Combining pyclen framework with conjugated antenna for the design of europium and samarium luminescent bioprobes

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Summary

- Ligand and complex synthesis ............................................................... page 2
- Materials and methods ........................................................................ page 2
- Synthesis of (3) ......................................................................................... page 2
- Synthesis of (L5b) ....................................................................................... page 3
- Synthesis of Eu complex [EuL5b] ............................................................. page 4
- Synthesis of Sm complex [SmL5b] ............................................................ page 4
- Figure S1: 1H NMR of compound 3 (500 MHz, CDCl3, 25°C) ................... page 5
- Figure S2: 13C NMR of compound 3 (75 MHz, CDCl3, 25°C) ................... page 5
- Figure S3: HRMS of compound 3 ............................................................ page 6
- Figure S4: 1H NMR of compound L5b (500 MHz, D2O, 25°C) .................. page 7
- Figure S5: 13C NMR of compound L5b (125 MHz, D2O, 25°C) ................ page 7
- Figure S6: HRMS of compound L5b ........................................................ page 8
- Figure S7: Analytical HPLC of the [EuL5b] complex ................................ page 9
- Figure S8: HRMS of the [EuL5b] complex ............................................... page 10
- Figure S9: Analytical HPLC of the [SmL5b] complex .............................. page 11
- Figure S10: HRMS of the [SmL5b] complex ............................................ page 12
- Computationnal Details .......................................................................... page 13

Table S1. Continuous shape measurements of the O3N6 coordination polyhedron in [YL5b+] performed with SHAPE 2 ........................................ page 13
Photophysical measurements in solution ................................................ page 14
- Figure S11: Determination of the quantum yield of [EuL5b] ..................... page 14
- Figure S12: absorption and emission spectra 3 ........................................ page 15
- Table S1. Relative intensity of the [EuL1] complexes ............................... page 15
- Cell culturing and treatment ................................................................. page 16
Ligand and complex synthesis
- Materials and methods

Reagents were purchased from ACROS Organics and from Aldrich Chemical Co and used without further purification. Dialysis membranes (cut-off 100-500 Da) were purchased from spectrumlabs. All solvents were dried and distilled prior to use according to standard methods. Methyl 3,6,9,15-tetraazabicyclo[9.3.1]pentadecane-1(15),11,13-triene-3-acetate 1 and methyl 4-(((4-(2-(2-methoxyethoxy)ethoxy)ethoxy)phenyl)ethynyl)-6-(((methylsulfonyl)oxy)methyl)picolinate 2 were synthesized as previously described. Analytic HPLC was performed on a Prominence Shimadzu HPLC/LCMS-2020 equipped with a UV SPD-20 A detector. The chromatographic system employs HPLC (Vision HT C18 HL 5 μ250×4.6 mm) with H₂O and MeCN as eluents [isocratic 100% H₂O (5 min), linear gradient from 0 to 90% MeCN (10 min), isocratic 90% MeCN (5 min)] at a flow rate of 1 mL/min and UV detection at 254 and 350 nm. NMR spectra were recorded at the “Services communs” of the University of Brest. 1H and 13C NMR spectra were recorded using Bruker Avance 500 (500 MHz), Bruker Avance 400 (400 MHz), or BrukerAMX-3 300 (300 MHz) spectrometers. HRMS analyses were realized on a HRMS Q-ToF MaXis, sources ESI, APCI, APPI, nano-ESI (at the Institute of Organic and Analytic Chemistry – ICOA in Orléans).

Synthesis of (3):

Dimethyl 9-[(methoxycarbonyl)methyl]-3,6,9,15-tetraazabicyclo[9.3.1]pentadecane-1(15),11,13-triene-3,6-dimethylene-4-((4-(2-(2-methoxyethoxy)ethoxy)ethoxy)phenyl)ethynyl)picolinate

A solution of compound 1 (106 mg, 0.38 mmol) and K₂CO₃ (211 mg, 1.52 mmol, 4 eq) in CH₃CN (9.5 mL) was stirred at room temperature for 30 min. To this solution was added dropwise a solution of compound 2 (396 mg, 0.78 mmol, 2.05 eq) in CH₃CN (14 mL). The reaction mixture was stirred at room temperature for 15h and solvents were evaporated to dryness. The residue was taken up in CH₂Cl₂ and the residual salts were filtered on celite.
Solvents were evaporated to dryness and the crude was purified by chromatography on neutral alumina (eluent: CH₂Cl₂/MeOH 100/0 to 100/1.5) to give 3 (166 mg, 0.15 mmol, 39%) as a brown oil. Rf (CH₂Cl₂/MeOH 100/3) = 0.29. ¹H NMR spectrum could not be described because of its complexity. ¹³C NMR (75 MHz, CDCl₃) δ 171.6, 165.2, 164.4, 160.1, 159.9, 159.8, 158.7, 158.4, 157.8, 147.4, 147.3, 137.9, 134.4, 134.3, 133.5, 133.4, 127.8, 127.6, 125.1, 124.9, 121.3, 120.7, 114.7, 114.6, 113.3, 113.2, 97.0, 96.6, 84.7, 84.6, 71.7, 70.6, 70.4, 70.3, 69.3, 67.4, 62.5, 61.9, 61.6, 61.3, 59.3, 58.8, 57.1, 55.6, 54.8, 52.9, 52.7, 51.5. ESI-HR-MS (positive, MeOH) m/z calcd. for [C₆₀H₇₃N₆O₁₄]⁺: 1101.5179, found: 1101.5183, [M+H]⁺; calcd. for [C₆₀H₇₄N₆O₁₄]²⁺: 551.2626, found: 551.2631, [M+2H]²⁺; calcd. for [C₆₀H₇₆Na₂O₁₄]⁺: 1123.4999, found: 1123.5003, [M+Na]⁺.

Synthesis of (L⁵⁵b):

9-[acetate]-3,6,9,15-tetraazabicyclo[9.3.1]pentadecane-1(15),11,13-triene-3,6-di[methylene-4-((4-(2-(2-methoxyethoxy)ethoxy)ethoxy)phenyl)ethynyl]picolinate]

To a solution of compound 3 (130 mg, 0.12 mmol) in THF/MeOH (8/2 v/v, 7.9 mL) was added 1M KOH (1.57 mL). The reaction mixture was stirred at reflux for 22h. Solvents were evaporated and the residue was submitted to a dialysis for 15h (cut-off 100-500 Da) to give L⁵⁵b as a brown oil. ¹H NMR (500 MHz, D₂O) δ 7.74 (d, J = 1.0 Hz, 1H), 7.61 (d, J = 0.7 Hz, 1H), 7.47 (t, J = 7.7 Hz, 1H), 7.18 (s, 1H), 7.13 (d, J = 8.6 Hz, 2H), 7.08 (d, J = 8.5 Hz, 2H), 6.93 (d, J = 7.7 Hz, 1H), 6.87 (s, 1H), 6.76 (dd, J = 8.8, 3.0 Hz, 4H), 6.73 (d, J = 7.6 Hz, 1H), 4.08-3.51 (m, 36H), 3.31 (s, 3H), 3.31 (s, 3H), some signals of the pyclen skeleton could not be observed. ¹³C NMR (126 MHz, D₂O) δ 181.2, 174.2, 174.0, 161.9, 161.8, 161.2, 160.2, 156.9, 156.4, 154.0, 140.9, 136.7, 136.4, 136.1, 135.7, 131.2, 129.7, 127.9, 126.7, 123.7, 123.3, 117.5, 116.6, 116.5, 98.8, 97.8, 88.7, 88.1, 73.8, 72.7, 72.4, 72.3, 71.7, 70.0, 69.9, 65.9, 61.1, 60.9, 60.0, 59.7, 59.2, 58.2, 57.3, 54.5, 50.5. ESI-HR-MS (positive, H₂O, M corresponds to the three carboxylic acids form of formula C₅₇H₆₆N₆O₁₄) m/z calcd. for [C₅₇H₆₄FeN₆O₁₄]⁺: 1112.3824,

**Synthesis of Eu complex [EuL₅b]**

Compound L⁵b (25 mg, 21.3 µmol) was dissolved in H₂O (10 mL). The pH was adjusted to 6.4 with HCl 0.2M before addition of EuCl₃.6H₂O (15 mg, 1.5 eq). The pH was then adjusted to 5.8 with KOH 1M. The reaction mixture was stirred at room temperature for 1h and the pH checked again. The reaction mixture was stirred at reflux for 20h and solvents were evaporated to dryness. Purification of the residue by flash chromatography (cartridge C18 from Reveleris, Gradient: H₂O/MeOH 100/0 to 2/8 over 35 min) gave the desired Eu-complex [EuL₅b] (8 mg, 6.6 µmol, 31%) as a yellow solid. ESI-HR-MS (positive, MeOH) m/z calcd. for [C₅₇H₆₆EuN₆O₁₄]⁺: 1209.3687, found: 1209.3708, [M+H]⁺; calcd. for [C₅₇H₆₅EuN₆O₁₄]²⁺: 605.1880, found: 605.1891, [M+2H]²⁺.

**Synthesis of Sm complex [SmL₅b]**

Compound L⁵b (22 mg, 18.7 µmol) was dissolved in H₂O (10 mL). The pH was controlled (8.3) before addition of SmCl₃.6H₂O (10 mg, 1.5 eq). The pH was then adjusted to 5.6 with KOH 1M. The reaction mixture was stirred at reflux for 1h and the pH checked again. The reaction mixture was stirred at reflux for 17h, resulting in the formation of a solid on the flask’s walls. The reaction mixture was filtered on cotton and rinsed with H₂O. The solid stuck on the cotton was dissolved with MeOH and combined with the solid on the flask’s walls to give the Sm complex [SmL₅b] (13 mg, 10.8 µmol, 58%) as a brown oil. ESI-HR-MS (positive, MeOH) m/z calcd. for [C₅₇H₆₄N₆O₁₄Sm]⁺: 1208.3672, found: 1208.3688, [M+H]⁺; calcd. for [C₅₇H₆₅N₆O₁₄Sm]²⁺: 604.6872, found: 604.6876, [M+2H]²⁺.
Figure S1: $^1$H NMR of compound 3 (500 MHz, CDCl$_3$, 25°C).

Figure S2: $^{13}$C NMR of compound 3 (75 MHz, CDCl$_3$, 25°C).
Figure S3: HRMS of compound 3.
**Figure S4**: $^1$H NMR of compound L$_{5b}$ (500 MHz, D$_2$O, 25°C).

**Figure S5**: $^{13}$C NMR of compound L$_{5b}$ (125 MHz, D$_2$O, 25°C).
Figure S6: HRMS of compound L⁵b. Peak at 338 is due to the presence of erucamide in Eppendorf’s.

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Figure S7: Analytical HPLC of the [EuL⁵b] complex. Column: Vision HT C18 HL 5 µ 250× 4.6 mm. Gradient: 100% H₂O 0-5 min, 0-90% ACN 5-15 min, 90% ACN 15-20 min, 100% H₂O 20-25 min. Flow: 1mL/min. retention time = 15.186 min.
Figure S8: HRMS of the [EuL₅b] complex. Peak at 338 is due to the presence of erucamide in Eppendorf’s.
Figure S9: Analytical HPLC of the [SmL<sub>5b</sub>] complex. Column: Vision HT C18 HL 5 μ 250× 4.6 mm. Gradient: 100% H<sub>2</sub>O 0-5 min, 0-90% ACN 5-15 min, 90% ACN 15-20 min, 100% H<sub>2</sub>O 20-25 min. Flow : 1mL/min. retention time = 15.278 min.
Figure S10: HRMS of the [SmL₅b] complex. Peak at 338 is due to the presence of erucamide in Eppendorf’s.
Computational details

DFT geometry optimizations were carried out with the Gaussian 09 (revision D.01) package\(^3\) tightening self-consistent field convergence thresholds (10-10 a.u.) and geometry optimization (10-5 a.u.) convergence thresholds. Calculations were realized on the \([Y\text{L}^{5b}']\) model. The hybrid PBE0 functional has been used.\(^4\) The “Stuttgart/Dresden” basis sets and effective core potentials were used to describe the yttrium atom,\(^5\) whereas all other atoms were described with the SVP basis sets.\(^6\)

Table S1. Continuous shape measurements of the \(O_3N_6\) coordination polyhedron in \([Y\text{L}^{5b}']\) performed with SHAPE 2.\(^7\)

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Photophysical measurements in solution

Absorption spectra were recorded on a JASCO V-650 spectrophotometer as solutions in spectrophotometric-grade methanol or water (ca. 10^{-5} or 10^{-6} mol.L^{-1}). Emission spectra were measured by using a Horiba–Jobin–Yvon Fluorolog-3 fluorimeter. Spectra were corrected for both excitation-source light-intensity variation and emission spectral responses. Luminescence lifetimes were obtained by pulsed excitation with a FL-1040 UP xenon lamp. Luminescence quantum yields, \( \Phi \), were measured with dilute solutions in water or organic solvents with an absorbance of less than 0.1 by using equation (1):

\[
\frac{\Phi_x}{\Phi_r} = \frac{A_r(\lambda) n_x^2 D_x}{A_x(\lambda) n_r^2 D_r}
\]

in which A is the absorbance at the excitation wavelength (\( \lambda \)), n is the refractive index, and D is the integrated luminescence intensity; r and x stand for reference and sample, respectively. The reference is quinine bisulfate in a 1N aqueous solution of sulfuric acid (\( \Phi_r = 0.546 \)). Excitation of reference and sample compounds was performed at the same wavelength. Practically, for each sample, series of measurements are performed with different absorbance ranging from 0.1 to 0.01. The plot of the integrated luminescence intensity vs. absorbance gives straight line with excellent correlation coefficients (figure S11) and the slope \( S \) can be determined. Equation (1) becomes (2):

\[
\frac{\Phi_x}{\Phi_r} = \frac{S_x(\lambda) n_x^2}{S_r(\lambda) n_r^2}
\]

The hydration number \( q \) is determined for europium complexes by measuring the lifetime in H\(_2\)O and D\(_2\)O according to the equation (3):8

\[ q = 1.11 (k(H_2O) - k(D_2O) - 0.31) \]
Figure S11. Determination of the quantum yield of [EuL₅b] in MeOH (red), H₂O (blue), CH₂Cl₂ (magenta) and the reference quinine sulfate in a 1N aqueous solution of sulfuric acid (black). The straight lines correspond to the linear fit.

Figure S12. Determination of the quantum yield of [SmL₅b] in MeOH (red), H₂O (blue), and the reference quinine sulfate in a 1N aqueous solution of sulfuric acid (black). The straight lines correspond to the linear fit.

Figure S13. Normalized absorption (blue), excitation (dotted) and emission spectra (red) of 3 in MeOH (λ_exc = 325nm) at room temperature. The harmonic scattering at 650 nm is indicated by the *.
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Cell culturing and treatment

We used the T24 human epithelial bladder cancer cell line (ATCC no. HBT-4). In our experiments, T24 cells were cultured in 25 cm² tissue-culture flasks (T25) at 37°C in a humidified atmosphere with 5% CO₂. They were incubated in RPMI 1640 supplemented with 100 U·mL⁻¹ penicillin, 100 µg·mL⁻¹ streptomycin, and 10% fetal calf serum (complete medium). Cells were grown to near confluence in the culture flasks and then suspended with a solution of 0.05% trypsin–ethylenediaminetetraacetic acid (EDTA; Sigma). Cells were placed on a LabTek I chambered cover glass (Nunc) at low cell density in complete culture medium 24 h before experiments. After being washed with phosphate-buffered saline (PBS), cells were fixed with PFA (3% in PBS) for 10 min, permeabilized with PBS containing 0.5% Triton X100 for 10 min, and then washed with PBS.

References

1 M. Le Fur, R. Tripier,; O. Rousseaux, M. Beyler, Preparation of macrocyclic ligands with picolinate group(s), their metal complexes, and their use for medical imaging or as radiopharmaceuticals in treating cancer ; PCT Int. Appl. (2017), WO 2017109217 A1 20170629.


