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Europium 2-benzofuranoate: Synthesis and use for bioimaging

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ABSTRACT

Europium 2-benzofuranoate $Eu(BFC)_3(H_2O)_3$ was successfully used for bioimaging *in cellulo* due to the combination of high solubility and high luminescence intensity in solution. It was possible due to the purposeful variation of the aromatic core of carboxylate anion.

1. Introduction

Unique luminescent properties make lanthanide coordination compounds (CCs) perfect materials for bioimaging due to long lifetimes of the excited state and the high values of the Stokes shift [1–3]. Europium complexes are among the mostly used due to they combine high photoluminescence quantum yields (PLQYs) with the emission in the long wavelength range, which can easily penetrate through the tissues [4–6]. Among these materials, aromatic carboxylates play important role due to the high PLQY values and high chemical stability [7–12]. However, the crucial drawback is that they usually possess low solubility, particularly in water, due to the polymerization, which is a result of the lanthanide coordination sphere saturation [13–18].

To increase the solubility in water, the ligand fluorination approach was recently used with great success [19–21]. This, however, leads to the ligand's optical absorption decrease, which is important for bright photoluminescence, because its intensity is proportional to $I \sim \varepsilon \times PLQY$ (ε is absorption coefficient) [17–22]. Also complex dissociation in water was observed due to the high acidity of fluorobenzoic acids.

The best approach to increase light absorption by ligand is to increase the size of conjugated system, that is the number of conjugated double bonds (conjugation degree) [22–31]. So, the molar absorption coefficient increases by three orders of magnitude on gosing from benzene to naphthalene, anthracene, and tetracene [28]. At the same time, it results in significant decrease of the excited state energy [28], which in the case of lanthanide complexes leads to the absence of sensitization of lanthanide luminescence by organic ligand if triplet state energy becomes lower than the lanthanide excited state one. To restrain triplet state decrease, aromatic ring constriction and heteroatom introduction can be used. For instance, the triplet state of picolinate (25773 cm^{-1}) is higher than those of benzoate (21300 cm^{-1}), and 2-furanoate triplet state is even higher (29200 cm^{-1}) [32].

Lanthanide complexes with this anion were obtained, and europium 2-benzofuranoate was studied in order to reveal if it answers four basic requirements for the biomarker materials [1,2,33,34], namely: 1) high luminescence intensity, 2) high solubility, 3) the absence of dissociation in solution, 4) high absorption.

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2. Methodology

2.1. Materials and methods

All solvents, including deuterated solvents (D_2O, DMSO-d_6), and chemicals were purchased from commercial sources.

^1H NMR spectra were recorded at 25 °C using Agilent 400 MR spectrometer with the operating frequency of 400.130 MHz. Chemical shifts are reported in ppm relative to Me_4Si (^1H). *Elemental analysis* was performed on the microanalytical device Heraeus Vario Elementar. *Thermal analyses* were carried out on a thermoanalyzer STA 409 PC Luxx (NETZSCH, Germany) in the temperature range of 20–1000 °C in argon atmosphere, heating rate 10°/min.

Single crystal X-ray diffraction (SC-XRD) studies were carried out using a Bruker APEX Duo CCD diffractometer (ω-scans) at 120 K, using Mo Kα radiation *(λ = 0.71073 Å)*. Using Olex2 [35], the structure was solved with the ShelXT [36] structure solution program using Intrinsic Phasing and refined with the ShelXL [37] refinement package using Least Squares minimization. CCDC number 1521877 contains all crystallographic information and can be obtained free of charge via www.ccdc.ac.uk.

X-ray powder diffraction (XRD) measurements were performed on a Rigaku D/MAX 2500 diffractometer in the *2θ* range 5–80° with Cu Kα radiation (*λ = 1,54046* Å).

Absorption spectra were recorded in the region 250–800 nm with a Perkin–Elmer Lambda 650 spectrometer. *Emission spectra* was measured with a Fluorolog 3 spectrofluorometer over excitation with a xenon lamp. *Luminescence lifetime* measurements were recorded and detected on the same system. Lifetimes are averages of at least three independent measurements. All luminescence decays proved to be perfect single-exponential functions.

2.2. MTT protocol

The cytotoxicity of the lanthanide complexes in water was assessed using an MTT assay. To determine the toxic effect of complexes on HeLa cells, the CellTiter 96® Non-Radioactive Cell Proliferation Assay (Promega) was used. This assay is based on the intracellular reduction of a tetrazolium salt (yellow) into a formazan product (blue), which only takes place in metabolic active cells. The generated formazan is detectable at wavelengths between 630 and 750 nm and is a direct measure for the viability of cells. For this assay, each well of a 96 well plate (Cstar 3596, 96 Well Cell Culture Cluster, sterile) was seeded with 1×10^4 HeLa cells in 100 μl Dulbecco's modified Eagle's medium (DMEM, high glucose, gibco) supplemented with 10% fetal calf serum (FCS, PAA), and 1 U/mL Penicillin/Streptomycin at 37 °C, 5% CO2 and 95% humidity. After 24 h cells were incubated with a DMEM-solution of each sample studied. For each concentration 6 wells were prepared and incubated for 72 h. A set of positive (cells treated with 5 μl of 20% triton) and negative (untreated cells) control wells, as well as the test samples, were treated with 15 μl of the dye solution and incubated for 4 h 100 μl Solubilization Solution/Stop Mix is then added to each well to solubilize the formazan product, according to the manufacturer's manual. After 24 h incubation the absorbance at 595 nm using a 96-well plate reader (Ultra Microplate Reader ELx808, BioTEK Instruments, INC) was measured. The data was averaged and the multiple determination of each substance and concentration made it possible to calculate the standard deviation.

2.3. Confocal protocol

Two hours after seeding 1×10^4 HeLa cells per well plate were transferred into 8-well ibiTreat chamber slides (ibidi, Martinsried, Germany) in 0.2 ml of the medium. After 24 h, cells were treated 3 with the complex at the desired concentrations. After 24 h the cells were washed and then investigated using confocal microscopy. The images were taken using confocal laser scanning fluorescence microscope (Leica TCS SP5). Fluorescence excitation was performed by the 405-nm line of an Ar-ion laser 15%, resolution 8 bit, line average 16, format 1024 × 1024 pixels, 200 Hz. Fluorescence detection took place in red channel in the wavelength rage of 550–800 nm. Additionally, bright field images were recorded in a second independent channel.

2.4. Synthesis

Benzofuran-2-carboxylic acid was synthesized by known two-stage procedure from coumarin with 60% yield [38].

^1H NMR (400 MHz, DMSO-d_6, 27 °C) δ = 7.35 (m, 1 H, C^4H), 7.50 (m, 1 H, C^5H), 7.67 (s, 1 H, C^3H), 7.69 (m, 1 H, C^6H), 7.79 (d, 1 H, C^7H), 13.55 (br.s, 1H, COOH) ppm.

2.5. Synthesis of lanthanide complexes

2.5.1. $Ln(BFC)_3(H_2O)_3$ (Ln = Eu, Gd, Tb)

Complexes $Ln(BFC)_3(H_2O)_3$ (Ln = Eu, Gd, Tb) were synthesized by reaction of the 50% excess of wet freshly prepared $Ln(OH)_3$ (from aqueous ammonia and $LnCl_3 \cdot 6H_2O$, 0.93 mmol) and H(BFC) (1.85 mmol) in acetone-methanol mixture (3:1, 20 ml). A hydroxide excess was filtered off, and the obtained solution was rotor evaporated (30 min, 60 °C, 10 mmHg). The solid product was recrystallized from water and dried in air. X-ray-quality crystals were grown from methanol-acetone mixture 1:3.

$Eu(BFC)_3(H_2O)_3$: clcd. for $EuC_{27}H_{21}O_{12}$ (M = 689), %: C 47,02, H 3,05 found, %: C 47,04, H 3,06.

$Tb(BFC)_3(H_2O)_3$: clcd. for $TbC_{27}H_{21}O_{12}$ (M = 696), %: C 46,55, H 3,02 found, %: C 46,56, H 3,00.

$Gd(BFC)_3(H_2O)_3$: clcd. for $GdC_{27}H_{21}O_{12}$ (M = 694), %: C 46,69, H 3,03 found, %: C 46,73, H 3,01.

3. Results and discussion

Lanthanide complexes $Ln(BFC)_3(H_2O)_3$ (Ln = Eu, Gd, Tb) were obtained by the interaction between freshly prepared lanthanide hydroxide and acid solution in acetone:methanol mixture (3:1), followed by the recrystallization from water [20]. During the reaction the hydroxide dissolution due to complex formation was observed, preliminary indicating that complexes $Ln(BFC)_3(H_2O)_3$ are indeed soluble in this solvent.

Luminescent properties investigation has revealed that $Eu(BFC)_3(H_2O)_3$ exhibits bright lanthanide-centred luminescence with the lifetime of 0.36 ms, sensitized by BFC ligand. The BFC^- triplet state energy of 20400 cm^{-1} was determined from the phosphorescence spectrum of $Gd(BFC)_3(H_2O)_3$ (Fig. 2). This value is rather low, resulting in Tb → BFC back energy transfer, which is vibrationally quenched at room temperature. At low temperatures, when vibrations “freeze”, no quenching is observed, and the intense terbium-centred luminescence of $Tb(BFC)_3(H_2O)_3$ was detected. The mechanism of this behaviour was recently studied in

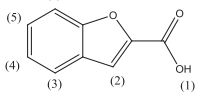

Fig. 1. Benzofurane-2-carboxylic acid (HBFC). Positions of H atoms are enumerated for signals in ^1H NMR spectra assignment.

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