Electronic Supplementary Information for

Nuclease and anti-proliferative activities of copper(II) complexes of N_3O tripodal ligands involving a sterically hindered phenolate

Nathalie Berthet, Véronique Frachet-Martel, Fabien Michel, Christian Philouze, Sylvain Hamman, Xavier Ronot, and Fabrice Thomas

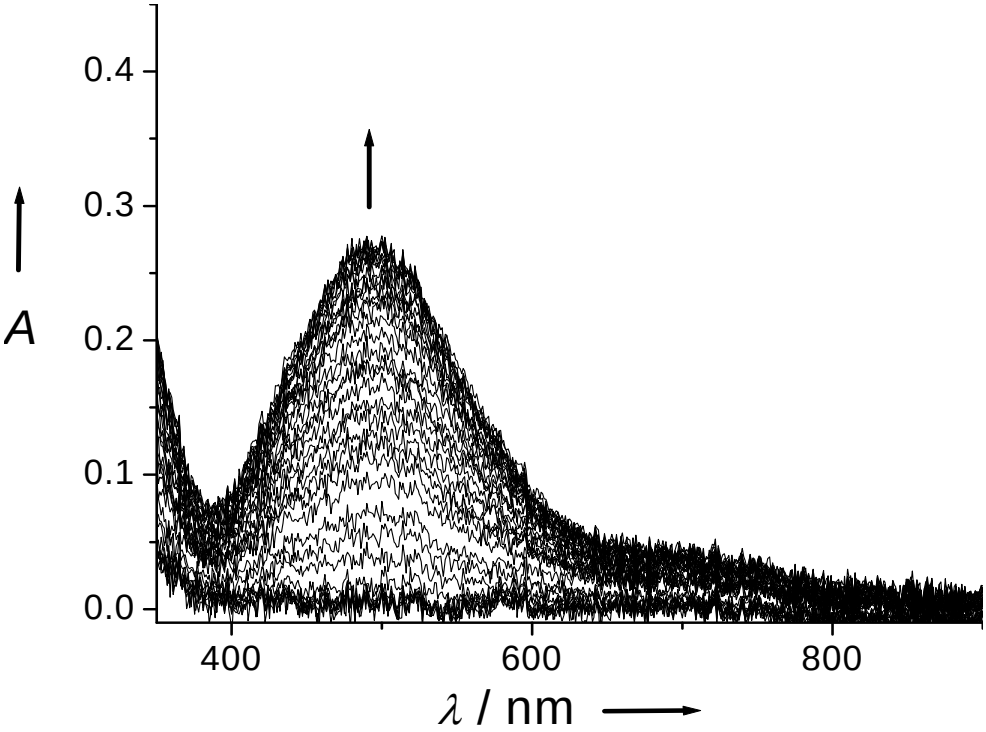

Fig. S1 Evolution of the UV-Vis spectra as a function of pH of a 0.23 mM solution of **3** in a water:DMF 90:10 mixture ([$NaClO_4$] = 0.1 M): T = 298 K, l = 1.000 cm. Arrows indicate spectral changes upon increase of the pH from 3.63 to 7.77.

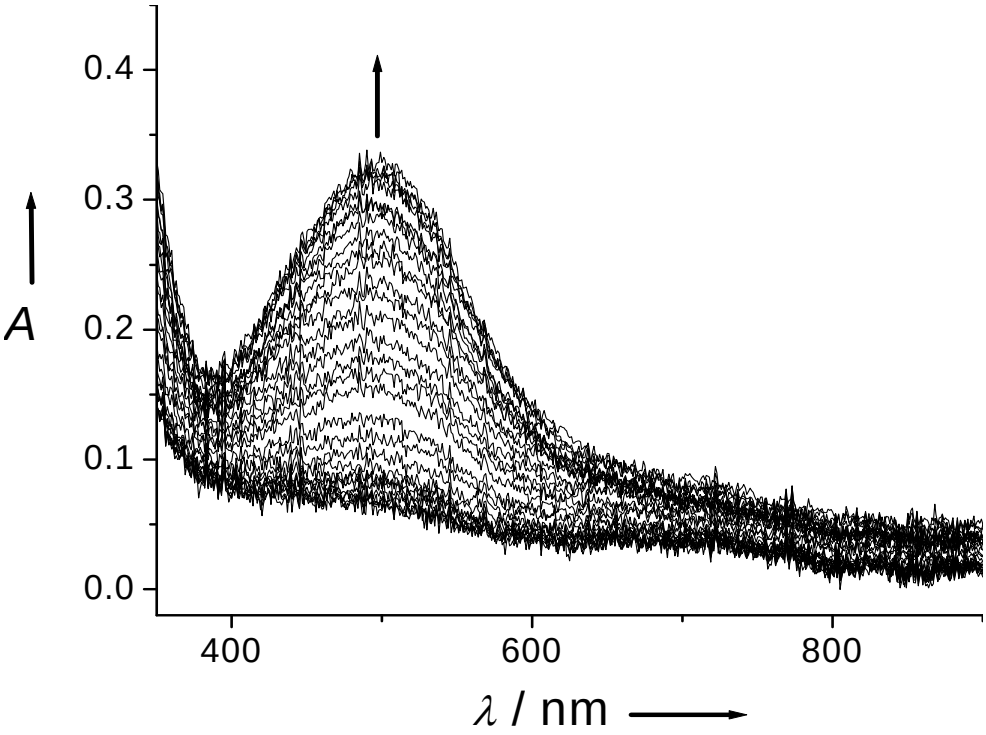

Fig. S2 Evolution of the UV-Vis spectra as a function of pH of a 0.3 mM solution of **4** in a water:DMF 90:10 mixture ([$NaClO_4$] = 0.1 M): T = 298 K, l = 1.000 cm. Arrows indicate spectral changes upon increase of the pH from 3.38 to 7.64.

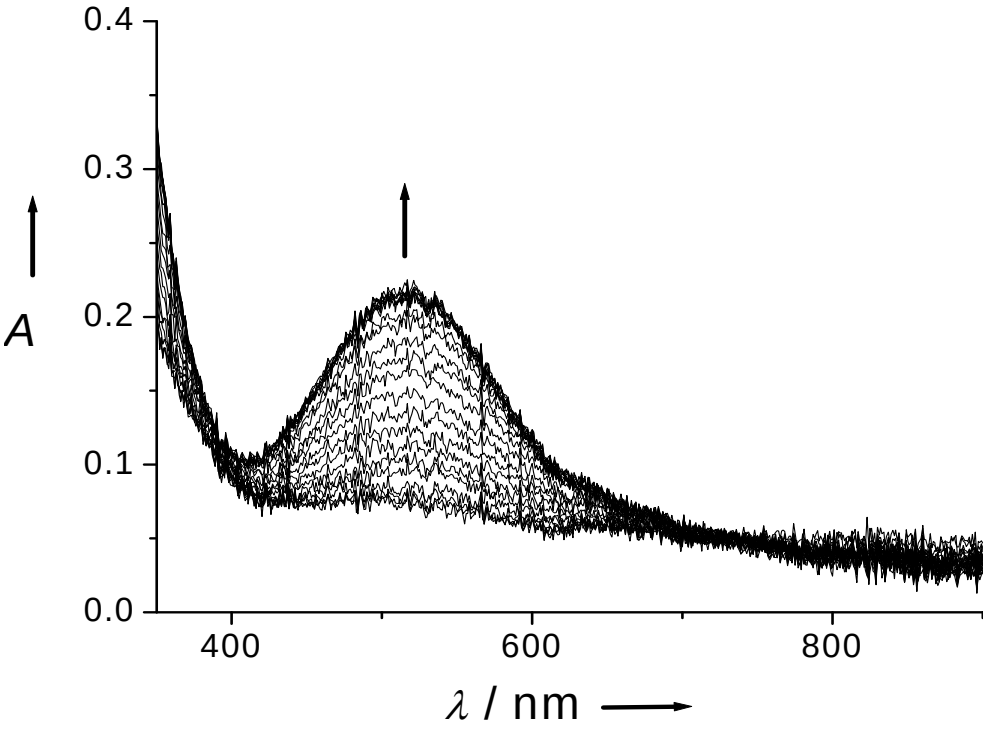

Fig. S3 Evolution of the UV-Vis spectra as a function of pH of a 0.23 mM solution of **6** in a water:DMF 90:10 mixture ([$NaClO_4$] = 0.1 M): T = 298 K, l = 1.000 cm. Arrows indicate spectral changes upon increase of the pH from 3.85 to 8.18.

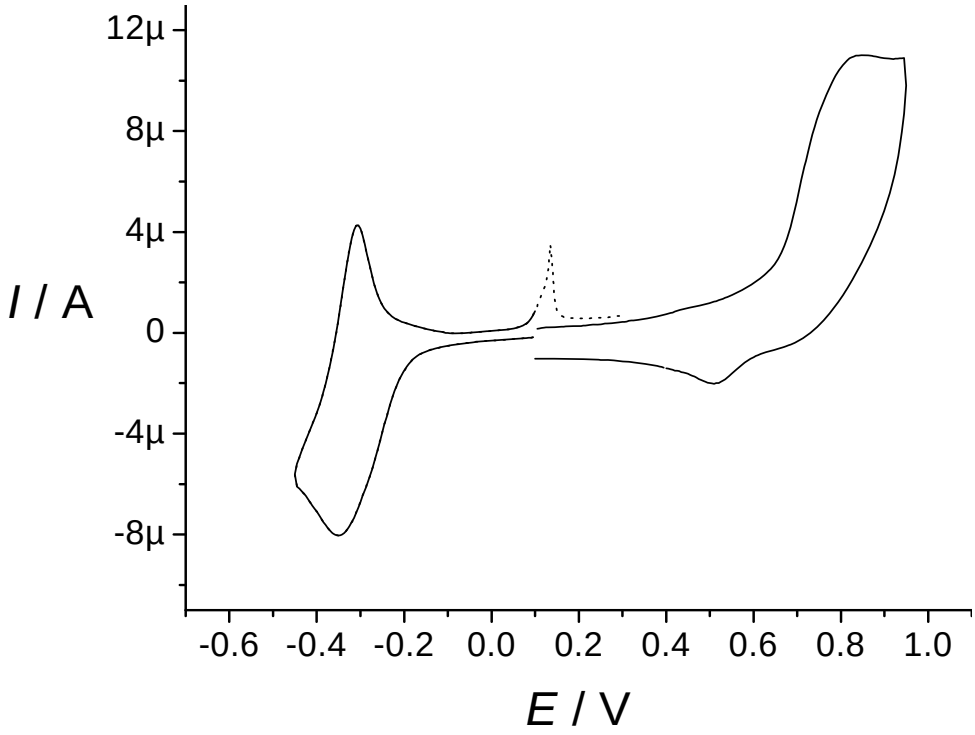

Fig. S4 Cyclic voltammetry curve of a 0.5 mM solution (+ 0.1 M $NaClO_4$) of **1** in a water:DMF 90:10 mixture at pH 3.7. Scan rate = 0.1 V/s. Potential are given *vs.* the SCE reference.

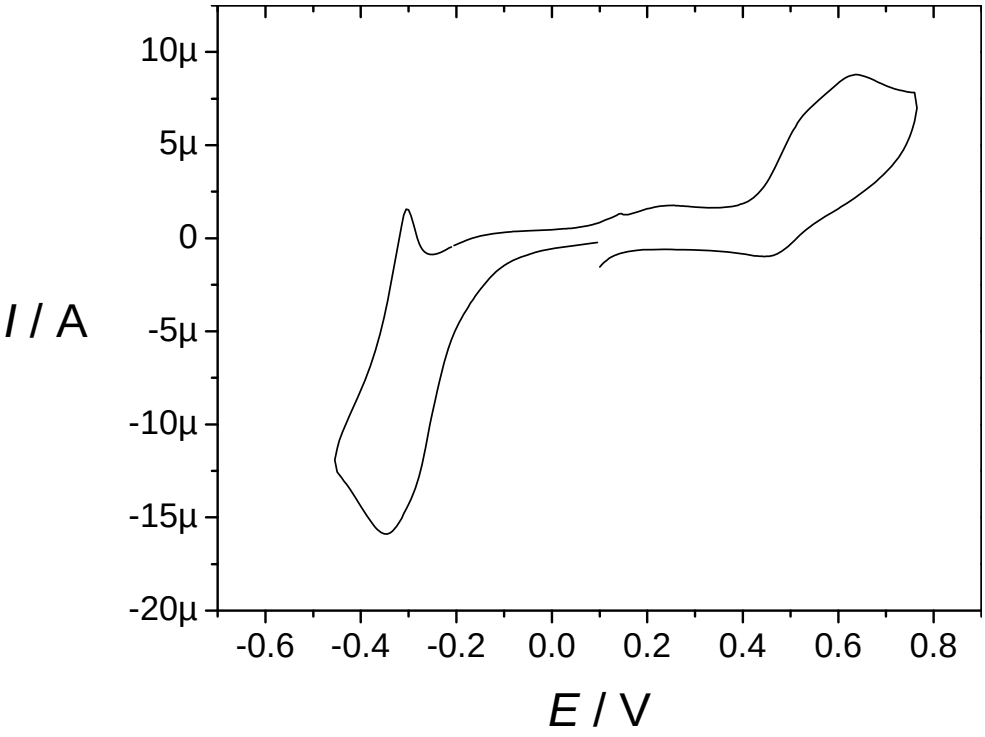

Fig. S5 Cyclic voltammetry curve of a 0.5 mM solution (+ 0.1 M $NaClO_4$) of **2** in a water:DMF 90:10 mixture at pH 3.7. Scan rate = 0.1 V/s. Potential are given *vs.* the SCE reference.

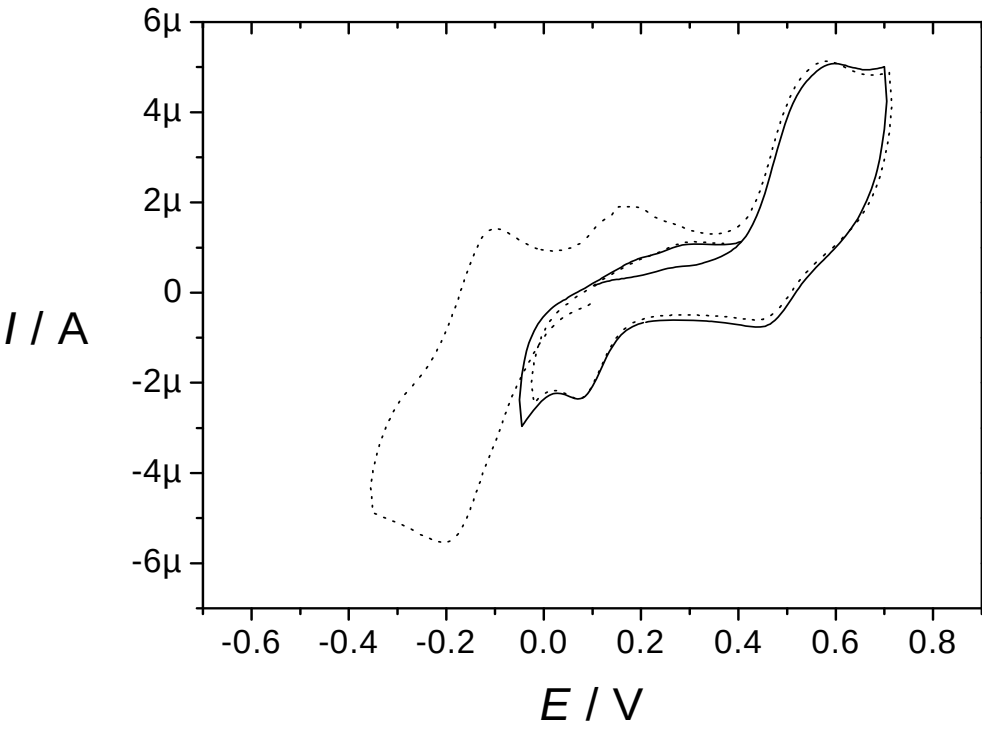

Fig. S6 Cyclic voltammetry curve of a 0.5 mM solution (+ 0.1 M $NaClO_4$) of **3** in a water:DMF 90:10 mixture at pH 3.3. Scan rate = 0.1 V/s (solid line: anodic scan, dotted line: cathodic scan). Potential are given *vs.* the SCE reference.

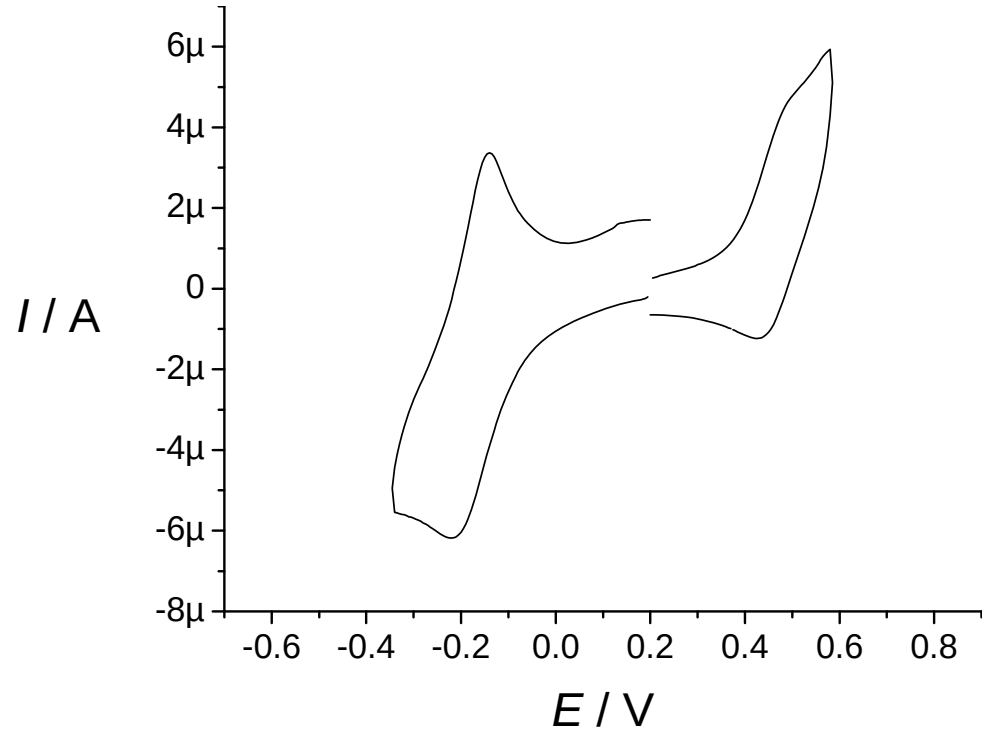

Fig. S7 Cyclic voltammetry curve of a 0.5 mM solution (+ 0.1 M $NaClO_4$) of **4** in a water:DMF 90:10 mixture at pH 3.5. Scan rate = 0.1 V/s. Potential are given *vs.* the SCE reference.

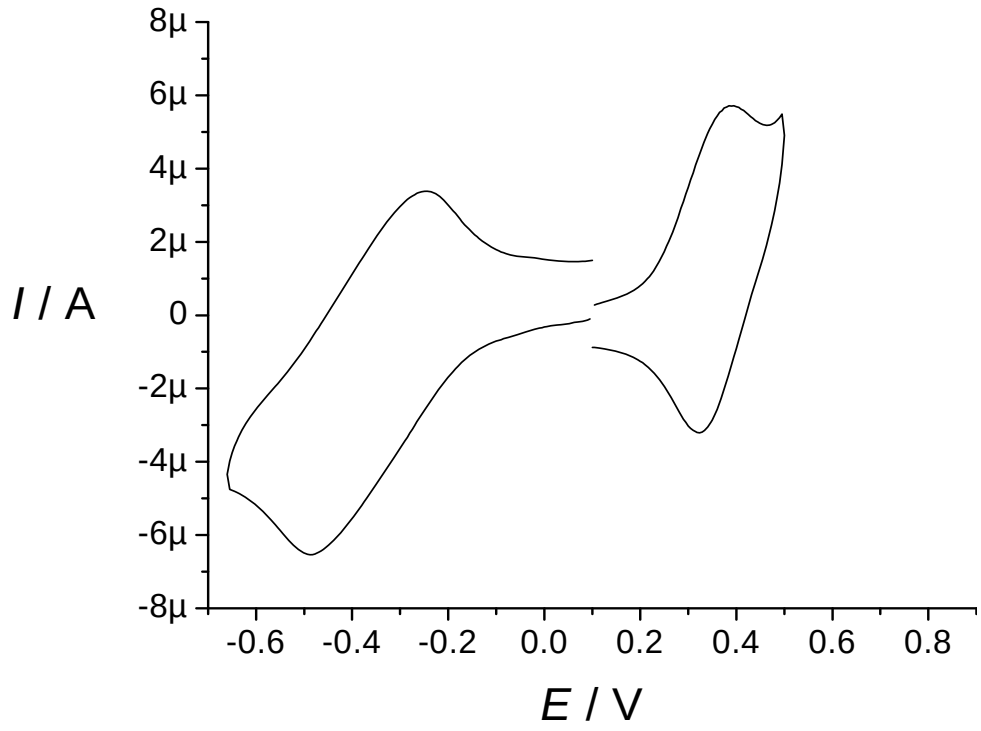

Fig. S8 Cyclic voltammetry curve of a 0.5 mM solution (+ 0.1 M $NaClO_4$) of **4** in a water:DMF 90:10 mixture at pH 7.3. Scan rate = 0.1 V/s. Potential are given *vs.* the SCE reference.

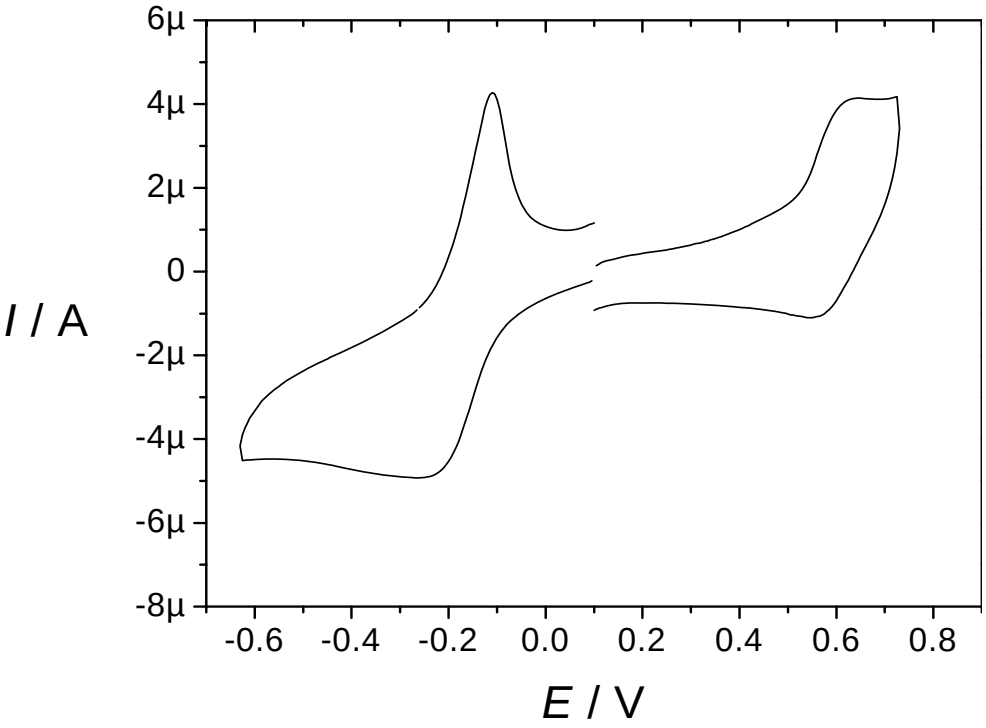

Fig. S9 Cyclic voltammetry curve of a 0.5 mM solution (+ 0.1 M $NaClO_4$) of **5** in a water:DMF 90:10 mixture at pH 3.5. Scan rate = 0.1 V/s. Potential are given *vs.* the SCE reference.

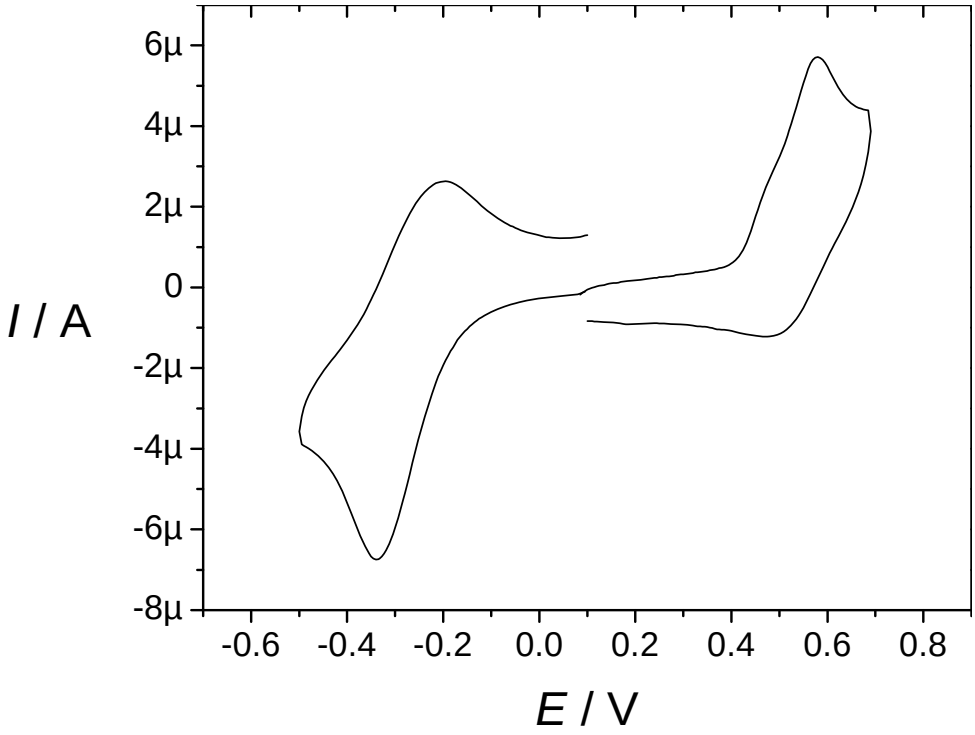

Fig. S10 Cyclic voltammetry curve of a 0.5 mM solution (+ 0.05 M Tris buffer containing 0.02 M NaCl) of **5** in a water:DMF 90:10 mixture at pH = 7.3. Scan rate = 0.1 V/s. Potential are given *vs.* the SCE reference.

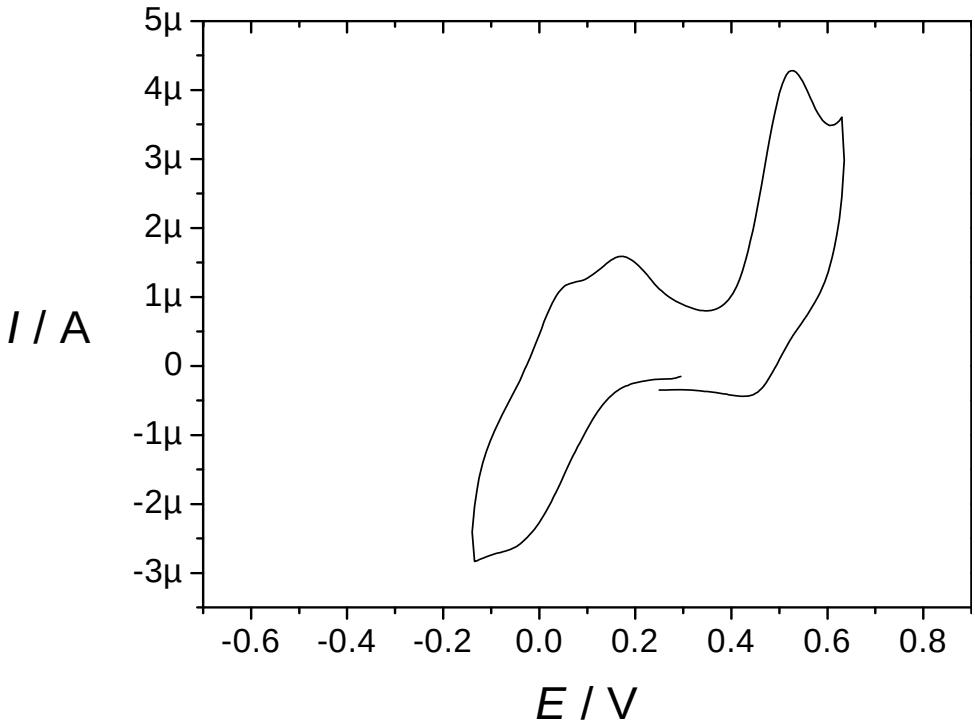

Fig. S11 Cyclic voltammetry curve of a 0.5 mM solution (+ 0.1 M $NaClO_4$) of **6** in a water:DMF 90:10 mixture at pH 3.5. Scan rate = 0.1 V/s. Potential are given *vs.* the SCE reference.

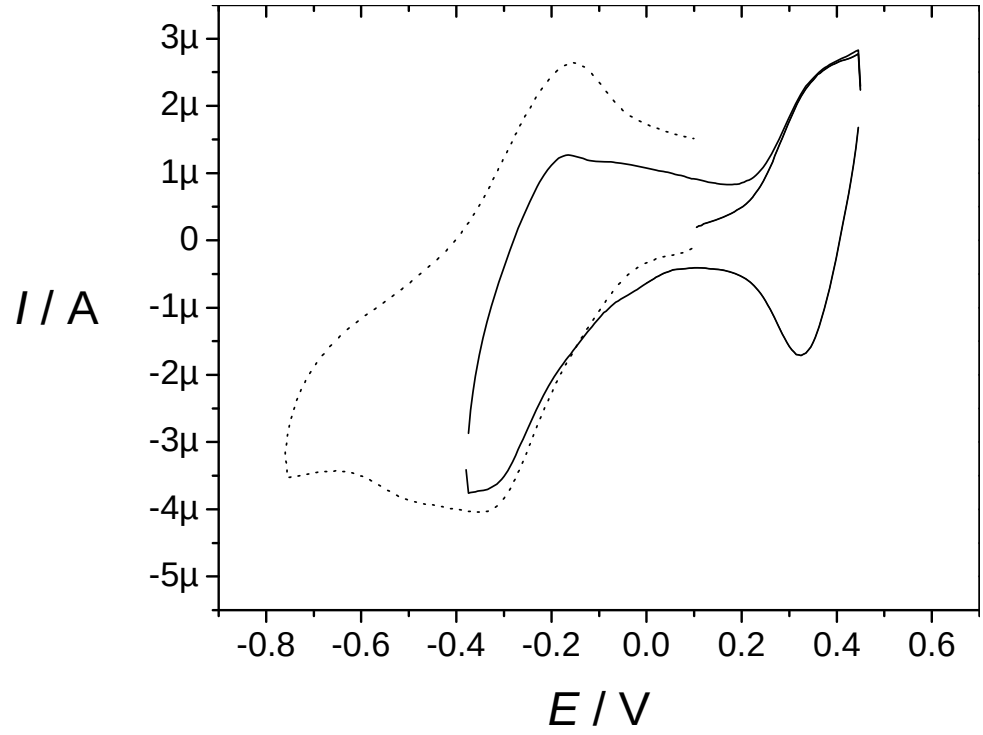

Fig. S12 Cyclic voltammetry curve of a 0.5 mM solution (+ 0.1 M $NaClO_4$) of **6** in a water:DMF 90:10 mixture at pH 7.3. Scan rate = 0.1 V/s (solid line: anodic scan, dotted line: cathodic scan). Potential are given *vs.* the SCE reference.

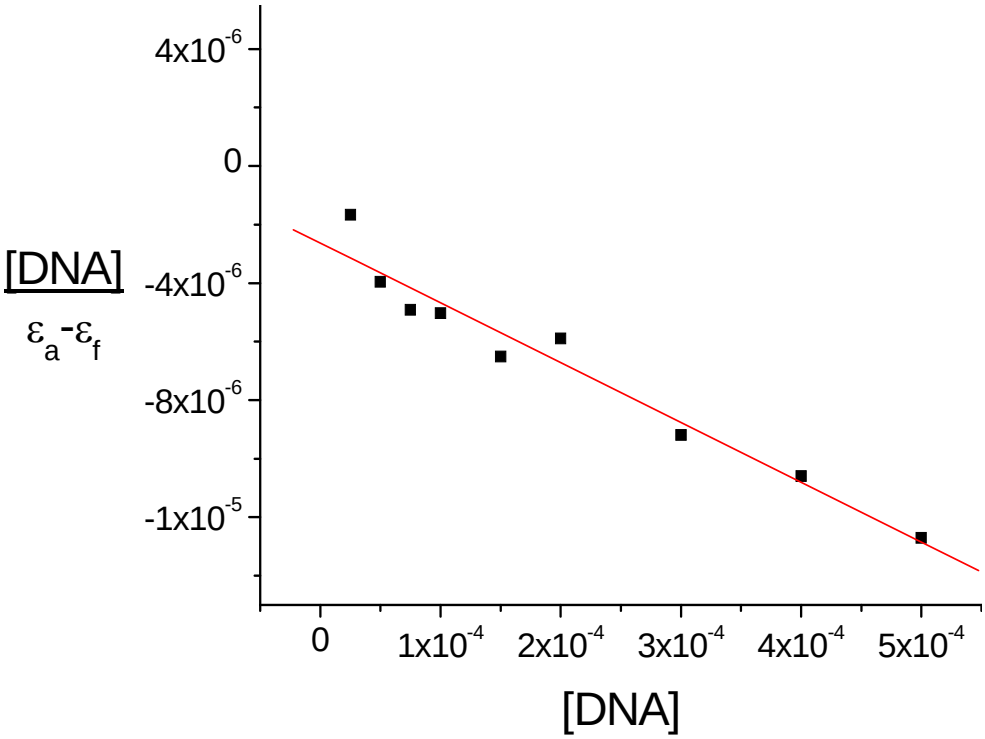

Fig. S13 Plot of [DNA]/(ε_a-ε_f) as a function of [DNA] for **1**

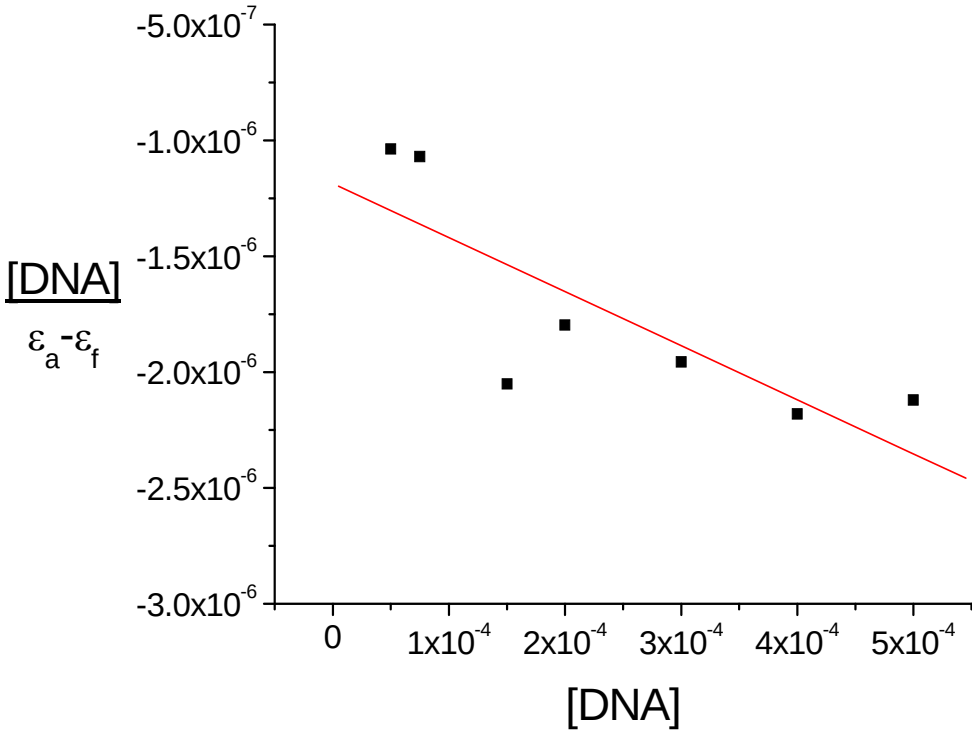

Fig. S14 Plot of [DNA]/(ε_a-ε_f) as a function of [DNA] for **3**

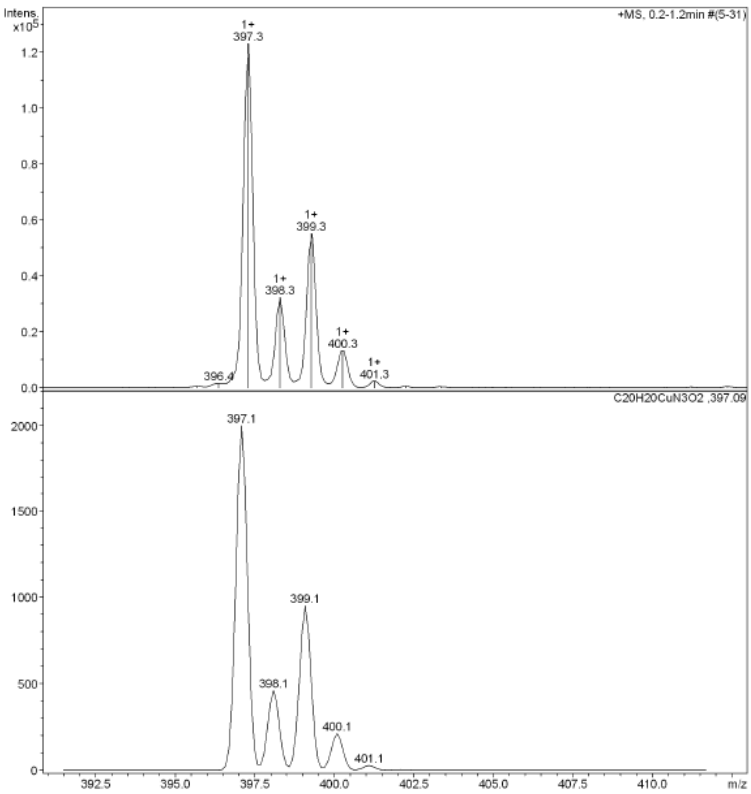

Fig. S15 ESI-MS spectra of $\mathbf{1}^{2+}$ in water:DMF 90:10 medium: experimental spectrum (top) and simulation by considering the formula $C_{20}H_{20}CuN_3O_2$ ($(\mathbf{1}^{2+})_{0.5}$, bottom).

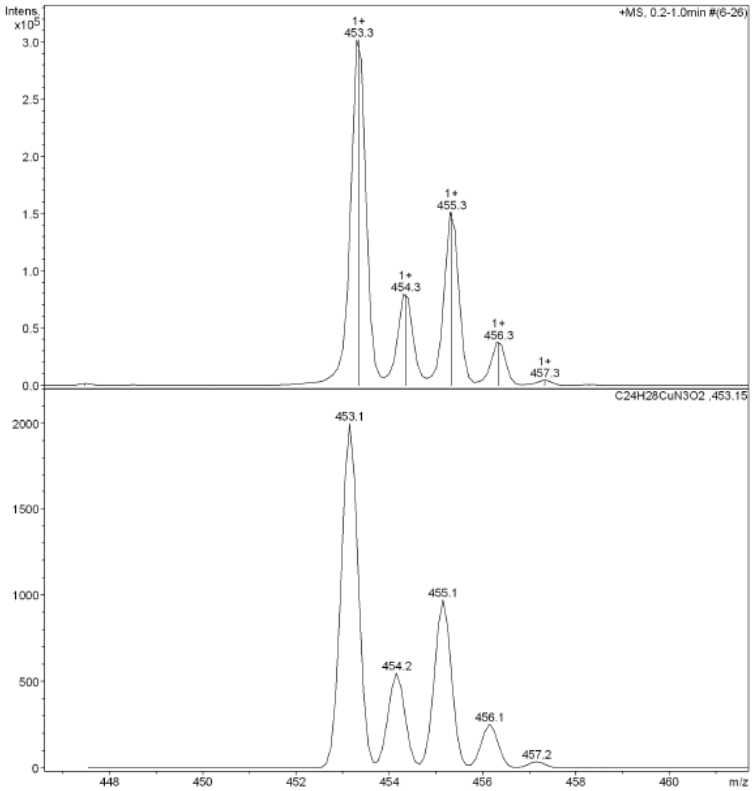

Fig. S16 ESI-MS spectra of $\mathbf{2}^{+}$ in water:DMF 90:10 medium: experimental spectrum (top) and simulation by considering the formula $C_{24}H_{28}CuN_3O_2$ ($\mathbf{2}^{+}$-H-CH_3CN-ClO_4, bottom).

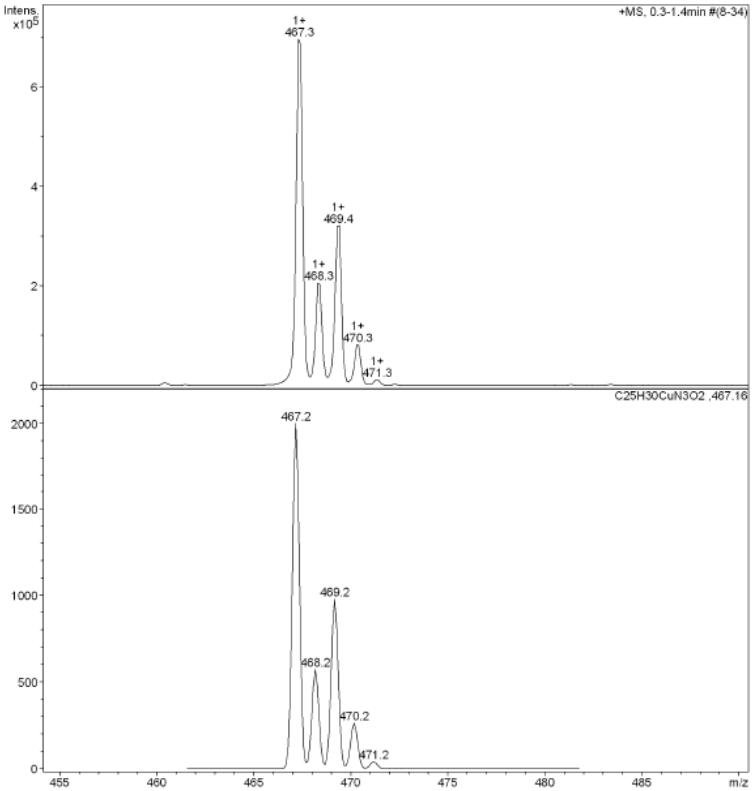

Fig. S17 ESI-MS spectra of $\mathbf{3}^{+}$ in water:DMF 90:10 medium: experimental spectrum (top) and simulation by considering the formula $C_{24}H_{28}CuN_3O_2$ ($\mathbf{3}^{+}$-H-CH_3CN-ClO_4, bottom).

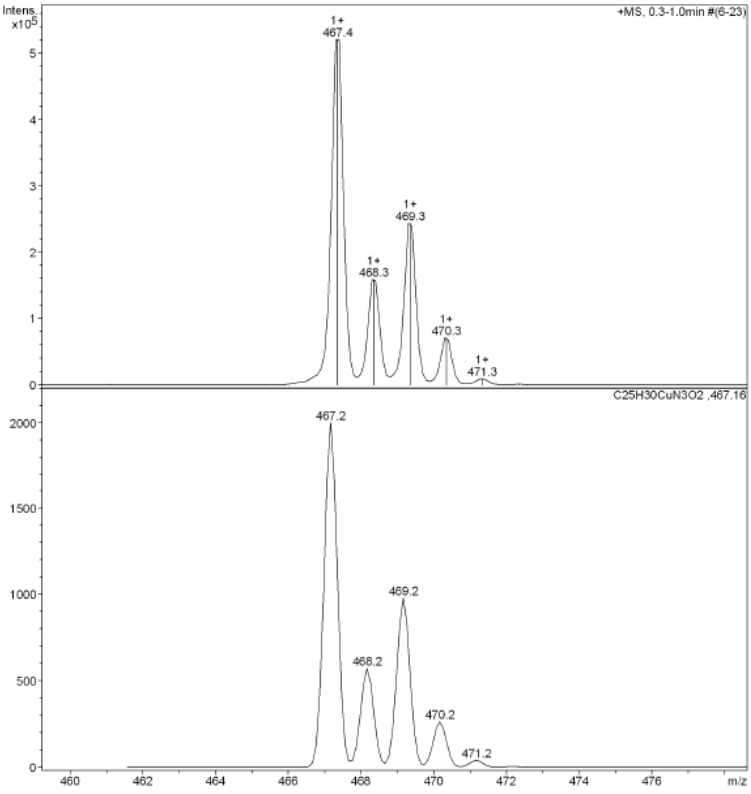

Fig. S18 ESI-MS spectra of $\mathbf{4}^{+}$ in water:DMF 90:10 medium: experimental spectrum (top) and simulation by considering the formula $C_{25}H_{30}CuN_3O_2$ ($\mathbf{4}^{+}$-H-CH_3CN-ClO_4, bottom).

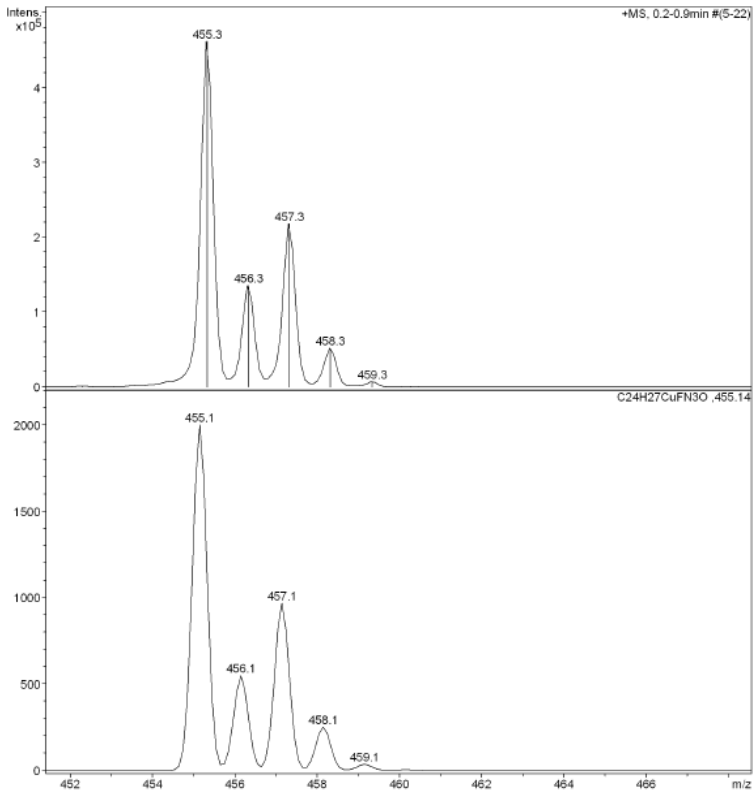

Fig. S19 ESI-MS spectra of $\mathbf{5}^{+}$ in water:DMF 90:10 medium: experimental spectrum (top) and simulation by considering the formula $C_{24}H_{27}CuFN_3O$ ($\mathbf{5}^{+}$-H-CH_3CN-ClO_4, bottom).

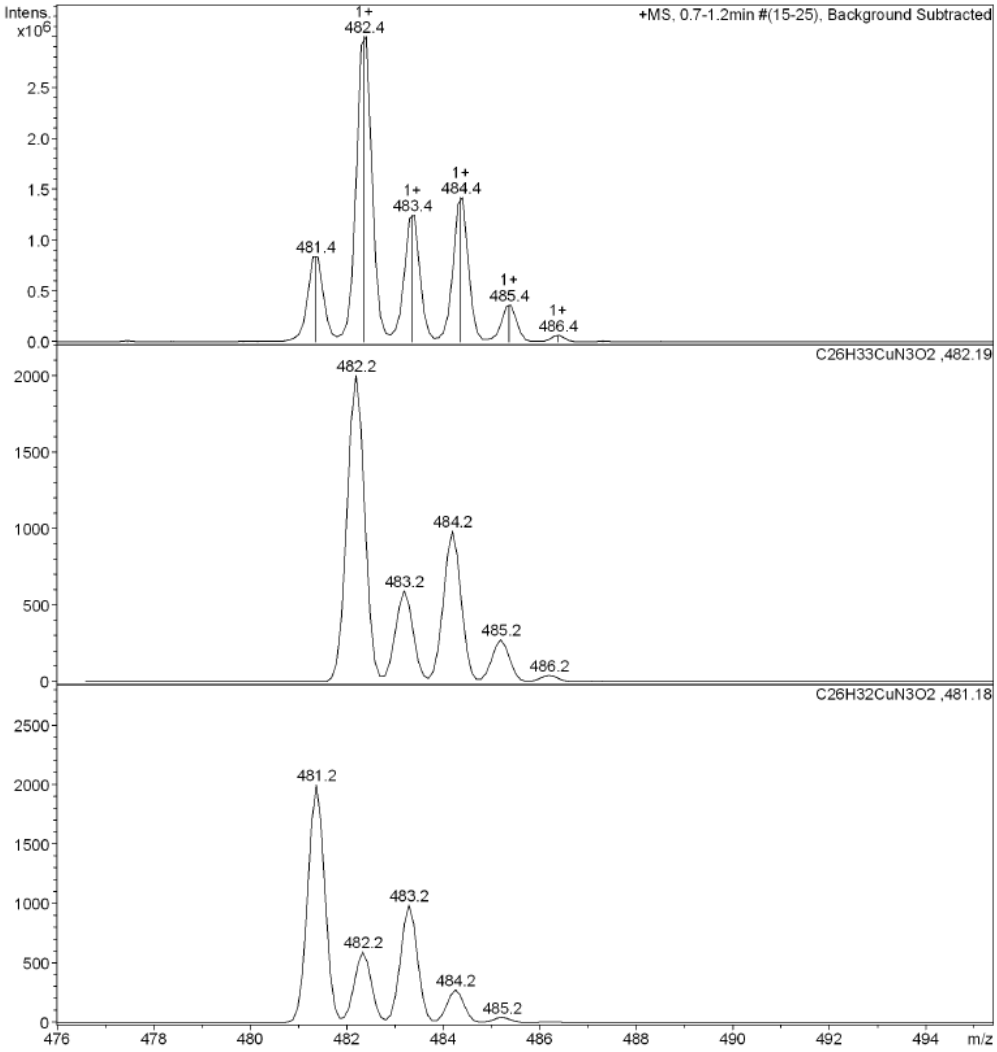

Fig. S20 ESI-MS spectra of $\mathbf{6}^+$ in water:DMF 90:10 medium: experimental spectrum (top) and simulations by considering the formula $C_{24}H_{27}CuFN_3O$ ($\mathbf{6}^+$-DMF, bottom) and $C_{24}H_{28}CuFN_3O$ ($\mathbf{6}^+$+H-DMF, center).*

*: Presence of the copper(I) form of the complex results from reduction of $\mathbf{6}^+$ in the gas phase. This is confirmed by the UV/Vis spectra of the sample before and after ESI-MS experiment, which systematically show the phenolate-to-copper(II) charge transfer band. This attests that reduction does not occur in solution, but in the gas phase. It is noteworthy that it is only in the case of $\mathbf{6}^+$ that a significant amount of Cu(I) species is produced in the gas phase. This fact correlates nicely with the solution reduction potential of $\mathbf{6}^+$, which is the highest in the series. Such behaviour has been previously reported for some copper complexes (see L. Gianelli, V. Amendola, L. Fabbrizzi, P. Pallavicini, G. G. Mellerio, *Rapid Commun. Mass Spectrom.* **2001**, *15*, 2347).

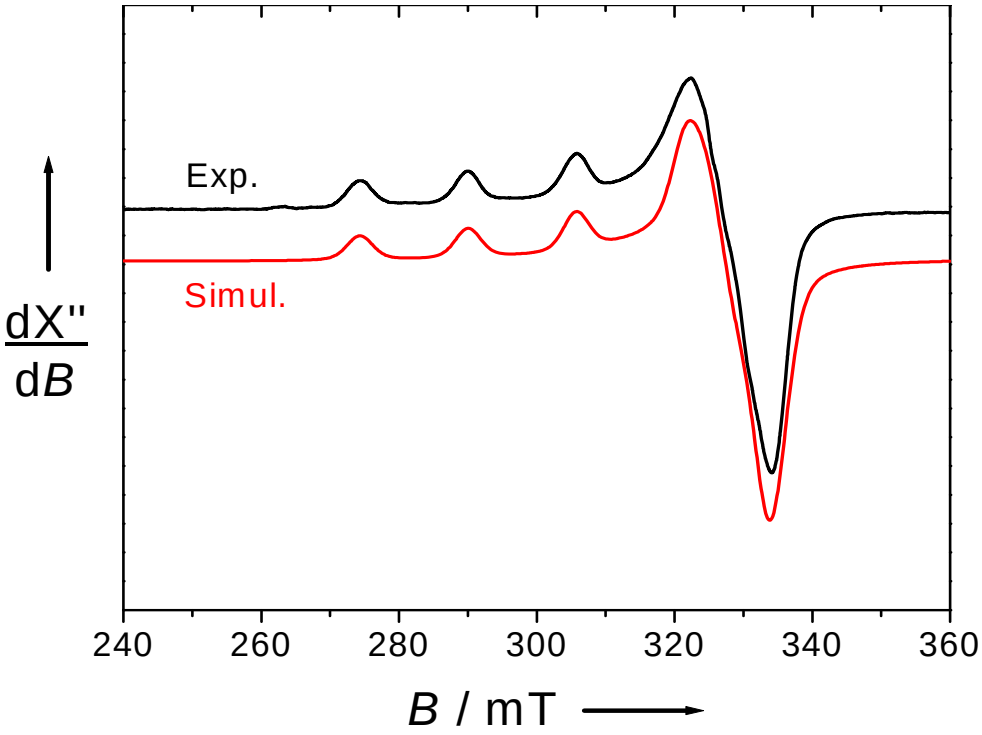

Fig. S21 X-Band EPR spectrum of 0.5 mM solutions of $\mathbf{6}^{+}$ generated *in situ* in DMF. The black line represents the experimental spectrum, the red line a simulation using parameters given in the text. T = 100 K, microwave frequency 9.44 GHz, power 10 mW, modulation frequency 100 KHz, amplitude 0.3 mT.

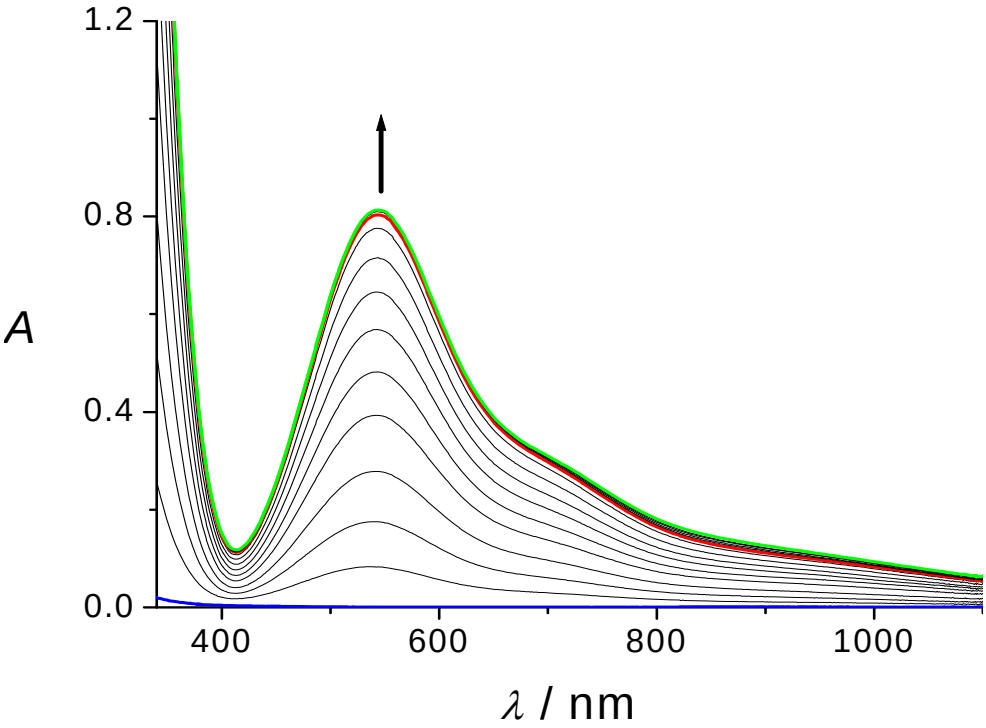

Fig. S22 UV-Vis titration of a 0.83 mM solution of 6L in DMF by $Cu(ClO_4)_2 \cdot 6\ H_2O$. Blue line: Before addition of copper; Red line: After 1 molar equivalent of copper added; Green line: After 1.5 molar equivalent of copper added. T = 298 K, l = 1.000 cm.

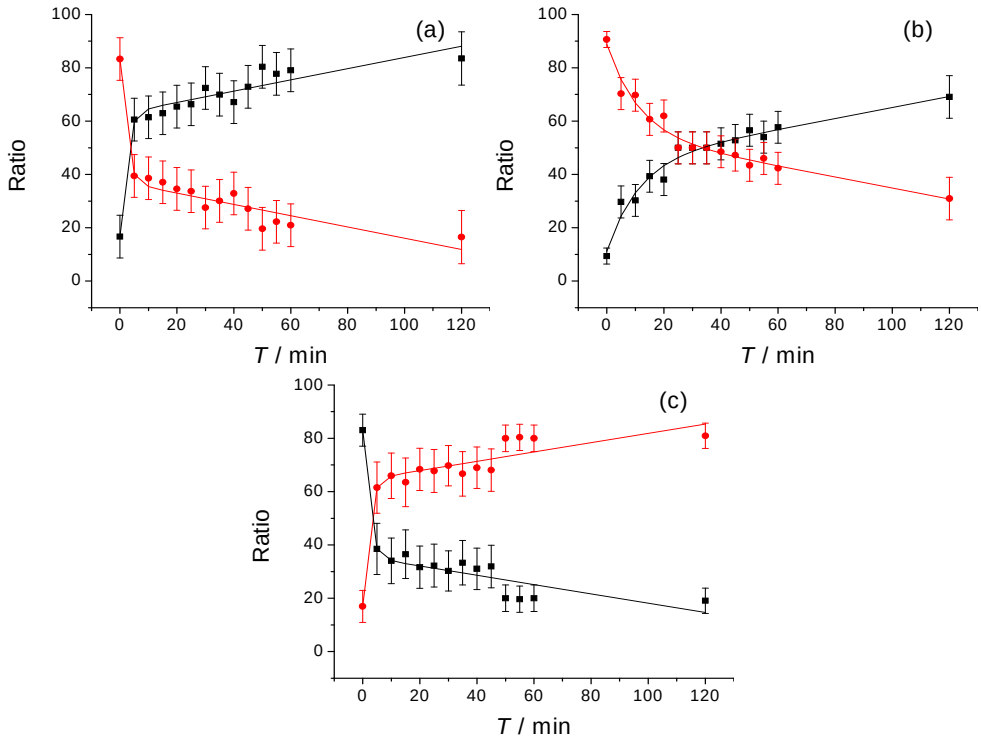

Figure S23 Ratio of nicked circular (red) and supercoiled (black) form of DNA as a function of time from agarose gel electrophoresis (and best fit using the rate constants given in the text). Supercoiled ϕX174 DNA (20 μM base pairs) was incubated for different times at 37°C with 300 μM of the copper complexes in a phosphate buffer 10 mM pH 7.2 (+ 10 % DMF): (a) $\mathbf{4}^{+}$, (b) $\mathbf{5}^{+}$, (c) $\mathbf{6}^{+}$.