Supporting Information
for

Strong Effect of Copper(II) Coordination on Antiproliferative Activity of Thiosemicarbazone-Piperazine and Thiosemicarbazone-Morpholine Hybrids

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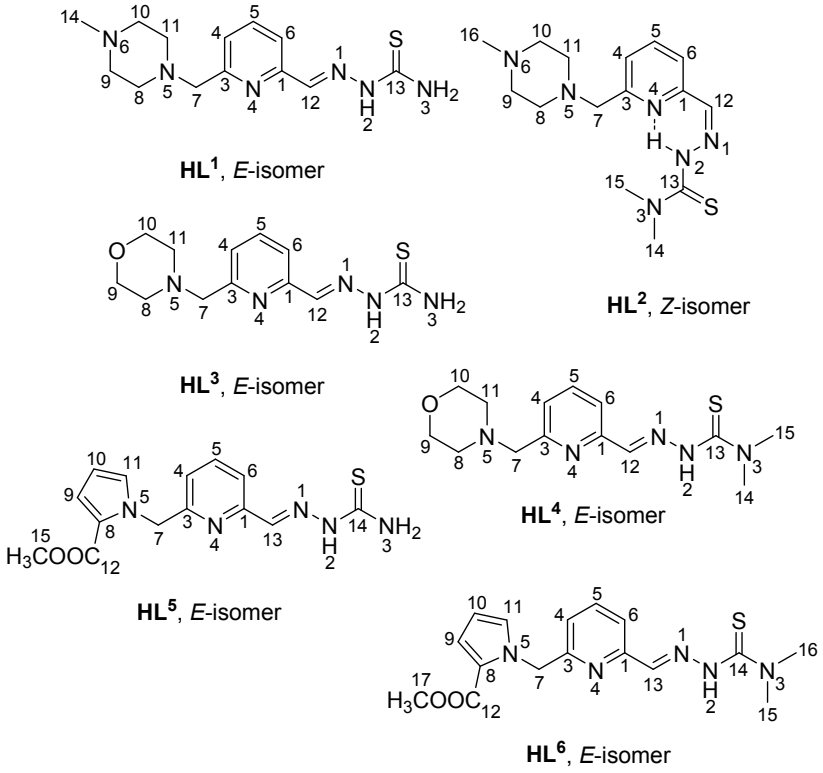
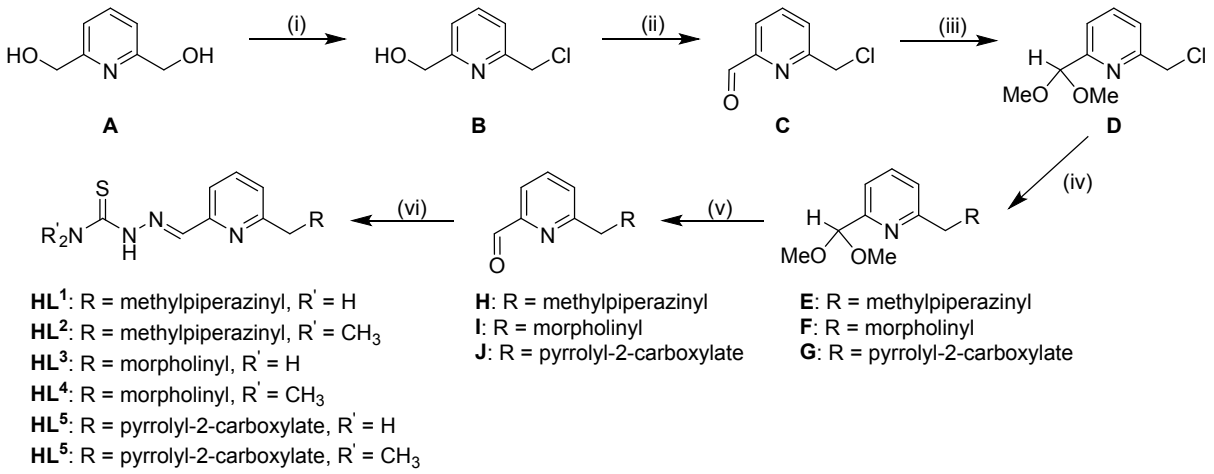
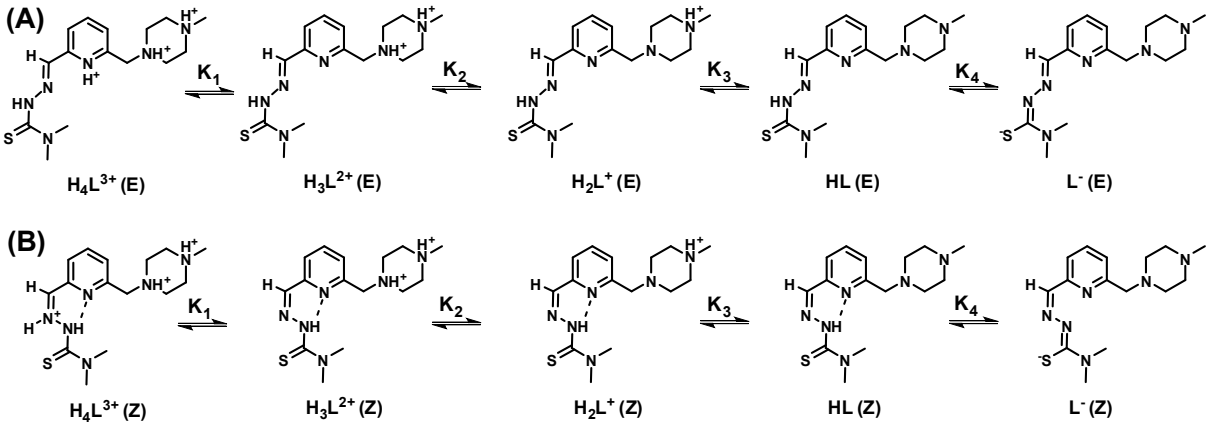


Scheme S1. Atom numbering scheme for ^{1}H and ^{13}C NMR data for **HL[1] – HL[6]**. **HL[2]** is depicted as *Z*-isomer for clarity reasons.



Scheme S2. Synthesis of **HL[1]-HL[6]**.[a]

[a]Reagents and conditions: (i) and (ii) see ref.1; (iii) trimethyl orthoformate, methanesulfonic acid, methanol, 78 °C, 3 h; (iv) **E:** methylpiperazine, triethylamine, THF/CH_2Cl_2 1:1, 40 °C, 12 h, purification by column chromatography; **F:** morpholine, triethylamine, THF/CH_2Cl_2 1:1, 40 °C, 12 h, purification by column chromatography; **G:** pyrrole-2-carboxylate, sodium hydride, DMF, 0° C, 1 h, than room temperature, 12 h, purification by column chromatography; (v) **H** and **I:** HCl, water, 60 °C, 12 h, sat. aqueous $NaHCO_3$, extraction with CH_2Cl_2; **J:** water/acetone 5:1, reflux, 12 h; (vi) **HL[1]:** thiosemicarbazide, EtOH, 78 °C, 12 h, purification on prep. HPLC; **HL[2]:** 4,4-dimethyl-3-thiosemicarbazide, EtOH, room temperature, 12 h, purification on prep. HPLC; **HL[3]:** thiosemicarbazide, EtOH, 78 °C, 12 h, recrystallization from water/methanol 5:1; **HL[4]:** 4,4-dimethyl-3-thiosemicarbazide, EtOH, room temperature, 12 h. **HL[5]:** thiosemicarbazide, EtOH/MeOH 1:1, 78 °C, 12 h, recrystallization from water/methanol 5:1. **HL[6]:** 4,4-dimethyl-3-thiosemicarbazide, EtOH/MeOH 1:1, room temperature, 6 h.



Scheme S3. Deprotonation steps of the H_4L^{3+} form of ligand mPip-dm-FTSC (**HL²**) for its *E* (A) and *Z* (B) isomers

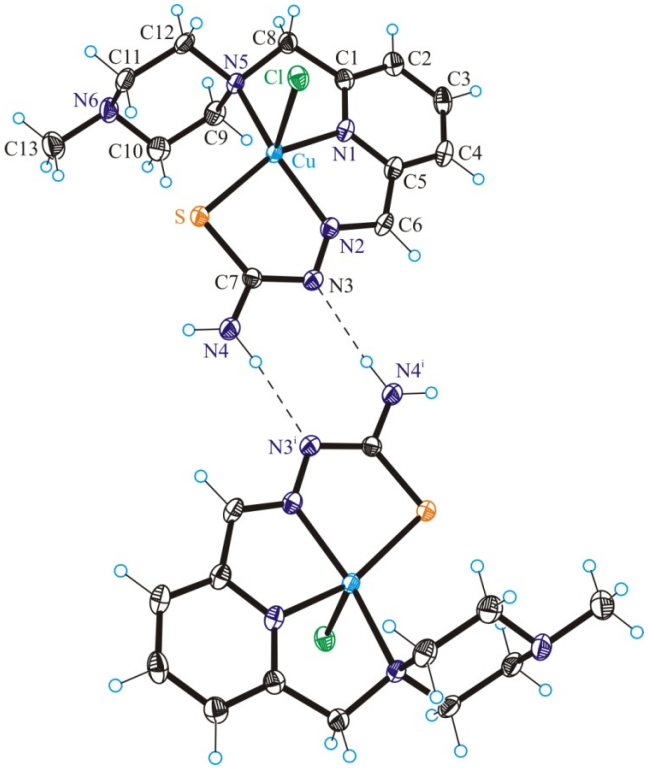


Figure S1. Part of the crystal structure of **1** showing complex pairing via intermolecular hydrogen bonding interactions. Donor···acceptor (D···A) distances (Å) and donor-hydrogen···acceptor (D–H···A) angles (deg) of the intermolecular hydrogen bonds: N4···N3^{i} 2.946(3) Å, N4–H···N3^{i} 173.7°; atoms marked with i are at symmetry position $–x + 2$, $–y + 1$, $–z + 1$.

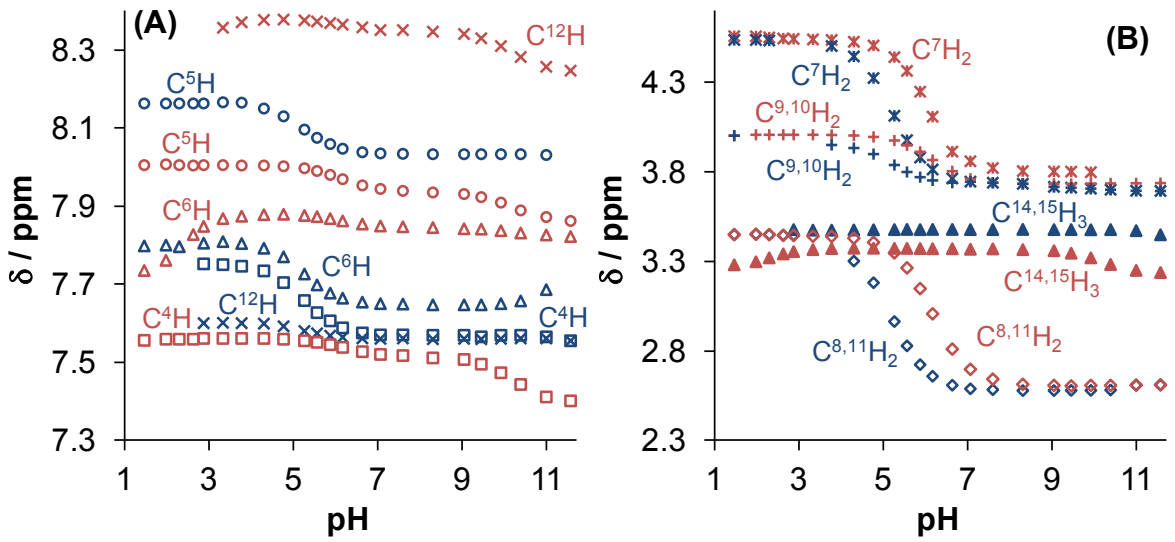


Figure S2. pH-Dependence of the chemical shifts of the various protons of the ligand Morph-dm-FTSC (**HL^4**) in the low- (A) and in the high-field (B) regions. **Red** and **blue** symbols denote the protons belonging to the major *E* and minor *Z* isomers, respectively. [c_L= 1.5 mM; *T* = 298 K; *I* = 0.10 M (KCl); 10% D_2O]

Figure S3. Low- (A) and high-field (B) regions of the ^{1}H NMR spectra of ligand mPip-dm-FTSC (**HL^2**) at different pH values, **red** and **blue** symbols denote the peaks belonging to the protons of the major *E* and minor *Z* isomers, respectively. [c_L= 3 mM; *T* = 298 K; *I* = 0.10 M (KCl); 10% D_2O]

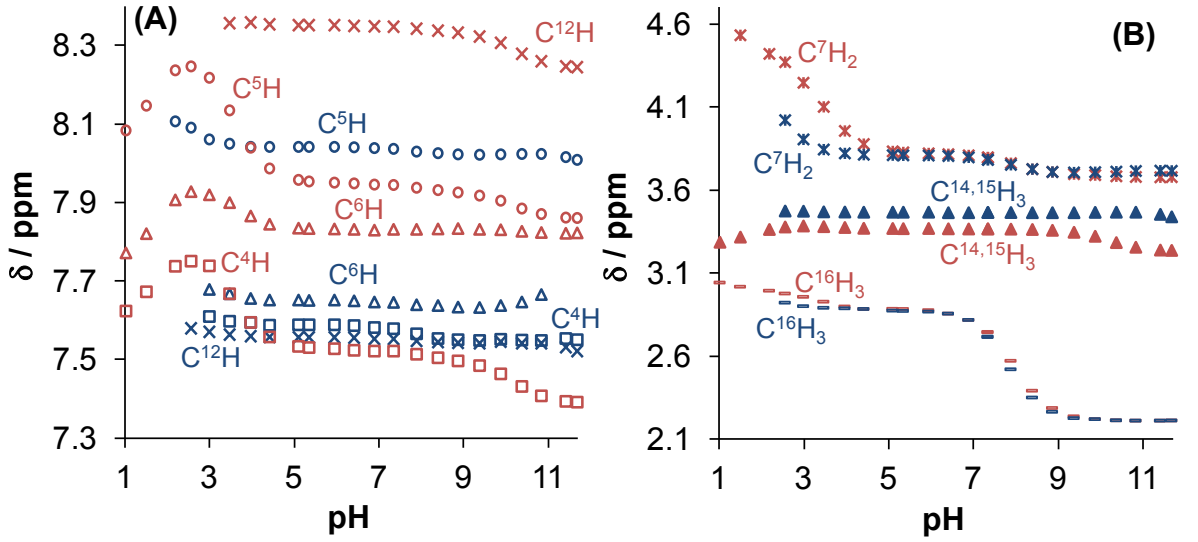


Figure S4. pH-Dependence of the chemical shifts of the various protons of the ligand mPip-dm-FTSC (**HL²**) in the low- (A) and in the high-field (B) regions. **Red** and **blue** symbols denote the protons belonging to the major *E* and minor *Z* isomers, respectively. [c_L= 3 mM; *T* = 298 K; *I* = 0.10 M (KCl); 10% D_2O]

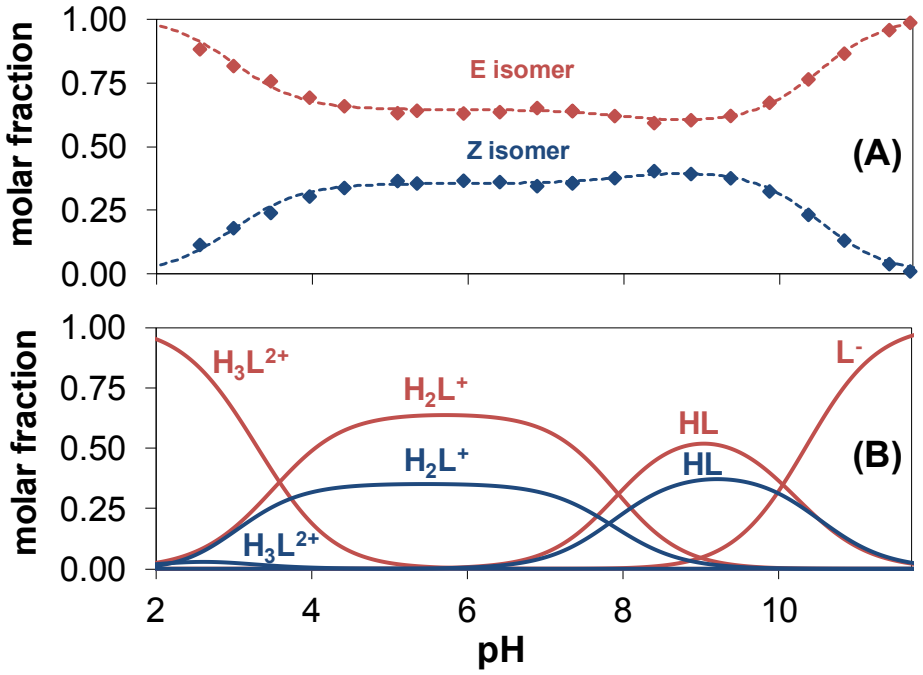


Figure S5. pH-Dependence of the molar fraction of the *E* (red symbols) and *Z* (blue symbols) isomers of the ligand mPip-dm-FTSC (**HL²**) calculated on the basis of the integrated areas of the signals of the various ligand protons (A). Concentration distribution curves for the isomeric ligand species (*E*: red letters; *Z*: blue letters) calculated with the aid of the microscopic proton dissociation constants (B). [c_L= 3 mM; *T* = 298 K; *I* = 0.10 M (KCl); 10% D_2O]

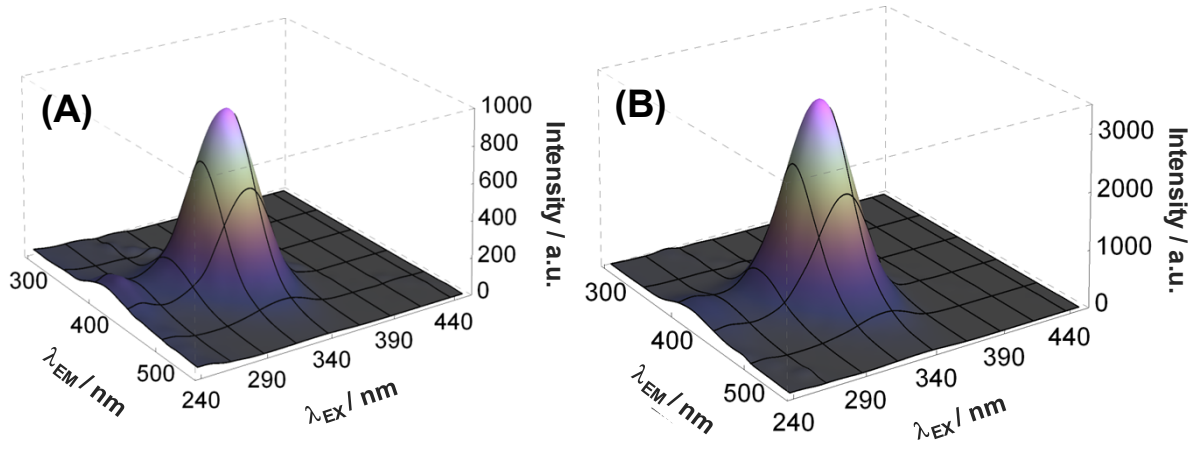


Figure S6. The 3-dimensional fluorescence spectrum of mPip-dm-FTSC (**HL²**) (A) and Morph-dm-FTSC (**HL⁴**) (B) at physiological pH in water. [c_L= 10 μM; T = 298 K; I = 0.10 M (KCl); pH = 7.4 (10 mM HEPES)]

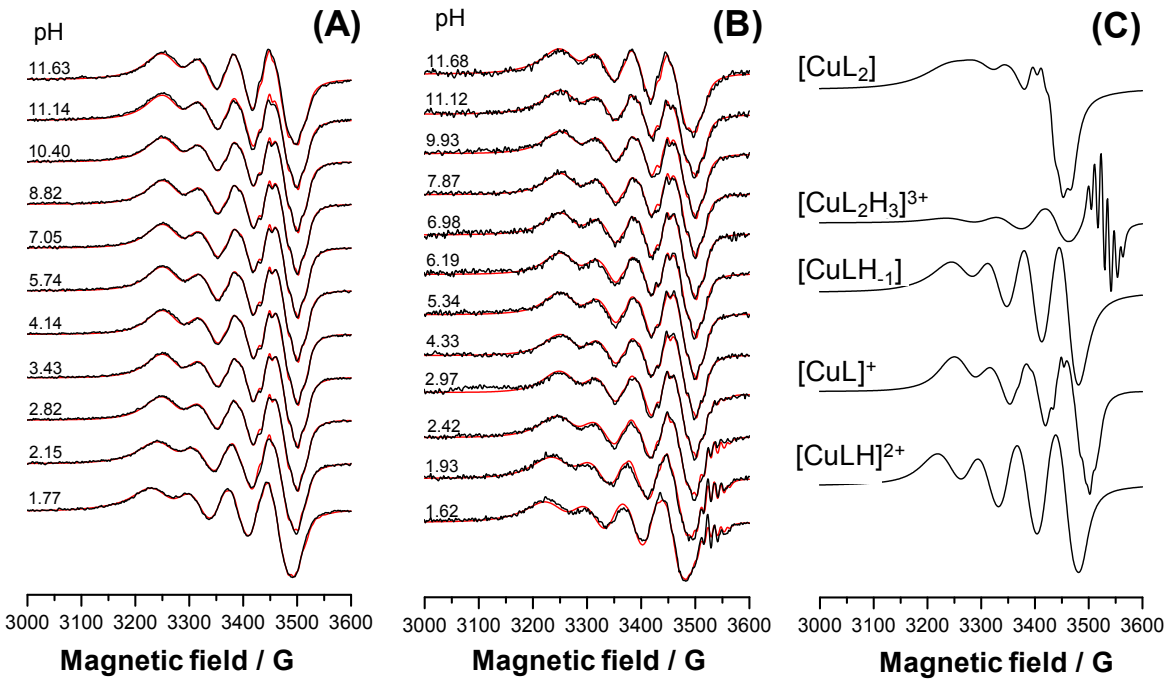


Figure S7. Experimental (black) and simulated (red) solution EPR spectra recorded for the copper(II) – Morph-dm-FTSC (**HL⁴**) system at 1:1 (A) and 1:2 (B) metal-to-ligand ratio. Calculated component EPR spectra obtained for the different copper(II) – Morph-dm-FTSC (**HL⁴**) species (C). [c_{ligand} = 1.0 mM; c_{Cu}= 1.0 mM (A) or c_{Cu}= 0.5 mM (B); T = 298 K; I = 0.10 M (KCl)]

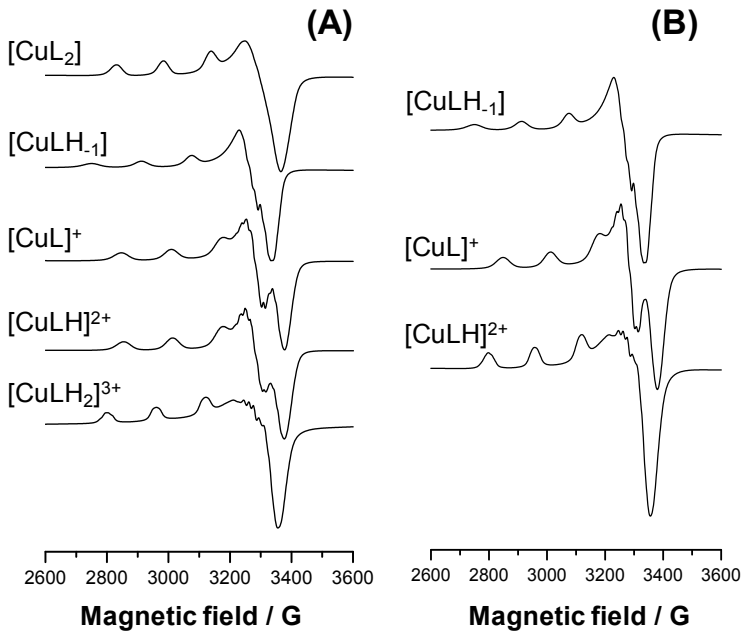


Figure S8. Calculated component EPR spectra obtained for the different copper(II) – mPip-dm-FTSC (**HL²**) (A) and Morph-dm-FTSC (**HL⁴**) (B) complexes in frozen solution. [T = 77 K; I = 0.10 M (KCl)]

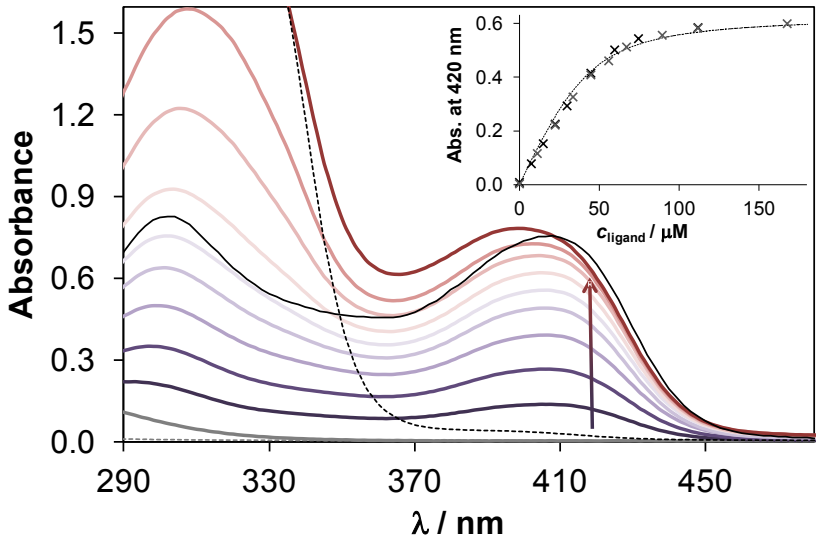


Figure S9. UV−vis spectra of the $[Cu(EDTA)]^{2-}$ complex (grey solid line, c = 50 μM) in the presence of ligand mPip-dm-FTSC (**HL²**) at increasing concentrations (solid lines in color, c = 7.5 - 167.6 μM). UV−vis spectra of EDTA (grey dashed line, c = 50 μM), mPip-dm-FTSC (black dashed line, c = 167.6 μM) and complex **2** (black solid line, c = 50 μM) are shown for comparison. Inset shows the absorbance values recorded at 420 nm for two parallel sets of measurements on the ternary copper(II) – EDTA − ligand system in dependence of the concentration of ligand mPip-dm-FTSC. [T = 298 K; I = 0.10 M (KCl); l = 1 cm; pH = 7.4 (10 mM HEPES)]

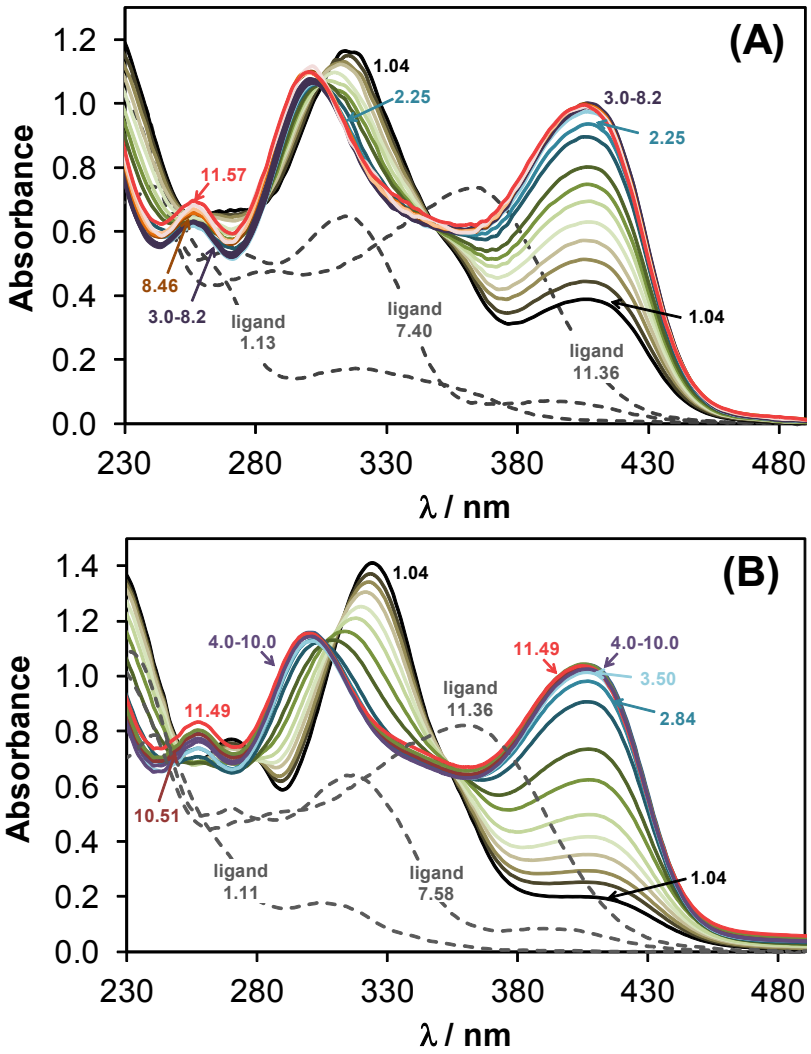


Figure S10. UV−vis spectra of the copper(II) – mPip-dm-FTSC (**HL²**) (A) and copper(II) – Morph-dm-FTSC (**HL⁴**) (B) systems at 1:1 metal-to-ligand ratio. (Grey dashed lines show the spectra of the ligands alone at chosen pH values.) [c_L= 153 μM (A) and 157 μM (B); T = 298 K; I = 0.10 M (KCl); l = 0.5 cm]

References

1. G. Paolucci, A. Zanella, M. Bortoluzzi, S. Sostero, P. Longo and M. Napoli, *J. Mol. Catal. A: Chem.*, 2007, **272**, 258-264.