Electronic Supplementary Information (ESI)

Camphor based 1,3-diamine Ru(II) terpyridine complex: Synthesis, characterization, kinetic investigation and DNA binding

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Experimental part:

General Experimental. All commercially available chemicals were used without further purification. Silica gel 60 (0.063-0.040 mm particle size) was used for column chromatography. Thin layer chromatography was performed on plates from Merck (Silica gel 60, F254). Substances were detected under UV-light at 254 nm. NMR spectra were recorded at 30 °C on a Bruker Avance 500 spectrometer (^{1}H-NMR: 700 MHz; ^{13}C-NMR: 175 MHz; ^{15}N-NMR: 70 MHz). The NMR signals are referenced to the residual proton or carbon signals of the deuterated solvent (^{1}H- and ^{13}C-NMR) and are reported in ppm relative to TMS. Liquid ammonia was used as an external reference for ^{15}N-NMR. Mass spectrometry was carried out on Waters Quadrupole-ToF Synapt 2G using electrospray ionization (ESI). Synthesis of the ligand is prepared according to the literature procedure.[S1]

Preparation of the ligand and complex

Ruthenium(II) terprydine complex **1** was synthesized by a literature method described earlier.[S2] $RuCl_3$ x 3 H_2O (1 mmol) was dissolved in 139.0 mL of ethanol and the solution was refluxed until the color of the solution changed from brown to green (*ca.* 2h). Terpyridine, tpy, (1 mmol) was added and reflux continued for 5 h where the color of the solution turned again to brown with a formation of the product as a brown solid. The brown solid is [Ru(tpy)Cl_3] complex **A** which was used without further purification. **A** (0.181 mmol), *N*-*N* ligand (0.218 mmol) **B**, LiCl (1,814 mmol) and Et_3N (0.544 mmol) were mixed in a 20 mL solution of EtOH/H_2O which afforded ruthenium(II) complex **1** as a dark purple liquid. The product was purified via column chromatography on silica gel using dichloromethane/methanol (75:25, *v/v*) as eluent. The purple fraction was collected and the solvent removed to give a purple solid of complex **1** (yield 68%, 0.123 mmol) (Scheme 1).

^{1}H-NMR (CD_3OD) δ [ppm] = 9.35 (d, *J* = 5 Hz, 1H, Dia2), 9.29 (d, *J* = 5 Hz, 1H, Dia1), 9.18 (d, *J* = 5 Hz, 1H, Dia2), 9.12 (d, *J* = 5 Hz, 1H, Dia1), 8.43-8.37 (m, 8H, Dia1+Dia2), 7.98-7.93 (m, 4H, Dia1+Dia2), 7.77-7.73 (m, 2H, Dia1+Dia2), 7.63-7.60 (m, 4H, Dia1+Dia2), 4.48 (s, br, NH), 3.80-3.78 (m, 1H, Dia1), 2.48-2.43 (m, 1H, Dia2), 2.40-2.39 (m, 1H, Dia2), 2.26-2.23 (m, 2H, Dia1), 2.07-2.02 (m, 1H, Dia2), 1.75 (s, 3H, Dia2), 1.70-1.67 (m, 1H, Dia2), 1.51-1.46 (m, 1H, Dia1), 0.93 (s, 3H, Dia1), 0.91 (s, 3H, Dia2), 0.90 (s, 3H, Dia1), 0.86 (s, 3H, Dia2), 0.83 (s, 3H, Dia1), 0.74-0.71 (m, 1H, Dia1), 0.47-0.42 (m, 1H, Dia2)

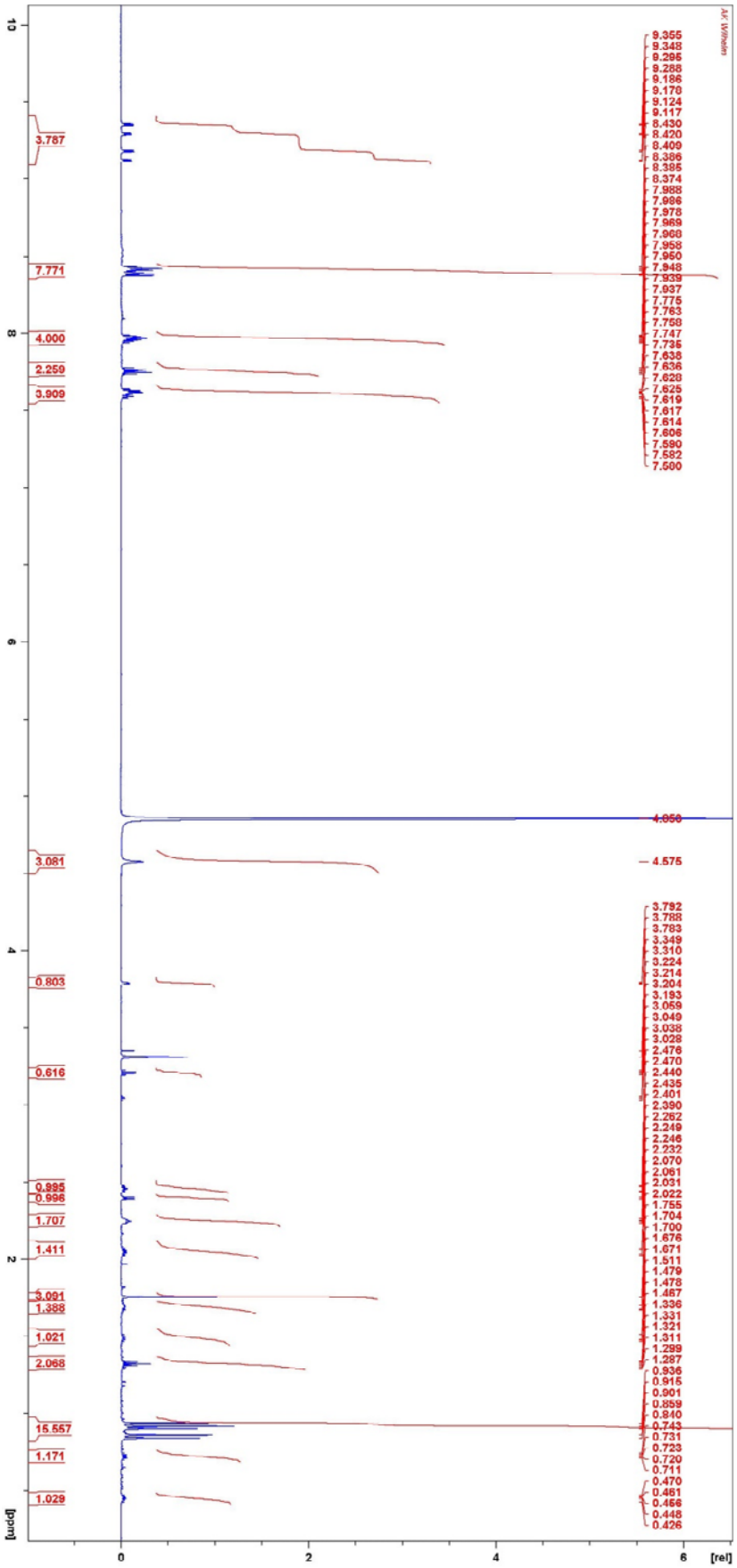
^{13}C-NMR (CD_3OD) δ [ppm] = 162.6 (q), 162.4 (q), 162.3 (q), 162.2 (q), 162.1 (q), 162.0 (q), 161.8 (q), 161.5 (q), 155.7 (+), 155.4 (+), 155.2 (+), 137.4 (+), 137.2 (+), 137.1 (+), 137.0 (+), 131.3 (+), 131.2 (+), 127.4 (+), 127.3 (+), 127.1 (+), 123.7 (+), 123.6 (+), 123.5 (+), 122.9 (+), 122.8 (+), 122.3 (+), 122.2 (+), 65.6 (+), 65.5 (q), 64.5 (q), 63.8 (+), 48.6 (+), 48.4 (+), 36.7 (-), 36.3 (-), 28.8 (-), 27.7 (-), 27.3 (+), 26.9 (+), 24.4 (+), 23.1 (+), 16.9 (+), 16.5 (+), 9.2 (q).

^{15}N-NMR (CD_3OD) δ [ppm] = 294, 248, 247, 19, −5.

HRMS (ESI) m/z calcd for $C_{23}H_{29}ClN_5Ru$ 512.1155 $[M]^+$, found: 512.1170.

Fig. S1 NMR spectrums of complex **1** in CD_3OD at ambient temperature.

^{1}H-NMR



^{13}C-NMR

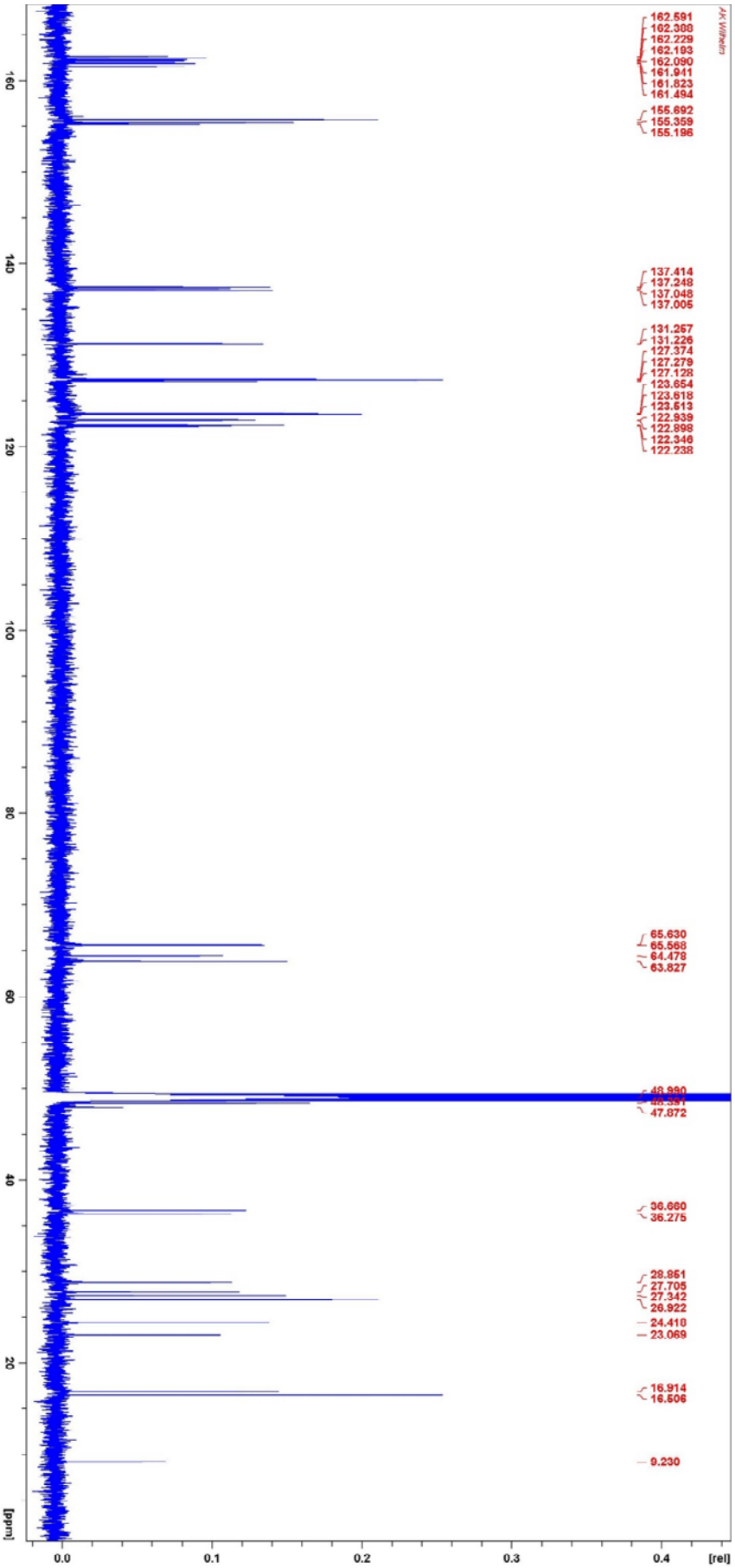
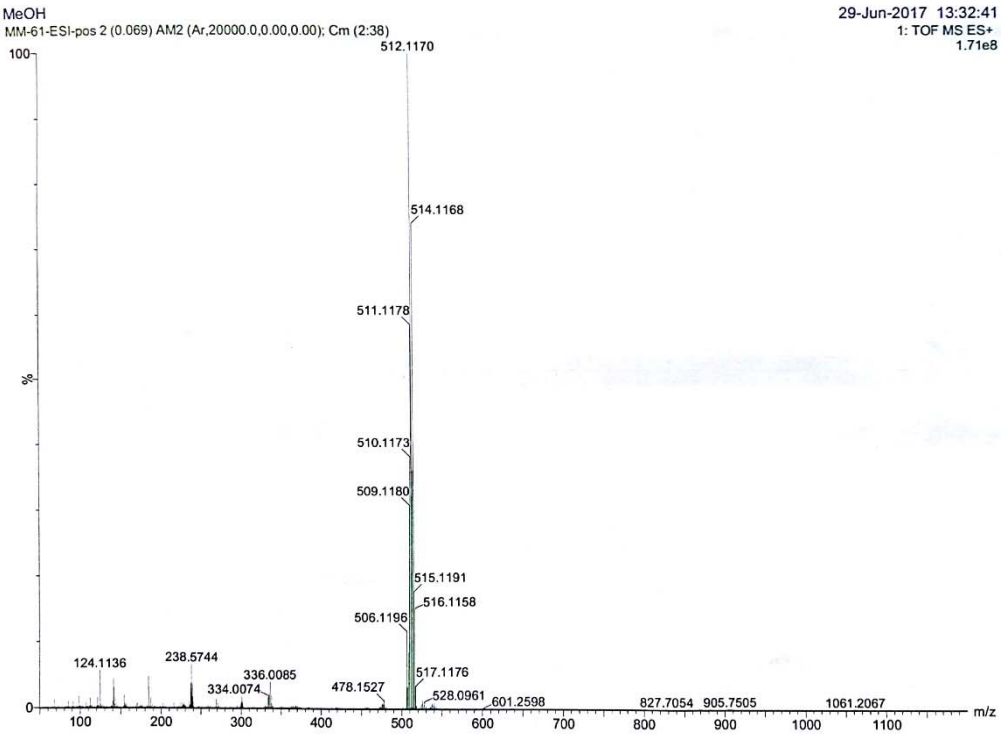

162.591
162.388
162.229
162.193
162.090
161.941
161.823
161.494
155.692
155.359
155.196
137.414
137.248
137.048
137.005
131.257
131.226
127.374
127.279
127.128
123.654
123.618
123.613
122.939
122.898
122.346
122.238
65.630
65.568
64.478
63.827
47.872
36.660
36.275
28.851
27.705
27.342
26.922
24.418
23.069
16.914
16.506
9.230
160
140
120
100
80
60
40
20
[ppm]
0.0
0.1
0.2
0.3
0.4
[rel]

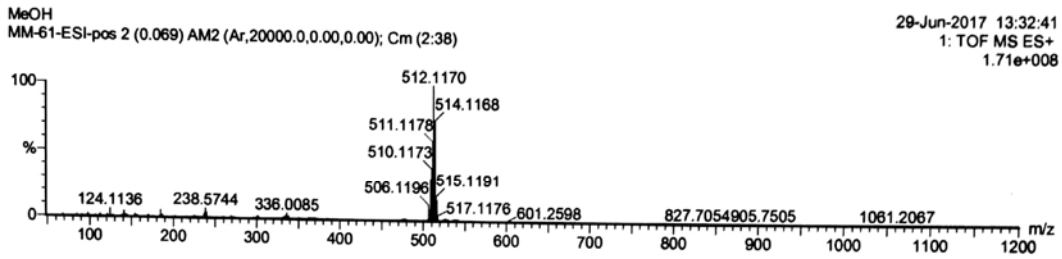
Fig. S2 Electrospray (ESI)-MS spectrum of complex **1**.



Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Odd and Even Electron Ions
22 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-23 H: 0-29 N: 0-5 Cl: 0-1 Ru: 0-1



Minimum:				-1.5				
Maximum:		5.0	10.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
512.1170	512.1155	1.5	2.9	11.5	695.3	n/a	n/a	C23 H29 N5 Cl Ru

Fig. S3 Time evolution of the UV-Vis (top row). Experimental conditions and UV-Vis difference spectra (bottom row, $\Delta A = A_t - A_0$, where A_t = absorbance at time t and A_0 = absorbance at the time at which the first spectrum was recorded) during the aquation of the complex **1** (0.1 mM) in H_2O at room temperature.

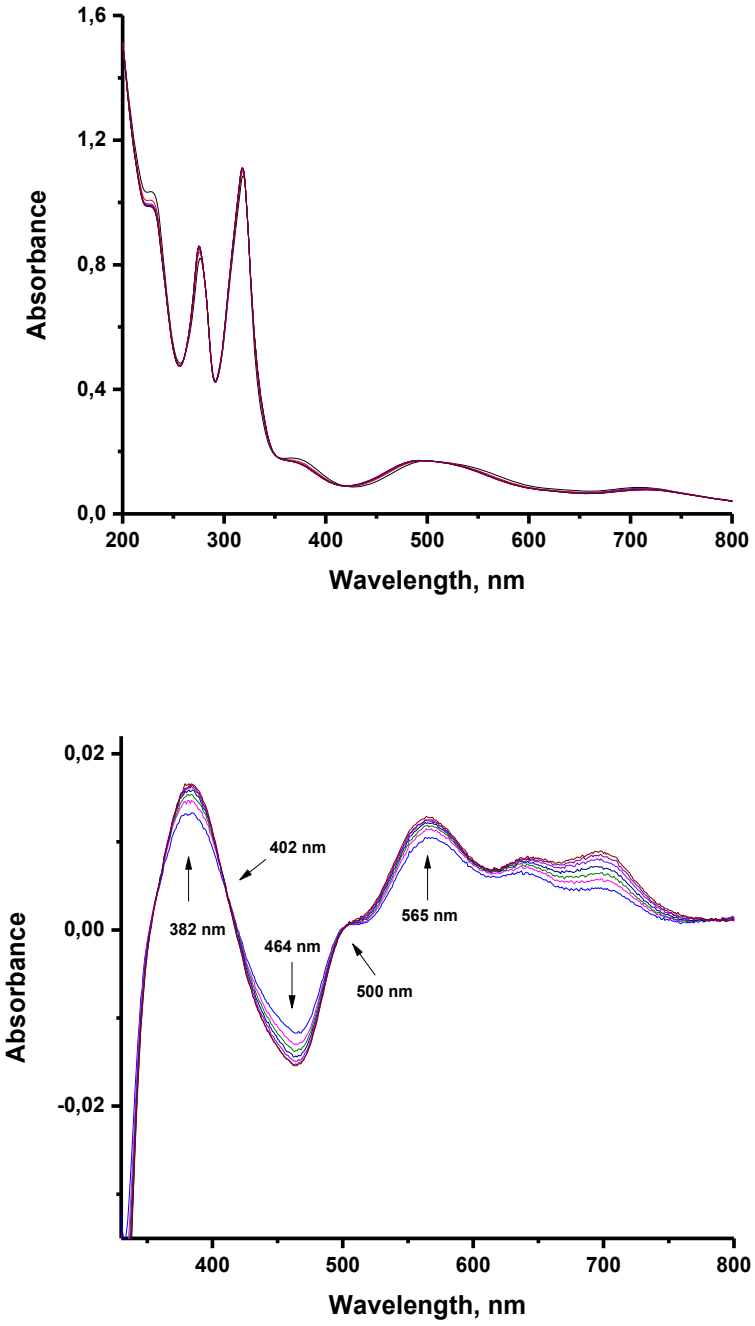
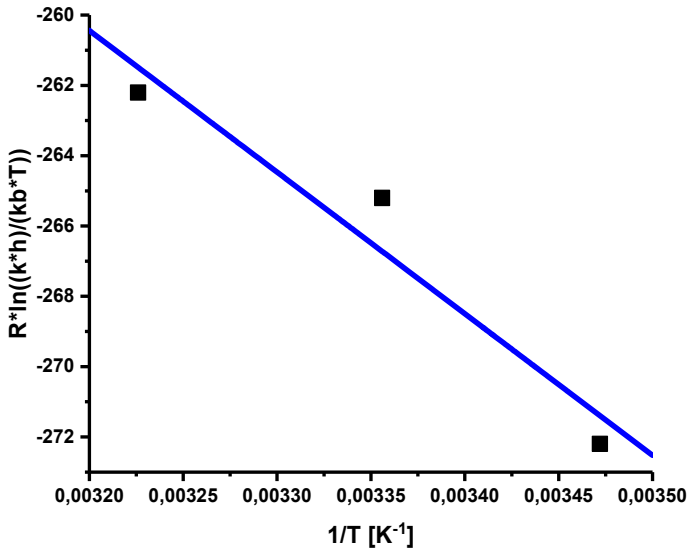


Fig. S4 *Eyring* plot for the reactions of complex **1** with 5'-GMP in H_2O.



DNA-binding studies

Calculation of DNA-binding constant

In order to compare quantitatively the binding strength of the complexes, the intrinsic binding constants K_b were determined by monitoring the changes in absorption at the MLCT band with increasing concentration of CT-DNA using the following equation (S1):[S3]

$$[DNA]/(\varepsilon_a - \varepsilon_f) = [DNA]/(\varepsilon_b - \varepsilon_f) + 1/[K_b(\varepsilon_b - \varepsilon_f)] \quad (S1)$$

K_b is given by the ratio of slope to the *y* intercept in plots $[DNA]/(\varepsilon_a - \varepsilon_f)$ *versus* [DNA] (**Fig. S6**), where [DNA] is the concentration of DNA in base pairs, $\varepsilon A = A_{obs}/[complex]$, ε_f is the extinction coefficient for the unbound complex and ε_b is the extinction coefficient for the complex in the fully bound form.

Stern-Volmer equation for EB competitive studies

The relative binding of complexes to CT-DNA is described by Stern-Volmer equation (S2):[S4]

$$I_0/I = 1 + K_{sv}[Q] \quad (S2)$$

Where, I_0 and I are the emission intensities in the absence and the presence of the quencher (complex **1**), respectively, [Q] is the total concentration of quencher, K_{sv} is the dynamic quenching constant. (**Fig. 5**).

Fig. S5 Absorption spectra of the complex **1** in PBS buffer upon addition of calf thymus DNA; [**1**] = 1.25 x 10^{-5} M, [CT-DNA] = (0.12-1.25) x 10^{-5} M. Arrow shows the absorbance changing upon increasing DNA concentrations.

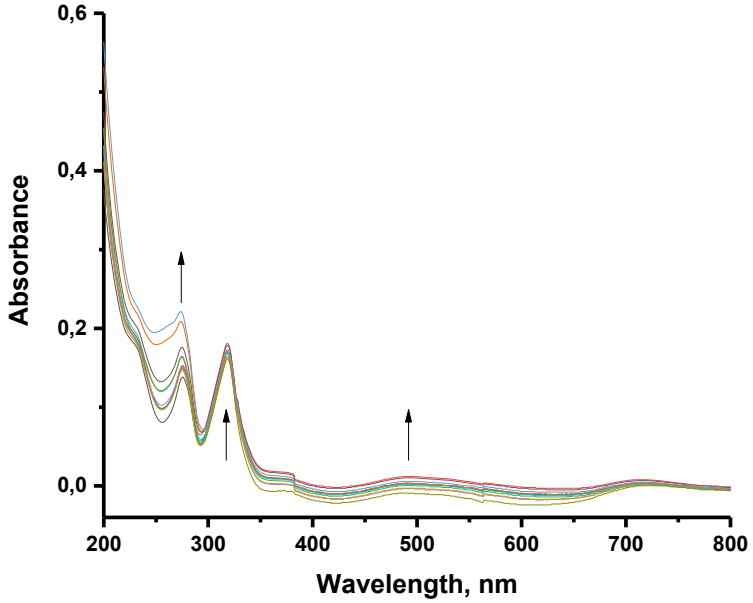
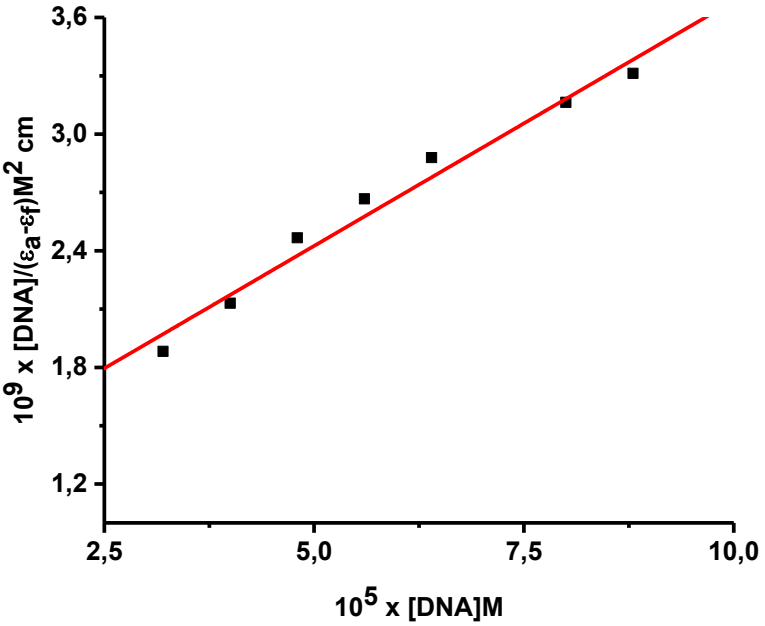


Fig. S6 Plot of [DNA]/($\varepsilon_a - \varepsilon_f$) versus [DNA] for the complexes **1**.



References

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S2. M. M. Milutinović, A. Rilak, I. Bratsos, O. Klisurić, M. Vraneš, N. Gligorijević, S. Radulović, Ž. D. Bugarčić, *J. Inorg. Biochem.*, 2017, **169**, 1.

S3. A. M. Pyle, J. P. Rehmann, R. Meshoyrer, C. V. Kumar, N. J. Turro and J. K. Barton, *J. Am. Chem. Soc.*, 1989, **111**, 3051-3058.

S4. R. Lakowicz and G. Weber, *Biochemistry*, 1973, **12**, 4161-4170.