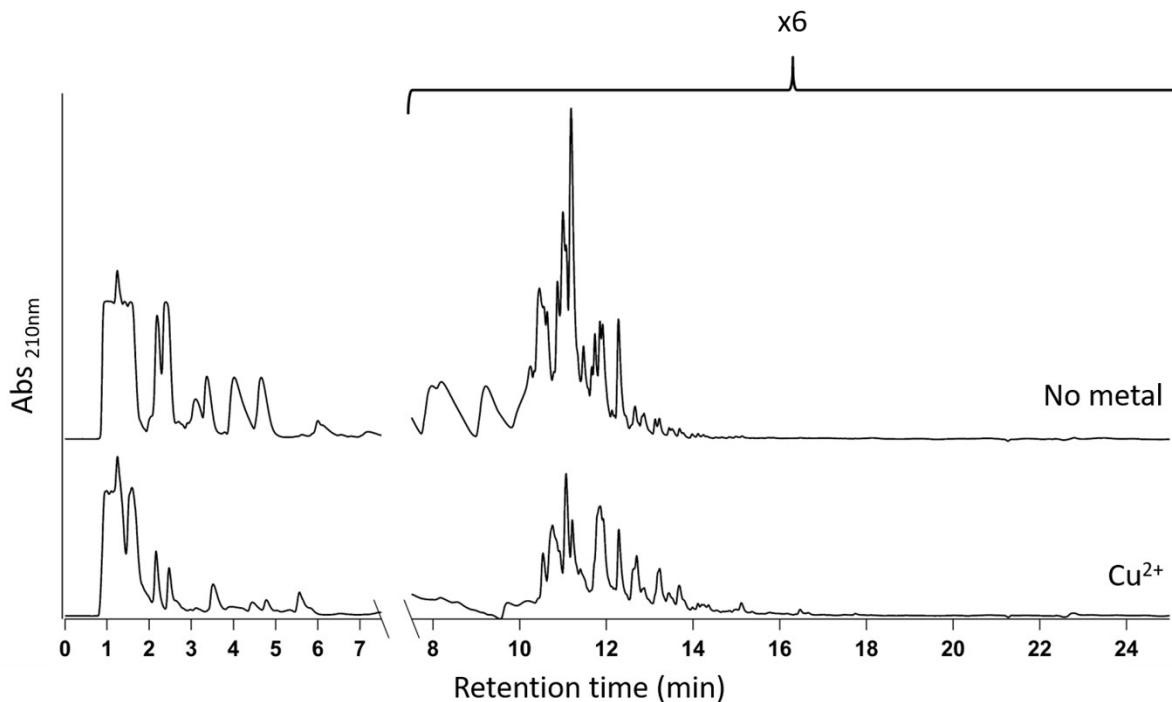


Figure S6. The effect of Cu²⁺ on oligomerization reactions of His-containing depsipeptides. Glycolic acid (glc) and His were dried at 85°C for seven days, at 1:1 molar ratio in the presence or absence of 1eq CuCl₂. Notably, the dry-down mixtures containing Cu²⁺ had precipitates. Only the supernatant was analyzed via the HPLC. Analysis of soluble product mixtures via C18-HPLC showed that Cu²⁺ increased the yields of various long His-containing depsipeptides.

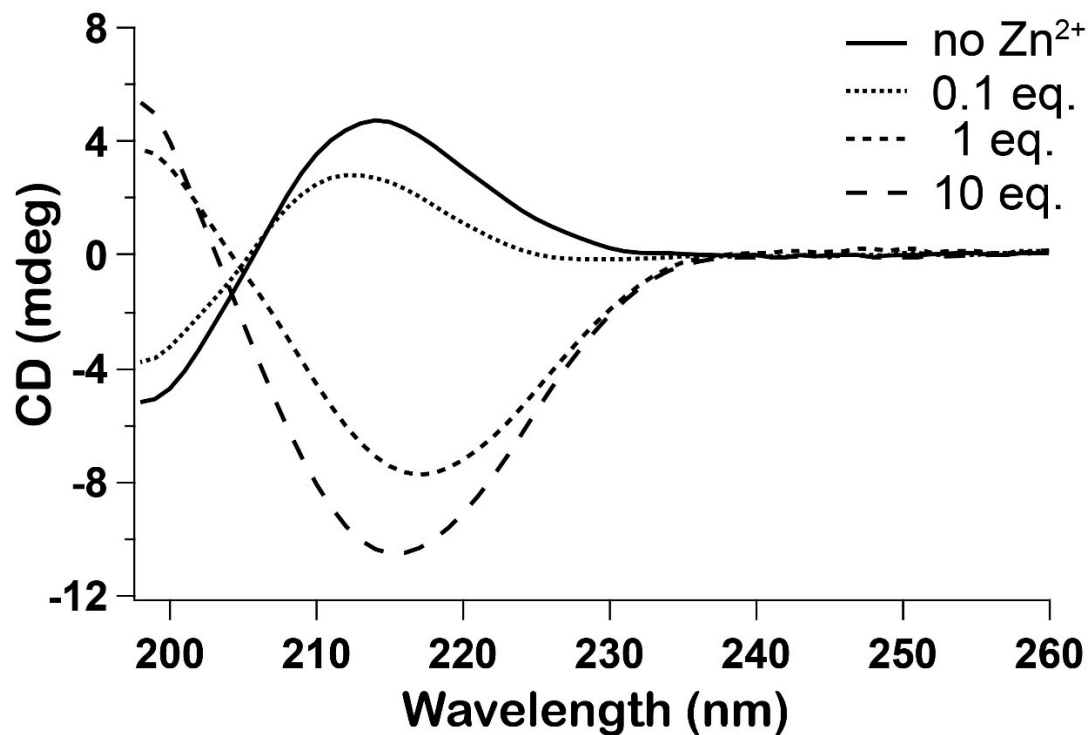


Figure S7. Dose-dependent spectral changes in circular dichroism of glc+His samples upon addition of zinc. Glycolic acid (glc) and His were mixed, at a 5:1 molar ratio in favor of glc, in 50mM Tris buffer. Circular dichroism (CD) spectra were collected for the mixture in the presence or absence of 0.1, 1, or 10 eq. of zinc (refers to His monomer). The conformational change appears to be dose dependent, and the inversion of the CD spectra of His is more evident at higher concentrations of Zn²⁺.

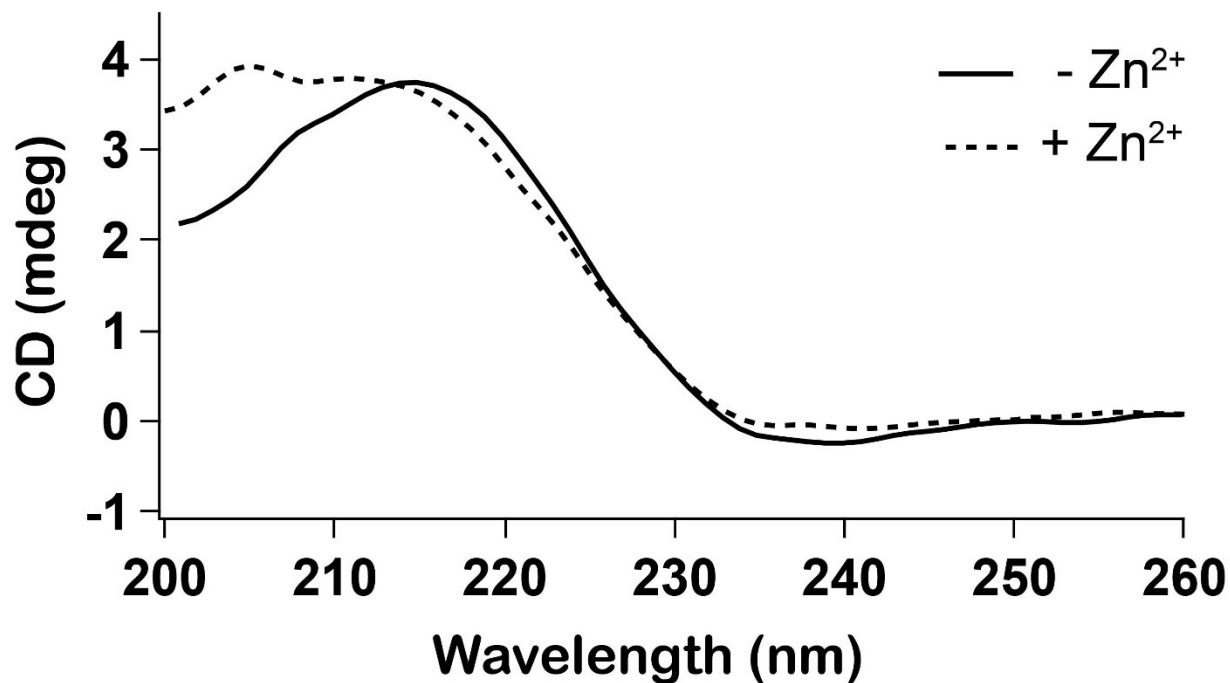


Figure S8. Minor spectral changes in circular dichroism of dried glc+His samples upon addition of zinc. Glycolic acid (glc) and His were dried at 85°C for seven days, at a 5:1 molar ratio in favor of glc, in the absence of zinc. Circular dichroism (CD) spectra were collected for the dried mixture in the presence or absence of 1 eq. of zinc (refers to His monomer) in 50mM Tris buffer. Slight shifts in the CD spectra are evident upon addition of zinc, which could be attributed to minor remaining of un-reacted His monomer that interacts with zinc.

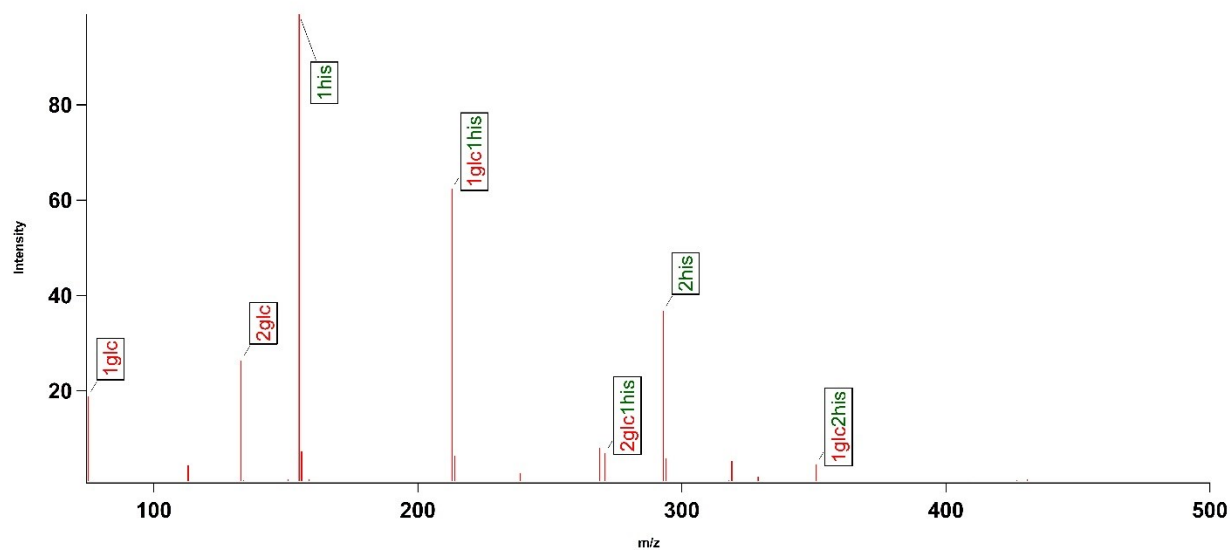


Figure S9. ESI-MS of a dry-down reaction of β -imidazole lactic acid and glc resulted in formation of polyesters. L- β -imidazole lactic acid (his) was dried with glycolic acid (glc) at a 1:1 molar ratio at 85°C for seven days and the resulting polyesters were analyzed by negative-mode ESI-MS, indicating a variety of polyesters formed. glc is labeled in red, his is labeled in green. Labeled species correspond to $[M-H]^-$ ions.

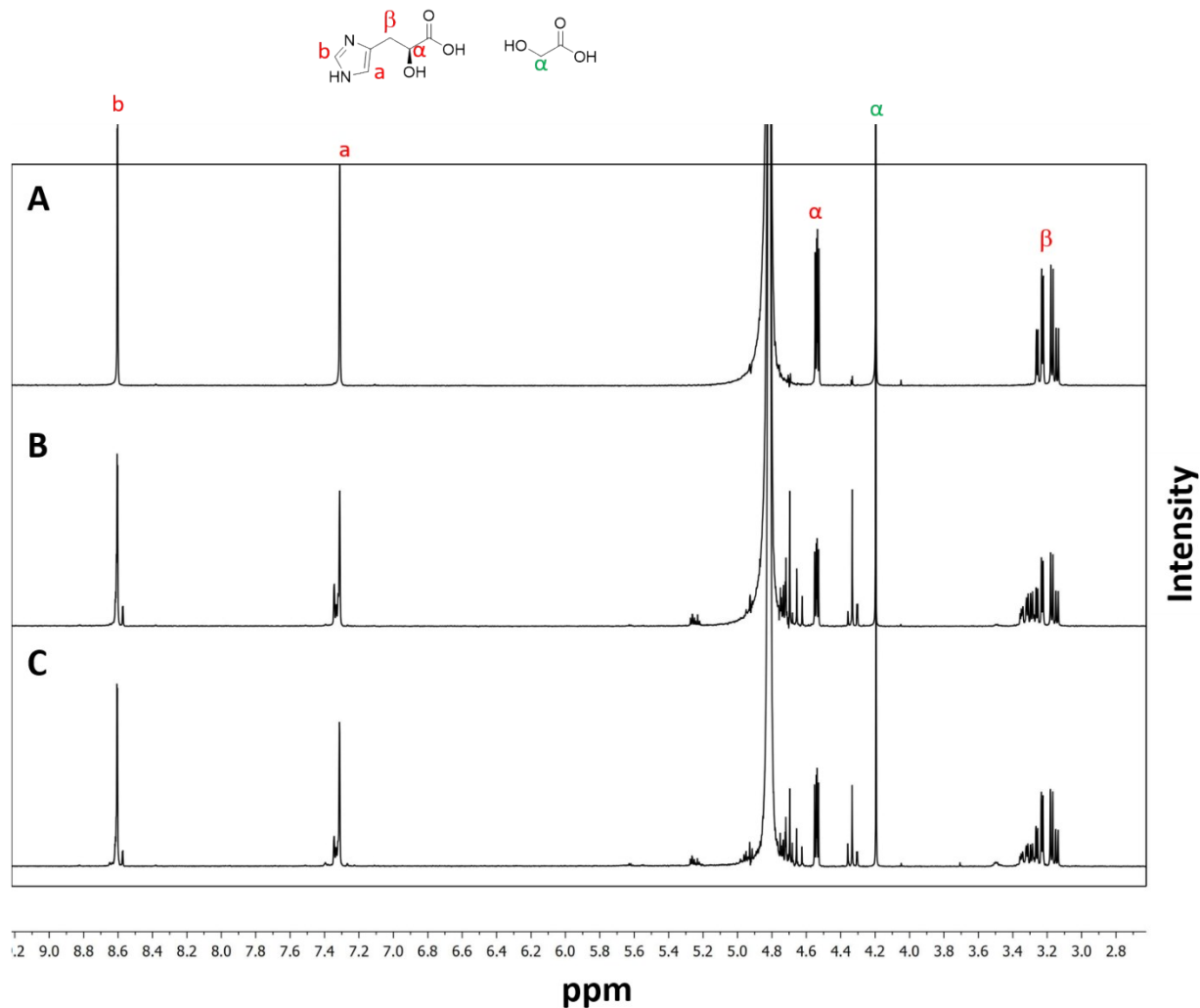


Figure S10. ^1H NMR spectrum supports the formation of polyesters upon dry-down reactions of glc and his. ^1H NMR spectrum of a mixture of L- β -imidazole lactic acid (his) and glycolic acid (glc) at a 1:1 molar ratio in D_2O before (A) and after (B-C) dry-down at 85°C for seven days in the absence (B) or presence (C) of 1 eq of zinc. Internal integration of the remaining non-reacted α -proton of his to its β -protons indicated similar conversion of his monomer into oligomers: about 39% of his has converted into oligomers in the absence of zinc and about 38% of his has converted into oligomers in its presence.