

Russian Nesting Doll Complexes of Molecular Baskets and Zinc Containing TPA Ligands

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SUPPORTING INFORMATION

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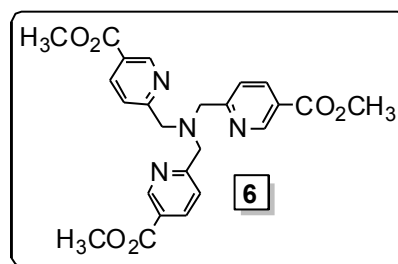
General Methods

All solvents were dried before use following standard procedures. Unless indicated otherwise, all starting materials were obtained from commercial suppliers and used without additional purification. Analytical thin-layer chromatography (TLC) was performed on silica-gel plates w/UV254. ^1H NMR and ^{13}C NMR spectra were recorded on 400, 600 or 700 MHz spectrophotometers. Chemical shifts are expressed in parts per million (δ , ppm) using residual solvent protons as an internal standard; for calibrating ^1H NMR spectra we used $\text{CDCl}_3 = 7.259$ ppm, $\text{CD}_3\text{OD} = 3.31$ ppm and $\text{D}_2\text{O} = 4.79$ ppm while for ^{13}C NMR spectra $\text{CDCl}_3 = 77.0$ ppm and $\text{CD}_3\text{OD} = 49.0$ ppm. The measurements of pH were completed with HI 2210 pH meter. Circular dichroism (CD) spectra were measured with JASCO-815 spectrometer. UV-Vis measurements were completed with Shimadzu CARY-100 spectrophotometer.

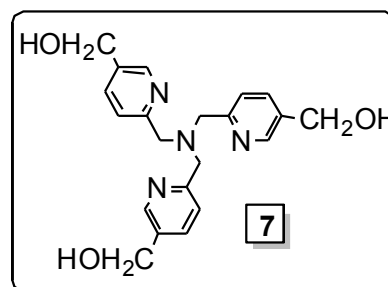
Synthetic Procedures

Compound 5 was prepared by following literature procedures¹ and from commercially available 6-methylnicotonate **4**; ^1H NMR data corresponding to **5** is in line with the literature.¹

Compound 6:² To compound **5** (10 mmol, 2.3 g), dissolved in dry dimethyl formamide (50 mL) and under an atmosphere of nitrogen, NH_4Br (3.33 mmol, 326.1 mg) and Na_2CO_3 (20 mmol, 1.68 g) were added. The reaction mixture was sealed and stirred at room temperature for 5 days, followed by heating at 60 °C for 8 hours. Subsequently, the mixture was poured into water (100 mL) and extracted with dichloromethane (3 x 50 mL). The combined organic layers were washed with saturated NaCl solution (3 x 20 mL). Upon removal of the organic solvent in vacuum, compound **6** was purified by column chromatography (SiO_2 , acetone:hexane:ethylacetate = 1:4:5) and recrystallized from ethylacetate/hexane = 1:2 to give light yellow solid (1.084 g, 70%). ^1H NMR (600 MHz, CDCl_3) δ (ppm): 9.13 (dd, $J = 2.4, 0.6$ Hz, 1H), 8.25 (dd, $J = 8.4, 1.8$ Hz, 1H), 7.63 (d, $J = 8.4$ Hz, 1H), 3.96 (s, 2H), 3.94 (s, 3H). ^{13}C NMR (150 MHz, CDCl_3) δ (ppm): 165.72, 163.41, 150.48, 137.58, 124.66, 122.54, 60.09, 52.34. HRMS (ESI): m/z : $[\text{M} + \text{H}]^+$ calculated for $\text{C}_{24}\text{H}_{25}\text{N}_4\text{O}_6$: 465.17686, found 465.17689.

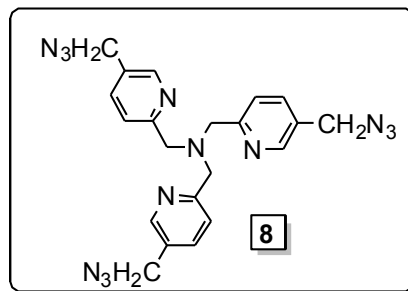


Compound 7:³ To compound **6** (2 mmol, 0.928 g) dissolved in dry tetrahydrofuran (40 mL) and at room temperature, LiBH_4 (12 mmol, 261.6 mg) was added in portions over 2 days. After stirring for an additional day, the reaction mixture was heated to reflux for 8 hours. The solvent was removed under reduced pressure, and crude product purified by column chromatography (SiO_2 , methanol:dichloromethane:aqueous ammonia = 1/3/0.05) to give **7** as a yellow solid (532.6 mg, 70%). ^1H NMR (600 MHz,

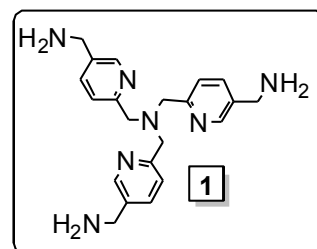


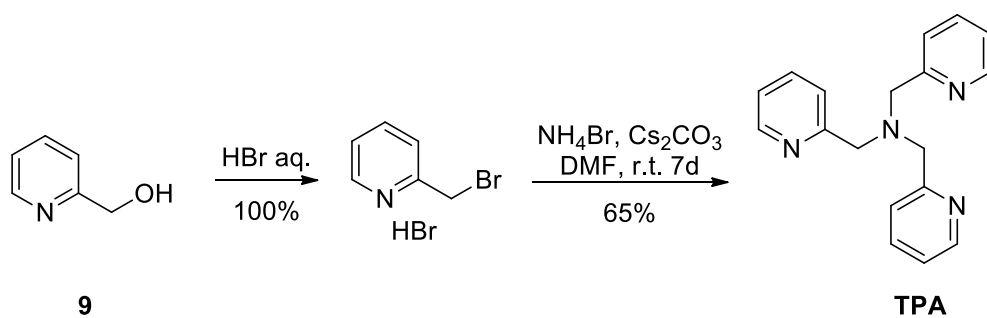
CD₃OD) δ (ppm): 8.42 (d, J = 1.8 Hz, 1H), 7.77 (dd, J = 8.4, 2.1 Hz, 1H), 7.61 (d, J = 8.4 Hz, 1H), 4.62 (s, 2H), 3.85 (s, 2H). ¹³C NMR (150 MHz, CD₃OD) δ (ppm): 158.87, 148.21, 137.50, 137.45, 124.62, 62.37, 60.89. HRMS (ESI): m/z : [M + H]⁺ calculated for C₂₁H₂₅N₄O₃: 381.19212, found 381.19219.

Compound 8:⁴ To neat SOCl₂ (5.08 mL, 70 mmol) kept at 0 °C, compound 7 (532.6 mg, 1.4 mmol) was added and the reaction mixture stirred at room temperature for 12 hours. The solvent was removed under reduced pressure and crude product was dissolved in anhydrous dimethyl formamide (40 mL) to which we added NaI (1.26 g, 8.4 mmol), NaN₃ (546 mg, 8.4 mmol) and NaHCO₃ (1.176 g, 14 mmol). The reaction mixture was stirred at 60 °C for 12 hours and under an atmosphere of nitrogen. Subsequently, the reaction mixture was quenched with water (70 mL) and extracted by ethyl acetate (3 x 100 mL). The combined organic phases were washed with saturated NaCl solution (3 x 15 mL) and dried with Na₂SO₄. Upon removal of the solvent in vacuum, the crude product was purified by column chromatography (SiO₂, dichloromethane/methanol/hexanes = 15:1:10) to give **8** as a yellow solid (522.9 mg, two steps 82%). ¹H NMR (600 MHz, CDCl₃) δ (ppm): 8.47 (d, J = 1.8 Hz, 1H), 7.61 (dd, J = 7.8, 2.4 Hz, 1H), 7.55 (d, J = 8.4 Hz, 1H), 4.34 (s, 2H), 3.92 (s, 2H). ¹³C NMR (150 MHz, CDCl₃) δ (ppm): 159.32, 148.62, 136.24, 129.45, 123.04, 59.93, 51.90. HRMS (ESI): m/z : [M + H]⁺ calculated for C₂₁H₂₂N₁₃: 456.21156, found 456.21164.

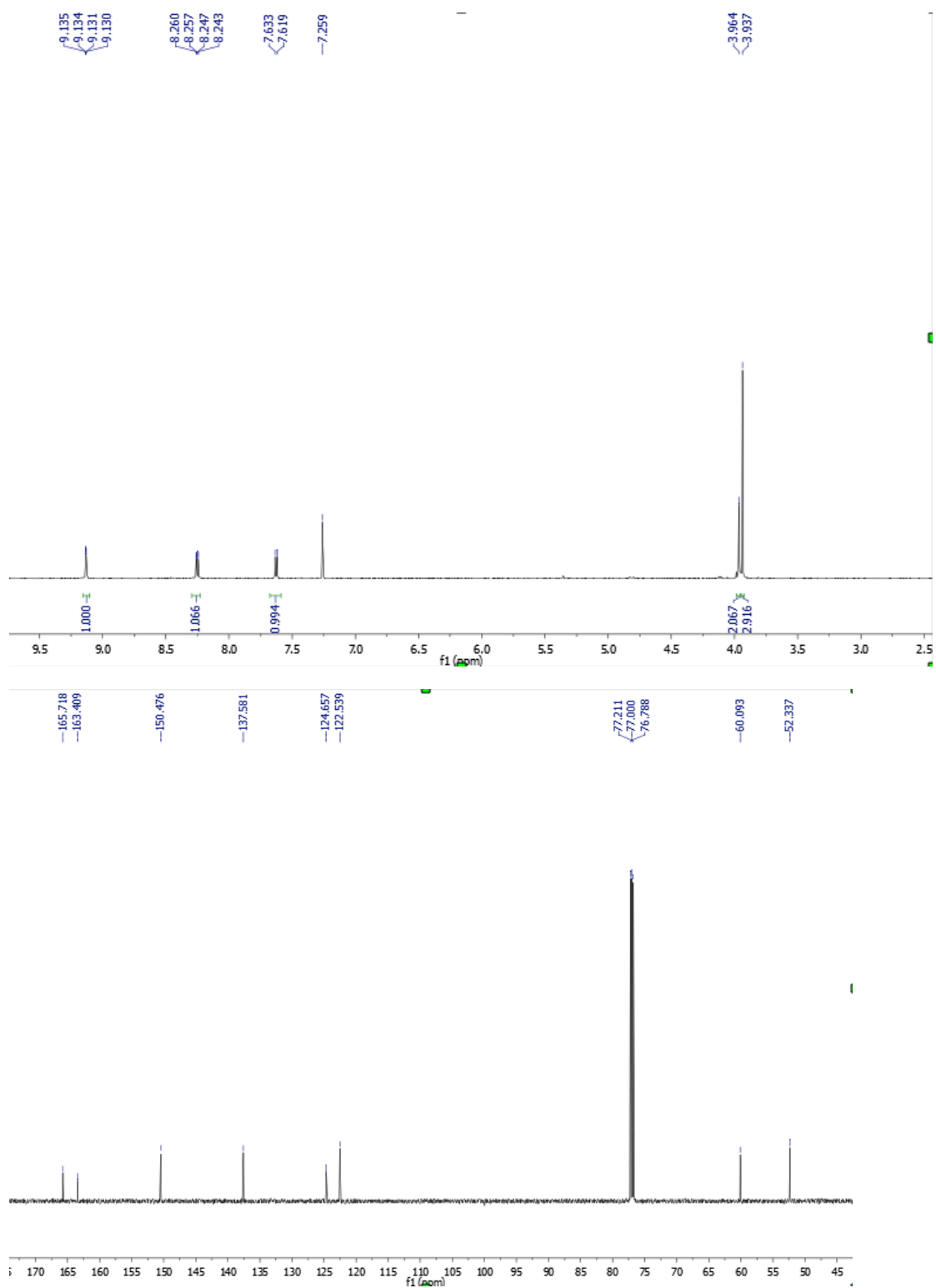
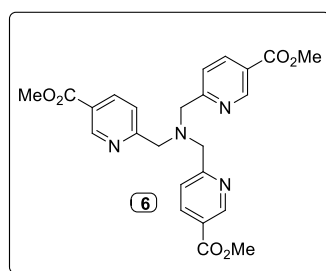


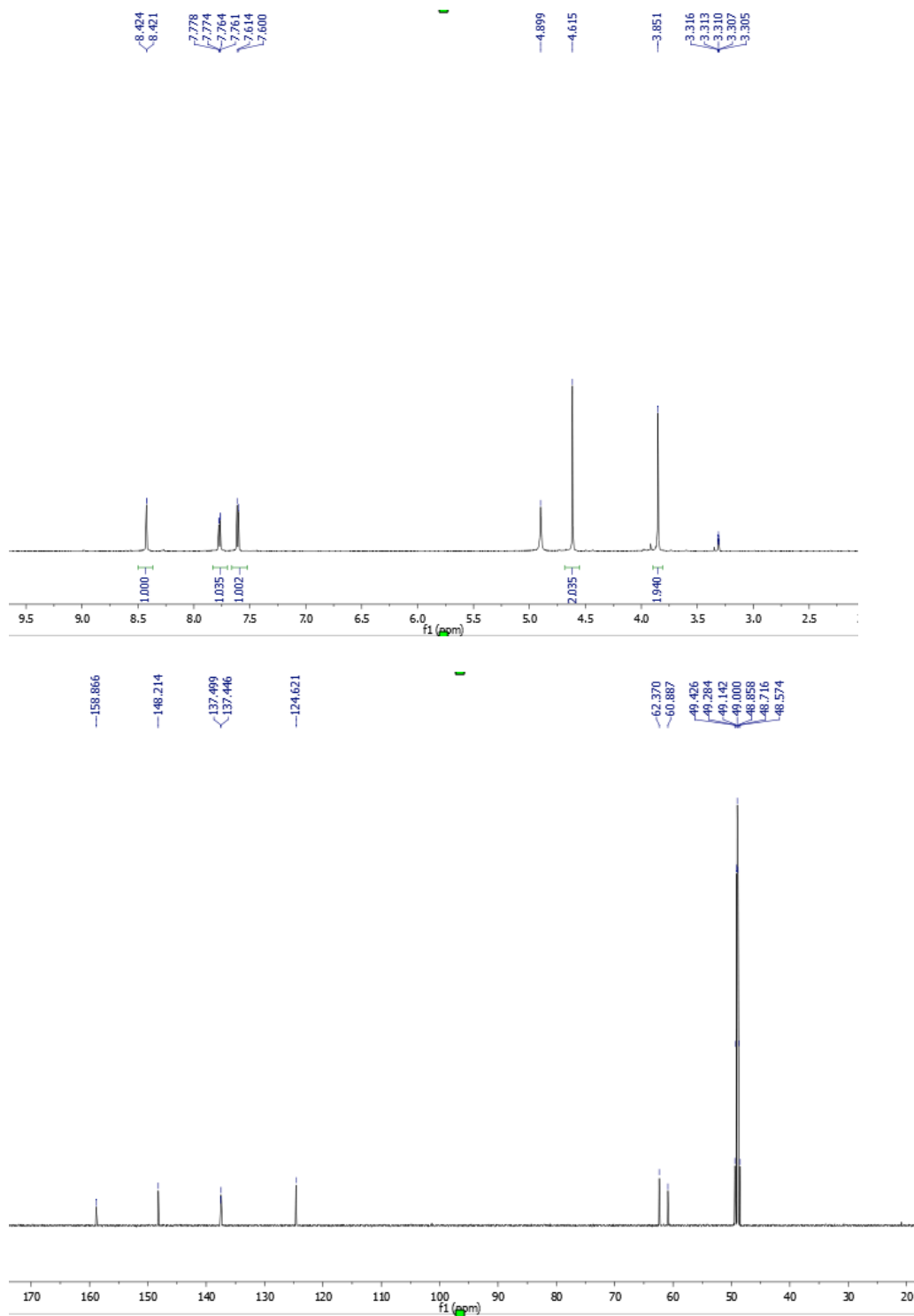
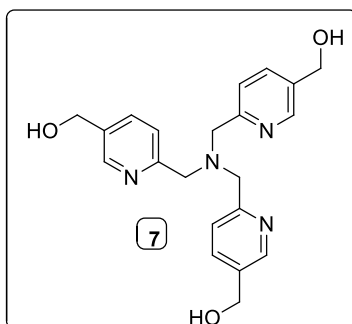
Compound 1:² To a solution of compound **8** (45 mg, 0.1 mmol) in tetrahydrofuran (4.8 mL) was added triphenylphosphine (131 mg, 0.5 mmol) and water (0.3 mL). The reaction mixture was stirred at 50 °C for 12 hours. The solvent was removed under reduced pressure, and the residue dissolved in water (10 mL). The pH of the aqueous solution was adjusted to 1 with 2 M HCl and then extracted with CH₂Cl₂ (10 mL), which was discarded. The aqueous layer was subsequently removed under reduced pressure and the remaining solid dissolved in methanol (10 mL). Basic ion-exchange resin (Amberlyst A26, OH-form; 300 mg) was added to this methanol solution of the product. After 3 hours of stirring, we filtered and washed the residue with methanol (20 mL). The combined organic solvents were removed under reduced pressure to give **1** as a yellow solid (67.9 mg, 90%). ¹H NMR (600 MHz, CD₃OD) δ (ppm): 8.41 (d, J = 1.2 Hz, 1H), 7.79 (dd, J = 7.8, 2.4 Hz, 1H), 7.63 (d, J = 7.8 Hz, 1H), 3.81 (s, 2H), 3.80 (s, 2H). ¹³C NMR (150 MHz, CD₃OD) δ (ppm): 158.60, 148.81, 138.40, 137.87, 124.59, 60.71, 43.80. HRMS (ESI): m/z : [M + H]⁺ calculated for C₂₁H₂₈N₇: 378.24007, found 378.24025.

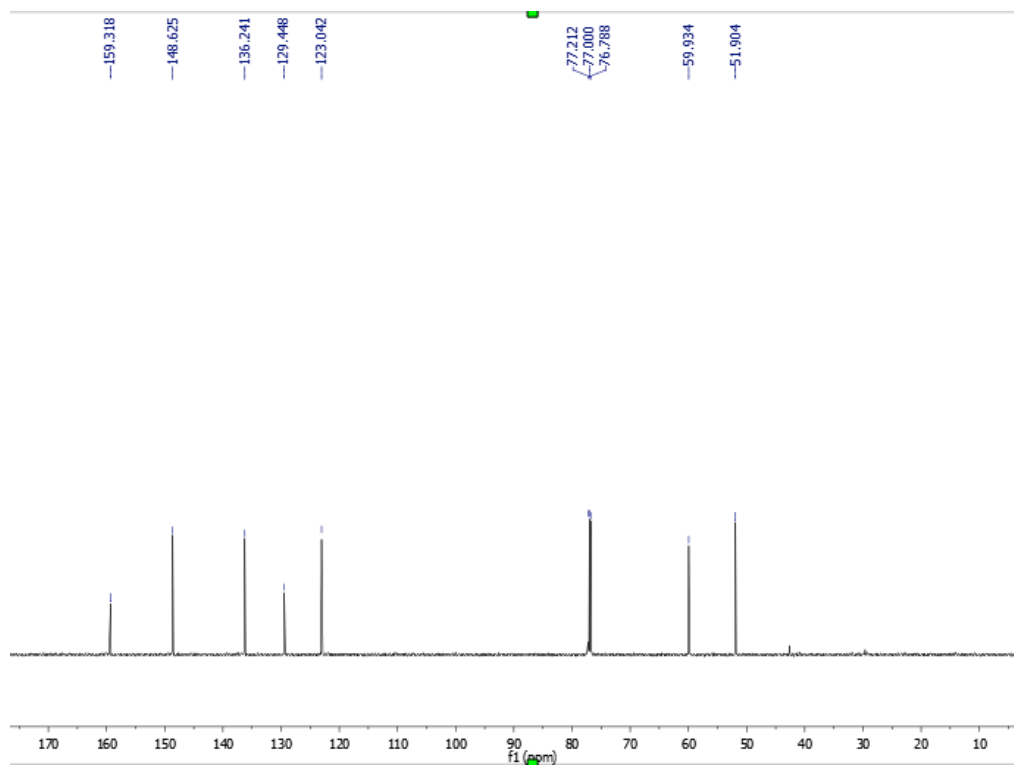
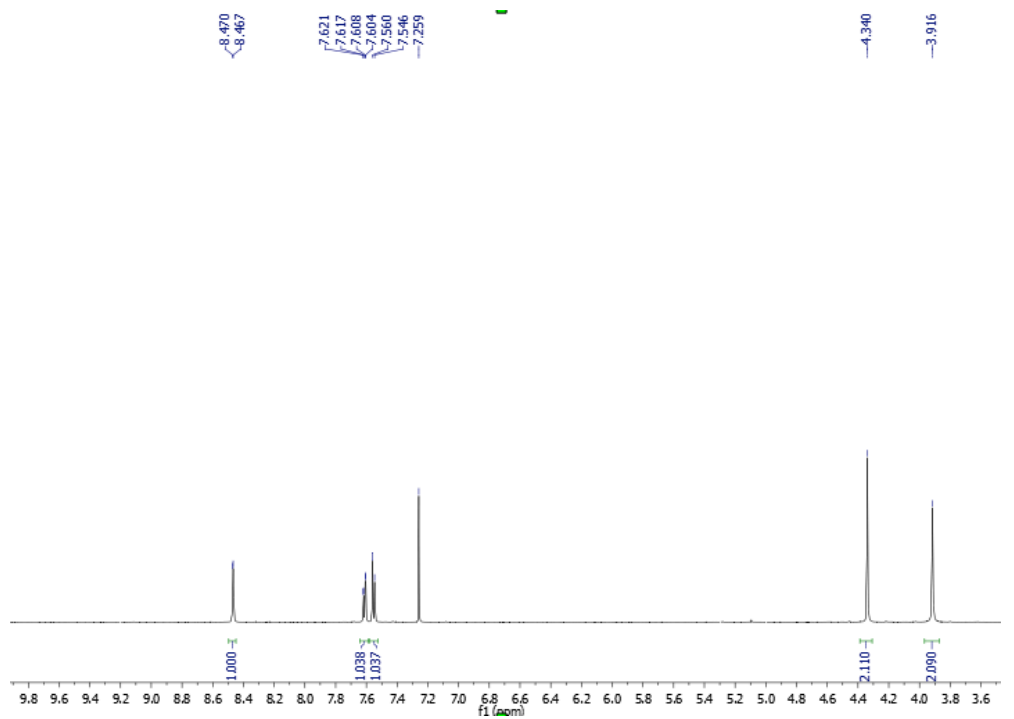
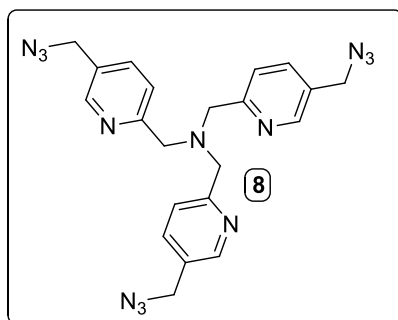


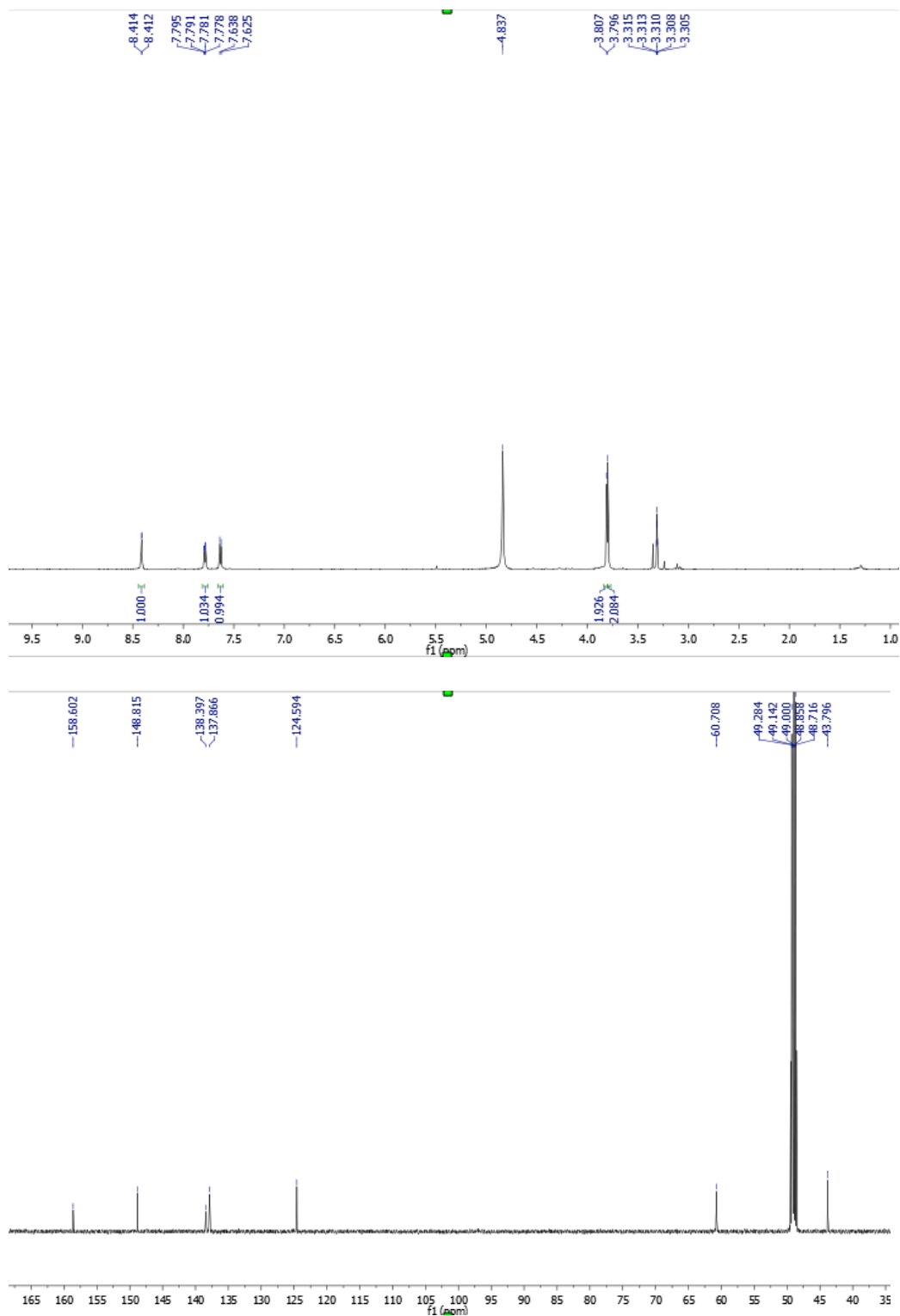
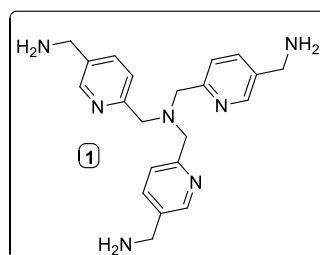


TPA ligand: An aqueous solution of HBr (69 %, 10 mL) was added to compound **9** (327.3 mg, 3 mmol) and the reaction mixture heated at reflux for 12 hours. The solvent was removed under reduced pressure and crude product dissolved in anhydrous dimethyl formamide (15 mL). To this solution, NH_4Br (98 mg, 1 mmol) and Cs_2CO_3 (977.4 mg, 3 mmol) were added and the reaction mixture was sealed and stirred at room temperature for seven days. The mixture was transferred to water (30 mL), extracted with dichloromethane (3 x 60 mL) and combined organic layers washed with saturated NaCl solution (3 x 20 mL). Crude TPA was purified with column chromatography (SiO_2 , acetone:hexanes:ethylacetate = 1:5:3) and then recrystallized from ethylacetate:hexanes = 1:1 to give TPA as a light yellow solid (188.5 mg, 65%). ^1H NMR spectrum of TPA is in agreement with the literature.⁵ ^1H NMR (600 MHz, CDCl_3) δ (ppm): 8.54-8.53 (m, 1H), 7.65 (td, J = 7.8, 1.8 Hz, 1H), 7.58 (d, J = 7.8 Hz, 1H), 7.14 (ddd, J = 7.2, 4.8, 1.2 Hz, 1H), 3.89 (s, 2H).









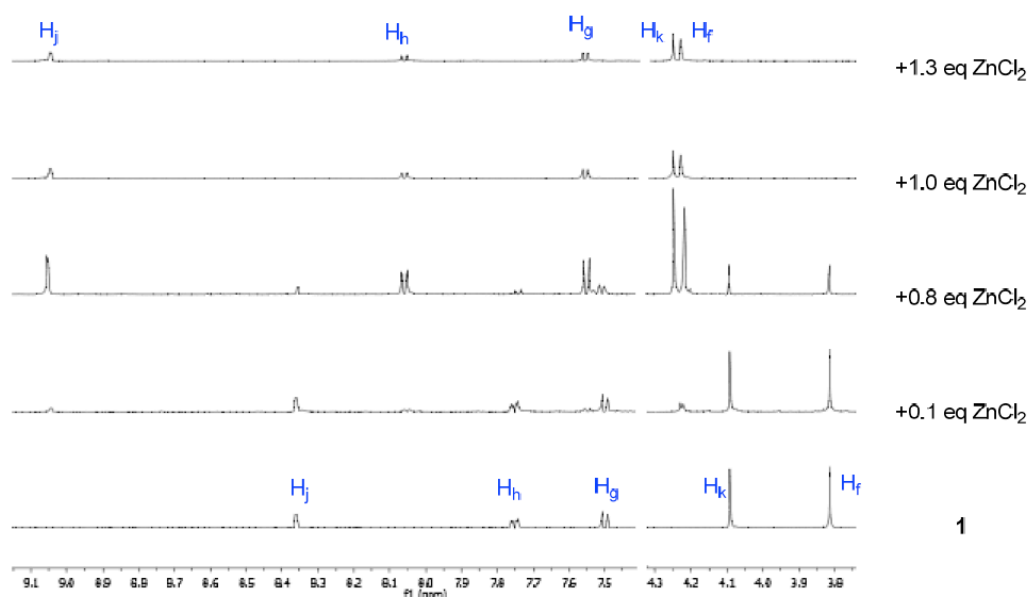


Figure S1. ^1H NMR spectra (600 MHz, 298 K) of a solution of **1** (0.2 mM in 10 mM phosphate buffer at pH = 7.2) obtained upon an incremental addition of ZnCl_2 (30 mM in 10 mM phosphate buffer at pH = 7.2).

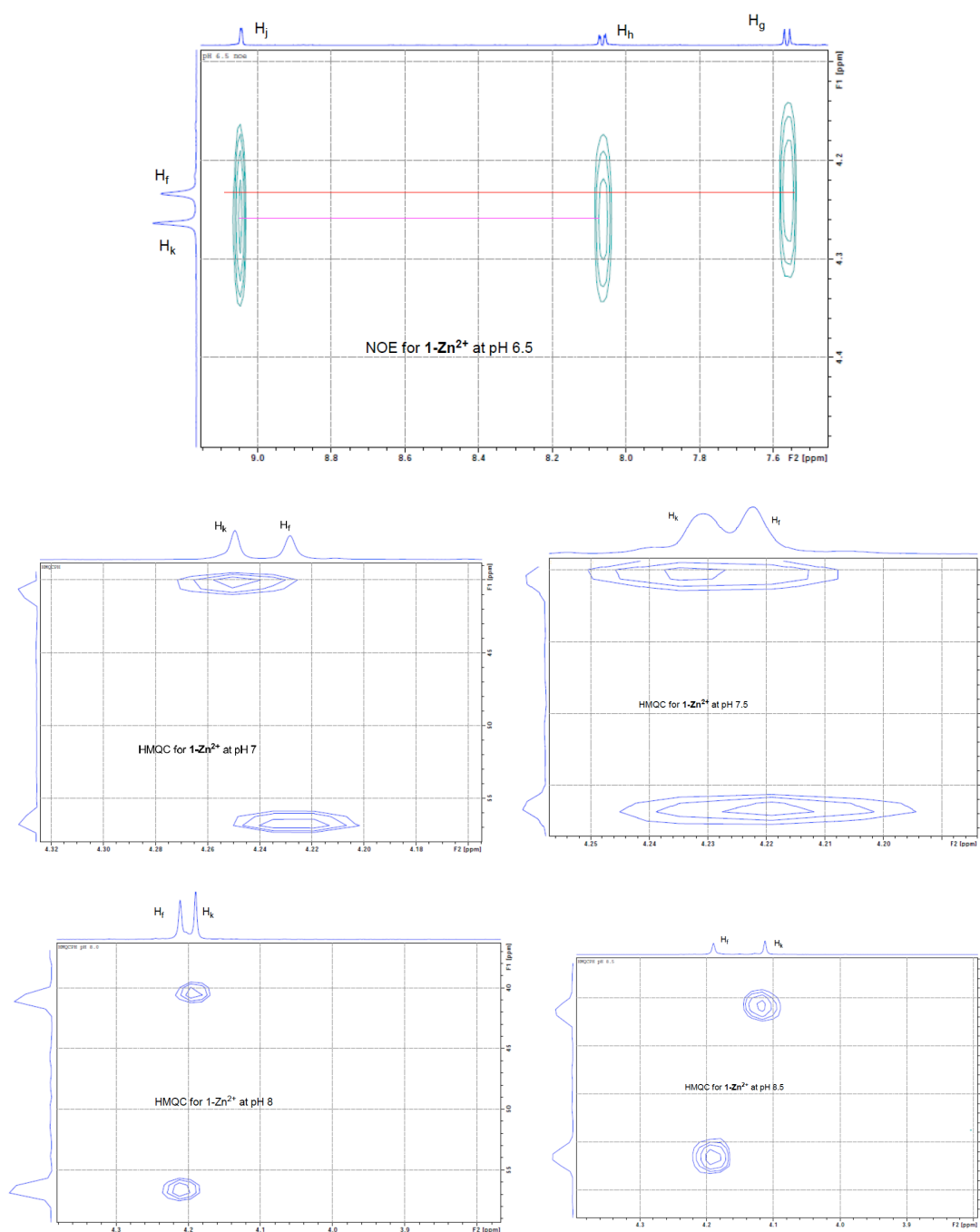


Figure S2. (Top) A segment of ^1H - ^1H NOESY spectrum (600 MHz, 298 K) of **1-Zn(II)** (0.5 mM) in 10 mM phosphate buffer at pH = 6.5, showing the magnetization transfer of $\text{H}_\text{k}/\text{H}_\text{j}/\text{h}$ and $\text{H}_\text{f}/\text{H}_\text{g}$ to permit an unambiguous assignment of these resonances. (Below) ^1H - ^{13}C HMQC spectra (600 MHz, 298 K) of **1-Zn(II)** (0.5 mM) in 10 mM phosphate buffer at pH = 7–8.5 showing cross correlations between H_k and $^{13}\text{C}_\text{k}$ (at a higher field) and H_f and $^{13}\text{C}_\text{f}$ (at a lower field).

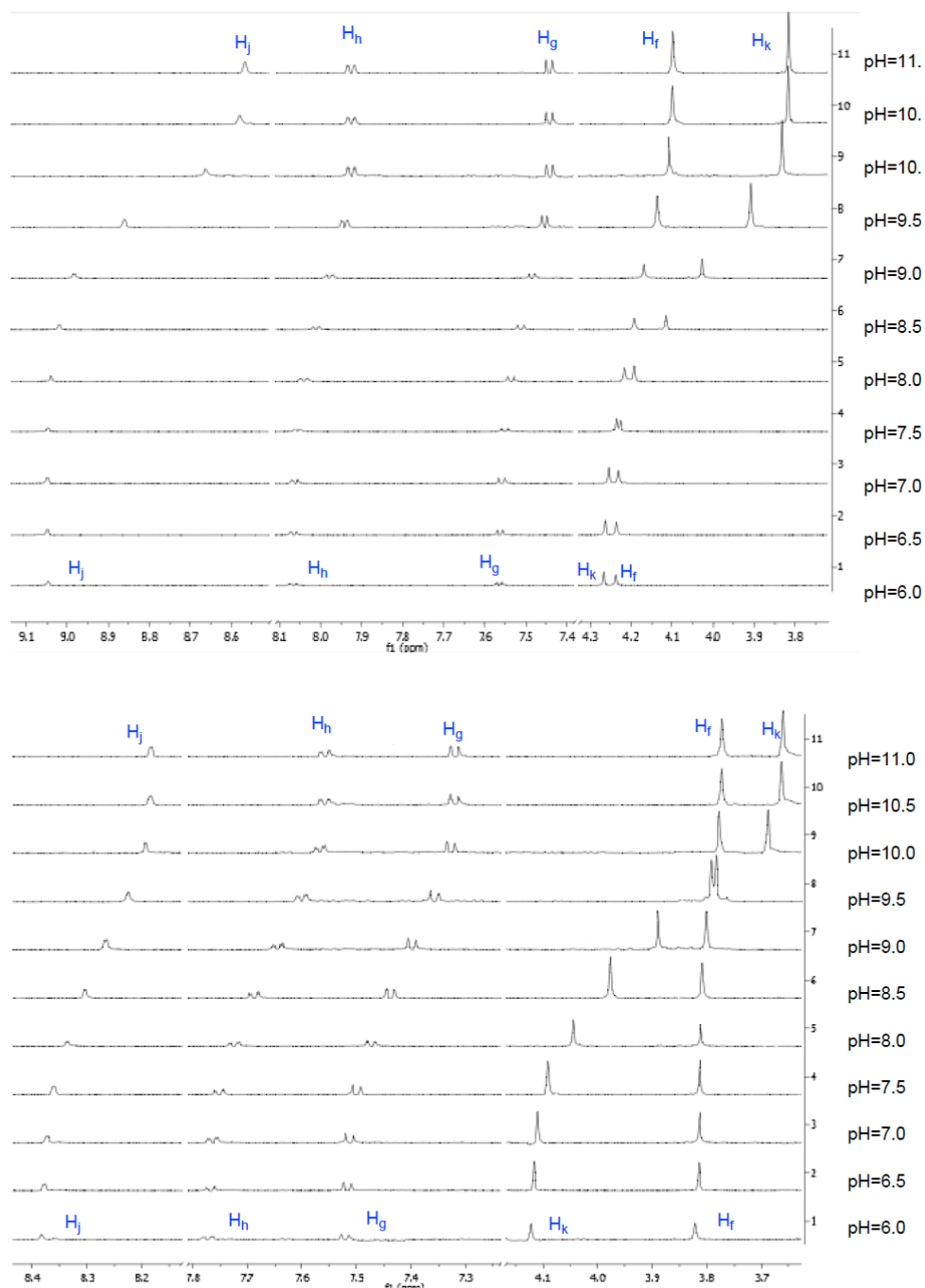


Figure S3. (Top) ¹H-NMR spectra (600 MHz, 298 K) of **1-Zn(II)** (0.5 mM) in 10 mM phosphate buffer solutions at pH = 6.0 – 11.0. (Bottom). ¹H-NMR spectra (600 MHz, 298 K) of **1** (0.5 mM) in 10 mM phosphate buffer solutions at pH = 6.0 – 11.0.

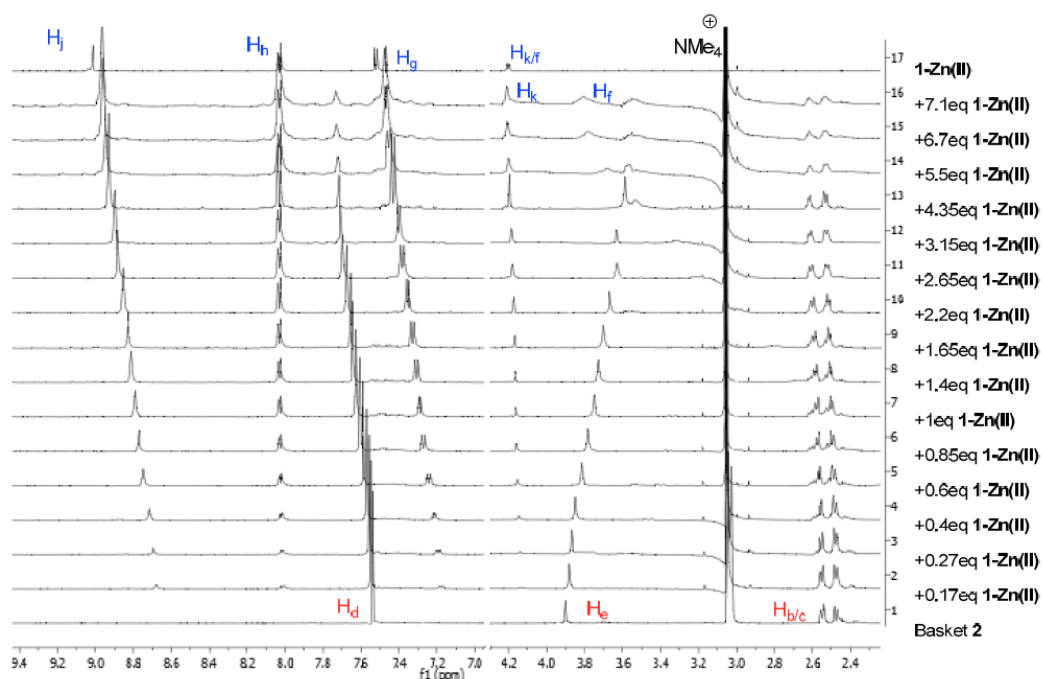


Figure S4. (A) ¹H-NMR spectra (600 MHz, 298 K) of basket **2** (0.31 mM) dissolved in 10 mM phosphate buffer at pH = 7.2 and obtained after incremental addition of a standard solution of **1**-Zn(II) (8.5 mM); the standard solution was obtained upon dissolving **1** in phosphate buffer at pH = 7.2 followed by one molar equivalent of ZnCl₂ and three equivalents of concentrated HCl.

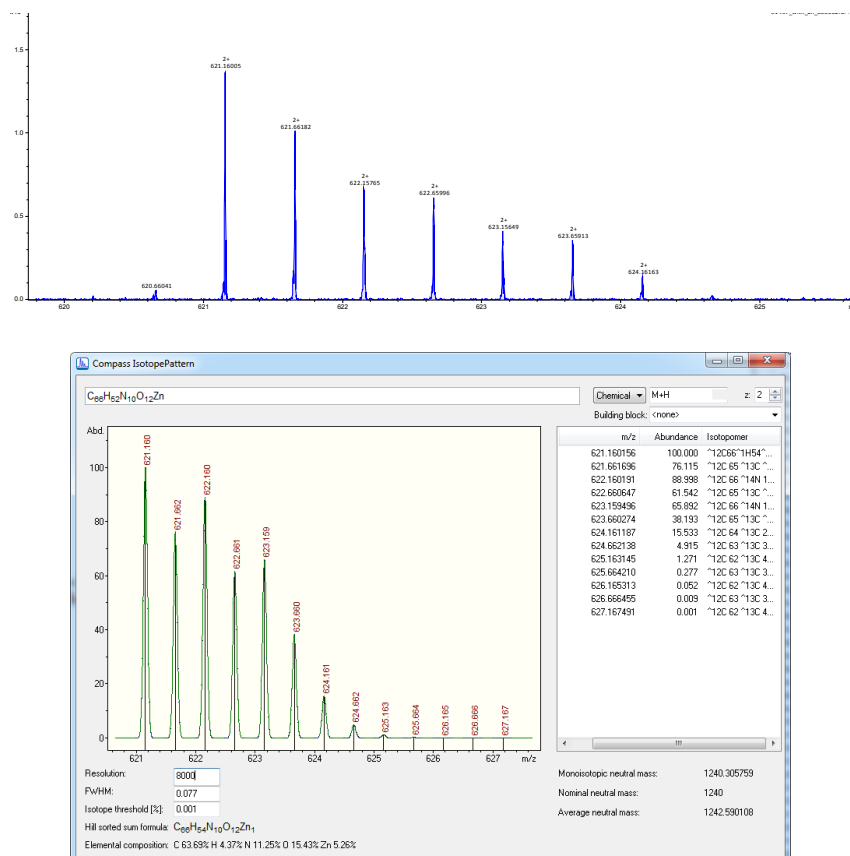


Figure S5. (Top) A segment of electrospray ionization (ESI) spectrum of **1**–Zn(II) dissolved in H₂O (0.1 mM) and containing basket **2** (0.1 mM): the observed signals correspond to doubly charged ion of **1**–ZnC**2** complex at 621.160 a.u. (Bottom) Computer simulated isotope distribution of doubly charged **1**–ZnC**2** of the composition C₆₆H₅₂N₁₀O₁₂Zn.

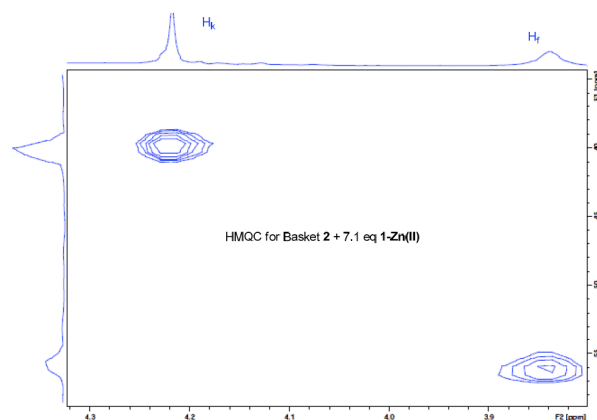


Figure S6. A segment of ¹H–¹³C HMQC spectrum (600 MHz, 298 K) of basket **2** (0.31 mM) containing **1**–Zn(II) (2.2 mM) in phosphate buffer at pH = 7.2. The apparent cross correlations between **H_k** and ¹³C_k (at a higher field) as well as **H_f** and ¹³C_f (at a lower field) permit the assignment of the proton resonances.

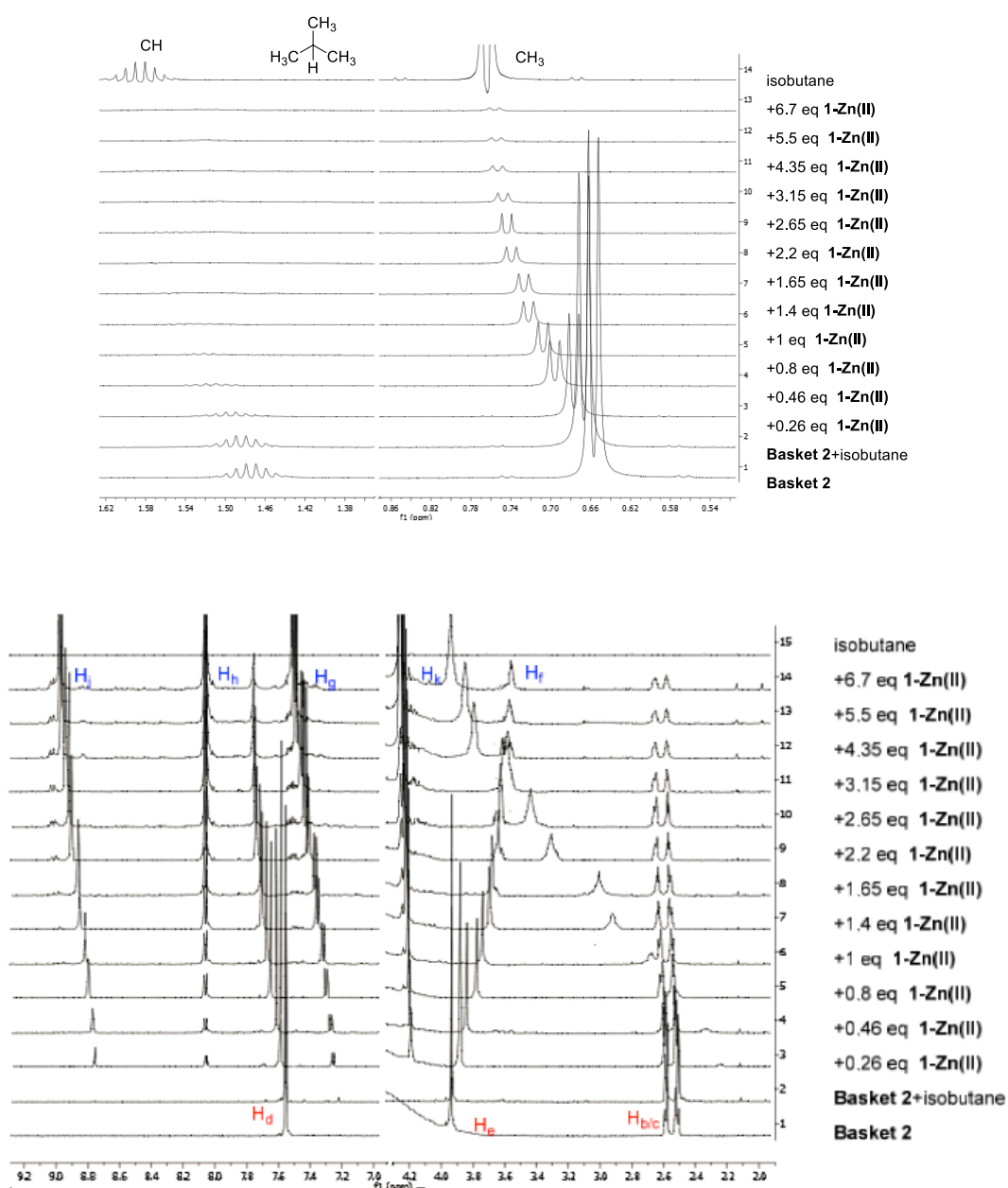


Figure S7. An incremental addition of a standard solution of **1-Zn(II)** (13.5 mM) to iso- C_4H_{10} (0.8 mM) and basket **2** (0.47 mM) in 10 mM phosphate buffer at pH = 7.2 was monitored with ^1H NMR spectroscopy (700 MHz, 298.0 K); the standard solution was obtained upon dissolving **1** in phosphate buffer at pH = 7.2 followed by one molar equivalent of ZnCl_2 and three equivalents of concentrated HCl.

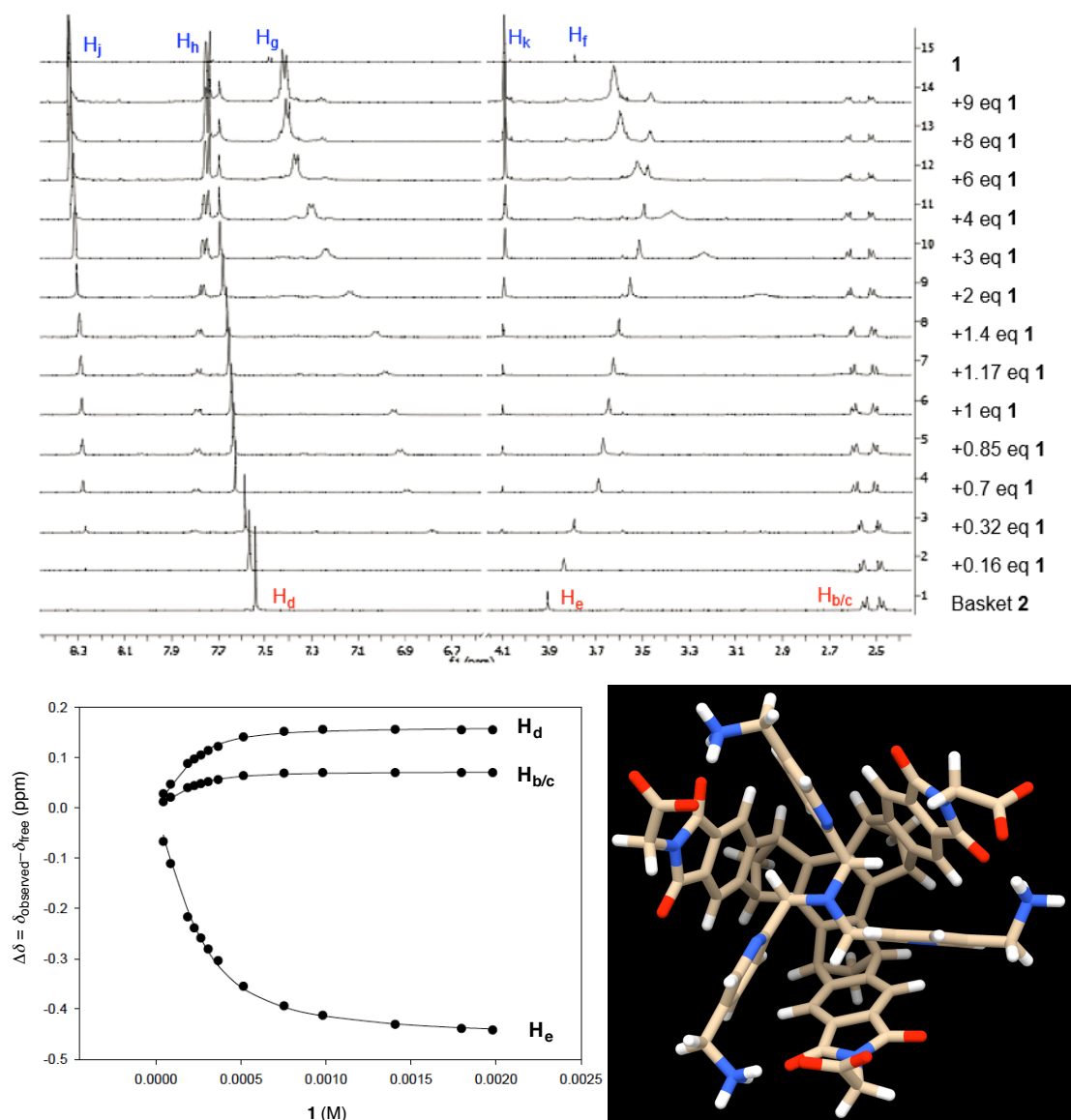


Figure S8. (Top) ¹H NMR spectra (600 MHz, 298.0 K) of a solution of basket **2** (0.236 mM in 10 mM phosphate buffer at pH = 7.2) obtained upon an incremental addition of standard solution of compound **1** (17.3 mM); the standard solution was obtained upon dissolving **1** in phosphate buffer at pH = 7.2 followed by the addition of three equivalents of concentrated HCl. (Bottom Left) The nonlinear least-square fitting of the experimental isotherms from H_{b-c} resonances to a 1:1 stoichiometric model gave the association constants in the range of $K = 1.0 - 2.4 \pm 0.1 \cdot 10^4 \text{ M}^{-1}$ ($R^2 = 0.99$, Sigma Plot) which on average amounts to $\Delta G^\circ = 5.8 \text{ kcal/mol}$ at 298 K. (Bottom Right) Top view of the energy-minimized structure of **1C2** (Amber Force Field) obtained from Monte Carlo conformational search (10000 steps) in the implicit water solvent.

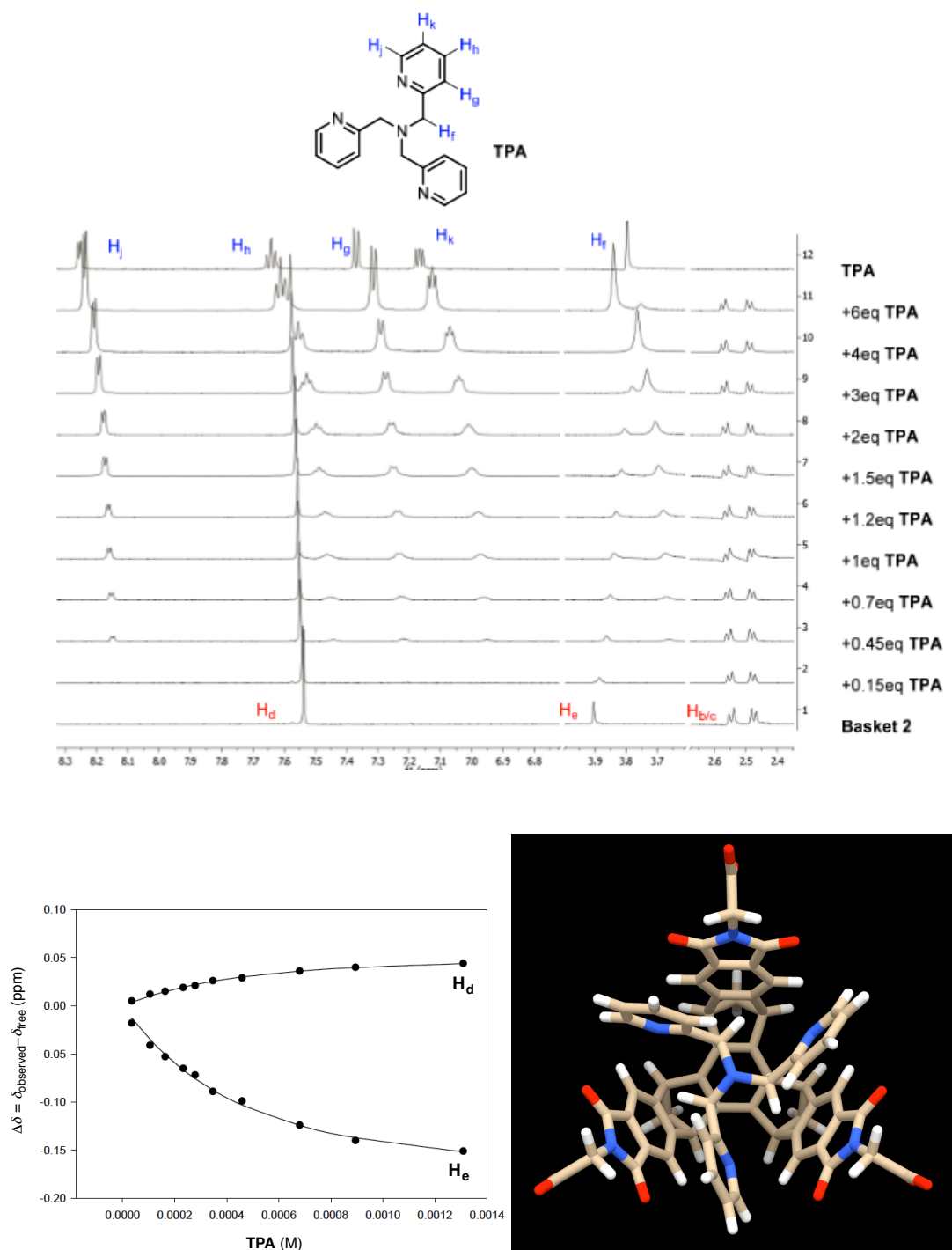


Figure S9. (Top) ^1H NMR spectra (600 MHz, 298.0 K) of a solution of basket **2** (0.236 mM in 10 mM phosphate buffer at pH = 7.2) obtained upon an incremental addition of standard solution of unfunctionalized **TPA** ligand (17.3 mM); the standard solution was obtained upon dissolving **TPA** in phosphate buffer at pH = 7.2 followed by the addition of three equivalents of concentrated HCl. (Bottom Left) The nonlinear least-square fitting of the experimental isotherms from $\text{H}_{\text{d-e}}$ resonances to a 1:1 stoichiometric model gave the association constants in the range of $K = 3.6 \pm 0.2 \cdot 10^3 \text{ M}^{-1}$ ($R^2 = 0.99$, Sigma Plot) which amounts to $\Delta G^\circ = 4.9 \text{ kcal/mol}$ at 298 K. (Bottom Right) Top view of the energy-minimized structure of **TPAC2** (Amber Force Field) obtained from Monte Carlo conformational search (10000 steps) in the implicit water solvent.

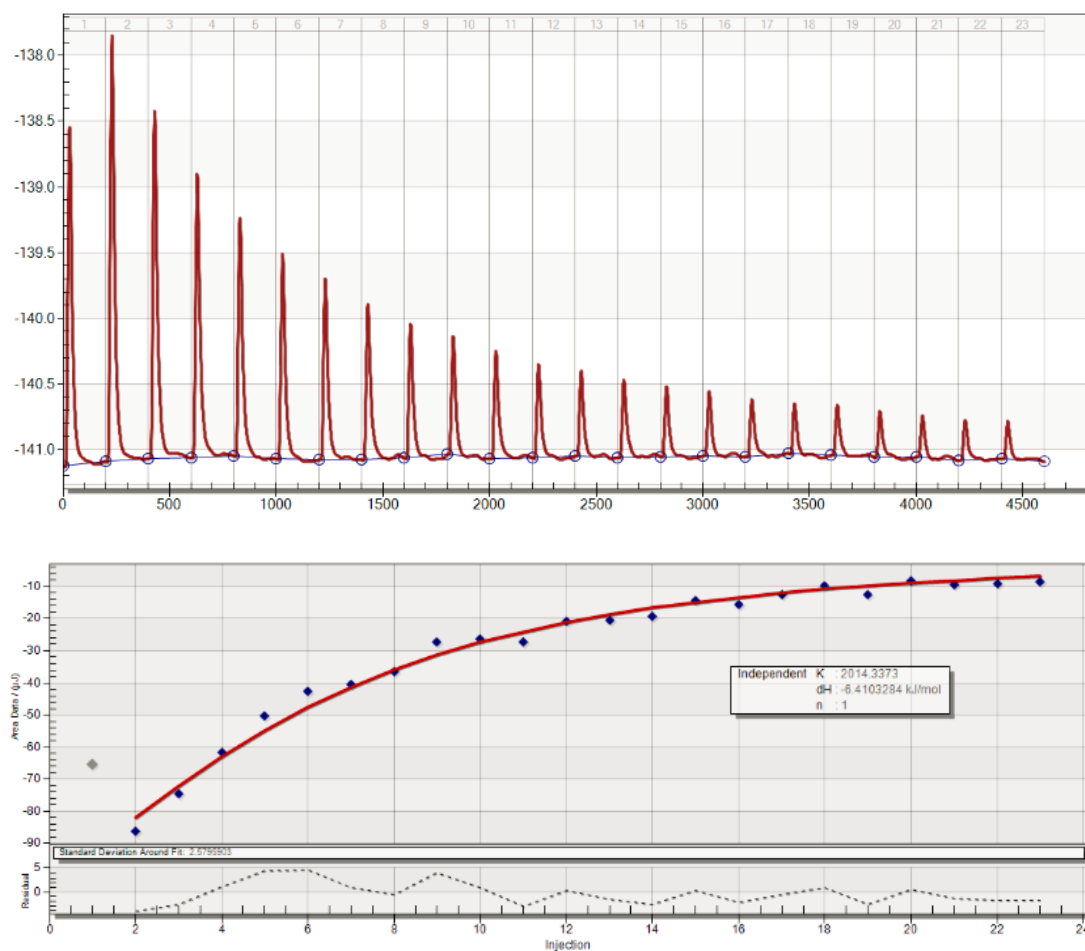


Figure S10. The experimental (top) and integrated (bottom) ITC data corresponding to incremental addition of a standard solution of **TPA** (2.02 μL per injection of its 15.0 mM solution) in phosphate buffer (30 mM, pH = 7.2) to basket **2** (0.5 mM) in 300.0 μL of PBS (30 mM at pH = 7.2) at 298.0 K. The nonlinear least-square analysis (bottom) of the binding data was completed with the assistance of single site-binding model (with the stoichiometric coefficient n fixed to 1) to give $K_a = 2.01 \pm 0.26 \times 10^3 \text{ M}^{-1}$ and $\Delta H^\circ = -1.53 \pm 0.08 \text{ kcal/mol}$ ($R^2 = 0.95$).

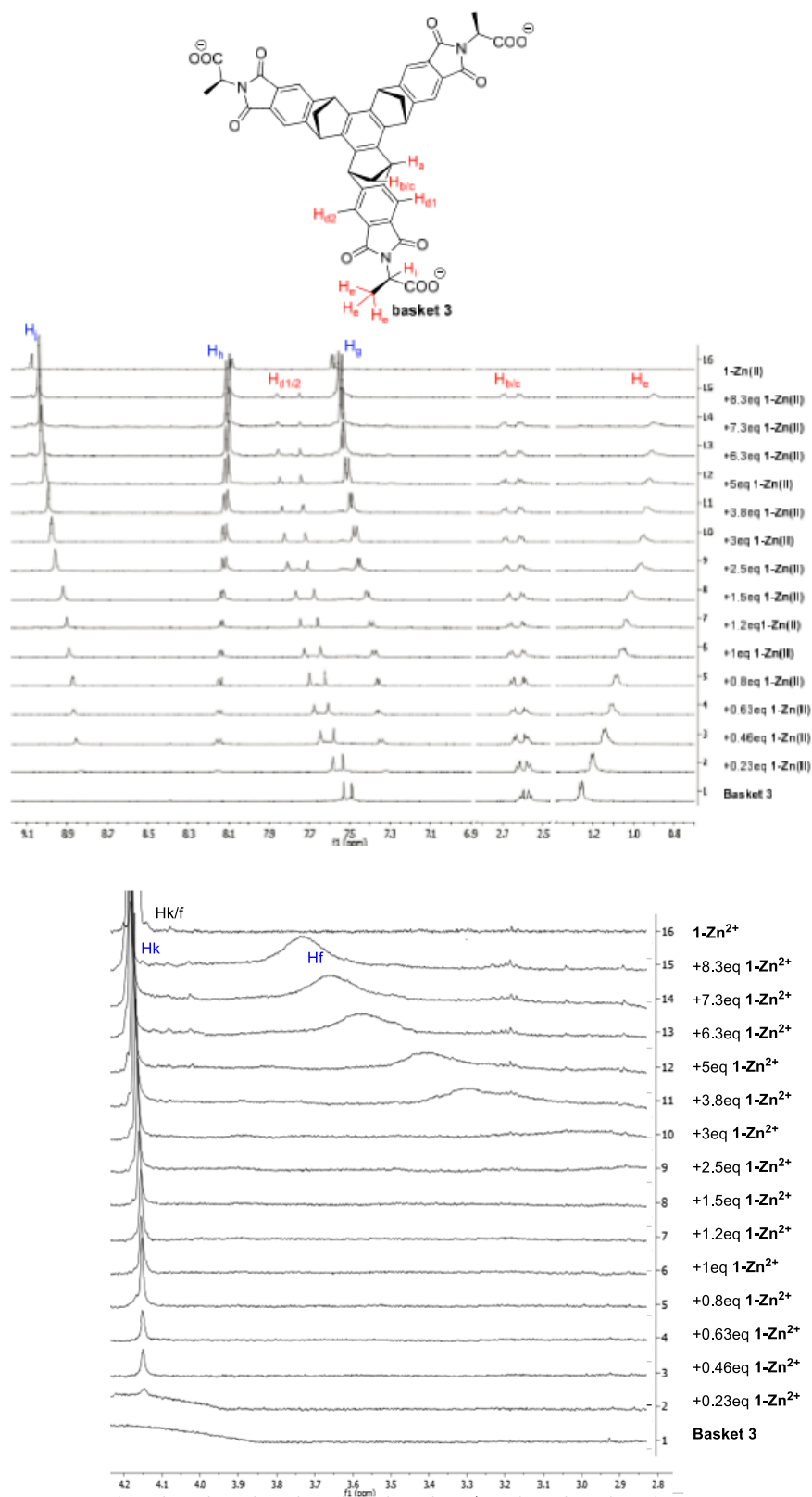


Figure S11. ^1H -NMR spectra (600 MHz, 298 K) of **basket 3** (0.31 mM) dissolved in 10 mM phosphate buffer at pH = 7.2 obtained after incremental addition of a standard solution of **1-Zn(II)** (8.5 mM); the standard solution was obtained upon dissolving **1** in phosphate buffer at pH = 7.2 followed by one molar equivalent of ZnCl_2 and three equivalents of concentrated HCl.

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