Stability and spectral properties of europium and zinc phenanthroline complexes as luminescent probes in high content cell-imaging analysis

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Summary

Novel phenanthroline derivatives and their europium(III) and zinc(II) complexes have been prepared in up to 92 %. In contrast to the stable zinc complexes, the europium compounds exhibit a strong luminescence in THF solution and complete dissociation of the complexes back to free ligands in DMSO. ¹H NMR studies of the Eu(III)-complexes **5** and **6** also confirmed the existence of different states depending on the solvent used. Moreover, it was found that the compound **5** is stable in EtOH-PBS solutions; here a strong signal in the emission spectra corresponding to the europium ion was detected. These metal complexes can be used as fluorescence markers for the diagnosis of esophageal squamous carcinoma (OE21) cells at low concentrations. Cell images were acquired using the compound **5**, **7-9** as luminescent markers. The first images were taken already after 20 min incubation time at a very low concentration range (0.7-1.6 μM).

Introduction

In the past decades, molecular imaging technology has become a central tool in biology, particularly in bioassays and cell imaging, cancer research, and clinical trials in medicine [1-4]. The development of novel luminescent probes for intracellular analysis, that are efficient and kinetically stable, is one of the vital goals. Rare earth metal complexes are now versatile targets in many areas of research such as photochemistry, biomedicine and nanotechnology [5-12]. Special attention has been given to the luminescence properties of lanthanide ions emitting at a millisecond lifetime-scale and in the visible region for europium(III) and terbium(III) complexes [13-16]. These remarkable photophysical properties are key factors to make them molecules of choice for cellular imaging and diagnostics [17-19]. A variety of the different Ln(III) complexes have been synthesized, however a main problem is their instability in aqueous media. The neutral ligands and the lanthanide ion are linked via weak coordination bonds and generally exist in an equilibrium “free ligand” $\rightleftharpoons$ “metal complex” that can be shifted in a medium via solvent exchange [20]. The metal complexes are prone to dissociation in the presence of solvent molecules. Disadvantageously, in some cases this can lead to an irreversible dissociation into the “free ligand” form in water through completion of the first coordination sphere [20-22] of a lanthanide ion with solvent molecules. This normally prevents the use of Ln(III) complexes as luminescent markers for bioassays. Nevertheless, only limited information is available on the real state of such metal complexes in different media.

Zinc compounds are known to be highly fluorescent and present an alternative to lanthanide complexes. Thus, many photochemical studies targeted at modeling the natural situation use the more stable zinc complexes. The propensity of zinc to act as an acceptor atom is used in many supramolecular approaches and/or for the modulation of chromophore properties *via* ligand binding. Due to their biological relevance, technical importance and good stability, they have found significant use as industrial pigments, electron transfer components, for photochemical transformations and in photobiotechnology [23].

Here, we have studied stability and photophysical efficiency of europium and zinc complexes of phenanthroline derivatives. These molecules are strongly absorbing chromophores often used as antennas for indirect excitation of metal ions, where direct excitation of a metal is inefficient. Phenanthroline itself is known to inhibit metalloproteases and its various metal complexes have been prepared and tested as bioprobes [24-29]. Here we present results based on the synthesis, behavior in the different solvents monitored by NMR spectroscopy, photophysical analysis and cellular imaging of novel metal complexes under low concentrations.

Results and Discussion

Synthesis

The neutral bidentate ligands **2-4** were synthesized using a simple two-step approach starting from commercially available 1,10-phenanthroline. 1,10-

Phenanthroline-5,6-dione **1** readily reacted with various aldehydes to give compounds **2-4** in up to 80 % (Fig. 1).

Figure 1. Synthesis of 1,10-phenanthroline-5,6-dione **1** and the derivatives **2-4**.

The metal complexes were prepared using the derivatives **2-4** and the corresponding metal salts in good yields. Formation of the europium(III) complexes achieved in ethanol in the presence of europium(III) trinitrate with the ligands **2-4** in a stoichiometric ratio of 1 to 3 yielding the compounds **5-7** in 58 %, 92 %, and 55 % yield, respectively (Fig. 2). The structure of the new europium compounds have been confirmed by MS-spectroscopy and ¹H NMR (in THF) analysis.

Figure 2. Synthesis of the Eu(III) complexes **5-7**.

The synthesis of the zinc(II) complexes **8-10** was carried out using ZnCl₂ in THF-MeOH in a similar manner to afford the compounds in up to 69 % yield (Fig. 3). Noteworthy, the different stoichiometric salt-ligand ratios result only in the formation of the 2 to 1 complexes **8** and **9**; neither 1:1 nor 3:1 complexes were observed, as reported in some cases.

Figure 3. Synthesis of the Zn(II) complexes **8-10**.

Photophysical behavior of compounds **5-10**

The influence of the structural differences on the photophysical properties in complexes **5-10** was investigated by comparing the spectroscopic profiles of the free ligands, zinc and europium complexes. The complexes **5-10** exhibited absorption bands between 280 and 400 nm that strongly depended on the ligation state and type of metal inserted. Slight shifts, intensity and shape differences were observed in the absorption spectra for the free ligand **2** and its europium **5** and zinc **9** complexes (Fig. 4).

Figure 4. Absorption spectra of the compounds **2**, **5** and **9** in THF (10^{-5} M).

Emission spectra for this series of compounds were recorded at various irradiation energies. All three types of compounds exhibited an emission band at 405 nm which is characteristic for the ligand's emission followed by an additional band corresponding to metal-ligand transitions (Fig. 5).

Figure 5. Comparison of the emission spectra of compound **2** and its europium **5** and zinc **9** complexes at excitation 280 nm in THF (10^{-6} M).

The europium complex **5** showed the characteristic bands of lanthanide ion emission between 670 nm and 710 nm. In the case of the zinc complex **9**, a split of the band at 410 nm was observed with the new maxima at 465 nm being the result of the complex formation (Table 1).

Table 1. Photophysical parameters of **5** and **6** in THF and **8-10** in MeOH.

Entry	Absorption maxima, nm	Emission maxima, nm ^{a)}
5	279; 325	405; 591; 617; 646; 681
6	282; 339	405; 591; 617; 650; 681
8	277; 319	400
9	283; 290; 318	560
10	294; 301	495

^{a)} Emission spectra recorded with 280 nm excitation

Luminescence: Solvents dependency and dissociation of the europium(III) complexes

A change in a photophysical profile of many optical materials such as a “switch-on” and “-off” can be achieved simply by varying a solvate medium through temporary quenching emission of one or more components of the system. However, in some cases these changes can be irreversible and can be used to detect a particular molecule or ion. We have analyzed the behavior of the phenanthrene-based europium complexes **5-7** under different conditions. Effects of the solvents can even be seen with the “naked eye”. Simple illumination of the different solutions of complex **5** with a standard laboratory UV-lamp revealed the differences (Fig. 5).

A detailed analysis of compounds **5-7** was performed in various solvents including THF, D₂O, EtOH and DMSO. The presence of the metal complexes was clearly verified for THF and D₂O solutions. Here the typical emission signals corresponding to $^5D_0 \rightarrow ^7F_{0,1,2,3,4}$ transitions of the europium (III) ion in **5** and **6** were observed (Fig. 6).

Figure 6. Solvent dependency of solutions of **5** in THF, EtOH, D₂O and DMSO (from left to right) irradiated with UV-light (365 nm).

Figure 7. Emission spectra of complex **5** in different solvents at 335 nm excitation and with a 420 nm filter.

No lanthanide emission was found for **5** and **6** in DMSO. Here only fluorescence corresponding to the ligand unit was detected. These results indicate that emission of the Eu(III) ion is either quenched by solvent molecules entering the first solvation sphere or indicate complete dissociation of the complex back to a free ligand and lanthanide salt with solvent molecules as new ligands:

Detailed studies on solvent exchange mechanisms had been carried out for the series of d- and f- metal complexes [20]. The solvent exchange rates differ greatly between harder and softer atom donors of a solvent and different solvent exchange mechanisms (associative vs. dissociative activation) had been proposed [20,30]. Certainly, other factors such as different steric and electronic characteristics of the system components, the presence of an extensive π -moiety on the *NN* part of the ligand, an ease of the π -system to interact with nonbonding electrons of the metal, flexibility and stretching of the chelate bite mode have to be taken in account.

NMR studies

As the luminescence studies indicated a possible decomposition of the europium (III) complexes **5** and **6** in DMSO we employed NMR analysis to determine the existence of different states for these materials. For this, the effects of the different deuterated solvents were analyzed by ^1H NMR spectroscopy. Figure 8 gives an example of the different behavior of the compound **5** in THF- d_8 , Methanol- d_4 and DMSO- d_6 .

Figure 8. ^1H NMR (400 MHz) of the Eu(III) complex **5** in different deuterated solvents.

Clearly, the europium complex **5** exists in its “metal complex”-form in THF solution. Here, the phenanthroline hydrogen atoms in closest proximity to the Eu(III)-centre are shifted up-field. This is in good agreement that europium(III) complexes have low paramagnetic relaxation enhancement (T_1 and T_2 shortening effect) and small paramagnetic shifts [20,30,31]. Remarkably, the methanolic solution represents a

middle stage of the dissociation, where the hydrogen atom signals of the phenanthroline are shifted and congregated together. The spectrum in DMSO represents an identical one for a “free ligand” **2**. Clearly, this is the only form that exists in DMSO for compound **5**. Similar results were reported for europium (III) complexes with phosphine oxides in DMSO- d_6 [32]. However, complex **7** showed a remarkable stability in comparison to **5** and **6** in a deuterated DMSO- D_2O solvent mixture.

Interestingly, the mass spectrometric analysis of compounds **5** and **6** carried out with a DMSO solution did not give any molecular ion corresponding to these complexes. Only the signal of the free ligand was detected in all cases. These results were observed for the complete series of the europium complexes and can be explained by a potential dissociation of the complexes through ligand exchange with solvent molecules. No such changes were observed for the zinc complexes **8-10** in various solvents, including MeOH and THF.

Aqueous solution

Analysis of biological systems requires work under aqueous conditions. Some luminescent materials can be reversibly quenched by water via formation of hydrogen bonds or directly by water molecules. Thus, it is important to establish the real state of the complex and its photophysical characteristics in aqueous solution before the substance enters a cell. It is necessary to establish whether any observed fluorescence quenching is the result of reduced emission of the europium centre or is due to irreversible decomposition of the metal complex. Here, the emission spectrum of compound **5** in EtOH-PBS buffer at physiological pH (7.4) is similar to one in THF. It clearly shows the strong signals of the europium-ion bonded to the phenanthroline ligand (Fig. 9). The materials **5** and **7** were stable in buffer solution for a quite some time (24-48 h). However, the compound **6** does not exhibit any lanthanide-corresponding emission under these conditions.

Figure 9. Emission spectrum of complex **5** in EtOH-PBS at pH 7.4.

Cell imaging

Esophageal cancer is a common and aggressive form of cancer and has shown a dramatic increase in Europe and North America in a past few years [33-36]. Survival rates in Europe are only about 10 % [37] and the incidence of this cancer, esp. in the UK and Eire, is rising [38]. Thus, we chose the esophageal SCC cell line OE21 for cell labeling studies [39].

The determination of the lowest staining concentration was performed in the 0.74 to 10 μM range. We have demonstrated that the complexes **5** and **7** are stable (up to 48 hours) in aqueous solutions (EtOH-PBS) and it still exhibits a strong luminescence (Fig. 9). The 1 mM stock ethanolic solutions of the compounds **5** and **7** were diluted with PBS buffer and an excess of crystals was filtered off to give the concentrations of the 0.74 μM for **5** and 8.2 μM for **7**. The fluorescence images were taken after 20 min and 1 h incubation times at 405 nm irradiation energy and detection at 535 nm (Fig. 10).

Figure 10. IN Cell images of the complexes **5** (a) and **7** (b) in OE21-fixed cells after 1h incubation (irradiation at 405 nm and detection at 535 nm).

The zinc complexes **8-10** were less fluorescent after one hour incubation at these concentrations, the fluorescent images were obtained for the zinc compounds **8** (1.6 μM) and **9** (0.84 μM) after 2 and 6 hours incubation (Fig. 11). Interestingly, the fluorescence of **9** diminished with time, and no intracellular fluorescence was observed for complex **10**.

Figure 11. IN Cell images of the complexes **8** (a) and **9** (b) in EtOH-PBS pH 7.4 for OE21-fixed cells after 2h incubation (irradiation at 405 nm and detection at 535 nm).

Experimental

Materials and methods

A 1 mM stock solution was prepared in THF, MeOH, DMSO and EtOH for each compound. Dilutions were carried out directly before starting the analyses. For cell stain imaging a stock solution prepared in EtOH was diluted with phosphate buffer saline (PBS) to different concentrations. Buffer solutions prepared at the desired concentrations were added directly to the cell culture. Total EtOH concentration was lower than 0.1 %. After adding compounds, cells were incubated at 37 °C in 5 % CO₂ for 20 minutes to 24 hours. Cells were routinely cultured in RPMI 1640 (Hyclone, USA) with 10 % inactivated foetal bovine serum (Hyclone, USA) and 1 % Penicillin/(Hyclone, USA). These cultures were grown in sterile filtered top cell culture flasks (Nunc, Denmark). Cultures were split 1:8 when 70-80 % confluency was reached. Cells were split using trypsin 0.25 % solution, with EDTA (Hyclone, USA) and kept at 37 °C and 5 % CO₂ in a humidified atmosphere. Culture medium was changed every 3 to 4 days until confluency. The cell lines were plated at a concentration of 1.0×10^5 cells.ml⁻¹ into sterile 96-well plates (Nunc, Denmark) and left to attach overnight.

Spectroscopy and analysis

¹H NMR spectra were recorded on a Bruker DPX 400 (400 MHz and 600 MHz for ¹H NMR). Chemical shifts are reported in ppm referred to TMS set at 0.00 ppm. All photophysical measurements were performed in THF, MeOH, EtOH, DMSO and PBS. All emission spectra were recorded on Fluorolog Horiba Jobin Yvon spectrometer. HRMS spectra were measured on MaldiQ-ToF Premier Micromass and Micromass/Waters Corp. USA liquid chromatography time-of-flight spectrometer equipped with ES source. UV-vis measurements were performed on Specord 250 Analytik Jena instrument. Melting points were acquired on Stuart SMP10 melting point apparatus and are uncorrected. Thin layer chromatography (TLC) was performed on silica gel 60F₂₅₄ (Merck) pre-coated aluminum sheets. Spectroscopy grade solvents were used in all cases. All commercial chemicals and solvents were supplied by Sigma-

Aldrich and used without further purification. Further details were as described before [40].

Syntheses

Synthesis of 1,10-phenanthroline-5,6-dione [41] 1: To a dry mixture of 1,10-phenanthroline (2 g, 0.011 mol) and KBr (10 g, 0.084 mol), H₂SO₄ (40 mL) followed by HNO₃ (20 mL) were added drop-wise at 0 °C. The resulting mixture was heated at 100 °C until the bromine vapors disappeared. The solution was poured carefully onto ice and slowly neutralized to pH>7 with Na₂CO₃ (saturated solution and powder). The product was extracted with dichloromethane and dried over Na₂SO₄. The solvents were evaporated to give a yellow solid that was dried under vacuum (88-95%). ¹H NMR (400 MHz, CDCl₃): δ 9.16 (dd, *J* = 4.6 Hz, *J* = 1.7 Hz, 2H); 8.54 (dd, *J* = 7.9 Hz, *J* = 1.6 Hz, 2H); 7.62 (dd, *J* = 7.9 Hz, *J* = 4.7 Hz, 2H).

Synthesis of imidazo[4,5-*f*]1,10-phenanthroline derivatives 2-4: 1,10-Phenanthroline-5,6-dione **1** (1 g, 4.76 mmol) and ammonium acetate (11.7 g, 0.152 mol) were dissolved in hot glacial acetic acid (50 mL) at ca. 70 °C and an appropriate aldehyde (4.76 mmol), dissolved in 20 mL of glacial acetic acid, was added. The resulting mixture was heated at 70-80 °C for 2 hours (TLC-control). Then the solution was allowed to cool to RT and was neutralized with 1N NaOH. The yellow compound was filtered, washed with water and dried under vacuum.

2-(4-Bromophenyl)imidazo[4,5-*f*]-1,10-phenanthroline 2: Yellow solid (1.43 g, 80%); analytical data were in accordance with the literature [42].

2-{4-[1'-(2'-Trimethylsilyl)ethynyl]phenyl}imidazo[4,5-*f*]-1,10-phenanthroline 3: Yellow solid (1.24 g, 66 %). Mp 258 °C; δ_H (400MHz, DMSO-*d*₆) 9.06 (m, 2H, H_{phenan}), 8.92 (d, *J* = 6.9 Hz, 2H, H_{phenan}), 8.29 (d, *J* = 8.4 Hz, 2H, H_{Ph}), 7.86 (dd, *J* = 8.0 Hz, *J* = 4.3 Hz, 2H, H_{phenan}), 7.70 (d, 2H, *J* = 8.4 Hz, H_{Ph}), 0.27 (s, 9H, TMS); δ_C (150.2 MHz, DMSO-*d*₆) 149.9, 148.3, 144.0, 132.6, 130.4, 129.9, 126.6, 123.3, 105.2, 96.5 (m); *m/z* (TOF MS) 393.1524 (M+H. C₂₄H₂₁N₄Si requires 393.1536); UV/Vis (CH₃OH): λ_{max} (lg ε) 283 (4.3), 335 (4.4).

2-(4-Pyridinyl)imidazo[4,5-*f*]-1,10-phenanthroline [23] 4: Brown solid (1.07 g, 76 %); δ_H (400MHz, DMSO-*d*₆) 9.20 (m, 2H, H_{phenan}), 8.92 (m, 4H, H_{phenan} and H_{Py}), 8.27 (m, 2H, H_{Py}), 7.97 (m, 2H, H_{phenan}). UV/Vis (CH₃OH): λ_{max} (lg ε) 273 (4.6), 322 (4.3), 387 (3.6).

Synthesis of the imidazo[4,5-*f*]-1,10-phenanthroline europium (III) complexes 5-7: To an appropriate imidazo[4,5-*f*]-1,10-phenanthroline derivative **2-4** (0.63 mmol) was dissolved in hot ethanol (100 mL) and an ethanolic solution of Eu(NO₃)₃•5H₂O (90 mg, 0.21 mmol) was added. The resulting mixture was heated at reflux until the starting material disappeared (TLC-control, ca. 30 hrs). The yellow solid formed was filtered, washed with ethanol and dried under vacuum to give the desired product **5-7**.

2-(4-Bromophenyl)imidazo[4,5-*f*]-1,10-phenanthroline europium(III) complex 5: Yellow solid (0.47 g, 58%). Mp > 300 °C; δ_H (400 MHz, THF-*d*₈, TMS) 13.19 (3H, br s, NH), 8.86 (6H, br m, H_{phenan}), 8.08 (16H, br m, H_{phenan} and H_{Ph}), 6.50 (8H, br m, H_{phenan}); *m/z* (TOF MS) 1274.9714 (M. C₅₇H₃₃Br₃EuN₁₂ requires 1274.9762).

2-{4-[1'-(2'-Trimethylsilyl)ethynyl]phenyl}imidazo[4,5-*f*]-1,10-

phenanthroline europium(III) complex 6: Yellow solid (0.77 g, 92 %). Mp > 300 °C; δ_{H} (400 MHz, THF- d_8 , TMS) 13.00 (br s, 3H, NH), 8.75 (br m, 6H, H_{phenan}), 8.18 (br m, 10H, H_{phenan} and H_{Ph}), 7.63 (m, 6H, H_{Ph}), 6.26 (br m, 8H, H_{phenan}), 0.20 (s, 27H, TMS); m/z (TOF MS) 1300.3098 (M-2CH₃+H. requires 1300.3193).

2-(4-Pyridinyl)imidazo[4,5-*f*]-1,10-phenanthroline europium(III) complex 7: Yellow solid (0.36 g, 55%). Mp >300 °C; δ_{H} (400 MHz, D₂O+DMSO- d_6 , TMS) 7.82 (br m, 30H, H_{phenan} and H_{Ph}), 0.52 (br, 3H, NH); m/z (ES) 968.17 (M-Py+H. C₅₀H₃₀EuN₁₄ requires 968.21).

Synthesis of imidazo[4,5-*f*]-1,10-phenanthroline zinc complexes 8-10: To an appropriate imidazo[4,5-*f*]-1,10-phenanthroline derivative **2** or **4** (0.54 mmol) dissolved in 10-20 mL of THF-MeOH (1:1, v/v) was added a solution of ZnCl₂ (0.27 mmol) in MeOH. A yellow precipitate was formed immediately after addition of the metal salt. The mixture was stirred at room temperature for 24 hours and filtered. The solid residue was washed with ethanol and dried *in vacuo*.

2-(4-Pyridinyl)imidazo[4,5-*f*]-1,10-phenanthroline zinc(II) complex 8: Pale yellow solid (36 mg, 20 %). Mp > 300 °C; δ_{H} (600 MHz, DMSO- d_6) 10.09 (s, 2H, NH), 9.16 (br, 8H, H_{phenan}), 8.96 (d, J = 4.6 Hz, 4H, H_{phenan}), 8.27 (br, 8H, H_{Py}); δ_{C} (150.2 MHz, DMSO- d_6) 162.1, 151.4, 144.1, 134.8, 133.3, 121.0; UV/Vis (CH₃OH): λ_{max} (lg ϵ) 277 (4.0), 319 (3.9).

2-(4-Bromophenyl)imidazo[4,5-*f*]-1,10-phenanthroline zinc(II) complex 9: Pale yellow solid (152 mg, 69 %). Mp > 300 °C. δ_{H} (600 MHz, DMSO- d_6) 14.22 (br, 2H, NH), 9.17 (br, 4H, H_{phenan}), 8.82 (m, 4H, H_{phenan}), 8.23 (d, 4H, H_{Ph}), 8.08 (dd, 4H, H_{Ph}), 7.84 (m, 4H, H_{phenan}); δ_{C} (150.2 MHz, DMSO- d_6) 150.8, 146.6 (m), 137.7, 132.1, 128.8, 125.6, 123.4; m/z (TOF MS) 812.9679 (M+H). C₃₈H₂₃N₈ZnBr₂ requires 812.9704; UV/Vis (CH₃OH): λ_{max} (lg ϵ) 283 (4.8), 390 (4.8), 318 (4.7).

(2-(4-Bromophenyl)-*N*-hexyl)imidazo[4,5-*f*]-1,10-phenanthroline zinc complex 10: To a solution of 2-(4-bromophenyl)imidazo[4,5-*f*]-1,10-phenanthroline **2** (230 mg, 0.605 mmol) in anhydrous DMF (10 mL), K₂CO₃ (170 mg, 1.21 mmol) was added under argon. The mixture was heated at 60 °C for 2 hours and 1-bromohexane (0.13 mL) was added. The reaction was refluxed at 80 °C for 48 hours and quenched with 10 mL of water. The product was extracted with 3 × 20 mL of CHCl₃, washed with water and dried over Na₂SO₄. The solvents were evaporated to give the crude product. To the residue (80 mg, 0.174 mmol) dissolved in 10 mL of THF-MeOH (1:1, v/v) was added a solution of ZnCl₂ (12 mg, 0.087 mmol) in MeOH. The reaction gave the complex **10** as a pale yellow solid (5 mg, 6 %). Mp 260 °C. δ_{H} (400 MHz, DMSO- d_6): 9.79 (d, J = 8.3 Hz, 2H, H_{phenan}), 9.54 (br, 2H, H_{phenan}), 9.23 (m, 4H, H_{phenan}), 8.47 (m, 2H, H_{phenan}), 8.32 (d, J = 8.3 Hz, 4H, H_{Ph}), 8.14 (m, 2H, H_{phenan}), 7.89 (d, J = 8.3 Hz, 4H, H_{Ph}), 5.92 (t, J = 7.0 Hz, 4H, CH₂), 2.09 (m, 4H, CH₂), 1.55 (m, 4H, CH₂), 1.37 (m, 8H, CH₂), 0.91 (t, J = 7.0 Hz, 6H, CH₃). m/z (TOF MS) 981.1537 (M+H. C₅₀H₄₇N₈ZnBr₂ requires 981.1582); UV/Vis (CH₃OH): λ_{max} (lg ϵ) 294 (4.2), 301 (4.2).

Conclusions

We have demonstrated that the new zinc(II) and europium(III) complexes **5-10** can be synthesized in the good yields. Emission spectra of the compounds **5-7** show a

strong signal corresponding to the Eu(III)-ion in solvents such as THF and D₂O. However, quenching of the emission is observed in DMSO. Moreover, NMR analysis also has confirmed that DMSO-d₆ solutions contain only the free ligand for the compounds **5** and **6**. Therefore, the materials **5** and **6** are prone to dissociation in media such as DMSO. Interestingly, that complex **7** is still present in the D₂O-DMSO-d₆ mixture. Analysis of the complexes **1-3** in EtOH-PBS aqueous buffer revealed an intense emission of the lanthanide ion for **5** and **7**, **6** however, exhibited a similar spectrum to the one in DMSO. No spectral changes were observed for the zinc(II) complexes, they were shown to be stable in the media. Fluorescence cell images were recorded at 20 min to 6 h incubation time points using the complexes **5-9**. The europium samples show a good intensity after 1h incubation and are photostable over 6h. In contrast, the zinc complexes required longer incubation times and are less photostable. Furthermore, a decrease in fluorescence over time occurred which is a result of photobleaching.

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References

- [1] Weissleder R, Pittet, MJ. Imaging in the era of molecular oncology. *Nature* 2008; 452:580-589.
- [2] Suhling K, French PMW, Phillips D. Time-resolved fluorescence microscopy. *Photochem Photobiol Sci* 2005, 4:13-22.
- [3] Berezin MY, Achilefu S. Fluorescence Lifetime Measurements and Biological Imaging. *Chem Rev* 2010; 110:2641-2684.
- [4] Kobayashi H, Ogawa M, Alford R, Choyke PL, Urano Y. New Strategies for Fluorescent Probe Design in Medical Diagnostic Imaging. *Chem Rev* 2010; 110:2620-2640.
- [5] dos Santos CMG, Harte AJ, Quinn SJ, Gunnlaugsson T. Recent developments in the field of supramolecular lanthanide luminescent sensors and self-assemblies. *Coord Chem Rev* 2008; 252:2512-2527.
- [6] Tsukube H, Shinoda S. Lanthanide complexes in molecular recognition and chirality sensing of biological substrates. *Chem Rev* 2002; 102:2389-2403.
- [7] Pandya S, Yu JH, Parker D. Engineering emissive europium and terbium complexes for molecular imaging and sensing. *Dalton Trans* 2006; 2757-2766.
- [8] Parker D, Dickins RS, Puschmann H, Cossland C, Howard JAK. Being excited by lanthanide coordination complexes: Aqua species, chirality, excited-state chemistry, and exchange dynamics. *Chem Rev* 2002; 102:1977-2010.

- [9] Bünzli JCG. Lanthanide-containing luminescent molecular edifices. *J Alloys Comp* 2006; 408:934-944.
- [10] Bünzli JCG. Luminescent lanthanide probes as diagnostic and therapeutic tools. *Metal Ions Biol Syst* 200; 42:39-75.
- [11] Hamacek J, Borkovec M, Piguet C. Simple thermodynamics for unravelling sophisticated self-assembly processes. *Dalton Trans* 2006; 1473-1490.
- [12] Piguet C, Borkovec M, Hamacek J, Zeckert K. Strict self-assembly of polymetallic helicates: the concepts behind the semantics. *Coord Chem Rev* 2005; 249:705-726.
- [13] Faulkner S, Pope SJA, Burton-Pye BP. Lanthanide complexes for luminescence imaging applications. *Appl Spectrosc Rev* 2005; 40:1-31.
- [14] Bünzli JCG, Piguet C. Taking advantage of luminescent lanthanide ions. *Chem Soc Rev* 2005; 34:1048-1077.
- [15] Binnemans K. Lanthanide-Based Luminescent Hybrid Materials. *Chem Rev* 2009; 109:4283-4374.
- [16] Moore EG, Samuel APS, Raymond KN. From Antenna to Assay: Lessons Learned in Lanthanide Luminescence. *Acc Chem Res* 2009; 42:542-552.
- [17] Bünzli JCG. Lanthanide Luminescence for Biomedical Analyses and Imaging. *Chem Rev* 2010; 110:2729-2755.
- [18] Barta CA, Sachs-Barrable K, Jia J, Thompson KH, Wasan KM, Orvig C. Lanthanide containing compounds for therapeutic care in bone resorption disorder. *Dalton Trans* 2007; 5019-5030.
- [19] Montgomery CP, Murray BS, New EJ, Pal R, Parker D. Cell-Penetrating Metal Complex Optical Probes: Targeted and Responsive Systems Based on Lanthanide Luminescence. *Acc Chem Res* 2009; 42:925-937.
- [20] Helm L, Merbach AE. Inorganic and bioinorganic solvent exchange mechanisms. *Chem Rev* 2005; 105:1923-1959.
- [21] Charbonnière LJ, Ziessel R, Montalti M, Prodi L, Zaccheroni N, Boehme C, Wipff G. Luminescent lanthanide complexes of a bis-bipyridine-phosphine-oxide ligand as tools for anion detection. *J Am Chem Soc* 2002; 124:7779-7788.
- [22] Sabbatini N, Guardigli M, Manet I, Bolletta F, Ziessel R. Synthesis and luminescence of lanthanide complexes of a branched macrocyclic ligand containing 2,2'-biopyridine and 9-methyl-1,10-phenanthroline subunits. *Inorg Chem* 1994; 33:955-959.
- [23] Senge MO, Sergeeva NN. Photochemical Transformations Involving Zinc Porphyrins and Phthalocyanines. In: Rappoport Z, Marek I, editors. *The Chemistry of Functional Groups. The chemistry of organozinc compounds*. John Wiley & Sons Ltd.: Chichester, 2006:395-419.
- [24] Ke HZ, Wang HD, Wong WK, Mak NK, Kwong DWJ, Wong KL, Tam HL. Responsive and mitochondria-specific ruthenium(II) complex for dual in vitro applications: two-photon (near-infrared) induced imaging and regioselective cell killing. *Chem Commun* 2010; 46:6678-6680.
- [25] Kieltyka R, Fakhoury J, Moitessier N, Sleiman HF. Platinum phenanthroimidazole complexes as G-quadruplex DNA selective binders. *Chem Eur J* 2008; 14:1145-1154.

- [26] Li YY, Cheng H, Zhang ZG, Wang C, Zhu JL, Liang Y, Zhang KL, Cheng SX, Zhang XZ, Zhuo RX. Cellular internalization and in vivo tracking of thermosensitive luminescent micelles based on luminescent lanthanide chelate. *ACS Nano* 2008; 2:125-133.
- [27] Neugebauer U, Pellegrin Y, Devocelle M, Forster RJ, Signac W, Moran N, Keyes TE. Ruthenium polypyridyl peptide conjugates: membrane permeable probes for cellular imaging. *Chem Commun* 2008; 5307-5309.
- [28] Cosgrave L, Devocelle M, Forster RJ, Keyes TE. Multimodal cell imaging by ruthenium polypyridyl labelled cell penetrating peptides. *Chem Commun* 2010; 46:103-105.
- [29] Puckett CA, Ernst RJ, Barton JK. Exploring the cellular accumulation of metal complexes. *Dalton Trans* 2010; **39**:1159-1170.
- [30] Peters JA, Huskens J, Raber DJ. Lanthanide induced shifts and relaxation rate enhancements. *Prog Nucl Magn Reson Spectrosc* 1996; 28:283-350.
- [31] Alpha B, Lehn JM, Mathis G. Luminescence probes – The Eu^{3+} -cryptates and Tb^{3+} -cryptates of polypyridine macrobicyclic ligands. *Angew Chem Int Ed* 1987; 26:1266-1267.
- [32] Iwanaga H, Amano A, Oguchi M. Study of molecular structures and properties of europium(III) complexes with phosphine oxides by NMR analysis. *Jap J Appl Phys* 2005; 44:3702-3705.
- [33] Umar SB, Fleischer DE. Esophageal cancer: epidemiology, pathogenesis and prevention. *Nature Rev. Gastroenterol. Hepatol.* 2008; 5:517-526.
- [34] La Vecchia C, Bosetti C, Lucchini F, Bertuccio P, Negri E, Boyle P, Levi F. Cancer mortality in Europe, 2000-2004, and an overview of trends since 1975. *Ann Oncol* 2010; 21:1323-1360.
- [35] Wild CP, Hardie LJ. Reflux, Barrett's oesophagus and adenocarcinoma: Burning questions. *Nature Rev Cancer* 2003; 3:676-U3.
- [36] Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. *CA Cancer J Clin* 2005; **55**:74-108.
- [37] Villette S, Pigaglio-Deshayes S, Vever-Bizet C, Validire P, Bourg-Heckly G. Ultraviolet-induced autofluorescence characterization of normal and tumoral esophageal epithelium cells with quantitation of NAD(P)H. *Photochem Photobiol Sci* 2006; 5:483-492.
- [38] O'Lorcain P, Deady S, Comber H. Mortality predictions for esophageal, stomach, and pancreatic cancer, Ireland, up to 2015. *Int J Gastrointest Cancer* 2006; 37:15-25.
- [39] Nishihira T, Hashimoto Y, Katayama M, Mori S, Kuroki T. Molecular and cellular features of esophageal cancer cells. *J Cancer Res Clin Oncol* 1993; 119:441-492.
- [40] Senge MO, Shaker YM, Pintea M, Ryppa C, Hatscher SS, Ryan A, Sergeeva Y. Synthesis of meso-Substituted ABCD-type Porphyrins via Functionalization Reactions. *Eur J Org Chem* 2010; 237-258.
- [41] Bodige S, MacDonnell FM. Synthesis of free and ruthenium coordinated 5,6-diamino-1,10-phenanthroline. *Tetrahedron Lett.* 1997; 38:8159-8160.

- [42] Xu H, Zheng KC, Chen Y, Li YZ, Lin LJ, Li H, Zhang PX, Ji LN. Effects of ligand planarity on the interaction of polypyridyl Ru(II) complexes with DNA. *Dalton Trans* 2003; 2260-2268.
- [43] Wang XL, Chen YQ, Gao Q, Lin HY, Liu GC, Zhang JX, Tian AX. Coordination Behavior of 5,6-Substituted 1,10-Phenanthroline Derivatives and Structural Diversities by Coligands in the Construction of Lead(II) Complexes. *Crystal Growth Des* 2010; 10:2174-2184.