Supporting information

Near Infrared Two photon Imaging using a Bright Cationic Yb(III) Bioprobe Spontaneously Internalized into Live Cells.

Anh Thy Bui,[a] Maryline Beyler,[b] Alexei Grichine,[c] Alain Duperray,[c] Jean-Christophe Mulatier,[a] Yannick Guyot,[d] Chantal Andraud,[a] Raphaël Tripier*,[b] Sophie Brasselet*,[e] and Olivier Maury*,[a]

[a] Univ. Lyon, Ecole Normale Supérieure de Lyon, CNRS UMR 5182, Université Claude Bernard Lyon 1, Laboratoire de Chimie, 46 allée d'Italie, 69364 Lyon (France).

[b] Univ. Brest, UMR-CNRS 6521, UFR des Sciences et Techniques, 6 avenue Victor le Gorgeu, C.S. 93837, 29238 Brest Cedex 3 (France).

[c] Univ. Lyon, Institut Lumière Matière, UMR 5306 CNRS–Université Claude Bernard Lyon 1, 10 rue Ada Byron, 69622 Villeurbanne Cedex (France)

[d] Univ. Grenoble Alpes, INSERM, U1209, F-38000 Grenoble;, IAB, F-38000 Grenoble (France).

[e] Univ. Aix Marseille, CNRS, Centrale Marseille, Institut Fresnel, UMR 7249, F-13013 Marseille (France)

Photophysical measurements in solution. Absorption spectra were recorded on a JASCO V-650 spectrophotometer in spectrophotometric grade methanol, water or dichloromethane solutions (ca 10^{-5} mol L^{-1}). Molar extinction coefficients (ε) were determined at least two times. Emission spectra were measured using Horiba-Jobin-Yvon Fluorolog-3 fluorimeters. The steady-state luminescence was excited by unpolarized light from a 450 W xenon CW lamp and detected at an angle of 90° for diluted solution measurements (10 mm quartz cuvette) by Hamamatsu R928 photomultiplier tube or by a nitrogen cooled InGaAs detector. Spectra were corrected for both the excitation source light intensity variation and the emission spectral response. The luminescence decay of ytterbium complexes was determined using a home–made set–up. The excitation of the Yb(III) luminescence decays was performed with an optical parametric oscillator from EKSPLA NT342, pumped with a pulsed frequency tripled YAG:Nd laser. The pulse duration was 6 ns at 10 Hz repetition rate. The detection was performed by a R1767 Hamamatsu photomultiplier through a Jobin–Yvon monochromator equipped with a 1 μm blazed grating. The signal was visualized and averaged with a Lecroy digital oscilloscope LT342.

Cell culture. We used T24 human epithelial bladder cancer cell line (ATCC No HBT-4). In our experiments, T24 cells were cultured in 25 cm^2 tissue-culture flasks (T25) at 37°C, in a humidified atmosphere with 5% CO_2. They were incubated in RPMI 1640 supplemented with 100 U/mL penicillin,

100 μg/mL streptomycin, and 10% fetal calf serum (complete medium). Cells were grown to near confluence in the culture flasks and then suspended with 0.05% trypsin–EDTA solution (Sigma). Twenty-four hours before experiments, cells were placed on LabTek I chambered coverslips (Nunc) at low cell density in complete culture medium. The complexes were added to the cell medium to get a ca. 10^{-5} M concentration.

Confocal and two-photon microscopy. All confocal experiments were performed using a LSM710 NLO (Carl Zeiss) confocal laser scanning microscope based on the inverted motorized stand (AxioObserver, Zeiss). The excitation was provided by either 561 nm DPSS cw laser or Ti:Sa femtosecond tunable laser (Chameleon, Ultra II, Coherent) for 2P excitation at 750 nm in descanned detection mode. In the former case the pinhole was closed to 1 Airy Unit and in the latter one it was fully open. Spectral imaging was realized using an internal Quasar detector in the range 577-723 nm with the resolution of 9.7 nm.

NIR-to-NIR microscope. The two–photon excitation microscope is based on an excitation source from a Ti:Sa laser (pulse width, 100 fs; repetition rate, 80 MHz) set at a wavelength of 745 nm, focused on the sample using a water immersion high numerical aperture objective (NA 1.15, 40) after reflection on a dichroic mirror (FF720–SDi01, Semrock). Images are formed by galvanometric scanning (typically 30 μm x 30 μm regions are scanned, sampled with 60nm per pixel, with integration time 100μs per pixel) in the sample plane. The fluorescence emission is collected by the objective and passes through the dichroic mirror in the epi detection path. Remaining laser light is further rejected by interferential filters (610/70nm or 1000/50nm) and the emission is finally focused on a photomultiplier working in the photon–counting mode. The lateral resolution of the imaging setup is about 300 nm. Complete description of the set-up has been previously published.[1]

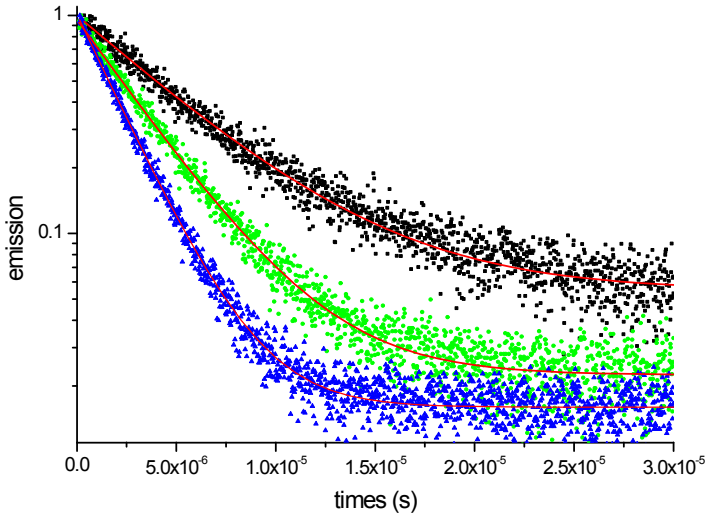


Figure S1. Room temperature luminescence decays of **3** measured at 980 nm in dichloromethane (black square), methanol (green dot) and water (blue triangle). λ_{ex} = 400 nm. In red is superimposed the best mono-exponential fit.

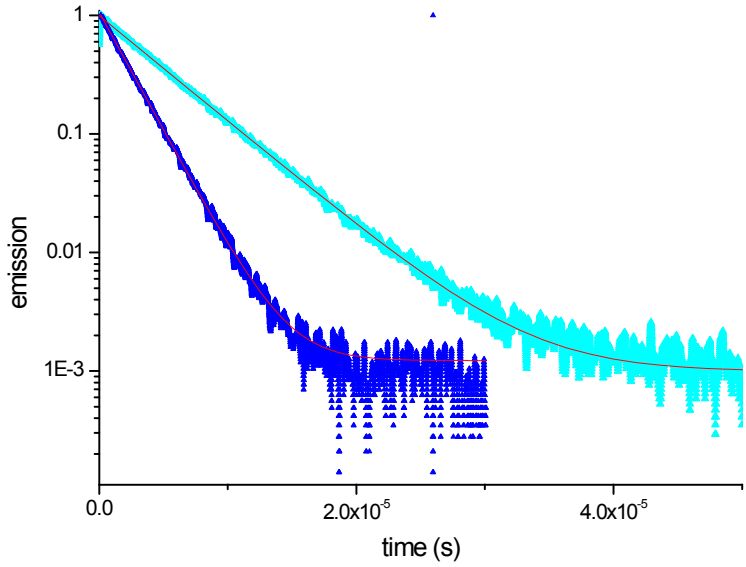
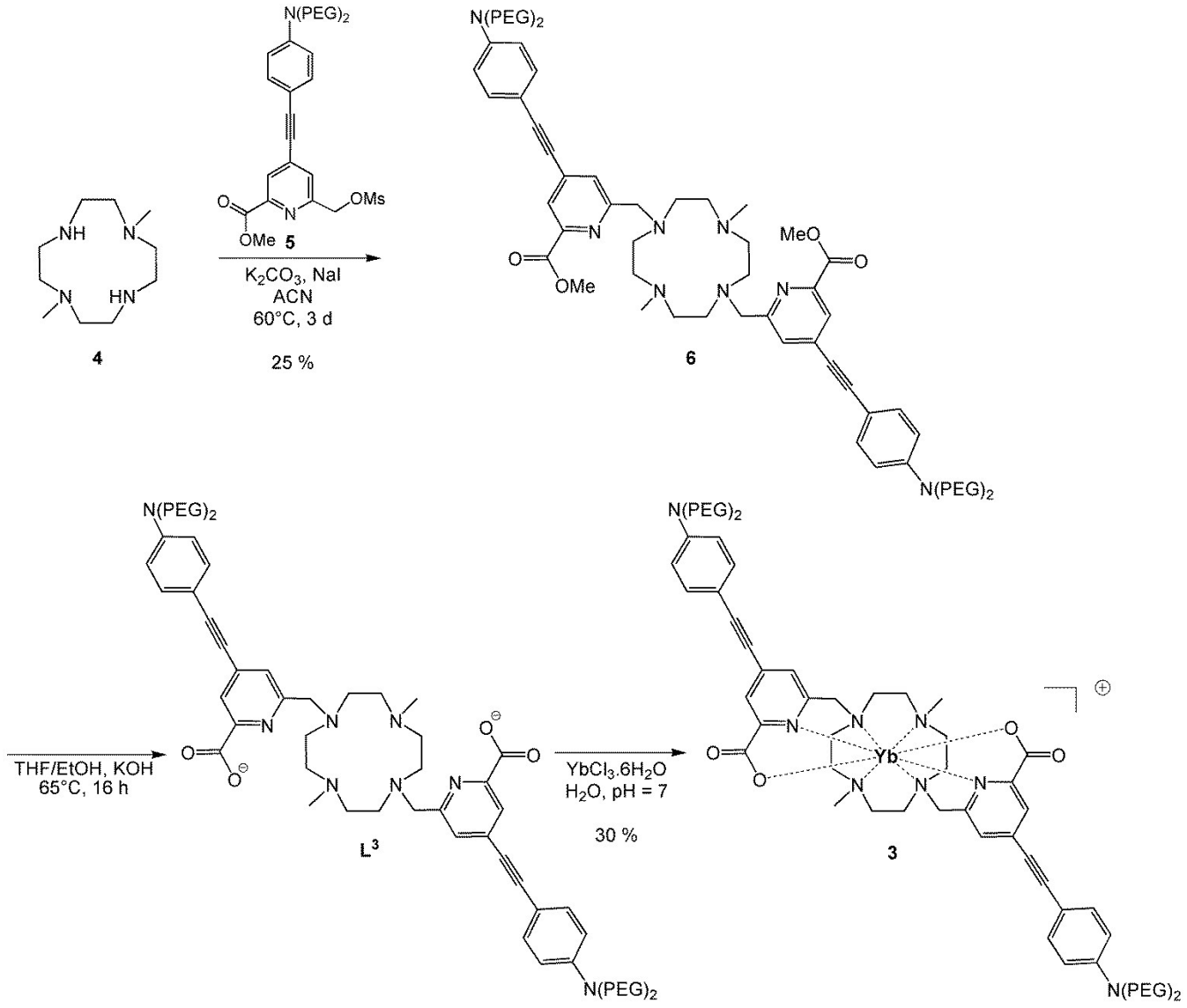


Figure S2. Room temperature luminescence decays of **3** measured at 1000 nm in water (blue triangle) and deuterated water (cyan triangle). λ_{ex} = 400 nm. In red is superimposed the best mono-exponential fit.

Synthesis. Reagents were purchased from ACROS Organics and from ALDRICH Chemical Co. Cyclen was purchased from Chematech (Dijon, France). Dimethyl-Cyclen (**4**)[2] and Pegylated mesylated picolinate chromophore (**5**)[1a] were synthesized as previously described. The solvents were freshly distilled prior to use and according to the standard methods. The analytic HPLC (High Performance Liquid Chromatography) was performed on a Prominence Shimadzu HPLC/LCMS-2020 equipped with a UV SPD-20 A detector. The chromatographic system employs HPLC (VisionHT C18 HL 5µ 250 × 4.6 mm for analytic HPLC and VisionHT C18 HL 5µ 250 × 10 mm for semi-preparative HPLC) with 0.1 % aq. trifluoroacetic acid - MeCN (*v*/*v*) as eluents [isocratic 10 % MeCN (4 min), linear gradient from 10 to 90 % MeCN (6 min), isocratic 90 % MeCN (4 min)] at a flow rate of 1 mL/min and UV detection at 254 and 350 nm. NMR spectra (^{1}H and ^{13}C) were recorded at the core facilities of the University of Brest, with Bruker Advanced 500 (500 MHz) or Bruker AMX-3 300 (300 MHz) spectrometers. The HR-MS analyses were performed at the Institute of Analytic and Organic Chemistry, ICOA in Orléans.



Scheme S1. Synthetic scheme of the ytterbium complex **3**.

Compound 6. A solution of compound **5** (530 mg, 0.81 mmol) in dry CH_3CN (15 mL) was added dropwise to a solution of dimethyl cyclen **4** (78 mg, 0.39 mmol), K_2CO_3 (216 mg, 4 equiv.) and NaI (58 mg, 1 equiv.) in the same solvent (10 mL). The mixture was stirred at 60°C for 3 days. The solution was cooled down to room temperature and K_2CO_3 was filtrated off and washed with CH_3CN (3×5 mL). The filtrate was evaporated and taken up in CH_2Cl_2 and washed with water (2 × 20 mL). The organic layer was dried over $MgSO_4$, filtrated and concentrated under vacuum. The mixture was purified by flash chromatography using Amino Flash Functionalized Silica gel column (20 g) eluted with CH_2Cl_2/MeOH (100:0 to 90:10) to give compound **6** as a yellow oil. (130 mg; y = 25 %). ^{1}H NMR (300 MHz; $CDCl_3$; 298 K) δ (ppm): 7.95 (s, 2 H), 7.52 (s, 2 H), 7.39 (d, 4 H, ^{3}J = 9 Hz), 6.69 (d, 4 H, ^{3}J = 9 Hz), 3.65-3.59

(m, 44 H), 3.56-3.51 (m, 16 H), 3.55-3.54 (m, 4 H), 3.36 (s, 12 H), 3.07 (bs, 2 H), 2.50 (vbs, 8 H), 1.86 (s, 6 H). ^{13}C NMR (Jmod; 75 MHz; $CDCl_3$; 298K) δ (ppm): 164.9 (CO), 159.1 (C_{quat} Pico), 148.9 (C_{quat} Pico), 146.6 (C_{quat} Pico), 135.0 (C_{quat} Ph), 133.9 (CH Ph), 127.7 (CH Pico), 125.1 (CH Pico), 111.5 (CH Ph), 107.3 (C_{quat} Ph), 99.0 (C_{quat} acetylene), 84.5 (C_{quat} acetylene), 71.8 (CH_2-Pico), 70.6 (CH_2-Peg), 70.5 (CH_2-Peg), 70.4 (CH_2-Peg), 68.2 (CH_2-Peg), 59.1 (CH_2-cyclen), 58.9 (O-CH_3 Peg), 52.7 (O-CH_3-Pico), 50.7 (CH_2-Peg), 44.0 (CH_3-cyclen), 29.6 (CH_2 cyclen). HR-MS: m/z: 657.3857 $[M+ 2H]^{2+}$ calcd. 657.3857 for $C_{70}H_{104}N_8O_{16} + 2H^+$.

Compound L^3. Compound **6** (70 mg, 0.053 mmol) was dissolved in 4 mL of a mixture of THF/EtOH (1/1) and an aqueous solution of KOH 4 M (3 mL) was added. The mixture was vigorously stirred at 65°C for 16 hours. The mixture was cooled down to room temperature, poured into a separating funnel and the organic layer was recovered, concentrated under vacuum and used without further purification.

Ytterbium complex 3. The previously hydrolysed ligand $\mathbf{L^3}$ was dissolved in water and the pH of the solution adjusted to 7 by addition of 1M HCl. $YbCl_3.6H_2O$ was added (31 mg, 1.5 eq). The solution was stirred under argon for 1 h at 60°C. The pH of the solution was measured and increased to 6 by addition of solid K_2CO_3. The mixture was further heated at 60°C overnight. The crude mixture was concentrated and purified by HPLC using the method described in the general methods to give compound **3** as a yellow oil. (25 mg; y = 30 % calculated from compound **6**). HR-MS: m/z: 486.2220 $[M]^{3+}$ calcd. 486.2214 for $C_{68}H_{100}N_8O_{16}Yb$.

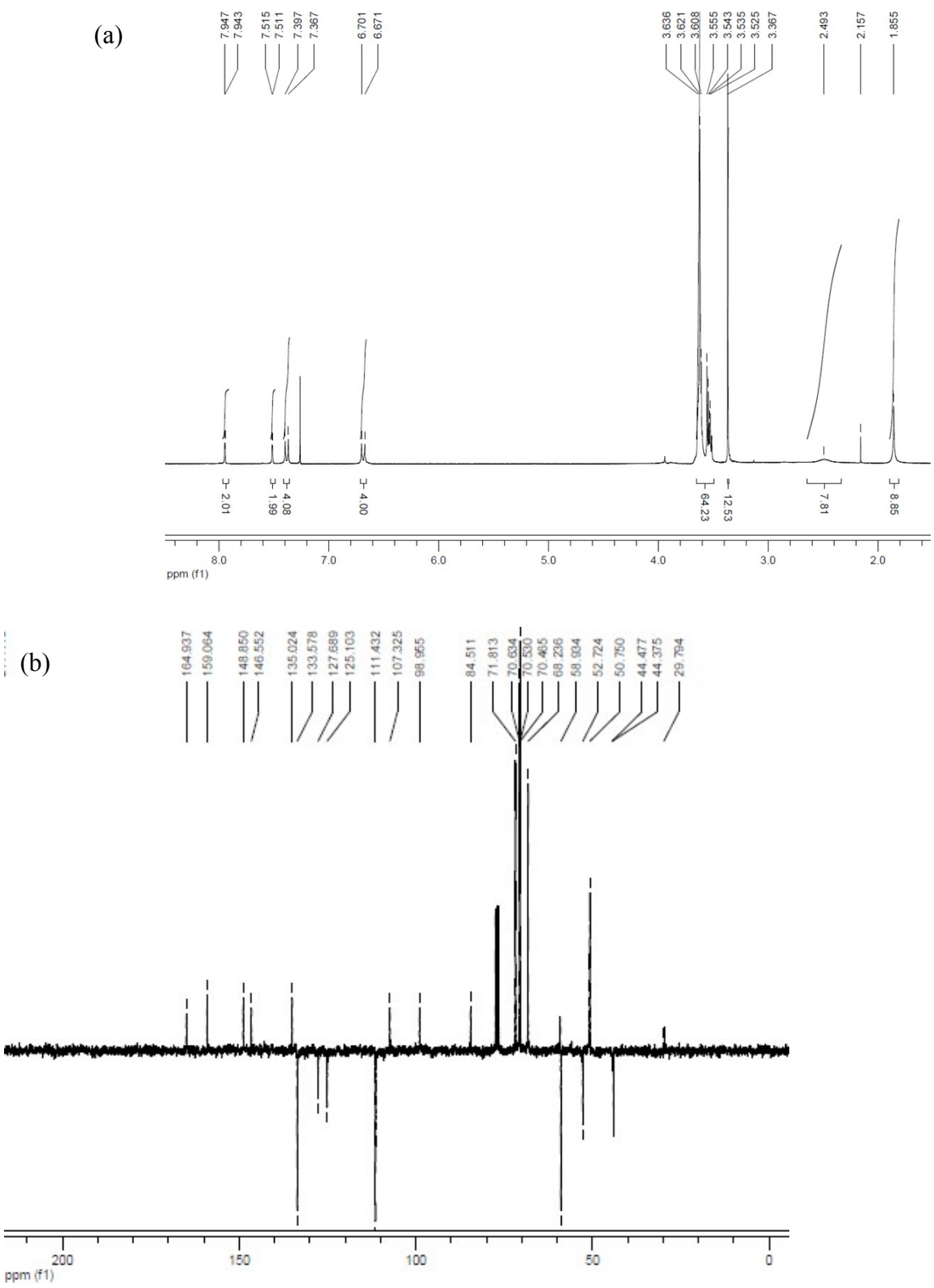


Figure S3. NMR spectra of compound **6** (298 K, $CDCl_3$) (a) ^{1}H NMR (300 MHz), (b) ^{13}C NMR (100 MHz).

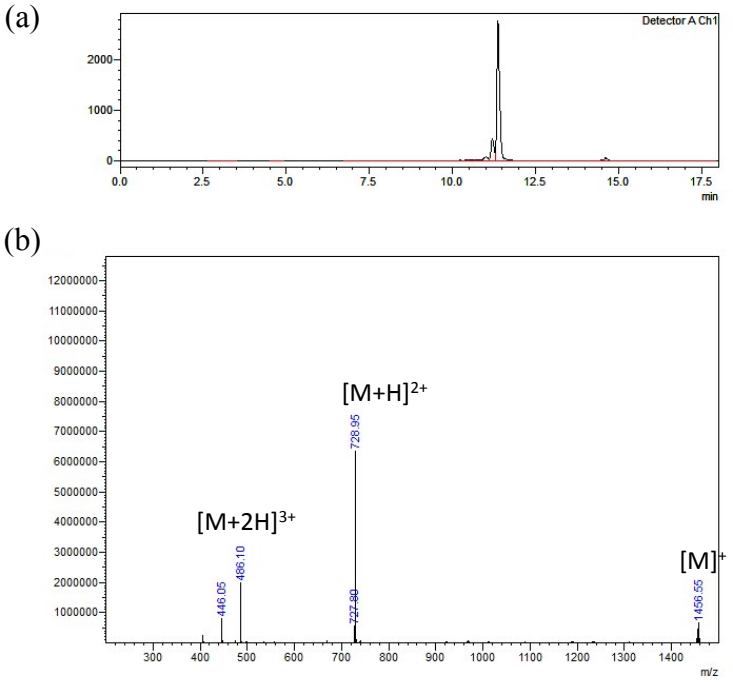


Figure S4. Semi-preparative analysis of complex ytterbium(III) complex **3**, retention time rt = 11.38 min (a) Chromatogram with UV detector (254 nm), (b) Mass spectrum from t= 11 min to t= 11.5 min.

References.

1. a) A. D'Aléo, A. Bourdolle, S. Brustlein, T. Fauquier, A. Grichine, A. Duperray, P. L. Baldeck, C. Andraud, S. Brasselet and O. Maury, *Angew. Chem. Int. Ed*. **2012**, *51*, 6622-6625; 1b) G. Lapadula, A. Bourdolle, F. Allouche, M. Conley, I. del Rosa, L. Maron, W. W. Lukens, Y. Guyot, C. Andraud, S. Brasselet, C. Copéret, O. Maury and R. A. Andersen, *Chem. Mater*., **2014**, *26*, 1062.
2. A. Rodríguez-Rodríguez, D. Esteban-Gomez, A. de Blas, T. Rodríguez-Blas, M. Fekete, M. Botta, R. Tripier and C. Platas-Iglesias, *Inorg. Chem.* **2012**, *51*, 2509-2521.