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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 (1H-NMR: 700 MHz; 13C-NMR: 175 MHz; 15N-NMR: 70 MHz). The NMR signals are referenced to the residual proton or carbon signals of the deuterated solvent (1H- and 13C-NMR) and are reported in ppm relative to TMS. Liquid ammonia was used as an external reference for 15N-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) terpyridine complex 1 was synthesized by a literature method described earlier.52 RuCl₃ x 3 H₂O (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)Cl₃] complex A which was used without further purification. A (0.181 mmol), N-N ligand (0.218 mmol) B, LiCl (1,814 mmol) and Et₃N (0.544 mmol) were mixed in a 20 mL solution of EtOH/H₂O 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).

1H-NMR (CD3OD) 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)

13C-NMR (CD3OD) 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 (+), 122.1 (+), 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).

15N-NMR (CD3OD) δ [ppm] = 294, 248, 247, 19, -5.

Fig. S1 NMR spectrums of complex 1 in CD₃OD at ambient temperature.

$^1$H-NMR
$^{13}$C-NMR
Fig. S2 Electrospray (ESI)-MS spectrum of complex 1.
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**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\(_2\)O at room temperature.
Fig. S4 *Eyring* plot for the reactions of complex 1 with 5'-GMP in H₂O.

**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):\(^{53}\)

$$
\frac{[\text{DNA}]}{(\varepsilon_a - \varepsilon_f)} = \frac{[\text{DNA}]}{(\varepsilon_b - \varepsilon_f)} + \frac{1}{[K_b(\varepsilon_b - \varepsilon_f)]}
$$

(S1)

$K_b$ is given by the ratio of slope to the $y$ intercept in plots $[\text{DNA}]/(\varepsilon_a - \varepsilon_f)$ versus $[\text{DNA}]$ (Fig. S6), where $[\text{DNA}]$ is the concentration of DNA in base pairs, $\varepsilon_A = A_{\text{obs}}/[\text{complex}]$, $\varepsilon_f$ is the extinction coefficient for the unbound complex and $\varepsilon_b$ 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):\(^{54}\)

$$
\frac{I_0}{I} = 1 + K_{sv}[Q]
$$

(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).
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**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.

![Absorption Spectra](image1)

**Fig. S6** Plot of [DNA]/(ε_a – ε_f) versus [DNA] for the complexes 1.

![Plot](image2)
References


