

## 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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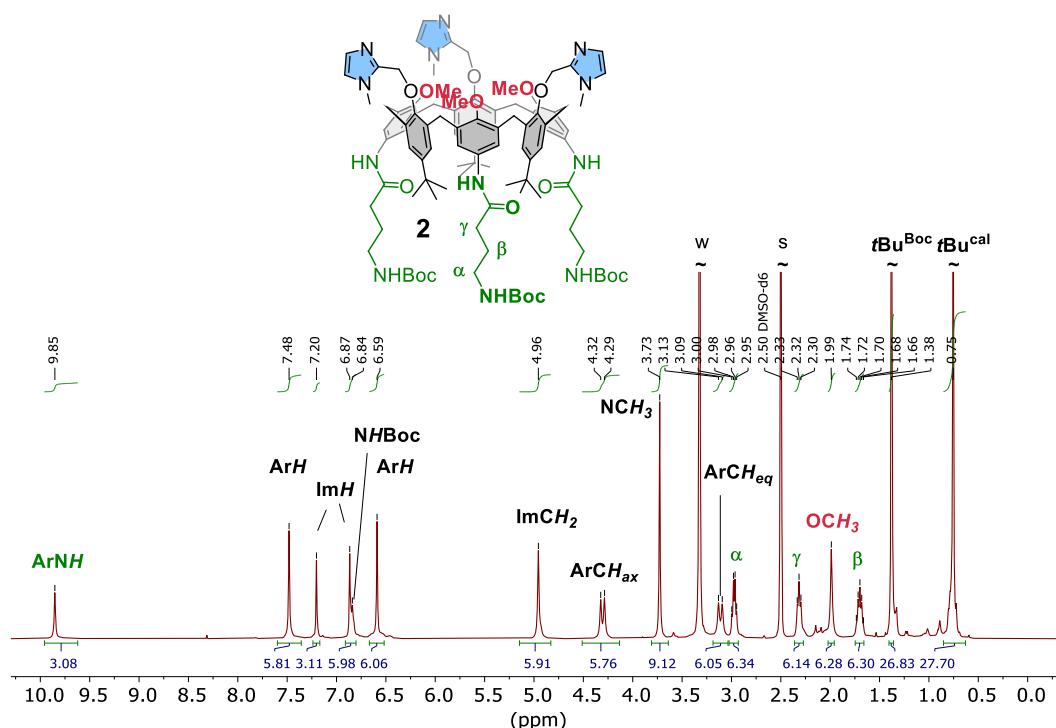
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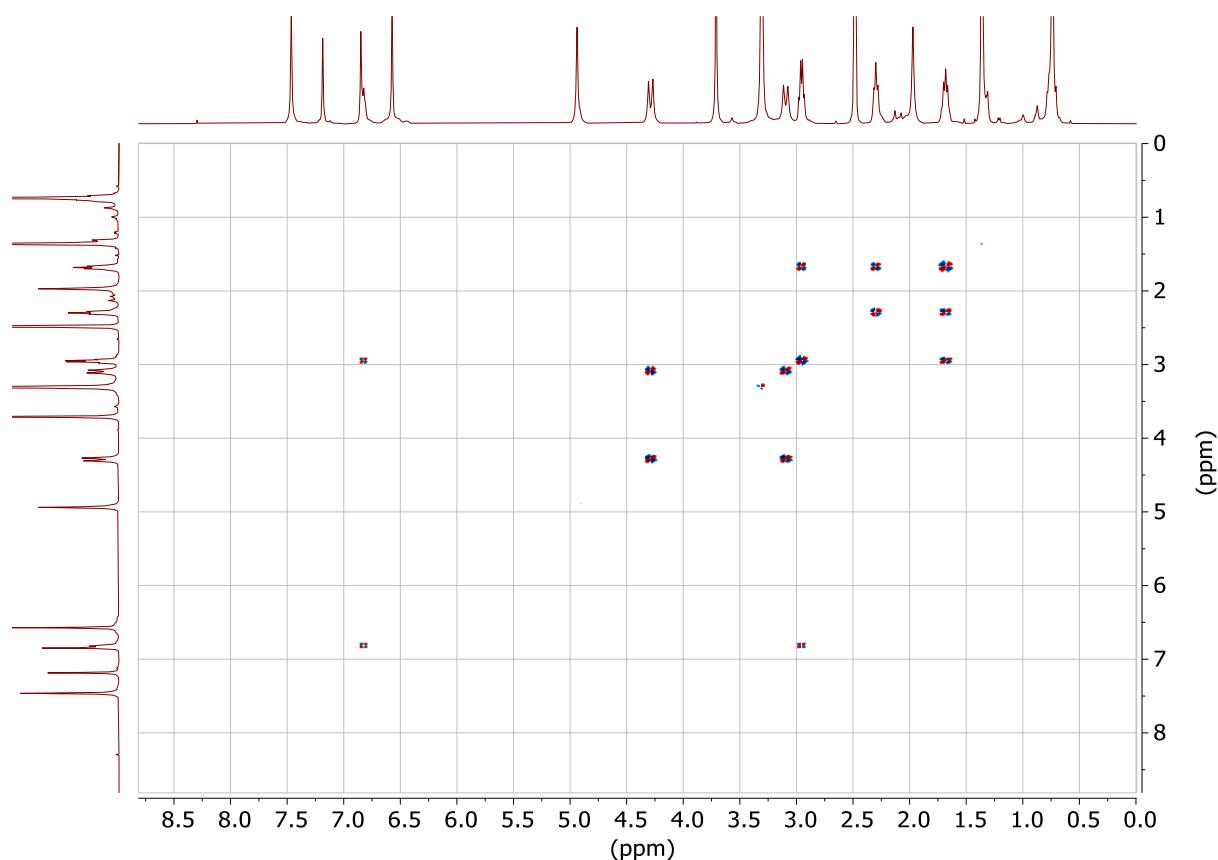
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## Characterization of 2

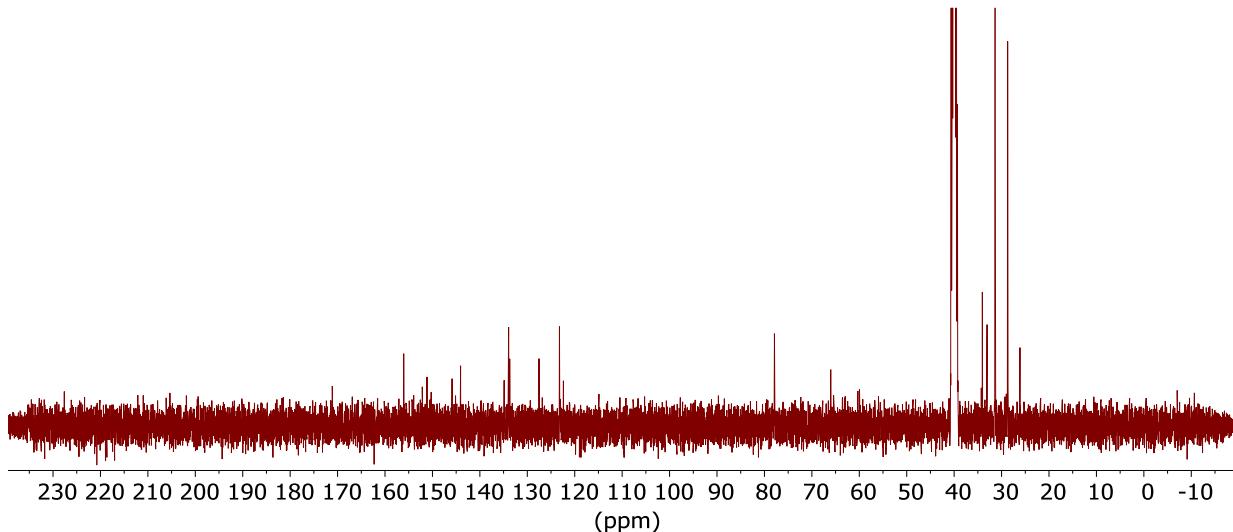


**Figure S1.**  $^1\text{H}$  NMR spectrum (298K, 400 MHz,  $\text{DMSO}-d_6$ ) of **2**. s: residual solvent, w: water.

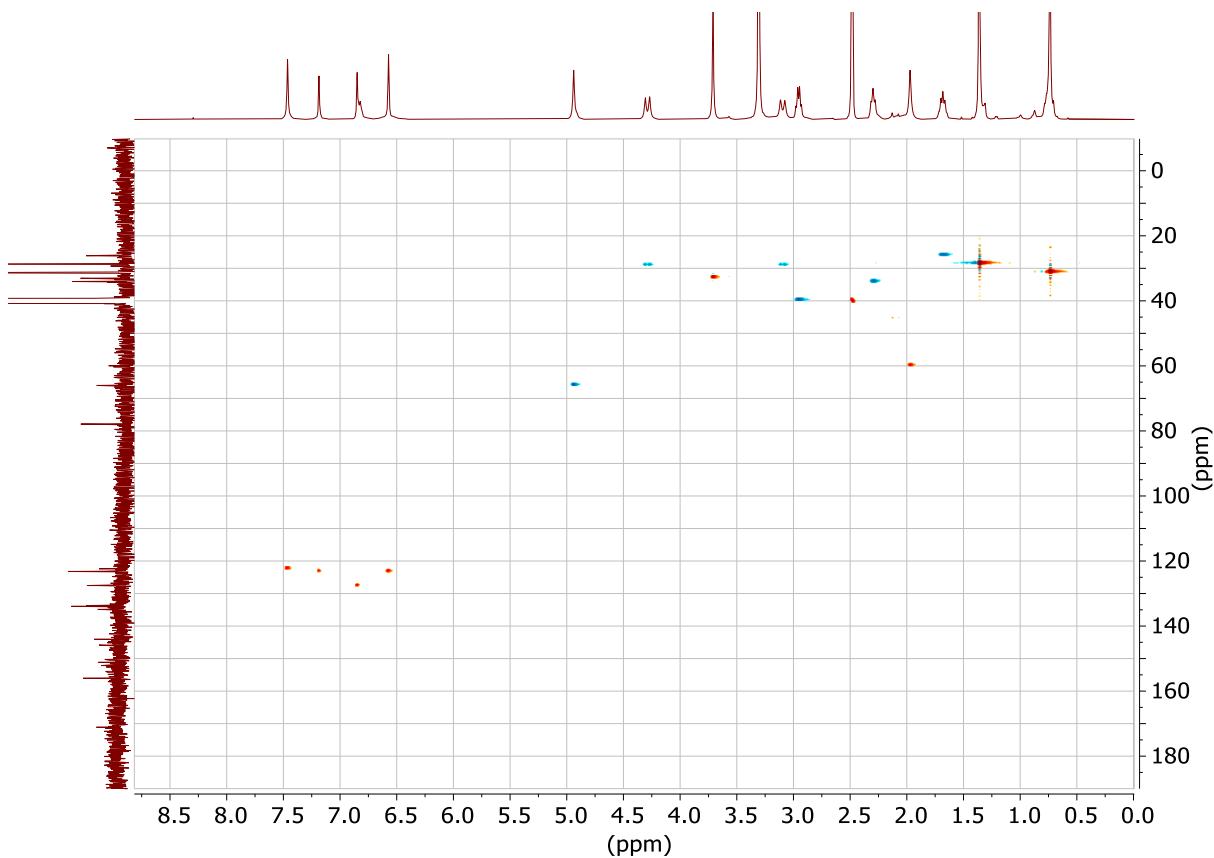


**Figure S2.** COSY NMR spectrum (298K, 400 MHz,  $\text{DMSO}-d_6$ ) of **2**.

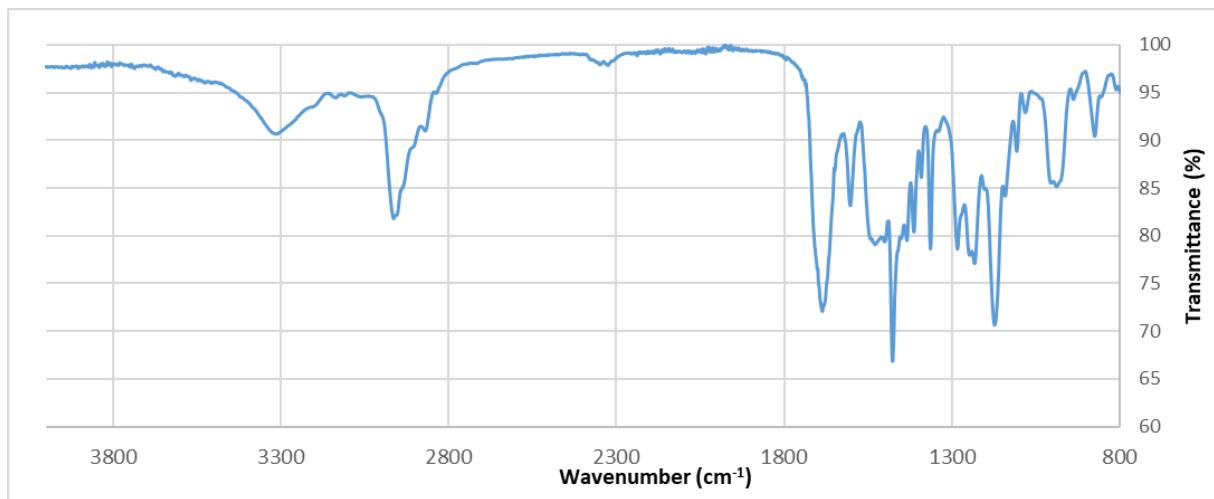
$^{13}\text{C}$  NMR characterization was attempted but only small signals could be detected after a prolonged analysis time and over 5000 scans (Figure S3). However, a HSQC spectrum allowed the assignment of some of the  $^{13}\text{C}$  signals (Figure S4).



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

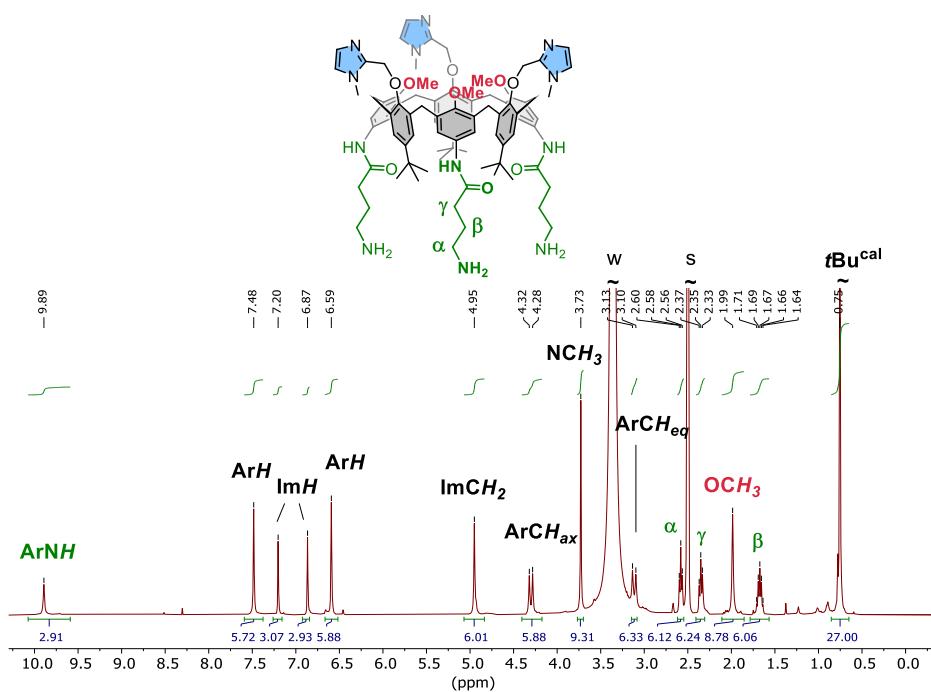


**Figure S4.** Edited HSQC NMR spectrum (298K, 400 MHz, DMSO- $d_6$ ) of **2**.

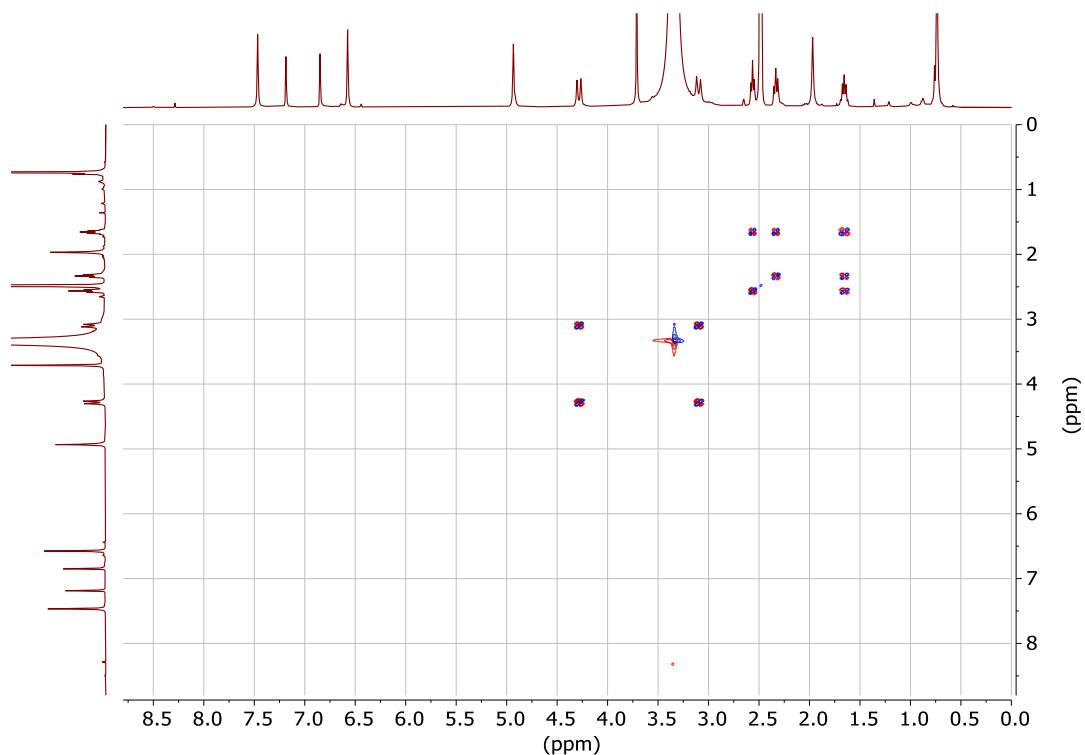


**Figure S5.** ATR-FTIR spectrum of **2**.

## Characterization of 3

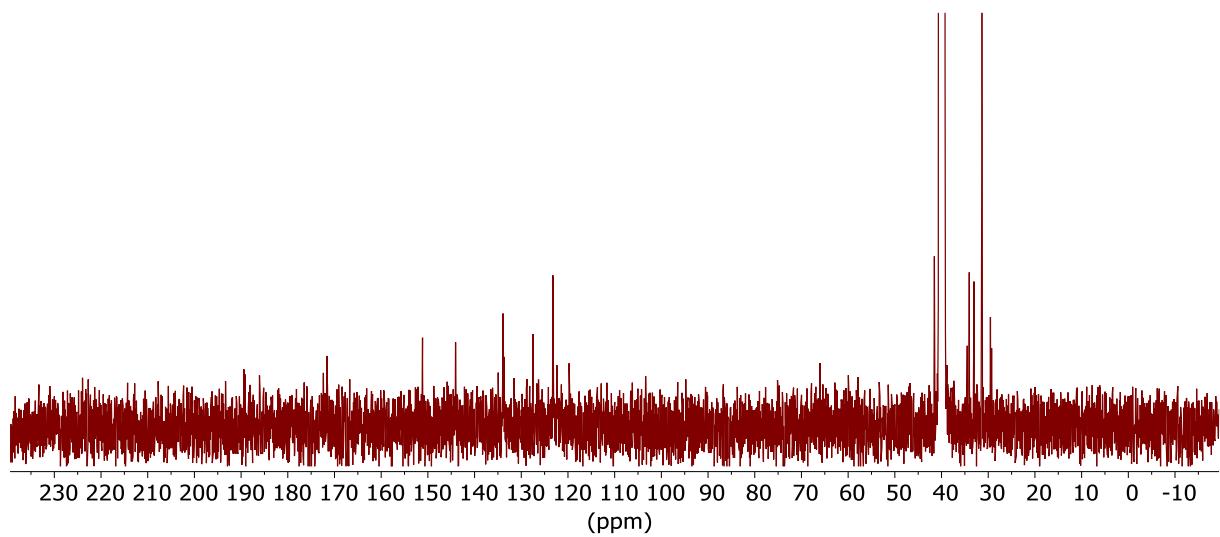


**Figure S6.** <sup>1</sup>H NMR spectrum (298K, 400 MHz, DMSO-*d*<sub>6</sub>) of 3. s: residual solvent, w: water.

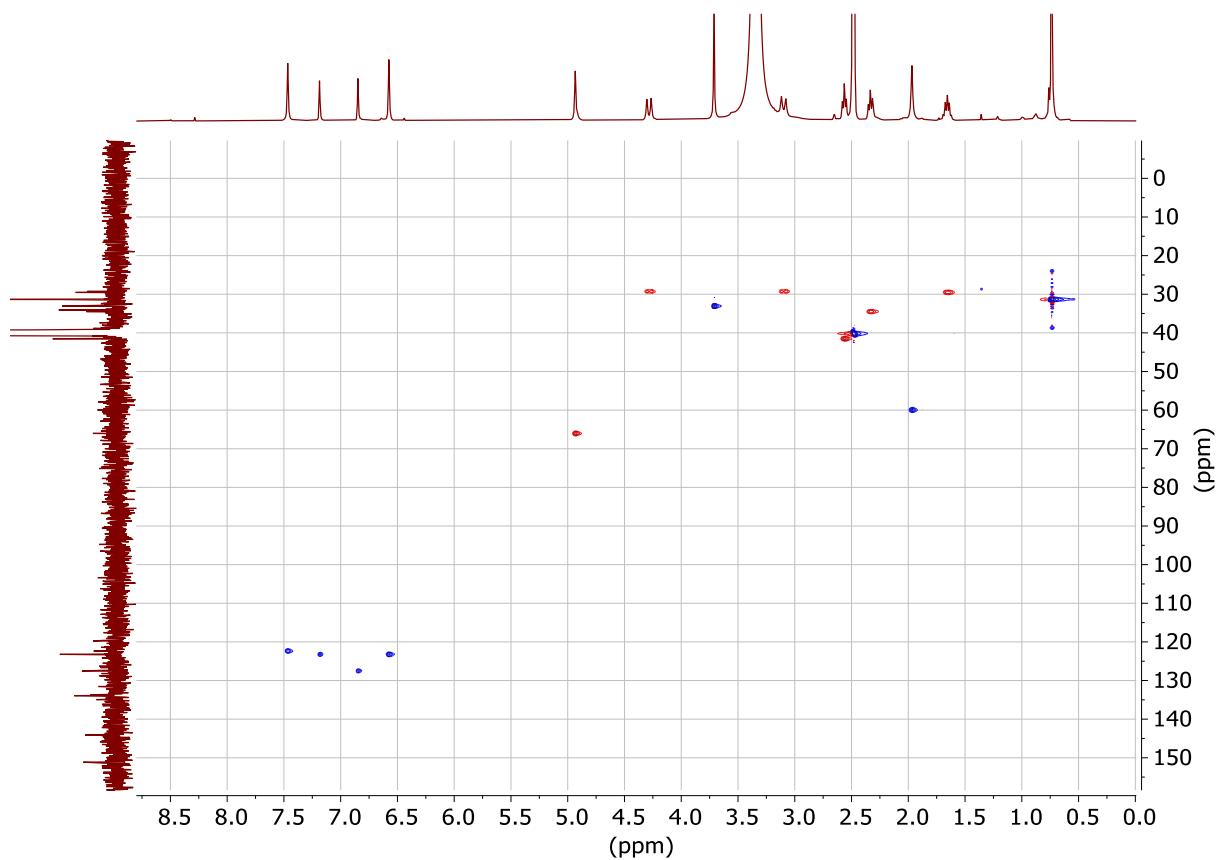


**Figure S7.** COSY NMR spectrum (298K, 400 MHz, DMSO-*d*<sub>6</sub>) of 3.

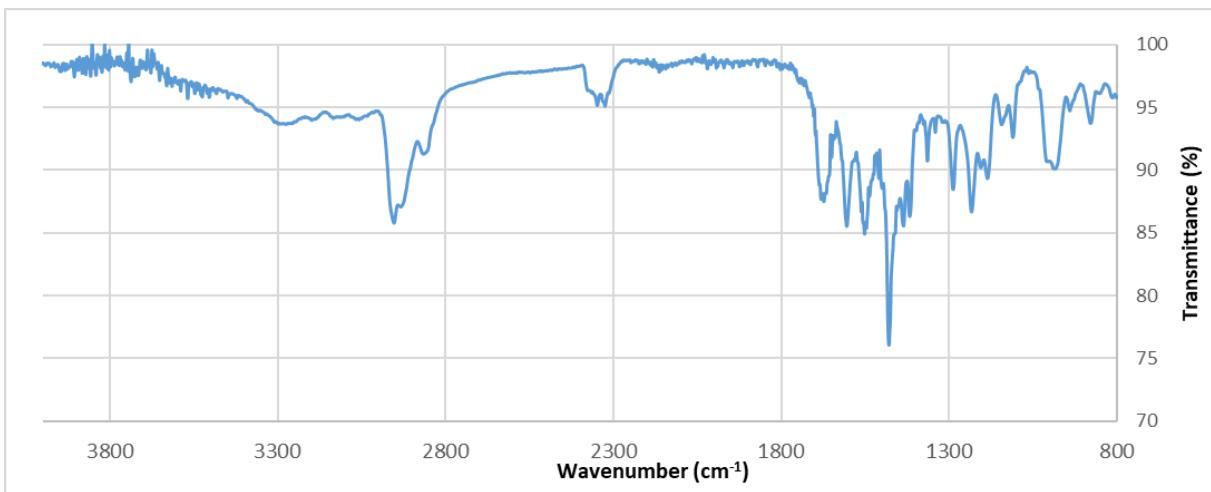
$^{13}\text{C}$  NMR characterization was attempted but only small signals could be detected after a prolonged analysis time and over 5000 scans (Figure S8). However, a HSQC spectrum allowed the assignment of some of the  $^{13}\text{C}$  signals (Figure S9).



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

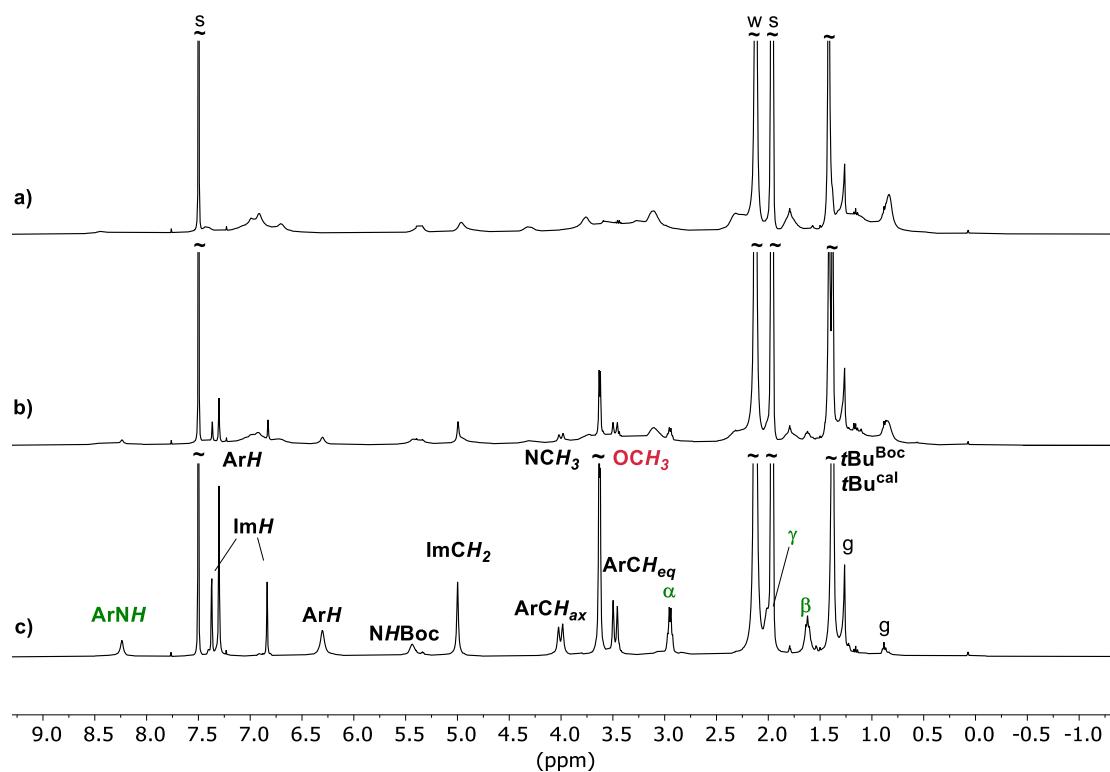


**Figure S9.** Edited HSQC NMR spectrum (298K, 400 MHz, DMSO- $d_6$ ) of **3**.

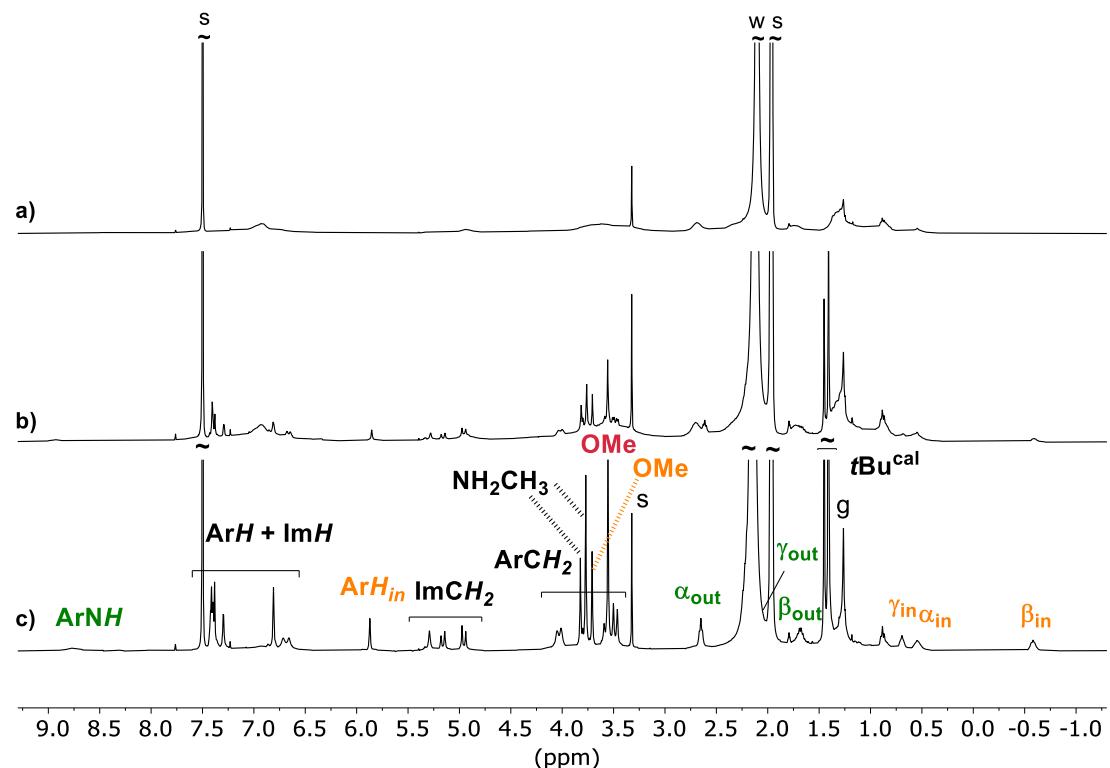


**Figure S10.** ATR-FTIR spectrum of **3**.

**Titrations for the formation of the complexes in  $\text{CDCl}_3/\text{CD}_3\text{CN}$  1:1**

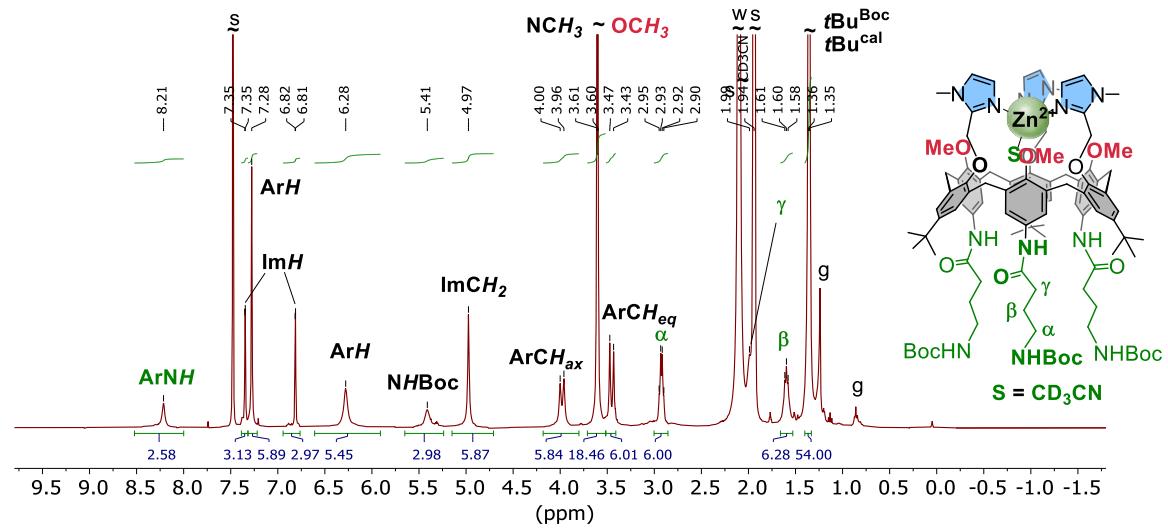


**Figure S11.**  $^1\text{H}$  NMR spectra (298 K, 400 MHz,  $\text{CDCl}_3/\text{CD}_3\text{CN}$  1:1) of a) **2** (1 mM), b) **2** (1 mM) + ~0.4 equiv. of  $\text{Zn}(\text{OTf})_2$ , c) **2** (1 mM) + ~1.0 equiv. of  $\text{Zn}(\text{OTf})_2$ . s = residual solvents, w = water, g = grease.

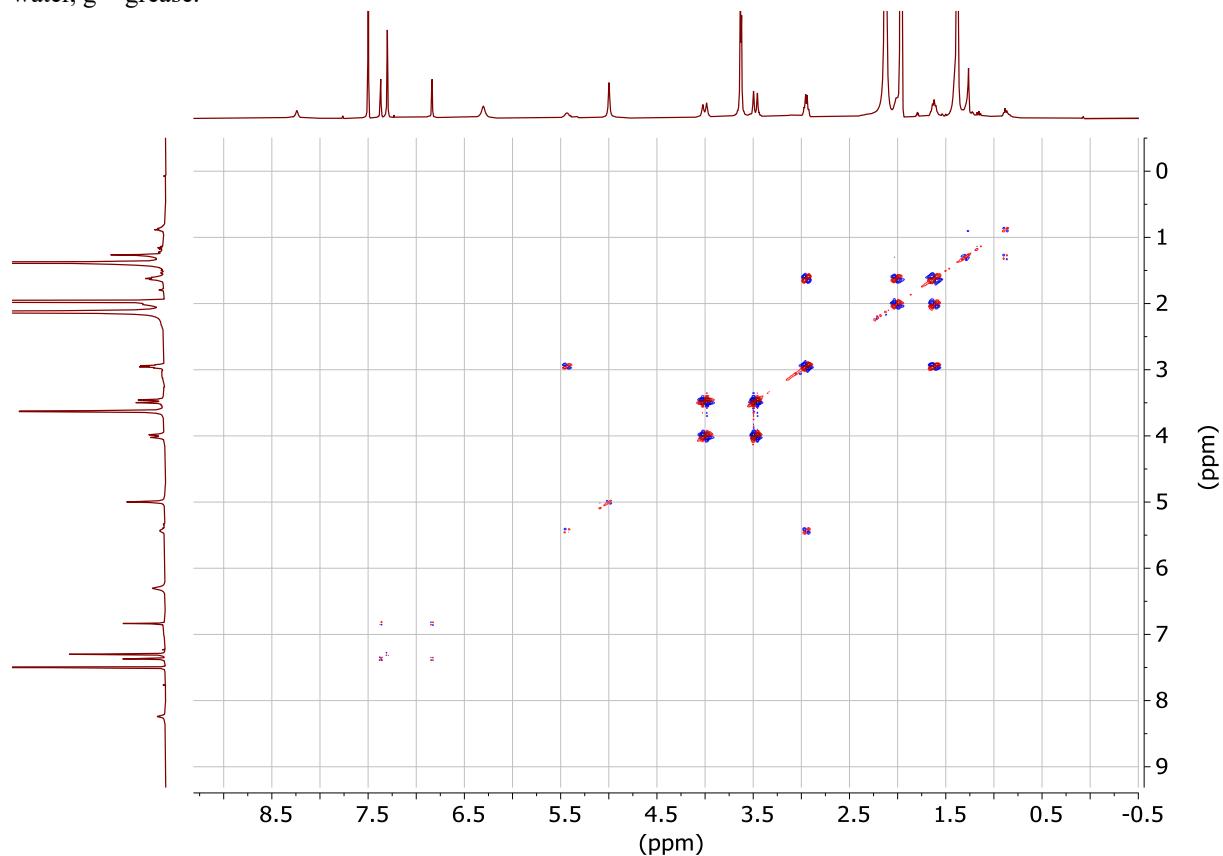


**Figure S12.**  $^1\text{H}$  NMR spectra (298 K, 400 MHz,  $\text{CDCl}_3/\text{CD}_3\text{CN}$  1:1) of a) **3** (1 mM), b) **3** (1 mM) + ~0.4 equiv. of  $\text{Zn}(\text{OTf})_2$ , c) **3** (1 mM) + ~1.0 equiv. of  $\text{Zn}(\text{OTf})_2$ . s = residual solvents, w = water, g = grease.

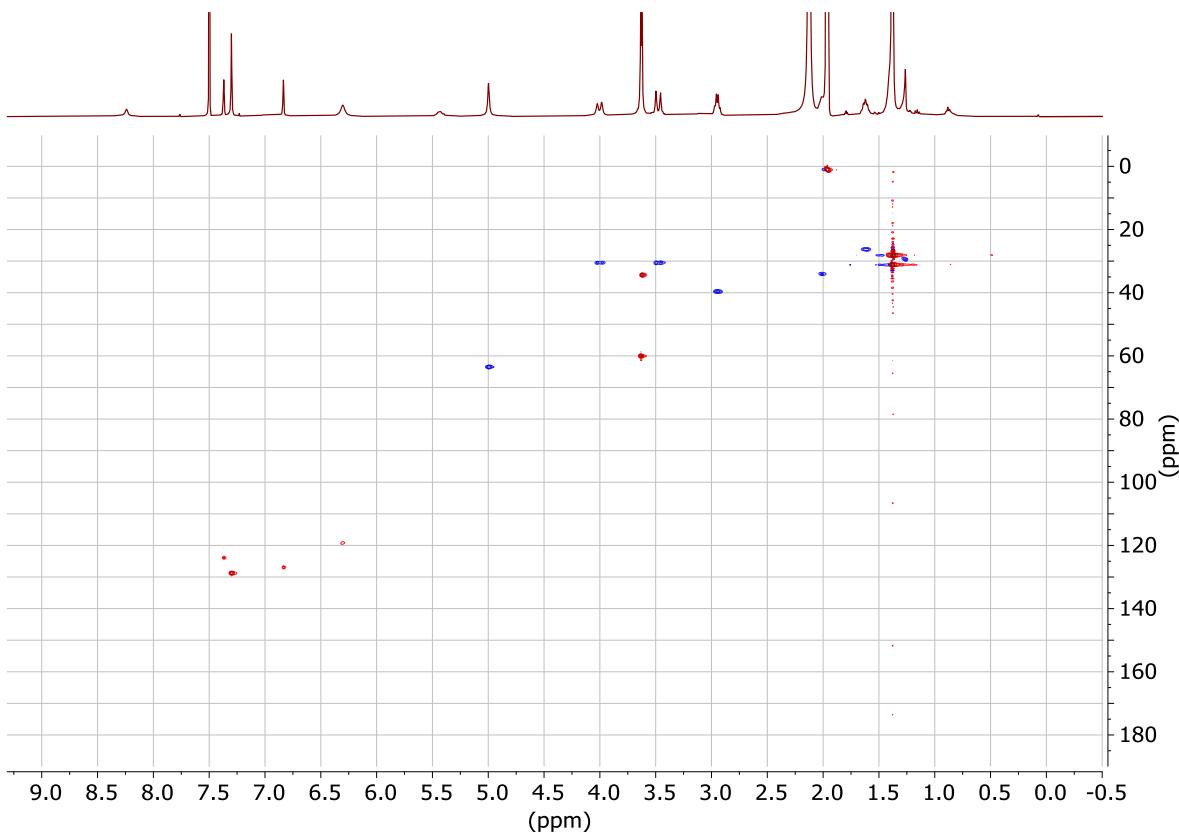
## Characterization of complex **2-Zn<sup>2+</sup>** in CDCl<sub>3</sub>/CD<sub>3</sub>CN 1:1



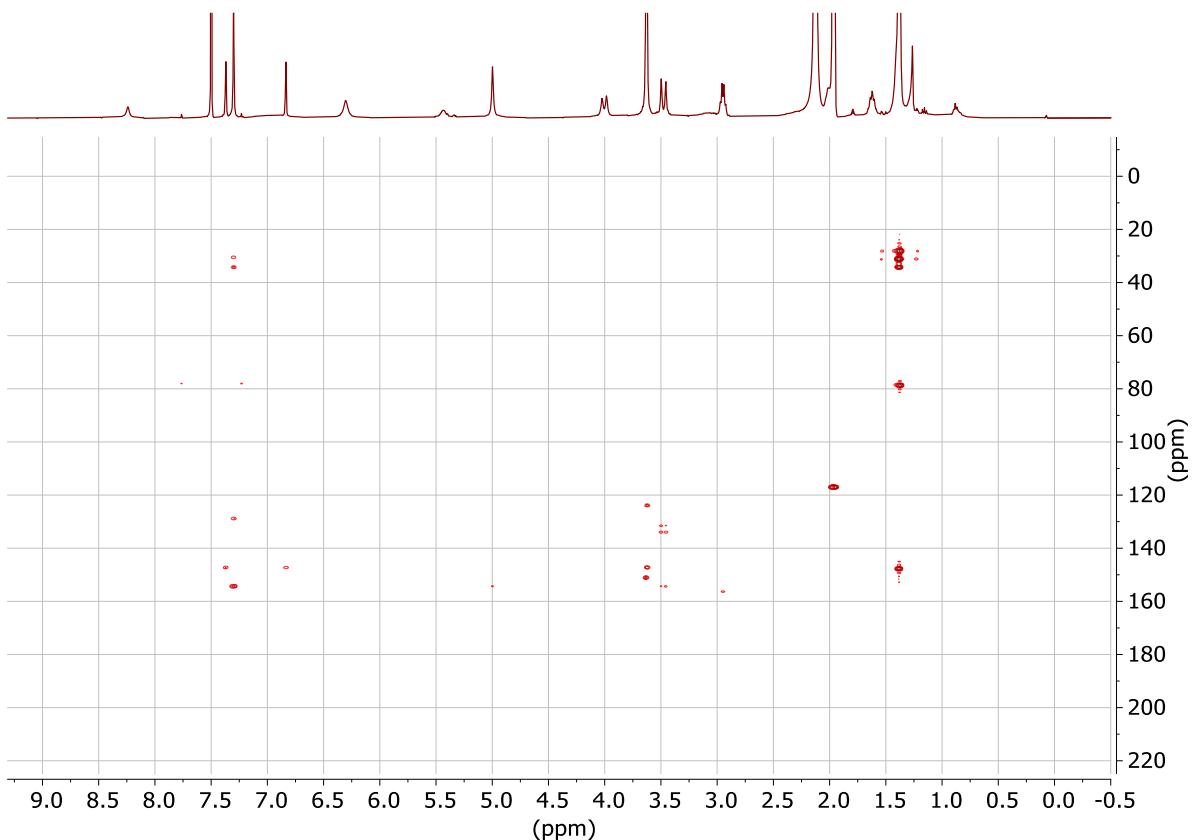
**Figure S13.** <sup>1</sup>H NMR spectrum (298K, 400 MHz, CDCl<sub>3</sub>/CD<sub>3</sub>CN 1:1) of **2-Zn<sup>2+</sup>** (1 mM). s: residual solvent, w: water, g = grease.



**Figure S14.** COSY NMR spectrum (298K, 400 MHz, CDCl<sub>3</sub>/CD<sub>3</sub>CN 1:1) of **2-Zn<sup>2+</sup>** (1 mM).

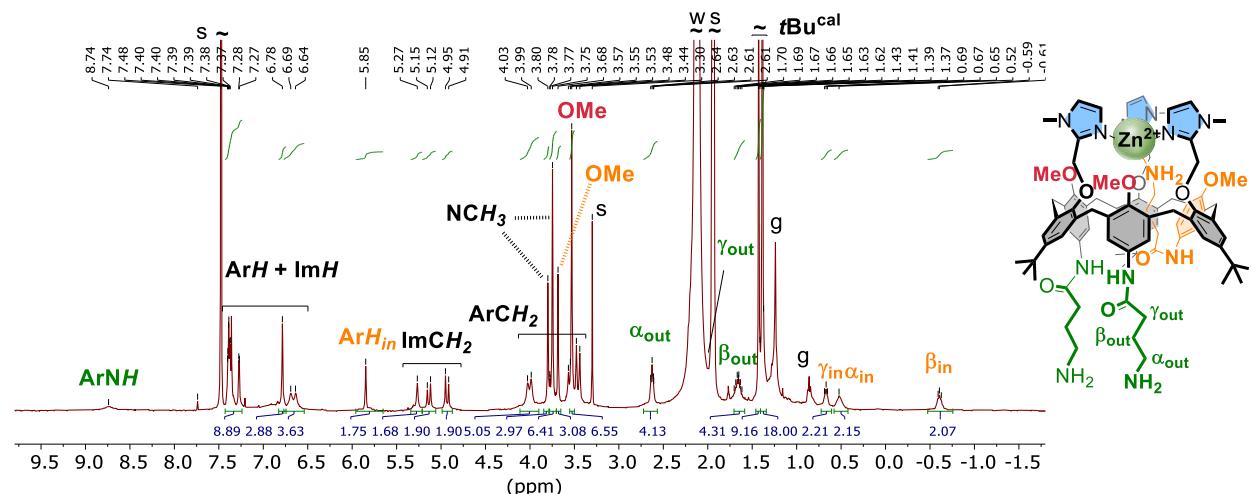


**Figure S15.** Edited HSQC NMR spectrum (298K, 400 MHz, CDCl<sub>3</sub>/CD<sub>3</sub>CN 1:1) of **2-Zn<sup>2+</sup>** (1 mM). The <sup>13</sup>C NMR spectrum has not been recorded for **2-Zn<sup>2+</sup>**.

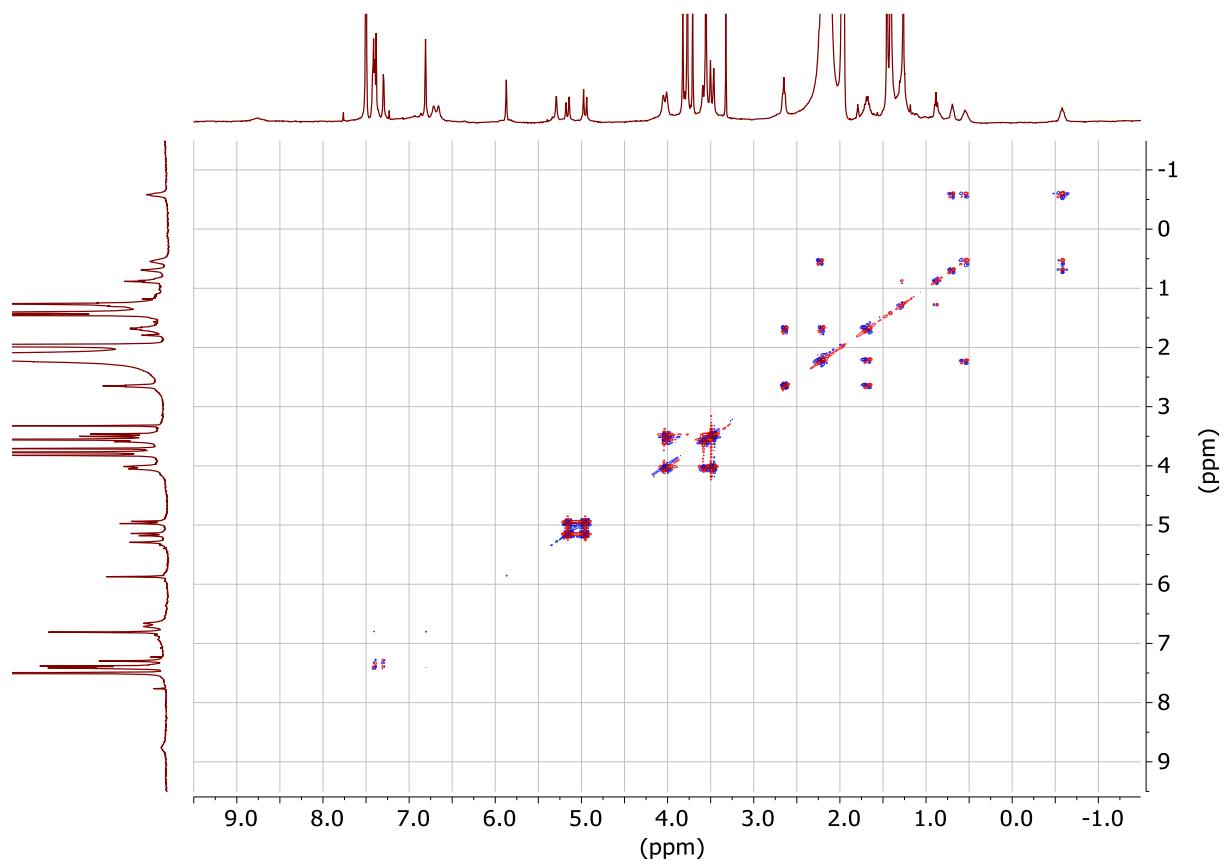


**Figure S16.** HMBC NMR spectrum (298K, 400 MHz, CDCl<sub>3</sub>/CD<sub>3</sub>CN 1:1) of **2-Zn<sup>2+</sup>** (1 mM). The <sup>13</sup>C NMR spectrum has not been recorded for **2-Zn<sup>2+</sup>**.

## Characterization of complex **3-Zn<sup>2+</sup>** in CDCl<sub>3</sub>/CD<sub>3</sub>CN 1:1

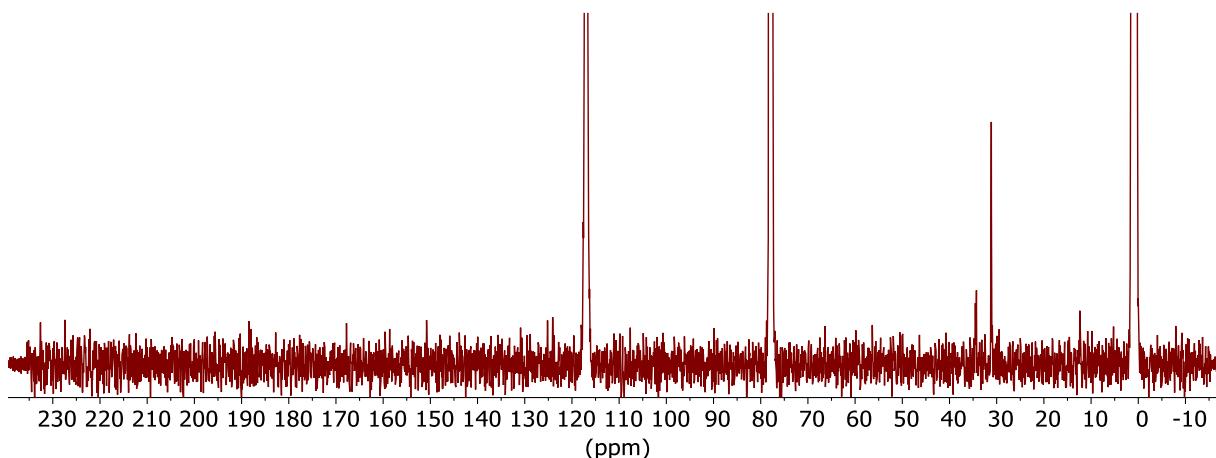


**Figure S17.** <sup>1</sup>H NMR spectrum (298K, 400 MHz, CDCl<sub>3</sub>/CD<sub>3</sub>CN 1:1) of **3-Zn<sup>2+</sup>** (1 mM). s: residual solvent, w: water, g = grease.

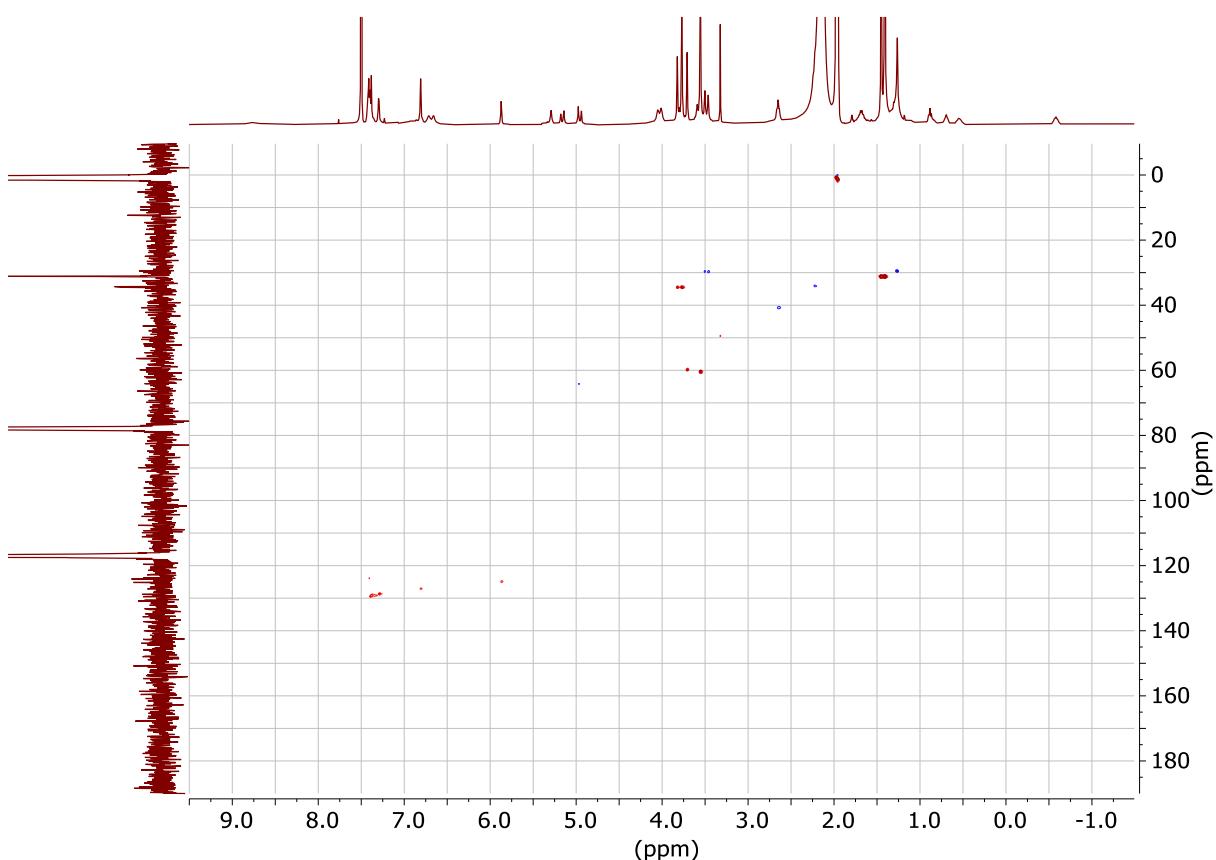


**Figure S18.** COSY NMR spectrum (298K, 400 MHz, CDCl<sub>3</sub>/CD<sub>3</sub>CN 1:1) of **3-Zn<sup>2+</sup>** (1 mM).

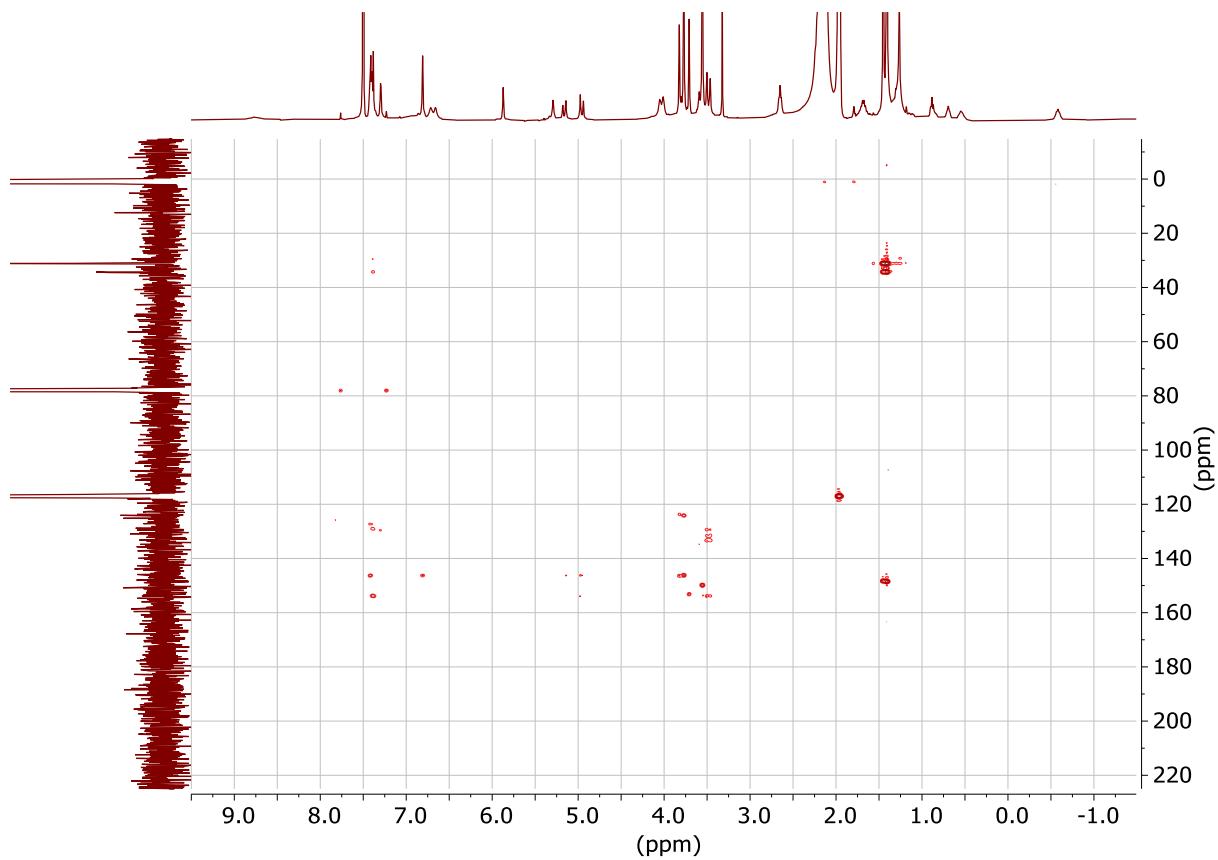
$^{13}\text{C}$  NMR characterization was attempted but only small signals could be detected after a prolonged analysis time and over 5000 scans (Figure S19). However, a HSQC spectrum allowed the assignment of some of the  $^{13}\text{C}$  signals (Figure S20).



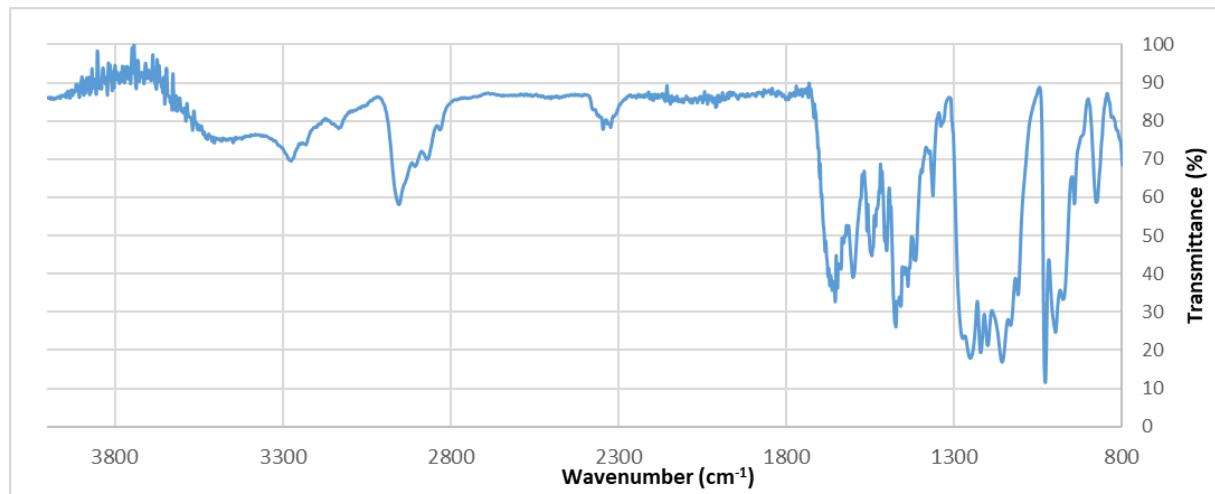
**Figure S19.**  $^{13}\text{C}$  NMR spectrum (298K, 400 MHz,  $\text{CDCl}_3/\text{CD}_3\text{CN}$  1:1) of **3-Zn<sup>2+</sup>**.



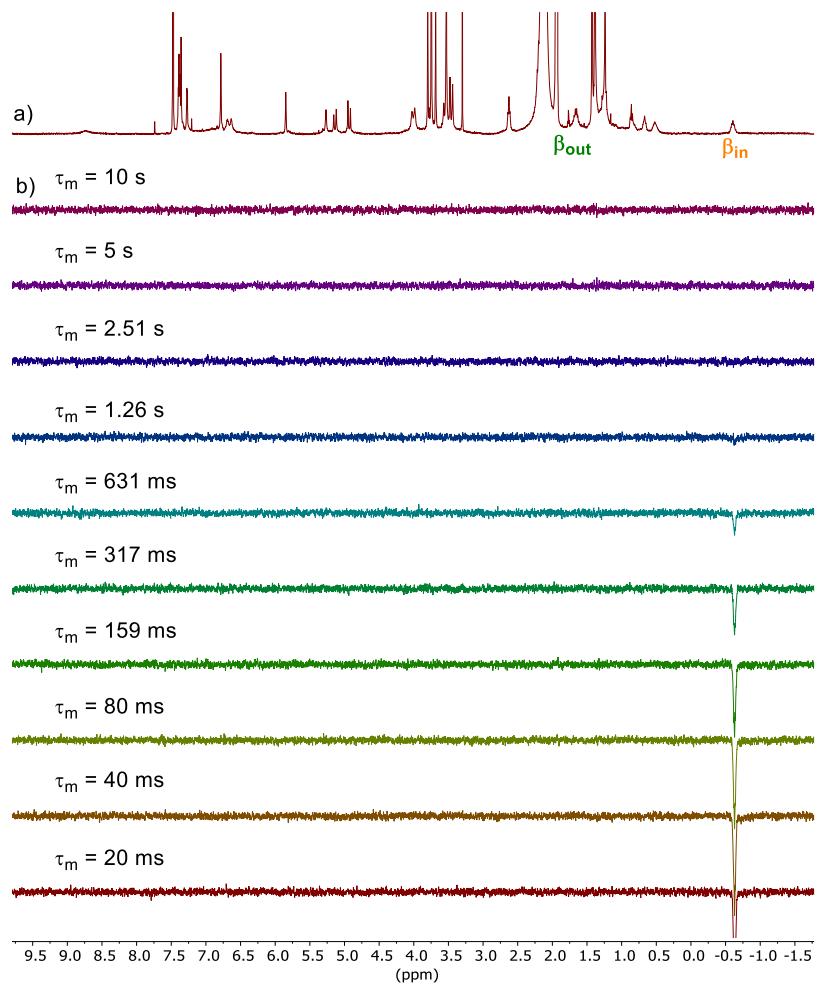
**Figure S20.** Edited HSQC NMR spectrum (298K, 400 MHz,  $\text{CDCl}_3/\text{CD}_3\text{CN}$  1:1) of **3-Zn<sup>2+</sup>** (1 mM).



**Figure S21.** HMBC NMR spectrum (298K, 400 MHz,  $\text{CDCl}_3/\text{CD}_3\text{CN}$  1:1) of **3-Zn<sup>2+</sup>** (1 mM).

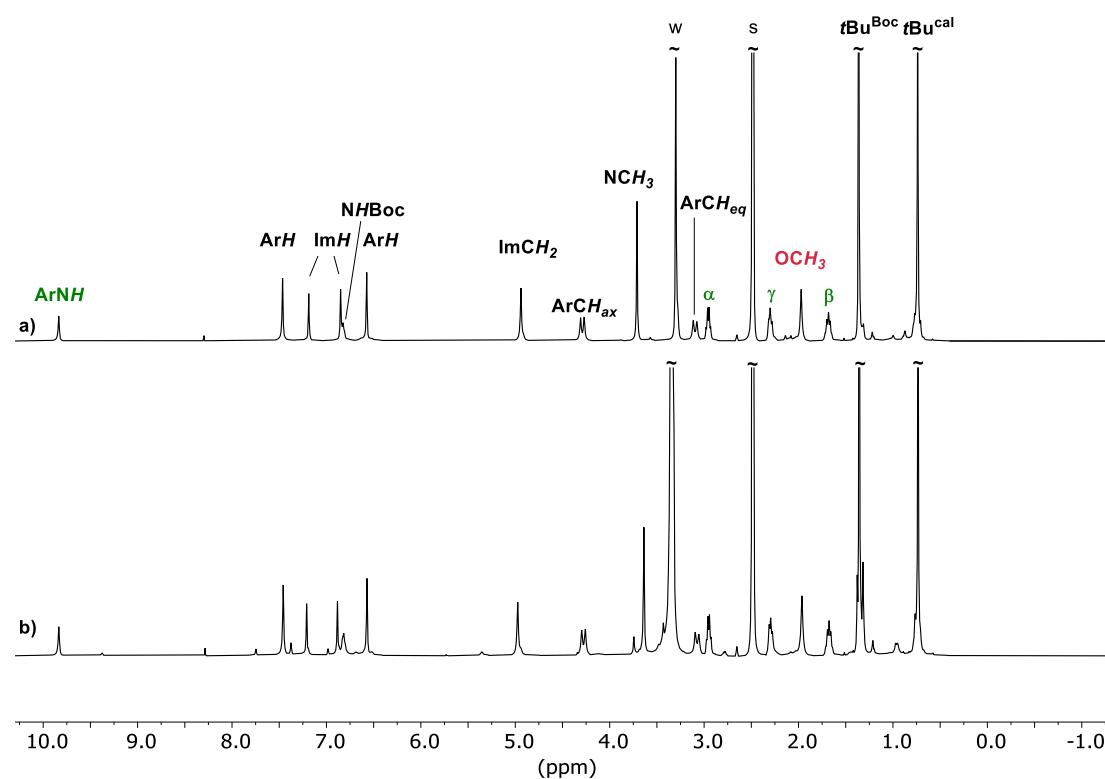


**Figure S22.** ATR-FTIR spectrum of **3-Zn<sup>2+</sup>**.

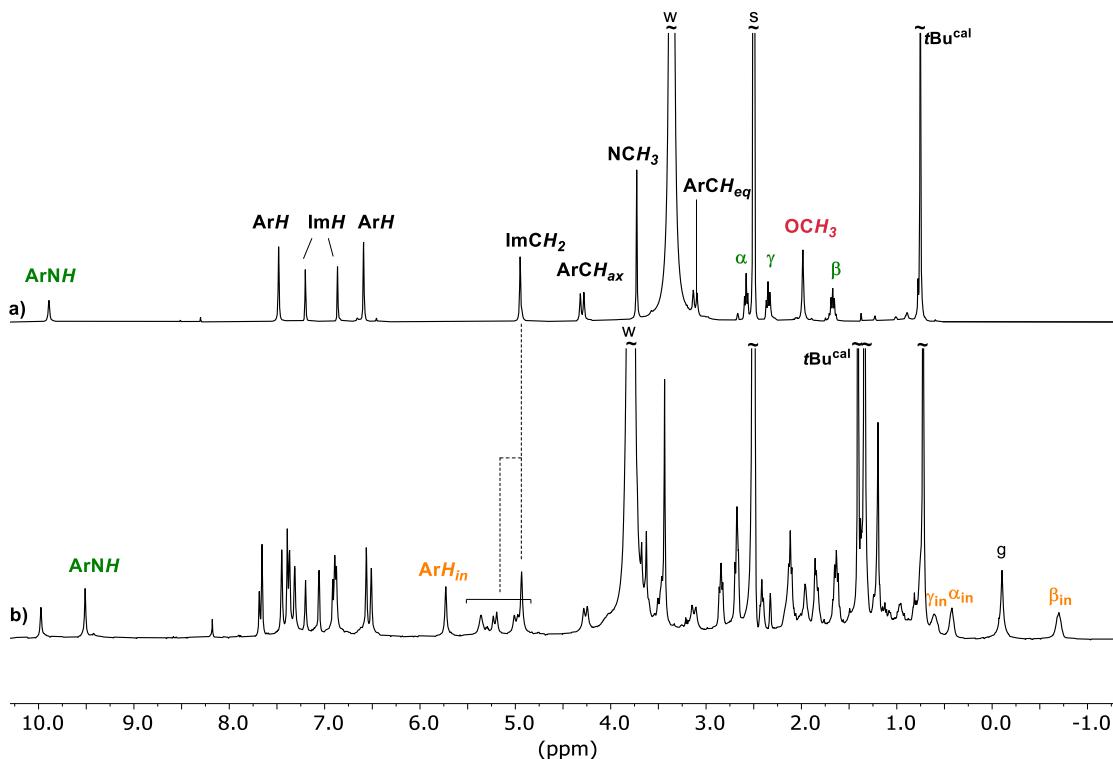


**Figure S23.**  $^1\text{H}$  NMR spectra (298 K, 600 MHz,  $\text{CDCl}_3/\text{CD}_3\text{CN}$  1:1) of a) **3-Zn**<sup>2+</sup>, b) 1D EXSY spectra (at various mixing times) after selective excitation of the  $\beta_{\text{in}}$  signal at  $-0.61\text{ ppm}$ .

## Titrations for the formation of the complexes in DMSO

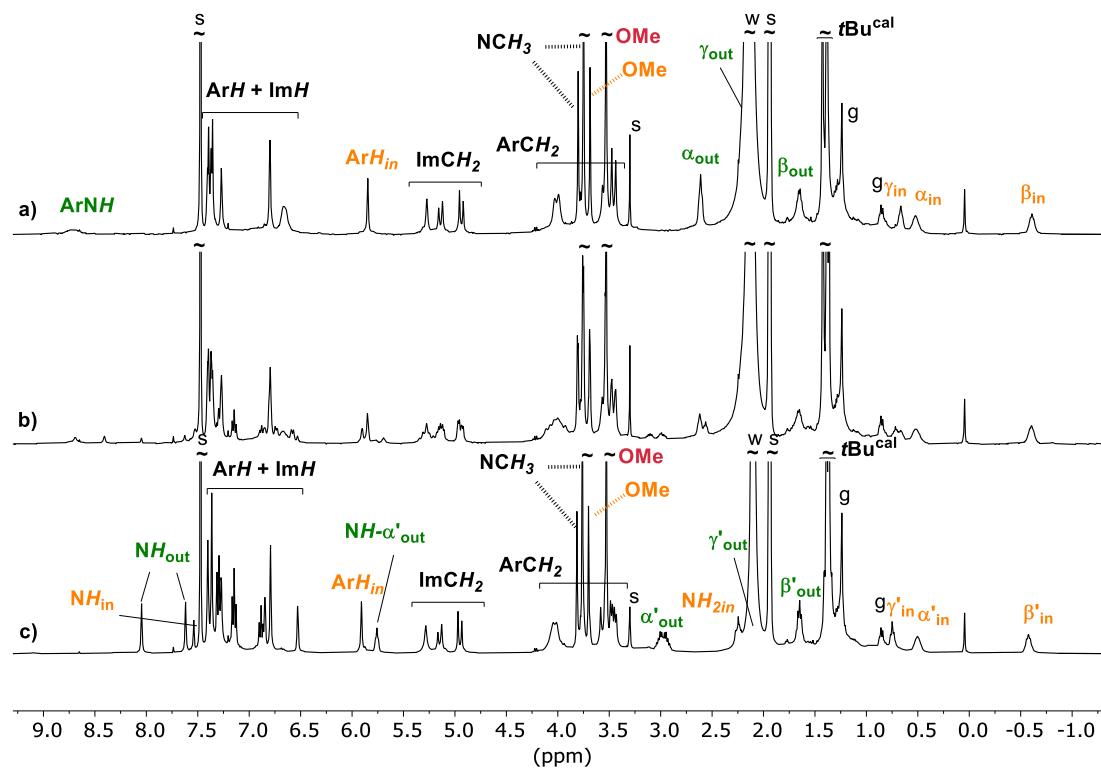


**Figure S24.** <sup>1</sup>H NMR spectra (298 K, 400 MHz, DMSO-*d*<sub>6</sub>) of a) **2** (2 mM), b) **2** (2 mM) + ~27 equiv. of Zn(OTf)<sub>2</sub>. s = residual solvents, w = water.

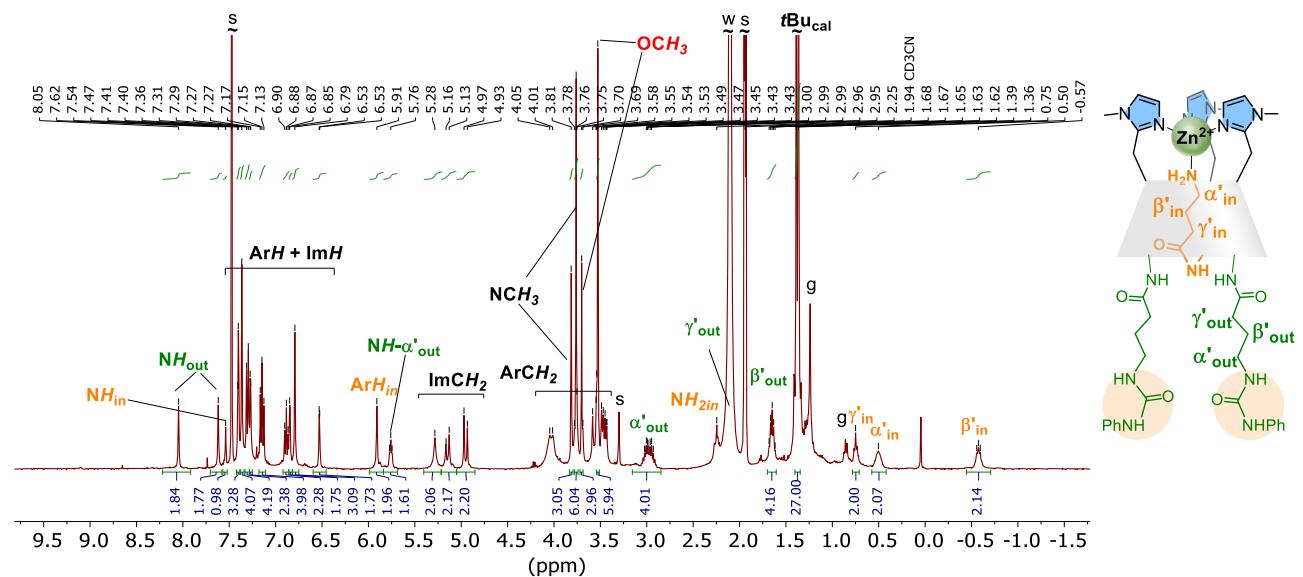


**Figure S25.** <sup>1</sup>H NMR spectra (298 K, 400 MHz, DMSO-*d*<sub>6</sub>) of a) **3** (2 mM), b) **3** (2 mM) + ~27 equiv. of Zn(OTf)<sub>2</sub>. s = residual solvents, w = water.

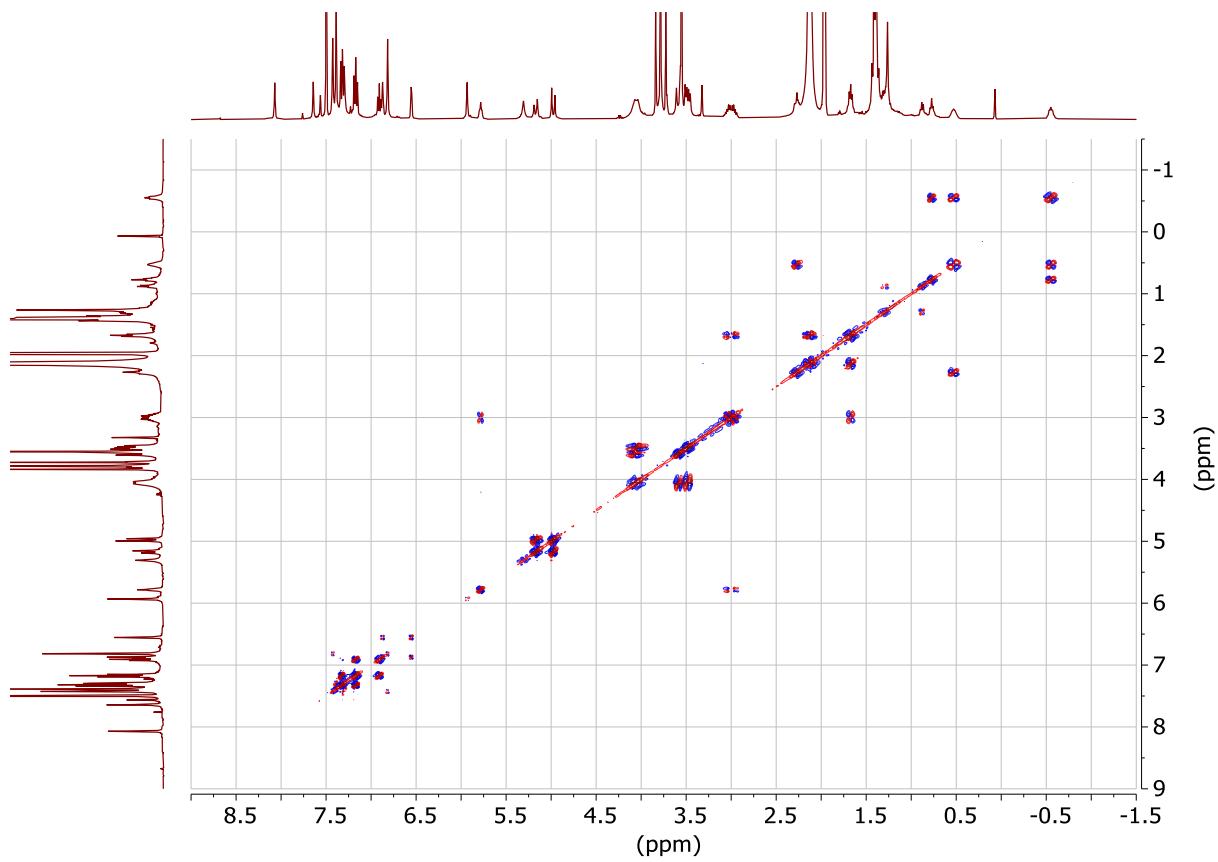
## Formation and characterization of complex **4-Zn<sup>2+</sup>** in CDCl<sub>3</sub>/CD<sub>3</sub>CN 1:1



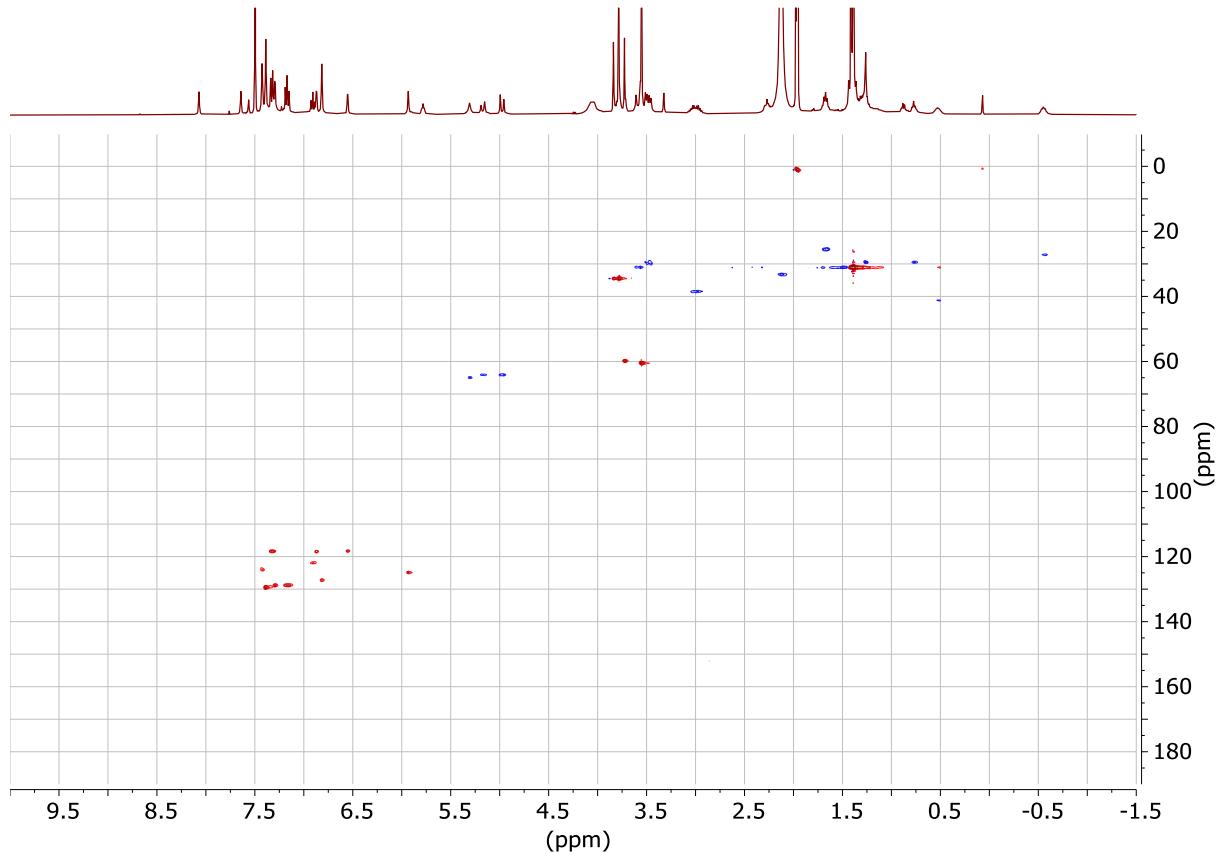
**Figure S26.** <sup>1</sup>H NMR spectra (298 K, 400 MHz, CDCl<sub>3</sub>/CD<sub>3</sub>CN 1:1) of a) **3** (2 mM) + ~1.0 equiv. of Zn(OTf)<sub>2</sub>, b) **3** (2 mM) + ~1.0 equiv. of Zn(OTf)<sub>2</sub> + ~0.6 equiv. phenylisocyanate, c) **3** (2 mM) + ~1.0 equiv. of Zn(OTf)<sub>2</sub> + ~2 equiv. phenylisocyanate → **4-Zn<sup>2+</sup>**. s = residual solvents, w = water, g = grease.



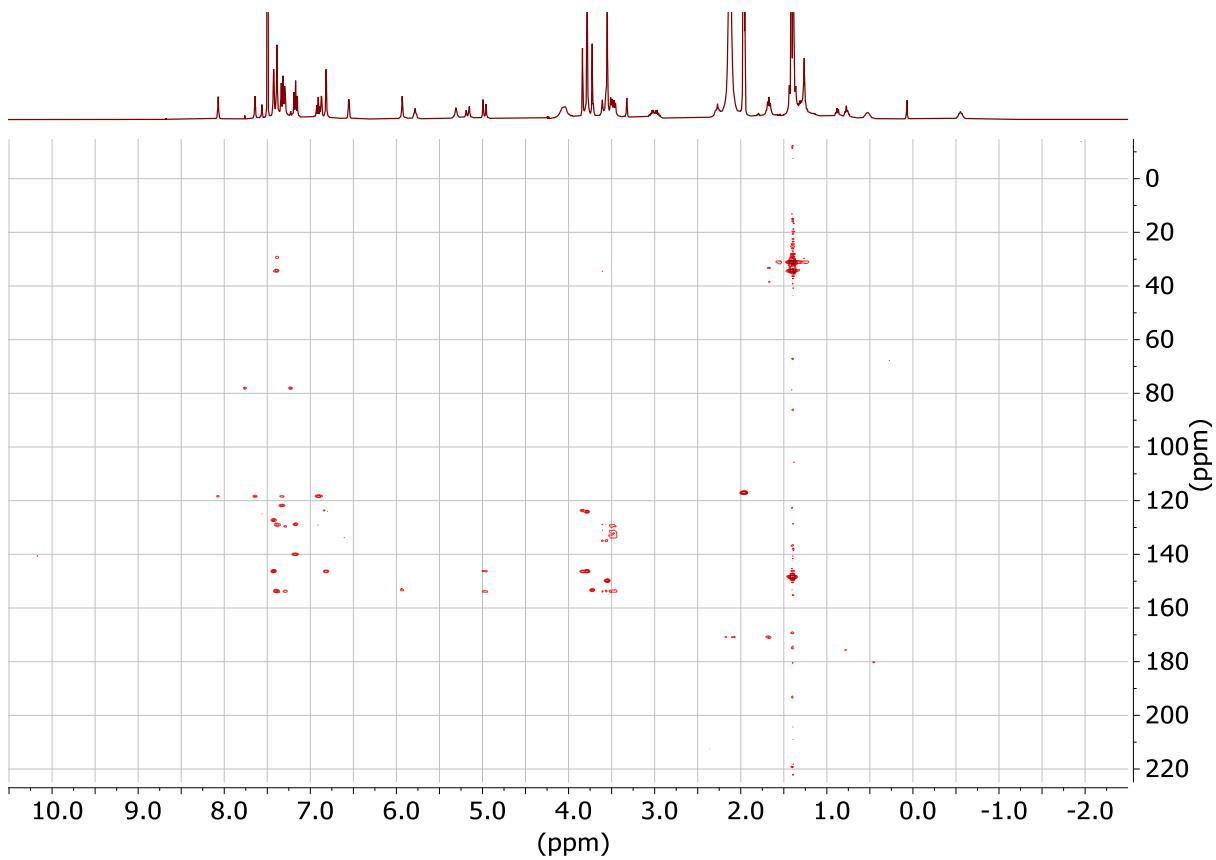
**Figure S27.** <sup>1</sup>H NMR spectrum (298K, 400 MHz, CDCl<sub>3</sub>/CD<sub>3</sub>CN 1:1) of **4-Zn<sup>2+</sup>** (2 mM). s: residual solvent, w: water, g = grease.



**Figure S28.** COSY NMR spectrum (298K, 400 MHz, CDCl<sub>3</sub>/CD<sub>3</sub>CN 1:1) of 4-Zn<sup>2+</sup> (2 mM).

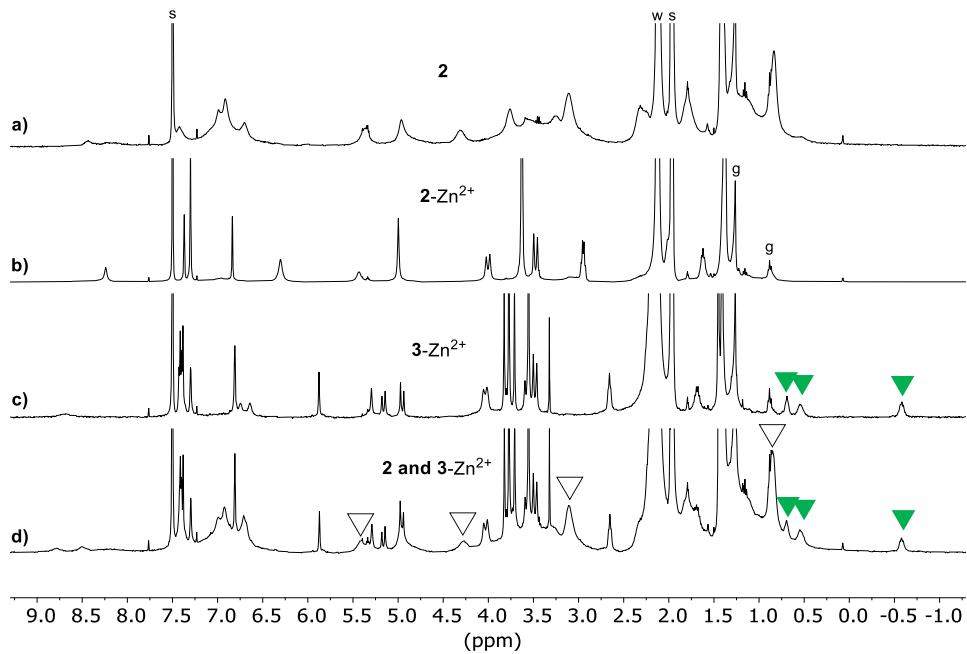


**Figure S29.** Edited HSQC NMR spectrum (298K, 400 MHz, CDCl<sub>3</sub>/CD<sub>3</sub>CN 1:1) of 4-Zn<sup>2+</sup> (2 mM). The <sup>13</sup>C NMR spectrum has not been recorded for 4-Zn<sup>2+</sup>.

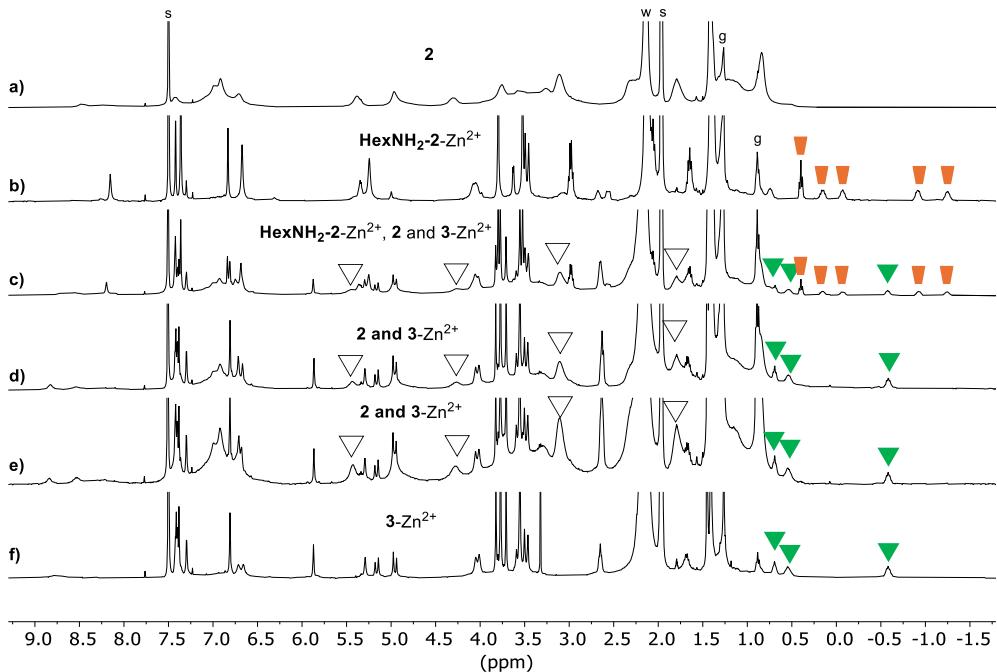


**Figure S30.** HMBC NMR spectrum (298K, 400 MHz,  $\text{CDCl}_3/\text{CD}_3\text{CN}$  1:1) of **4**- $\text{Zn}^{2+}$  (2 mM). The  $^{13}\text{C}$  NMR spectrum has not been recorded for **4**- $\text{Zn}^{2+}$ .

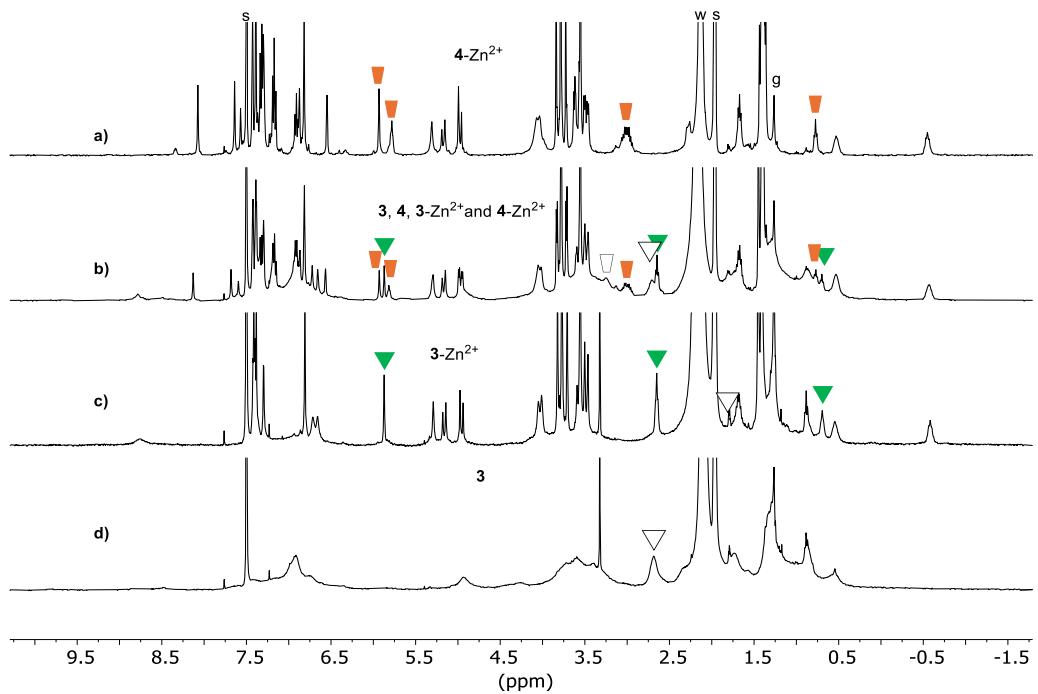
## Competition experiments in $\text{CDCl}_3/\text{CD}_3\text{CN}$ 1:1



**Figure S31.**  $^1\text{H}$  NMR spectra (298 K, 400 MHz,  $\text{CDCl}_3/\text{CD}_3\text{CN}$  1:1) of a) **2** (1 mM), b) **2** (1 mM) + 1 equiv.  $\text{Zn}(\text{OTf})_2 \rightarrow \mathbf{2}\text{-Zn}^{2+}$ , c) **3** (1 mM) + 1 equiv.  $\text{Zn}(\text{OTf})_2 \rightarrow \mathbf{3}\text{-Zn}^{2+}$ , d) **2** (1 mM) + **3** (1 mM) + 1 equiv.  $\text{Zn}(\text{OTf})_2$  (1 mM)  $\rightarrow$  only **3-Zn**<sup>2+</sup>. s = residual solvents, w = water, g = grease.  $\blacktriangledown$  = **3-Zn**<sup>2+</sup> signals (some of them, of interest) and  $\bigtriangleup$  = free **2**.

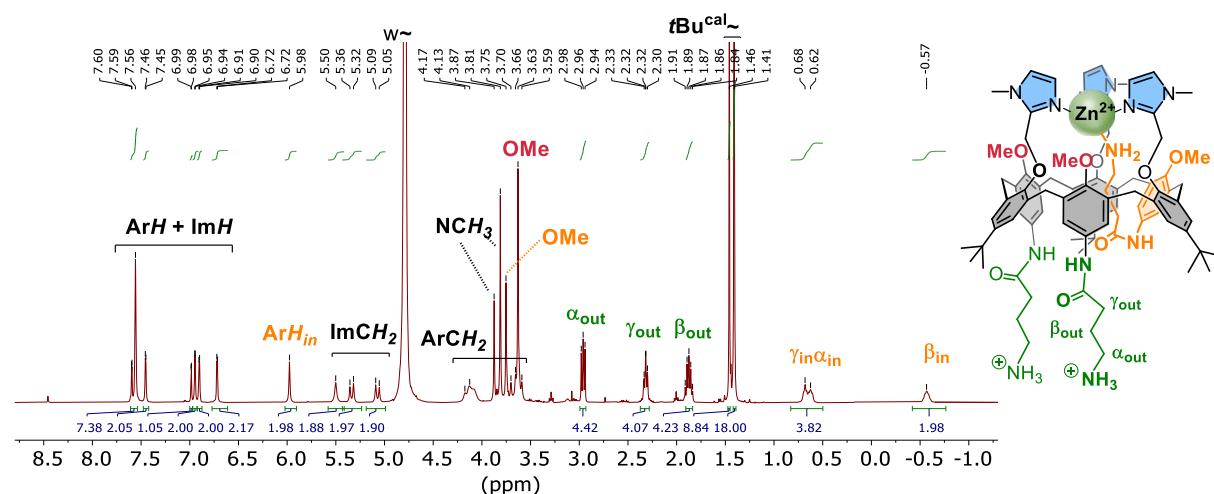


**Figure S32.**  $^1\text{H}$  NMR spectra (298 K, 400 MHz,  $\text{CDCl}_3/\text{CD}_3\text{CN}$  1:1) of a) **2** (1 mM), b) **2** (1 mM) + 1.6 equiv.  $\text{HexNH}_2$  + 1 equiv.  $\text{Zn}(\text{OTf})_2 \rightarrow \text{HexNH}_2\text{-}\mathbf{2-Zn}^{2+}$ , c) **2** (1 mM) + 1 equiv.  $\text{HexNH}_2$  + 1 equiv.  $\text{Zn}(\text{OTf})_2$  + 0.5 equiv. **3** (0.5 mM)  $\rightarrow$   $\text{HexNH}_2\text{-}\mathbf{2-Zn}^{2+}$  (50%) and **3-Zn**<sup>2+</sup> (50%), d) **2** (1 mM) + 1 equiv.  $\text{HexNH}_2$  + 1 equiv.  $\text{Zn}(\text{OTf})_2$  + 1 equiv. **3** (1 mM)  $\rightarrow$  only **3-Zn**<sup>2+</sup>, e) **2** (2 mM) + 1 equiv.  $\text{HexNH}_2$  (2 mM) + 0.5 equiv.  $\text{Zn}(\text{OTf})_2$  + 0.5 equiv. **3** (1 mM)  $\rightarrow$  only **3-Zn**<sup>2+</sup>, f) **3** (1 mM) + 1 equiv.  $\text{Zn}(\text{OTf})_2 \rightarrow \mathbf{3-Zn}^{2+}$  (spectrum for comparison). s = residual solvents, w = water, g = grease.  $\blacksquare$  =  $\text{HexNH}_2\text{-}\mathbf{2-Zn}^{2+}$ ,  $\blacktriangledown$  = **3-Zn**<sup>2+</sup> signals (some of them, of interest) and  $\bigtriangleup$  = free **2**.

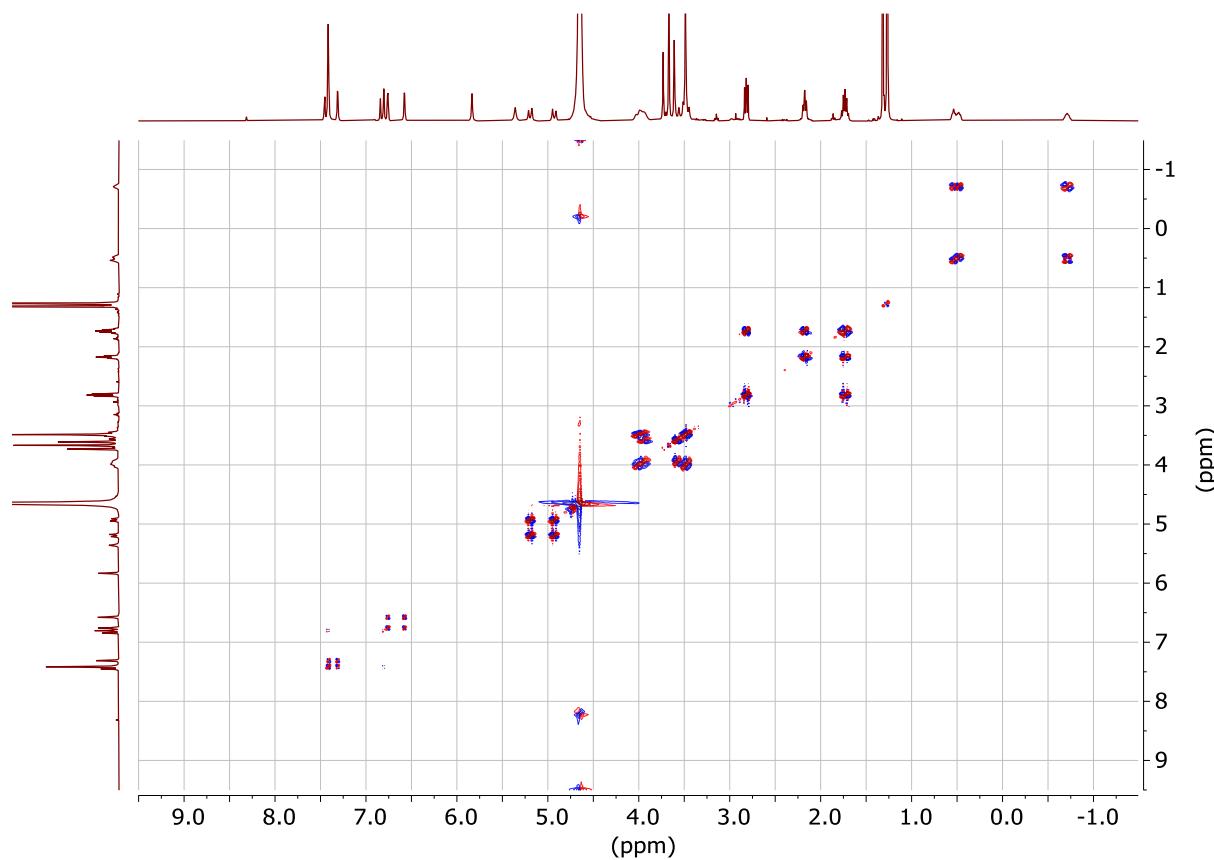


**Figure S33.** <sup>1</sup>H NMR spectra (298 K, 400 MHz,  $\text{CDCl}_3/\text{CD}_3\text{CN}$  1:1) of a) **4** (2 mM) + 1 equiv.  $\text{Zn}(\text{OTf})_2 \rightarrow \mathbf{4-Zn}^{2+}$ , b) **4** (2 mM) + 1 equiv.  $\text{Zn}(\text{OTf})_2$  + 1 equiv. **3**  $\rightarrow \mathbf{4-Zn}^{2+}$  (50%) and **3-Zn**<sup>2+</sup> (50%), c) **3** (1 mM) + 1 equiv.  $\text{Zn}(\text{OTf})_2 \rightarrow \mathbf{3-Zn}^{2+}$ , d) **3** (1 mM). s = residual solvents, w = water, g = grease. □ = **4-Zn**<sup>2+</sup>, ▽ = free **4**, ▼ = **3-Zn**<sup>2+</sup>, △ = free **3**.

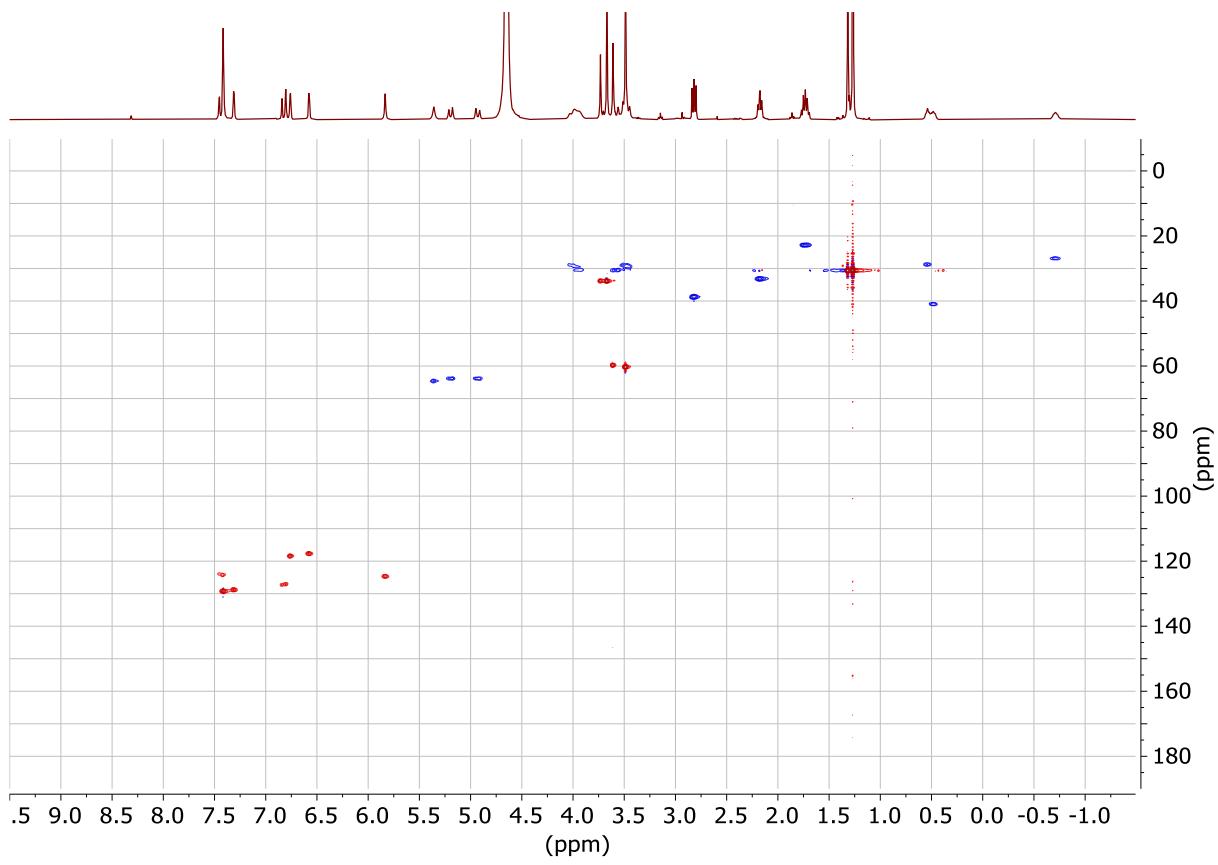
## Characterization of complex $\mathbf{3}\text{-Zn}^{2+}$ in $\text{D}_2\text{O}$



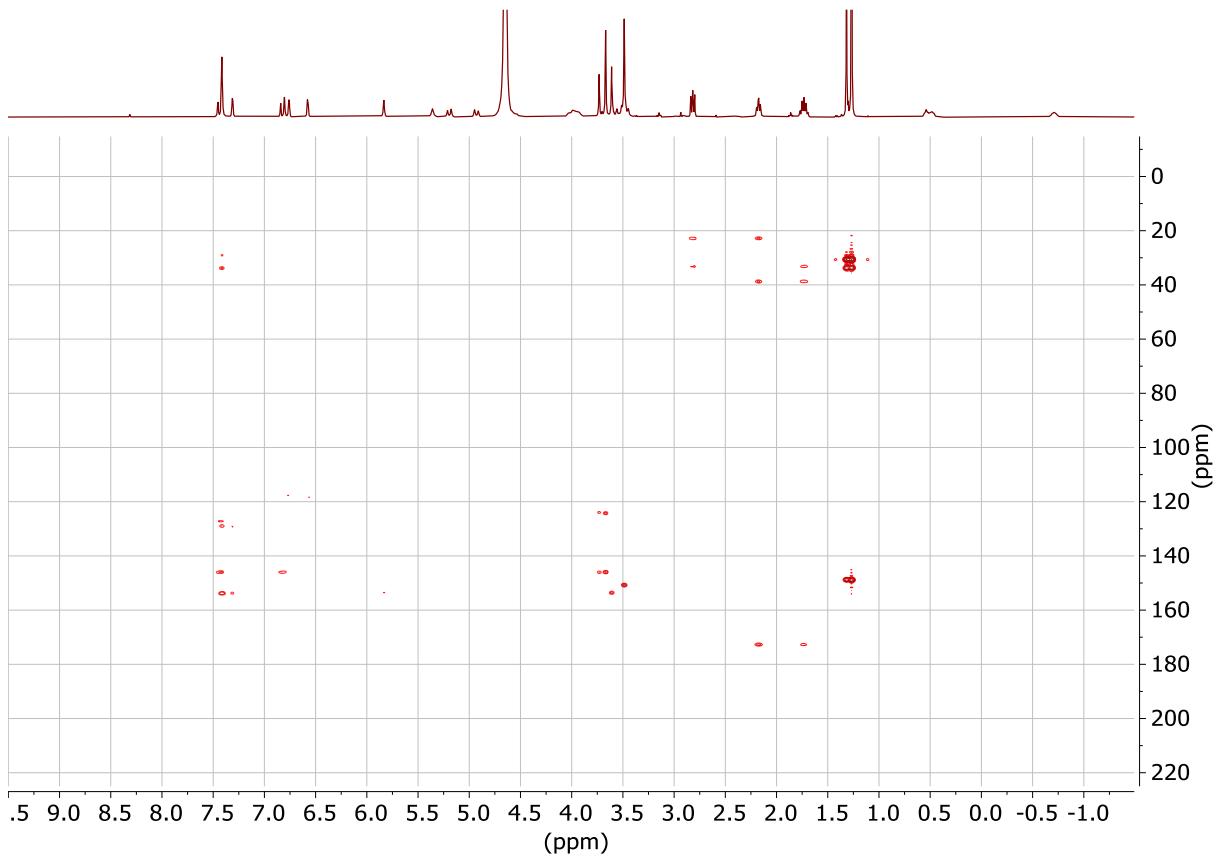
**Figure S34.**  $^1\text{H}$  NMR spectrum (298K, 400 MHz,  $\text{D}_2\text{O}$ ) of  $\mathbf{3}\text{-Zn}^{2+}$  (5 mM). w: water.



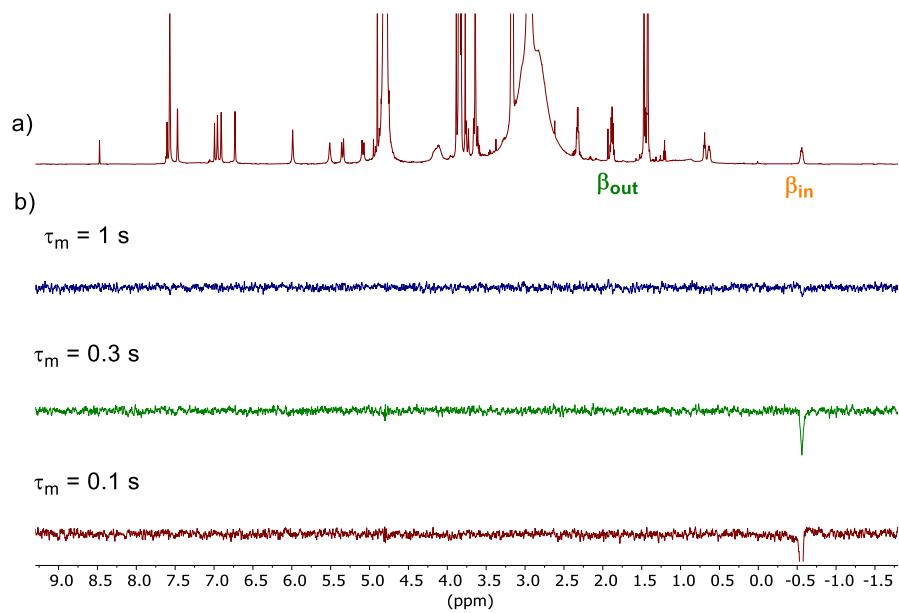
**Figure S35.** COSY NMR spectrum (298K, 400 MHz,  $\text{D}_2\text{O}$ ) of  $\mathbf{3}\text{-Zn}^{2+}$  (5 mM).



**Figure S36.** Edited HSQC NMR spectrum (298K, 400 MHz,  $\text{D}_2\text{O}$ ) of **3**- $\text{Zn}^{2+}$  (5 mM).

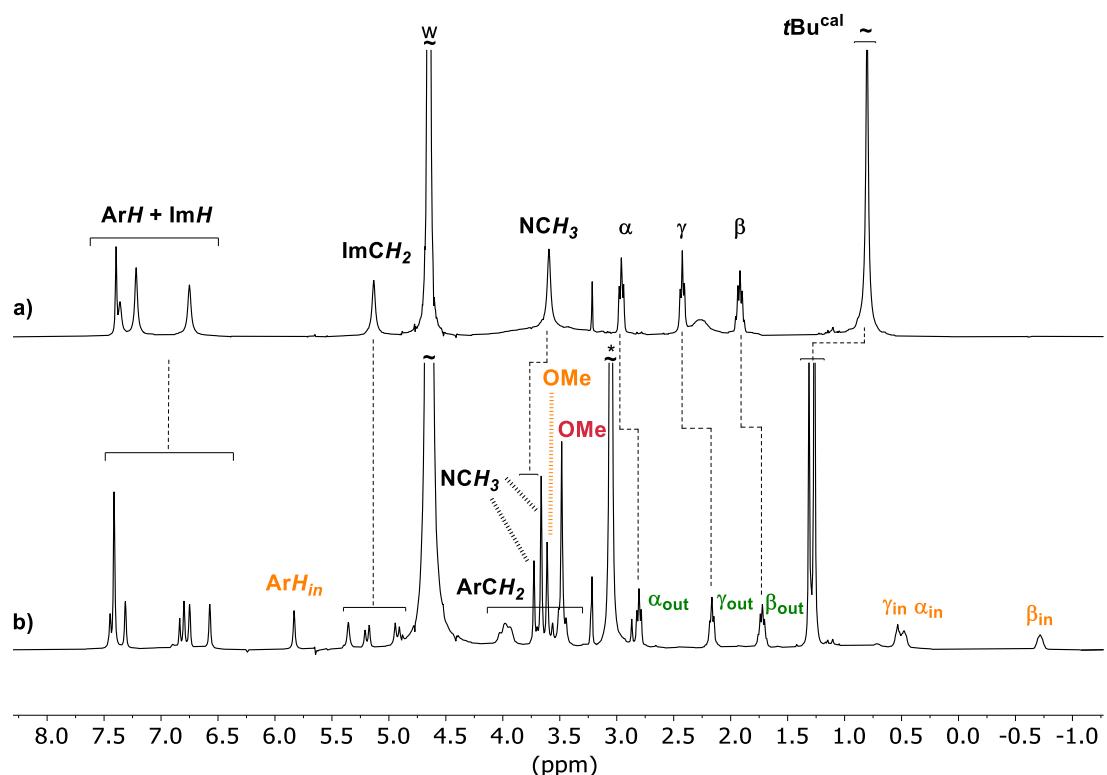


**Figure S37.** HMBC spectrum (298K, 400 MHz,  $\text{D}_2\text{O}$ ) of **3**- $\text{Zn}^{2+}$  (5 mM).

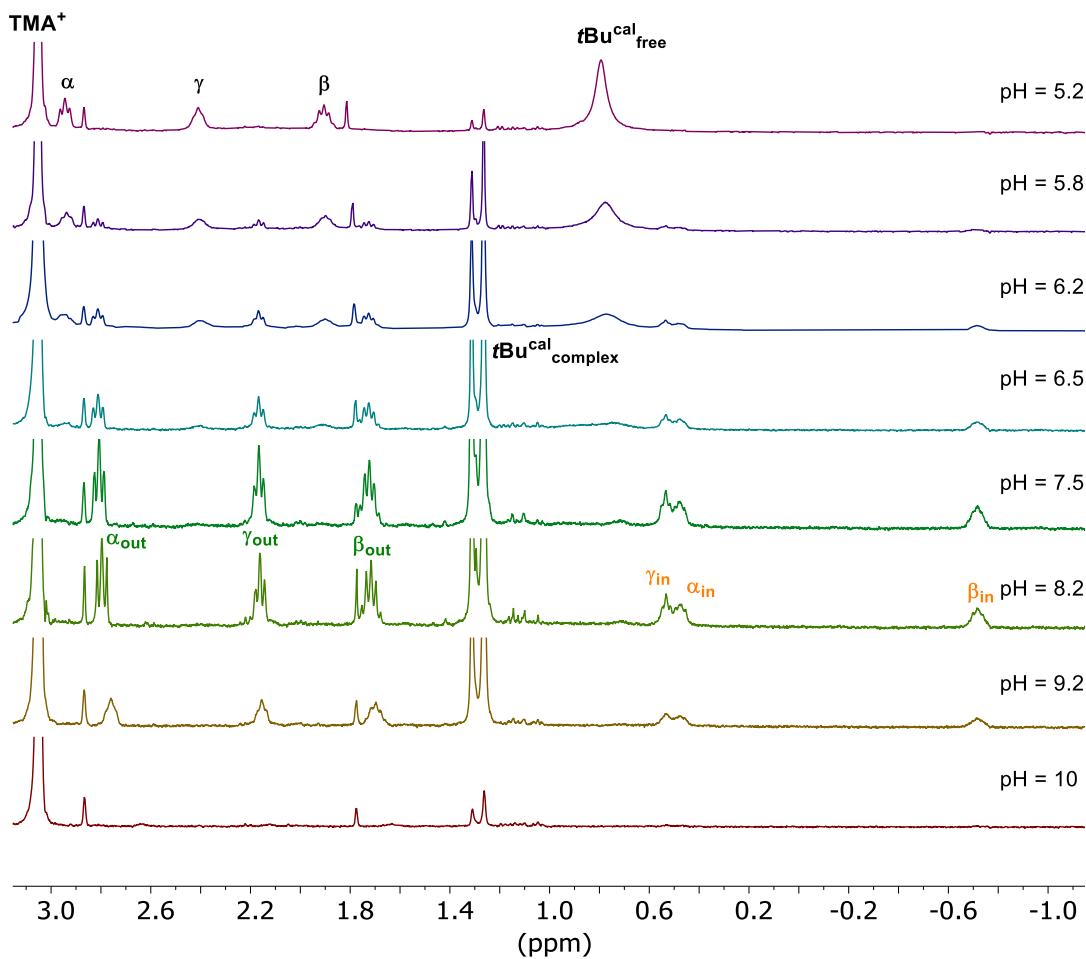


**Figure S38.** <sup>1</sup>H NMR spectra (298 K, 600 MHz, D<sub>2</sub>O) of a) 3-Zn<sup>2+</sup>, b) 1D EXSY spectra (at various mixing times) after selective excitation of the β<sub>in</sub> signal at -0.57 ppm.

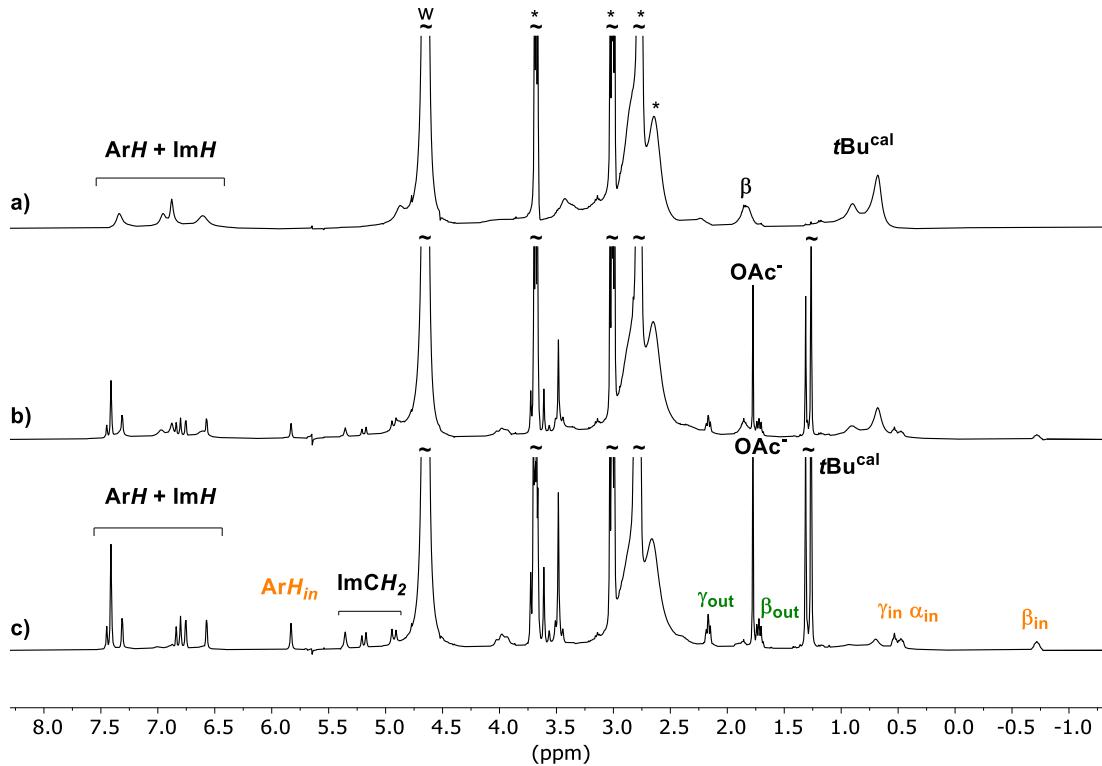
**Titration for the formation of the complexes in D<sub>2</sub>O**



**Figure S39.** <sup>1</sup>H NMR spectra (298 K, 400 MHz, D<sub>2</sub>O) of a) **3** (1 mM) + ~1 equiv. of Zn(ClO<sub>4</sub>)<sub>2</sub> at pH = 3–4, b) **3** (1 mM) + ~1 equiv. of Zn(ClO<sub>4</sub>)<sub>2</sub> + TMAOH (pH = 7.8). \* = TMA<sup>+</sup>, w = water.

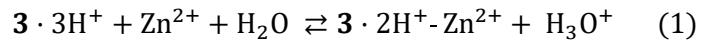


**Figure S40.** High field region of <sup>1</sup>H NMR spectra (298 K, 400 MHz, D<sub>2</sub>O) of **3** (1 mM) + ~1 equiv. of Zn(ClO<sub>4</sub>)<sub>2</sub> at various pH, changed by addition of TMAOH/DCl aliquots. At pH 9.2 and 10, a precipitate was observed in the NMR tube.



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

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



$$K_a^{\text{eff}} = \frac{[\mathbf{3} \cdot 2\text{H}^+-\text{Zn}^{2+}][\text{H}_3\text{O}^+]}{[\text{Zn}^{2+}][\mathbf{3} \cdot 3\text{H}^+]}$$

and

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

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 (**3** $\cdot$ 2 $\text{H}^+$ - $\text{Zn}^{2+}$ , **3** $\cdot$ 3 $\text{H}^+$  and  $\text{AcO}^-$ ). The  $^1\text{H}$  NMR signals of **3** $\cdot$ 3 $\text{H}^+$  being rather broad, the experimental error was estimated on a wide range.

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

Knowing the pH and therefore  $[\text{H}_3\text{O}^+]$ , we were able to calculate  $K_a^{\text{eff}} = 5.4 \cdot 10^{-4}$  and  $3.8 \cdot 10^{-4}$ . Therefore,  $pK_a^{\text{eff}} = 3.4 \pm 0.3$  as the pseudo-p $K_a$  of the amino leg.