# Supporting Information

# Dicopper(II) and Dizinc(II) Complexes with Nonsymmetric Dinucleating Ligands Based on Indolo[3,2-*c*]quinolines: Synthesis, Structure, Cytotoxicity and Intracellular Distribution

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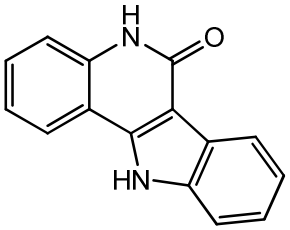
**Synthesis and NMR characterization of starting compounds and intermediates**

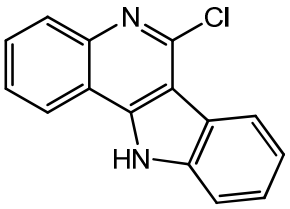
Indolo[3,2-*c*]quinoline precursors were synthesized as described by Filak et al. (*Organometallics* **2011**, *30*, 273–283) and Primik et al. ( *Inorg. Chem.* **2010**, *42*, 11084–11095).

5*H*-Indolo[3,2-*c*]quinolin-6(11*H*)-one




A mixture of isatin (10.2 g, 69.3 mmol) and 2-aminobenzylamine (9.0 g, 73.7 mmol) in glacial acetic acid (85 mL) was heated at 144 °C for 4 h under argon atmosphere. The solution was allowed to cool to room temperature generating a brown precipitate which was filtered, washed with cold acetic acid (3 × 5 mL) and water (twice 20 mL), and dried at 95 °C *in vacuo*. Yield: 12.3 g (80%). $^{1}$H NMR 500.13 MHz (DMSO-$d_6$, $\delta_H$, ppm): 12.55 (s, 1H, N11), 11.42 (s, 1H, N5), 8.23-8.18 (m, 2H), 7.63 (d, 1H, $^{3}J$ = 7.9 Hz), 7.54 – 7.45 (m, 2H), 7.40-7.35 (m, 1H), 7.33-7.25 (m, 2H).

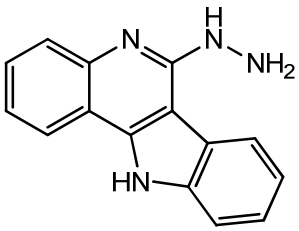
6-Chloro-11*H*-indolo[3,2-*c*]quinoline




A suspension of 5*H*-Indolo[3,2-*c*]quinolin-6(11*H*)-one (3.0 g, 13.0 mmol) in $POCl_3$ (40 mL) was refluxed at 130 °C for 26 h under argon atmosphere. The solution was allowed to cool to room temperature and was poured onto ice (300 mL) and slowly neutralized with solid sodium hydroxide until a pH of 7–8 was reached. The resulting aqueous suspension was extracted with ethyl acetate (5 × 200 mL). The combined organic phases were washed with 5% $NaHCO_3$ aqueous solution (2 × 250 mL) and dried over $Na_2SO_4$. The solvent light beige product was isolated by removal of the solvent under reduced pressure and dried *in vacuo* overnight. Yield: 3.1 g (95%). $^{1}$H NMR 500.13 MHz (DMSO-$d_6$,

$\delta_H$, ppm): 13.13 (s, 1H, N11), 8.57 (d, 1H, $^3J$ = 8.2 Hz), 8.45 (d, 1H, $^3J$ = 7.8 Hz), 8.07 (d, 1H, $^3J$ = 8.3 Hz), 7.84-7.73 (m, 3H), 7.61 – 7.56 (m, 1H), 7.46 – 7.41 (m, 1H).

6-Hydrazinyl-11*H*-indolo[3,2-*c*]quinoline




To 6-chloro-11*H*-indolo[3,2-*c*]quinoline (3.0 g, 11.9 mmol) hydrazine hydrate (50 mL) as reagent and solvent was added and the reaction mixture heated at 115 °C for 24 h under argon atmosphere. After cooling to room temperature, the resulting beige precipitate was filtered off, washed with water (2 × 10 mL), ethyl acetate and dried *in vacuo*. Yield: 2.8 g (95%). $^1$H NMR 500.13 MHz (DMSO-$d_6$, $\delta_H$, ppm): 12.48 (bs, 1H, N11), 8.35 (d, 1H, $^3J$ = 8.2 Hz), 8.28 (d, 1H, $^3J$ = 7.8 Hz), 8.03 (bs, 1H, N12), 7.76 (d, 1H, $^3J$ = 7.8 Hz), 7.65 (d, 1H, $^3J$ = 7.3 Hz), 7.57-7.51 (m, 1H), 7.43 – 7.37 (m, 1H), 7.36 – 7.30 (m, 1H), 7.29-7.23 (m, 1H), 4.73 (bs, 1H, N13).

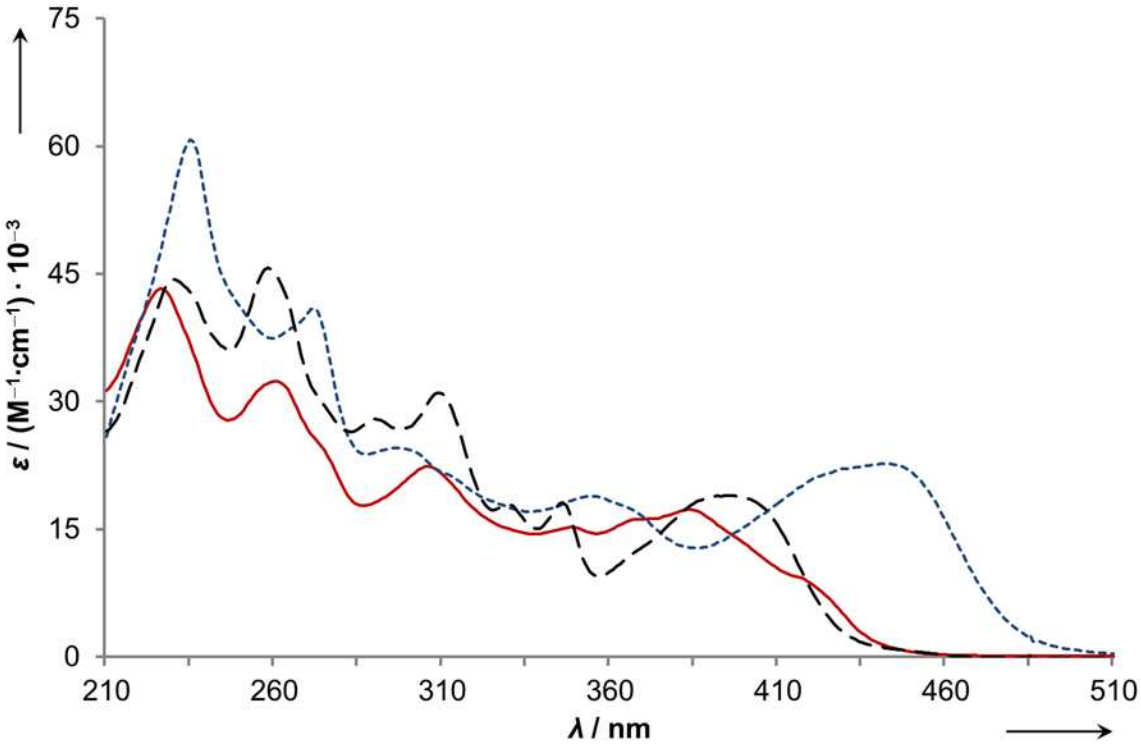



**Figure S1**. UV–vis spectra of 10 μM solutions of $^{EtOOC}$**HL**$^{COOEt}$ (red/solid trace), **1** (blue/shortly dashed trace) and **2** (black, long dashed trace) in methanol.

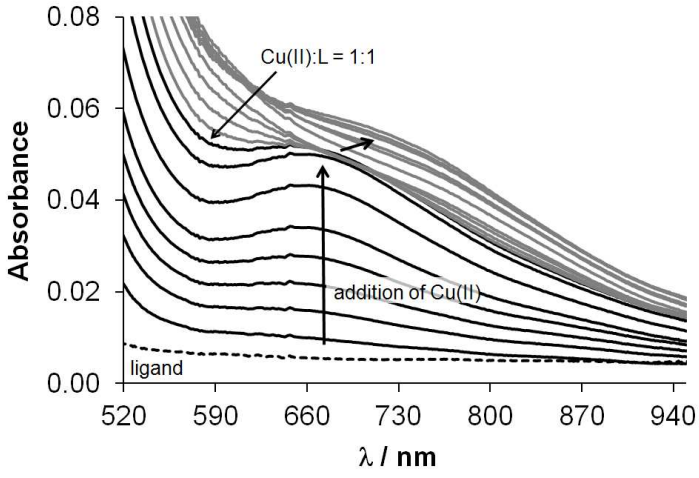



**Figure S2.** UV–vis absorbance spectrum of the ligand $^{EtOOC}$**HL**$^{COOEt}$ (dashed trace) and its changes by the addition of copper(II) (solid traces) in methanol ($c_L$ = 250 μM; $c_{Cu}$ = 0 – 562.5 μM; $T$ = 298 K; $l$ = 1 cm).

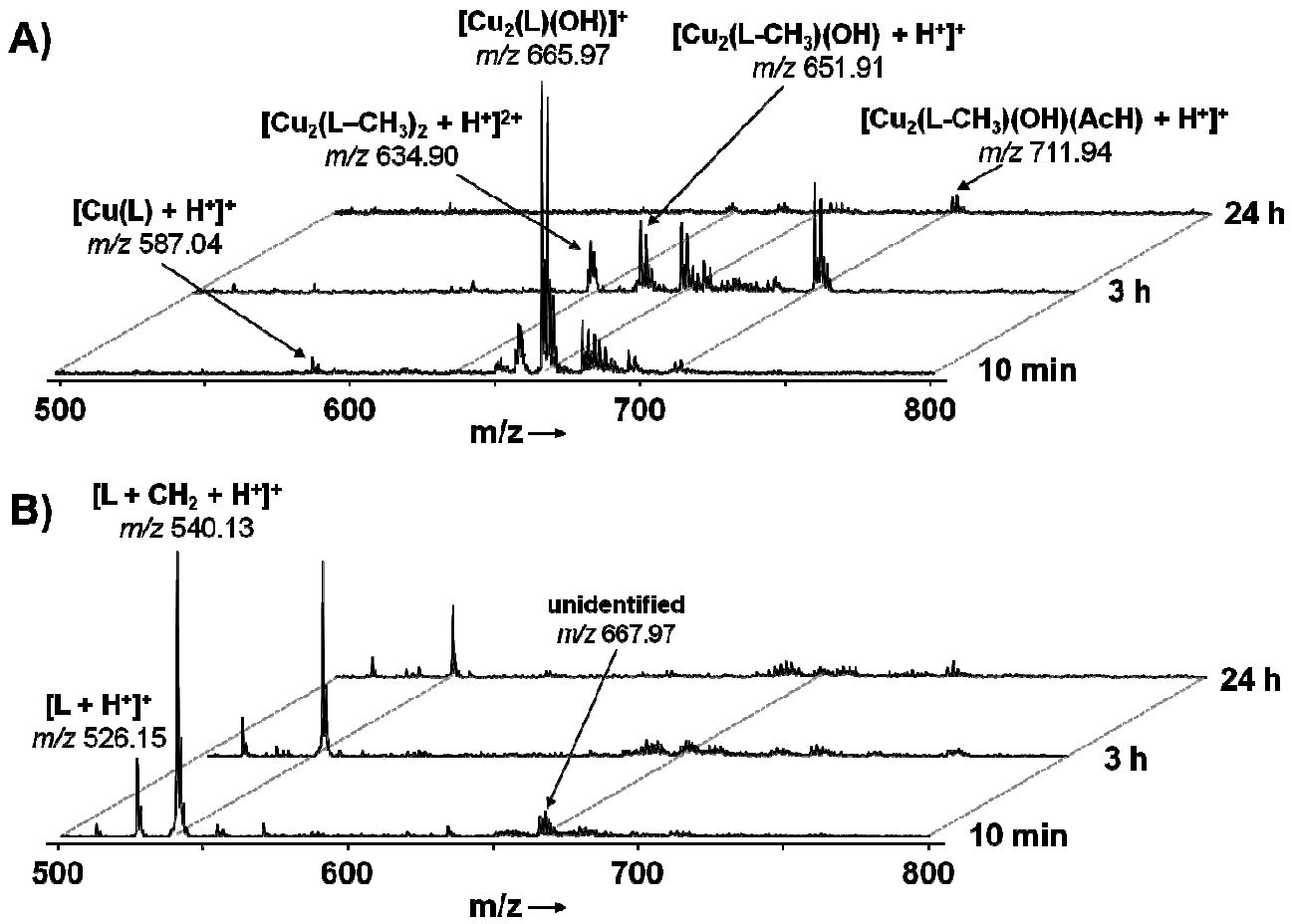



**Figure S3**. The ESI mass spectra in the positive ion mode of **1** (A) and **2** (B) are shown. Complex **2** does not ionize in the positive ion mode. Positive ion mode (HV –4.5 kV, RF level 89%, trap drive 74.4, dry temperature 250 °C, nebulizer 8 psi, dry gas 6 L/min and average accumulation time 144 μs), diluted with water : methanol (50 : 50).

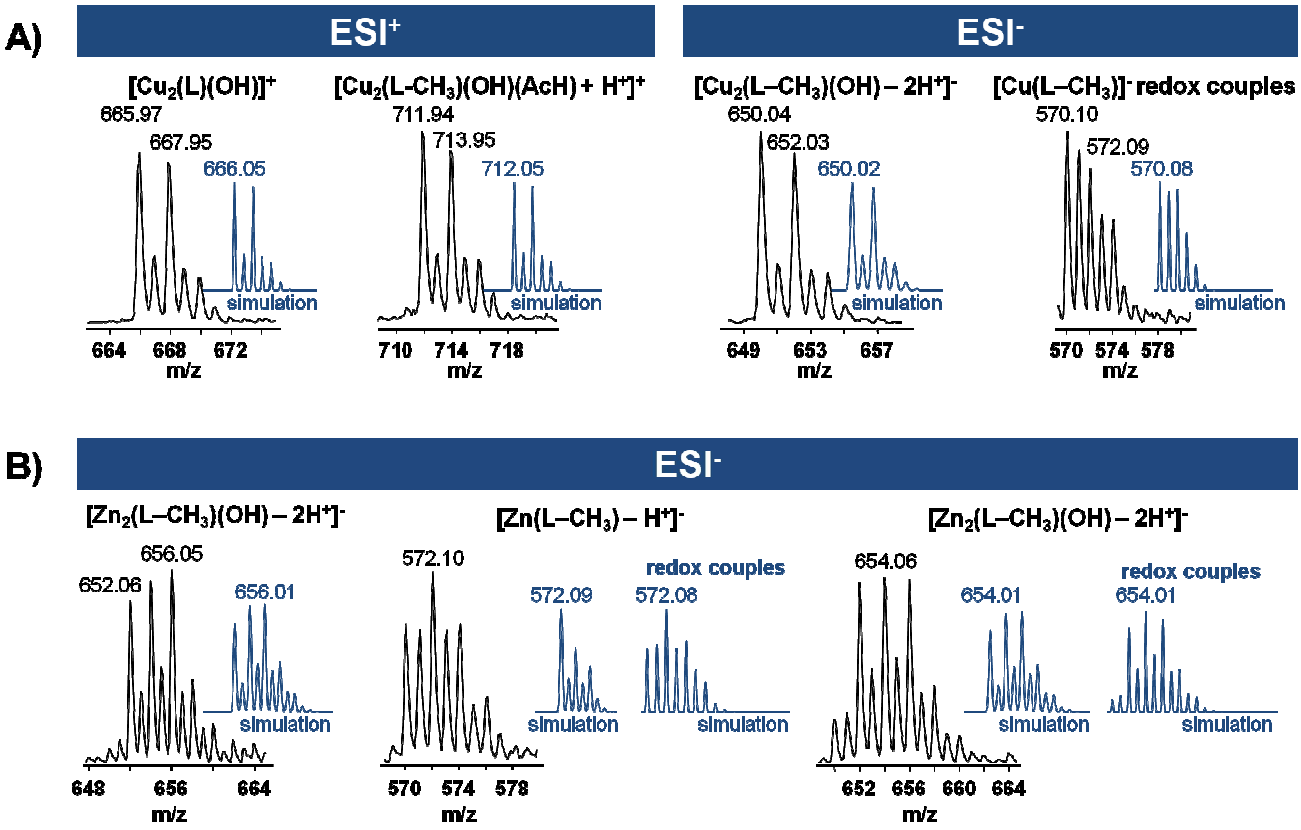



**Figure S4**. Details on isotopic distributions of the major detected mass signals of **1** (A) and **2** (B) and respective simulations (*grey*).

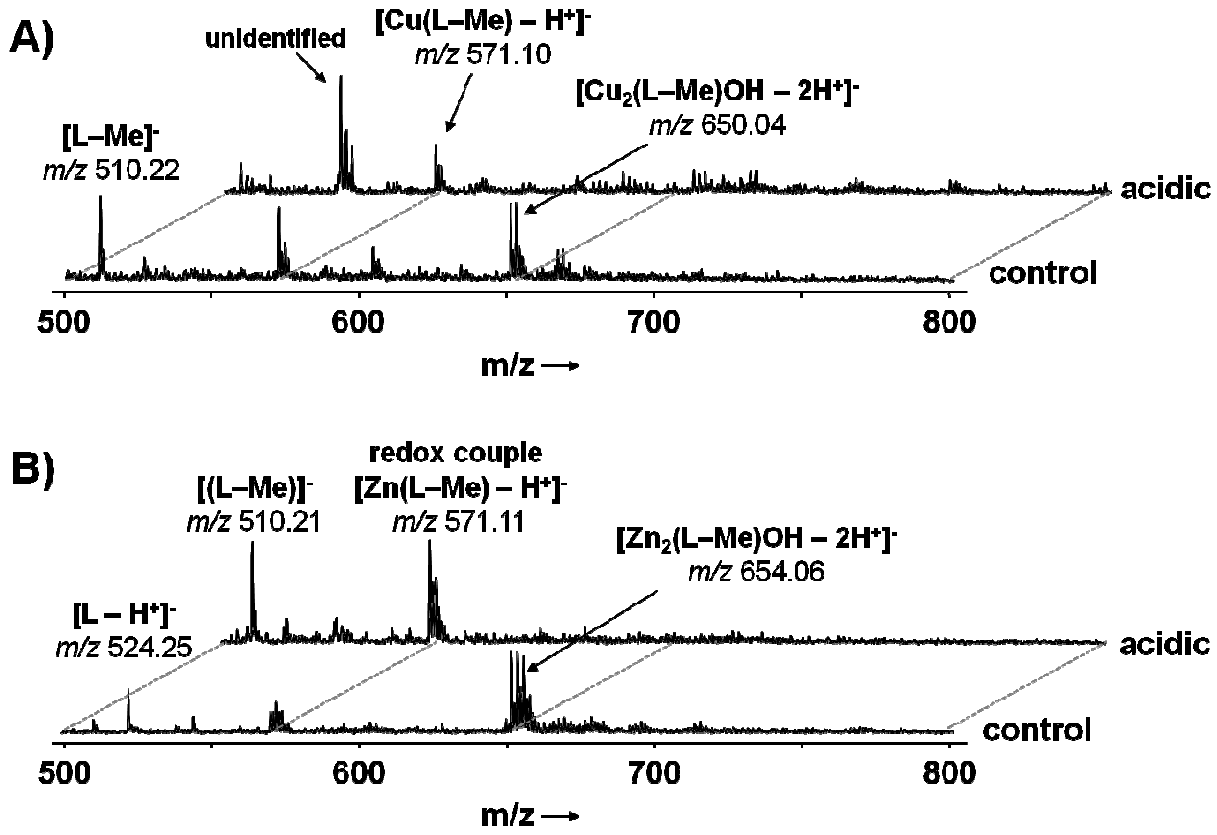



**Figure S5**. The impact of lowering the pH of the incubation solution is shown for **1** (A) and **2** (B). The control mass spectrum is recorded after 24 h of incubation, followed by acidification of solutions with formic acid and immediate measuring thereafter. Acidic conditions seem to lead to release of one metal-equivalent. Negative ion mode (HV 4.5 kV, RF level 89%, trap drive 63.8, dry temperature 250 °C, nebulizer 8 psi, dry gas 6 L/min and average accumulation time 2 ms), diluted with water : methanol : formic acid (50 : 50 : 0.2).

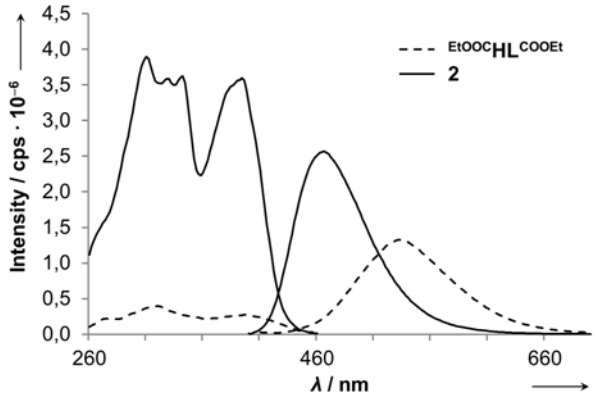



**Figure S6.** Fluorescence excitation ($\lambda_{em}$ = 470 nm) and emission ($\lambda_{ex}$ = 395 nm) spectra of 10 μM solutions of $^{\mathbf{EtOOC}}\mathbf{HL}^{\mathbf{COOEt}}$ (dashed traces) and **2** (solid traces) at physiological pH (20 mM HEPES buffer, 1% v/v DMSO).

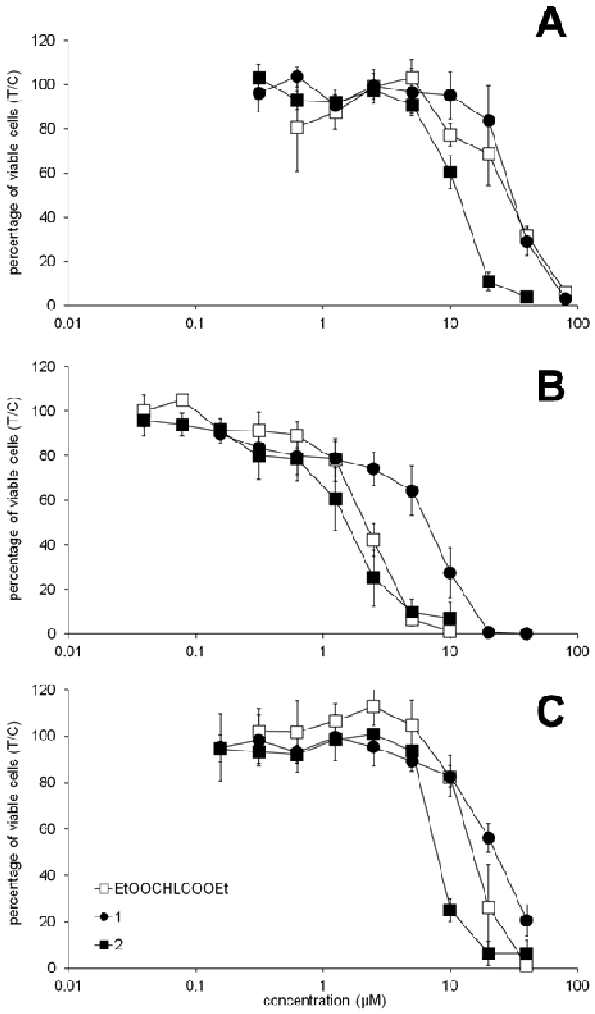



**Figure S7.** Concentration-effect curves of $^{EtOOC}HL^{COOEt}$, complexes **1** and **2**, in the human cancer cell lines A549 (A), CH1 (B) and SW480 (C), determined by the MTT assay using continuous exposure for 96 h.

**Table S1**. The experimental masses and their assignment.

| | ion | *m/z* | $m_{theor}$ |
|---|---|---|---|
| $ESI^+$ | $[(L–Me) + H^+]^+$ | 512.17 ± 0.04 | 512.19 |
| | $[L + H^+]^+$ | 526.21 ± 0.01 | 526.21 |
| | unidentified | 540.12 ± 0.01 | 540.22 |
| | $[Cu(L) + H^+]^+$ | 587.04 ± 0.03 | 587.12 |
| | $[Cu_2(L–Me)_2]^{2+}$ | 634.90 ± 0.02 | 635.02 |
| | $[Cu_2(L–Me)(OH) + H^+]^+$ | 651.92 ± 0.03 | 652.03 |
| | $[Cu_2(L)(OH)]^+$ | 665.97 ± 0.01 | 666.05 |
| | $[Cu_2(L)(CH_3O)]^+$ | 679.97 ± 0.02 | 680.06 |
| | $[Cu_2(L–Me)(CH_3O)(HOAc)+ H^+]^+$ | 711.92 ± 0.06 | 712.05 |
| $ESI^-$ | $[L–Me]^-$ | 510.22 ± 0.04 | 510.14 |
| | $[L – H^+]^-$ | 524.24 ± 0.01 | 524.19 |
| | unidentified | 538.25 ± 0.02 | 538.21 |
| | $[Cu(L–Me)]^-$ | 571.10 ± 0.06 | 571.09 |
| | $[Zn(L–Me)]^-$ | 572.13 ± 0.01 | 572.09 |
| | $[Cu(L) + H^+]^-$ | 585.15 ± 0.02 | 585.11 |
| | $[Cu_2(L–Me)(OH)^+]^-$ | 650.04 ± 0.02 | 650.02 |
| | $[Zn_2(L) – H^+]^-$ | 654.06 | 654.03 |
| | $[Zn_2(L–Me)(OH)]^-$ | 656.04 ± 0.01 | 656.01 |
| | $[Cu_2(L)(OH) – 2H^+]^-$ | 664.05 | 664.03 |
| | $[Zn_2(L) – OH]^-$ | 668.05 | 668.03 |
| | $[Zn_2(L–Me)(OAc)]^-$ | 696.03 | 696.01 |