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Water-soluble lanthanide complexes with bispidine-substituted benzoic acid for luminescent thermometry in physiological range

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Experimental details

Thermal analysis was carried out on a thermoanalyzer STA 449 F1 Jupiter (NETZSCH, Germany) in the temperature range of 40–1000 °C in air, heating rate 10 °/min. The evolved gases were simultaneously monitored during the TA experiment using a coupled QMS 403 Aeolos quadrupole mass spectrometer (NETZSCH, Germany). The mass spectra were registered for the species with following m/z values: 18 (corresponding to H_2O), 28 (N_2), and 44 (CO_2). **The IR spectra** were recorded on an Thermo Scientific™ Nicolet™ iS50 FTIR Spectrometer as powdered at ATR.

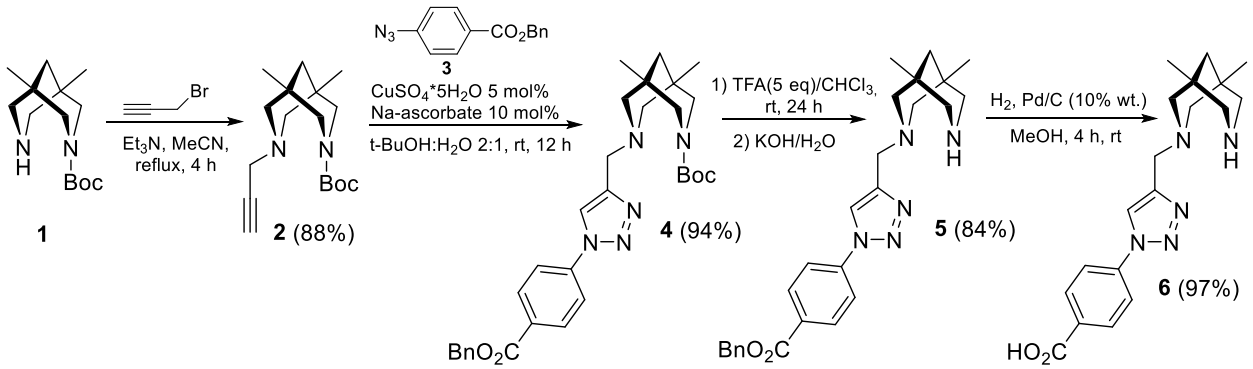
^{1}H and ^{13}C NMR spectra were recorded at 298 K on Bruker Avance-400 and Bruker AM-300 spectrometers. The spectrometer frequency is denoted in parentheses for each spectral data set. For ^{1}H and ^{13}C nuclei TMS was used as internal standard. **The purity of the obtained compounds** was controlled by TLC on Merck Silicagel 60G F_{254} plates. For column chromatography Carl Roth Silica gel 60, 0.04–0.063 mm was used. **High-resolution mass spectra** were recorded on a Bruker MicroOTOFII mass spectrometer with electrospray ionization.

Absorption spectra were recorded in the range 200-800 nm using Perkin-Elmer Lambda 650 spectrometers to determine the maximum absorption of the ligand, as well as to estimate the molar extinction coefficient of the ligand. **Emission, excitation spectra and lifetimes** were measured using FluoroMax-Plus fluorometer (HORIBA) with 1905-OFR 150-W Xenon Lamp as excitation source.

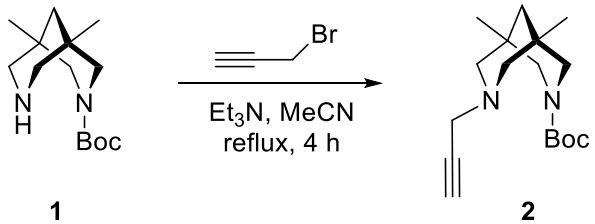
Synthesis of ligand

All reagents and solvents used in the work (purity 90.0–99.9+ %) were purchased from commercial sources (Sigma Aldrich, abcr, Acros Organics, Fisher Scientific), and, if necessary, subjected to further purification by standard routines immediately before use to achieve analytical purity. *N*-Boc-1,5-dimethylbispidine **1** and 4-azidobenzoic acid were synthesized according to procedures [1] and [2], respectively.

The synthesis of the ligand (**HL**, **6**) was carried out according to the following scheme:



*tert-Butyl 1,5-dimethyl-7-(prop-2-yn-1-yl)-3,7-diazabicyclo[3.3.1]nonane-3-carboxylate **(2)***



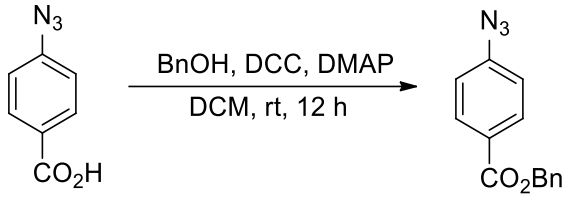
1.712 g (6.73 mmol) of *N*-Boc-1,5-dimethylbispidine **1**, 0.725 mL (6.73 mmol) of propargyl bromide (80% wt. solution in toluene) and 0.92 mL (6.73 mmol) of Et_3N were dissolved in 10 mL of MeCN. The reaction mixture was stirred under reflux for 4 hours. The reaction mixture was evaporated to dryness and diluted with water. The mixture was extracted with DCM. The organic phase was separated, dried over anhydrous Na_2SO_4 and evaporated to dryness. The residue was purified with column chromatography on silica (eluent: petroleum ether:EtOAc 10:1). Colourless oil. Yield: 88% (1.728 g).

^{1}H NMR (300 MHz, $CDCl_3$) δ 0.82 (s, 6H), 1.13 (d, *J* = 12.3 Hz, 1H), 1.22 (d, *J* = 12.0 Hz, 1H), 1.44 (s, 9H), 2.08 – 2.23 (m, 3H), 2.44 (dd, *J* = 13.2, 2.3 Hz, 1H), 2.50 – 2.69 (m, 3H), 3.23 – 3.29 (m, 2H), 3.93 (d, *J* = 13.0 Hz, 1H), 4.14 (d, *J* = 13.6 Hz, 1H).

^{13}C NMR (76 MHz, $CDCl_3$) δ 25.18, 28.61, 31.37, 31.54, 46.61, 47.06, 53.13, 54.31, 61.65, 61.99, 73.21, 78.47, 78.54, 155.21.

Found $[M+H]^+$: 293.2234. $C_{17}H_{29}N_2O_2^+$. Calculated $[M+H]^+$: 293.2224.

*Benzyl 4-azidobenzoate (**3**)*



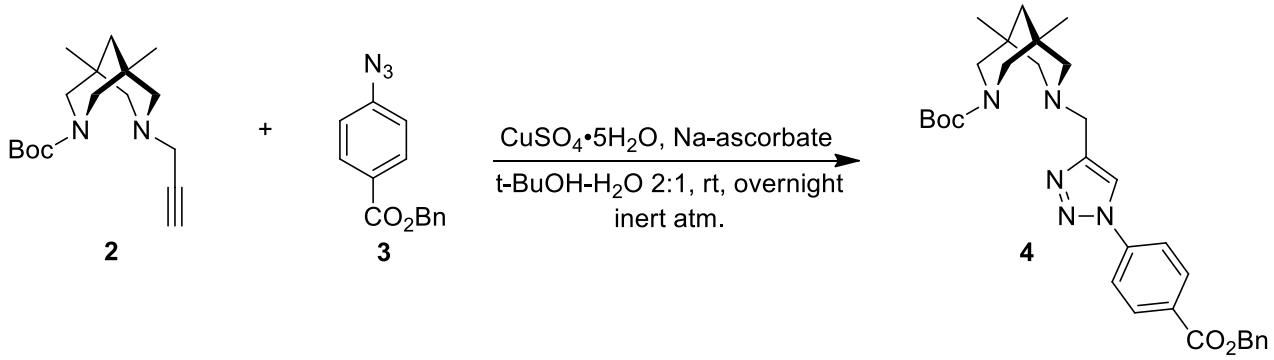
3

To the suspension of 4-azidobenzoic acid (6.5 g, 39.88 mmol) in 50 mL of dry DCM, a solution of DCC (8.215 g, 39.88 mmol) in 50 mL of DCM was dropwise added under cooling (0 °C). Benzyl alcohol (4.14 mL, 39.88 mmol) and DMAP (0.487 g, 3.988 mmol) were added to the resulting yellow suspension. The reaction mixture was stirred at RT for 24 hours. The reaction mixture was filtered through Celite and filtrate was evaporated to dryness. The residue was purified with flash chromatography on silica (eluent: petroleum ether:EtOAc 10:1). Yellow oil. Yield: 87% (8.812 g).

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.37 (s, 2H), 7.05 – 7.10 (m, 2H), 7.34 – 7.48 (m, 5H), 8.06 – 8.11 (m, 2H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 66.39, 109.57, 118.44, 126.23, 127.83, 127.93, 128.23, 131.14, 135.54, 144.45, 165.21.

*Tert-butyl 7-((1-(4-((benzyloxy)carbonyl)phenyl)-1H-1,2,3-triazol-4-yl)methyl)-1,5-dimethyl-3,7-diazabicyclo[3.3.1]nonane-3-carboxylate **(4)***



2 **3** **4**

1.693 g (5.79 mmol) of bispidine **2** and 1.466 g (5.79 mmol) of benzyl 4-azidobenzoate (**3**) were dissolved in mixture of 20 mL t-BuOH and 10 mL water. To the reaction mixture 0.072 g (0.289 mmol, 5 mol. %) of

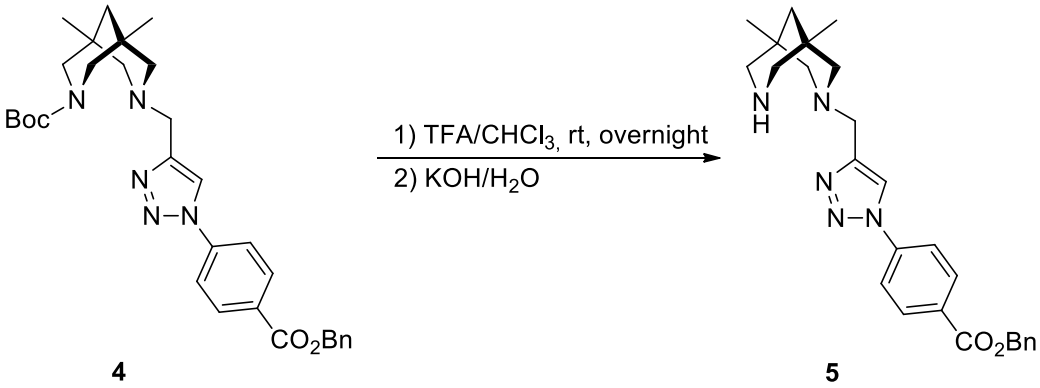
$CuSO_4 \cdot 5H_2O$ and 0.115 g (0.579 mmol, 10 mol. %) of sodium ascorbate were added under argon atmosphere. The reaction mixture was stirred at RT overnight. Then the reaction mixture was evaporated to dryness. The residue was dissolved in DCM and diluted with water. The organic phase was separated and washed with water solution of EDTA. The organic phase was separated, dried over anhydrous Na_2SO_4 and evaporated to dryness. The residue was purified with column chromatography on silica (eluent: petroleum ether:EtOAc 2:1). Yellow oil. Yield: 94% (2.957 g).

^{1}H NMR (300 MHz, $CDCl_3$) δ 0.80 (s, 3H), 0.88 (s, 3H), 1.18 – 1.34 (m, 2H), 1.44 (s, 9H), 1.70 (dd, *J* = 11.0, 2.5 Hz, 1H), 2.14 (dd, *J* = 10.9, 2.5 Hz, 1H), 2.53 (dd, *J* = 13.3, 2.5 Hz, 1H), 2.65 (dd, *J* = 13.2, 2.5 Hz, 1H), 2.87 (d, *J* = 10.6 Hz, 2H), 3.39 (d, *J* = 14.8 Hz, 1H), 3.80 (d, *J* = 14.8 Hz, 1H), 4.05 (d, *J* = 13.3 Hz, 1H), 4.15 (d, *J* = 13.5 Hz, 1H), 5.42 (s, 2H), 7.35 – 7.51 (m, 5H), 8.05 (d, *J* = 8.7 Hz, 2H), 8.26 (d, *J* = 8.7 Hz, 2H), 8.73 (s, 1H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 25.18, 25.27, 28.91, 31.28, 31.82, 47.43, 52.60, 54.40, 54.57, 63.17, 65.75, 67.05, 79.00, 119.24, 121.06, 128.30, 128.44, 128.72, 129.60, 131.54, 135.88, 140.61, 147.53, 155.49, 165.53.

Found $[M+H]^+$: 546.3088. $C_{31}H_{40}N_5O_4^+$. Calculated $[M+H]^+$: 546.3075.

Benzyl 4-(4-((1,5-dimethyl-3,7-diazabicyclo[3.3.1]nonan-3-yl)methyl)-1H-1,2,3-triazol-1-yl)benzoate ***(5)***



4 **5**

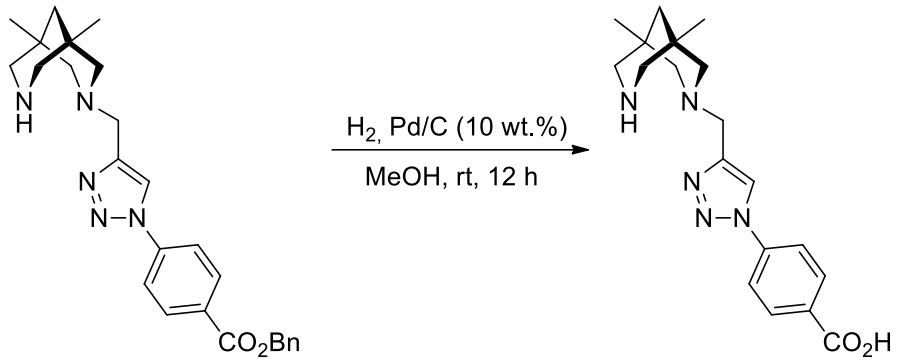
TFA (0.79 mL, 10.308 mmol) was added to the stirring solution of Boc-bispidine **4** (1.125 g, 2.062 mmol) in $CHCl_3$ at 0 °C and allowed to stir at room temperature overnight. Then reaction mixture was made alkaline by adding 10% aq. solution of KOH and resulting mixture was extracted with $CHCl_3$. The organic phase was separated, dried over anhydrous Na_2SO_4 and evaporated to dryness. Beige solid. Yield: 84% (0.729 g).

^{1}H NMR (300 MHz, $CDCl_3$) δ 0.74 (s, 6H), 1.22 (d, *J* = 12.3 Hz, 1H), 1.32 (d, *J* = 12.1 Hz, 1H), 2.03 (dd, *J* = 11.0, 2.8 Hz, 2H), 2.45 (dd, *J* = 13.5, 2.8 Hz, 2H), 2.85 (ddd, *J* = 11.2, 6.5, 2.2 Hz, 4H), 3.61 (s, 2H), 3.65 (br.s, 1H), 5.41 (s, 2H), 7.34 – 7.50 (m, 5H), 7.89 (d, *J* = 8.8 Hz, 2H), 8.01 (s, 1H), 8.24 (d, *J* = 8.8 Hz, 2H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 25.35, 31.29, 47.29, 53.56, 57.02, 64.69, 67.12, 119.77, 120.71, 128.32, 128.45, 128.68, 130.03, 131.42, 135.70, 140.24, 145.96, 165.26.

Found $[M+H]^+$: 446.2553. $C_{26}H_{32}N_5O_2^+$. Calculated $[M+H]^+$: 446.2551.

4-(4-((1,5-Dimethyl-3,7-diazabicyclo[3.3.1]nonan-3-yl)methyl)-1H-1,2,3-triazol-1-yl)benzoic acid ***(6)***



5 **6**

0.183 g of Pd/C (10 wt.%) was carefully added to the solution of bispidine **5** (0.613 g, 1.38 mmol) in MeOH (15 mL) under argon atmosphere. The reaction mixture was evacuated and put under hydrogen (1 atm) three times then stirring was continued under a hydrogen pressure (1 atm) at RT for 12 h. The reaction mixture was filtered through Celite and the solvent was evaporated to give the product **6**. White solid. Yield: 98% (0.479 g).

^{1}H NMR (300 MHz, D_2O) δ 0.83 (s, 6H), 1.17 (d, *J* = 12.8 Hz, 1H), 1.43 (d, *J* = 13.0 Hz, 1H), 2.00 (d, *J* = 9.5 Hz, 2H), 2.74 – 2.90 (m, 4H), 3.25 (d, *J* = 12.0 Hz, 2H), 3.64 (s, 2H), 7.50 (d, *J* = 8.6 Hz, 2H), 7.84 (d, *J* = 8.7 Hz, 2H), 8.22 (s, 1H).

^{13}C NMR (75 MHz, D_2O) δ 23.20, 29.98, 43.80, 50.61, 52.81, 62.09, 119.40, 122.68, 130.86, 133.08, 138.39, 143.09, 170.55.

Found $[M+H]^+$: 356.2080. $C_{19}H_{26}N_5O_2^+$. Calculated $[M+H]^+$: 356.2081.

Characterization of CCs

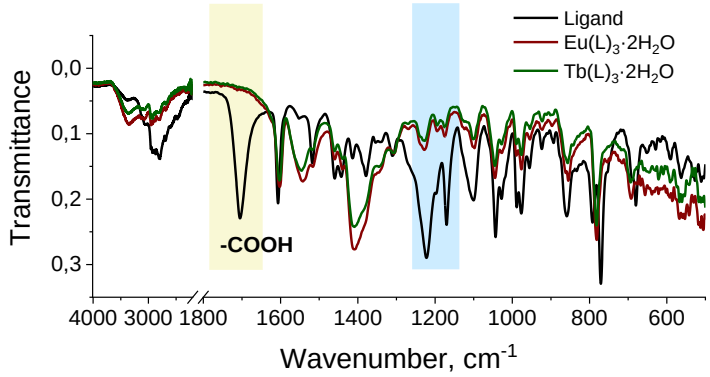


Figure S1. FTIR spectra of ligand and corresponding complexes (ATR, RT, air)

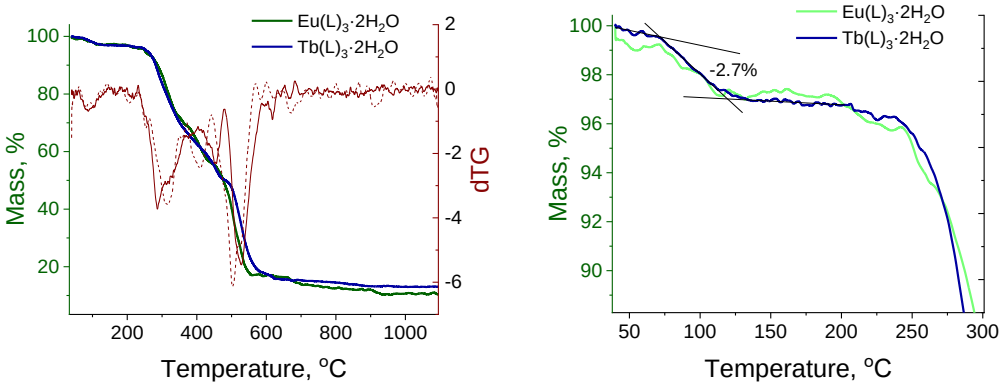


Figure S2. TGA data of $Ln(L)_3 \cdot 2H_2O$ (Ln = Tb, Eu) (Air, 10°/min)

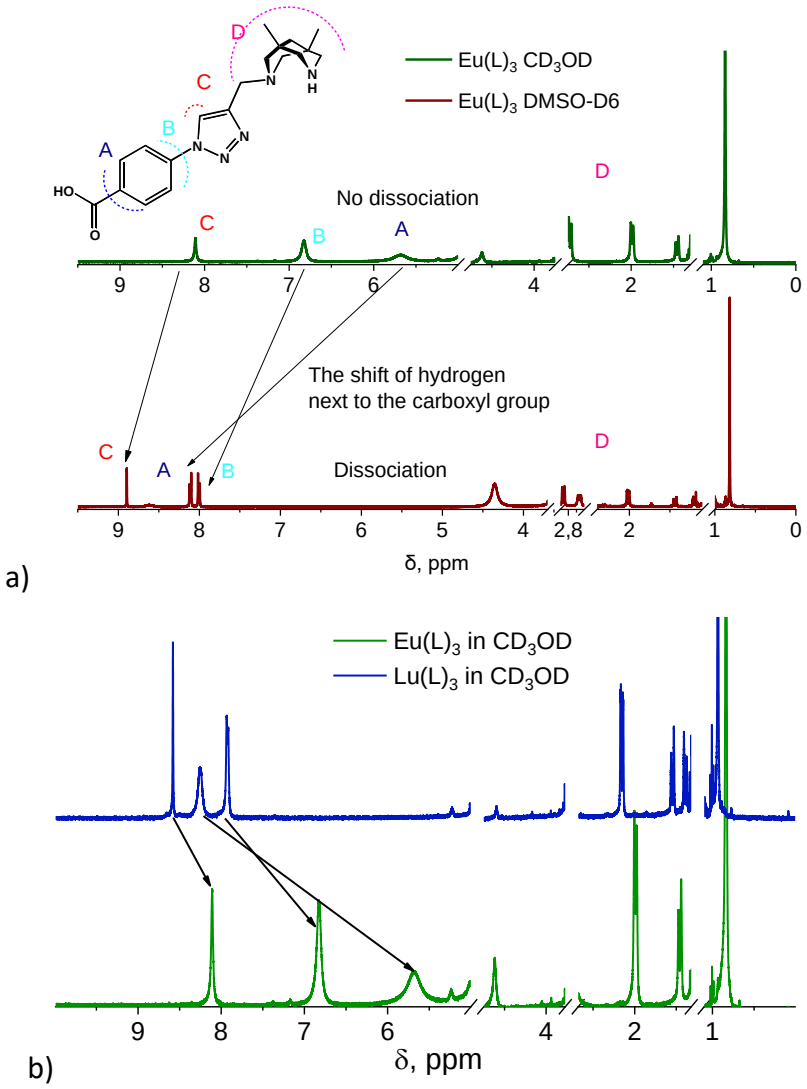


Figure S3. NMR spectra of a) $Eu(L)_3$ in DMSO-D6 and CD_3OD, b) $Eu(L)_3$ and $Lu(L)_3$ in CD_3OD (300 MHz, RT)

Photophysical properties of ligand

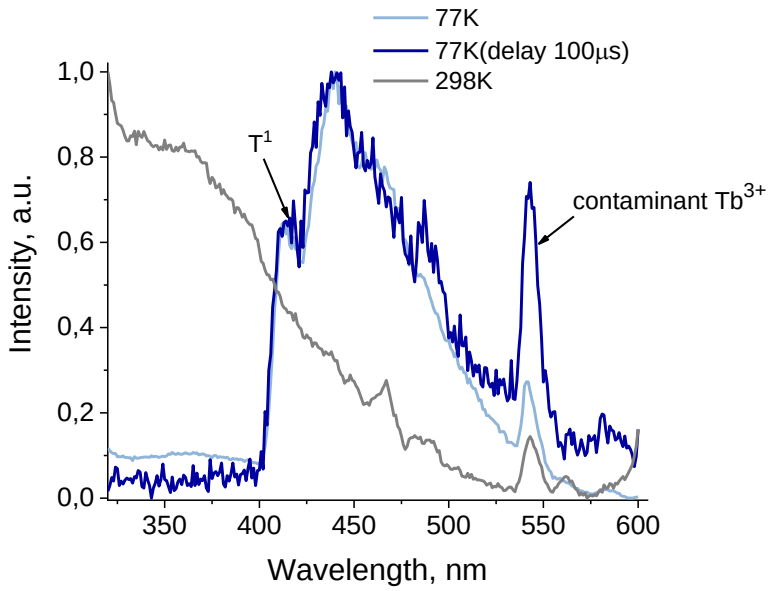
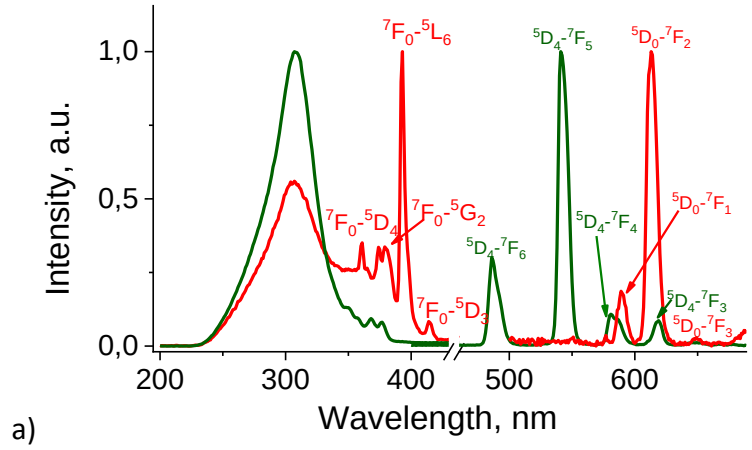
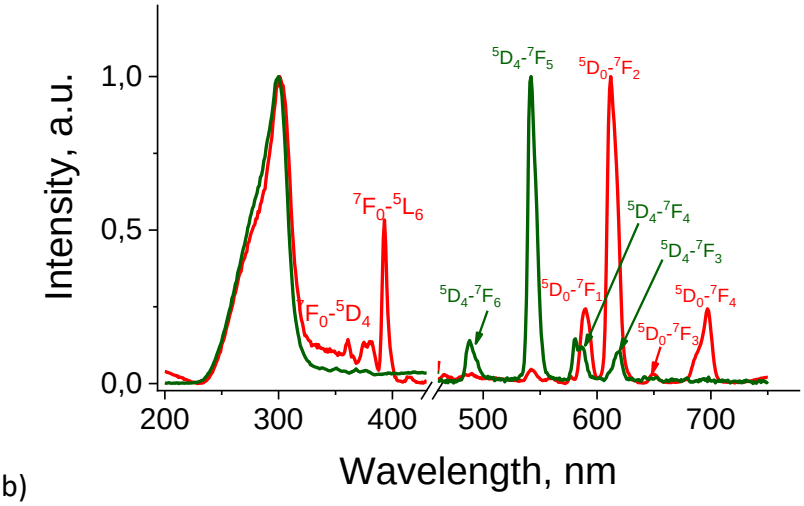


Figure S4. Luminescence spectra of $Gd(L)_3 \cdot 2H_2O$ (powder, air, λ_{ex}=303nm)

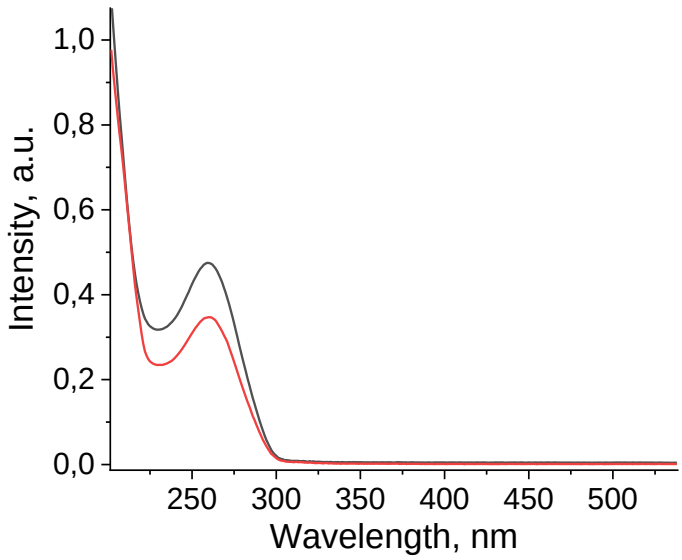
Luminescent properties



a)



b)



c)

Figure S5. Emission (λ_{ex}=303nm) and excitation (λ_{em}=545 nm for Tb complex and 612 nm for Eu complex) spectra of $Tb(L)_3 \cdot 2H_2O$ (green) and $Eu(L)_3 \cdot 2H_2O$ (red) (a – powder, b – solution, RT); c) absorption spectra (black – Tb complex, red – ligand)

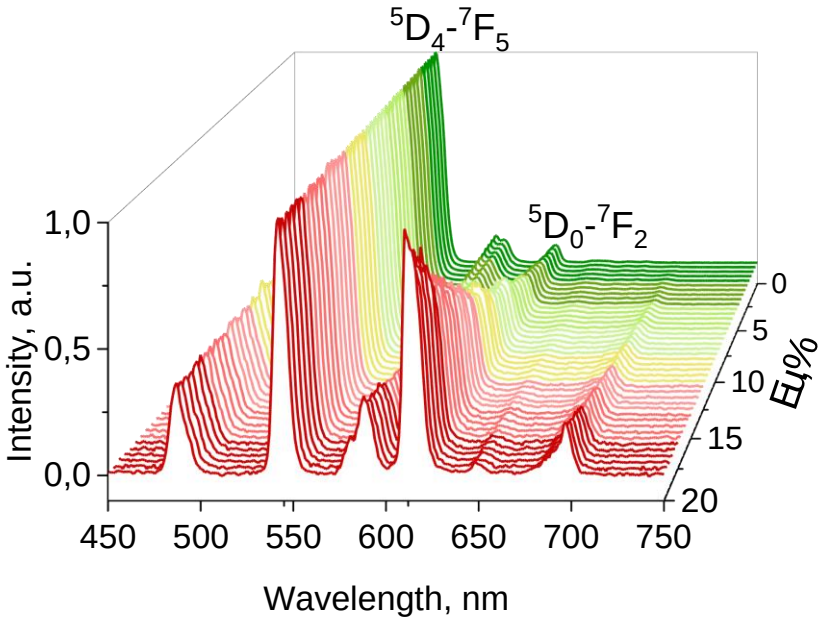


Figure S6. Luminescence titration of $Tb(L)_3$ water solution by $Eu(L)_3$ (RT, λ_{ex}=303nm)

Thermometric properties

In ethanol, two methods of measuring temperature using luminescent spectroscopy data were used: measuring the ratio of the intensity bands of europium and terbium and analyzing lifetimes. In the first case, linear LIR dependences were obtained (ratios of integral intensities, formula LIR = I (Tb)/ I (Eu)) from temperature with a maximum sensitivity of 2.03%/K. In the second case, linear dependences of the europium lifetime on temperature were obtained with a maximum sensitivity of 0.53%/K.

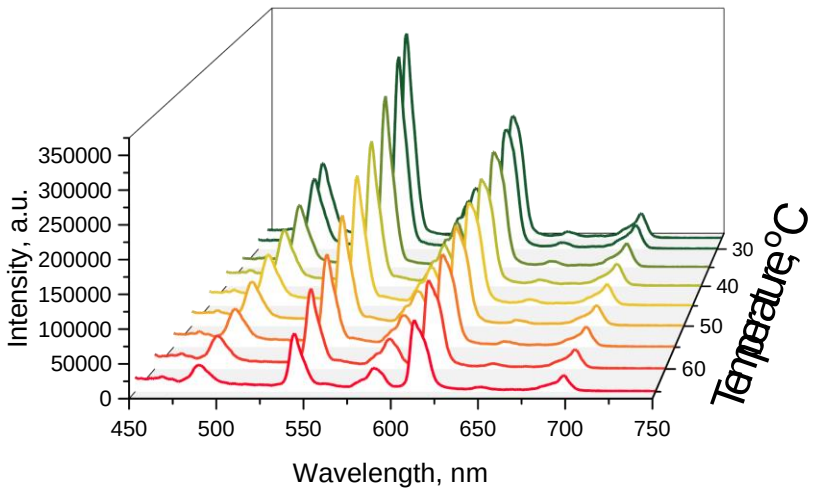


Figure S7. Luminescence spectra of $(Eu,Tb)(L)_3$ in ethanol at different temperatures (λ_{ex}=303nm)

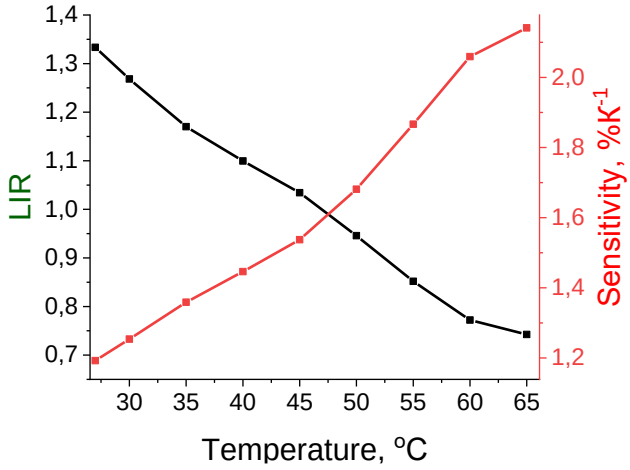


Figure S8. Temperature dependence of LIR and sensitivity of $(Eu,Tb)(L)_3$ in ethanol

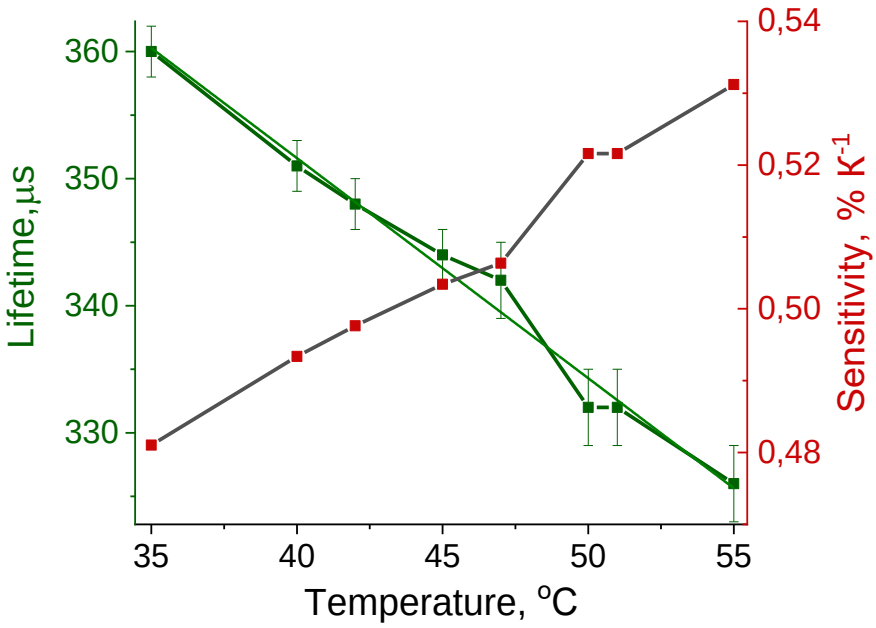


Figure S9. Temperature dependence of Eu lifetimes and sensitivity of Eu $(Eu,Tb)(L)_3$ in ethanol (λ_{ex}=303nm, λ_{em}=612nm)

Cell study

Experimental details

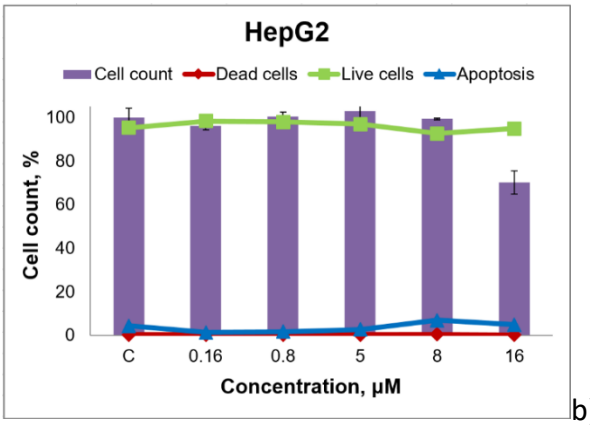
Cell lines of human hepatocellular carcinoma HepG2 and non-tumor human lung fibroblasts MRC5 were purchased from the State Scientific Center for Virology and Biotechnology "Vector" (Russia). Cell viability was assessed by double staining with fluorescent dyes Hoechst 33342 and propidium iodide (PI) according to the standard method [3]. Cells were seeded in 96-well plates at $5 \cdot 10^3$ cells per well in DMEM medium and cultured for 24 hours under standard conditions (37°C, 5% CO_2, humidified atmosphere). Then the cells were treated with the water solution of $Eu(L)_3 \cdot 2H_2O$ and $Tb(L)_3 \cdot 2H_2O$ (1:4 mol) complexes in concentrations 0.2-20μg/ml and DMEM medium (dilution of the original solution, 2mg/ml, in DMEM with in the ratios of 1:100, 1:200, 1:400, 1:2000, 1:10000) and incubated for 48 hours under standard conditions. After incubation, cells were stained with a mixture of Hoechst 33342 (Sigma-Aldrich) and PI (Invitrogen) dyes for 30 min at 37°C. The recording was carried out on an IN Cell Analyzer 2200 device (GE Healthcare, UK) in automatic mode, 4 fields per well. The resulting images were analyzed using the In Cell Investigator software (version 1.5, GE Healthcare, UK) to identify live, dead and apoptotic cells in the entire population. The result is presented as the percentage of cells from three independent experiments with standard deviation.

Results

Exposure to the minimum tested dilution of the complex ($Eu(L)_3 \cdot 2H_2O$:$Tb(L)_3 \cdot 2H_2O$=1:4, water solution) had a cytostatic effect (a decrease in the rate of cell division and, as a consequence, a decrease in the total number of cells compared to the control) on HepG2 cells, but did not have a significant effect on cell death (the percentage of cells in apoptosis is comparable to the control).

Cytotoxicity was studied on both tumor and non-tumor human cell lines MRC5; it was shown that the complex does not exhibit pronounced cytotoxic activity towards these cells (Fig. 10).

a)



b)

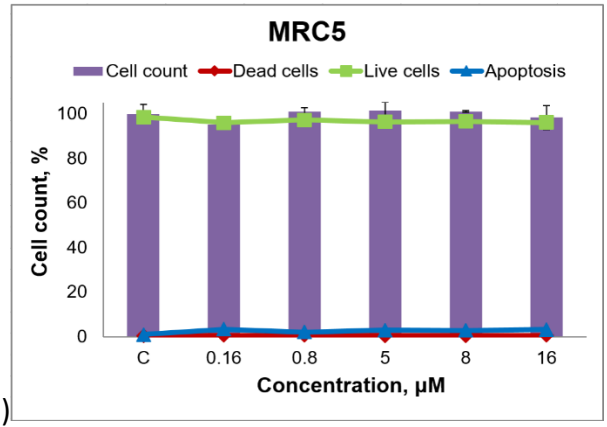


Figure S10. Effect of $Eu(L)_3 \cdot 2H_2O$ and $Tb(L)_3 \cdot 2H_2O$ (1:4 mol) complexes water solution on the viability of HepG-2 (a) and MRC5 (b) cells determined by dual staining with Hoechst 33342/propidium iodide. The incubation time was 48 hours.

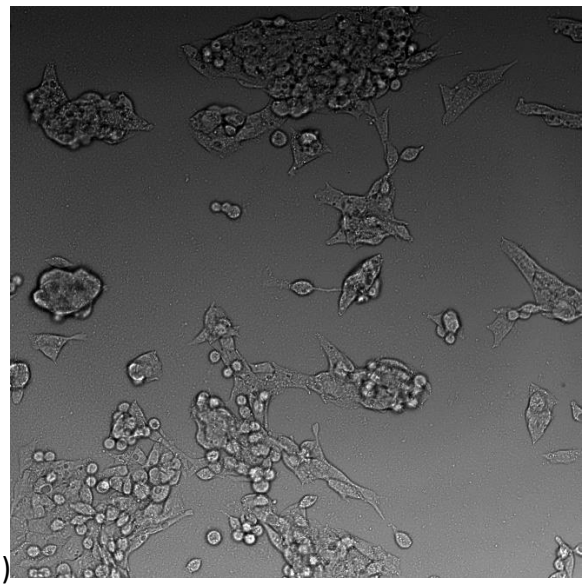
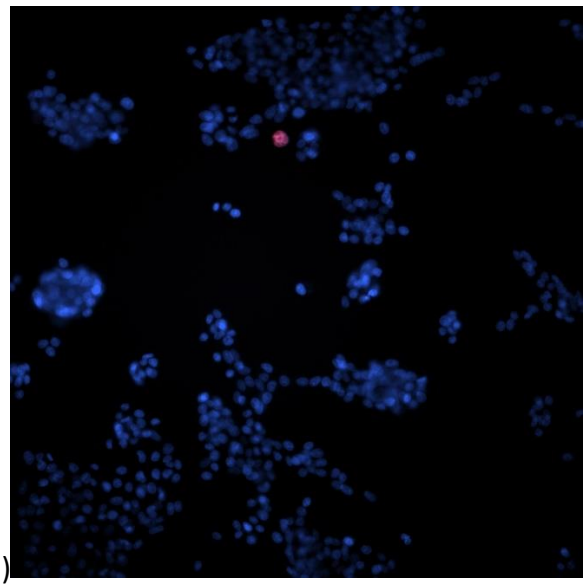
a)  b) 

Figure S11. Representative images of HepG2 cells obtained in the bright field (a) and fluorescent channels (b). Scale bar: 60 µm. Cells were treated with the $Eu(L)_3 \cdot 2H_2O$ and $Tb(L)_3 \cdot 2H_2O$ (1:4 mol) complexes water solution (c=20µg/ml) for 48 hours. Cells were classified as live (normal nuclei – uncondensed chromatin distributed evenly throughout the nucleus), apoptotic (round cells, condensed or fragmented chromatin) or dead (PI-stained due to damage to the cell membrane).

NMR data

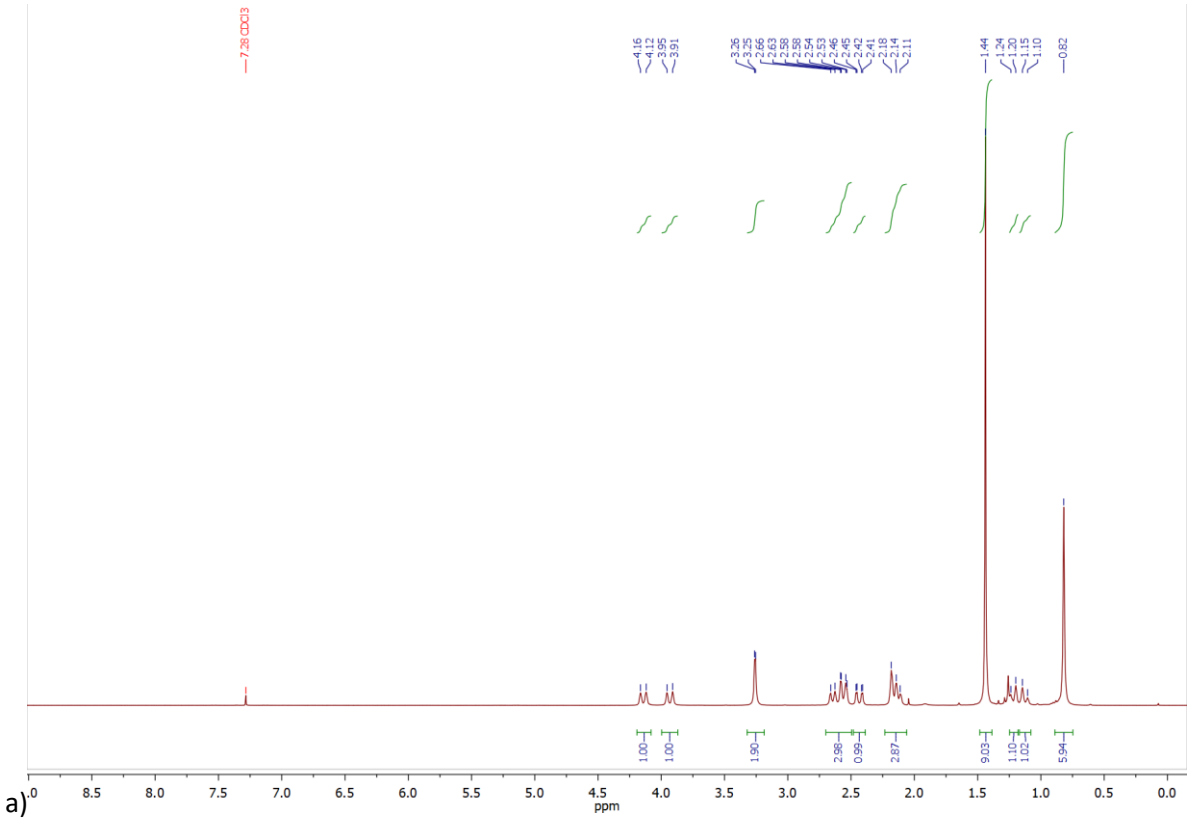
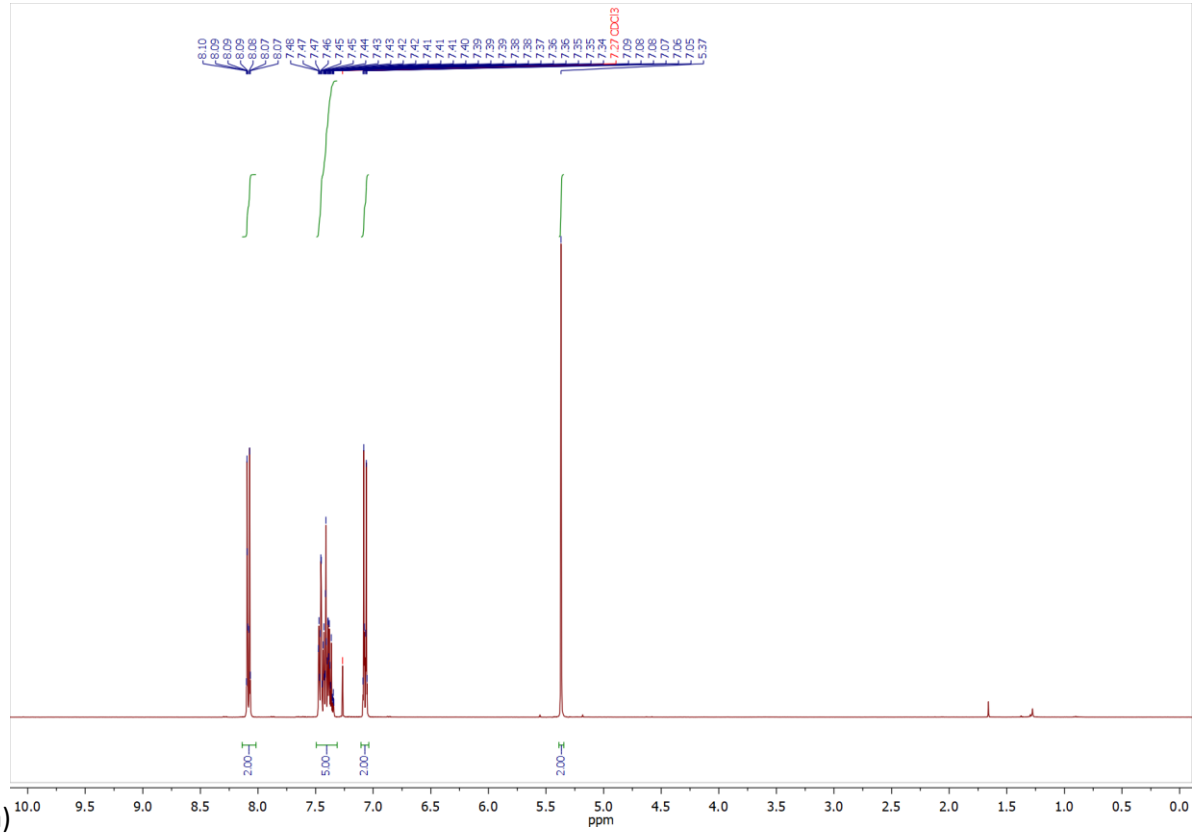


Figure S12. ^{1}H NMR (300 MHz, $CDCl_3$) – *(a)* and ^{13}C NMR (75 MHz, $CDCl_3$) – *(b)* of compound 2

a)



b)

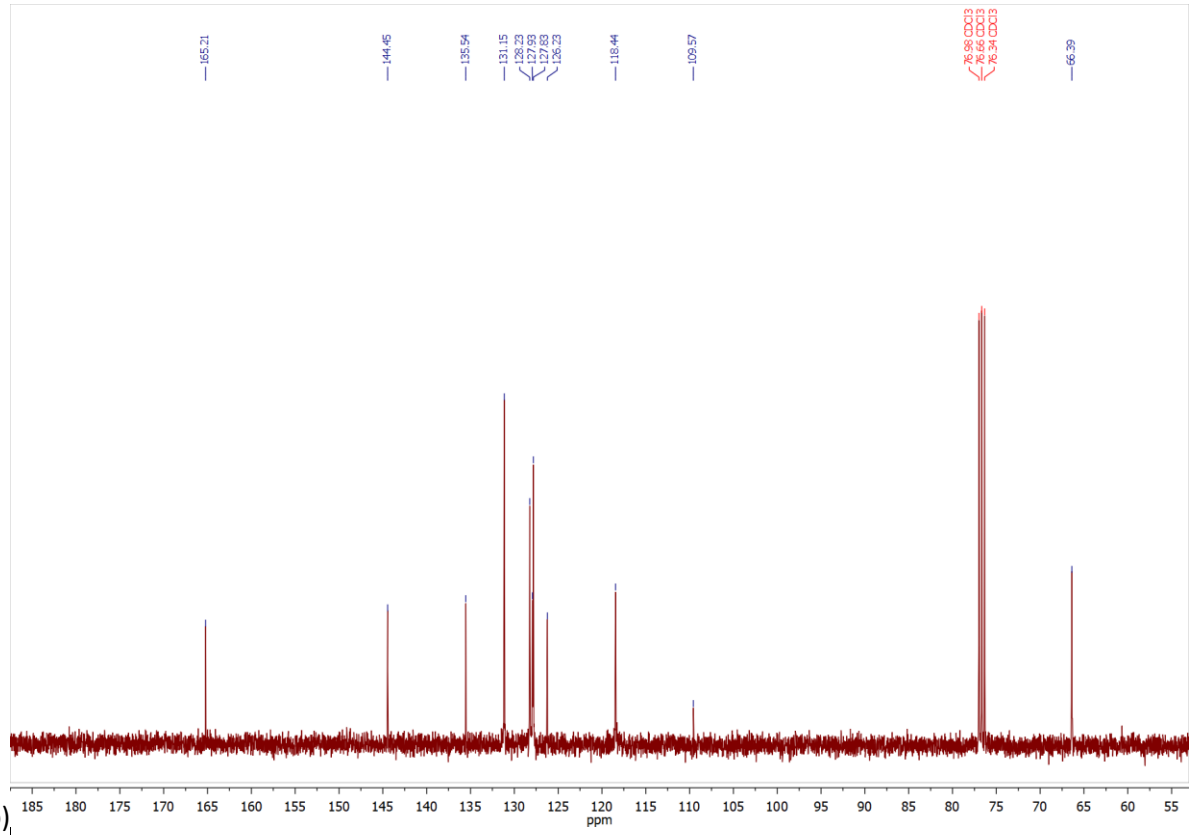


Figure S13. ^{1}H NMR (400 MHz, $CDCl_3$) – *(a)* and ^{13}C NMR (101 MHz, $CDCl_3$) – *(b)* of compound **3**

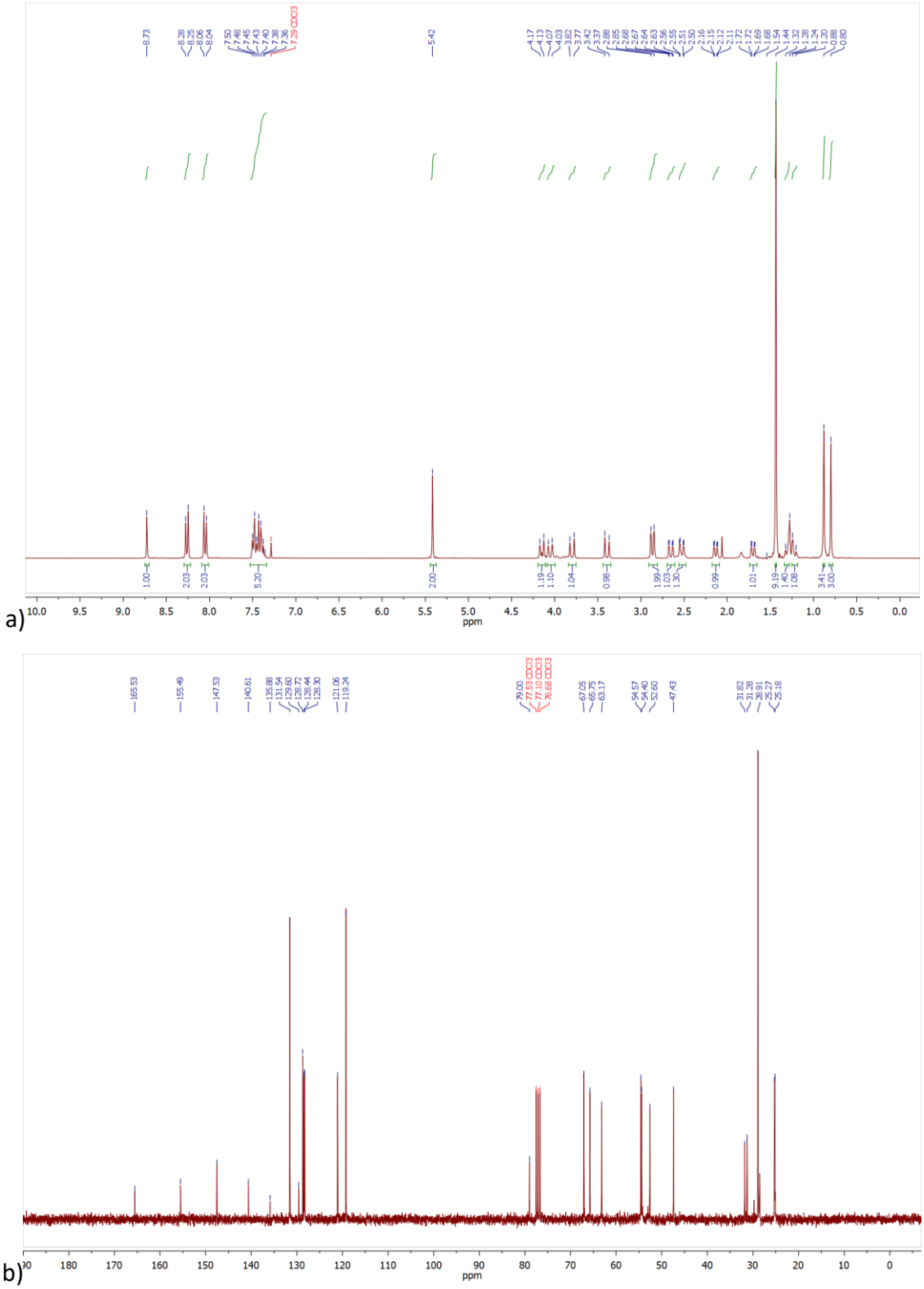


Figure S14. ^{1}H NMR (300 MHz, $CDCl_3$) – *(a)* and ^{13}C NMR (75 MHz, $CDCl_3$) – *(b)* of compound **4**

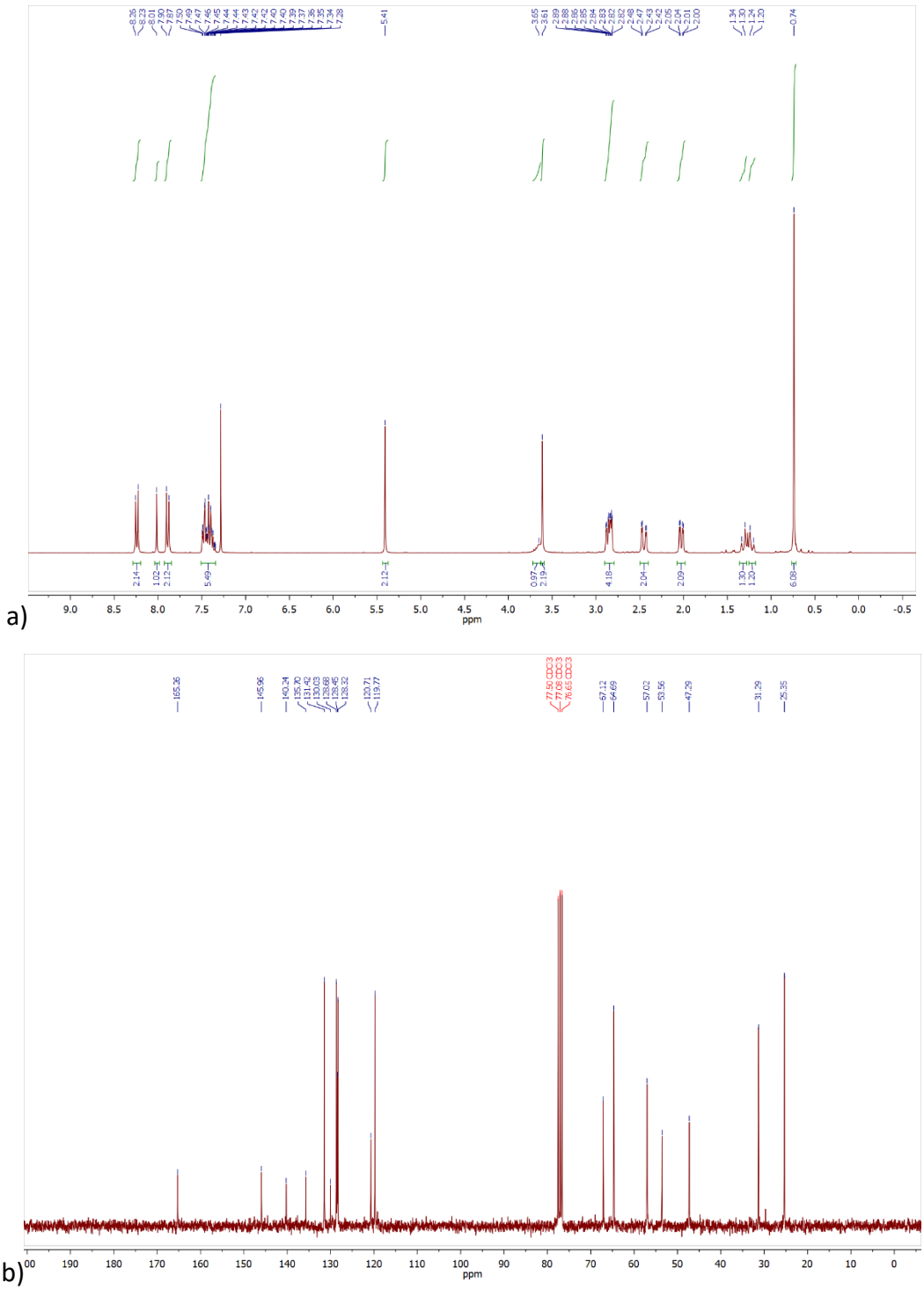
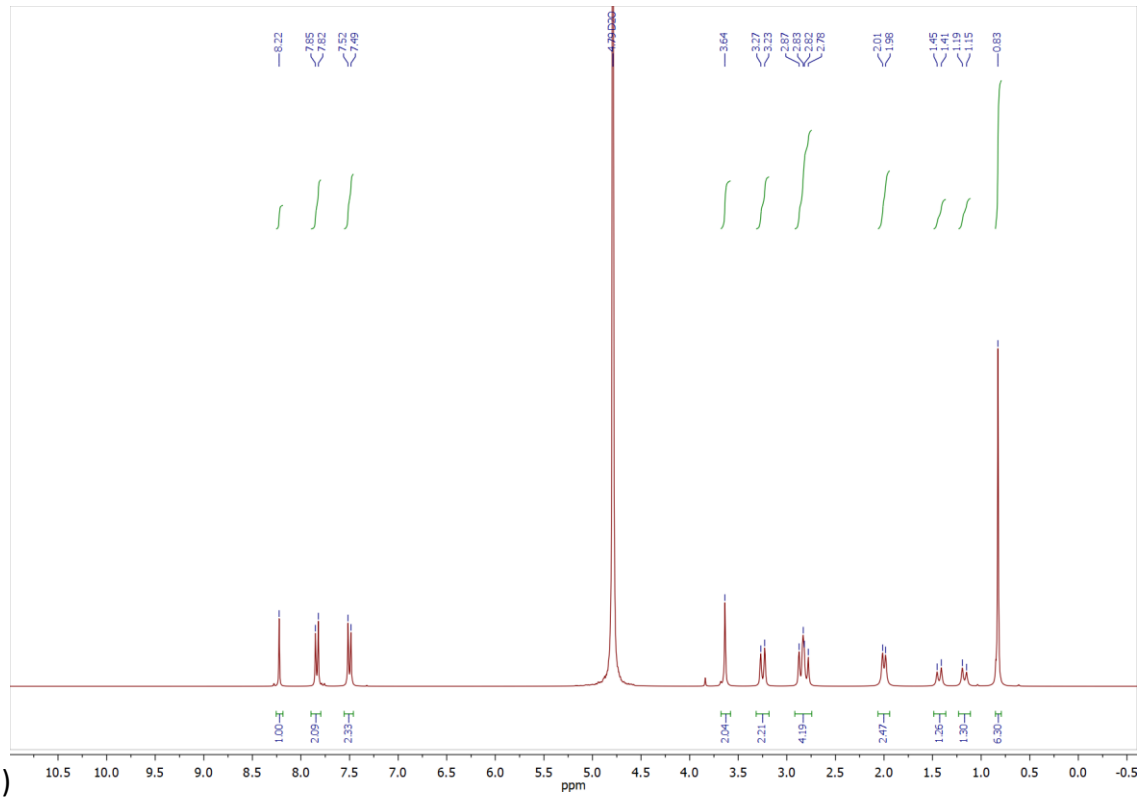
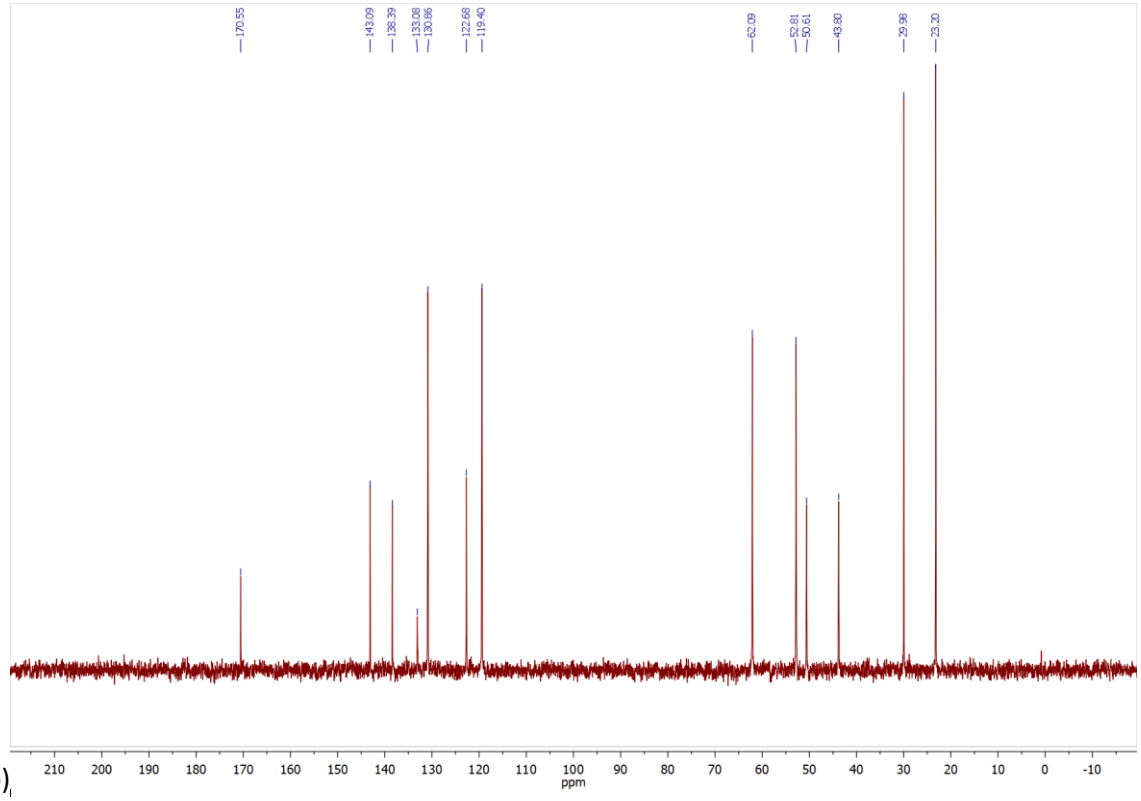


Figure S15. ^{1}H NMR (300 MHz, $CDCl_3$) – *(a)* and ^{13}C NMR (75 MHz, $CDCl_3$) – *(b)* of compound **5**



a)



b)

Figure S16. ^{1}H NMR (300 MHz, D_2O) – *(a)* and ^{13}C NMR (75 MHz, D_2O) – *(b)* of compound ***6 (HL)***

References

1. E. V Suslov, K.Y. Ponomarev, O.S. Patrusheva, S.O. Kuranov, A.A. Okhina, A.D. Rogachev, A.A. Munkuev, R. V Ottenbacher, A.I. Dalinger, M.A. Kalinin, S.Z. Vatsadze, K.P. Volcho and N.F. Salakhutdinov, *Molecules*, 2021, **26**, 7539; https://doi.org/10.3390/molecules26247539.

2. S. Suwal and M.K.H. Pflum, *Angew. Chemie, Int. Ed*., 2010, **49**, 1627; https://doi.org/10.1002/anie.200905244.

3. J. A. Eremina, E. V. Lider, N. V. Kuratieva, D. G. Samsonenko, L. S. Klyushova, D. G. Sheven', R. E. Trifonov and V. A. Ostrovskii, *Inorg. Chim. Acta*, 2021, **516,** 120169; https://doi.org/10.1016/j.ica.2020.120169.