CHEMISTRY
A European Journal

Supporting Information

One-Step Assembly of Visible and Near-Infrared Emitting Metallacrown Dimers Using a Bifunctional Linker

Tu N. Nguyen,[a] Chun Y. Chow,[a] Svetlana V. Eliseeva,*[b] Evan R. Trivedi,[a] Jeff W. Kampf,[a] Ivana Martinić,[b] Stéphane Petoud,*[b, c] and Vincent L. Pecoraro*[a]

chem_201703911_sm_miscellaneous_information.pdf

Supporting Information

One-step Assembly of Visible and Near-Infrared Emitting Metallacrown Dimers Using a Bifunctional Linker

Tu N. Nguyen,[†] Chun Y. Chow,[†] Svetlana V. Eliseeva* [‡] Evan R. Trivedi,[†] Jeff W. Kampf,[†] Ivana Martinić,[‡] Stéphane Petoud,*,[‡,§] and Vincent L. Pecoraro*,[†]

[†]Department of Chemistry, Willard H. Dow Laboratories, University of Michigan, Ann Arbor, Michigan 48109, United States

[‡]Centre de Biophysique Moléculaire, CNRS UPR 4301, F-45071 Orléans Cedex 2, France

[§] Department of Inorganic, Applied and Analytical Chemistry, University of Geneva, 1211 Geneva 4, Switzerland

Synthesis and characterizations of $[Ga_8Ln_2(shi)_8(ip)_4(DMF)_2(H_2O)_2](NH_4^+)_2 \cdot xDMF \cdot yH_2O$ complexes (Ln = Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, and Yb), and the Yttrium analogue

All reagents and chemicals were purchased from commercial sources and used without further purification. All reactions were carried under aerobic conditions. Elemental analysis was performed by Atlantic Microlabs Inc. ESI-MS spectra were collected with a QTOF mass spectrometer in negative ion mode on samples dissolved in DMSO.

H_3shi (306.3 mg, 2.0 mmol), isophthalic acid (166.1 mg, 1.0 mmol), $Ga(NO_3)_3 \cdot xH_2O$ (511.5 mg, 2.0 mmol), and $Ln(NO_3)_3 \cdot xH_2O$ (0.50 mmol) were dissolved in 18.0 mL of DMF. NH_4HCO_3 (632.5 mg, 8.0 mmol) was added to the solution and the reaction mixture was stirred for 30 mins. The solution was filtered and left for slow evaporation. Pure crystals of $[Ga_8Ln_2(shi)_8(ip)_4(DMF)_2(H_2O)_2](NH_4^+)_2 \cdot xDMF \cdot yH_2O$ (**$[LnGa_4]_2$**) were formed after two weeks and then collected by filtration and dried in air. Single crystals were screened for unit cell parameters.

$[Ga_8Pr_2(shi)_8(ip)_4(DMF)_2(H_2O)_2](NH_4^+)_2 \cdot 12DMF$ (**$[PrGa_4]_2$**). Yield: 33.9 mg (3.6%). ESI-MS, calc. for $[M]^{2-}$, $Pr_2Ga_8C_{88}H_{48}N_8O_{40}$, 1347.7; found, 1347.7. Anal. Calcd. for $Pr_2Ga_8C_{130}H_{158}N_{24}O_{56}$: C, 41.17; H, 4.20; N, 8.86. Found: C, 40.98; H: 4.46; N: 9.22. Single-crystal unit-cell parameters: a = 14.193Å, b = 17.696 Å, c = 19.169 Å; α = 113.22°, β = 103.24°, γ = 97.65°; V = 4170.37 Å^3.

$[Ga_8Nd_2(shi)_8(ip)_4(DMF)_2(H_2O)_2](NH_4^+)_2 \cdot 12DMF$ (**$[NdGa_4]_2$**). Yield: 98.0 mg (10.4%). ESI-MS, calc. for $[M]^{2-}$, $Nd_2Ga_8C_{88}H_{48}N_8O_{40}$, 1349.7; found, 1349.7. Anal. Calcd. for $Nd_2Ga_8C_{130}H_{158}N_{24}O_{56}$: C, 41.10; H, 4.19; N, 8.85. Found: C, 40.96; H: 4.39; N: 9.36. Single-crystal unit-cell parameters: a = 14.218 Å, b = 17.725 Å, c = 19.251 Å; α = 113.19°, β = 103.33°, γ = 97.63°; V = 4201.57 Å^3.

$[Ga_8Sm_2(shi)_8(ip)_4(DMF)_2(H_2O)_2](NH_4^+)_2 \cdot 12DMF$ (**$[SmGa_4]_2$**). Yield: 195.6 mg (20.5%). ESI-MS, calc. for $[M]^{2-}$, $Sm_2Ga_8C_{88}H_{48}N_8O_{40}$, 1357.9; found, 1357.9. Anal. Calcd. for $Sm_2Ga_8C_{130}H_{158}N_{24}O_{56}$: C, 40.97; H, 4.18; N, 8.82. Found: C, 40.67; H: 4.51; N: 8.96. Single-crystal unit-cell parameters: a = 14.152 Å, b = 17.741 Å, c = 19.188 Å; α = 112.91°, β = 102.71°, γ = 98.27°; V = 4185.38 Å^3.

$[Ga_8Eu_2(shi)_8(ip)_4(DMF)_2(H_2O)_2](NH_4^+)_2$ · 12DMF **([EuGa$_4$]$_2$)**. Yield: 196.0 mg (20.6%). ESI-MS, calc. for $[M]^{2-}$, $Eu_2Ga_8C_{88}H_{48}N_8O_{40}$, 1358.7; found, 1358.7. Anal. Calcd. for $Eu_2Ga_8C_{130}H_{158}N_{24}O_{56}$: C, 40.93; H, 4.18; N, 8.81. Found: C, 40.75; H: 4.49; N: 9.25. Single-crystal unit-cell parameters: *a* = 14.123 Å, *b* = 17.759 Å, *c* = 19.157 Å; α = 112.94°, *β* = 102.70°, γ = 98.36°; V = 4171.28 Å^3.

$[Ga_8Gd_2(shi)_8(ip)_4(DMF)_2(H_2O)_2](NH_4^+)_2$ · 12DMF (**[GdGa$_4$]$_2$**). Yield: 321.0 mg (33.7%). ESI-MS, calc. for $[M]^{2-}$, $Gd_2Ga_8C_{88}H_{48}N_8O_{40}$, 1364.7; found, 1364.7. Anal. Calcd. for $Gd_2Ga_8C_{130}H_{158}N_{24}O_{56}$: C, 40.82; H, 4.16; N, 8.79. Found: C, 40.98; H: 4.28; N: 8.99. Single-crystal unit-cell parameters: *a* = 14.104 Å, *b* = 17.581 Å, *c* = 19.217 Å; α = 113.09°, *β* = 102.60°, γ = 98.35°; V = 4134.44Å^3.

$[Ga_8Tb_2(shi)_8(ip)_4(DMF)_2(H_2O)_2](NH_4^+)_2$ · 12DMF (**[TbGa$_4$]$_2$**). Yield: 329.8 mg (34.6%). ESI-MS, calc. for $[M]^{2-}$, $Tb_2Ga_8C_{88}H_{48}N_8O_{40}$, 1366.7; found, 1366.7. Anal. Calcd. for $Tb_2Ga_8C_{130}H_{158}N_{24}O_{56}$: C, 40.79; H, 4.16; N, 8.78. Found: C, 40.62; H: 4.45; N: 8.81. Single-crystal unit-cell parameters: *a* = 14.105 Å, *b* = 17.595 Å, *c* = 19.248 Å; α = 113.24°, *β* = 102.63°, γ = 98.16°; V = 4142.24 Å^3.

$[Ga_8Dy_2(shi)_8(ip)_4(DMF)_2(H_2O)_2](NH_4^+)_2$· 12DMF (**[DyGa$_4$]$_2$**). Yield: 280.1 mg (29.2%). ESI-MS, calc. for $[M]^{2-}$, $Dy_2Ga_8C_{88}H_{48}N_8O_{40}$, 1369.7; found, 1369.7. Anal. Calcd. for $Dy_2Ga_8C_{130}H_{158}N_{24}O_{56}$: C, 40.71; H, 4.15; N, 8.76. Found: C, 40.75; H: 4.45; N: 8.87. Single-crystal unit-cell parameters: *a* = 14.1080 Å, *b* = 17.5806 Å, *c* = 19.2197 Å; α = 113.107°, *β* = 102.699°, γ = 98.218°; V = 4135.39 Å^3.

$[Ga_8Ho_2(shi)_8(ip)_4(DMF)_2(H_2O)_2](NH_4^+)_2$ · 12DMF (**[HoGa$_4$]$_2$**). Yield: 322.0 mg (33.7%). ESI-MS, calc. for $[M]^{2-}$, $Ho_2Ga_8C_{88}H_{48}N_8O_{40}$, 1371.7; found, 1371.7. Anal. Calcd. for $Ho_2Ga_8C_{130}H_{158}N_{24}O_{56}$: C, 40.66; H, 4.15; N, 8.75. Found: C, 40.31; H: 4.38; N: 9.02. Single-crystal unit-cell parameters: *a* = 14.114 Å, *b* = 17.658 Å, *c* = 19.239 Å; α = 113.06°, *β* = 102.65°, γ = 98.26°; V = 4161.85 Å^3.

$[Ga_8Er_2(shi)_8(ip)_4(DMF)_2(H_2O)_2](NH_4^+)_2$ · 12DMF (**[ErGa$_4$]$_2$**). Yield: 276.2 mg (28.7%). ESI-MS, calc. for $[M]^{2-}$, $Er_2Ga_8C_{88}H_{48}N_8O_{40}$, 1373.7; found, 1373.7. Anal. Calcd. for $Er_2Ga_8C_{130}H_{158}N_{24}O_{56}$: C, 40.61; H, 4.14; N, 8.74. Found: C, 40.36; H: 4.42; N: 8.98. Single-crystal unit-cell parameters: *a* = 14.092 Å, *b* = 17.504 Å, *c* = 19.208Å; α = 113.16°, *β* = 102.51°, γ = 98.25°; V = 4112.80Å^3.

$[Ga_8Tm_2(shi)_8(ip)_4(DMF)_2(H_2O)_2](NH_4^+)_2$ · 12DMF (**[TmGa$_4$]$_2$**). Yield: 235.0 mg (24.4%). ESI-MS, calc. for $[M]^{2-}$, $Tm_2Ga_8C_{88}H_{48}N_8O_{40}$, 1375.7; found, 1375.7. Anal. Calcd. for $Tm_2Ga_8C_{130}H_{158}N_{24}O_{56}$: C, 40.57; H, 4.14; N, 8.73. Found: C, 40.18; H: 4.26; N: 8.98. Single-crystal unit-cell parameters: *a* = 14.148 Å, *b* = 17.718 Å, *c* = 19.296 Å; α = 113.08°, *β* = 102.58°, γ = 98.30°; V = 4198.67Å^3.

$[Ga_8Yb_2(shi)_8(ip)_4(DMF)_2(H_2O)_2]$ $(NH_4^+)_2$ · 12DMF · H_2O (**[YbGa$_4$]$_2$**). Yield: 200.5 mg (20.7%). ESI-MS, calc. for $[M]^{2-}$, $Yb_2Ga_8C_{88}H_{48}N_8O_{40}$, 1379.7; found, 1379.7. Anal. Calcd. for $Yb_2Ga_8C_{130}H_{160}N_{24}O_{57}$: C, 40.30; H, 4.16; N, 8.68. Found: C, 39.92; H: 4.27; N: 8.88. Single-crystal unit-cell parameters: *a* = 14.110 Å, *b* = 17.700 Å, *c* = 19.242 Å; α = 113.05°, *β* = 102.48°, γ = 98.35°; V = 4173.97 Å^3.

$[Ga_8Y_2(shi)_8(ip)_4(DMF)_2(H_2O)_2]$ $(NH_4^+)_2$ · 8DMF · $3H_2O$ ([**YGa$_4$]$_2$**). ESI-MS, calc. for $[M]^{2-}$, $Y_2Ga_8C_{88}H_{48}N_8O_{40}$, 1295.7; found, 1295.7. Anal. Calcd. for $Yb_2Ga_8C_{130}H_{160}N_{24}O_{57}$: C, 41.08; H, 3.97; N, 8.12. Found: C, 40.73; H: 3.72; N: 7.79.

X-ray Crystallography

Colorless needles of **[DyGa$_4$]$_2$** were grown from a DMF solution at room temperature. A crystal of dimensions 0.10 x 0.08 x 0.03 mm was mounted on a Rigaku AFC10K Saturn 944+ CCD-based X-ray diffractometer equipped with a low temperature device and Micromax-007HF Cu-target micro-focus rotating anode (λ = 1.54187 A) operated at 1.2 kW power (40 kV, 30 mA). The X-ray intensities were measured at 85(1) K with the detector placed at a distance of 42.00 mm from the crystal. A total number of 2028 images were collected with an oscillation width of 1.0^o in ω. The exposure times were 1 sec. for the low angle images and 4 sec. for high angle images. Rigaku d*trek images were exported to CrysAlisPro for processing and corrected for absorption. The crystal was determined to be a two-component non-merohedral twin. Components from both domains as well as overlaps were used as the basis for a HKLF5 reflection file for refinement. The integration of the data yielded a total of 92383 reflections to a maximum 2θ value of 139.24^o of which 31908 were independent and 26389 were greater than 2σ(I). The final cell constants (Table 1) were based on the xyz centroids 36919 reflections above 10σ(I). Analysis of the data showed negligible decay during the data collection. The structure was solved and refined with the Bruker SHELXTL (version 2014/6) software package, using the space group $P2_1/n$ with Z = 2 for the formula $C_{97}H_{70}N_{11}O_{45}Ga_8Dy_2$. All non-hydrogen atoms were refined anisotropically with the hydrogen atoms placed in idealized positions. The structure is also fully disordered in two orientations at a 0.83/0.17 ratio. Full matrix least-squares refinement based on F^2 converged at R_1 = 0.0786 and wR_2 = 0.2157 [based on I > 2σ(I)], R_1 = 0.0845 and wR_2 = 0.2205 for all data. Additional details are presented in Table S1 and are given as Supporting Information in a CIF file (CCDC 1557091).

Table S1. Crystal data and structure refinement for $[DyGa_4]_2$	
Empirical formula	$C_{97}\ H_{70}\ Dy_2\ Ga_8\ N_{11}\ O_{45}$
Formula weight	2992.40
Temperature	85(2) K
Wavelength	1.54184 Å
Crystal system, space group	Monoclinic, $P2_1/n$
Unit cell dimensions	a = 14.8201(3) Å α = 90°
	b = 22.0890(3) Å β = 107.252(2)°
	c = 22.0033(4) Å γ = 90°
Volume	6879.0(2) $Å^3$
Z	2
Calculated density	1.445 g/cm^3
Absorption coefficient	8.061 mm^{-1}
F(000)	2938
Crystal size	0.100 x 0.080 x 0.030 mm
θ range for data collection	2.902 to 69.628°
Limiting indices	-17<=h<=17, -26<=k<=26, -26<=l<=26
Reflections collected / unique	31908 / 31908 [R(int) = 0.1003]
Completeness to θ = 67.684	100.0%
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.7940 and 0.5000
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	31908 / 2089 / 1470
Goodness-of-fit on F^2	1.041
Final R indices [I>2sigma(I)]	R_1 = 0.0786, wR_2 = 0.2157
R indices (all data)	R_1 = 0.0845, wR_2 = 0.2205
Largest diff. peak and hole	1.784 and -0.618 e. $Å^{-3}$

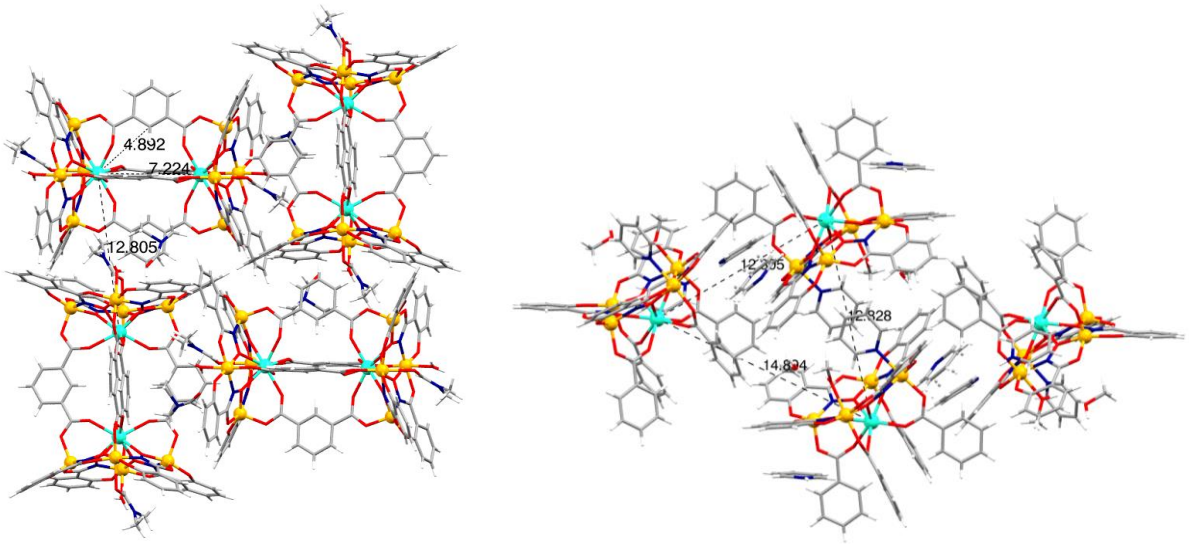

Figure S1. Distances between Dy ions in $[DyGa_4]_2$ (left) and $DyGa_4$ (right)[1]. The distance from the Dy ion to the nearest C-H bond is also included (4.892 Å).

UV-vis Absorption Spectroscopy

Solution UV−vis spectra of the compounds dissolved in DMSO were recorded on a Cary 100Bio UV−vis spectrophotometer in absorbance mode. Solid-state UV-vis spectra were collected using an Agilent-Cary 5000 spectrophotometer equipped with a Praying Mantis diffuse reflectance accessory. Spectra were collected in the reflectance (*R*) mode with the signal of pure $BaSO_4$ used as the baseline. Samples (10 wt.%) were milled in $BaSO_4$ (90 wt.%). The spectra were then converted to represent absorbance using Kubelka-Munk function $((1-R)^2/(2R))$.

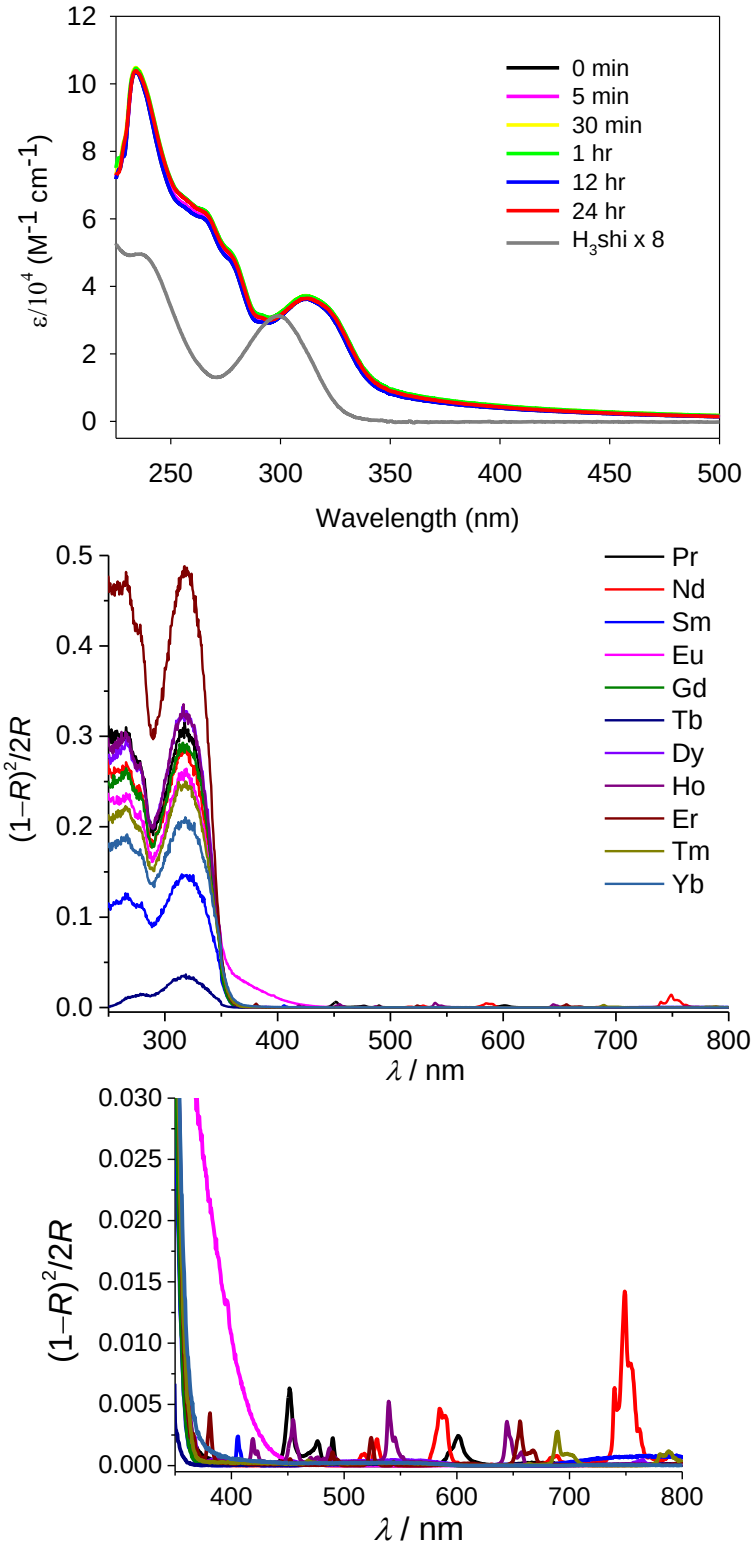

Figure S2. (Top) Time-dependent UV/Vis absorption spectra of $[TbGa_4]_2$ and the spectrum of H_3shi (multiplied by a factor of 8 to match the number of ligands present in the MCs). (Middle and bottom) Diffuse reflectance spectra represented as Kubelka-Munk function *vs.* wavelength of the $[LnGa_4]_2$ MCs at room temperature.

Photophysical Measurements

Luminescence data were collected on samples placed in 2.4 mm i.d. quartz capillaries. Emission and excitation spectra were measured on a custom-designed Horiba Scientific Fluorolog 3 spectrofluorimeter equipped with either a visible photomultiplier tube (PMT) (220-850 nm, R928P; Hamamatsu), a NIR solid-state InGaAs detector cooled to 77 K (800-1600 nm, DSS-IGA020L; Horiba Scientific), or a NIR PMT (950-1650 nm, H10330-75; Hamamatsu). Excitation and emission spectra were corrected for instrumental functions. Luminescence lifetimes were determined under excitation at 355 nm provided by a Nd:YAG laser (YG 980; Quantel). Signals were detected in the visible or NIR with the help of Hamamatsu R928P or H10330-75 PMTs. The output signals from the detectors were fed into a 500 MHz bandpass digital oscilloscope (TDS 754C; Tektronix), transferred to a PC for data processing with the program Origin 8®. Luminescence lifetimes are averages of at least three independent measurements. Quantum yields were determined with the Fluorolog 3 spectrofluorimeter based on an absolute method with the use of an integration sphere (Model G8, GMP SA, Renens, Switzerland). Each sample was measured several times under comparable experimental conditions, varying the position of samples. Estimated experimental error for quantum-yield determination is ~10%.

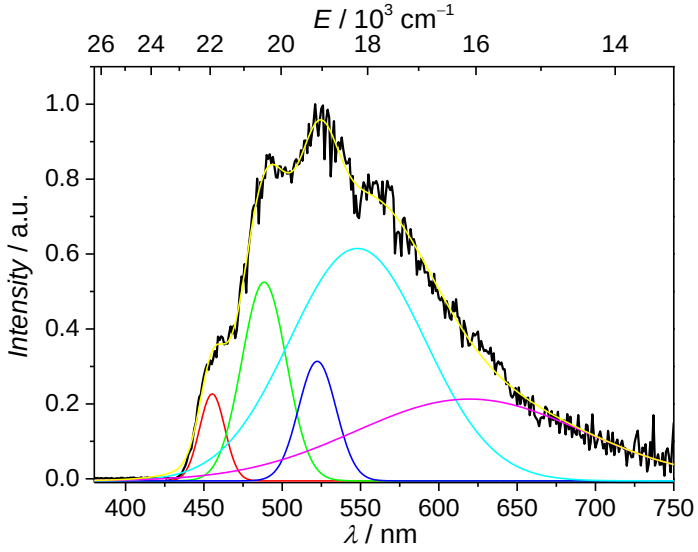

Figure S3. Phosphorescence spectrum of $[GdGa_4]_2$ recorded in the solid state under ligand excitation at 325 nm (black trace, 77 K, 100 µs delay after the excitation flash) and its Gaussian decomposition (colored traces).

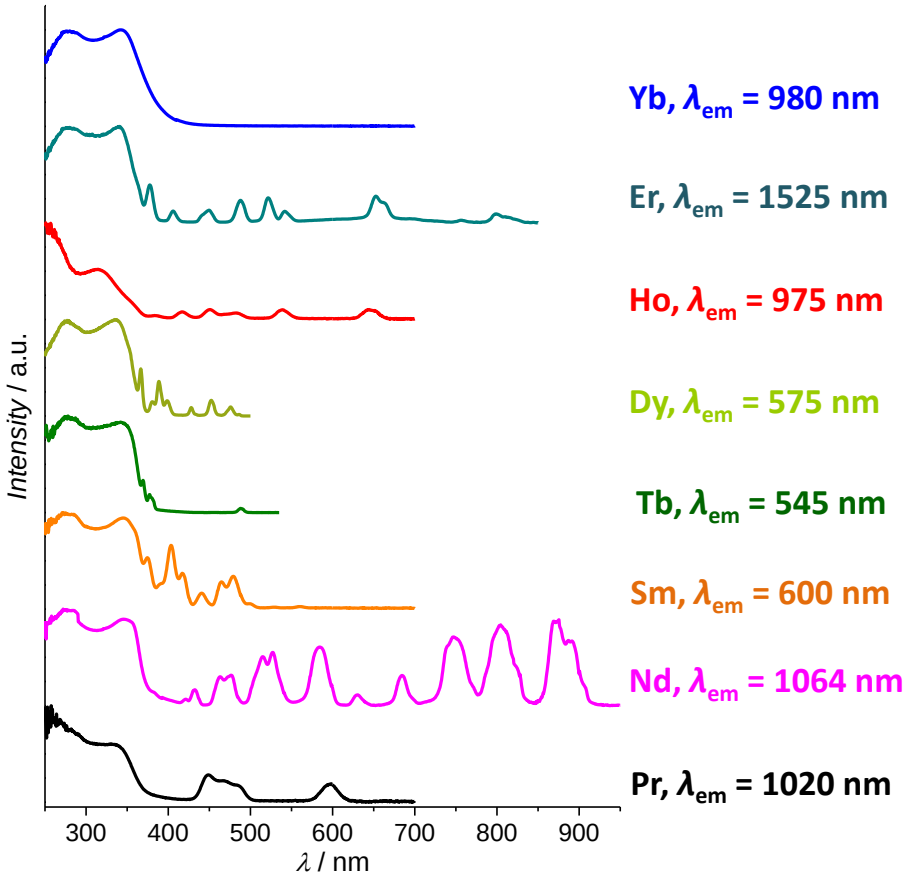

Figure S4. Corrected and normalized excitation spectra of $[LnGa_4]_2$ recorded in the solid state upon monitoring the emission bands resulting from the main transitions of the corresponding Ln^{3+}.

Cytotoxicity Test with AlamarBlue® Assay

Cytotoxicity tests were performed with the alamarBlue® assay (Invitrogen, France). HeLa cells were seeded in 96-wale plates at the density of 1×10^4 cells per well and cultured at 37 °C in a 5% humidified CO_2 atmosphere. After 24 h of attachment, cells were incubated with different concentrations of $[YbGa_4]_2$ during 24 h followed by the incubation with the alamarBlue® (10% v/v) during 3-4 h at 37 °C in a 5% humidified CO_2 atmosphere. The fluorescence of alamarBlue® was measured with a plate reader (Victor 3V, Perkin Elmer, France) under an excitation wavelength of 530 nm and collecting the emission at 590 nm. Control cells were prepared under the same experimental conditions but without addition of $[YbGa_4]_2$. For all cytotoxicity experiments, a 5 mM stock solution of $[YbGa_4]_2$ in DMSO was prepared which was then diluted in cell culture media to the desired concentration while the content of DMSO in solution was kept constant (4%).

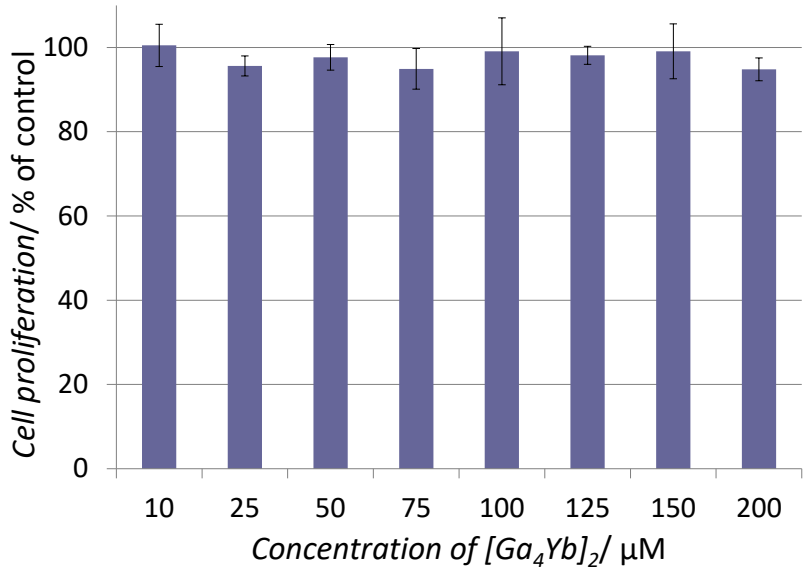

Figure S5. Plots of results of the cytotoxicity test performed with the help of the alamarBlue® assay for the $[YbGa_4]_2$ complex incubated with HeLa cells during 24 h.

NIR Epifluorescent Microscopy of HeLa Cells Incubated with the $[YbGa_4]_2$ Complex

HeLa (Human Epithelial Ovarian Carcinoma) cell line obtained from ATCC (Molsheim, France) was cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS), 1% of 100x non-essential aminoacid solution, 1% of L-glutamine (GlutaMAX) and 1% of streptomycin/penycilin antibiotics. Cells were seeded in a 8-well Lab Tek Chamber coverglass (Nunc, Dutsher S.A., Brumath, France) at a density of 6×10^4 cells/well and cultured at 37 °C in a 5% humidified CO_2 atmosphere. After 24 h cell culture media was removed, cells were washed twice with OptiMEM (room temperature). Stock solution of $[YbGa_4]_2$ complex in DMSO (5 mM) was diluted in OptiMEM cell culture media supplemented with 2% of FBS to a 10 µM concentration and used for incubation with HeLa cells at 37 °C in 5% CO_2 atmosphere during 12 h. Prior to epifluorescence imaging experiments, cells were washed twice with OptiMEM (room temperature). The cells were observed with a Zeiss Axio Observer Z1 fluorescence inverted microscope (Zeiss, Le Pecq, France) equipped with an EMCCD Evolve 512 (Roper Scientific) photometric camera. The light source, Zeiss HXP 120, was combined with the following filter cubes: 377 nm band pass 50 nm filter for the excitation and long pass filter 805 nm for the Yb^{3+} emission in the NIR range.

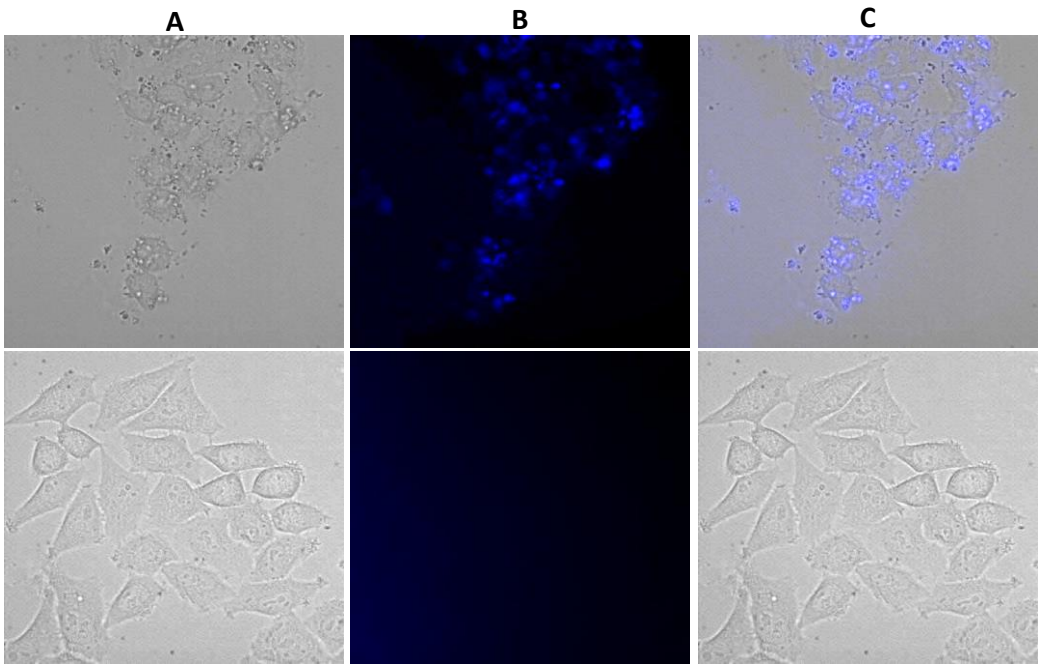

Figure S6. Images obtained from the epifluorescence microscopy experiments conducted on HeLa cells (top) incubated with a 10 µM solution of $[YbGa_4]_2$ MC during 12 h and (bottom) control cells. (A) Brightfield. (B) NIR signal from $[YbGa_4]_2$ MC (λ_{ex}: 377 nm band bass 50 nm, λ_{em}: long pass 805 nm, exposure time: 25 s). (C) Merged image obtained by the combination between $[YbGa_4]_2$ MC and brightfield. 40× objective.

References

[1] C. Y. Chow, S. V. Eliseeva, E. R. Trivedi, T. N. Nguyen, J. W. Kampf, S. Petoud, V. L. Pecoraro, *J. Am. Chem. Soc.* 2016, *138*, 5100-5109.