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

Anastasia V. Orlova,[a] Diana S. Polyakova,[b] Alexander I. Dalinger,[b,c] Vladislava Yu. Kozhevnikova,[a,b] Elizaveta V. Lider,[d] Sergey Z. Vatsadze*[b,c] and Valentina V. Utochnikova*[a,b]

[a] *Department of Materials Science, M. V. Lomonosov Moscow State University, 119991 Moscow, Russian Federation. E-mail: valentina.utochnikova@gmail.com*

[b] *Department of Chemistry, M. V. Lomonosov Moscow State University, 119991 Moscow, Russian Federation. E-mail: zurabych@gmail.com*

[c] *N. D. Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, 119991 Moscow, Russian Federation*

[d] *A. V. Nikolaev Institute of Inorganic Chemistry, Siberian Branch of the Russian Academy of Sciences, 630090 Novosibirsk, Russian Federation*

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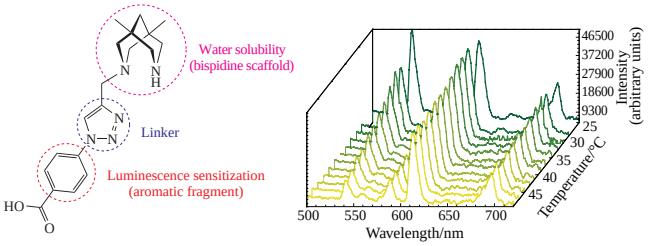
A novel material based on europium and terbium complexes with a bispidine-substituted benzoate anion for luminescent thermometry in the physiological temperature range was obtained. The obtained complexes demonstrated intense visible luminescence with quantum yields up to 75%, high water solubility, and high luminescence sensitivity toward temperature (S_r = 2.1% K^{-1}).



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Noninvasive precise thermometers working at a nanoscale with high spatial resolution, where conventional methods are ineffective, have emerged recently as a very active field of research.[1–5] Temperature is among the most important and fundamental parameters for cellular activities.[6–8] Variation of temperature at the single-cell level happens due to many biochemical reactions inside the cell. Cancer cells have higher temperatures than those of normal tissues because of their enhanced metabolic activity. Moreover, cellular thermal sensing will be beneficial to understand the influence of hyperthermia therapy to cells.[9,10]

Earlier, conventional thermometers were used to detect temperature changes of cell groups.[11,12] However, the conventional thermometers cannot be used for the accurate intracellular thermosensing of living cells in the culture medium. On the contrary, luminescent thermometers ensure high spatial resolution, high thermal sensitivity, and short acquisition times.[13–16] Among the distinct luminescent thermal probes, lanthanide-based materials play a central role in the field due to their unique thermometric response and intriguing emission features (high quantum yield, narrow bandwidth, long-lived emission, large Stokes shifts, and ligand-dependent luminescence sensitization).

Lanthanides can be used within inorganic compounds, such as fluorides,[17,18] which are, however, poorly emissive due to the low oscillation strength of the Laport forbidden *f–f* transitions. Greater accuracy can be achieved using highly emissive complexes with organic antenna ligands.[19–21] Among those, substituted benzoates have demonstrated ultimately high quantum yields, which sometimes reached 90–100%.[22,23] At the same time, they are usually insoluble in water and even in organic solvents due to the tendency of coordination polymer, and even MOF, formation.[24–27]

In order to obtain a brightly emissive intracellular luminescent thermometer, we selected lanthanide complexes with a bispidine substituted benzoate anion [HL, Figure 1(*a*)]. The introduction of heteroaromatic substituents is also expected to increase absorption and participate in the coordination to ensure enhanced complex stability in a biological environment.[28–30] Bispidine moiety is also known to provide the SARS-CoV-2 main viral protease inhibitor ability, which can further allow one to obtain a theranostic agent based on the studied system.[31,32] Red-emitting europium and green-emitting terbium ions were selected as light-emitting ions. The novel HL ligand was obtained by the introduction of a C≡C bond into the structure of bispidine by alkylation of *N*-Boc-bispidine (**1**) with propargyl bromide to form *N*-Boc, *N*′-propargylbispidine (**2**) followed by conjugation with benzyl 4-azidobenzoate (**3**) to form the conjugate (**4**) and stepwise removal of Boc and benzyl protecting groups [compounds (**5**) and HL (**6**), respectively] (see Online Supplementary Materials, section Synthesis of ligand, p. S2).

Coordination compounds of lanthanides were obtained by the exchange reaction between the ligand and 50% excess of freshly precipitated $Ln(OH)_3$ in water. Then, the obtained complexes were filtered from the excess of hydroxides and evaporated to dryness. The resulting complexes were soluble in water, DMSO, and ethanol and slightly soluble in acetonitrile and THF. In DMSO, we observed complete dissociation of the complexes,

while the coordination of the ligand to the lanthanide was preserved in water and alcohol. This was confirmed by the high luminescence intensity of the complexes in water and alcohol at an extremely low luminescence intensity in DMSO. In addition, comparing the ^{1}H NMR spectra measured in different solvents, it was clear that the signals corresponding to the hydrogens of the benzene ring (designated as A and B in Figure S3; see Online Supplementary Materials) are shifted and broadened for the complex in methanol, while the shift for hydrogens located closer to the carboxyl group is greater. Note that no shift or broadening of the signals corresponding to the bispidine fragment was observed, which indicated that this fragment of the complex remained available for binding to the substrate.

According to IR spectroscopy data (Figure S1), ligand binding to lanthanide ions predominantly through the carboxyl group was shown, as evidenced by the extinction of a band at 1700 cm^{-1}; weak binding to the nitrogen of the triazole fragment is also evidenced by a slight shift, broadening, and a decrease in the intensity of the bands (marked in blue). A wide band at 3000–3500 cm^{-1} corresponding to skeletal vibrations of OH groups indicated the presence of water in the coordination sphere of lanthanide. Thermogravimetric analysis (Figure S2) confirmed the presence of two water molecules in the complex by a weight loss at 100 °C (–2.7 wt%). The subsequent weight loss was observed at temperatures above 250 °C due to the decomposition of the organic ligand; the final weight of the residue corresponded to Eu_2O_3 and Tb_4O_7 oxides.

The study of luminescent properties included the determination of the energy of the ligand triplet state (T_1) from the low-temperature spectra of Gd(L)$_3$·2H$_2$O (the complex was prepared and characterized in the same manner as the terbium and europium complexes), which were recorded with time delay (Figure S4). The triplet state equaled 24400 cm^{-1}, according to which we expected that there would be intense luminescence with both europium and terbium. Indeed, both powders and aqueous solutions of Ln(L)$_3$·2H$_2$O (Ln = Tb, Eu) demonstrated intense luminescence of the corresponding lanthanides (Figure S5). The luminescence efficiency of terbium compounds in powder was significantly higher than that of europium ones, which was most likely due to the high position of the triplet state and the presence of water in the coordination sphere of lanthanide, leading to europium luminescence quenching. Quantum yields in powders were 75% for Tb(L)$_3$·2H$_2$O and only 9% for Eu(L)$_3$·2H$_2$O. In water solution, the quantum yield of Eu(L)$_3$·2H$_2$O did not change, while it decreased down to 46% for Tb(L)$_3$·2H$_2$O (Table 1). Nonetheless, this is a very high value for the water solution.

To find an optimal ratio of Eu(L)$_3$·2H$_2$O and Tb(L)$_3$·2H$_2$O for the luminescent thermometry, we varied them by dropping an aqueous solution of Eu(L)$_3$·2H$_2$O to a solution of Tb(L)$_3$·2H$_2$O and measuring the luminescence spectra of each mixture. A comparable intensity of the terbium and europium luminescence was obtained at the ratio Eu : Tb = 1 : 4 [Figure 1(*b*)]. The mixture was further tested for the luminescent thermometry in

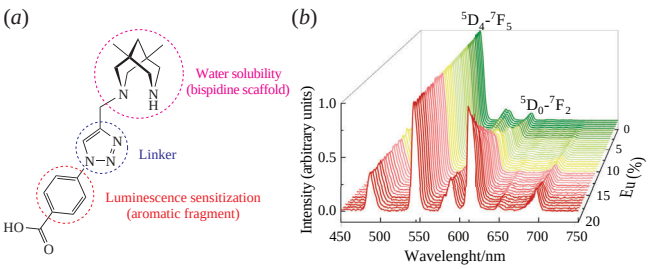

Figure 1 (*a*) Structural formula of the ligand HL; (*b*) luminescence spectra of Eu(L)$_3$·2H$_2$O and Tb(L)$_3$·2H$_2$O mixtures in various ratios in water (λ_{ex} = 303 nm, room temperature).

Table 1 Photophysical properties of Ln(L)$_3$·2H$_2$O (Ln = Tb, Eu) (λ_{ex} = = 303 nm, room temperature).

Complex	QY (%) powder/water solution	τ/μs powder/water solution
Eu(L)$_3$·2H$_2$O	9/9	485 ± 5 / 473 ± 3
Tb(L)$_3$·2H$_2$O	75/46	928 ± 3 / 976 ± 3

water. Upon heating in a temperature range of 30–50 °C, we observed a monotonic decrease in the intensity of terbium luminescence with temperature, while the luminescence intensity of europium did not change [Figures 2(*b*,*c*)].

This was surprising taking in mind that the europium luminescence is usually more subject to temperature quenching than that of terbium. We suggested that this effect can be due to the following: (a) sensitization of europium luminescence through the terbium ion because Tb–Eu energy transfer is highly effective or (b) the Tb–L back energy transfer. To verify the former suggestion, we analyzed the time-resolved luminescence data as Tb–Eu energy transfer should result in non-monoexponential character of the decay curve of europium emission [Figure 2(*a*)]. However, we found that europium luminescence demonstrated a perfect monoexponential character; therefore, the former suggestion was not the case.

To verify the latter suggestion, we analyzed the sole terbium emission as a function of temperature using the Boltzmann equation [Figure 2(*d*)]

$$I = \frac{1}{1 + A\exp(-\Delta E/T)}.$$

It demonstrated that the terbium luminescence intensity is subject to quenching by a state with ΔE = 4300 cm^{-1}, which almost exactly corresponds to the energy gap between the terbium emitting $^5D_4$ state (20400 cm^1) and the triplet state of the ligand (24400 cm^{-1}). In turn, such a transition between the resonance level of europium ($^5D_0$, 17200 cm^{-1}) and the triplet state of the ligand does not occur due to the large difference in energy. Therefore, we observed only a small decrease in europium intensity caused by vibrational quenching.

Using the terbium intensity, which varies significantly with temperature and normalized to the europium intensity (LIR, luminescence intensity ratio), as a temperature-dependent parameter, we obtained a thermometer with a relative sensitivity (S_r) of up to 3.2% K^{-1} in water solution.

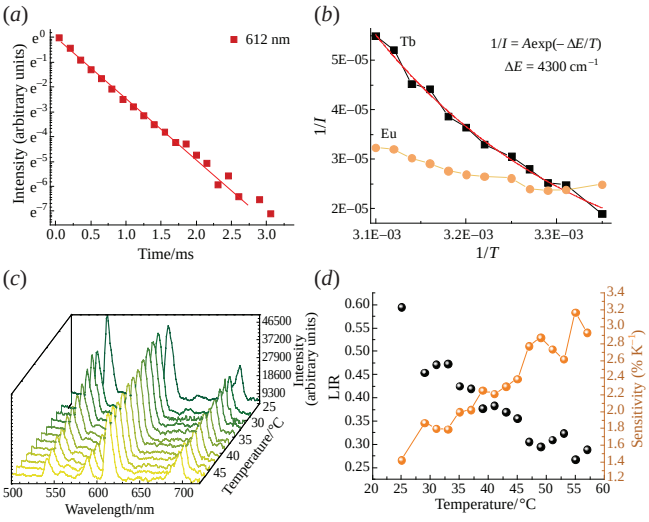

Figure 2 (*a*) Eu decay curve of Eu(L)$_3$·2H$_2$O and Tb(L)$_3$·2H$_2$O, 1 : 4 mixture (λ_{ex} = 303 nm, λ_{em} = 613 nm for Eu and 542 nm for Tb, T = 42 °C); (*b*) temperature dependence of terbium and europium luminescence intensity; (*c*) temperature dependence of a Eu(L)$_3$·2H$_2$O and Tb(L)$_3$·2H$_2$O mixture in water; and (*d*) LIR and S_r values for a Eu(L)$_3$·2H$_2$O and Tb(L)$_3$·2H$_2$O mixture in water.

Additionally, the thermometric properties of the above mixture were also studied in ethanol (see Figures S7–S9). The pronounced temperature dependence of the LIR with S_r of up to 2.1% K^{-1} allowed us to suggest using this system for systems other that biological objects.

Finally, we examined the effect of a $Eu(L)_3{\cdot}2H_2O$ and $Tb(L)_3{\cdot}2H_2O$ solution on the viability of HepG2 cells. Cell viability was assessed by double staining with the fluorescent dyes Hoechst 33342 and propidium iodide (PI) according to a standard method.[33] The complexes did not exhibit cytotoxicity in the test concentration range (0.2–20 μM), and the half-maximal lethal concentration (LC_{50} is the concentration of a compound at which the number of living cells is reduced by 50% compared to the control) was not reached. An increase in the number of apoptotic and dead cells was not observed in the test concentration range compared to control. The cytotoxic effect of the complexes was also studied in human fibroblasts MRC-5. Similarly to the effect on cancer cells, the complexes did not show cytotoxic activity (see Online Supplementary Materials for details).

Thus, this paper describes the synthesis of a novel bispidine-substituted benzoic acid ligand and its lanthanide complexes $Ln(L)_3{\cdot}2H_2O$ (Ln = Eu, Gd, and Tb). These complexes demonstrated very high solubility in water and intense luminescence in the visible range with quantum yields of up to 75% in powders and 46% in water solution. The mixture of $Eu(L)_3{\cdot}2H_2O$ and $Tb(L)_3{\cdot}2H_2O$ demonstrated a temperature dependence of luminescence in water solution, while the cellular studies revealed that the complexes did not show cytotoxic activity.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2024.10.012.

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