Supporting Information

Efficient Two-Photon-Sensitized Luminescence of a Novel Europium(III) β -Diketonate Complex and Application in Biological Imaging

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1. Synthesis and Characterization

The ¹H NMR spectra recorded on at 25°C using Bruker Avance spectrometer were reported as parts per million (ppm) from TMS (δ). Coupling constants J were given in Hertz. Mass spectra were determined with a Micromass GCT-MS (EI source).

All chemicals were available commercially and every solvent was purified as conventional methods before use. 9-hexyl-carbazole, ethyl trifluoroacetate, $Eu(NO_3)_3$ ·6H₂O were purchased from Aldrich. 1-bromopentane, NaOH, acetone, EtOH and dichloromethane were purchased from Shanghai Reagents.



9-hexyl-carbazole: To a solution of 9-H-carbazole (16.7 g, 100 mmol) and NaOH (6.0 g, 150 mmol) in acetone (100 mL) was refluxed for 2 hours. After cool to room temperature, 14 mL 1-bromopentane (16.5 g, 100 mmol) were added and refluxed for 24 hours. The solvent was removed by rotary evaporation and 200 mL water was

added with stirring. The residual solid was filtered and recrystallized from EtOH and water to give colorless crystal product (20.1 g , yield 80.0 %). Ms m/z (%): 251.17, 166.03(100) Anal. Calc. for C₁₈H₂₁N: C 86.01, H 8.42, N 5.57 %; found C 85.93, H 8.38, N 5.67 %.

3-acetyl-9-hexyl-carbazole: To a solution of 9-hexyl-carbazole (5.0 g, 20 mmol) in dichloromethane (80 mL) was rapidly added AlCl₃ (5.2 g, 40 mmol) with stirring. After cooling to 0°C, a solution of acetic anhydride (2.04 g, 20 mmol) in 20 mL of dichloromethane was added dropwise over 10 min under vigor stirring. After stirring overnight at room temperature, a large amount of water and HCl was added into the mixture and extracted with dichloromethane twice, washed by 1M NaHCO₃ and water. The combined organic phase was dried over anhydrous MgSO₄ and then filtered. The organic solvent was completely removed by rotary evaporation. The residue was purified by column chromatograph using petroleum ether and ethyl acetate as eluent to give the purified title compound (4.2 g, 71.6 %). Ms m/z (%): 293.17, 222.09(100). Anal. Calc. for C₂₀H₂₃NO: C 81.87, H 7.90, N 4.77 %; found C 81.89, H 7.78, N 4.90 %.

4,4,4-trifluoro-1-(9-hexylcarbazole-3-yl)-1,3-butanedione (HTHA): То а solution of ethyl trifluoroacetate (1.71 g, 12 mmol) in anhydrous EtOH (20 mL) was added t-BuOK (3.33 g, 15 mmol). A solution of 3-acetyl-9-hexyl-carbazole (2.93 g, 10 mmol) in 10 mL of anhydrous EtOH was added dropwise over 5 min, and then stirring for 6 hours at room temperature. After 3 M HCl was added to the mixture with stirring, the mixture was extracted with dichloromethane and the organic layer was collected. The organic layer was wished with brine, dried over anhydrous MgSO4 and filtered. The organic solvent was completely removed by rotary evaporation. The residue was purified by recrystalized from the petroleum ether and ethyl acetate to give the yellowish crystal of the title compound (4.51 g, 85.6 %). MS m/z (%): 389.16, 318.08(100). ¹H NMR (400 MHz, CDCl₃) δ : 15.76 (s, 1H), 8.77 (d, J = 1.45 Hz, 1H), 8.20 (d, J = 7.75 Hz, 1H), 8.09-8.11 (m, 1H), 7.55-7.59 (m, 1H), 7.46-7.48 (m, 2H), 7.36 (t, J = 7.44 Hz, 1H), 6.73(s, 1H), 4.35 (t, J = 7.28 Hz, 2H), 1.88-1.36 (m, 2H), 1.36 (m, 6H), 0.89 (t, J = 7.07 Hz, 3H). Anal. Calc. for C₂₂H₂₂F₃NO₂: C 67.86,

H 5.69, N 3.60; Found C 67.89, H 5.75, N 3.57 %.

(1,10-phenanthroline)tris[4,4,4-trifluoro-1-(9-hexylcarbazole-3-yl)-1,3-butaned ione] europium(III) (Eu(THA)₃Phen): Eu(THA)₃Phen was prepared by the conventional method. To a solution of HTHA (541.6 mg, 1.5 mmol) and 1,10-phenanthroline(99.1 mg, 0.5mmol) in EtOH (15 mL), which had been neutralized with 1.5 mL aqueous NaOH solution (1 M), was added dropwised an aqueous of Eu(NO₃)₃:6H₂O (223.0 mg, 0.5 mmol). Then, the mixture was stirred at 80 °C for 12 hours. The precipitated product was filtered and washed by water. The products were dried at 60 °C in vacuum for 12 hours. The pale red solid was obtained (0.53 g, 71 %). The crystal suit for single crystal X-ray determination was obtained from acetone/EtOH (1:20) for two weeks. ¹H NMR (d_6 -DMSO, 500 MHz), δ : 9.08 (d, J = 3.15 Hz, 2H), 8.56 (s, 1H), 8.48 (d, J = 7.78 Hz, 2H), 8.08-8.10 (m, 2H), 7.98 (s, 2H), 7.75-7.78 (m, 2H), 7.63-7.69 (m, 3H), 7.58(d, J = 8.16 Hz, 4H), 7.52 (t, J = 7.82 Hz, 4H), 7.36 (d, J = 7.52, 3H), 7.29 (d, J = 7.18, 2H), 7.06 (s, 3H), 6.88 (t, J = 8.40, 1H), 6.73 (t, J = 8.41, 1H), 4.41 (t, J = 11.72, 6H), 1.64-1.75 (m, 6H), 1.03-1.21 (m, 18H), 0.76 (t, J = 5.45, 9H). Anal. Calcd. for C₇₆H₇₁O₆N₅F₉Eu: C 62.57, H 4.78, N 4.68 %; found C 62.42, H 4.96, N 4.57 %.

2. Determination of Single Crystals

X-Ray diffraction data of single crystals were collected on a Siemens Smart 1000 CCD diffractometer. The determination of unit cell parameters and data collections were performed with Mo-K α radiation ($\lambda = 0.71073$ Å). Unit cell dimensions were obtained with least-squares refinements, and all structures were solved by direct methods using SHELXS-97. The other non-hydrogen atoms were located in successive difference Fourier syntheses. The final refinement was performed by full-matrix least-squares methods with anisotropic thermal parameters for non-hydrogen atoms on F2. The hydrogen atoms were added theoretically and riding on the concerned atoms.



Fig. S1 The molecular structure of the ligand and complex: a) **HTHA** obtained from a crystal grain of $0.49 \times 0.24 \times 0.20$ mm; b) Eu(**THA**)₃Phen obtained from a crystal grain of $0.50 \times 0.20 \times 0.10$ mm.

Some hydrogen atoms omitted for clarity.

The single crystals of HTHA and Eu(**THA**)₃Phen were obtsained by crystallization from their solutions of ethyl acetate/petroleum ether (1:3) and acetone/ethanol (1:20), respectively. The crystallographic data are listed in Table S1. An ORTEP diagram for the common repeating unit is shown in Fig. S1a. The molecular structure of **HTHA** reveals a planar arrangement of non-hydrogen atoms (O1, C13, C14, C15, O2, mean deviation of 0.0106 Å), and H1 lies 0.0401 Å from this plane. The carbonyl bond lengths for C13–O1 and C15–O2 are 1.278(8) and 1.255(8) Å, respectively. There is an intramolecular hydrogen bond between the atoms O1 and O2 (O1–H1^{...}O2, 2.502 Å, 151.05°). These data suggest that, in the ring consisting of O1/O2, the enolic proton is not shared symmetrically with the carbonyl oxygens; that is, **HTHA** exists in the enol form in the solid state. N-alkyl substituted carbazole ring (consisting of C1~C17 and N1) is highly planar (deviation of 0.0217 Å). The dihedral angle between two defined planes is about 7.48°, revealing the whole molecule is almost coplanar with slightly deviation of C16.

As shown in Fig. S1b, the asymmetric unit contains a mononuclear Eu(III) ion coordinated to three bidentate THA⁻ ligand anions and to two nitrogen atoms of a 1,10-phenanthroline, in a typical eight-coordination fashion. The symmetry can be approximately described as a distorted square antiprism, with the two "square" planes being defined by O1~O2~O5~O6 and by O3~O4~N4~N5. The dihedral angle between two planes is 3.82°. The Eu–O bond lengths range from 2.332(3) to 2.409(4) Å, and there is significant deviation from the average Eu–O bond length of 2.359 Å, slightly smaller than the sum of ionic radii of Eu^{3+} 1.07 Å (eight-coordinated) and O^{2-} 1.42 Å); [1] the anionic **THA** appear as typical O,O-chelates via the ketone functional groups, forming six-membered rings with an average bite angle of 72.12°, which is in accordance with those found in related Eu(III)- β -diketonate complexes.[2] These features indicate that novel β -diketonate **THA**⁻ has a strong coordinating ability. On the other hand, the two Eu-N bonds are 2.573(4) and 2.607(5) Å, respectively. Eight bonds with different lengths give rise to a strongly distorted square anitprism environment around the Eu(III) site. For a regular square antiprism, the two square planes are parallel to each other and one square is rotated by 45° with respect to the other. However, for Eu(THA)₃Phen, relative to the bottom plane, the top plane is rotated by the smaller angle of 36.72°. So the Eu(III) cation factually has a geometrical environment intermediate between a square antiprism (D_{4d} symmetry) and a dodecahedron (D_{2d} symmetry). This kind of coordination configuration around Eu(III) leads to the very low structural symmetry of Eu(THA)₃Phen.[3] One of three -CF₃ groups in complex shows disorder, which maybe affect the polarization of the complex.[4]

Compound reference	НТНА	Eu(THA) ₃ Phen
Empirical formula	$C_{22}H_{22}F_3NO_2$	C ₇₈ H ₇₁ O ₆ N ₅ F ₉ Eu
Formula Mass	389.41	1497.36
Crystal system	monoclinic	triclinic
a/Å	5.027(2)	12.548(1)
b/Å	14.846(3)	16.139(1)
$c/{ m \AA}$	26.355(3)	21.028(1)
$\alpha/^{\circ}$	90	69.106(1)
$\beta^{ m o}$	92.576(2)	87.515(1)
$\gamma/^{o}$	90	86.340(1)
Unit cell Volume/Å ³	1964.8(10)	3969.3(4)
Temperature/K	298(2)	298(2)
Space group	$P2_{1}/c$	$P\overline{\iota}$
No. of formula units per unit cell, Z	4	2
Radiation type	Μο-Κα	Μο-Κα
Absorption coefficient, μ/mm^{-1}	0.103	0.861
No. of reflections measured	8354	28929
No. of independent reflections	3010	13957
R _{int}	0.1596	0.0332
Final R_I indices $[I > 2\sigma(I)]$	0.0959	0.0464
Final $wR(F_2)$ values $(I > 2\phi(I))$	0.1870	0.1339
Final R_1 values (all data)	0.2766	0.0655
Final $wR(F_2)$ values (all data)	0.2361	0.1481
Goodness of fit on F_2	1.000	0.963
CCDC Number	CCDC 838899	CCDC 838898

 Table S1. Crystallographic data and refinement details

Table S2. Selected bond lengths (Å) and angles (°) for ligand and complex

НТНА					
C13-O1	1.278(8)	C15-O2	1.255(8)	C13-C14	1.420(9)
C14-C15	1.323(9)	C15-C16	1.515(10)	C13-C4	1.494(9)
H1-O1	0.820	H1-O2	1.746		
Eu(THA) ₃ Phen					
Eu1-O1	2.395(4)	Eu1-O2	2.337(4)	Eu1-O3	2.332(3)
Eu1-O4	2.409(4)	Eu1-O5	2.347(4)	Eu1-O6	2.338(3)
Eu1-N4	2.573(4)	Eu1-N5	2.607(5)	O2-Eu1-O1	71.38(13)
O5-Eu1-O6	73.33(13)	O3-Eu1-O4	71.65(12)	N4-Eu1-N5	62.86(16)

3. Computational

Optimization was performed by B3LYP [6-31G(d)] without any symmetry restraints. The TDDFT {B3LYP[6-31G(d)]} calculations were then performed on the optimized structure [5]. All the calculations, including optimizations and TDDFT, were conducted with the G03 software [6]. Geometry optimization of the singlet ground state and the TDDFT calculation of the lowest 25 singlet–singlet excitation energies were calculated with a basis set composed of 6-31G* for C N O [7]. An analytical frequency analysis provides evidence that the calculated species represents a true minimum without imaginary frequencies on the respective potential energy surface.

Table S3. TD-DFT calculated Singlet	→Singlet Excitation	Energies(E), and	Oscillator Strengths(f) of
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$\lambda(nm)$	<i>E</i> (eV)	f	Composition	Exp.
367.8	3.37	0.6422	$101 \rightarrow 103(17\%), 102 \rightarrow 103(83\%)$	360
335.7	3.69	0.0314	101→103(62%), 102→104(38%)	345
301.6	4.12	0.2940	100→103(11%), 101→103(20%)	
			101→104(7%), 102→104(32%)	
293.4	4.23	0.0260	100→103(61%), 101→104(16%)	278
			102→104(23%)	
262.0	4.73	0.1350	100→103(11%), 100→104(14%)	
			101→104(45%), 102→105(31%)	

HTHA

4. Photophysical Properties

UV-Vis absorption spectra were recorded on UV-3100 spectrophotometer (Shimadzu). Fluorescence measurements were carried out with an Edinburgh FLS920 fluorescence spectrometer equipped with a 450 W Xe lamp and a time correlated single-photon counting (TCSPC) card. All the fluorescence spectra were collected. The single-photon excited fluorescence (SPEF) quantum yields Φ were measured by using a standard method under the same experimental conditions for all compounds. Rhodamine B in ethanol ($\Phi_{ref} = 0.69$), at the same concentration as the other sample, was used as the standard. [8]

$$\Phi = \Phi_{ref} \frac{F}{F_{ref}} \frac{A_{ref}}{A} \frac{n^2}{n_{ref}^2}$$

Here, the subscripts *ref* stands for the reference molecule. Φ is the fluorescence quantum yield. A is the absorption at excitation wavelength, n is the refractive index of the solution, F is the integrated area of the detected fluorescence signal.

Lifetime value of **HTHA** was obtained by re-convolution fit analysis of the decay profiles with the aid of F900 analysis software. The fitting results were judged by their values of "reduced chi-aquared". Lifetime of Eu(**THA**)₃Phen was recorded at ambient temperature with a steady-state/lifetime spectrofluorometer (FLUOROLOG -3-TAU, Jobin Yvon)



Fig. S2 Time resolved fluorescence decay of Eu(THA)3Phen. The signals were monitored at 613

Two-photon excited fluorescence (TPEF) spectra were measured using a mode-locked Ti:sappire femtosecond laser (Spectra-Physics, Tsunami 3941, 700-910 nm, 80 MHz, <120fs) which was pumped by a compact cw prolite diode laser (Spectra-phusics, Millennia Pro 5S). The fluorescence signal was recorded by a fluorescence spectrophotomter (Ocean Optics, USB2000). All measurements were carried out in air at ambient temperature. The TPA cross-sections have been measured using the two-photon-induced fluorescence measurement technique with the following equation:[9]

$$\sigma = \sigma_{ref} \frac{\Phi_{ref}}{\Phi} \frac{c_{ref}}{c} \frac{n_{ref}}{n} \frac{F}{F_{ref}}$$

Here, the subscripts *ref* stands for the reference molecule. σ is the TPA cross-section value, *c* is the concentration of solution, *n* is the refractive index of the solution, *F* is the integrated area of the detected two-photon induced fluorescence signal, and Φ is the fluorescence quantum yield. σ_{ref} values for fluorescein in H₂O (pH~13) were taken from reference for comparison.[10]



Fig. S3 The linear dependence of Lg I_{out} on Lg I_{in} of two initiators. Excitation carried out at 720 nm

5. Cell culture and Two-Photon Laser Confocal Microscopy

MCF-7 cells were seeded in 6 well plates at a density of 2×10^5 cells per well and grown for 96 hours. The complex was dissolved in DMSO at high concentration in 20 mM and diluted by PBS (Phosphate Buffer Solution) to working concentration. For live cell imaging cell cultures were incubated with the complex (10% PBS: 90% cell media) at concentrations 400 and 200 μ M and maintained at 37°C in an atmosphere of 5% CO₂ and 95% air for incubation times ranging for 1 hour. The cells were then washed with PBS (3 × 3 mL per well) and 3 ml of PBS was added to each well. The cells were imaged using a Zeiss LSM 510 META upright confocal laser scanning microscope using magnification 40x water-dipping lenses for monolayer cultures. Excitation energy of 770 nm was used and the fluorescence emission measured at 615nm. MCF-7 cells were luminescently imaged on image data acquisition and processing was performed using Zeiss LSM Image Browser, Zeiss LSM Image Expert.

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