

Electronic Supplementary Information

Lanthanide complexes with phenanthroline-based ligands: insights into mechanisms of cell death by imaging tools

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[Sm(DBM)₃(Phen)] (1)

Anal. Calc. for :C₅₇H₄₁N₂O₆Sm: C,68.44; H,4.13; N, 2.80%; Found C, 68.40; H, 4.43; N, 2,83%.

IR (KBr, ν/cm^{-1}) : 3056 (w), 1595(s), 1551 (s) 1518 (s), 1478 (s), 1458 (m), 1411(s), 1311 (m), 1284 (m,sh), 1220 (m), 1178 (w), 1067 (w), 1023 (m), 941 (w), 783 (w), 750 (m,sh), 721 (s), 689 (m), 521 (w), 426 (w).

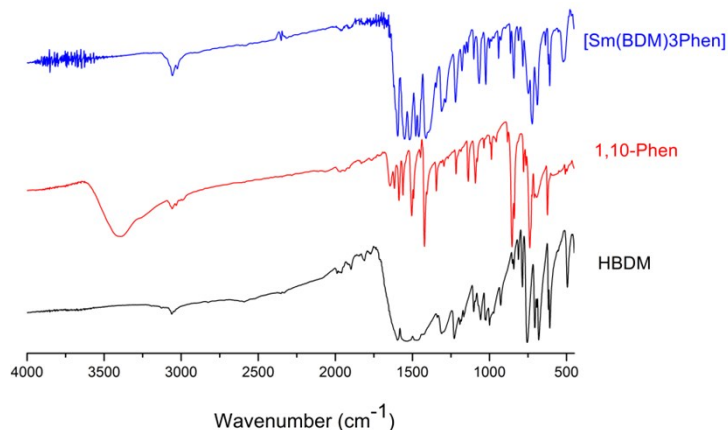


Figure S1. IR spectra of **1** and the co-ligands, 1,10-Phen and HDBM, in the region 4000-400cm⁻¹.

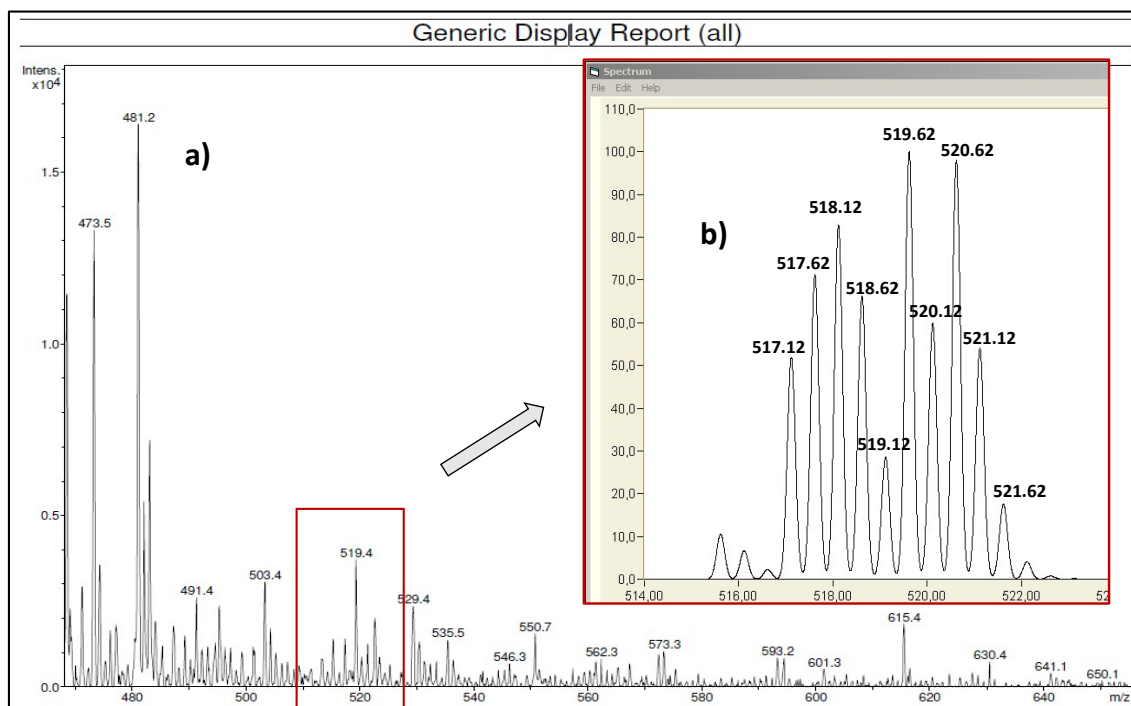


Figure S2. Characterization of [Sm(DBM)₃(Phen)] by mass spectrometry: **a)** experimental molecular-ion ([M+2H₂O]²⁺); **b)** calculated [M+2H₂O]²⁺.

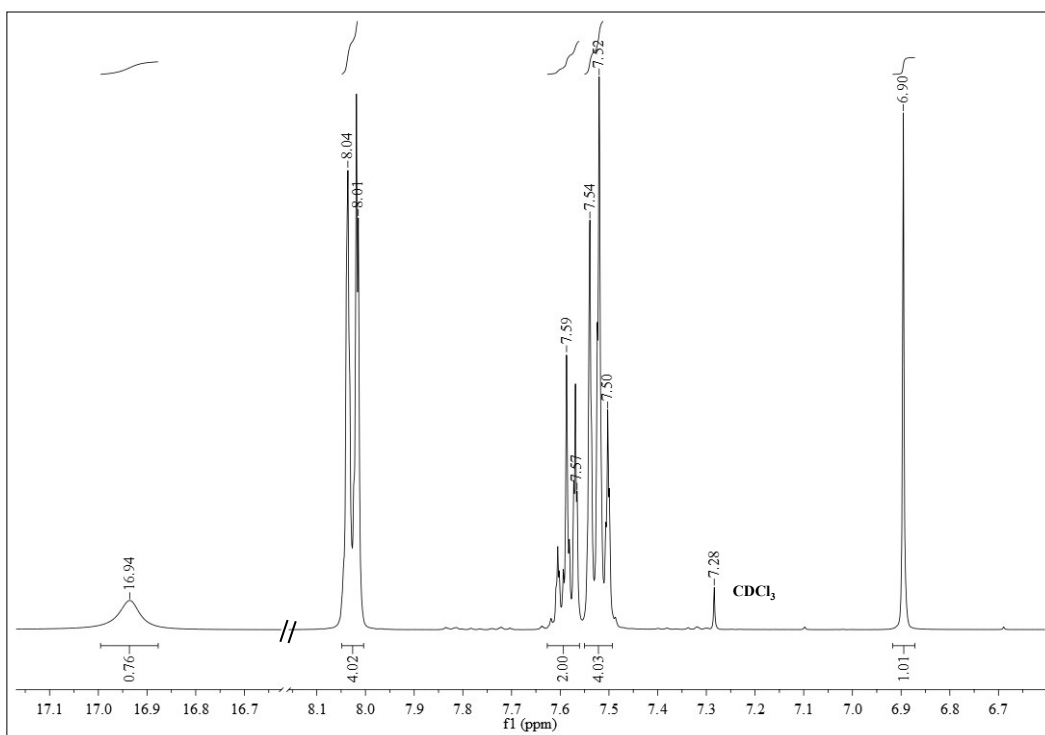


Figure S3. ^1H NMR (400 MHz, CDCl_3) of HDBM free ligand.

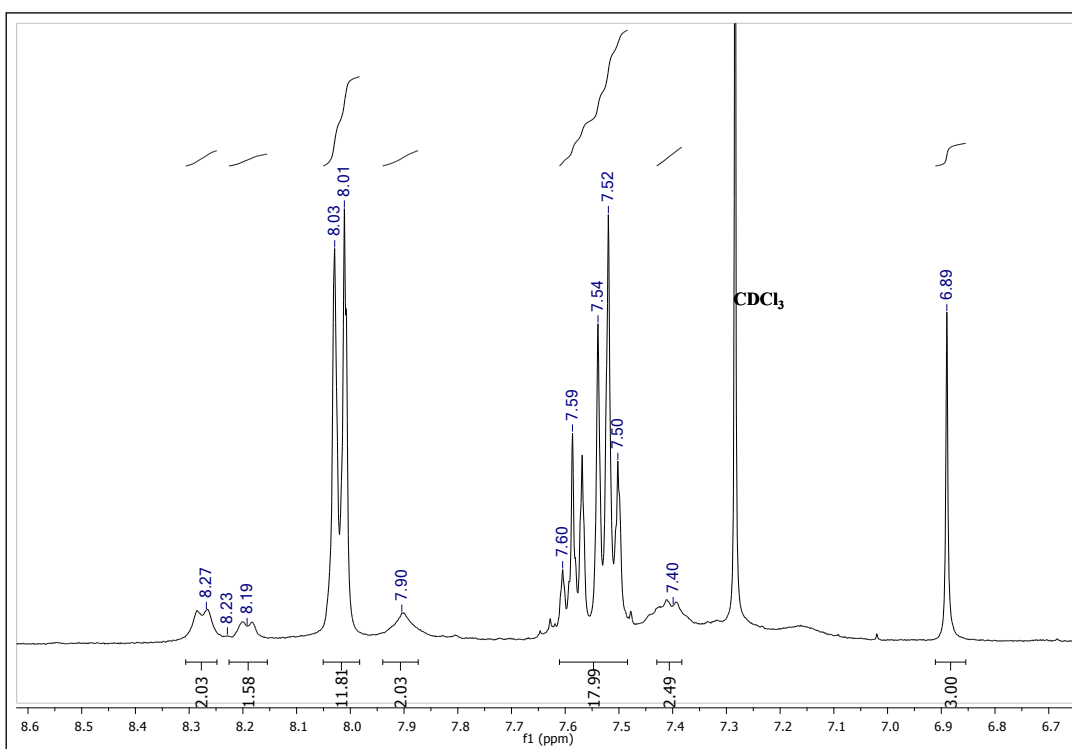


Figure S4. ^1H NMR (400 MHz, CDCl_3) of $[\text{Sm}(\text{DBM})_3(\text{Phen})]$ in CDCl_3 , and inset of zoom from 8.5 to 6.8 ppm. ^1H NMR in CDCl_3 (δ , ppm) 8.27 (d, 2H, Phen), 8.20 (d, 2H, Phen), 8.03 (d, $J = 7.0$ Hz, 12H, *o*-Ph), 7.90 (d, $J = 7.2$ Hz, 2H, Phen), 7.60-7.50 (m, 18H, *m*-Ph and *p*-Ph), 7.41 (d, $J = 7.2$ Hz, 2H, Phen), 6.89 (s, 3H, CH).

[Sm(DBM)₃NH₂Phen] (2)

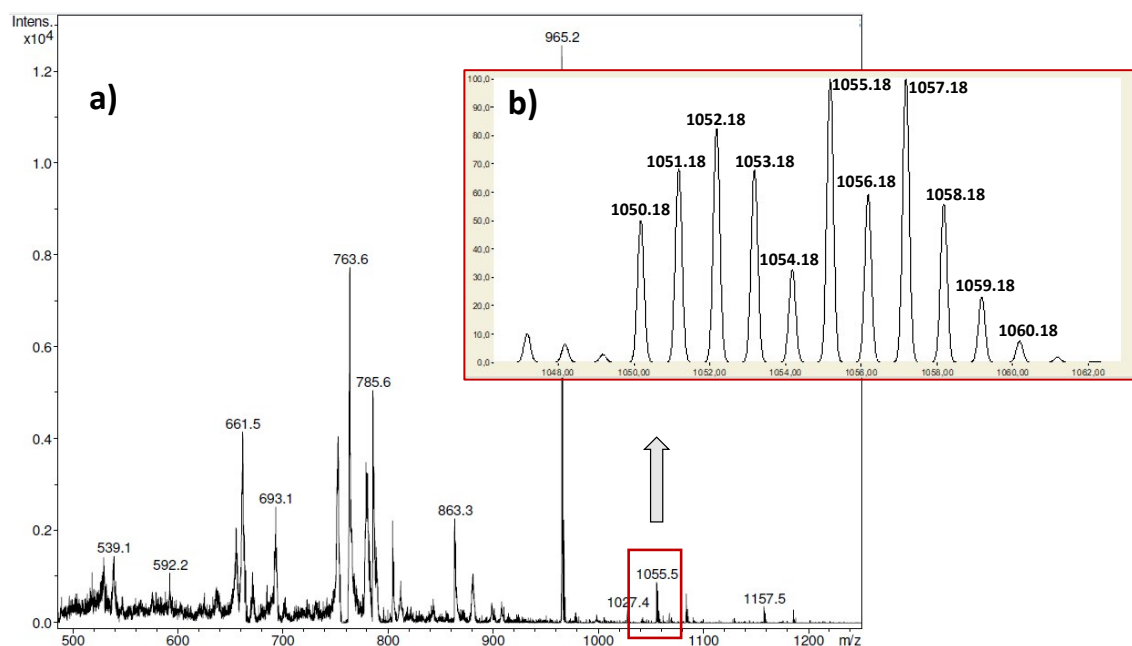


Figure S5. Characterization of [Sm(DBM)₃NH₂Phen] by mass spectrometry: **a)** experimental molecular-ion ($[M+K]^+$); **b)** calculated $[M+K]^+$.

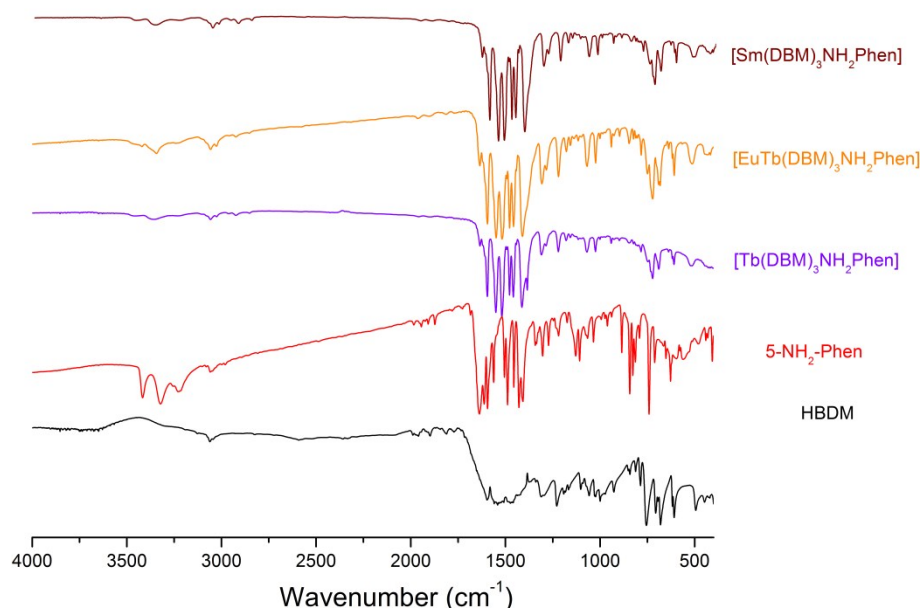


Figure S6. IR spectra of 2, 3, 4 and the co-ligands 5-NH₂Phen and HDBM, in the region 4000-400 cm^{-1} .

Table S1. Selected IR bands assignment of HDBM, NH₂Phen and its lanthanide complexes (cm⁻¹)

	HDBM	5 NH ₂ Phen	1	2	3	4
ν C-C	1598	1616	1595	1594	1594	1595
ν_a C=C-C=O	1540	-	1518	1516	1518	1518
ν_a O-C=C-C	1474	-	1480	1456	1458	1458
ν C=N	-	1428, 1407	1411	1409	1409	1411
ν C-N (CNH ₂)	-	1303	1311	1310	1308	1309
δ CH α	1229	-	1220	1220	1219	1220
γ CH+ π (C H)	756	741	750, 722	743,722	749, 721	747, 722
Ln-O	-	-	521	520	513	522

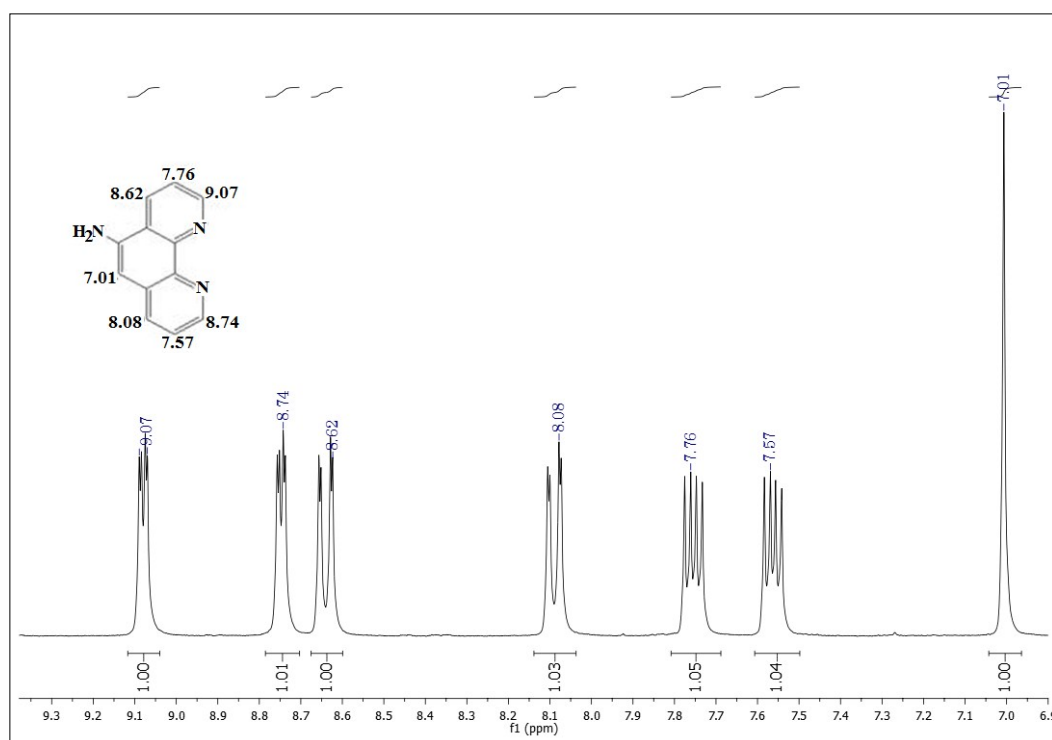


Figure S7. ¹H NMR (400 MHz, CD₃OD) of 5-NH₂ Phen in CD₃OD.

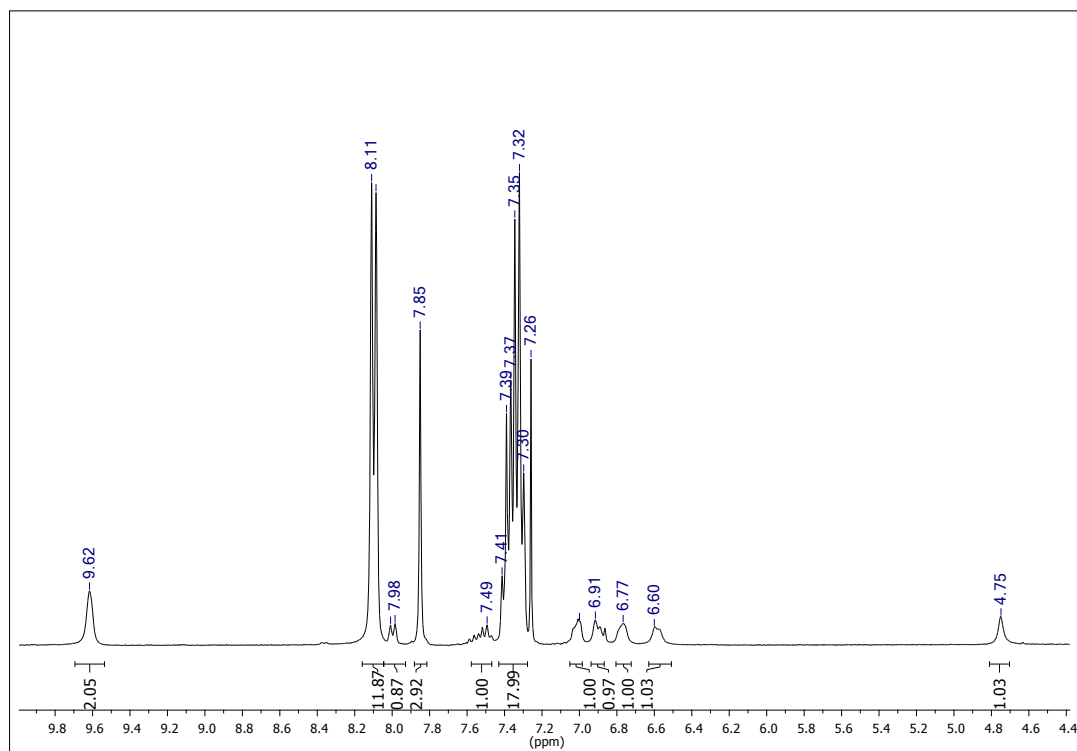


Figure S8. ^1H NMR (400 MHz, CDCl_3) [$\text{Sm}(\text{DBM})_3\text{NH}_2\text{Phen}$]

[$\text{Eu}(\text{DBM})_3\text{NH}_2\text{Phen}$] (3)

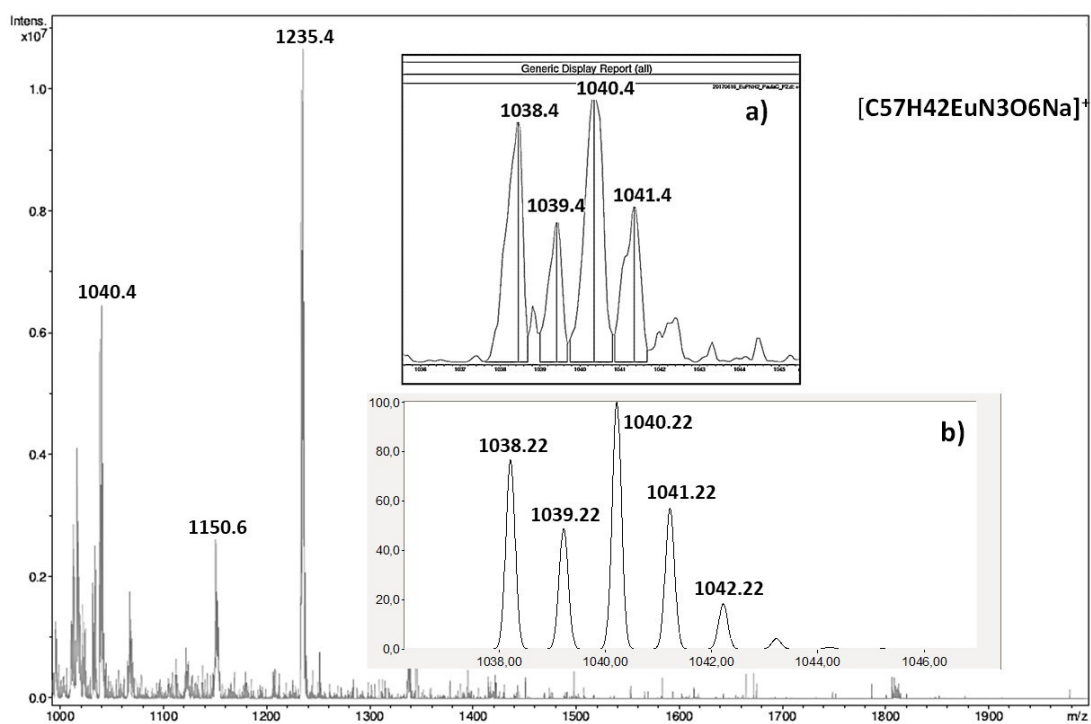


Figure S9. Characterization of [$\text{Eu}(\text{DBM})_3\text{NH}_2\text{Phen}$] by mass spectrometry: **a)** experimental molecular-ion ($[\text{M}+\text{Na}]^+$); **b)** calculated $[\text{M}+\text{Na}]^+$.

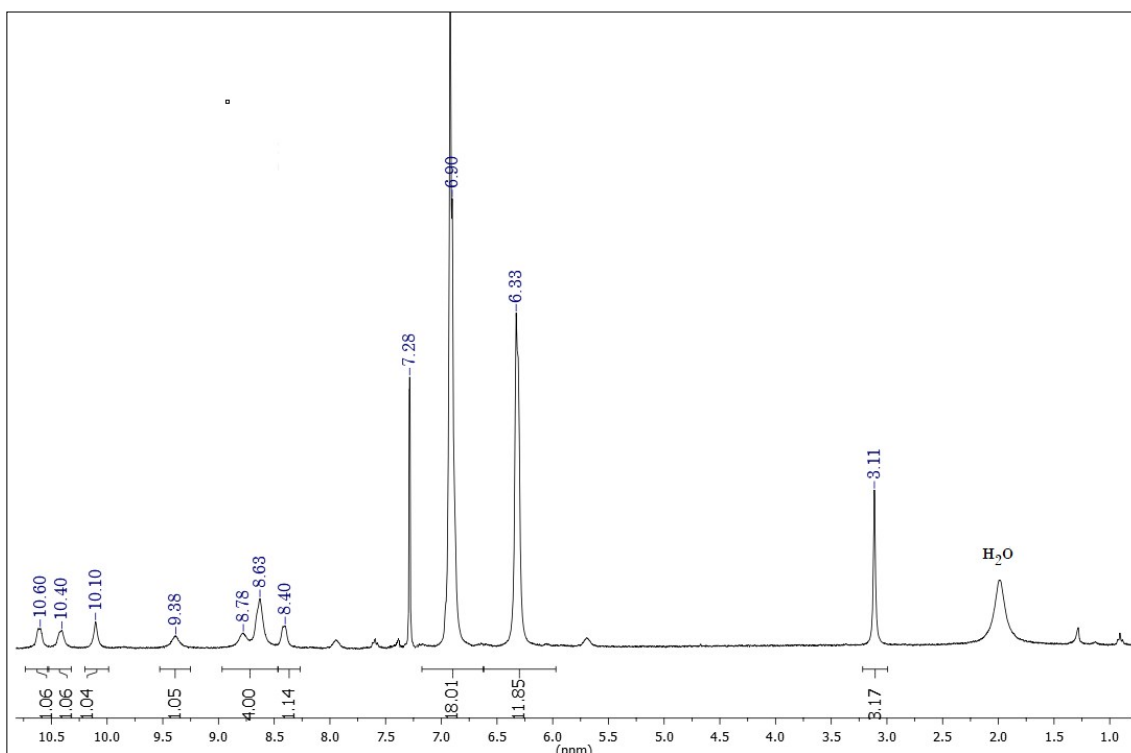


Figure S10. ^1H NMR (400 MHz, CDCl_3) of $[\text{Eu}(\text{DBM})_3\text{NH}_2\text{Phen}]$.

$[\text{Tb}(\text{DBM})_3\text{NH}_2\text{Phen}]$ (4)

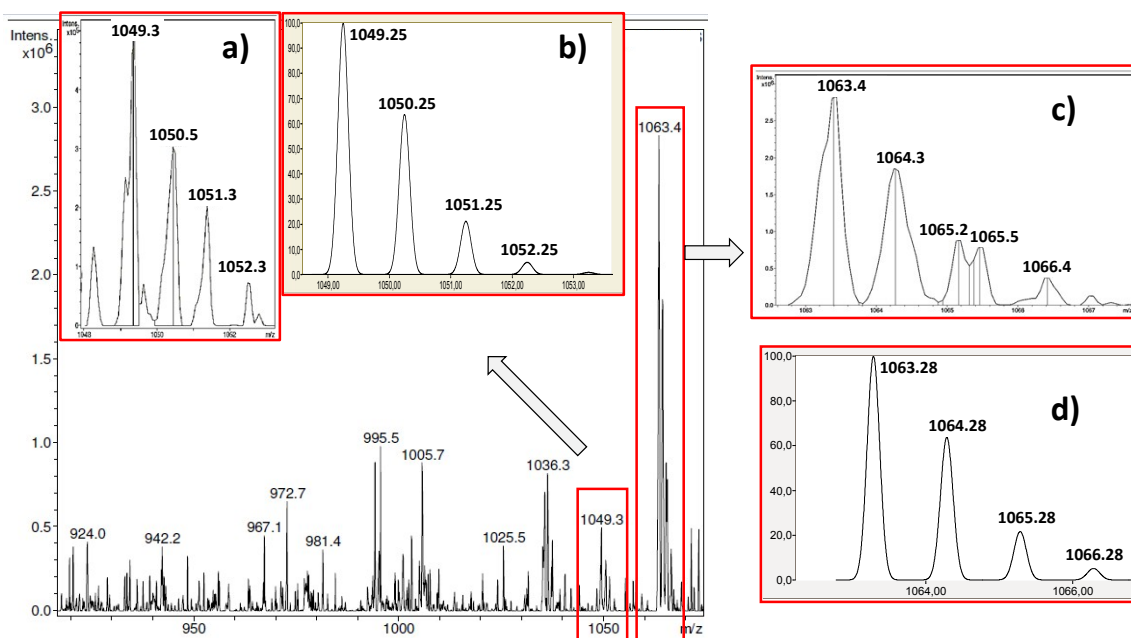
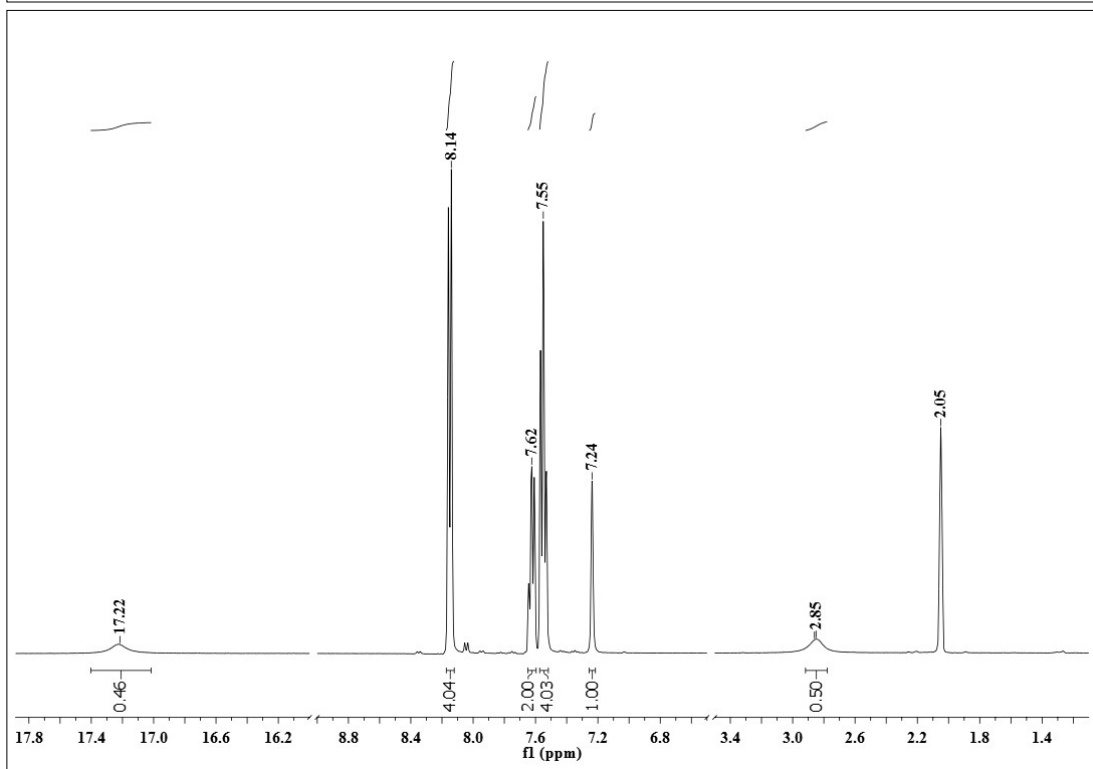
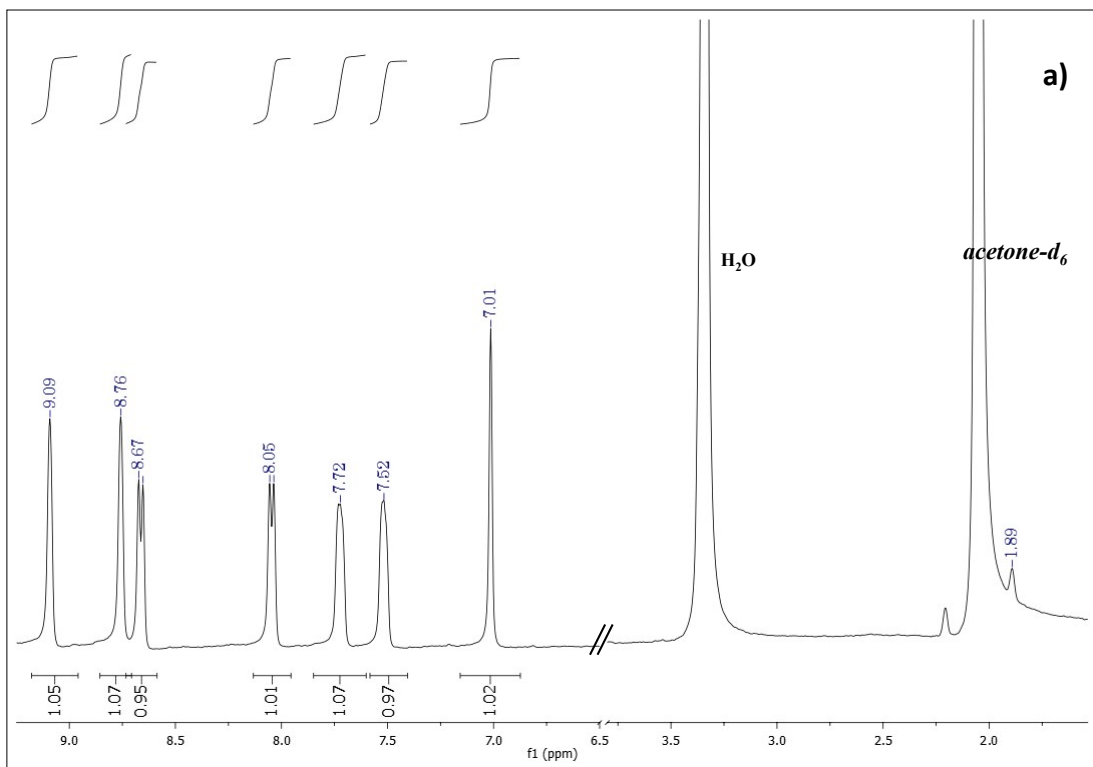


Figure S11. Characterization of $[\text{Tb}(\text{DBM})_3\text{NH}_2\text{Phen}]$ by mass spectrometry: **a)** experimental molecular-ion ($[\text{M}+\text{Na}]^+$; **b)** calculated $[\text{M}+\text{Na}]^+$; **c)** experimental molecular-ion $[\text{M}+\text{H}+2\text{H}_2\text{O}]^+$; **d)** calculated $[\text{M}+\text{H}+2\text{H}_2\text{O}]^+$.



Fi

Figure S12. ¹H NMR free ligands (400 MHz, Acetone-*d*₆) **a)** 5-NH₂Phen; **b)** HDBM.

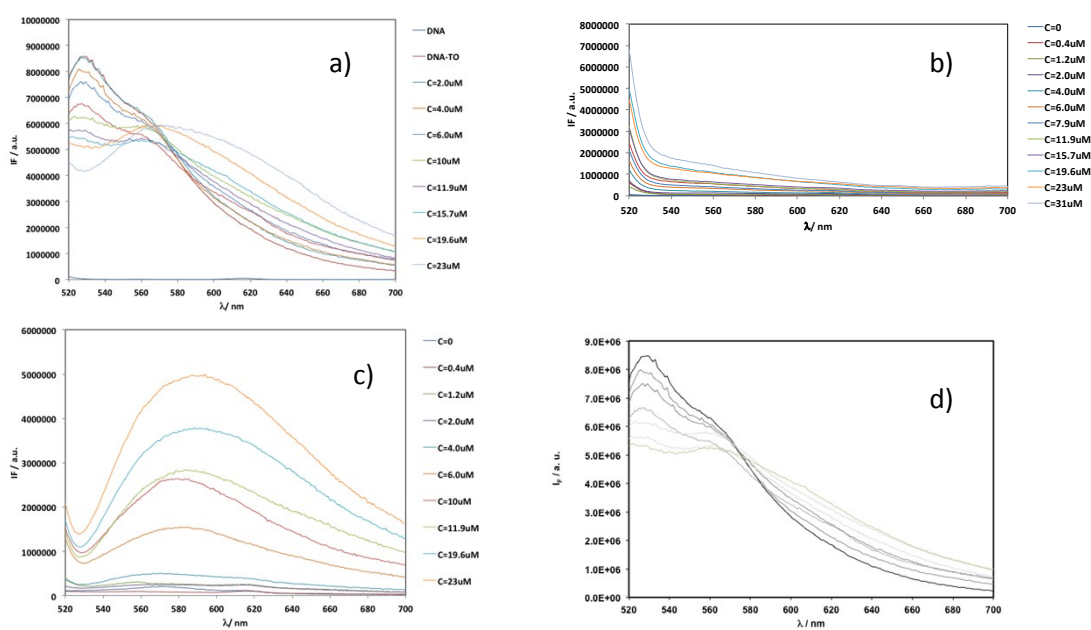
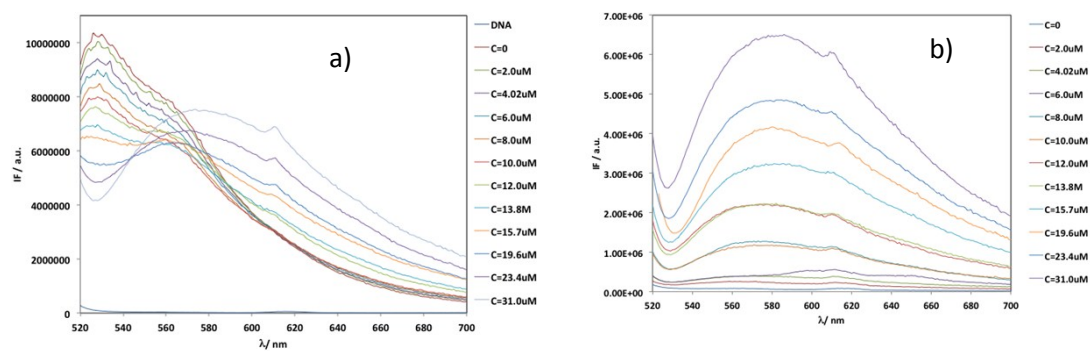


Figure S13A - Fluorescence emission spectra measured for solutions containing a) 2.0 μM of *ctDNA*, a molar ratio of TO:*ctDNA* = 0.8 and using $[\text{Sm}(\text{DBM})_3(\text{NH}_2\text{Phen})]$ concentrations from 0 to 23 μM ; b) $[\text{Sm}(\text{DBM})_3(\text{NH}_2\text{Phen})]$ from 0 to 23 μM ; c) 1.6 μM of TO and $[\text{Sm}(\text{DBM})_3(\text{NH}_2\text{Phen})]$ concentrations from 0 to 23 μM ; d) 2.0 μM of *ctDNA*, a molar ratio of TO:*ctDNA* = 0.8 and using $[\text{Sm}(\text{DBM})_3(\text{NH}_2\text{Phen})]$ concentrations from 0 to 23 μM , after subtraction of the corresponding spectra included in c).



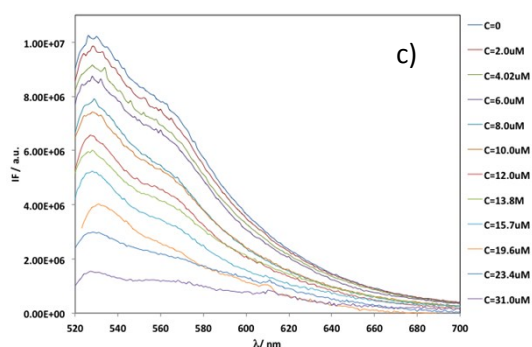


Figure S13B - Fluorescence emission spectra measured for solutions containing a) 2.0 μM of *ctDNA*, a molar ratio of TO:*ctDNA* = 0.8 and using $[\text{Eu}(\text{DBM})_3(\text{NH}_2\text{Phen})]$ concentrations from 0 to 31 μM . b) 1.6 μM of TO and $[\text{Eu}(\text{DBM})_3(\text{NH}_2\text{Phen})]$ concentrations from 0 to 31 μM ; d) 2.0 μM of *ctDNA*, a molar ratio of TO:*ctDNA* = 0.8 and using $[\text{Eu}(\text{DBM})_3(\text{NH}_2\text{Phen})]$ concentrations from 0 to 31 μM , after subtraction of the corresponding spectra included in b)

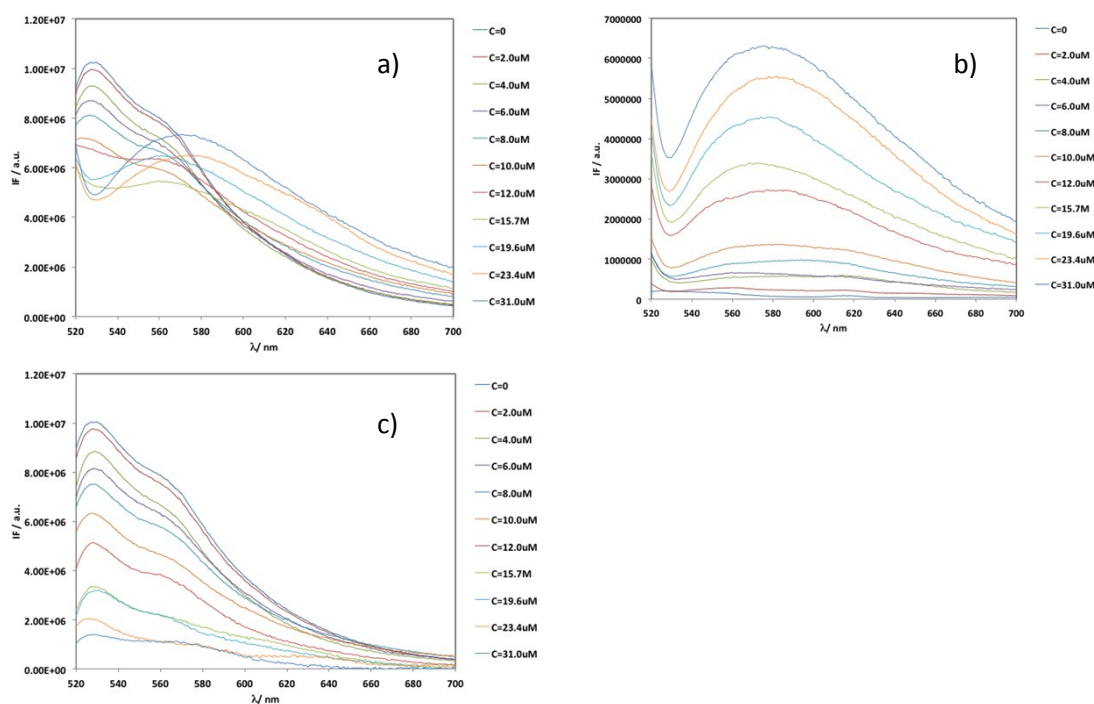


Figure S13C - Fluorescence emission spectra measured for solutions containing a) 2.0 μM of *ctDNA*, a molar ratio of TO:*ctDNA* = 0.8 and using $[\text{Tb}(\text{DBM})_3(\text{NH}_2\text{Phen})]$ concentrations from 0 to 31 μM . b) 1.6 μM of TO and $[\text{Tb}(\text{DBM})_3(\text{NH}_2\text{Phen})]$ concentrations from 0 to 31 μM ; c) 2.0 μM of *ctDNA*, a molar ratio of TO:*ctDNA* = 0.8 and using $[\text{Tb}(\text{DBM})_3(\text{NH}_2\text{Phen})]$ concentrations from 0 to 31 μM , after subtraction of the corresponding spectra included in b).

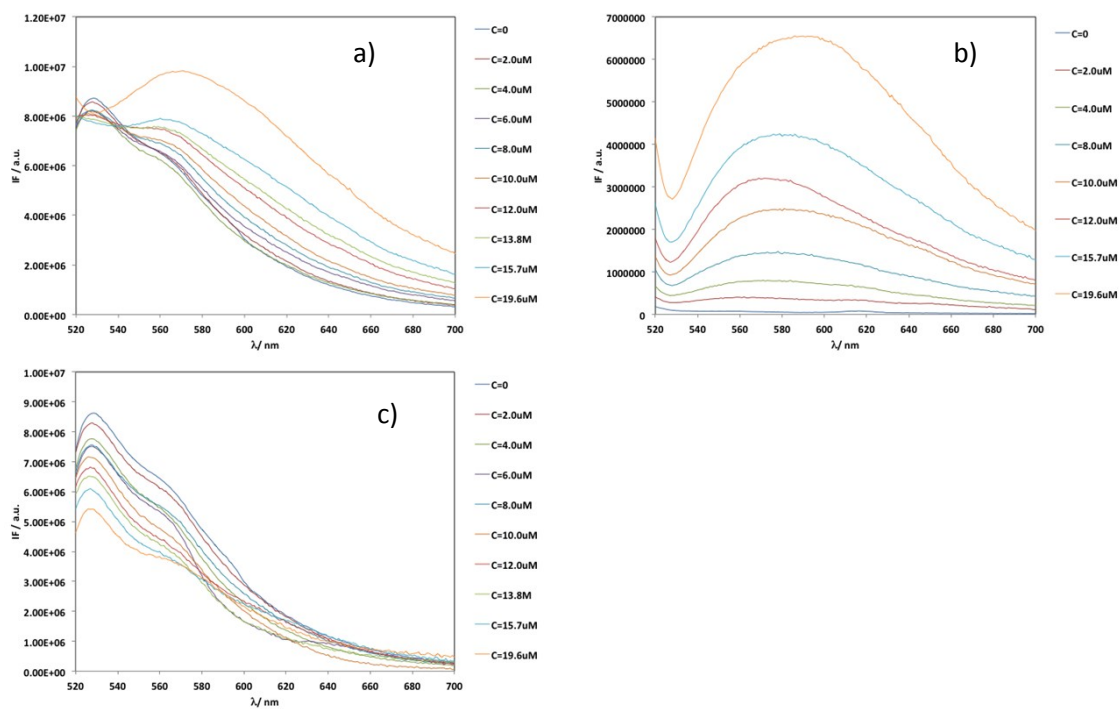


Figure S13D - Fluorescence emission spectra measured for solutions containing a) 2.0 μM of *ctDNA*, a molar ratio of TO:*ctDNA* = 0.8 and using [Sm(DBM)₃(Phen)] concentrations from 0 to 20 μM. b) 1.6 μM of TO and [Sm(DBM)₃(Phen)] concentrations from 0 to 20 μM; c) 2.0 μM of *ctDNA*, a molar ratio of TO:*ctDNA* = 0.8 and using [Sm(DBM)₃(Phen)] concentrations from 0 to 20 μM, after subtraction of the corresponding spectra included in b).

A



B

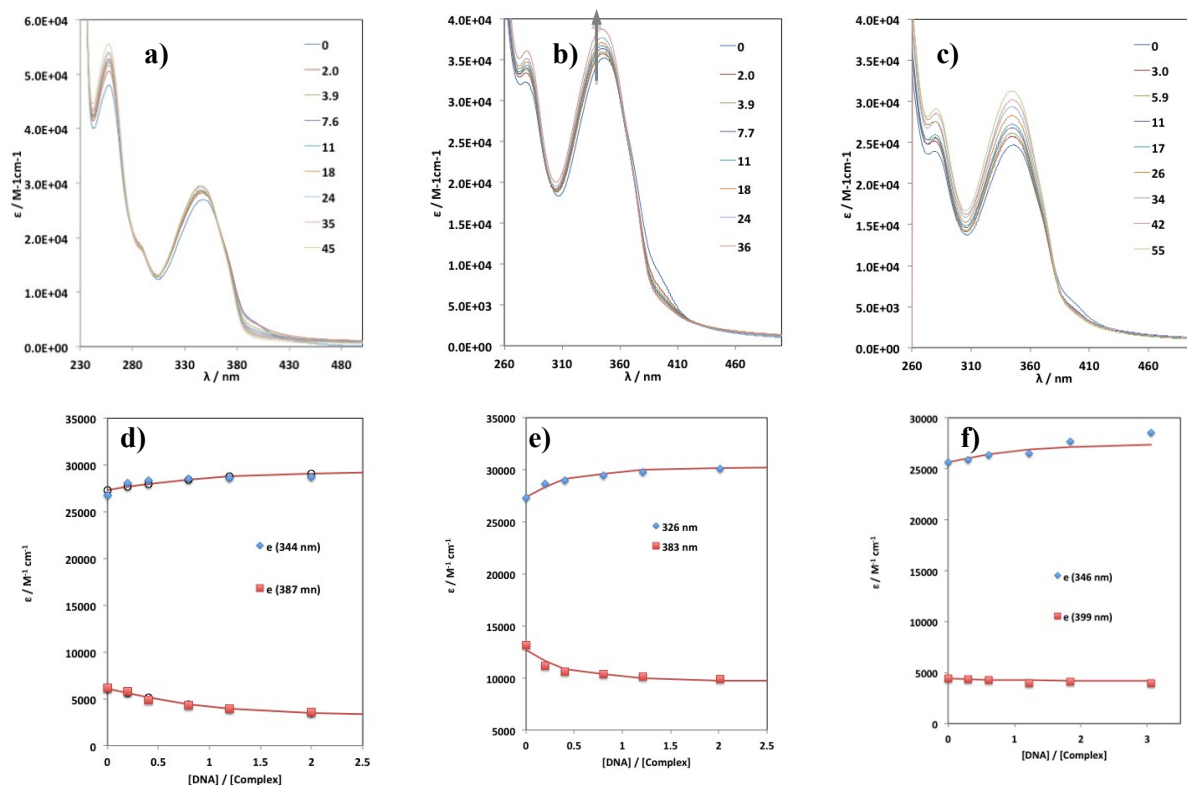


Figure S14. A) UV-vis spectra measured for the titration of solutions of the complexes, $1.0 \times 10^{-5} \text{M}$, with increasing amounts of ctDNA after correction for the dilution (concentrations indicated in the legend in μM , arrows indicate changes with DNA addition). The same amount of DNA was added in the reference cuvette. a) $[\text{Sm}(\text{DBM})_3(\text{Phen})]$, b) $[\text{Eu}(\text{DBM})_3(\text{NH}_2\text{Phen})]$ and c) $[\text{Tb}(\text{DBM})_3(\text{NH}_2\text{Phen})]$. B) Variation of the molar absorptivity at selected wavelengths with $[\text{DNA}]/[\text{complex}]$ ratios. d) $[\text{Sm}(\text{DBM})_3(\text{Phen})]$, e) $[\text{Eu}(\text{DBM})_3(\text{NH}_2\text{Phen})]$ and f) $[\text{Tb}(\text{DBM})_3(\text{NH}_2\text{Phen})]$. The curves in red show the fitted model to determine binding constants of Ln complexes with DNA, as described in the text.

The results shown in Fig. S14d-f were fitted assuming the formation of a 1:1 association complex between the DNA and Ln complexes,



The total concentration of DNA and of Ln complex are the sum of the concentrations of free and associated forms, respectively,

$$C_{\text{DNA}} = [\text{DNA}] + [\text{Cpx:DNA}] \quad (1)$$

$$C_{\text{Cpx}} = [\text{Cpx}] + [\text{Cpx:DNA}] \quad (2)$$

and these can be used to rewrite the concentration of associated form as a function of the total concentrations, C_{DNA} and C_{Cpx} , and of the binding constant, K_a ,

$$[Cpx:DNA] = \frac{1}{2} \left(C_{DNA} + C_{Cpx} + \frac{1}{K_a} \right) - \frac{1}{2} \sqrt{\left(C_{DNA} + C_{Cpx} + \frac{1}{K_a} \right)^2 - 4 \cdot C_{DNA} \cdot C_{Cpx}} \quad (3)$$

The apparent molar absorptivity of a given solution of Ln complex and DNA is obtained from the molar fraction average of the molar absorptivity of the individual species,

$$\bar{\varepsilon}^\lambda = \frac{[Cpx]}{C_{Cpx}} \varepsilon_{Cpx}^\lambda + \frac{[Cpx:DNA]}{C_{Cpx}} \varepsilon_{Cpx:DNA}^\lambda \quad (4)$$

The experimental results in Fig. S14d-f were fitted using equations 2, 3 and 4 by adjusting the parameter values of molar absorptivity of the individual species $\varepsilon_{Cpx}^\lambda$ and $\varepsilon_{Cpx:DNA}^\lambda$ at each wavelength selected and the value of the binding constant, K_a , for each Ln complex studied – Table S2.

Table S2. Values of the binding constant (K_a) for the association of Ln complexes with ctDNA obtained from fitting the variation of molar absorptivity from the UV-Vis absorption data.

		K_a (M^{-1})
[Sm(DBM) ₃ (Phen)]	1	4.21×10^5
[Sm(DBM) ₃ (NH ₂ Phen)]	2	3.21×10^5
[Eu(DBM) ₃ (NH ₂ Phen)]	3	4.41×10^5
[Tb(DBM) ₃ (NH ₂ Phen)]	4	3.10×10^5

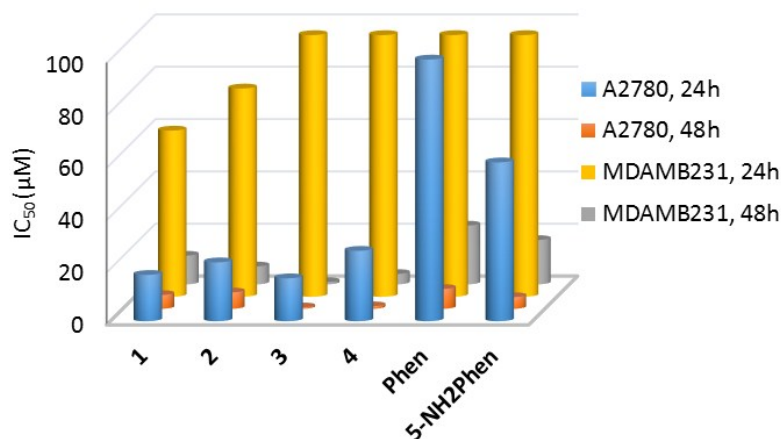


Figure S15. IC₅₀ values obtained for the ligands and the lanthanide complexes after 24 h and 48 h incubation using the MTT assay.

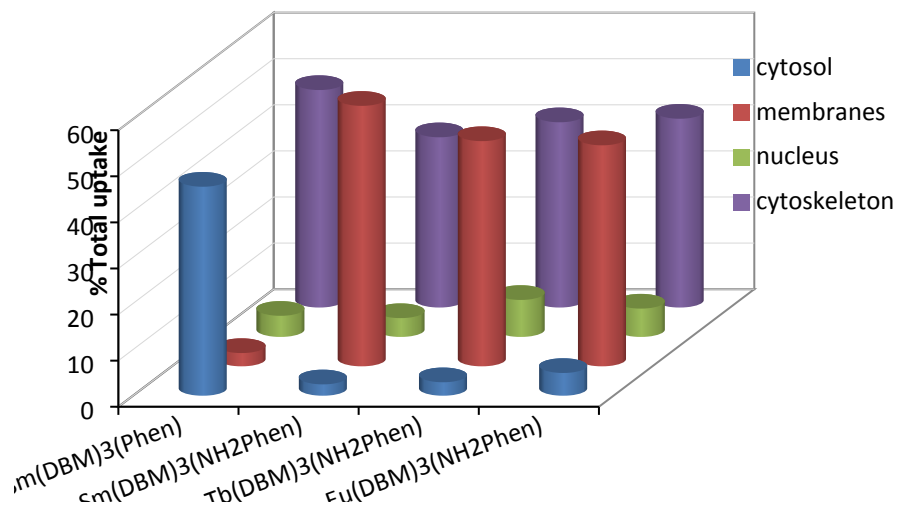


Figure S16. Percent total cellular uptake of the lanthanide complexes (metal content) in the A2780 cellular fractions.