Supporting information

to

Development of a water-soluble ouroboros-like calix[6]arene-trisimidazole-based ligand for enhanced binding of zinc

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Characterization of 2

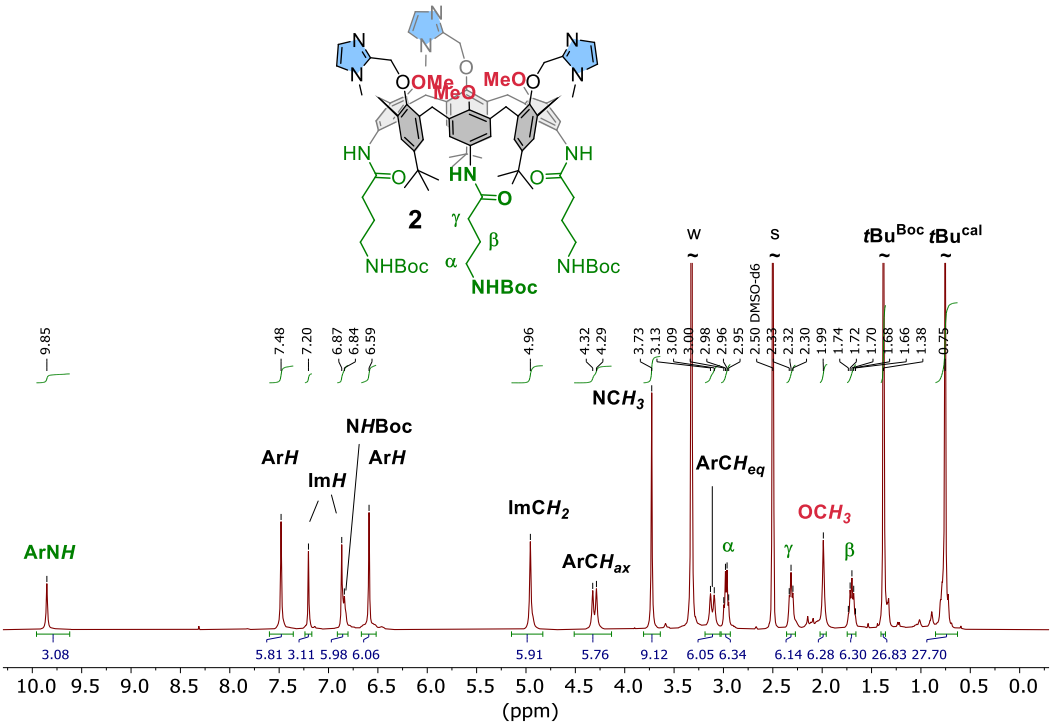

Figure S1. ^{1}H NMR spectrum (298K, 400 MHz, DMSO-d_6) of **2**. s: residual solvent, w: water.

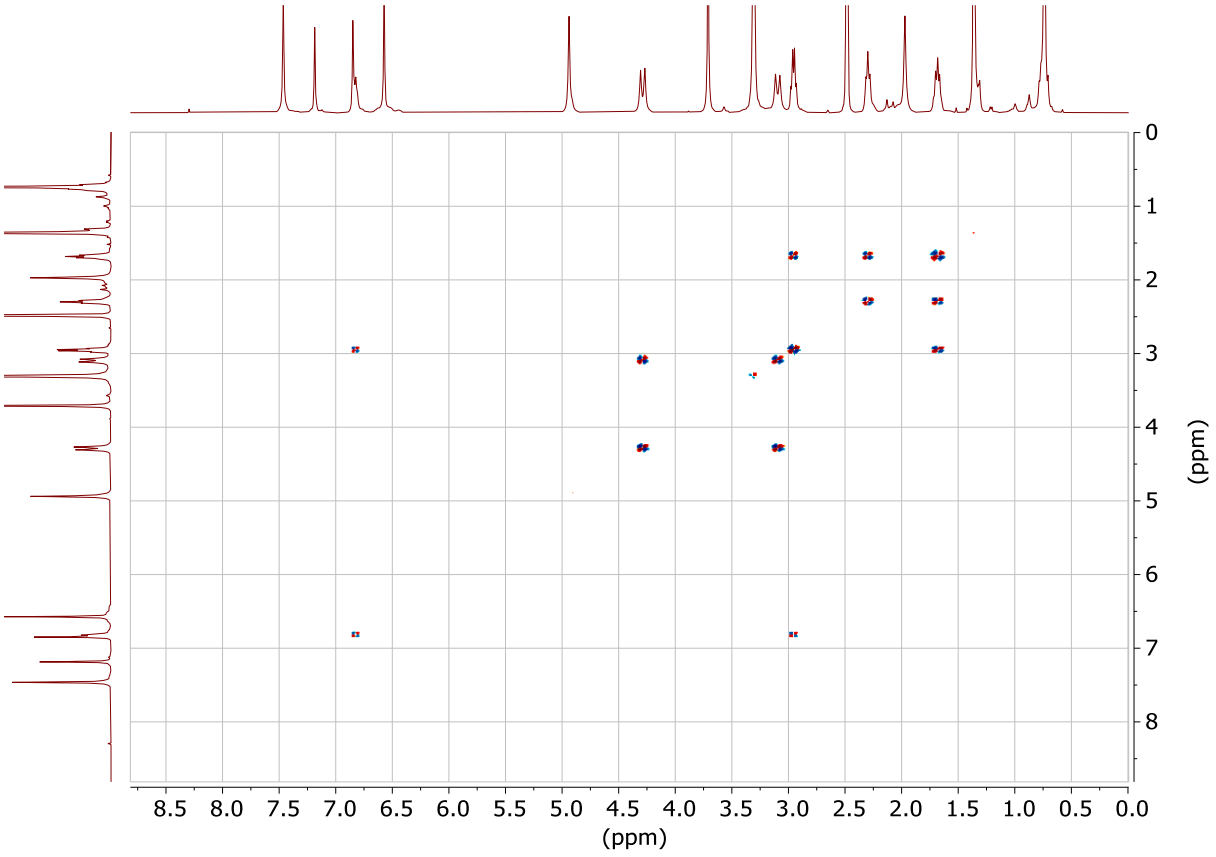

Figure S2. COSY NMR spectrum (298K, 400 MHz, DMSO-d_6) of **2**.

^{13}C NMR characterization was attempted but only small signals could be detected after a prolonged analysis time and over 5000 scans (Figure S3). However, a HSQC spectrum allowed the assignment of some of the ^{13}C signals (Figure S4).

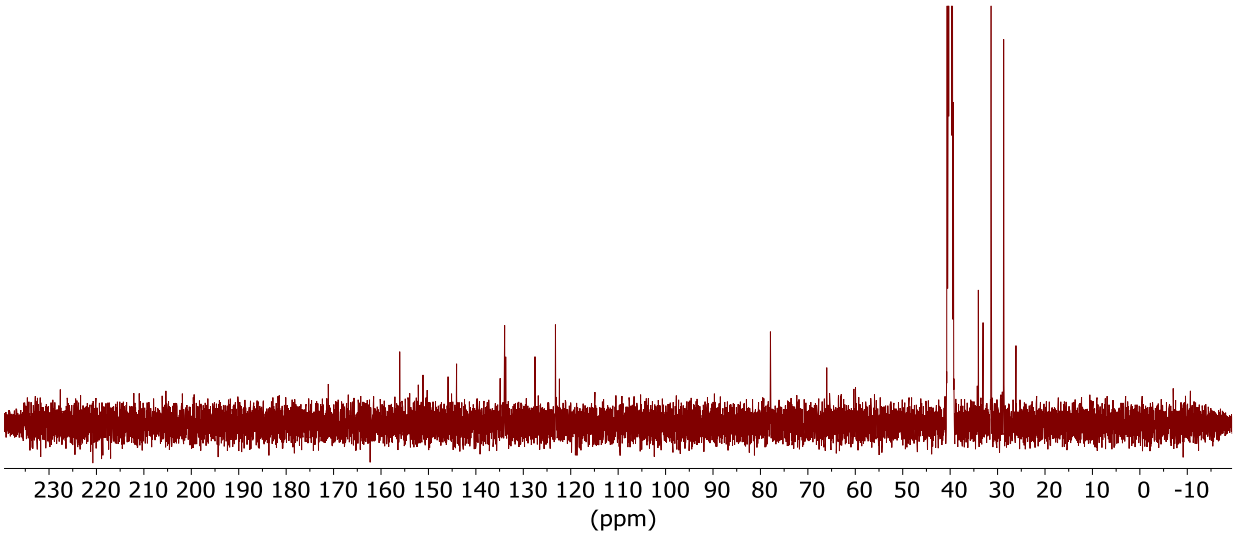

Figure S3. ^{13}C NMR spectrum (298K, 400 MHz, DMSO-d_6) of **2**.

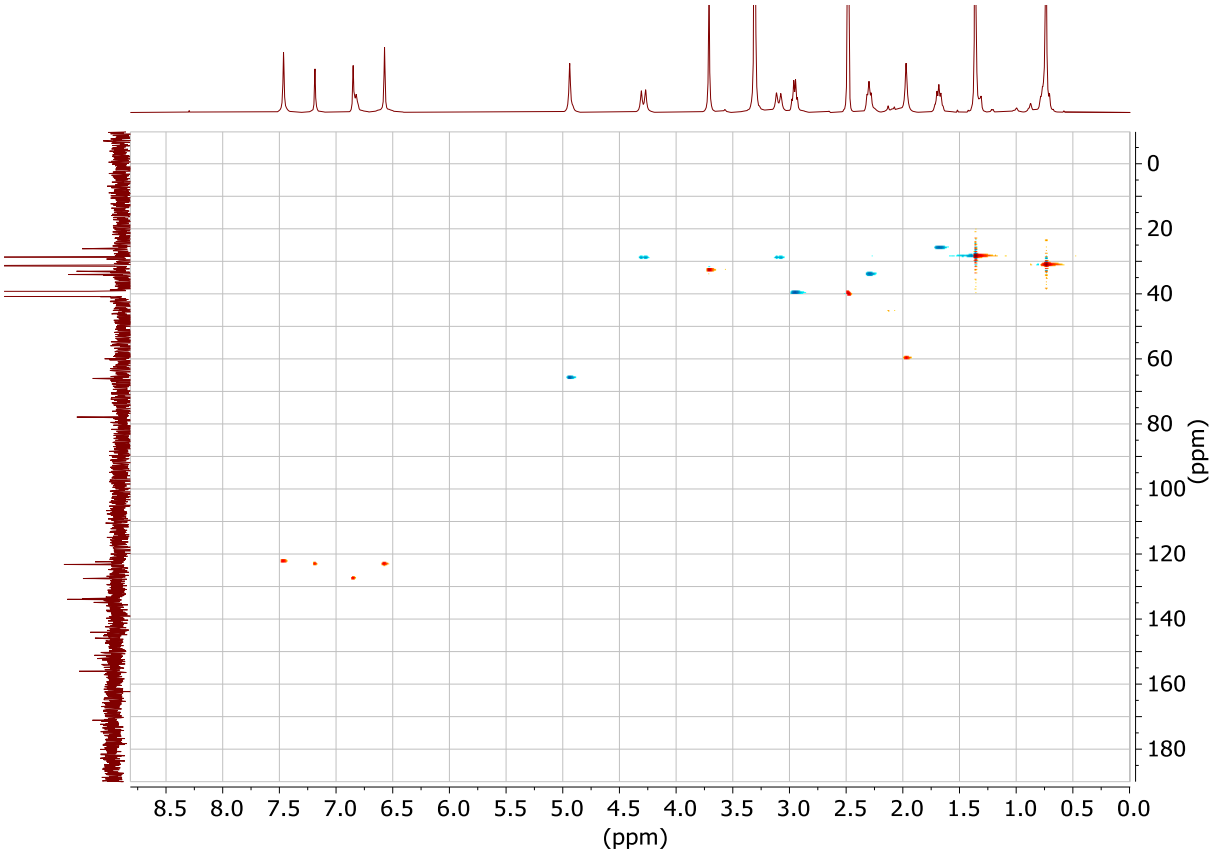

Figure S4. Edited HSQC NMR spectrum (298K, 400 MHz, DMSO-d_6) of **2**.

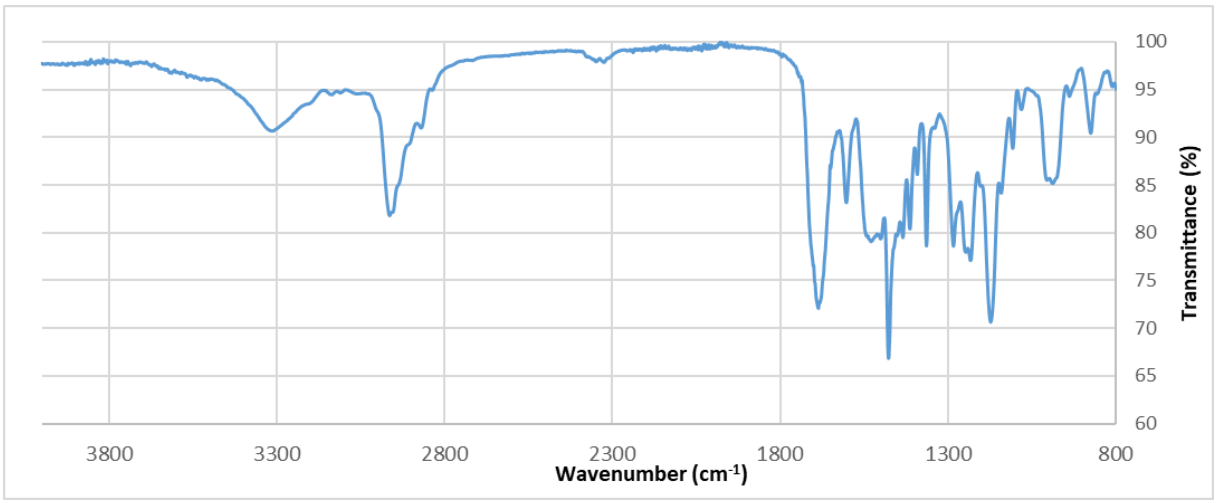

Figure S5. ATR-FTIR spectrum of **2**.

Characterization of 3

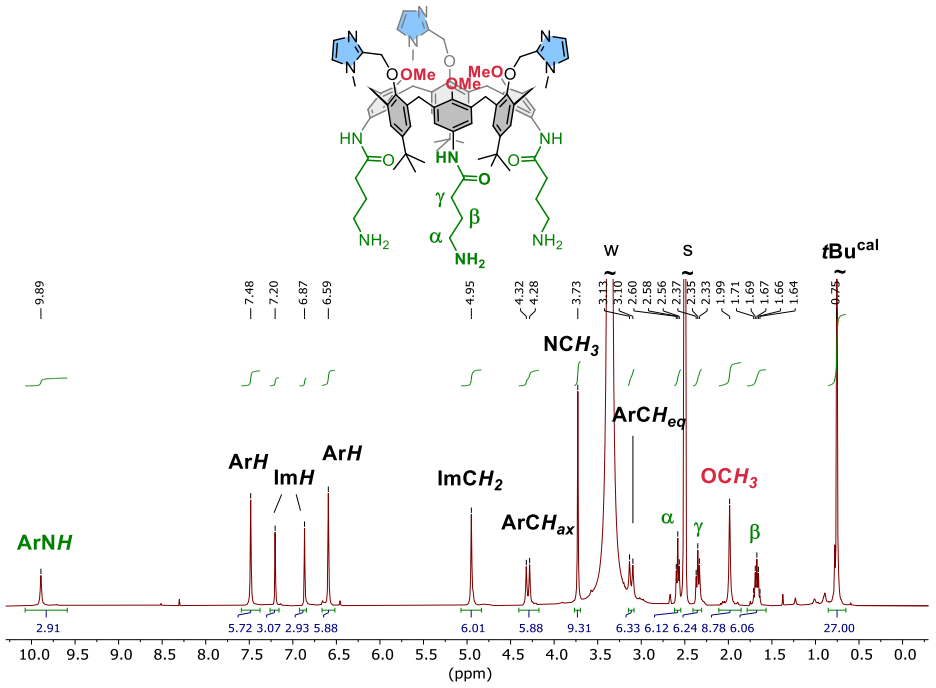

Figure S6. ^{1}H NMR spectrum (298K, 400 MHz, DMSO-d_6) of **3**. s: residual solvent, w: water.

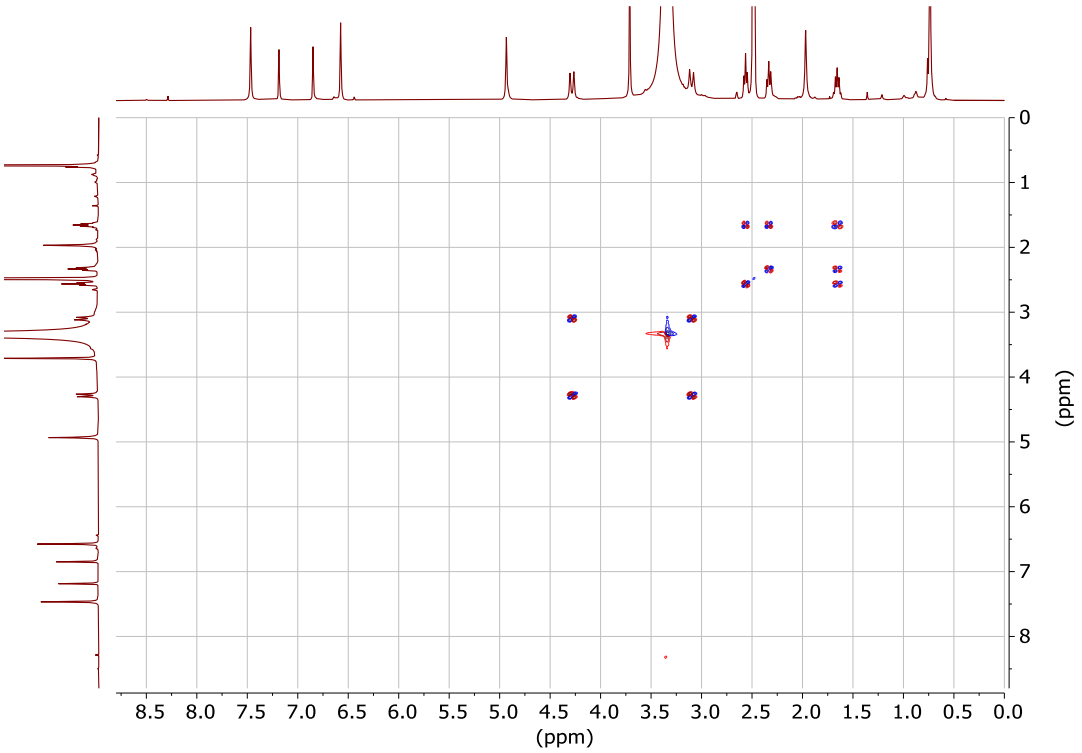

Figure S7. COSY NMR spectrum (298K, 400 MHz, DMSO-d_6) of **3**.

^{13}C NMR characterization was attempted but only small signals could be detected after a prolonged analysis time and over 5000 scans (Figure S8). However, a HSQC spectrum allowed the assignment of some of the ^{13}C signals (Figure S9).

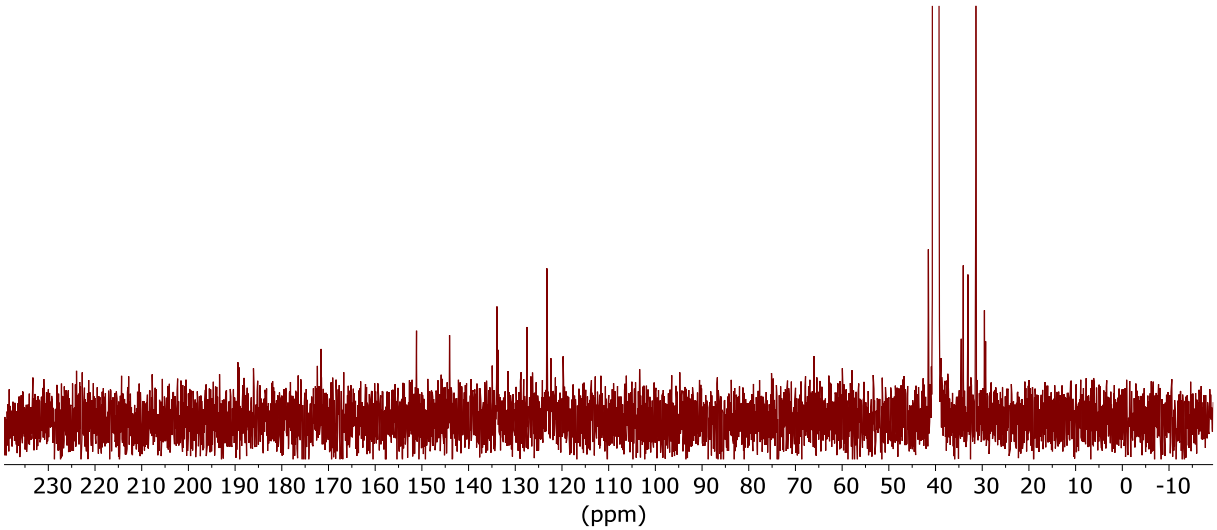

Figure S8. ^{13}C NMR spectrum (298K, 400 MHz, DMSO-d_6) of **3**.

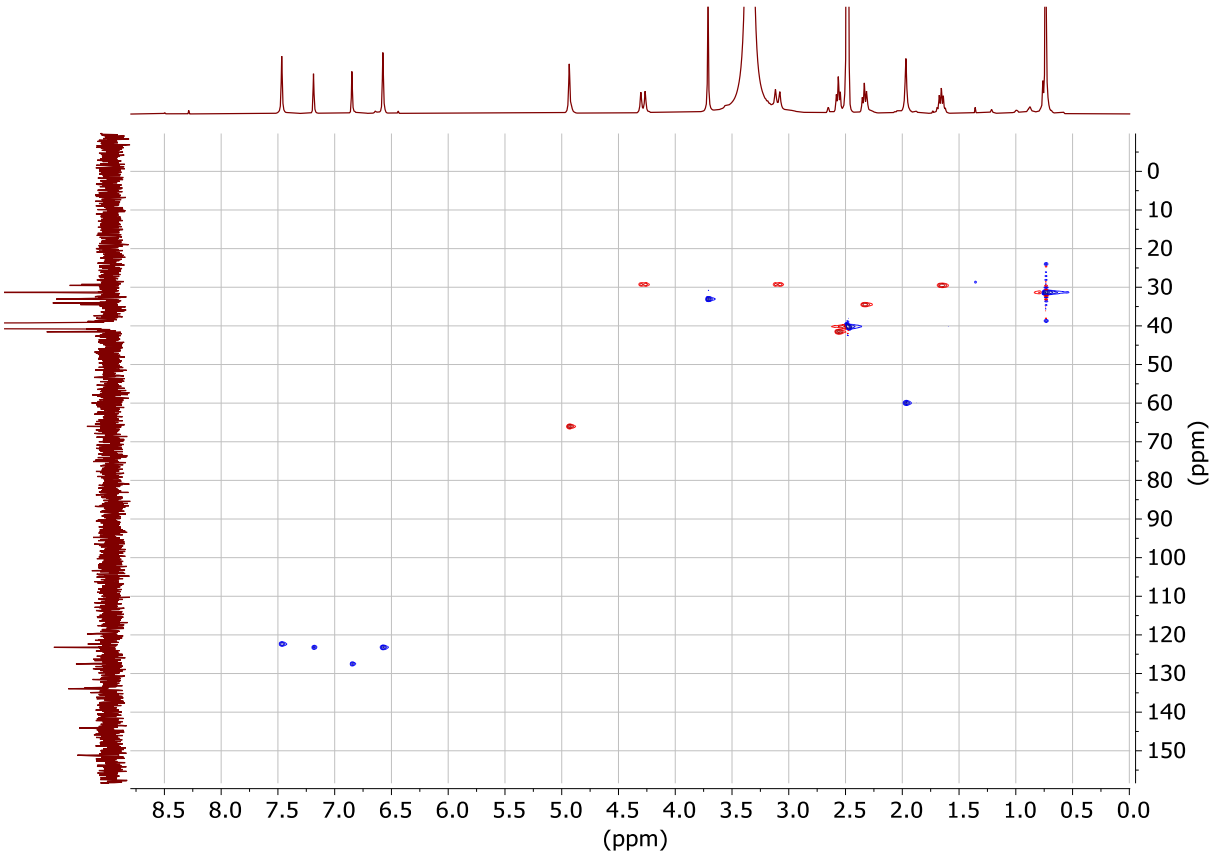

Figure S9. Edited HSQC NMR spectrum (298K, 400 MHz, DMSO-d_6) of **3**.

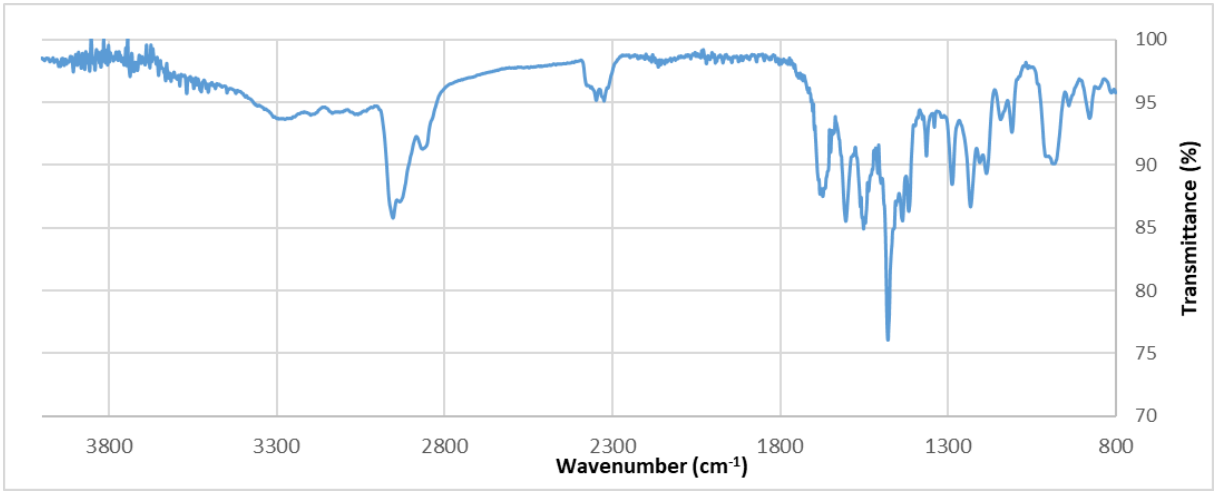

Figure S10. ATR-FTIR spectrum of **3**.

Titrations for the formation of the complexes in $CDCl_3/CD_3CN$ 1:1

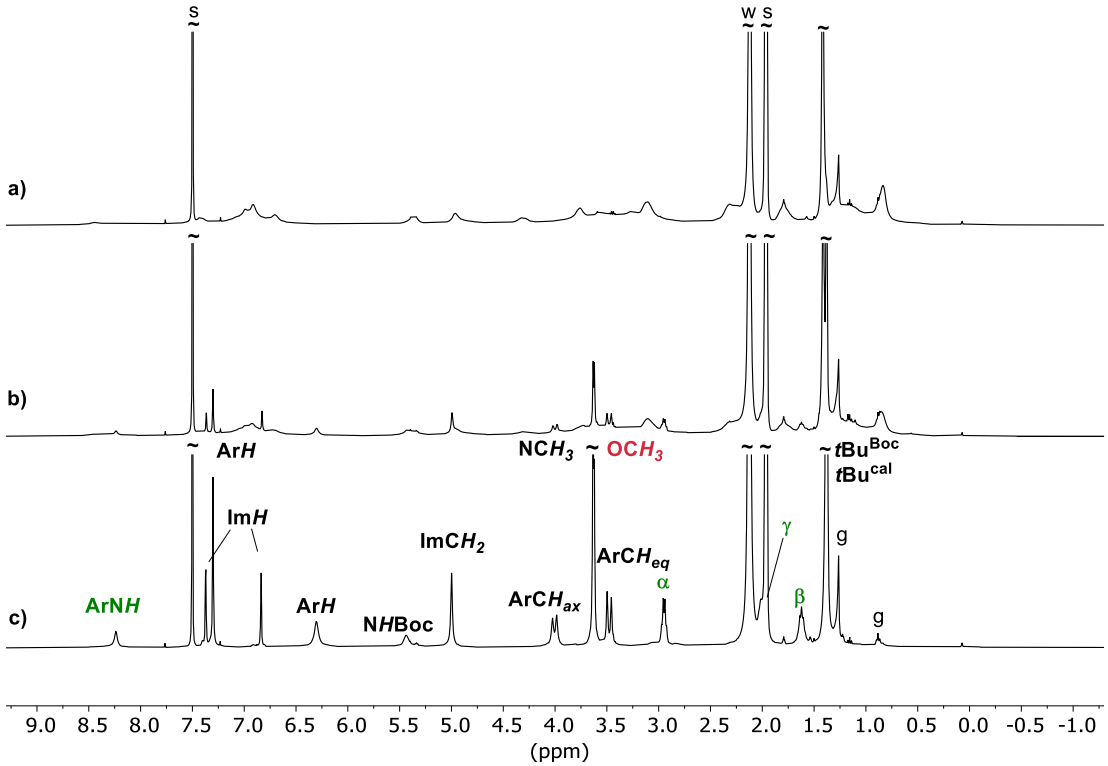

Figure S11. ^{1}H NMR spectra (298 K, 400 MHz, $CDCl_3/CD_3CN$ 1:1) of a) **2** (1 mM), b) **2** (1 mM) + ~0.4 equiv. of $Zn(OTf)_2$, c) **2** (1 mM) + ~1.0 equiv. of $Zn(OTf)_2$. s = residual solvents, w = water, g = grease.

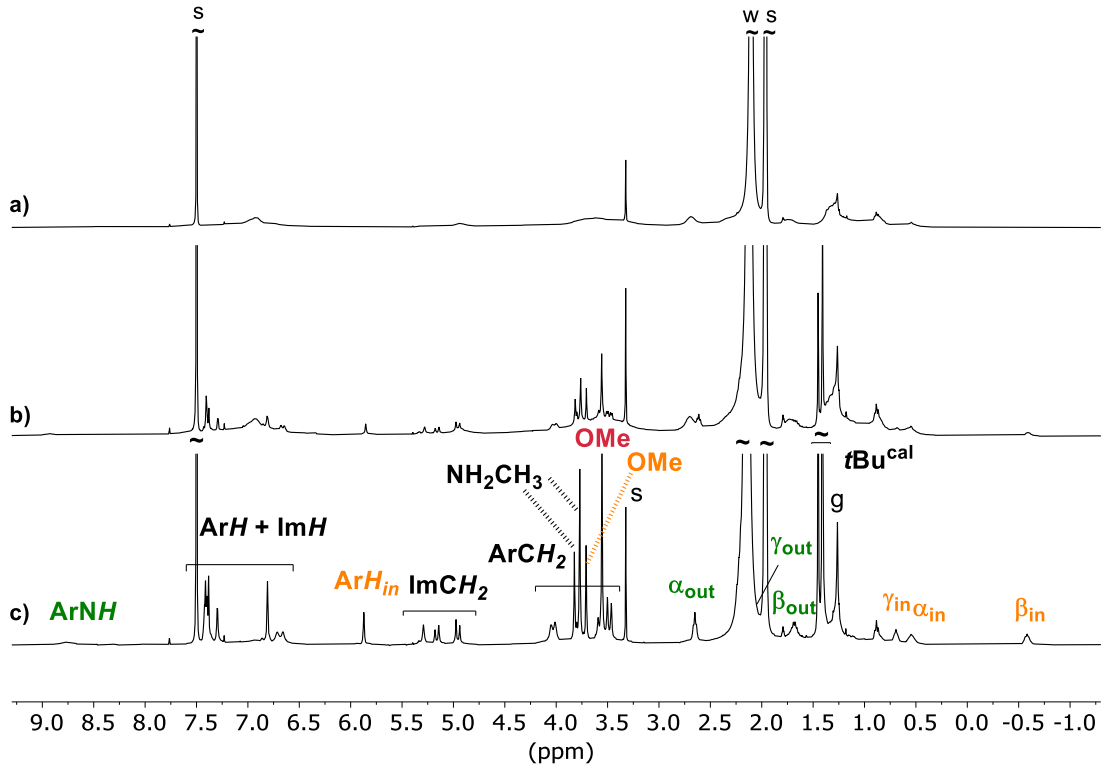

Figure S12. ^{1}H NMR spectra (298 K, 400 MHz, $CDCl_3/CD_3CN$ 1:1) of a) **3** (1 mM), b) **3** (1 mM) + ~0.4 equiv. of $Zn(OTf)_2$, c) **3** (1 mM) + ~1.0 equiv. of $Zn(OTf)_2$. s = residual solvents, w = water, g = grease.

Characterization of complex 2-Zn^{2+} in $CDCl_3/CD_3CN$ 1:1

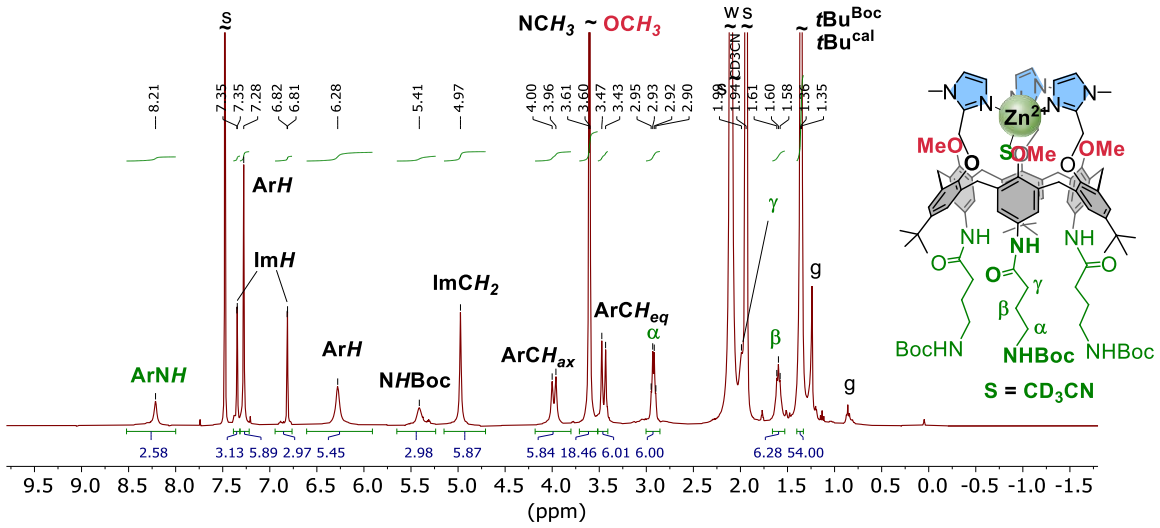

Figure S13. ^{1}H NMR spectrum (298K, 400 MHz, $CDCl_3/CD_3CN$ 1:1) of **2**-Zn^{2+} (1 mM). s: residual solvent, w: water, g = grease.

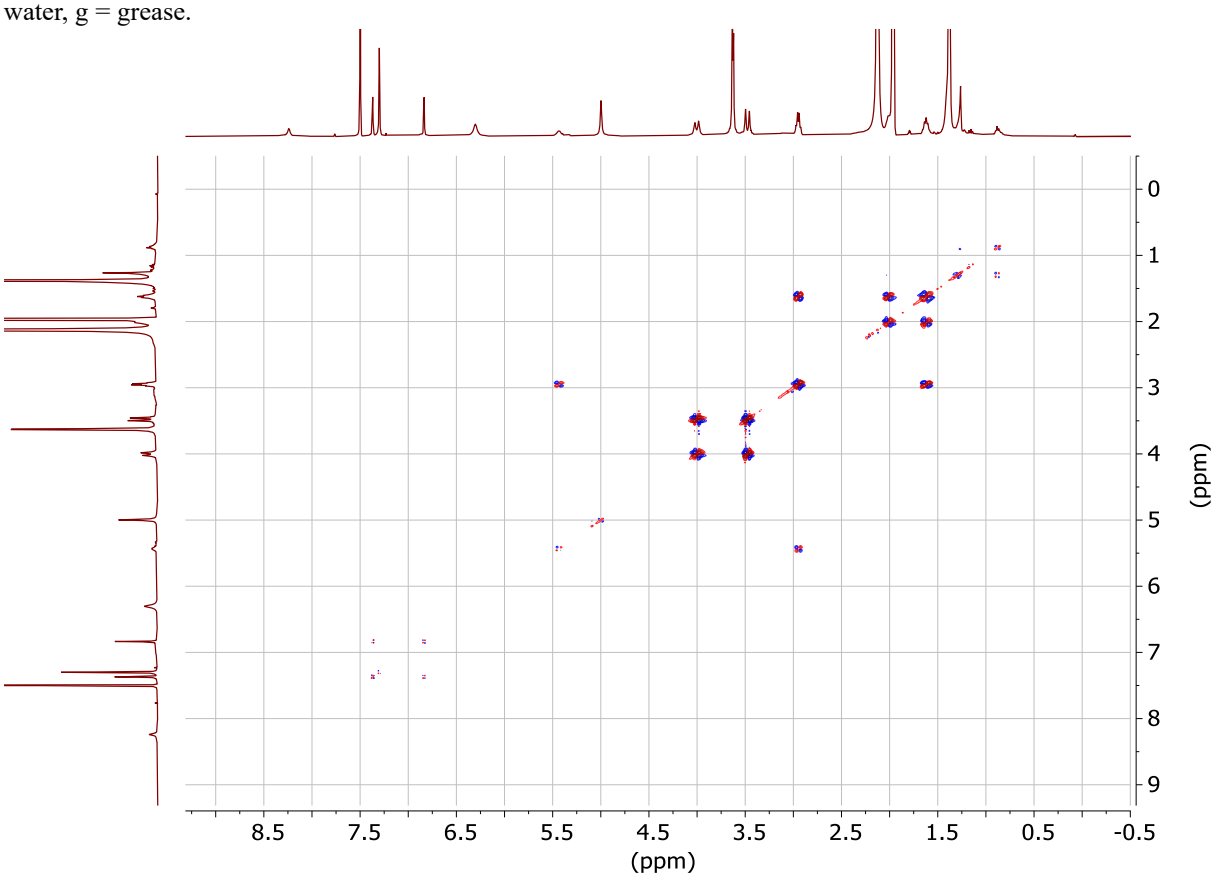

Figure S14. COSY NMR spectrum (298K, 400 MHz, $CDCl_3/CD_3CN$ 1:1) of **2**-Zn^{2+} (1 mM).

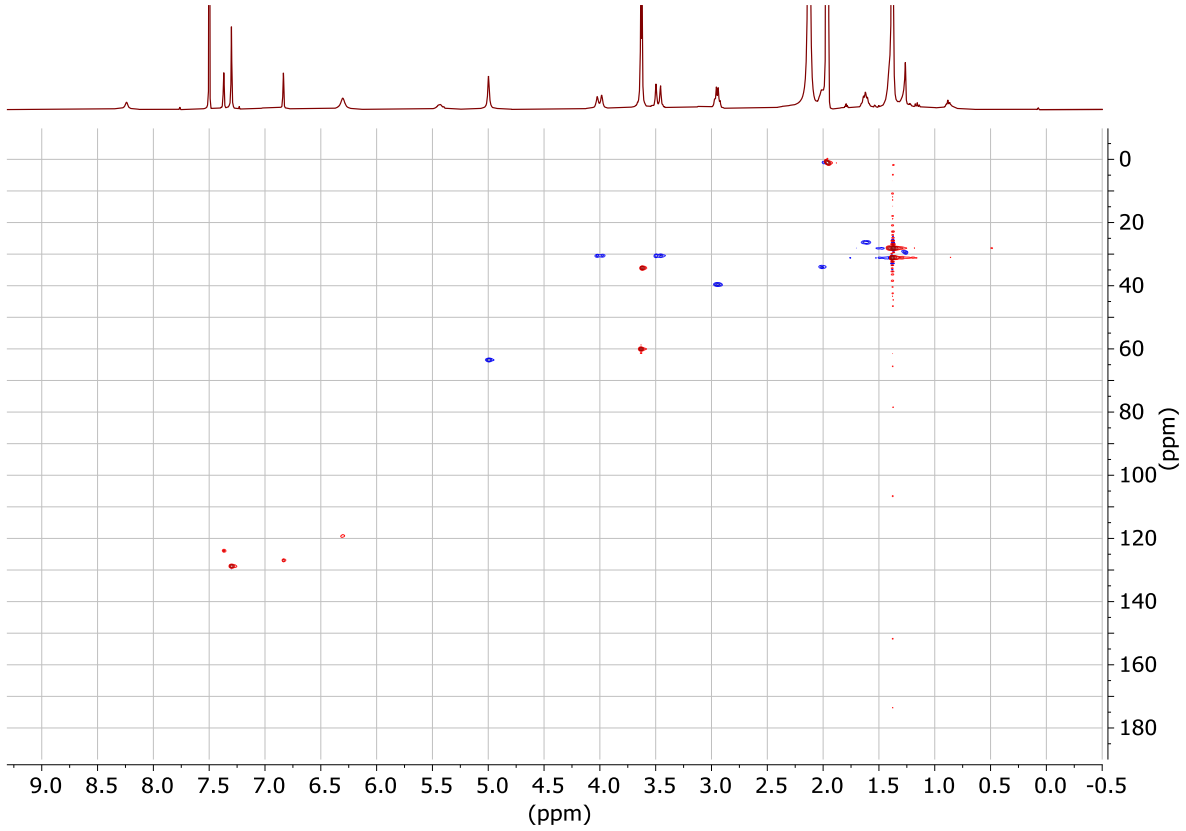

Figure S15. Edited HSQC NMR spectrum (298K, 400 MHz, $CDCl_3/CD_3CN$ 1:1) of **2**-Zn^{2+} (1 mM). The ^{13}C NMR spectrum has not been recorded for **2**-Zn^{2+}.

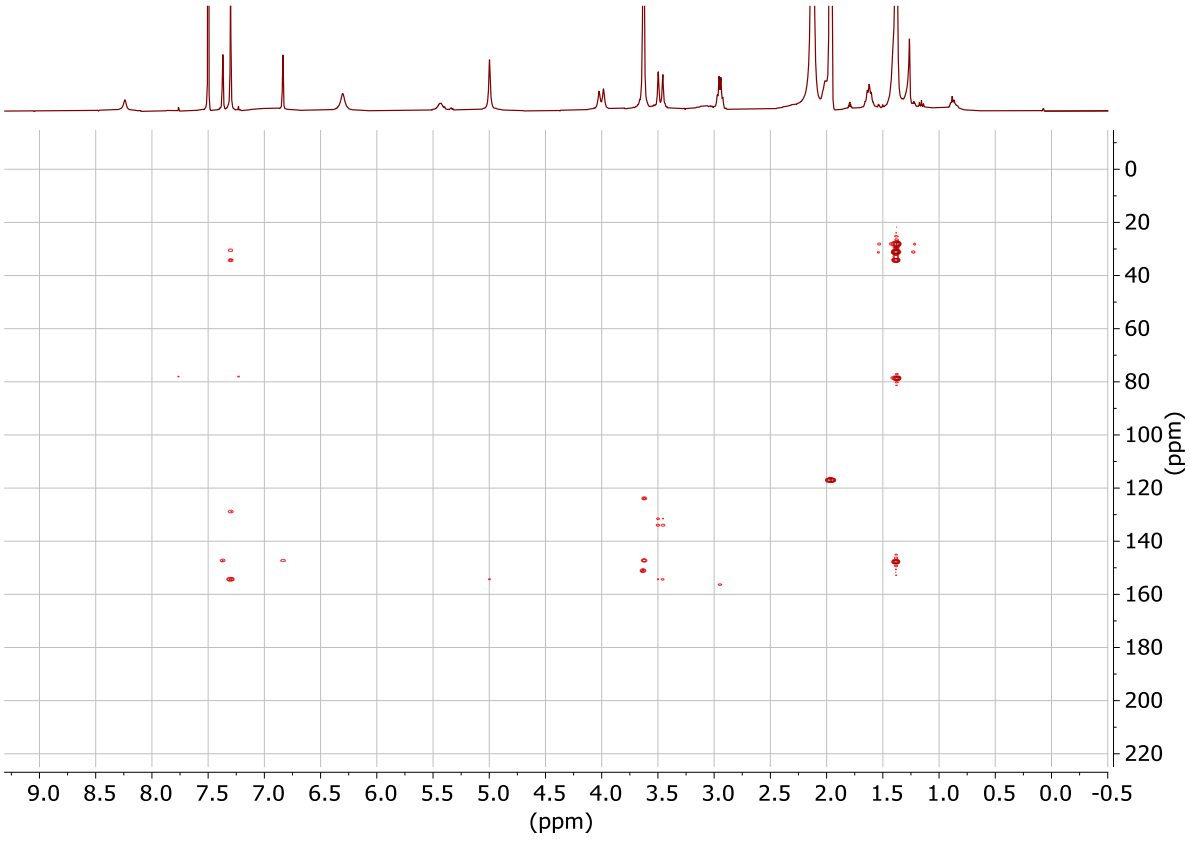

Figure S16. HMBC NMR spectrum (298K, 400 MHz, $CDCl_3/CD_3CN$ 1:1) of **2**-Zn^{2+} (1 mM). The ^{13}C NMR spectrum has not been recorded for **2**-Zn^{2+}.

Characterization of complex 3-Zn^{2+} in $CDCl_3/CD_3CN$ 1:1

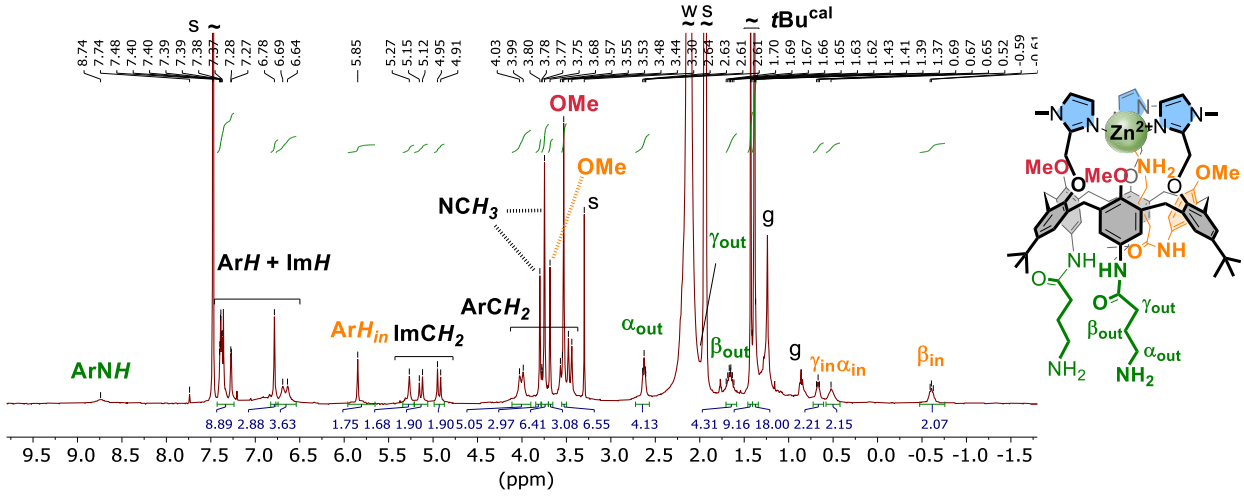

Figure S17. ^{1}H NMR spectrum (298K, 400 MHz, $CDCl_3/CD_3CN$ 1:1) of **3**-Zn^{2+} (1 mM). s: residual solvent, w: water, g = grease.

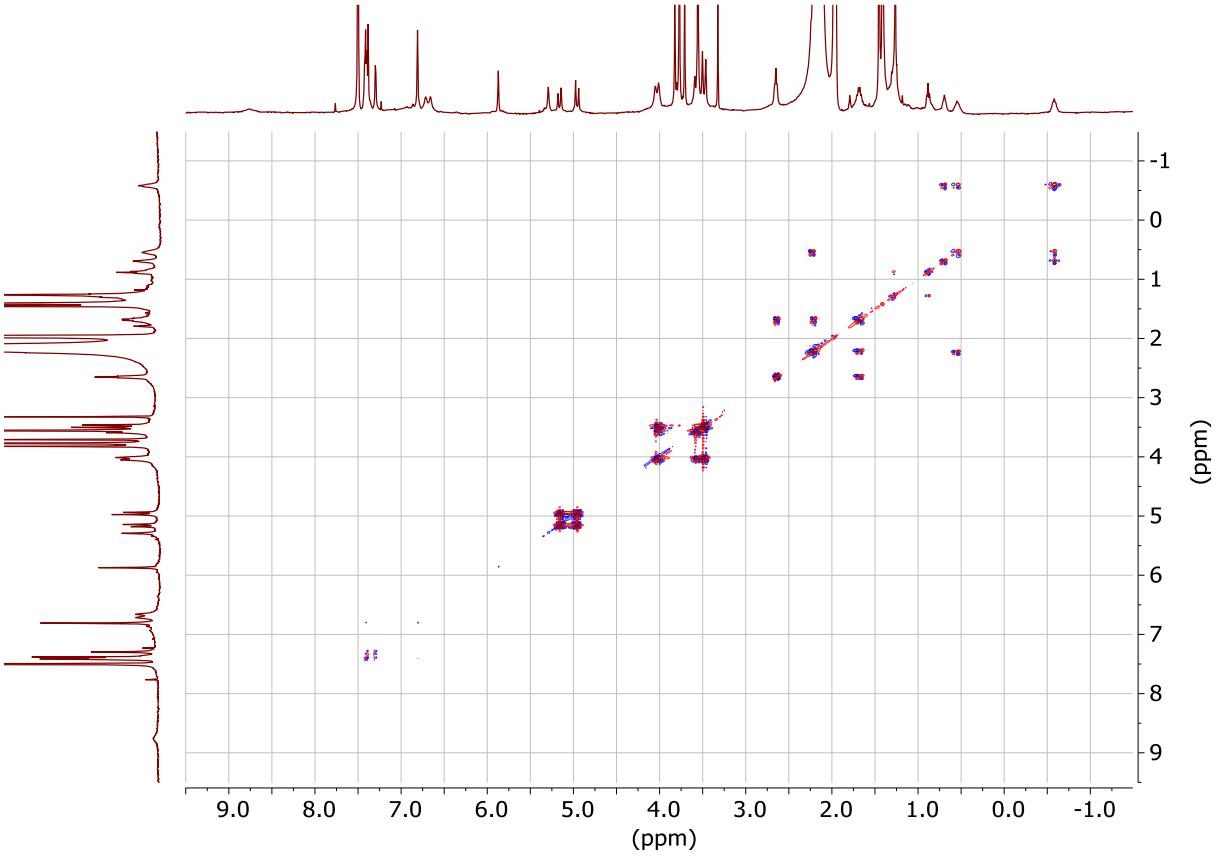

Figure S18. COSY NMR spectrum (298K, 400 MHz, $CDCl_3/CD_3CN$ 1:1) of **3**-Zn^{2+} (1 mM).

^{13}C NMR characterization was attempted but only small signals could be detected after a prolonged analysis time and over 5000 scans (Figure S19). However, a HSQC spectrum allowed the assignment of some of the ^{13}C signals (Figure S20).

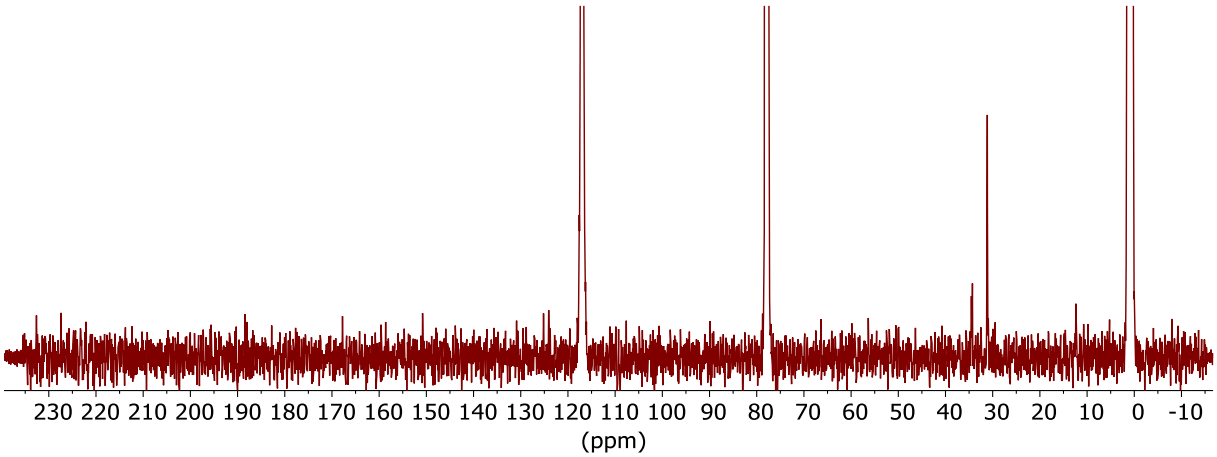

Figure S19. ^{13}C NMR spectrum (298K, 400 MHz, $CDCl_3/CD_3CN$ 1:1) of **3-Zn^{2+}**.

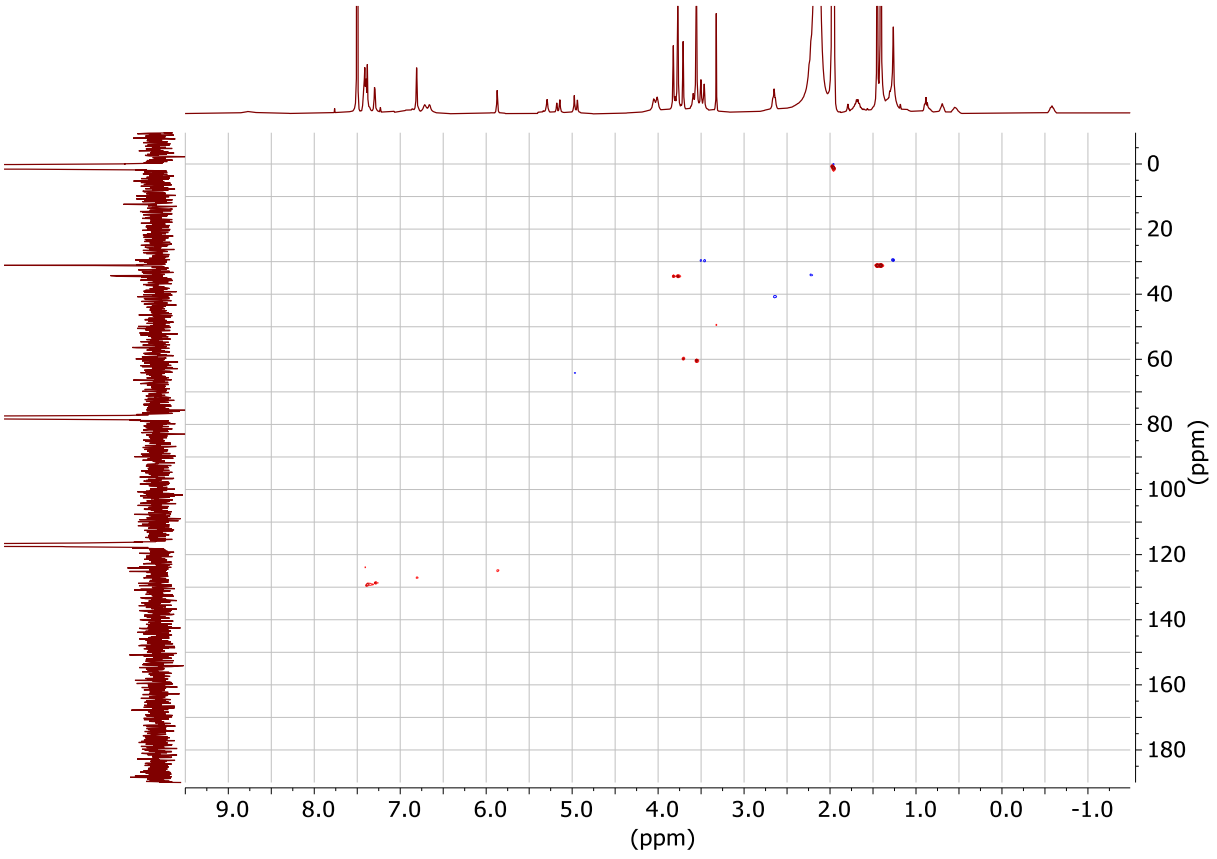

Figure S20. Edited HSQC NMR spectrum (298K, 400 MHz, $CDCl_3/CD_3CN$ 1:1) of **3**-Zn^{2+} (1 mM).

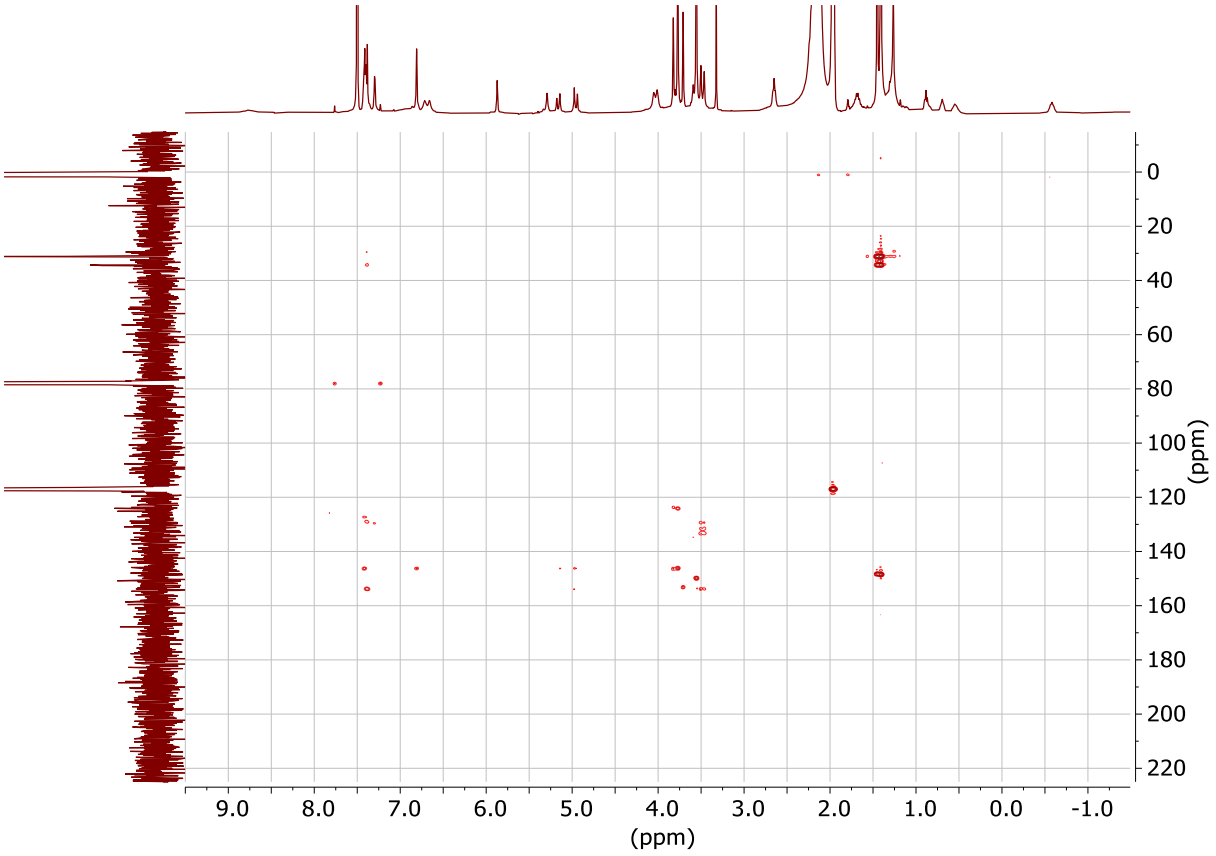

Figure S21. HMBC NMR spectrum (298K, 400 MHz, $CDCl_3/CD_3CN$ 1:1) of **3**-Zn^{2+} (1 mM).

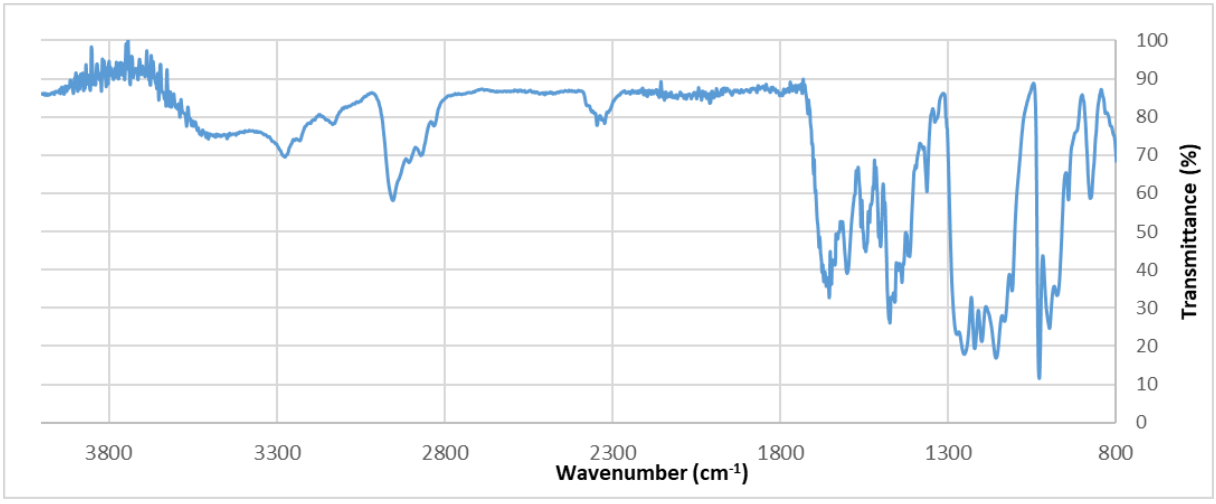

Figure S22. ATR-FTIR spectrum of **3-Zn**$^{2+}$.

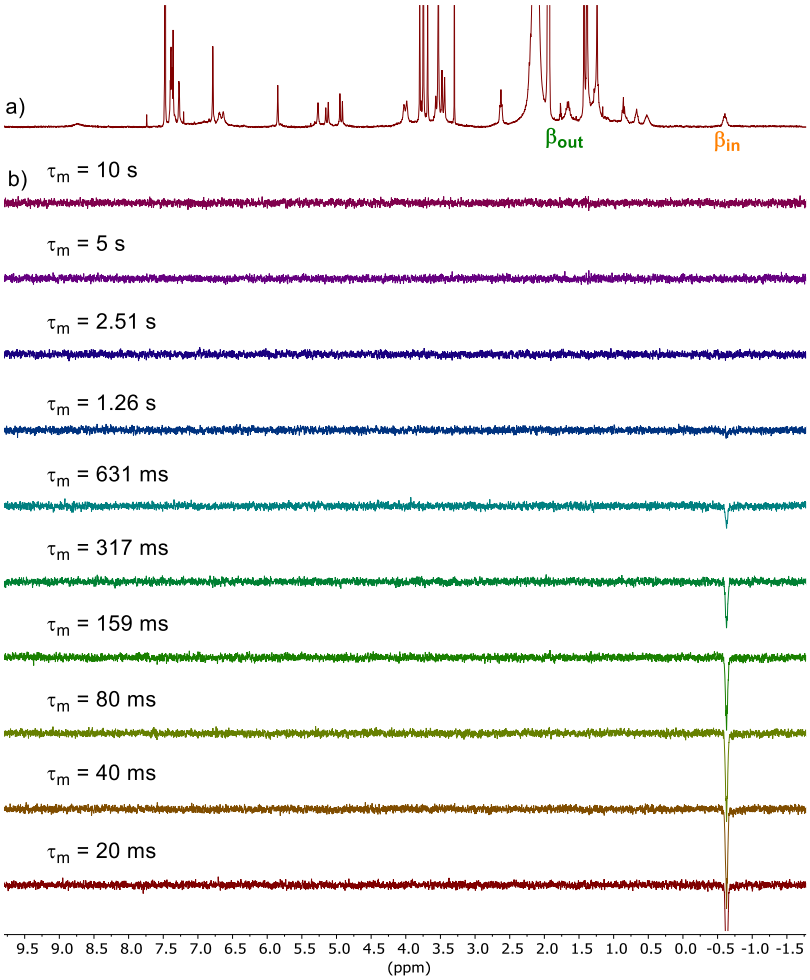

Figure S23. ^{1}H NMR spectra (298 K, 600 MHz, $CDCl_3/CD_3CN$ 1:1) of a) **3**-Zn^{2+}, b) 1D EXSY spectra (at various mixing times) after selective excitation of the β_{in} signal at −0.61 ppm.

Titrations for the formation of the complexes in DMSO

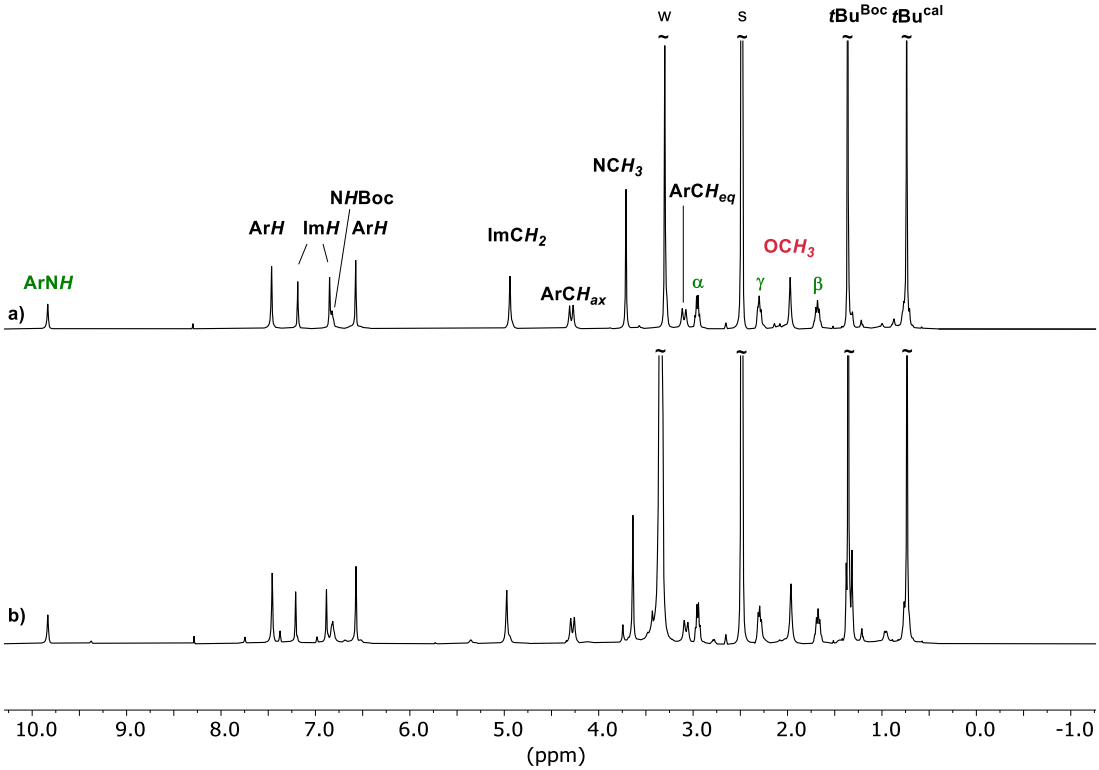

Figure S24. ^{1}H NMR spectra (298 K, 400 MHz, DMSO-d_6) of a) **2** (2 mM), b) **2** (2 mM) + ~27 equiv. of $Zn(OTf)_2$. s = residual solvents, w = water.

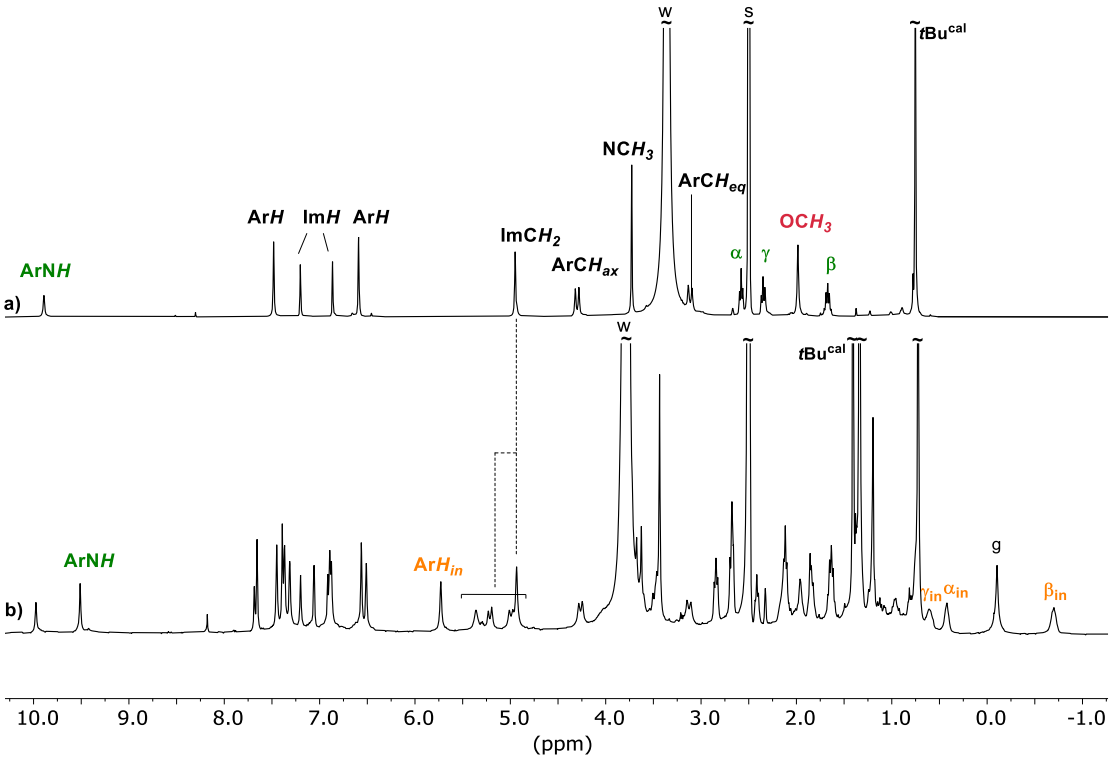

Figure S25. ^{1}H NMR spectra (298 K, 400 MHz, DMSO-d_6) of a) **3** (2 mM), b) **3** (2 mM) + ~27 equiv. of $Zn(OTf)_2$. s = residual solvents, w = water.

Formation and characterization of complex 4-Zn^{2+} in $CDCl_3/CD_3CN$ 1:1

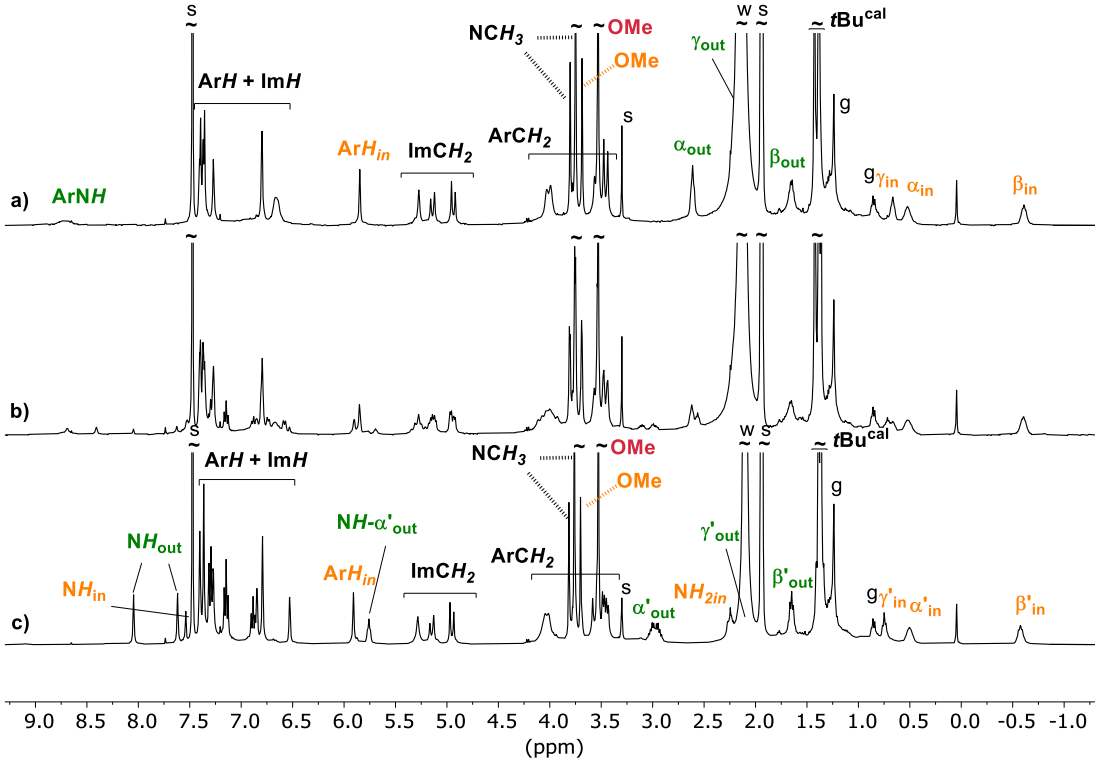

Figure S26. ^{1}H NMR spectra (298 K, 400 MHz, $CDCl_3/CD_3CN$ 1:1) of a) **3** (2 mM)) + ~1.0 equiv. of $Zn(OTf)_2$, b) **3** (2 mM)) + ~1.0 equiv. of $Zn(OTf)_2$ + ~0.6 equiv. phenylisocyanate, c) **3** (2 mM)) + ~1.0 equiv. of $Zn(OTf)_2$ + ~2 equiv. phenylisocyanate → **4**-Zn^{2+}. s = residual solvents, w = water, g = grease.

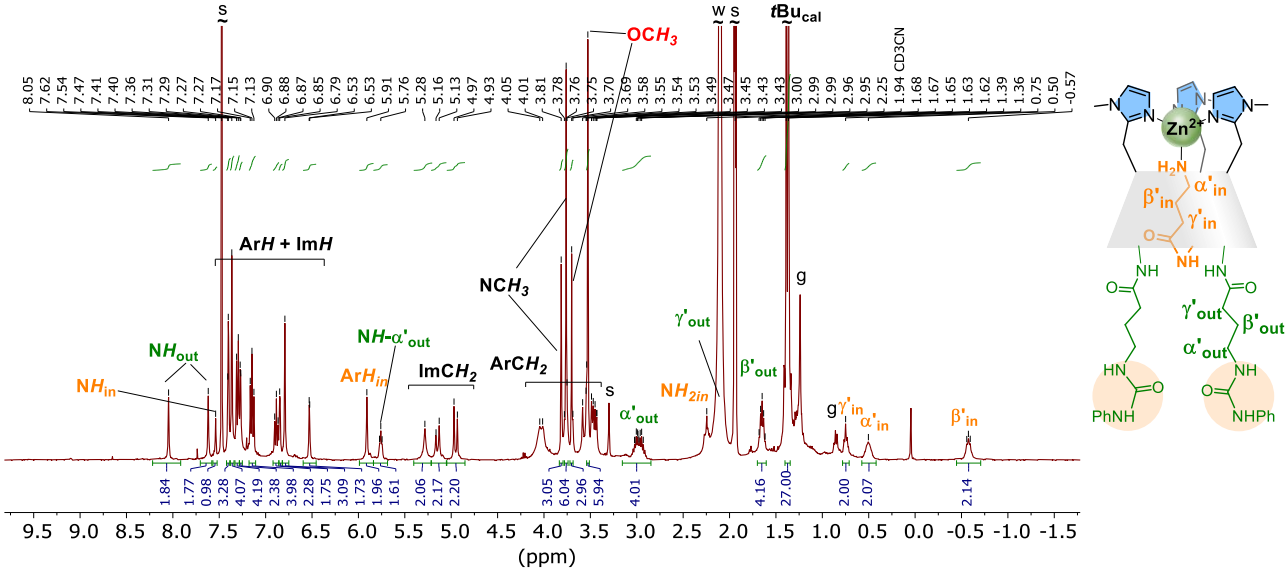

Figure S27. ^{1}H NMR spectrum (298K, 400 MHz, $CDCl_3/CD_3CN$ 1:1) of **4**-Zn^{2+} (2 mM). s: residual solvent, w: water, g = grease.

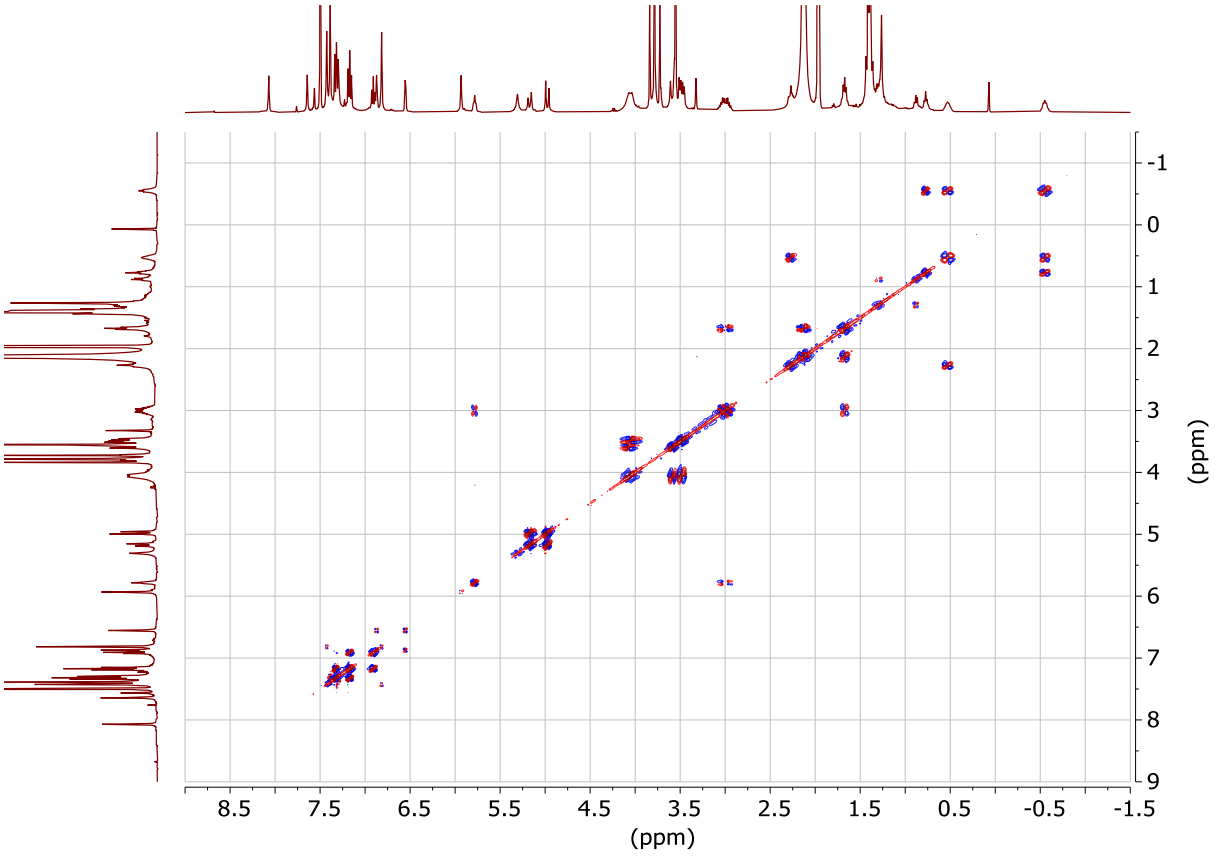

Figure S28. COSY NMR spectrum (298K, 400 MHz, $CDCl_3/CD_3CN$ 1:1) of **4**-Zn^{2+} (2 mM).

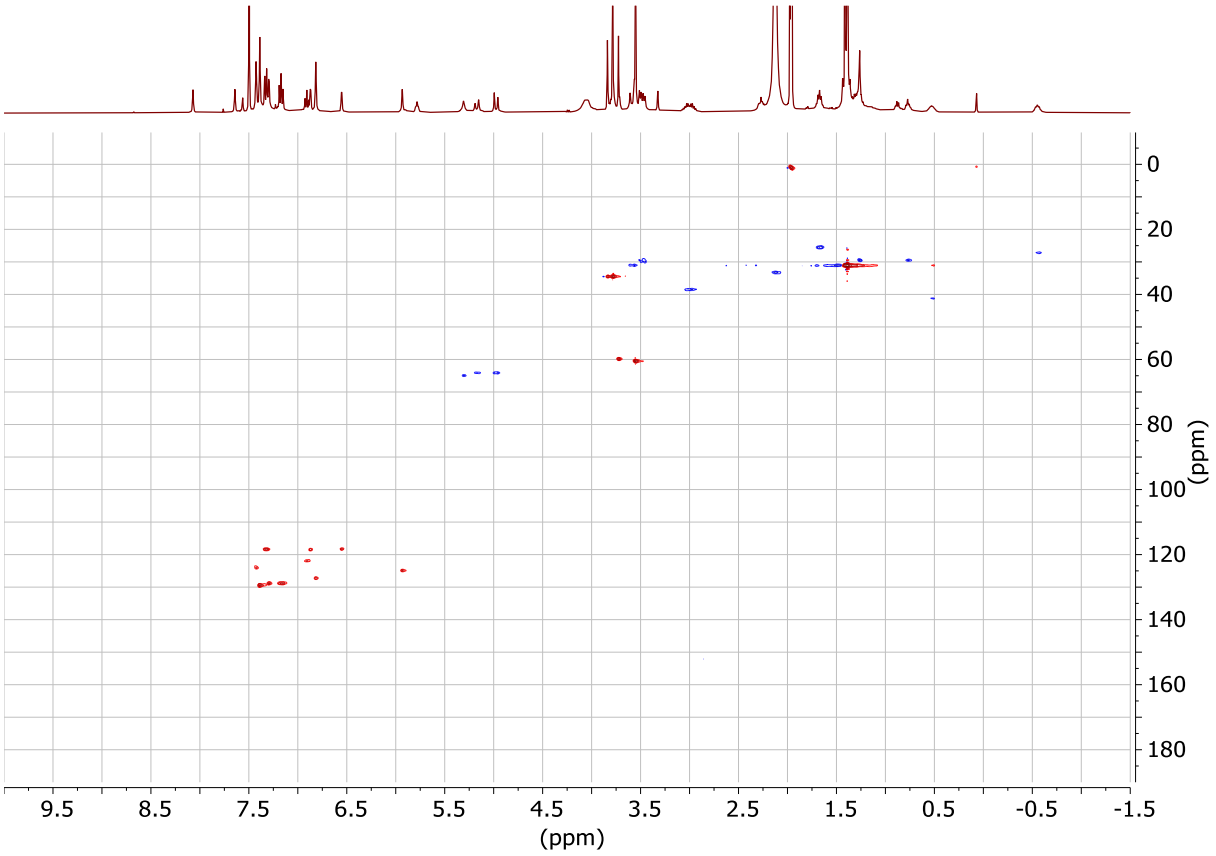

Figure S29. Edited HSQC NMR spectrum (298K, 400 MHz, $CDCl_3/CD_3CN$ 1:1) of **4**-Zn^{2+} (2 mM). The ^{13}C NMR spectrum has not been recorded for **4**-Zn^{2+}.

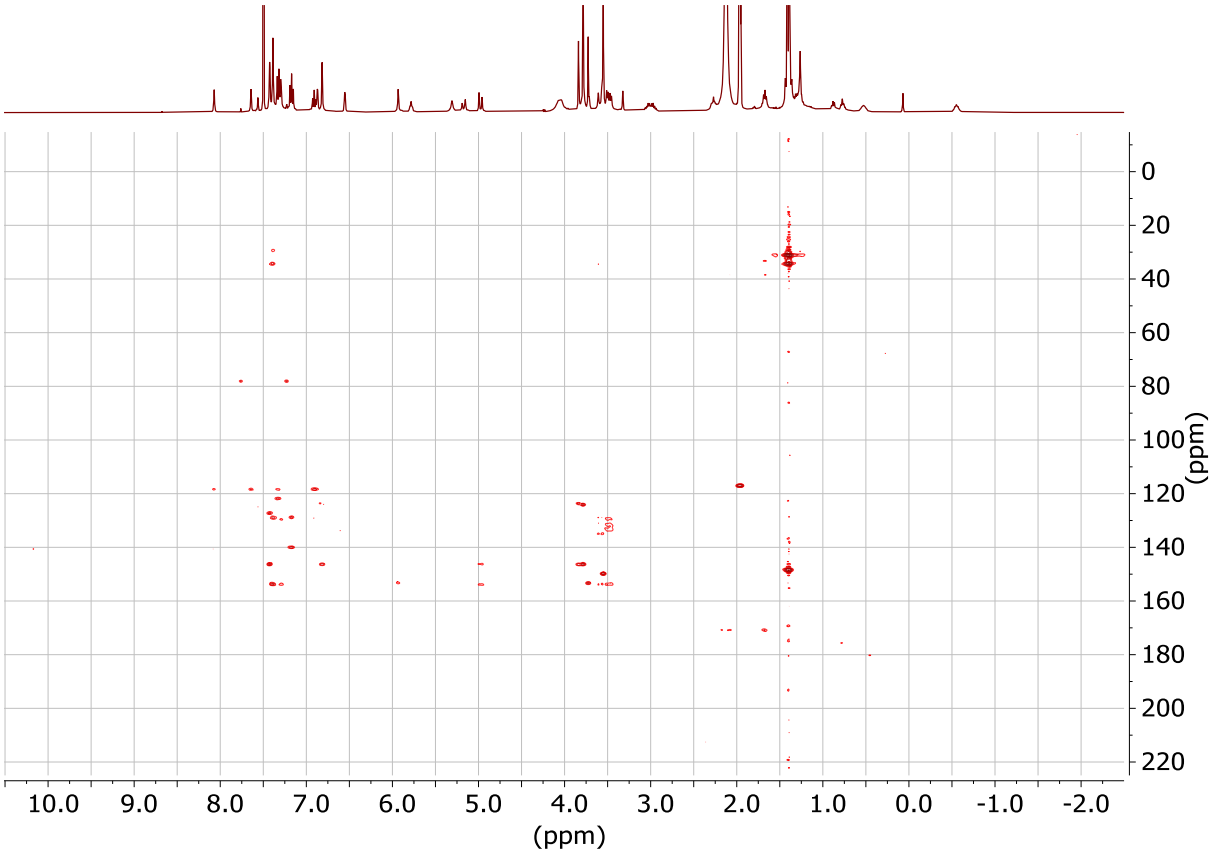

Figure S30. HMBC NMR spectrum (298K, 400 MHz, $CDCl_3/CD_3CN$ 1:1) of **4**-Zn^{2+} (2 mM). The ^{13}C NMR spectrum has not been recorded for **4**-Zn^{2+}.

Competition experiments in $CDCl_3/CD_3CN$ 1:1

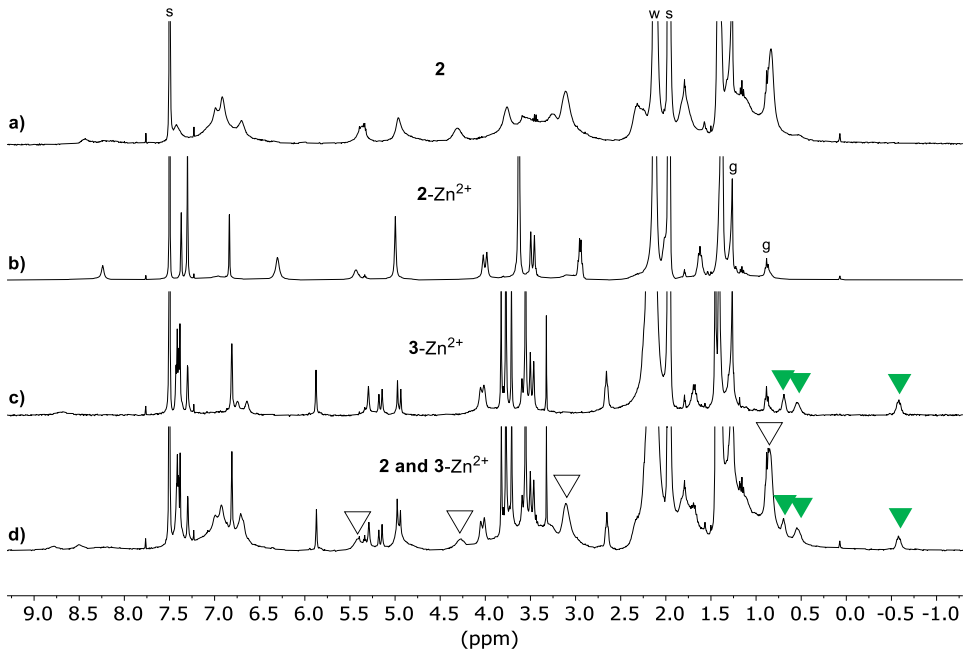

Figure S31. ^{1}H NMR spectra (298 K, 400 MHz, $CDCl_3/CD_3CN$ 1:1) of a) **2** (1 mM), b) **2** (1 mM) + 1 equiv. $Zn(OTf)_2$ → **2**-Zn^{2+}, c) **3** (1 mM) + 1 equiv. $Zn(OTf)_2$ → **3**-Zn^{2+}, d) **2** (1 mM) + **3** (1 mM) + 1 equiv. $Zn(OTf)_2$ (1 mM) → only **3**-Zn^{2+}. s = residual solvents, w = water, g = grease. ▼= **3**-Zn^{2+} signals (some of them, of interest) and ▽ = free **2.**

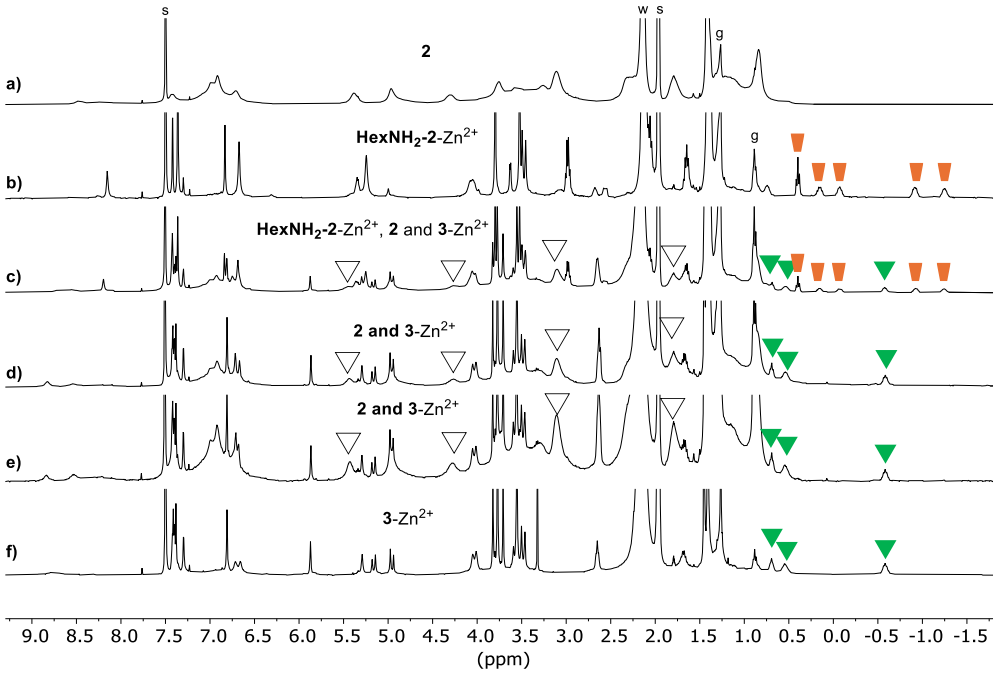

Figure S32. ^{1}H NMR spectra (298 K, 400 MHz, $CDCl_3/CD_3CN$ 1:1) of a) **2** (1 mM), b) **2** (1 mM) + 1.6 equiv. $HexNH_2$ + 1 equiv. $Zn(OTf)_2$ → $HexNH_2$⊂**2**-Zn^{2+}, c) **2** (1 mM) + 1 equiv. $HexNH_2$ + 1 equiv. $Zn(OTf)_2$ + 0.5 equiv. **3** (0.5 mM) → $HexNH_2$⊂**2**-Zn^{2+} (50%) and **3**-Zn^{2+} (50%), d) **2** (1 mM) + 1 equiv. $HexNH_2$ + 1 equiv. $Zn(OTf)_2$ + 1 equiv. **3** (1 mM) → only **3**-Zn^{2+}, e) **2** (2 mM) + 1 equiv. $HexNH_2$ (2 mM) + 0.5 equiv. $Zn(OTf)_2$ + 0.5 equiv. **3** (1mM) → only **3**-Zn^{2+}, f) **3** (1 mM) + 1 equiv. $Zn(OTf)_2$ → **3**-Zn^{2+} (spectrum for comparison). s = residual solvents, w = water, g = grease. ▼= $HexNH_2$⊂**2**-Zn^{2+}, ▼= **3**-Zn^{2+} signals (some of them, of interest) and ▽ = free **2.**

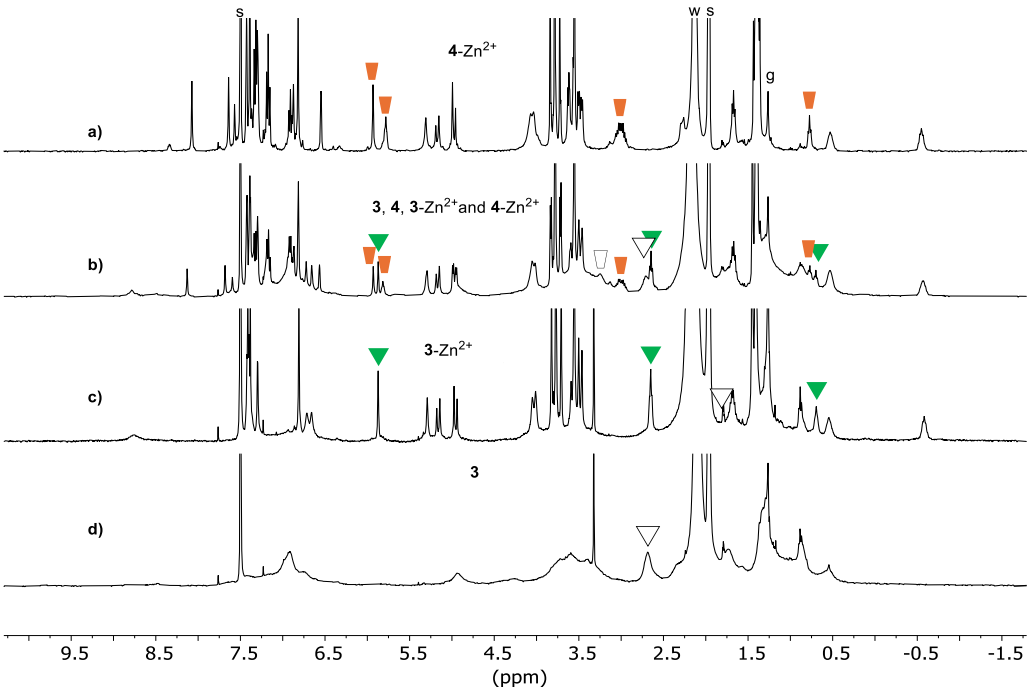

Figure S33. ^{1}H NMR spectra (298 K, 400 MHz, $CDCl_3/CD_3CN$ 1:1) of a) **4** (2 mM) + 1 equiv. $Zn(OTf)_2$ → **4**-Zn^{2+}, b) **4** (2 mM) + 1 equiv. $Zn(OTf)_2$ + 1 equiv. **3** → **4**-Zn^{2+} (50%) and **3**-Zn^{2+} (50%), c) **3** (1 mM) + 1 equiv. $Zn(OTf)_2$ → **3**-Zn^{2+}, d) **3** (1 mM). s = residual solvents, w = water, g = grease. ▼= **4**-Zn^{2+}, ▽ = free **4**, ▼= **3**-Zn^{2+} ▽ = free **3.**

Characterization of complex 3-Zn^{2+} in D_2O

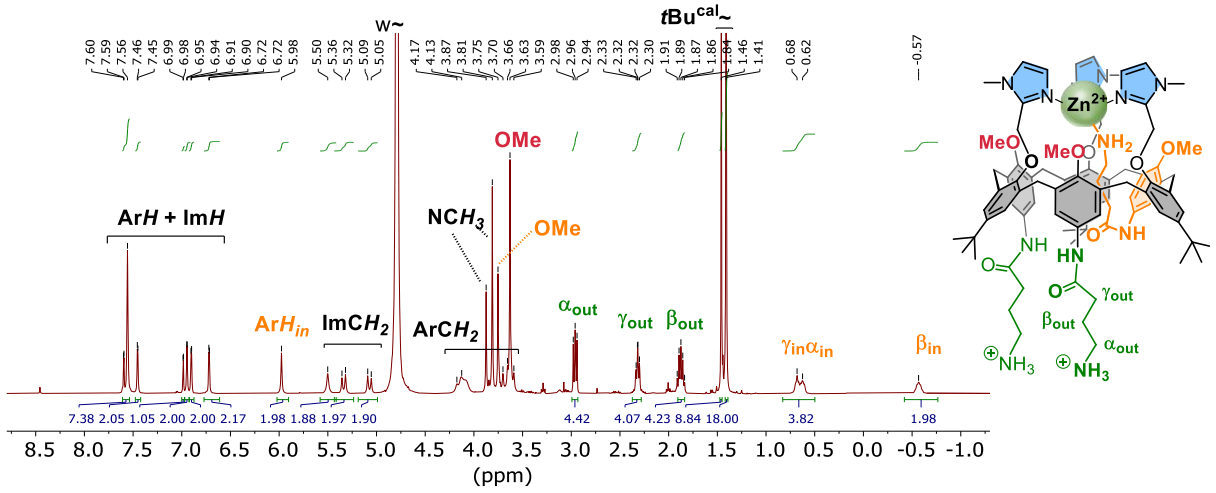

Figure S34. ^{1}H NMR spectrum (298K, 400 MHz, D_2O) of **3**-Zn^{2+} (5 mM). w: water.

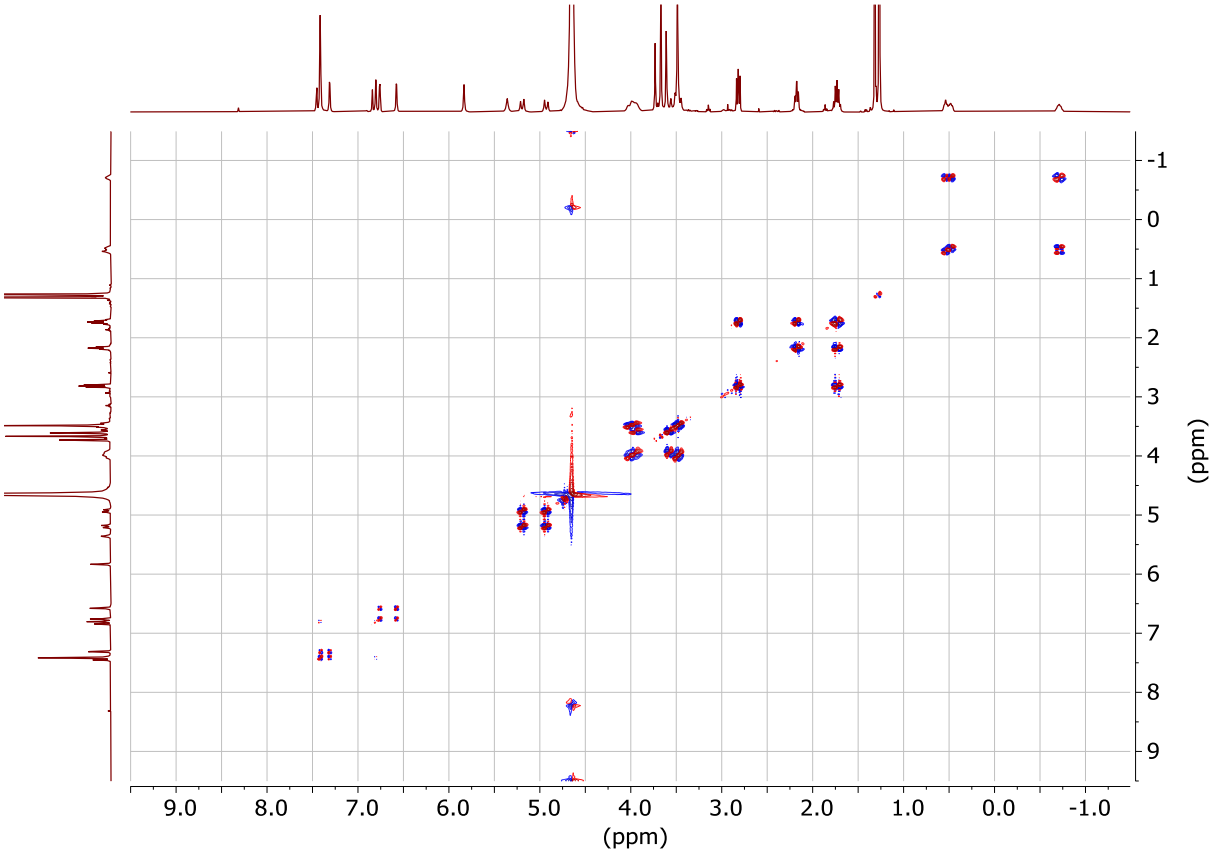

Figure S35. COSY NMR spectrum (298K, 400 MHz, D_2O) of **3**-Zn^{2+} (5 mM).

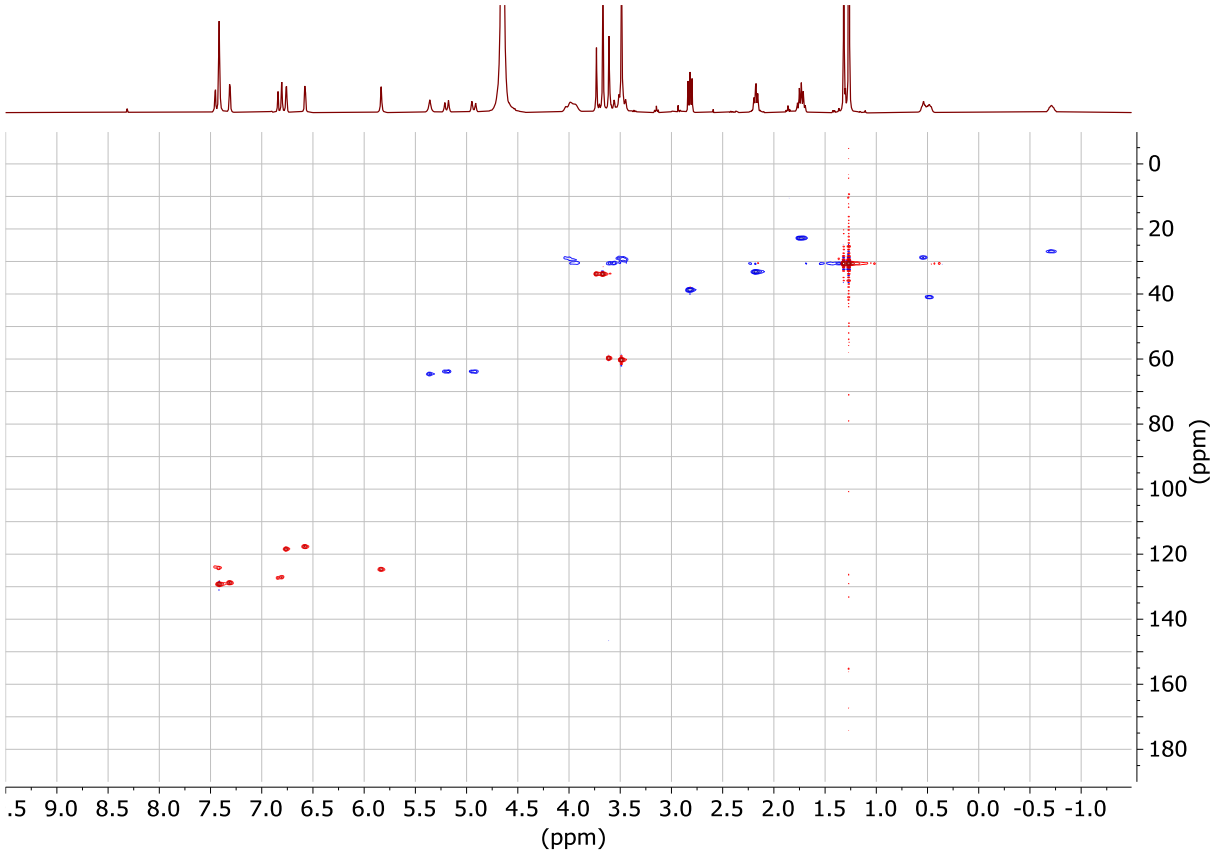

Figure S36. Edited HSQC NMR spectrum (298K, 400 MHz, D_2O) of **3**-Zn^{2+} (5 mM).

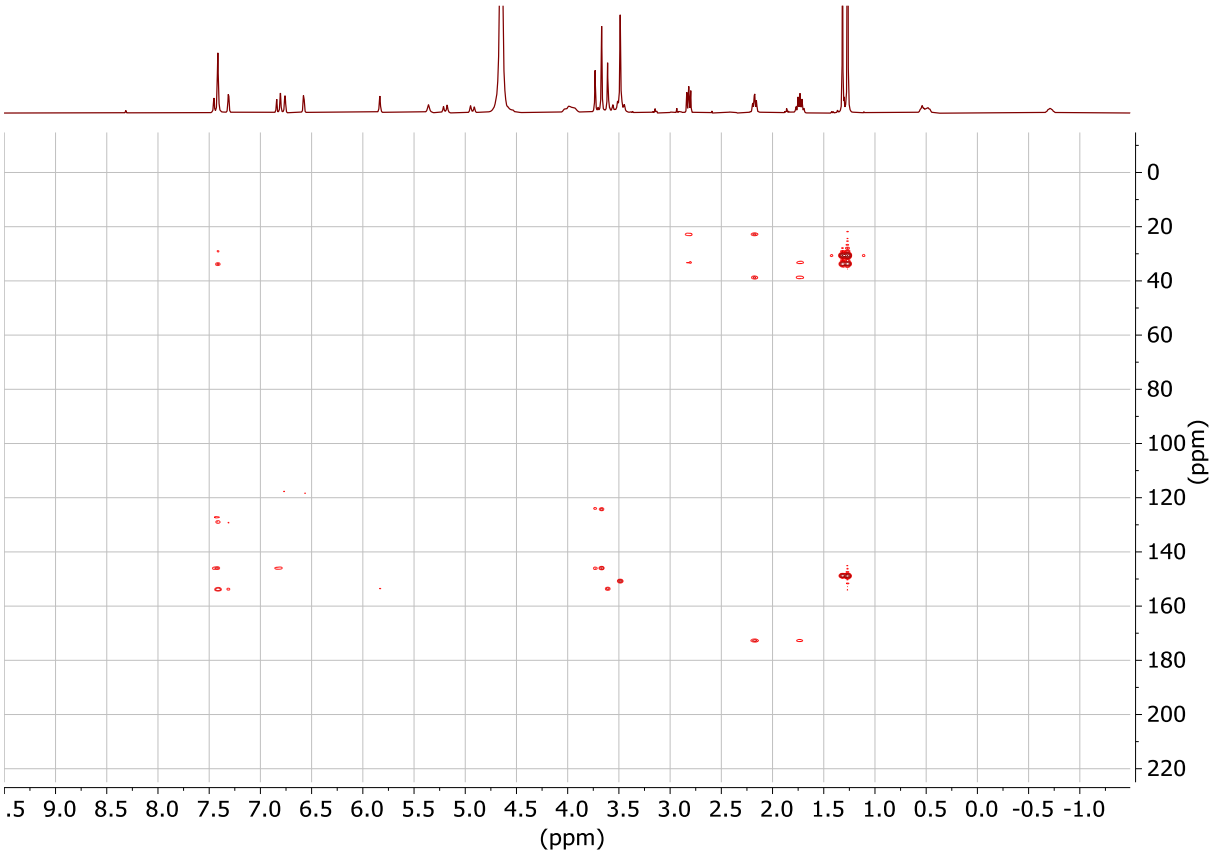

Figure S37. HMBC spectrum (298K, 400 MHz, D_2O) of **3**-Zn^{2+} (5 mM).

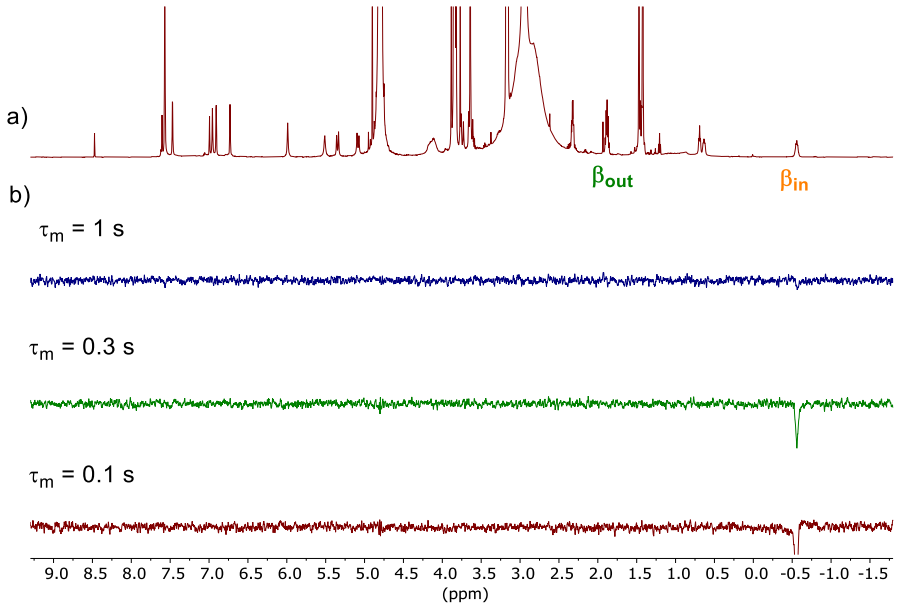

Figure S38. ^{1}H NMR spectra (298 K, 600 MHz, D_2O) of a) **3**-Zn^{2+}, b) 1D EXSY spectra (at various mixing times) after selective excitation of the β_{in} signal at −0.57 ppm.

Titrations for the formation of the complexes in D_2O

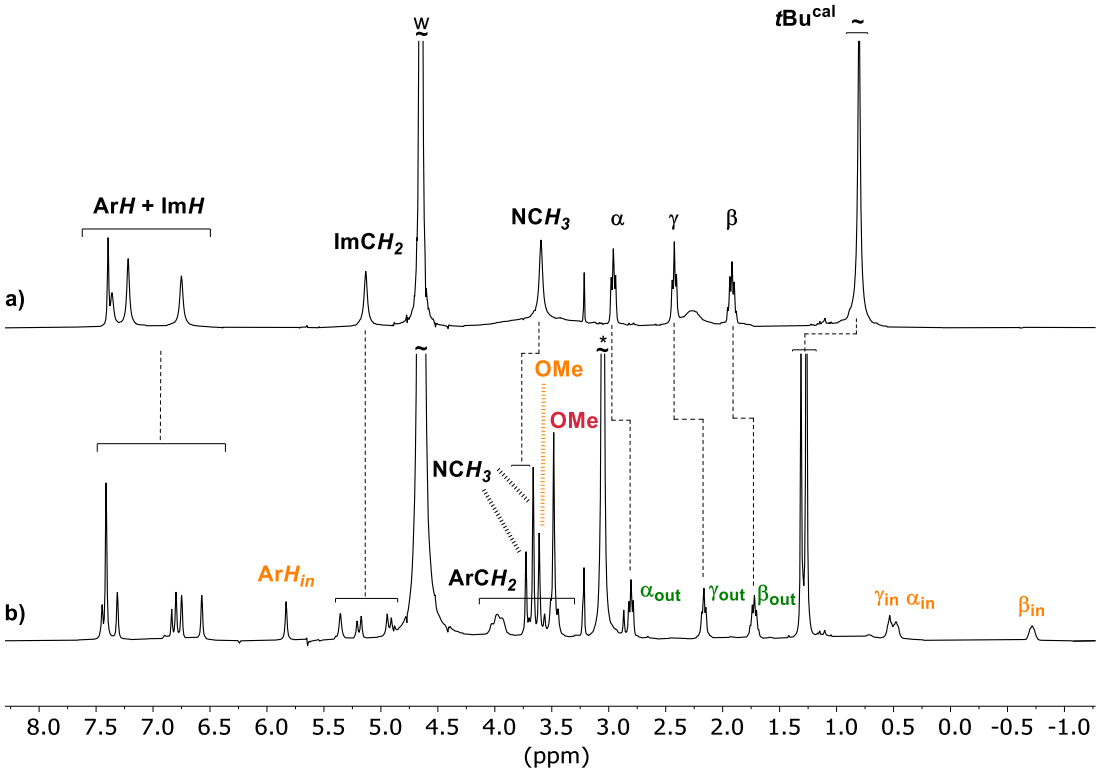

Figure S39. ^{1}H NMR spectra (298 K, 400 MHz, D_2O) of a) **3** (1 mM) + ~1 equiv. of $Zn(ClO_4)_2$ at pH = 3–4, b) **3** (1 mM) + ~1 equiv. of $Zn(ClO_4)_2$ + TMAOH (pH = 7.8). * = TMA^+, w = water.

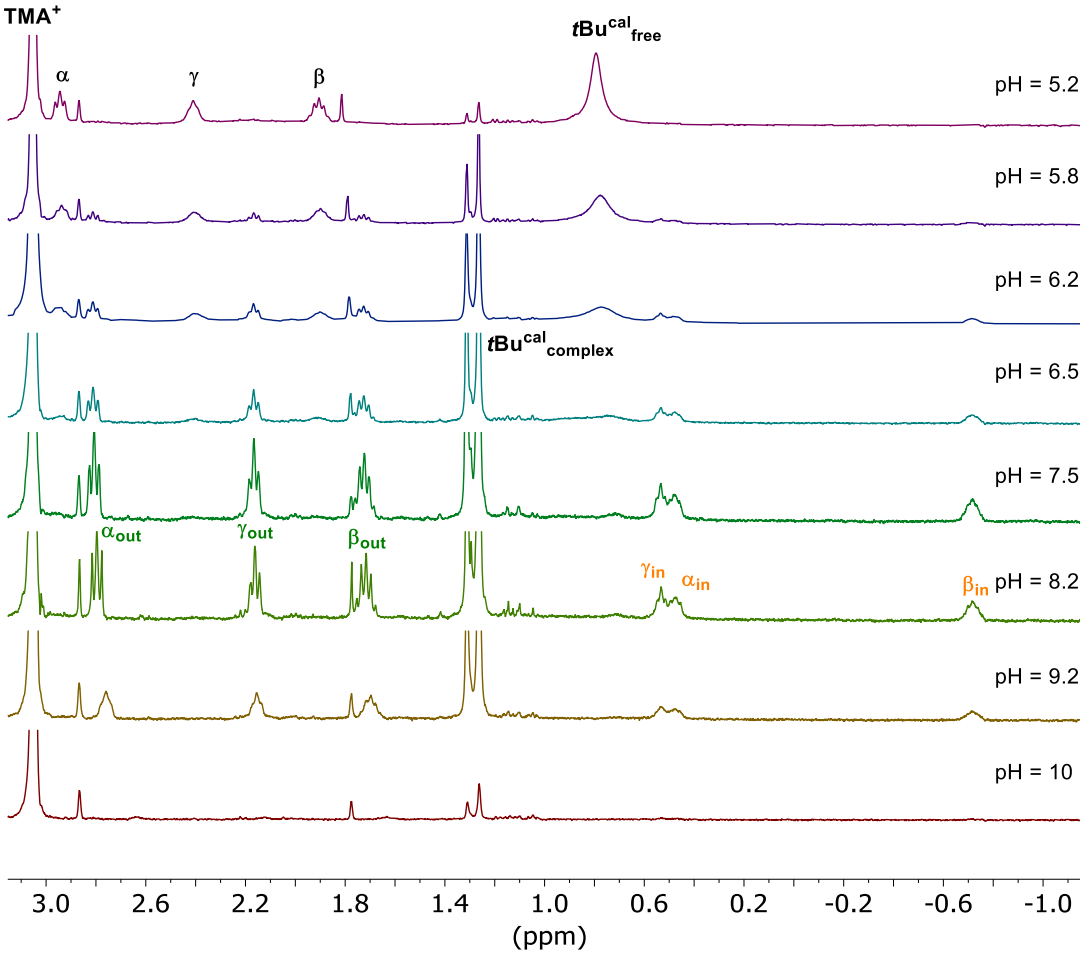

Figure S40. High field region of ^{1}H NMR spectra (298 K, 400 MHz, D_2O) of **3** (1 mM) + ~1 equiv. of $Zn(ClO_4)_2$ at various pH, changed by addition of TMAOH/DCl aliquots. At pH 9.2 and 10, a precipitate was observed in the NMR tube.

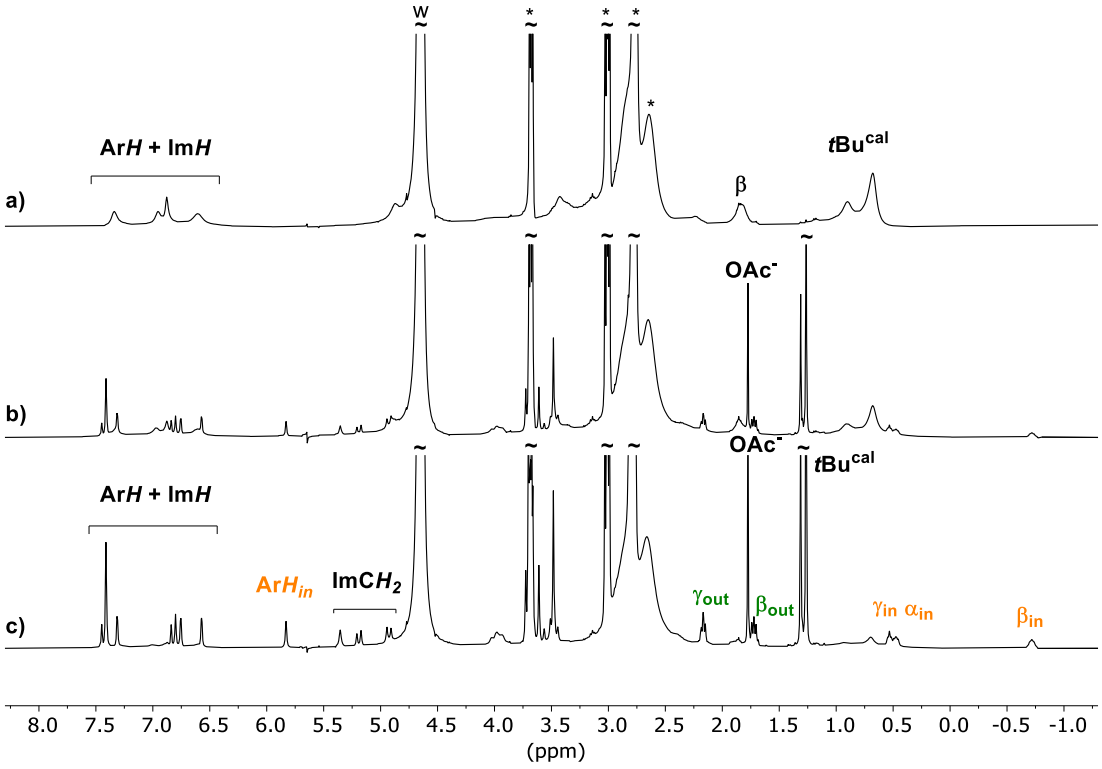

Figure S41. ^{1}H NMR spectra (298 K, 400 MHz, D_2O) of a) **3** (1 mM), b) **3** (1 mM) + ~0.4 equiv. of $Zn(OAc)_2$, b) **3** (1 mM) + ~1.1 equiv. of $Zn(OAc)_2$, all in HEPES 10 mM, pH 7.9. * = HEPES signals, w = water.

The formation constants, K_a^{eff} and K'_a(pH), of the complex $\mathbf{3}\cdot 2H^+\text{-}Zn^{2+}$ are defined according to equilibrium (1):

$$\mathbf{3}\cdot 3H^+ + Zn^{2+} + H_2O \rightleftarrows \mathbf{3}\cdot 2H^+\text{-}Zn^{2+} + H_3O^+ \qquad (1)$$

$$K_a^{eff} = \frac{[\mathbf{3}\cdot 2H^+\text{-}Zn^{2+}][H_3O^+]}{[Zn^{2+}][\mathbf{3}\cdot 3H^+]}$$

and

$$K'_a(\text{pH}) = \frac{[\mathbf{3}\cdot 2H^+\text{-}Zn^{2+}]}{[Zn^{2+}][\mathbf{3}\cdot 3H^+]}$$

From the two above spectra in Figure S41, the binding affinity $K'_a(7.9)$ can be calculated by integration of the different signals of interests ($\mathbf{3}\cdot 2H^+\text{-}Zn^{2+}$, $\mathbf{3}\cdot 3H^+$ and AcO^-). The ^{1}H NMR signals of $\mathbf{3}\cdot 3H^+$being rather broad, the experimental error was estimated on a wide range.

Therefore, $K'_a(7.9) = 42900$ and $30700\ M^{-1}$ were determined respectively for spectra b and c. Taking in account the experimental error, we estimated $logK'_a(7.9) = 4.5 \pm 0.3$.

Knowing the pH and therefore $[H_3O^+]$, we were able to calculate $K_a^{eff} = 5.4\,.\,10^{-4}$ and $3.8\,.\,10^{-4}$. Therefore, $pK_a^{eff} = 3.4 \pm 0.3$ as the pseudo-pK_a of the amino leg.