Electronic Supplementary Information for

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

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Summary

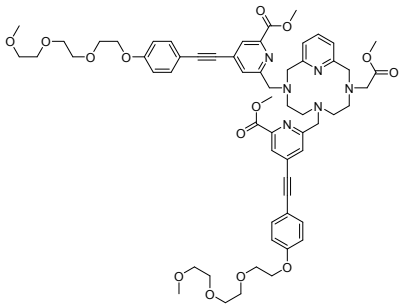
Ligand and complex synthesis

- 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**[1] and methyl 4-((4-(2-(2-(2-methoxyethoxy)ethoxy)ethoxy)phenyl)ethynyl)-6-(((methylsulfonyl)oxy)methyl)picolinate **2**[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 µ 250× 4.6 mm) with H_2O and MeCN as eluents [isocratic 100% H_2O (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 ^{13}C 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-di[methylene-4-((4-(2-(2-(2-methoxyethoxy)ethoxy)ethoxy)phenyl) ethynyl)picolinate]

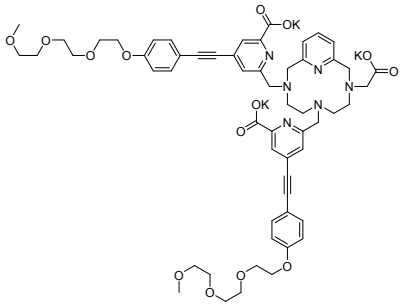


A solution of compound **1** (106 mg, 0.38 mmol) and K_2CO_3 (211 mg, 1.52 mmol, 4 eq) in CH_3CN (9.5 mL) was stirred at room temperature for 30 min. To this solution was added dropwise a solution of compound **2** (396 mg, 0.78 mmol, 2.05 eq) in CH_3CN (14 mL). The reaction mixture was stirred at room temperature for 15h and solvents were evaporated to dryness. The residue was taken up in CH_2Cl_2 and the residual salts were filtered on celite.

Solvents were evaporated to dryness and the crude was purified by chromatography on neutral alumina (eluent: CH_2Cl_2/MeOH 100/0 to 100/1.5) to give **3** (166 mg, 0.15 mmol, 39%) as a brown oil. Rf (CH_2Cl_2/MeOH 100/3) = 0.29. ^{1}H NMR spectrum could not be described because of its complexity. ^{13}C NMR (75 MHz, $CDCl_3$) 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 $[C_{60}H_{73}N_6O_{14}]^+$: 1101.5179, found: 1101.5183, $[M+H]^+$; calcd. for $[C_{60}H_{74}N_6O_{14}]^{2+}$: 551.2626, found: 551.2631, $[M+2H]^{2+}$; calcd. for $[C_{60}H_{72}N_6NaO_{14}]^+$: 1123.4999, found: 1123.5003, $[M+Na]^+$.

Synthesis of (L^{5b}):

9-[acetate]-3,6,9,15-tetraazabicyclo[9.3.1]pentadecane-1(15),11,13-triene-3,6-di[methylene-4-((4-(2-(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 **L^{5b}** as a brown oil. ^{1}H NMR (500 MHz, D_2O) 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.^{13}C NMR (126 MHz, D_2O) 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, H_2O, M corresponds to the three carboxylic acids form of formula $C_{57}H_{66}N_6O_{14}$) *m/z* calcd. for $[C_{57}H_{64}FeN_6O_{14}]^+$: 1112.3824,

found: 1112.3837, $[M-2H+Fe]^+$; calcd. for $[C_{57}H_{67}N_6O_{14}]^+$: 1059.4709, found: 1059.4720, $[M+H]^+$; calcd. for $[C_{57}H_{65}FeN_6O_{14}]^{2+}$: 556.6949, found: 556.6965, $[M-H+Fe]^{2+}$; calcd. for $[C_{57}H_{68}N_6O_{14}]^{2+}$: 530.2391, found: 530.2397, $[M+2H]^{2+}$

Synthesis of Eu complex [Eu**L**^{5b}]

Compound **L**^{5b} (25 mg, 21.3 µmol) was dissolved in H_2O (10 mL). The pH was adjusted to 6.4 with HCl 0.2M before addition of $EuCl_3.6H_2O$ (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: H_2O/MeOH 100/0 to 2/8 over 35 min) gave the desired Eu-complex [Eu**L**^{5b}] (8 mg, 6.6 µmol, 31%) as a yellow solid. ESI-HR-MS (positive, MeOH) *m/z* calcd. for $[C_{57}H_{64}EuN_6O_{14}]^+$: 1209.3687, found: 1209.3708, $[M+H]^+$; calcd. for $[C_{57}H_{65}EuN_6O_{14}]^{2+}$: 605.1880, found: 605.1891, $[M+2H]^{2+}$.

Synthesis of Sm complex [Sm**L**^{5b}]

Compound **L**^{5b} (22 mg, 18.7 µmol) was dissolved in H_2O (10 mL). The pH was controlled (8.3) before addition of $SmCl_3.6H_2O$ (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 H_2O. 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 [Sm**L**^{5b}] (13 mg, 10.8 µmol, 58%) as a brown oil. ESI-HR-MS (positive, MeOH) *m/z* calcd. for $[C_{57}H_{64}N_6O_{14}Sm]^+$: 1208.3672, found: 1208.3688, $[M+H]^+$; calcd. for $[C_{57}H_{65}N_6O_{14}Sm]^{2+}$: 604.6872, found: 604.6876, $[M+2H]^{2+}$.

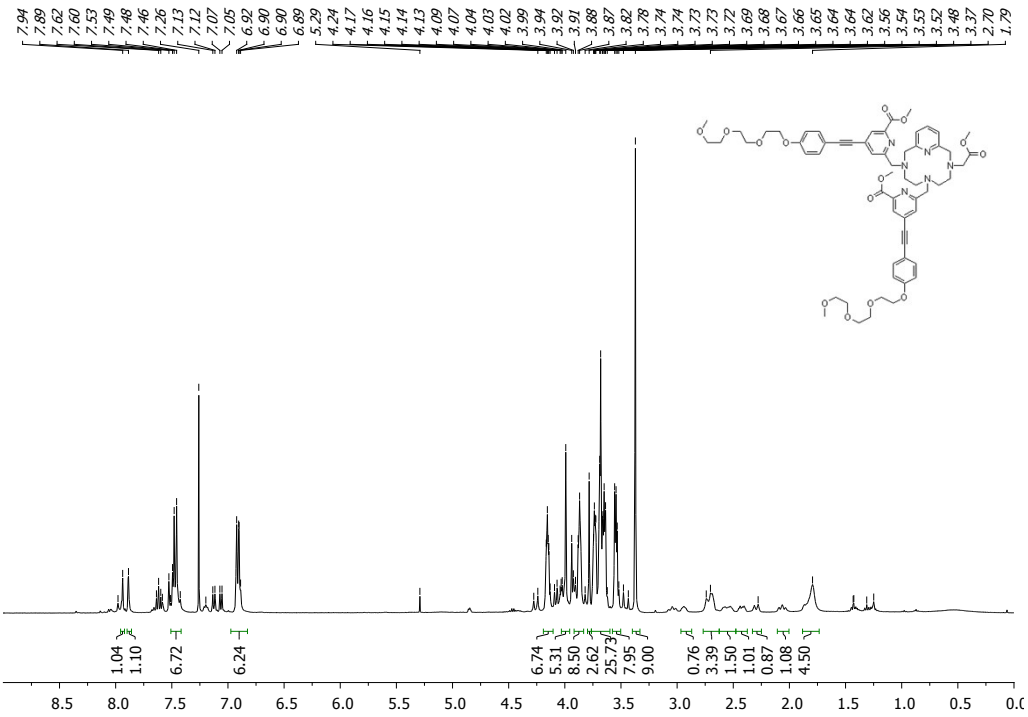


Figure S1: ^{1}H NMR of compound **3** (500 MHz, $CDCl_3$, 25°C).

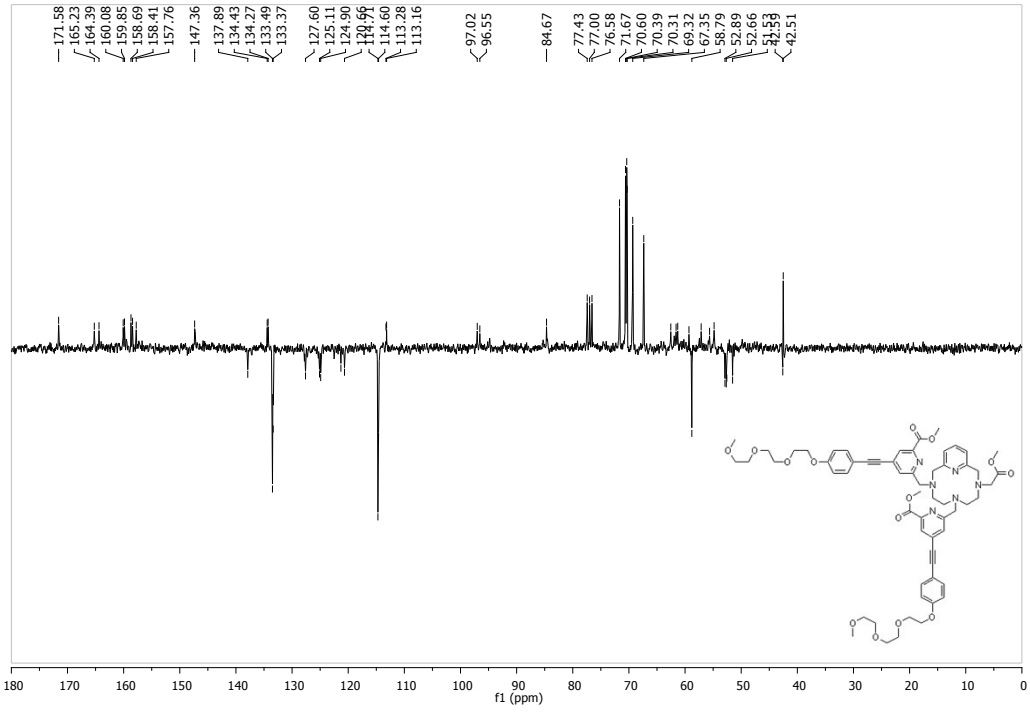


Figure S2: ^{13}C NMR of compound **3** (75 MHz, $CDCl_3$, 25°C).

Analysis Info

Sample Name	**NH27-f5**	Acquisition Date	10/07/2017 14:40:18
Analysis Name	X035627CYC.d	Instrument / Ser#	maXis 255552.00086
		Method	Positif.m

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.6 Bar
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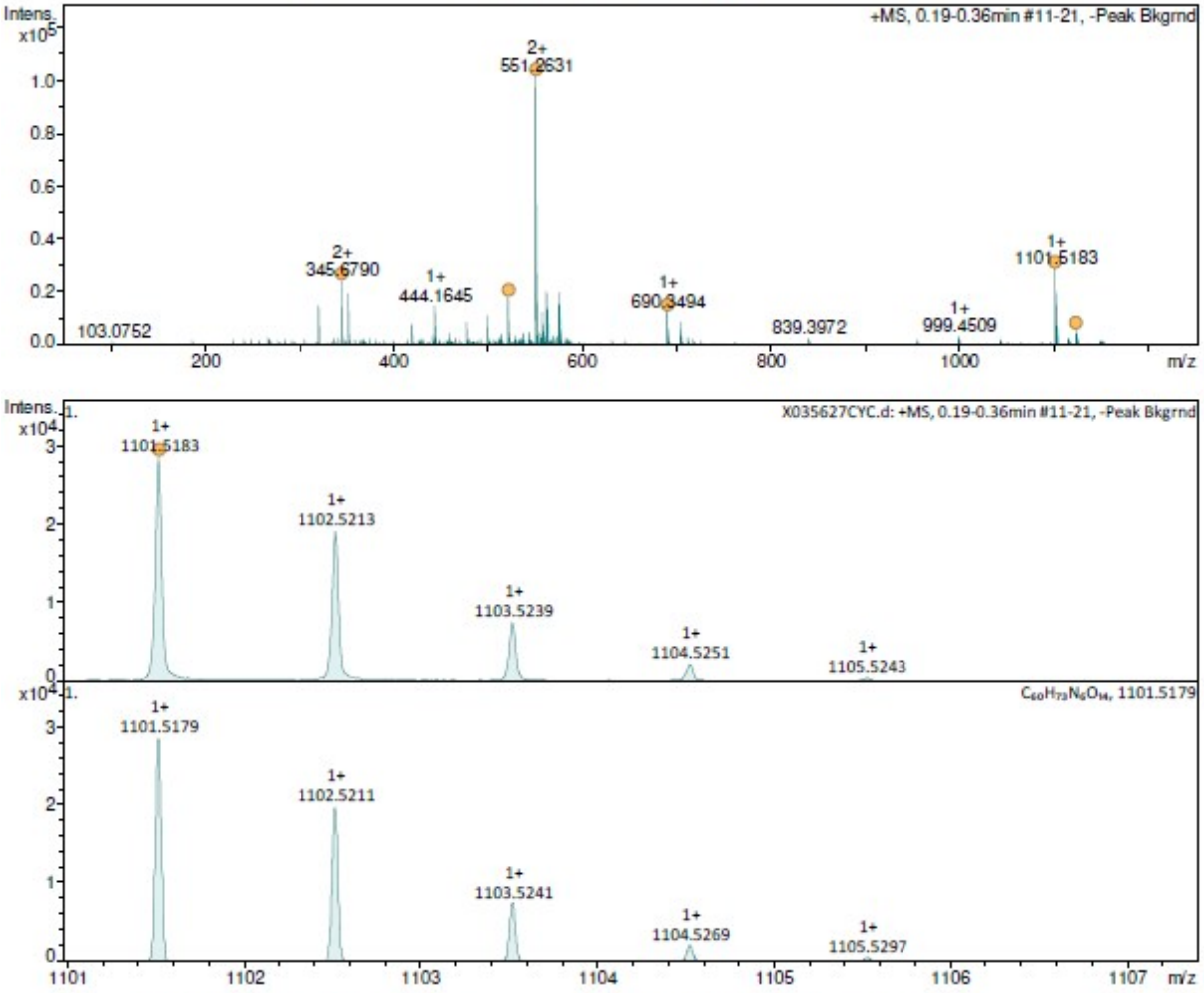

Meas. m/z	z	#	Ion Formula	m/z	err [ppm]	mSigma	rdb	e⁻ Conf
345.679008	2+	1	C37H49N5O8	345.678508	-1.4	21.6	17.0	even
522.242319	2+	1	C57H68N6O13	522.241670	-1.2	20.1	28.0	even
	2+	2	C61H72O15	522.243012	1.3	22.9	27.0	even
	2+	3	C70H60N8O2	522.241413	-1.7	78.3	46.0	even
	2+	4	C74H64N2O4	522.242756	0.8	91.2	45.0	even
551.263063	2+	1	C64H78O16	551.263945	1.6	6.9	27.0	even
	2+	2	C60H74N6O14	551.262602	-0.8	13.7	28.0	even
	2+	3	C59H78N2O18	551.261933	-2.0	24.3	23.0	even

Meas. m/z	z	#	Ion Formula	m/z	err [ppm]	mSigma	rdb	e⁻ Conf
690.349428	1+	1	C37H48N5O8	690.349740	0.5	10.1	17.0	even
	1+	2	C36H52NO12	690.348403	-1.5	14.8	12.0	even
1101.518254	1+	1	C59H77N2O18	1101.516590	-1.5	6.1	23.0	even
	1+	2	C60H73N6O14	1101.517927	-0.3	8.0	28.0	even
1123.500327	1+	1	C60H72N6NaO14	1123.499872	-0.4	35.7	28.0	even
	1+	2	C59H76N2NaO18	1123.498534	-1.6	37.8	23.0	even
	1+	3	C64H76NaO16	1123.502557	2.0	39.1	27.0	even

Figure S3: HRMS of compound **3.**

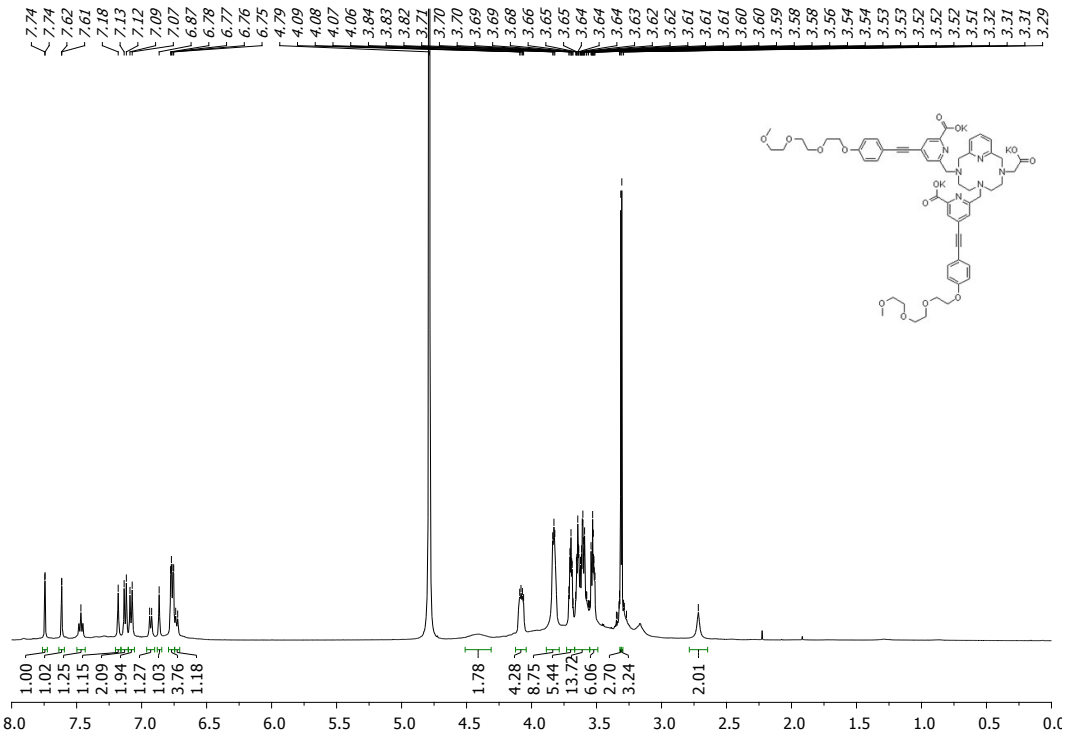

Figure S4: ^{1}H NMR of compound **L^{5b}** (500 MHz, D_2O, 25°C).

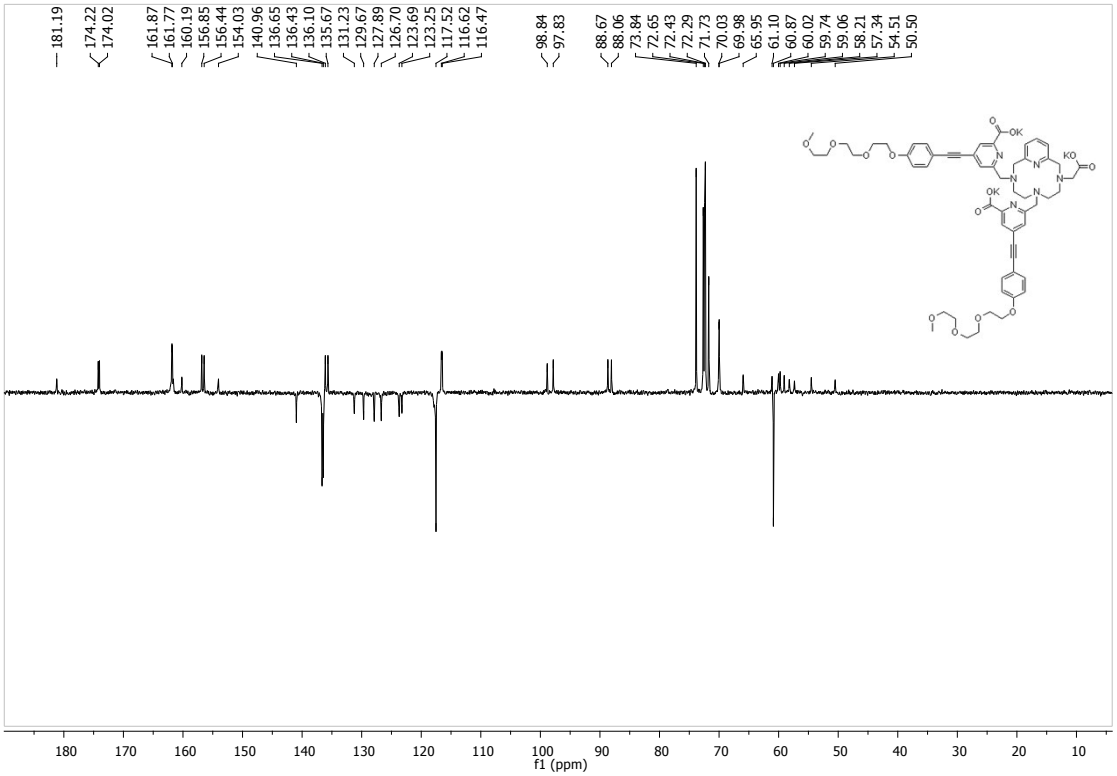

Figure S5: ^{13}C NMR of compound **L^{5b}** (125 MHz, D_2O, 25°C).

Analysis Info

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		Method	Positif.m

Acquisition Parameter

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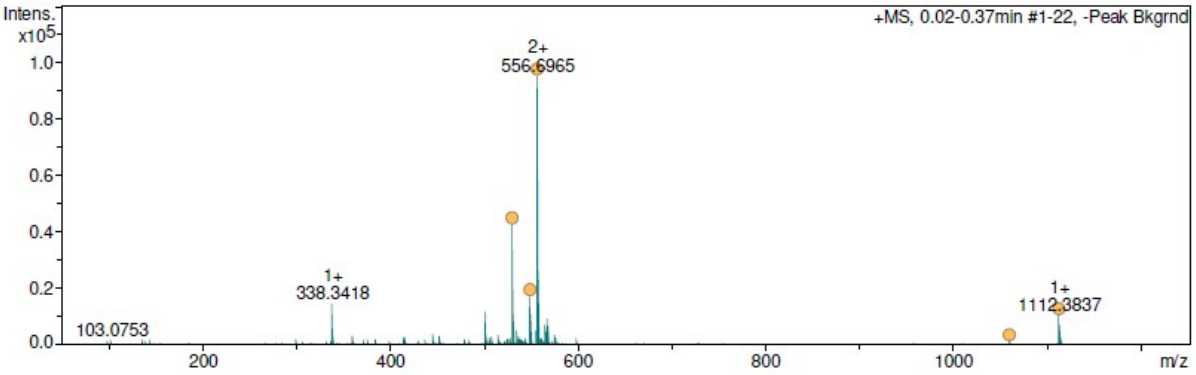
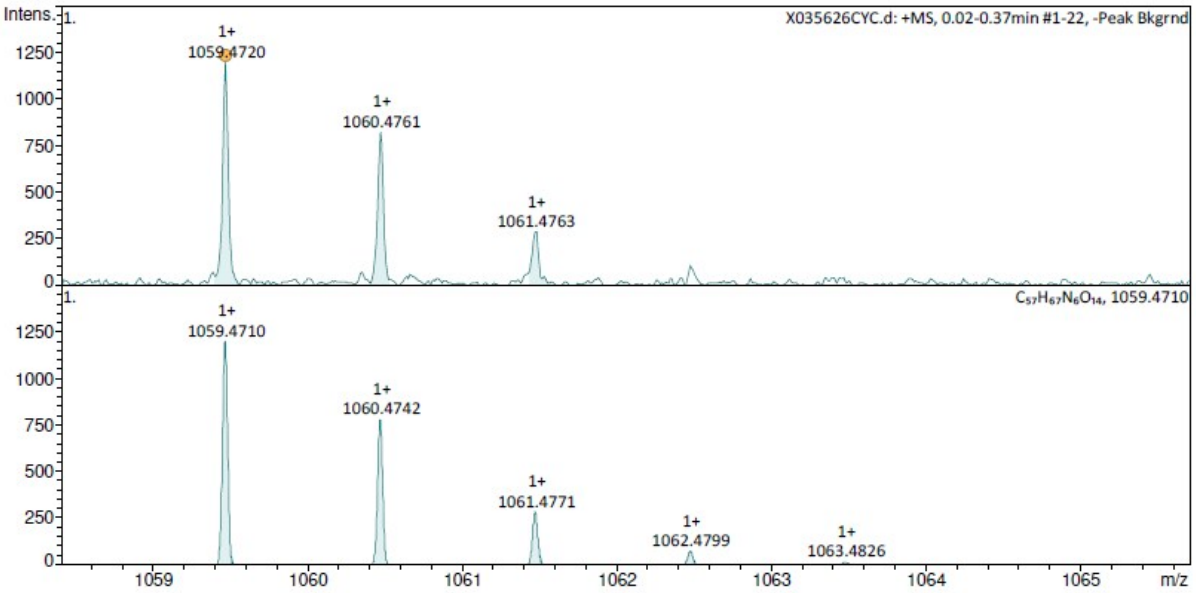




Meas. m/z	z	#	Ion Formula	m/z	err [ppm]	mSigma	rdb	e⁻ Conf
530.239702	2+	1	C61H72O16	530.240470	1.4	2.3	27.0	even
	2+	2	C57H68N6O14	530.239127	-1.1	12.1	28.0	even
	2+	3	C70H60N8O3	530.238870	-1.6	62.6	46.0	even
	2+	4	C74H64N2O5	530.240213	1.0	76.6	45.0	even
549.213642	2+	1	C57H66CaN6O14	549.212597	-1.9	12.8	29.0	even
	2+	2	C61H70CaO16	549.213940	0.5	15.4	28.0	even
	2+	3	C62H66CaN4O12	549.214609	1.8	23.5	33.0	even
	2+	4	C74H62CaN2O5	549.213683	0.1	74.8	46.0	even

Meas. m/z	z	#	Ion Formula	m/z	err [ppm]	mSigma	rdb	e⁻ Conf
	2+	5	C75H58CaN6O	549.214352	1.3	84.5	51.0	even
556.696527	2+	1	C61H69FeO16	556.696201	-0.4	8.9	28.5	odd
	2+	2	C57H65FeN6O14	556.694858	-2.9	11.6	29.5	odd
	2+	3	C62H65FeN4O12	556.696869	0.8	15.5	33.5	odd
	2+	4	C63H61FeN8O8	556.697538	2.0	23.9	38.5	odd
	2+	5	C74H61FeN2O5	556.695944	-0.8	66.2	46.5	odd
	2+	6	C75H57FeN6O	556.696613	0.4	75.7	51.5	odd
	2+	7	C79H61FeO3	556.697956	2.8	87.6	50.5	odd
1059.472006	1+	1	C57H67N6O14	1059.470977	-1.0	32.9	28.0	even
	1+	2	C61H71O16	1059.473663	1.6	33.7	27.0	even
	1+	3	C70H59N8O3	1059.470464	-1.5	70.2	46.0	even
	1+	4	C74H63N2O5	1059.473150	1.1	84.7	45.0	even
1112.383700	1+	1	C61H68FeO16	1112.385125	1.4	8.3	28.5	odd
	1+	2	C57H64FeN6O14	1112.382440	-1.0	15.3	29.5	odd
	1+	3	C70H56FeN8O3	1112.381927	-1.4	50.1	47.5	odd
	1+	4	C74H60FeN2O5	1112.384612	1.0	61.5	46.5	odd
	1+	5	C75H56FeN6O	1112.385949	-889.9	663.5	51.5	odd

Figure S6: HRMS of compound **L^{5b}.** Peak at 338 is due to the presence of erucamide in Eppendorf's.

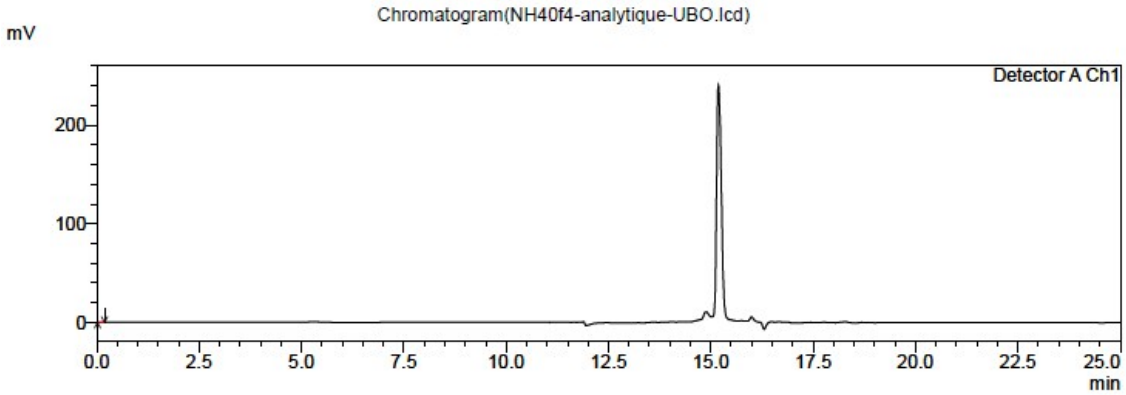
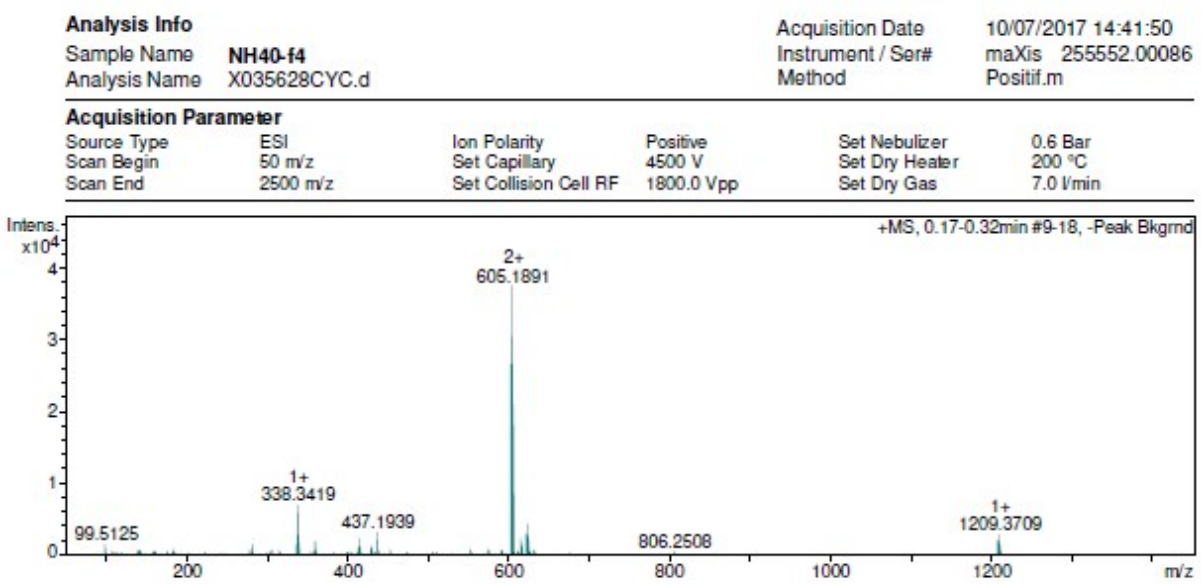
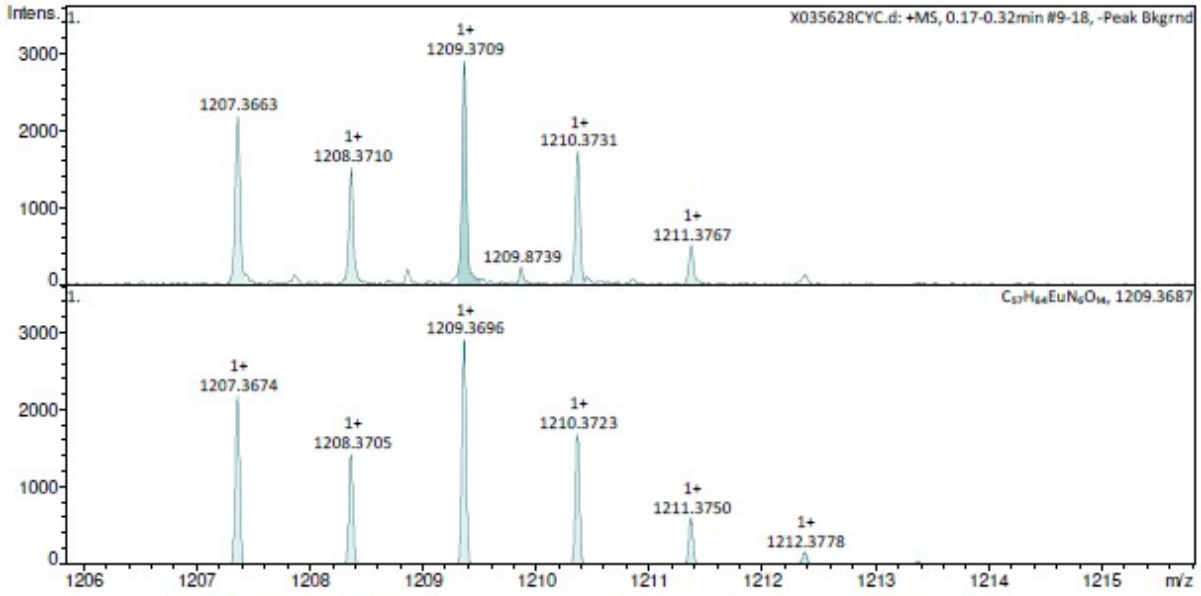


Figure S7: Analytical HPLC of the [Eu**L**^{5b}] complex. Column: Vision HT C18 HL 5 µ 250× 4.6 mm. Gradient: 100% H_2O 0-5 min, 0-90% ACN 5-15 min, 90% ACN 15-20 min, 100% H_2O 20-25 min. Flow : 1mL/min. retention time = 15.186 min.





Meas. m/z	z	#	Ion Formula	m/z	err [ppm]	mSigma	rdb	e⁻ Conf
605.189109	2+	1	C57H65EuN6O14	605.188004	-1.1	22.7	29.5	odd
	2+	2	C61H69EuO16	605.189347	1.2	24.9	28.5	odd
	2+	3	C45H69EuN8O21	605.188930	0.2	47.3	16.5	odd
	2+	4	C44H73EuN4O25	605.188261	-0.9	55.7	11.5	odd
624.162540	2+	1	C61H67CaEuO16	624.162818	1.2	38.1	29.5	odd
	2+	2	C57H63CaEuN6O14	624.161475	-1.1	40.3	30.5	odd
	2+	3	C74H59CaEuN2O5	624.162561	1.0	58.2	47.5	odd
1209.370862	1+	1	C57H64EuN6O14	1209.368732	-1.1	28.9	29.5	odd

Meas. m/z	z	#	Ion Formula	m/z	err [ppm]	mSigma	rdb	e⁻ Conf
	1+	2	C61H68EuO16	1209.371418	1.3	29.9	28.5	odd
	1+	3	C45H68EuN8O21	1209.370583	0.3	64.4	16.5	odd
	1+	4	C74H60EuN2O5	1209.370905	1.1	64.7	46.5	odd

Figure S8: HRMS of the [Eu**L**^{5b}] complex. Peak at 338 is due to the presence of erucamide in Eppendorf's.

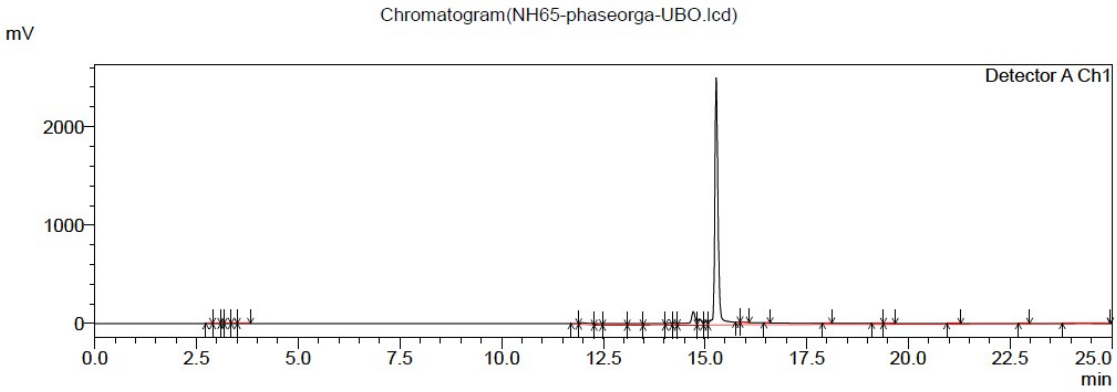
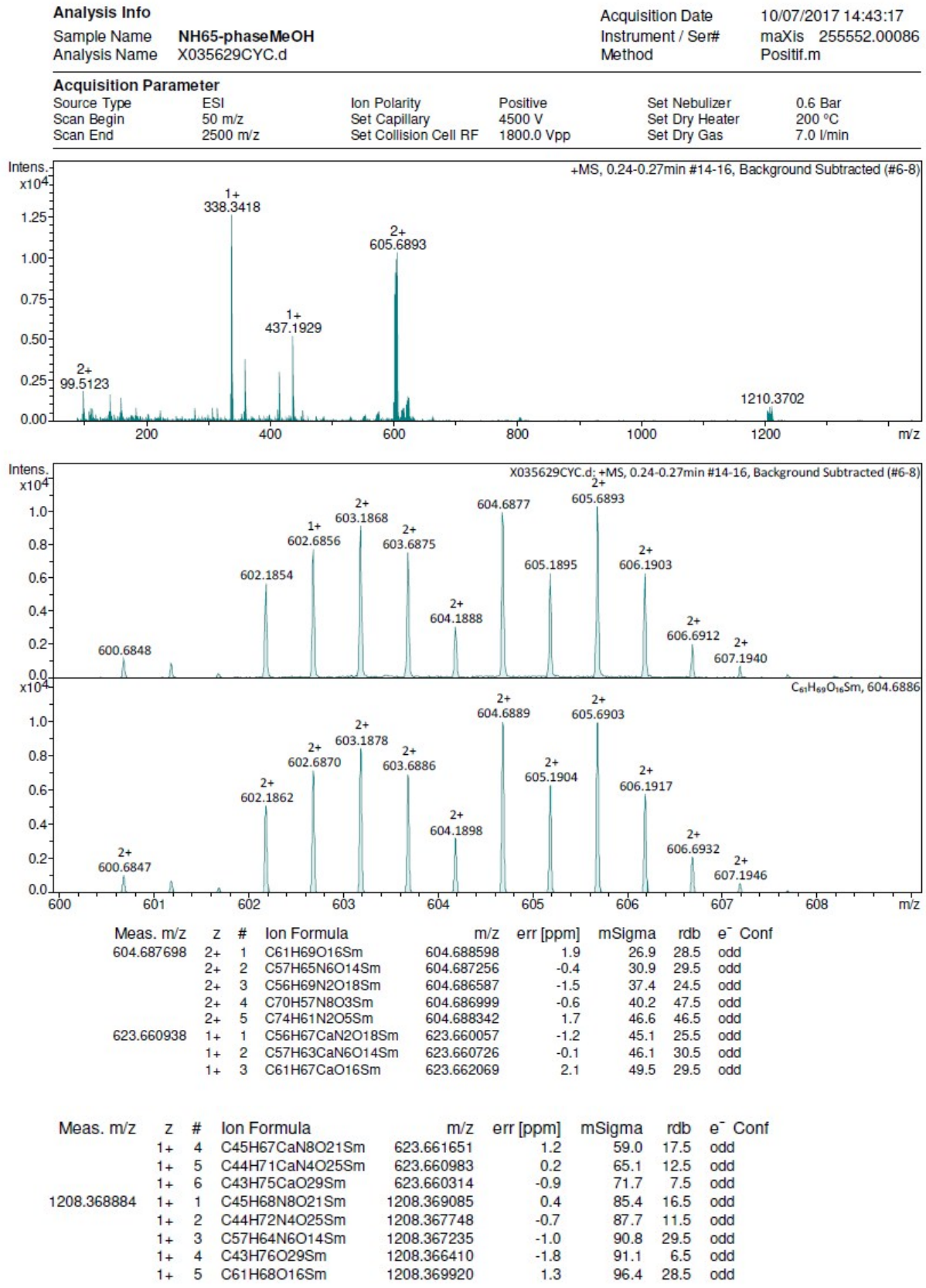


Figure S9: Analytical HPLC of the [Sm**L**^{5b}] complex. Column: Vision HT C18 HL 5 µ 250× 4.6 mm. Gradient: 100% H_2O 0-5 min, 0-90% ACN 5-15 min, 90% ACN 15-20 min, 100% H_2O 20-25 min. Flow : 1mL/min. retention time = 15.278 min.



Meas. m/z	z	#	Ion Formula	m/z	err [ppm]	mSigma	rdb	e⁻ Conf
604.687698	2+	1	C61H69O16Sm	604.688598	1.9	26.9	28.5	odd
	2+	2	C57H65N6O14Sm	604.687256	-0.4	30.9	29.5	odd
	2+	3	C56H69N2O18Sm	604.686587	-1.5	37.4	24.5	odd
	2+	4	C70H57N8O3Sm	604.686999	-0.6	40.2	47.5	odd
	2+	5	C74H61N2O5Sm	604.688342	1.7	46.6	46.5	odd
623.660938	1+	1	C56H67CaN2O18Sm	623.660057	-1.2	45.1	25.5	odd
	1+	2	C57H63CaN6O14Sm	623.660726	-0.1	46.1	30.5	odd
	1+	3	C61H67CaO16Sm	623.662069	2.1	49.5	29.5	odd

Meas. m/z	z	#	Ion Formula	m/z	err [ppm]	mSigma	rdb	e⁻ Conf
	1+	4	C45H67CaN8O21Sm	623.661651	1.2	59.0	17.5	odd
	1+	5	C44H71CaN4O25Sm	623.660983	0.2	65.1	12.5	odd
	1+	6	C43H75CaO29Sm	623.660314	-0.9	71.7	7.5	odd
1208.368884	1+	1	C45H68N8O21Sm	1208.369085	0.4	85.4	16.5	odd
	1+	2	C44H72N4O25Sm	1208.367748	-0.7	87.7	11.5	odd
	1+	3	C57H64N6O14Sm	1208.367235	-1.0	90.8	29.5	odd
	1+	4	C43H76O29Sm	1208.366410	-1.8	91.1	6.5	odd
	1+	5	C61H68O16Sm	1208.369920	1.3	96.4	28.5	odd

Figure S10: HRMS of the [Sm**L**^{5b}] 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**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**L**$^{5b'}$] performed with SHAPE 2.[7]

EP-9 (Enneagon) D_{9h}	OPY-9 (Octagonal pyramid) C_{8v}	HBPY-9 (Heptagonal bipyramid) D_{7h}	JTC-9 (Triangular cupola) C_{3v}	JCCU-9 (Capped cube) C_{4v}	CCU-9 (Capped cube) C_{4v}	JCSAPR-9 (Capped sq. antiprism) C_{4v}
34.033	22.836	16.619	10.540	9.239	8.281	2.614
CSAPR-9 (Capped sq. antiprism) C_{4v}	JTCTPR-9 (Tricapped trigonal prism) D_{3h}	TCTPR-9 (Tricapped trigonal prism) D_{3h}	JTDIC-9 (Tridiminished icosahedron) C_{3v}	HH-9 (Hula-hoop) C_{2v}	MFF-9 (Muffin) C_s	
1.896	2.68843	2.05745	10.238	10.617	2.197	

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, Φ, were measured with dilute solutions in water or organic solvents with an absorbance of less than 0.1 by using equation (1):

$$\frac{\Phi_x}{\Phi_r} = \frac{A_r(\lambda)}{A_x(\lambda)} \frac{{n_x}^2}{{n_r}^2} \frac{D_x}{D_r} \quad (1)$$

in which A is the absorbance at the excitation wavelength (λ), 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 (Φ_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):

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

The hydration number q is determined for europium complexes by measuring the lifetime in H_2O and D_2O according to the equation (3):[8]

$$q = 1.11\ (k(H_2O) - k(D_2O) - 0.31)$$

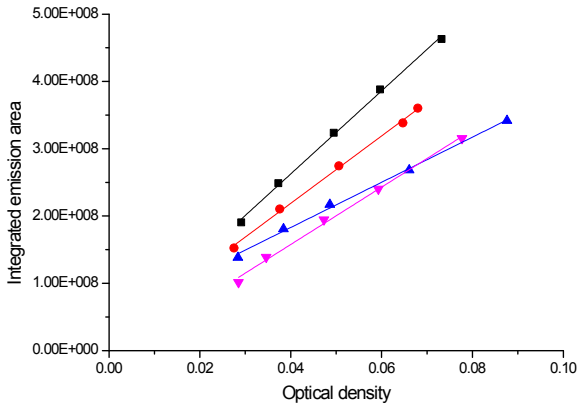


Figure S11. Determination of the quantum yield of [Eu**L**^{5b}] in MeOH (red), H_2O (blue), CH_2Cl_2 (magenta) and the reference quinine sulfate in a 1N aqueous solution of sulfuric acid (black). The straight lines correspond to the linear fit.

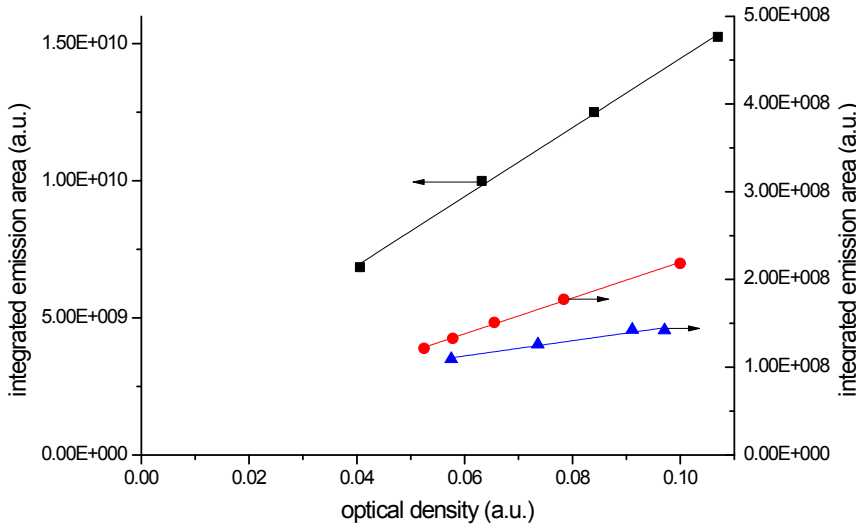


Figure S12. Determination of the quantum yield of [Sm**L**^{5b}] in MeOH (red), H_2O (blue), and the reference quinine sulfate in a 1N aqueous solution of sulfuric acid (black). The straight lines correspond to the linear fit.

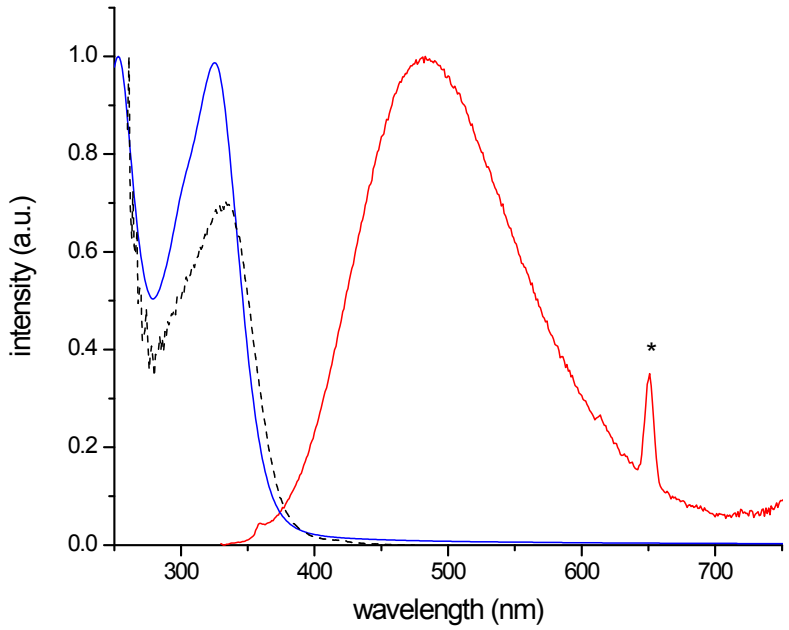


Figure S13. Normalized absorption (blue), excitation (dotted) and emission spectra (red) of **3** in MeOH (λ_{exc} = 325nm) at room temperature. The harmonic scattering at 650 nm is indicated by the *.

Table S2. Relative intensity of the [Eu**L**^{i}] complexes measured in MeOH at room temperature. r^{i} represents the ratio of area of the j=2 over j=4 band for the [Eu**L**^{i}] complex and %j=2 and %j=4 represent the percentages of the area of the j band over the entire spectrum.

	A_j							
complex	j =0	j = 1	j = 2	j = 3	j = 4	r^{i}	% j=2	% j=4
[Eu**L**^{5b}]	0.0	1.0	7.2	0.4	2.4	3.0	65.4	21.6
[Eu**L**^{3b}]$^{+}$	0.1	1.0	5.2	0.9	2.1	2.5	56.5	22.5
[Eu**L**^{2b}]	0.1	1.0	9.1	0.4	0.8	10.9	79.2	7.3
[Eu**L**^{1a}]$^{+}$	0.1	1.0	2.7	0.2	2.0	1.4	45.0	33.3

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 cm^2 tissue-culture flasks (T25) at 378C in a humidified atmosphere with 5% CO_2. They were incubated in RPMI 1640 supplemented with 100 UmL^{-1} penicillin, 100 $\mu g.mL^{-1}$ 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.

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