Supporting Information

Breaking the 1,2-HOPO barrier with a cyclen backbone for more efficient sensitization of Eu(III) luminescence and unprecedented two-photon excitation properties

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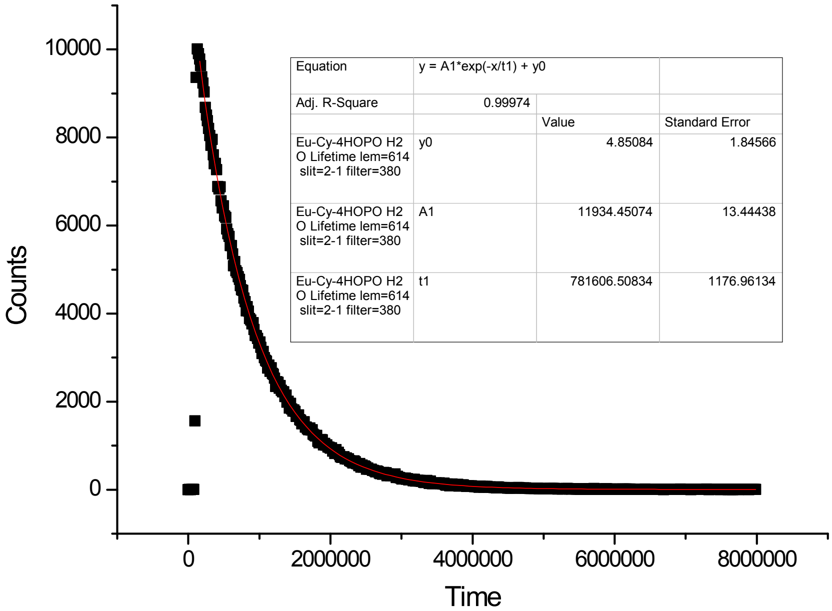


Figure S1. Luminescence decay curve of $^5D_0 \rightarrow {}^7F_2$ transition of **Eu-Cy-HOPO** in water (λ_{ex} = 350 nm, λ_{em} = 614 nm).

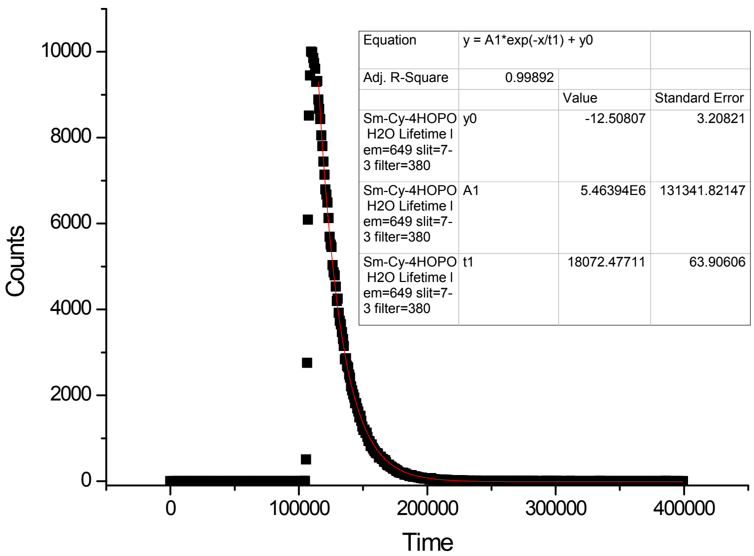


Figure S2. Luminescence decay curve of $^5D_0 \rightarrow {}^7F_2$ transition of **Eu-Cy-HOPO** in water (λ_{ex} = 350 nm, λ_{em} = 614 nm).

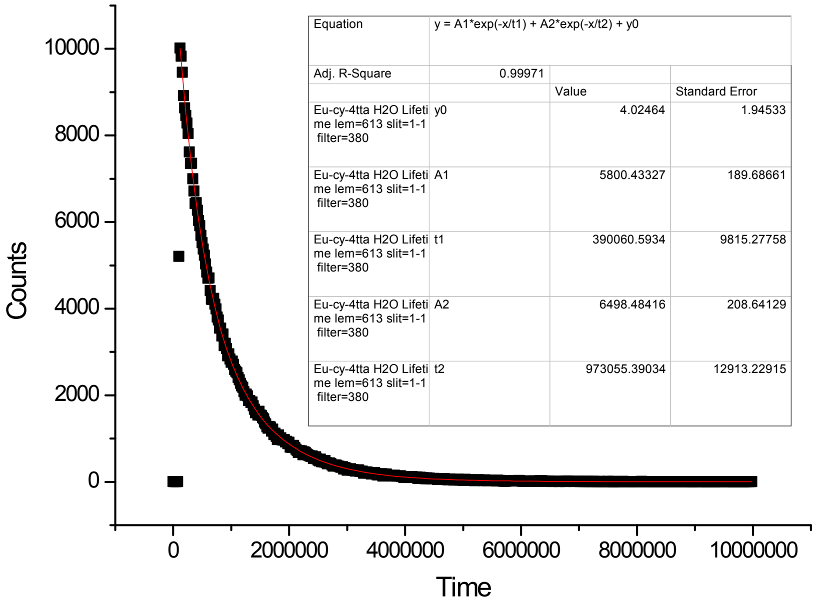


Figure S3. Luminescence decay curve of $^5D_0 \rightarrow {}^7F_2$ transition of **Eu-Cy-TTA** in water (λ_{ex} = 350 nm, λ_{em} = 613 nm).

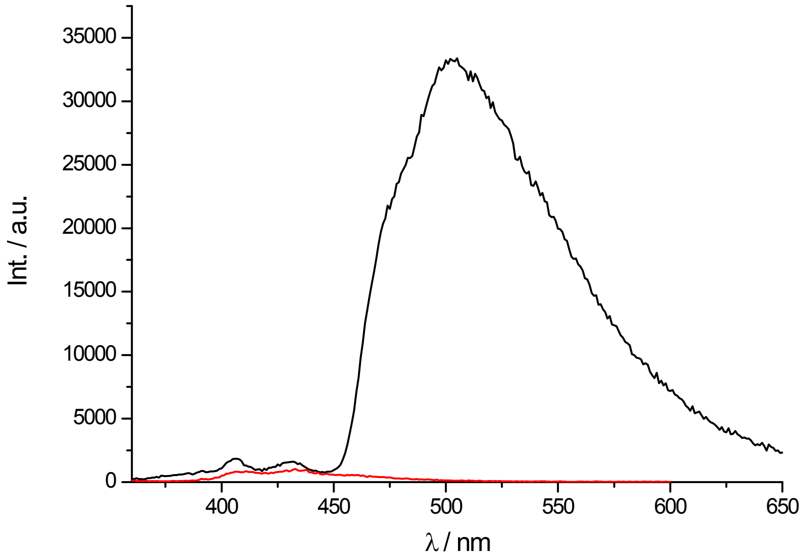


Figure S4. Emission spectra of **Gd-Cy-HOPO** at room temperature (red) and 77K (black) in water/glycerol (1:1) mixture (λ_{ex} = 350 nm).

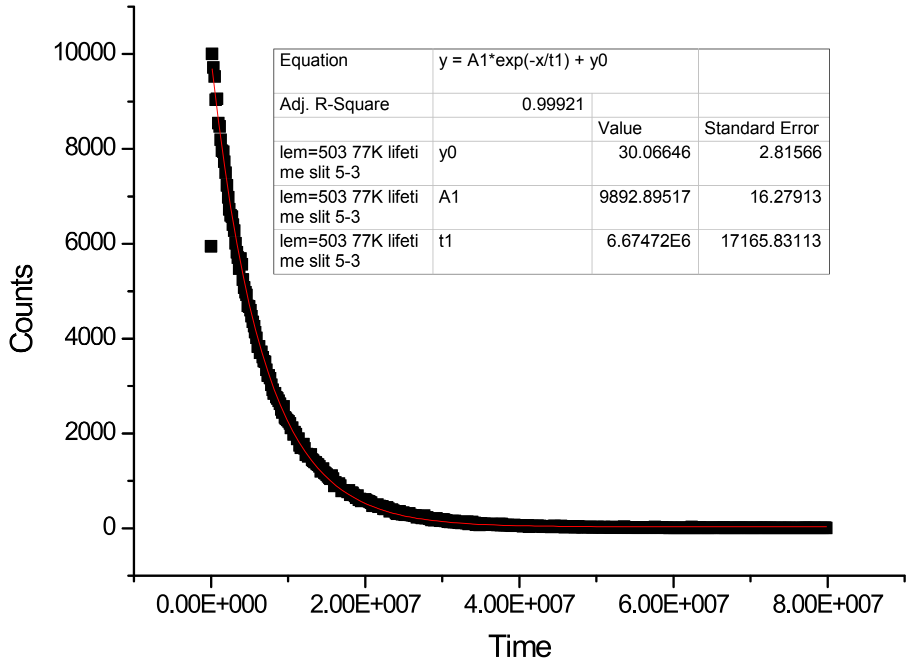


Figure S5. Phosphorescence decay curve of **Gd-Cy-HOPO** in water:glycerol (1:1) mixture (λ_{ex} = 350 nm, λ_{em} = 503 nm).

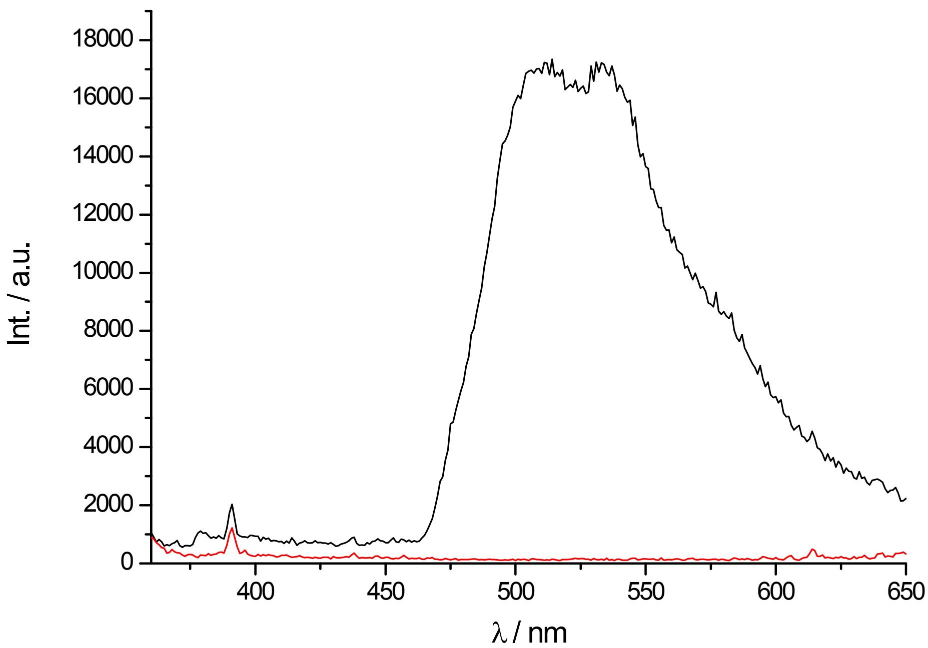


Figure S6. Emission spectra of **Gd-Cy-TTA** at room temperature (red) and 77K (black) in 2-methyltetrahydrofuran (λ_{ex} = 350 nm).

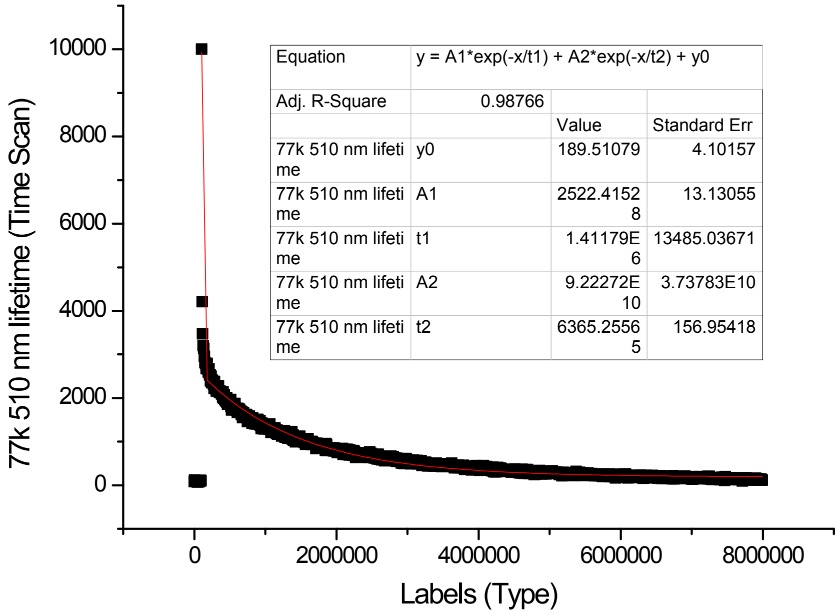


Figure S7. Phosphorescence decay curve of **Gd-Cy-TTA** in 2-methyltetrahydrofuran (λ_{ex} = 350 nm, λ_{em} = 510 nm).

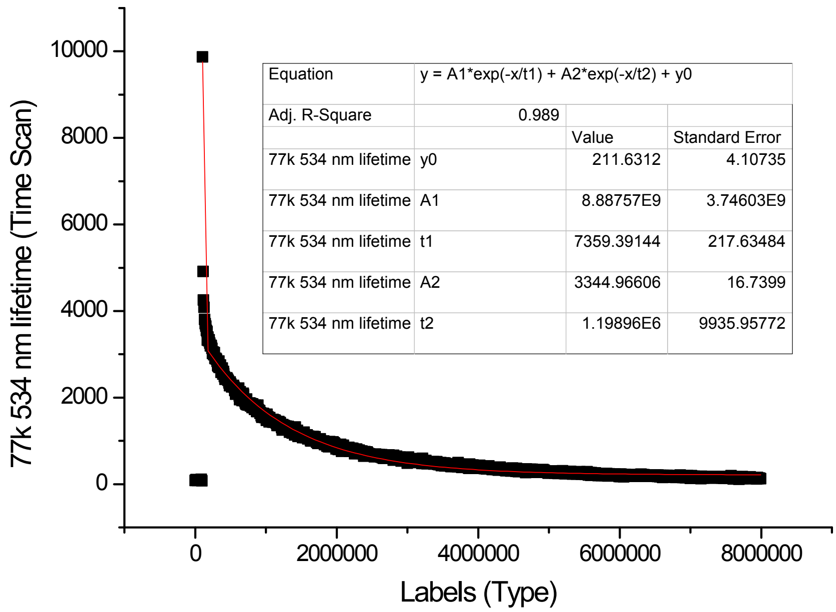


Figure S8. Phosphorescence decay curve of **Gd-Cy-TTA** in 2-methyltetrahydrofuran (λ_{ex} = 350 nm, λ_{em} = 534 nm).

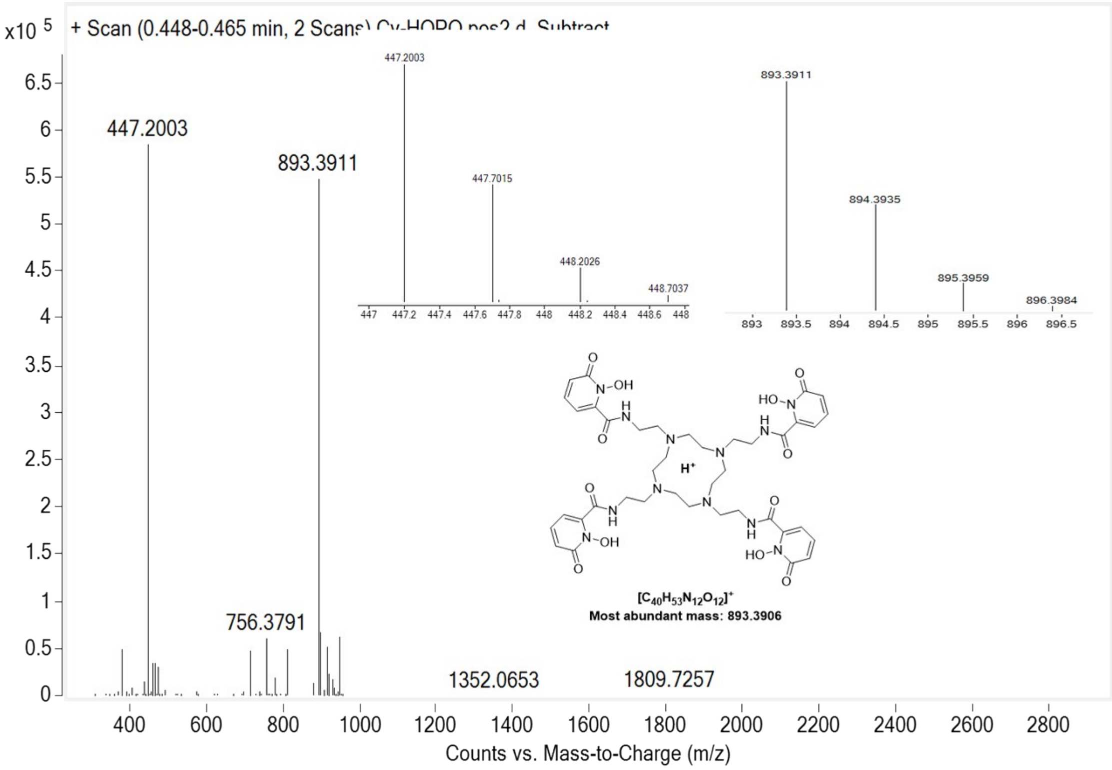


Figure S9. Mass spectrum of ligand **4**. Ion peaks at 447.2003 and 893.3911 (m/z) which correspond to $[M+2H]^{2+}/2$ and $[M+H]^{+}$ respectively.

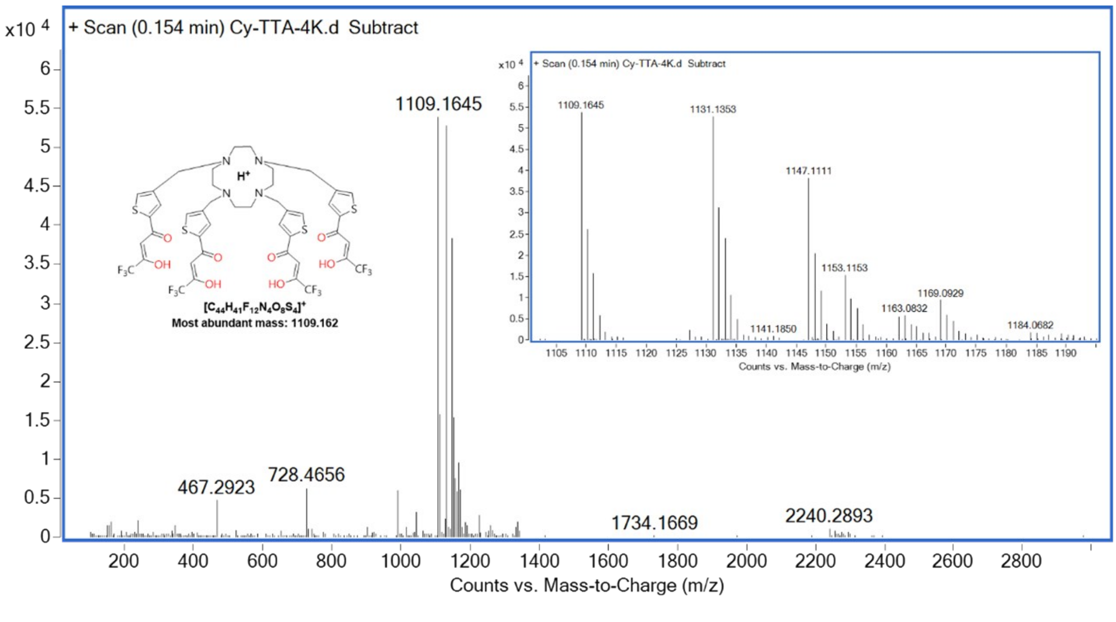


Figure S10. Mass spectrum of ligand **6**. Ion peaks at 1109.1645, 1131.1353, 1147.1111, 1153.1153, and 1169.0929 (m/z) which correspond to $[M-4K+5H]^{+}$, $[M-4K+4H+Na]^{+}$, $[M-3K+4H]^{+}$, $[M-4K+3H+2Na]^{+}$, $[M-3K+3H+Na]^{+}$ respectively.

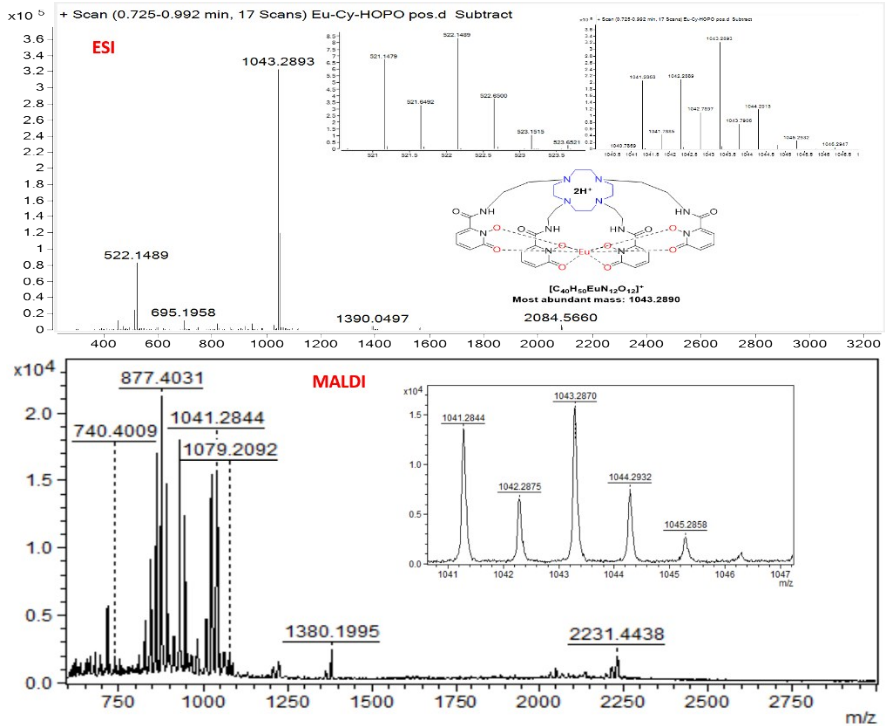


Figure S11. Mass spectrum of **Eu-Cy-HOPO**. Ion peaks at 522.1489 and 1043.2893 (ESI m/z) which correspond to $[M+3H]^{2+}/2$ and $[M+2H]^{+}$ respectively, ion peak at 1043.2870 (MALDI m/z) which corresponds to $[M+2H]^{+}$.

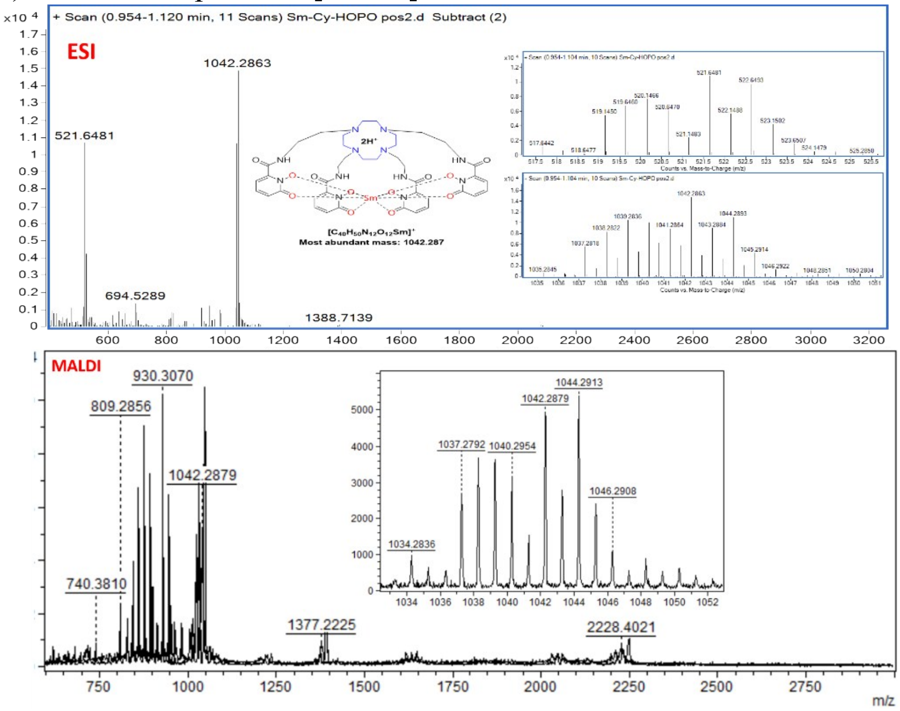


Figure S12. Mass spectrum of **Sm-Cy-HOPO**. Ion peaks at 521.6481 and 1042.2863 (ESI m/z) which correspond to $[M+3H]^{2+}/2$ and $[M+2H]^{+}$ respectively, ion peak at 1042.2879 (MALDI m/z) which corresponds to $[M+2H]^{+}$.

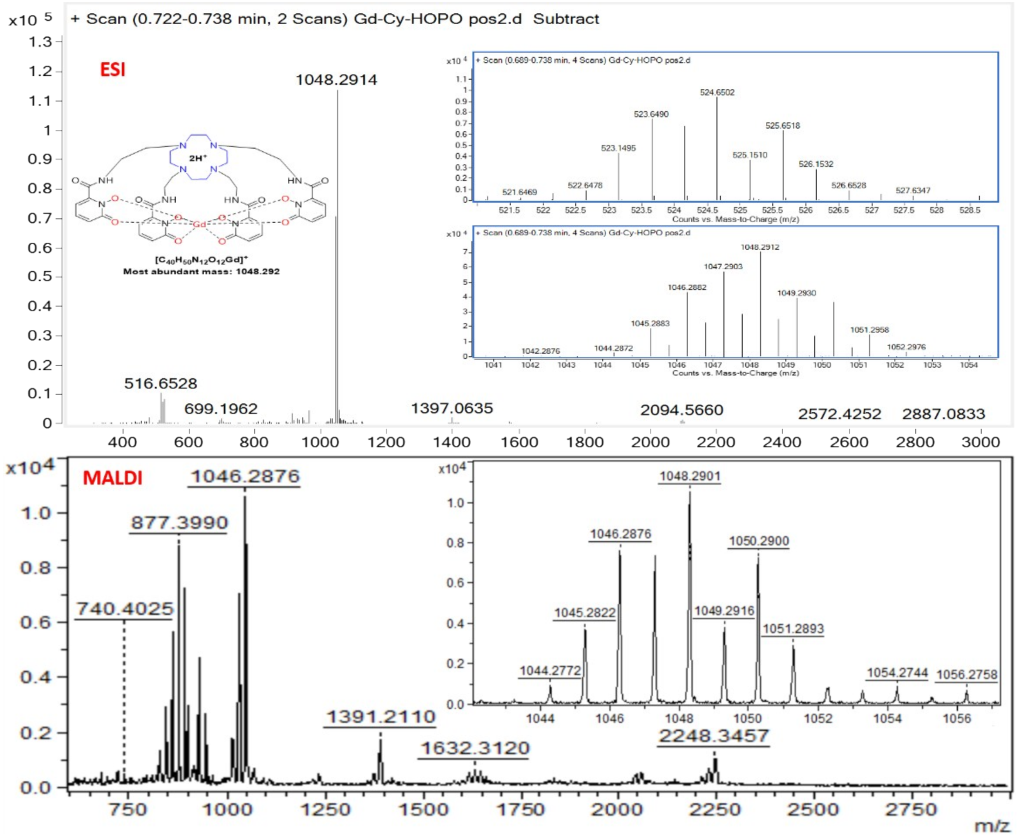


Figure S13. Mass spectrum of **Gd-Cy-HOPO**. Ion peak 1048.2914 (ESI m/z) which corresponds to $[M+2H]^+$, ion peak at 1048.2901 (MALDI m/z) which corresponds to $[M+2H]^+$.

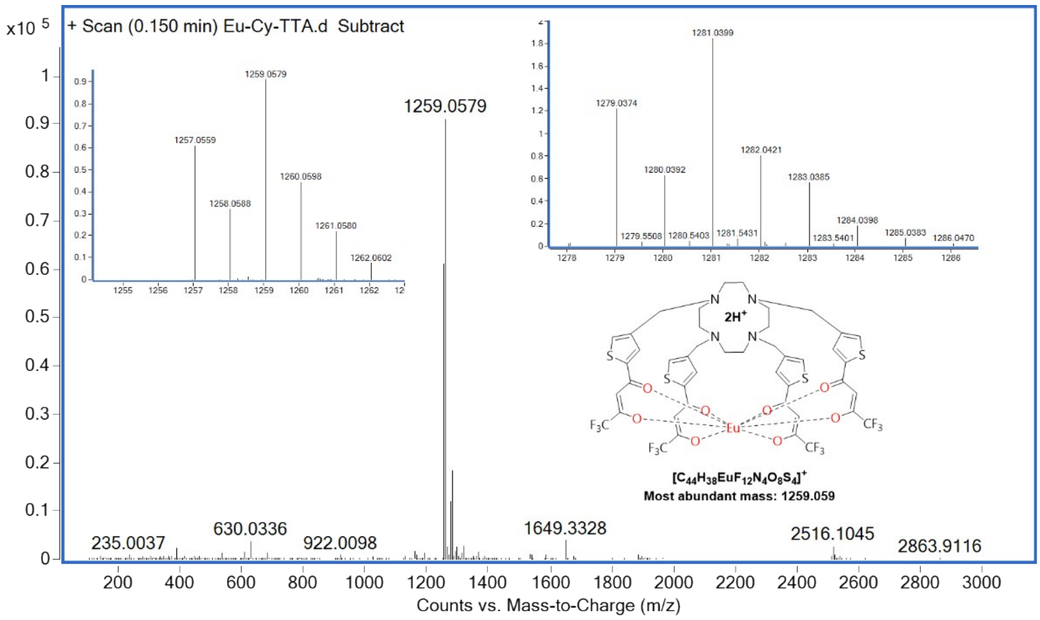


Figure S14. Mass spectrum of **Eu-Cy-TTA**. Ion peaks 1259.0579 and 1281.0399 (m/z) which correspond to $[M-K+2H]^+$ and $[M-K+H+Na]^+$ respectively.

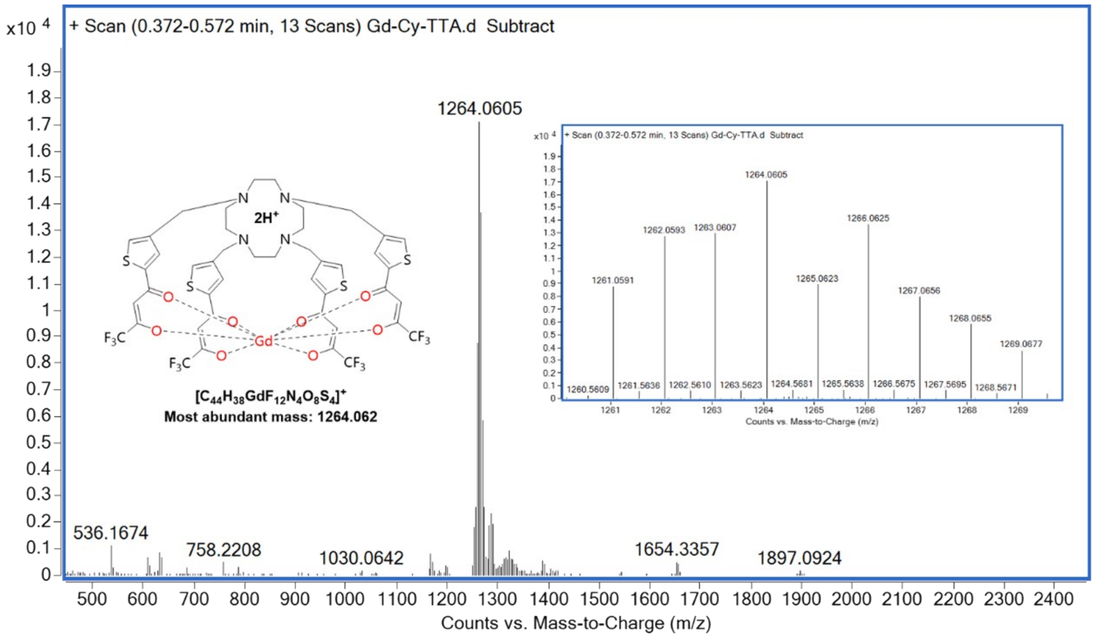


Figure S15. Mass spectrum of **Gd-Cy-TTA**. Ion peak 1264.0605 (m/z) which corresponds to $[M\text{-}K+2H]^{+}$.

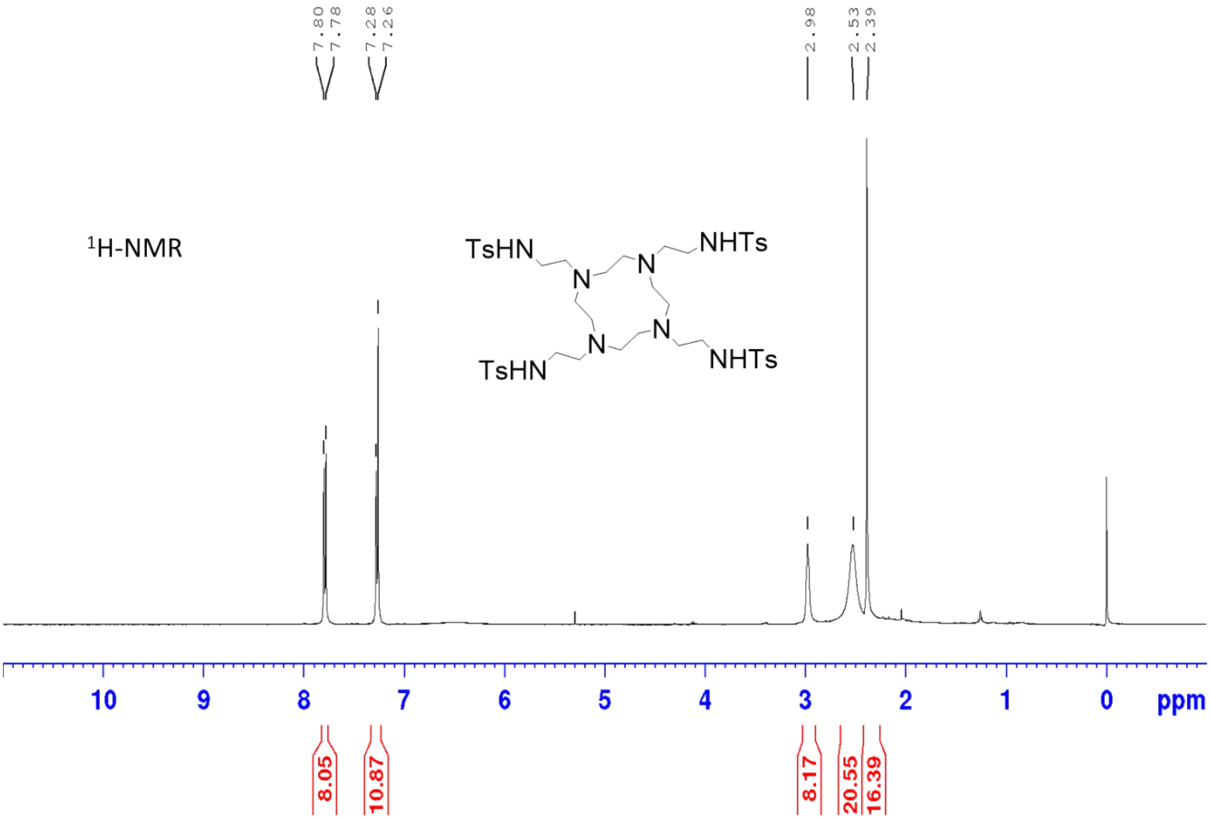


Figure S16. ^{1}H NMR spectrum of intermediate of compound **1** in $CDCl_3$.

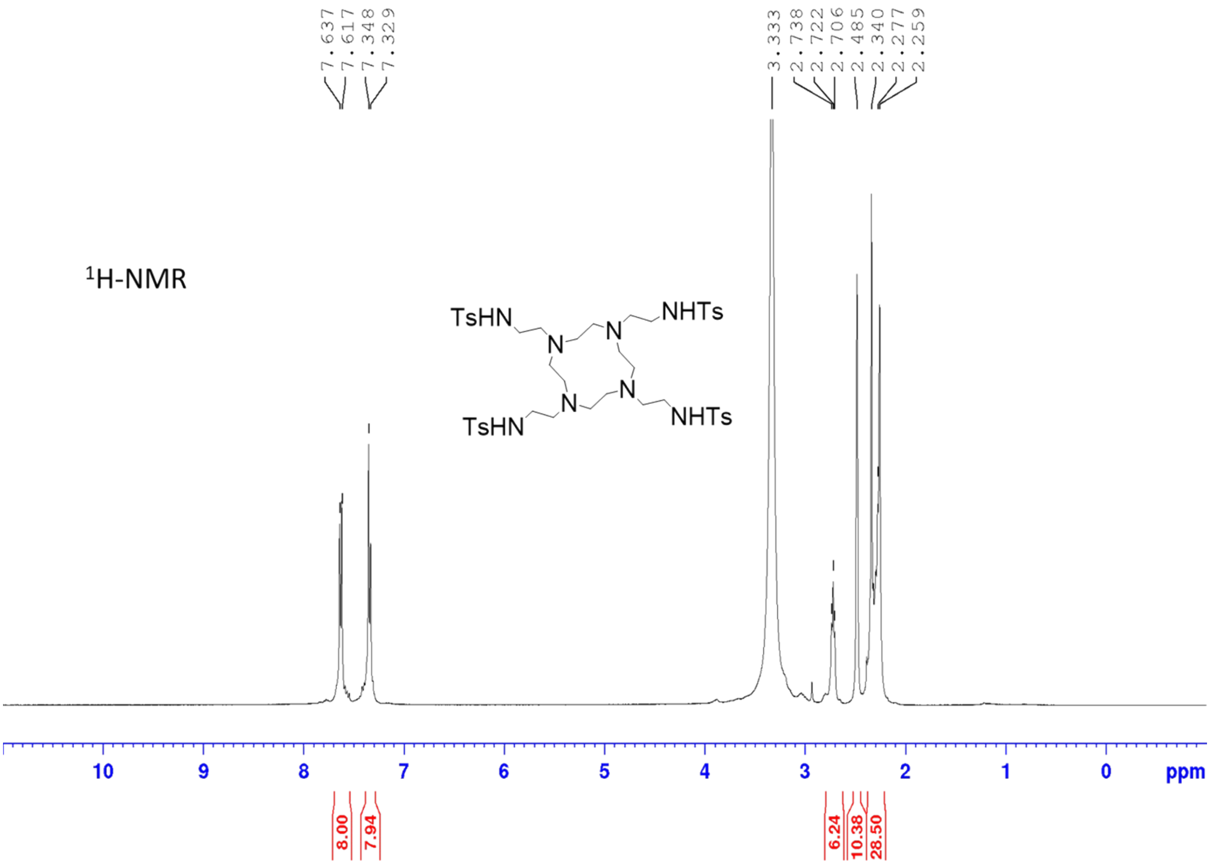


Figure S17. [1]H NMR spectrum of intermediate of compound **1** in d[6]-DMSO.

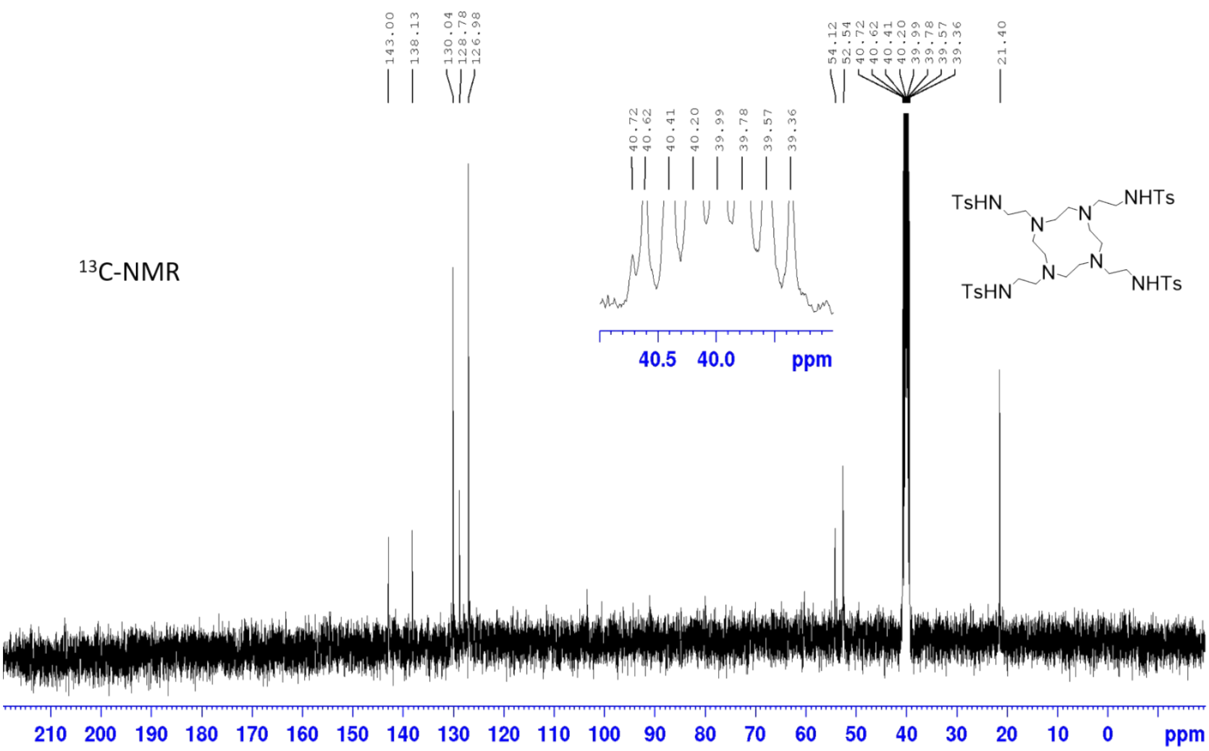


Figure S18. [13]C NMR spectrum of intermediate of compound **1** in d[6]-DMSO.

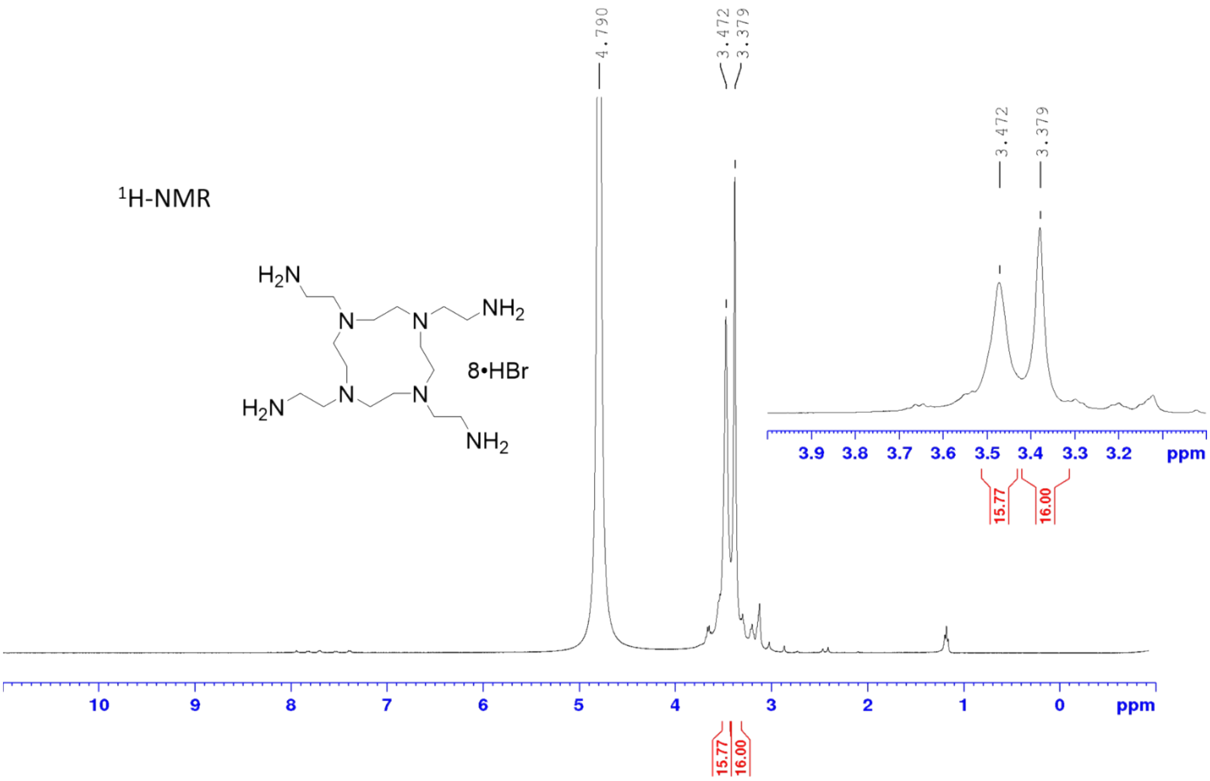


Figure S19. ^{1}H NMR spectrum of compound **1** in D_2O.

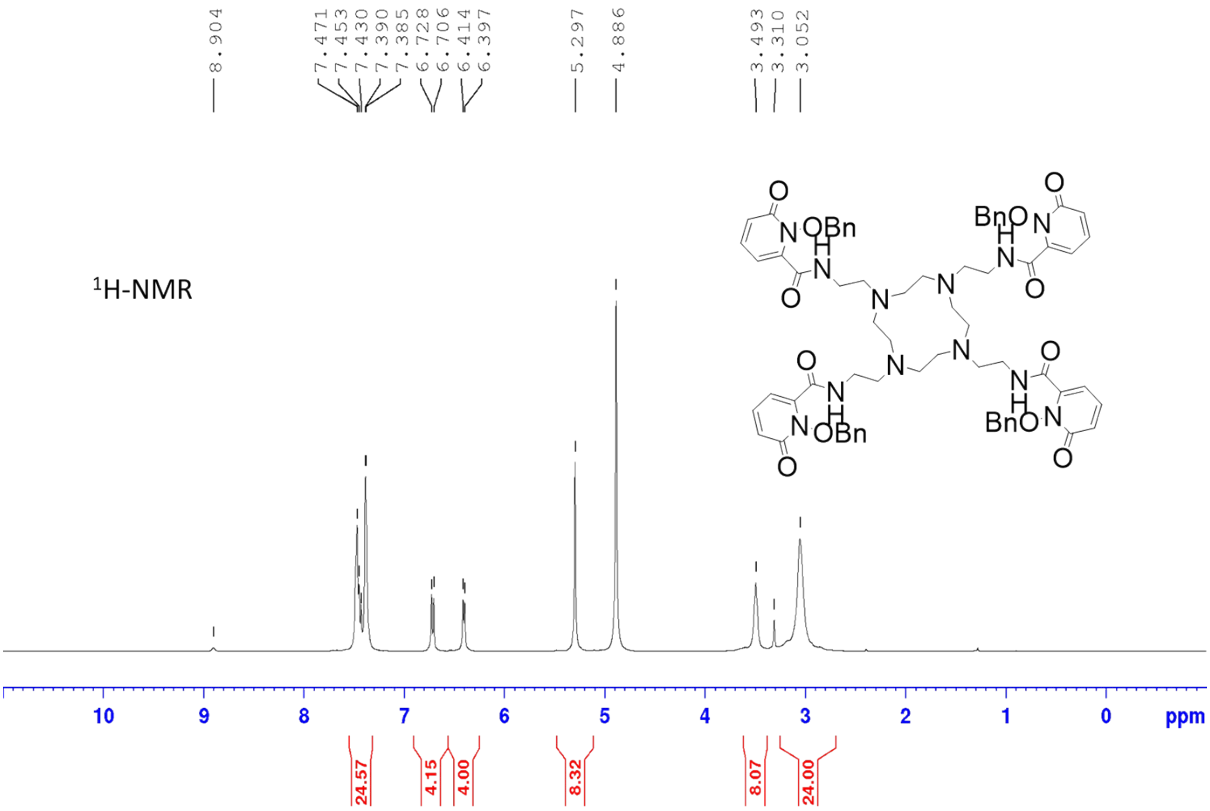


Figure S20. ^{1}H NMR spectrum of compound **3** in CD_3OD.

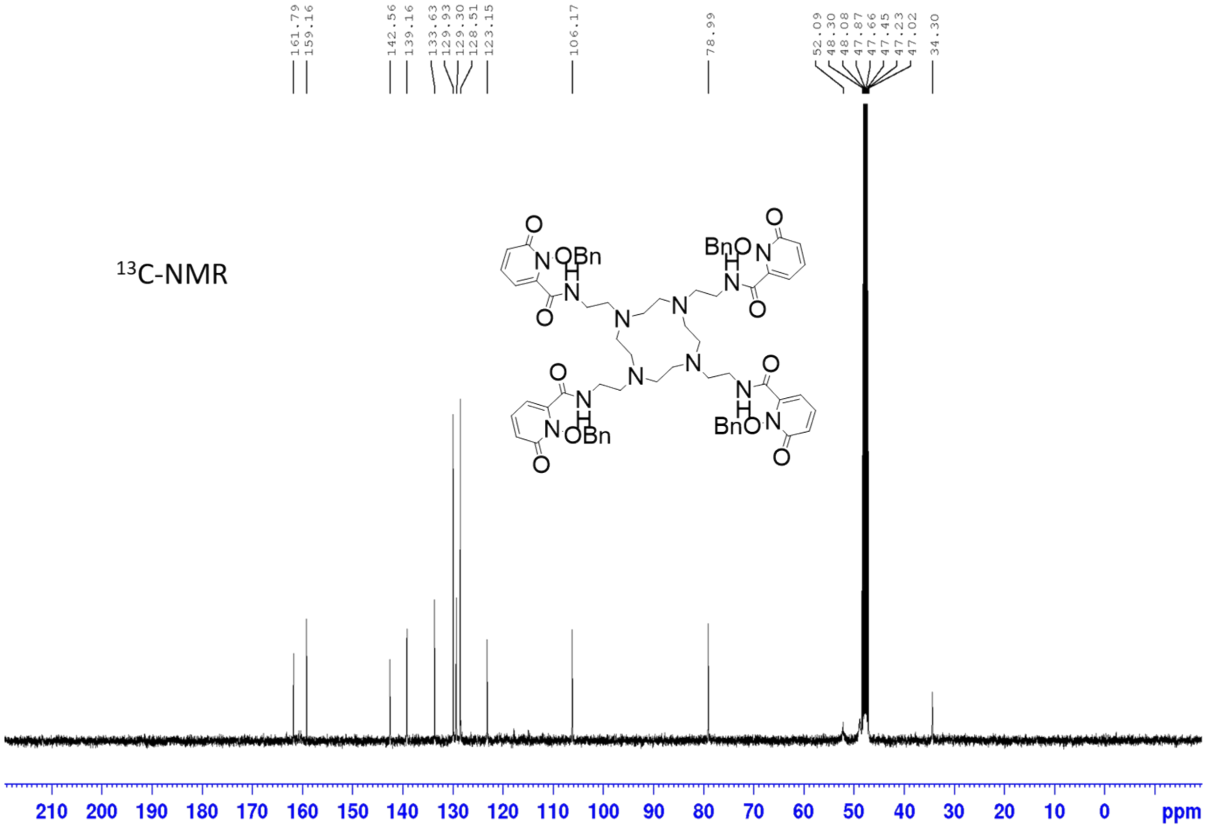


Figure S21. ^{13}C NMR spectrum of compound **3** in CD_3OD.

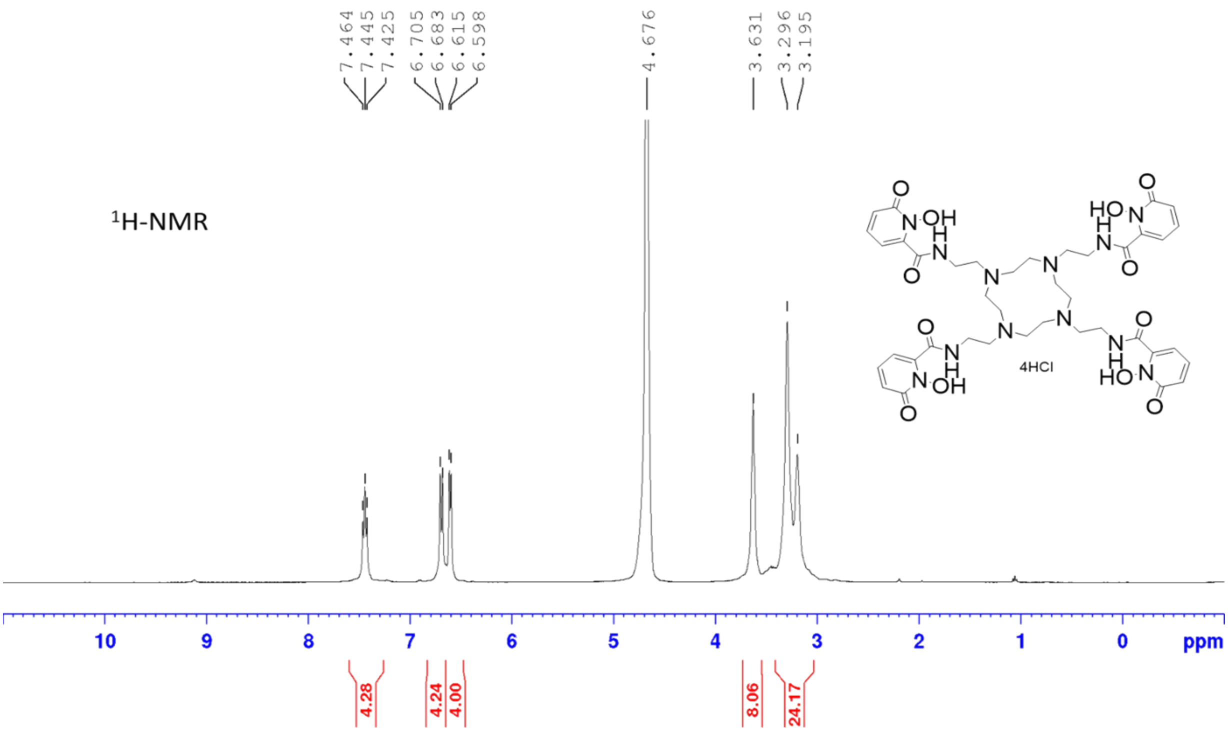


Figure S22. ^{1}H NMR spectrum of compound **4** in D_2O.

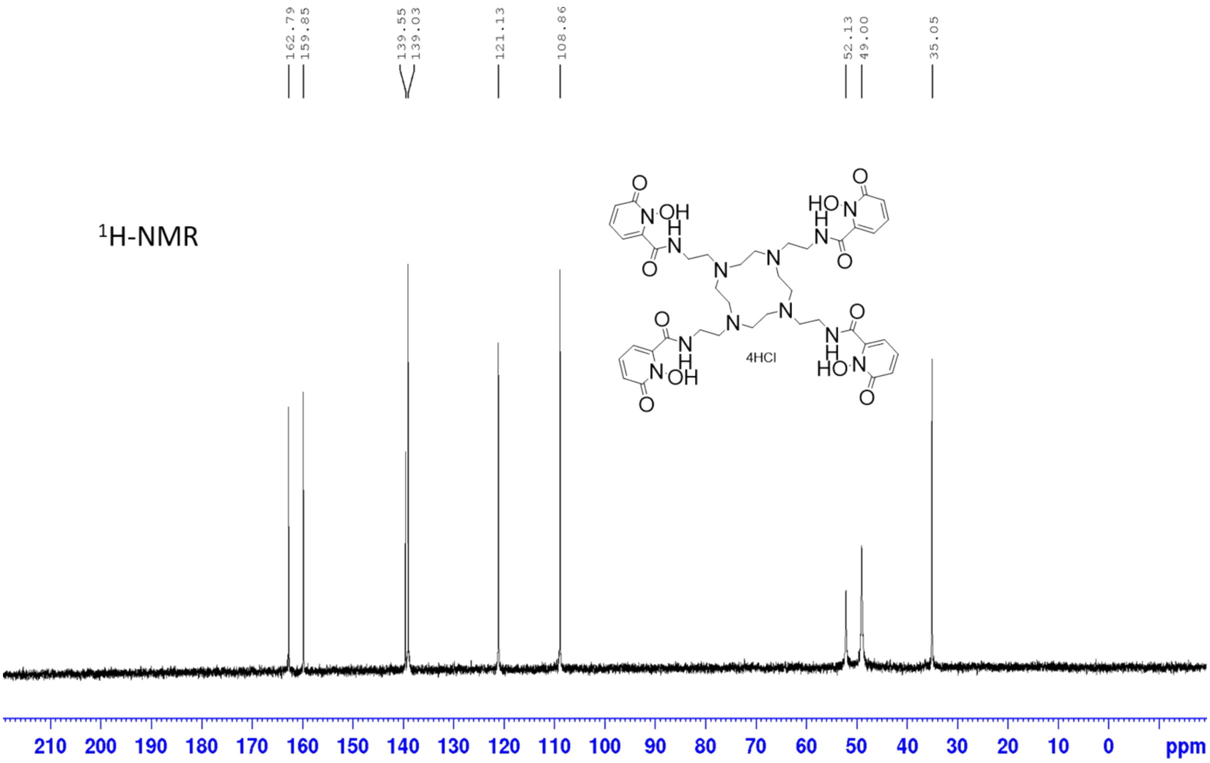


Figure S23. [1]C NMR spectrum of compound **4** in D_2O.

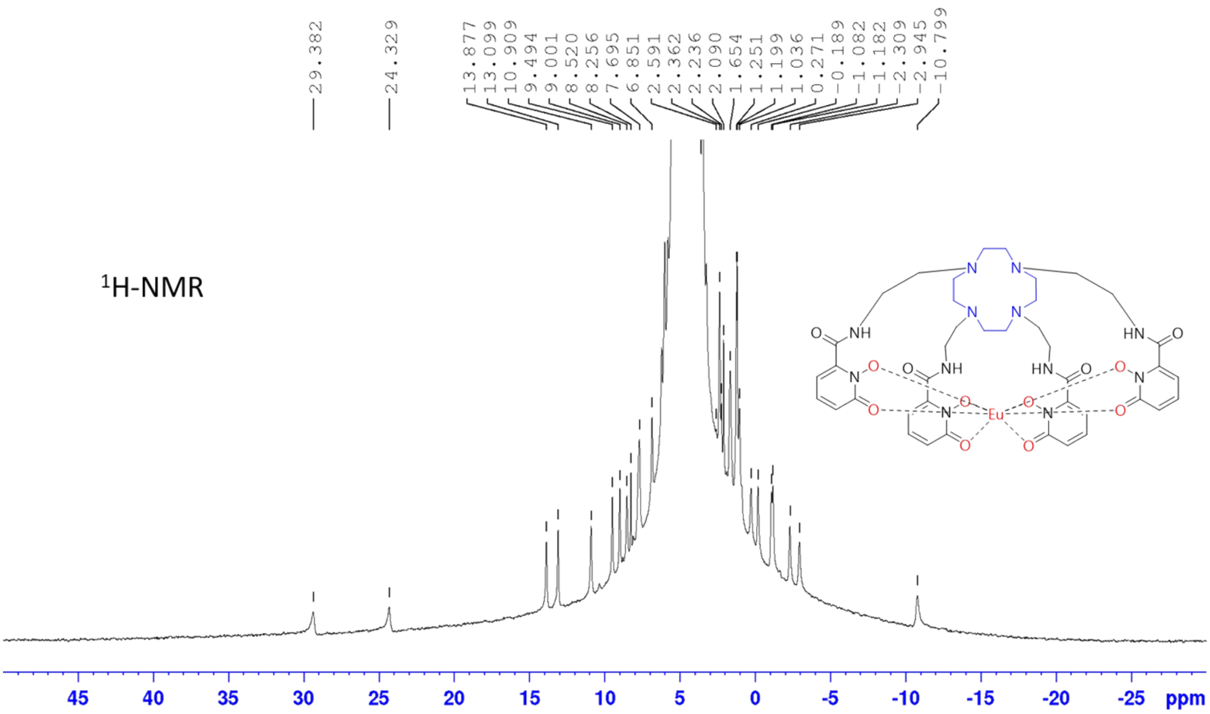


Figure S24. [1]H NMR spectrum of complex **Eu-Cy-HOPO** in D_2O.

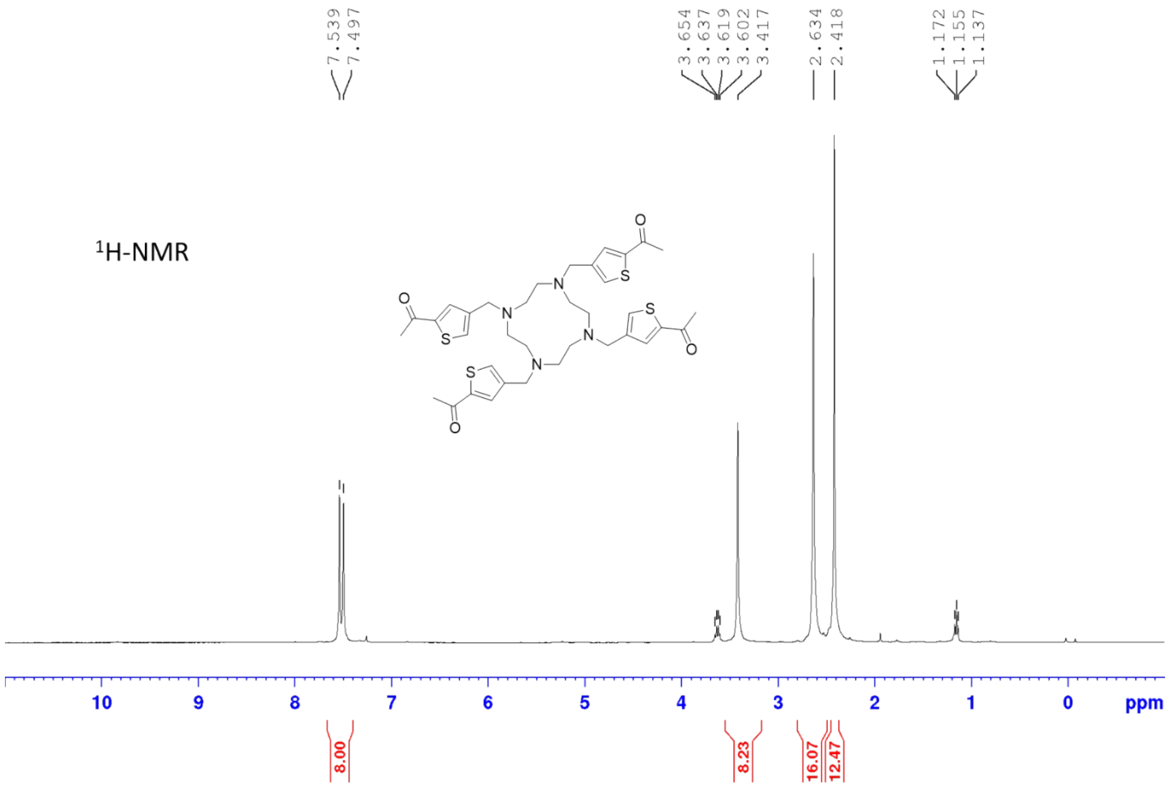


Figure S25. ^{1}H NMR spectrum of compound **5** in $CDCl_3$.

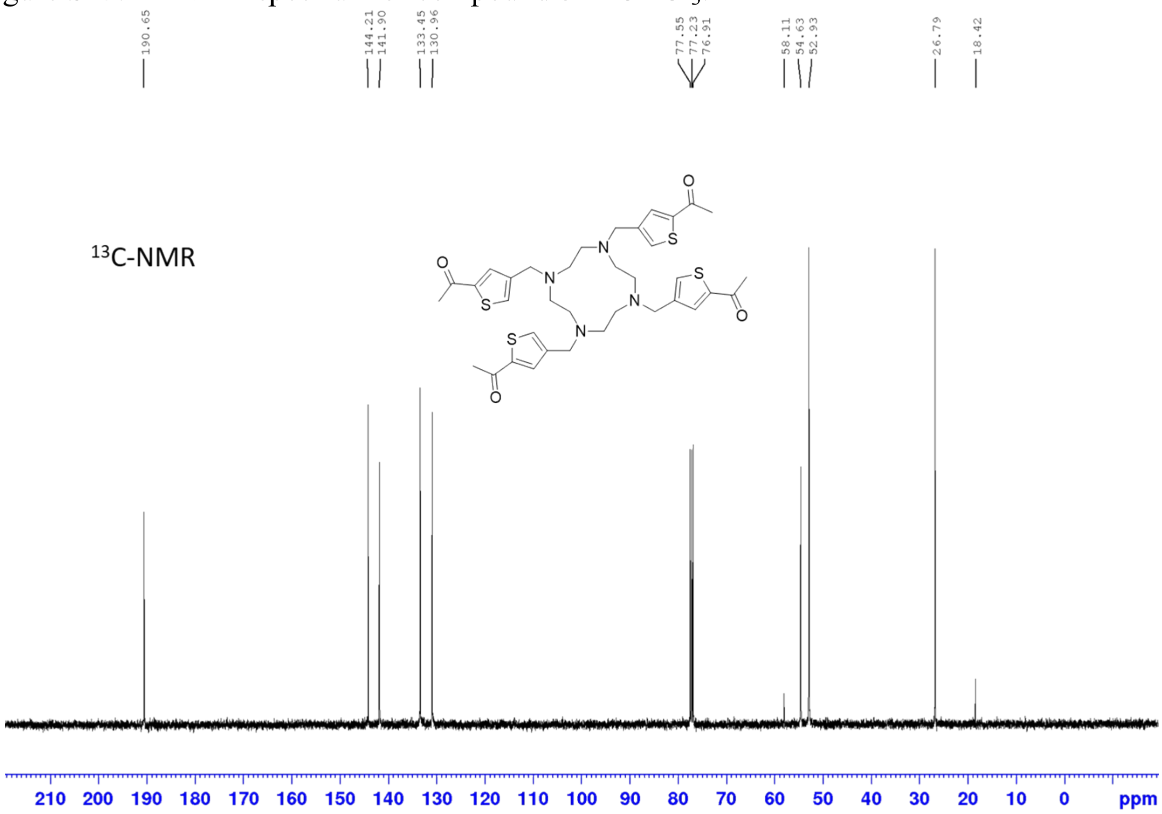


Figure S26. ^{13}C NMR spectrum of compound **5** in $CDCl_3$.

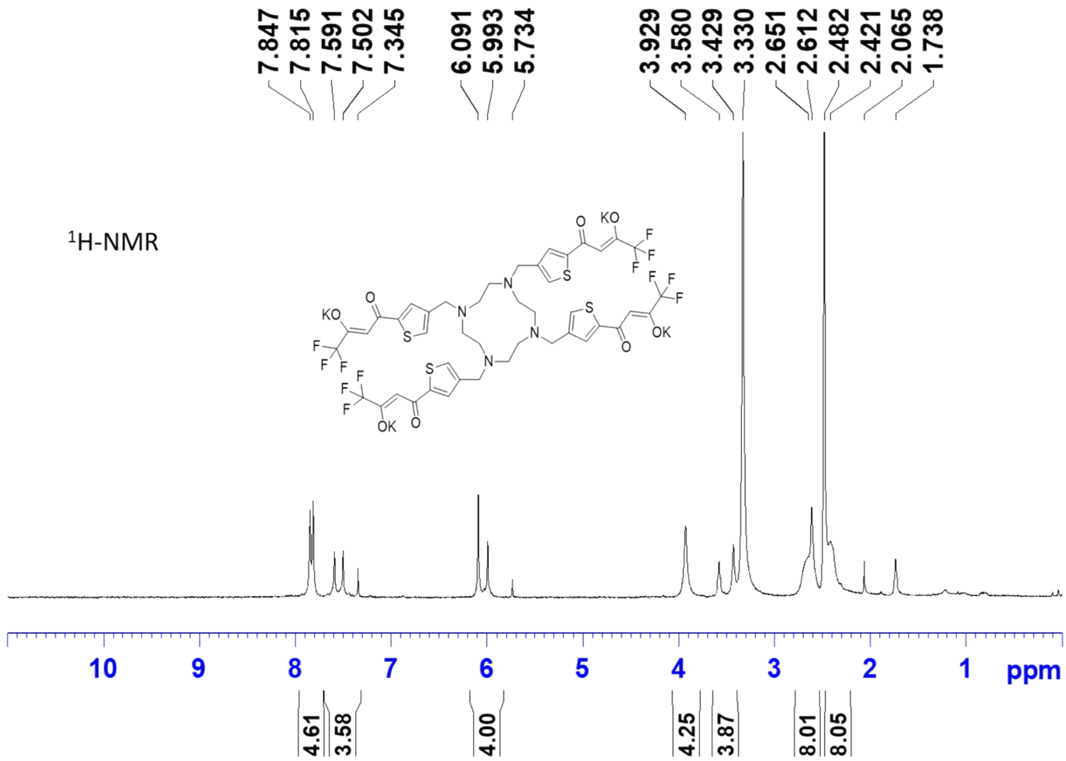


Figure S27. [1]H NMR spectrum of compound **6** in d[6]-DMSO.

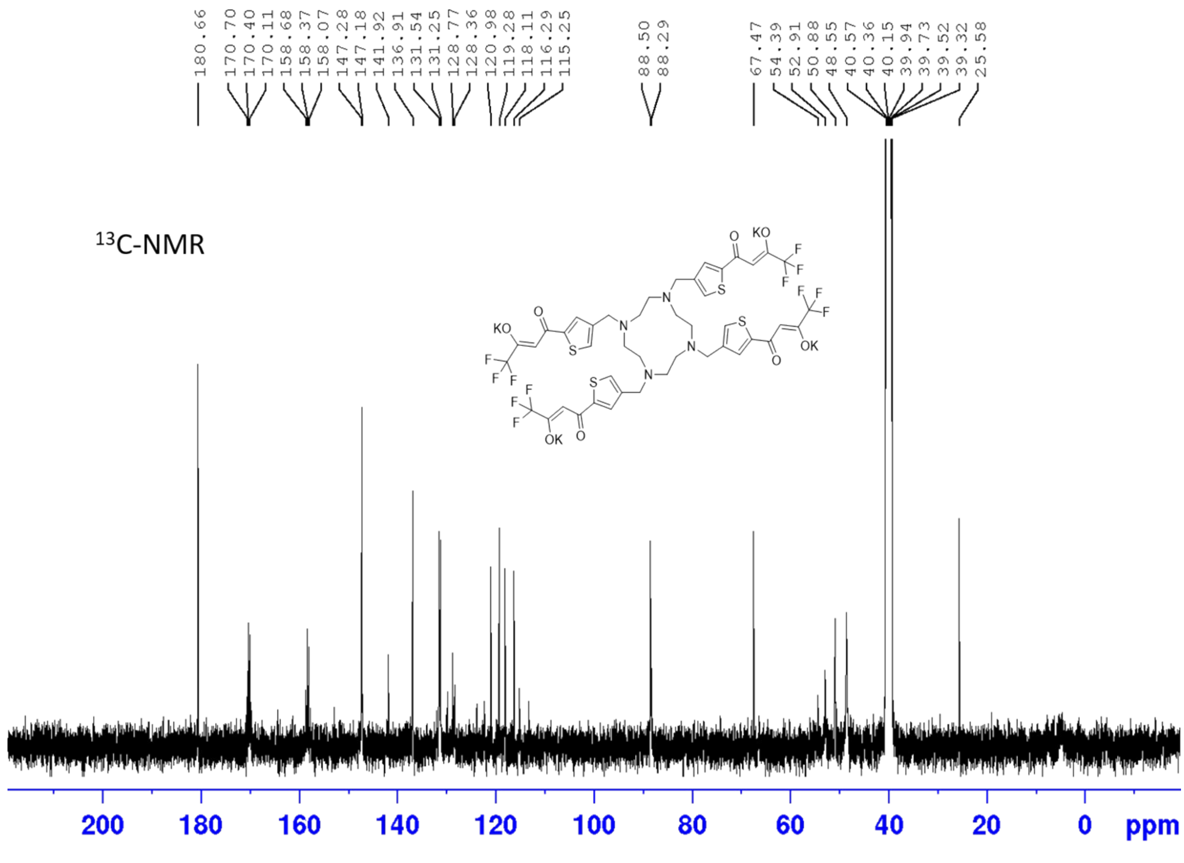


Figure S28. [13]C NMR spectrum of compound **6** in d[6]-DMSO.

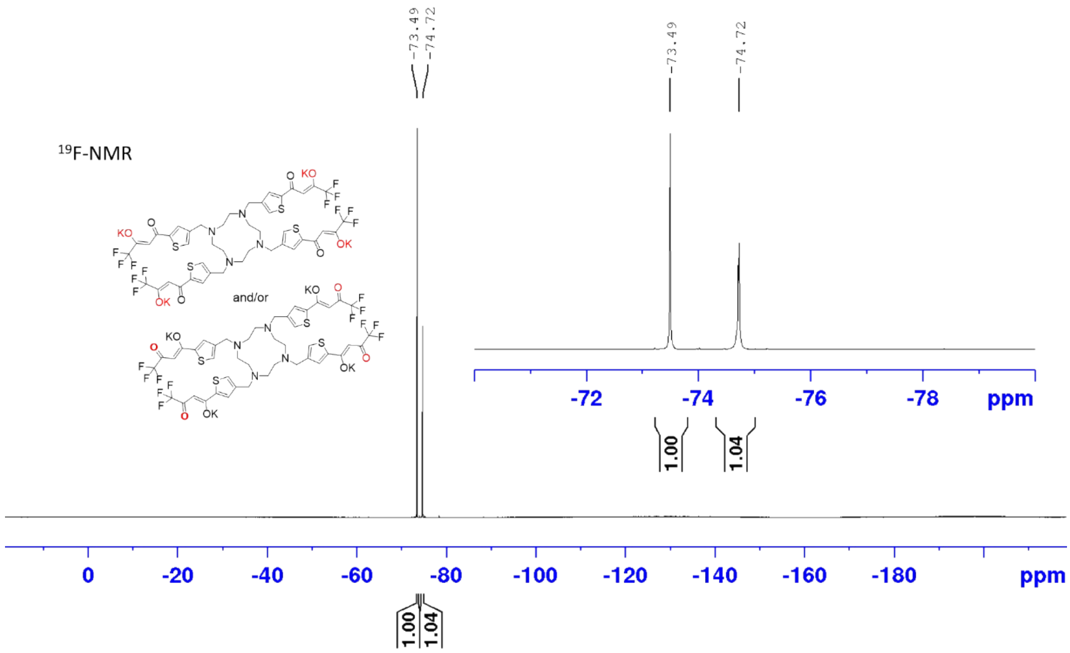
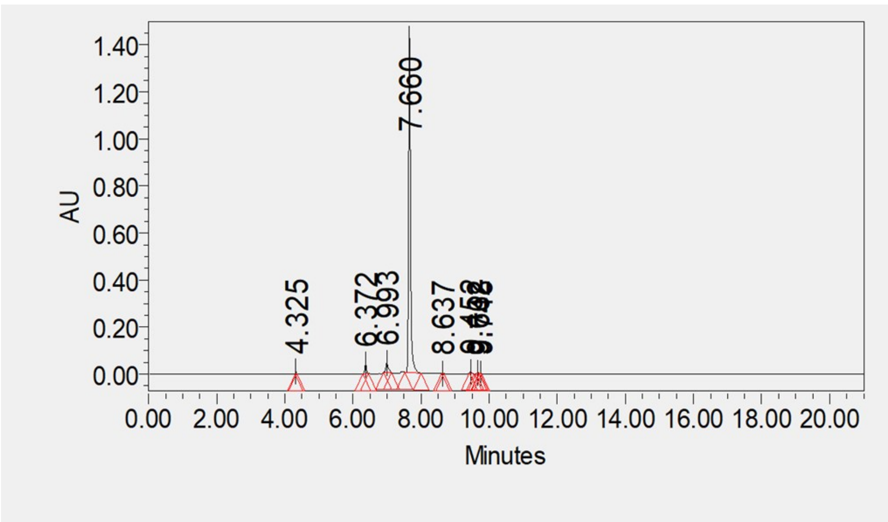
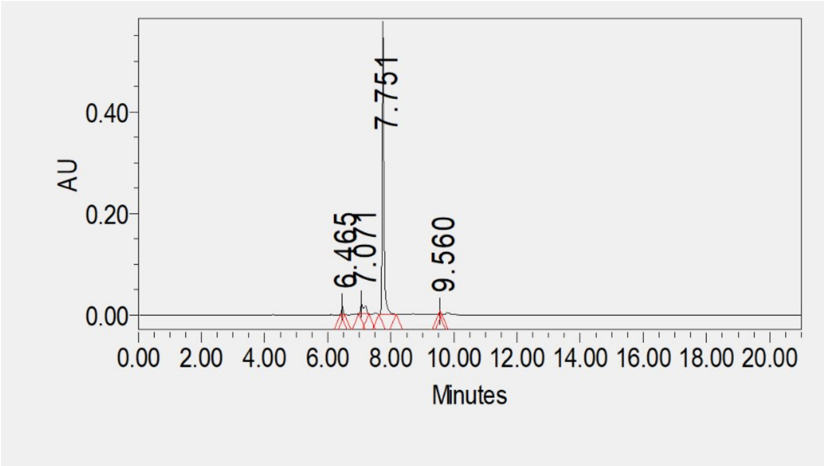


Figure S29. ^{19}F NMR spectrum of compound **6** in d^6-DMSO.



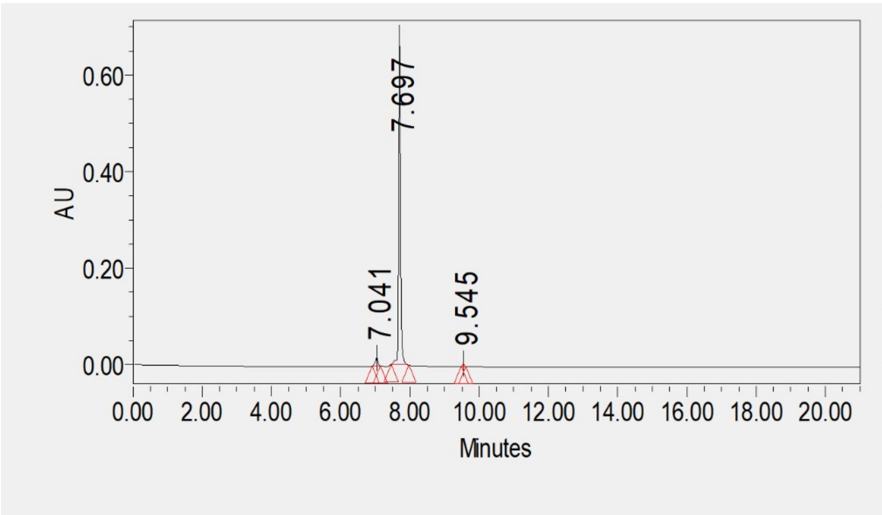
Retention Time (min)	% Area
4.325	0.21
6.372	2.27
6.993	3.38
7.660	93.60
8.637	0.07
9.452	0.29
9.662	0.15
9.748	0.03

Figure S30. RP-HPLC trace of complex **Eu-Cy-HOPO** (350 nm)



Retention Time (min)	% Area
6.465	2.14
7.071	7.53
7.751	89.64
9.560	0.68

Figure S31. RP-HPLC trace of complex **Sm-Cy-HOPO** (350 nm).



Retention Time (min)	% Area
7.041	3.26
7.697	95.97
9.545	0.76

Figure S32. RP-HPLC trace of complex **Gd-Cy-HOPO** (350 nm).

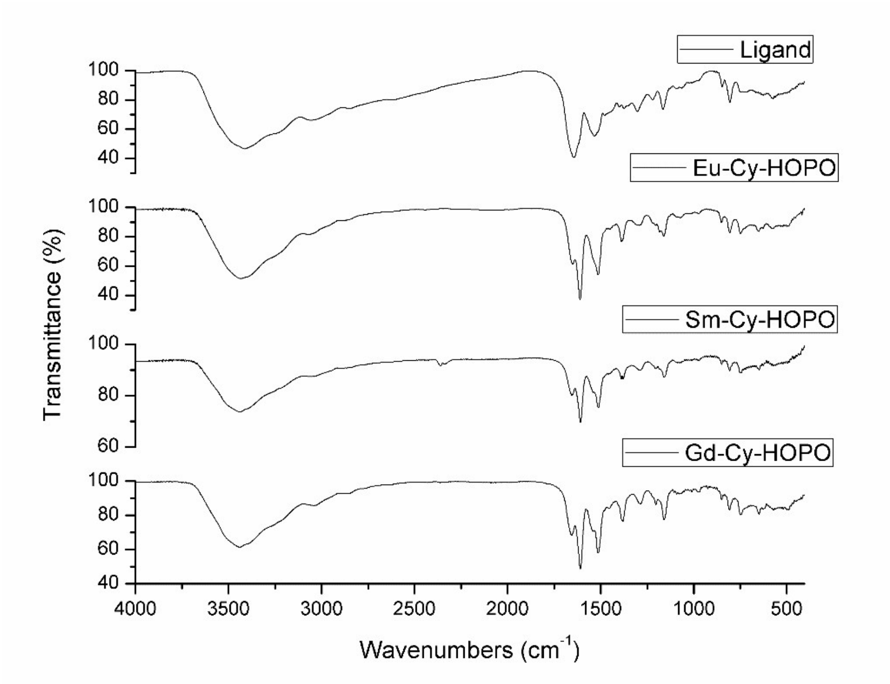


Figure S33. Comparison of IR spectra of ligand **4** and complexes of **Eu-Cy-HOPO**, **Sm-Cy-HOPO** and **Gd-Cy-HOPO**.

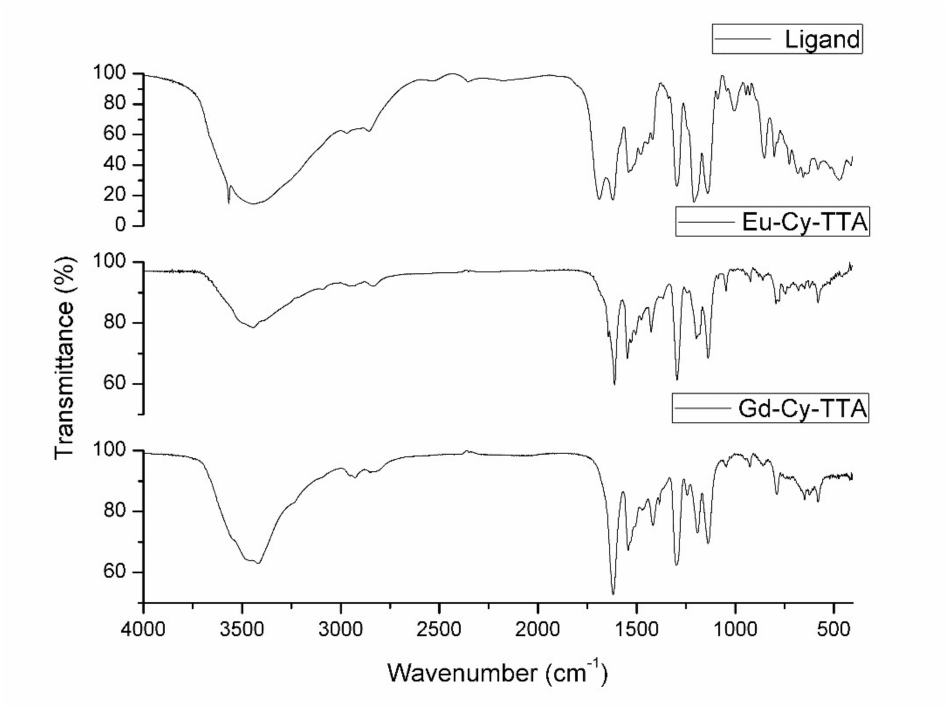


Figure S34. Comparison of IR spectra of ligand **6** and complexes of **Eu-Cy-TTA** and **Gd-Cy-TTA**.

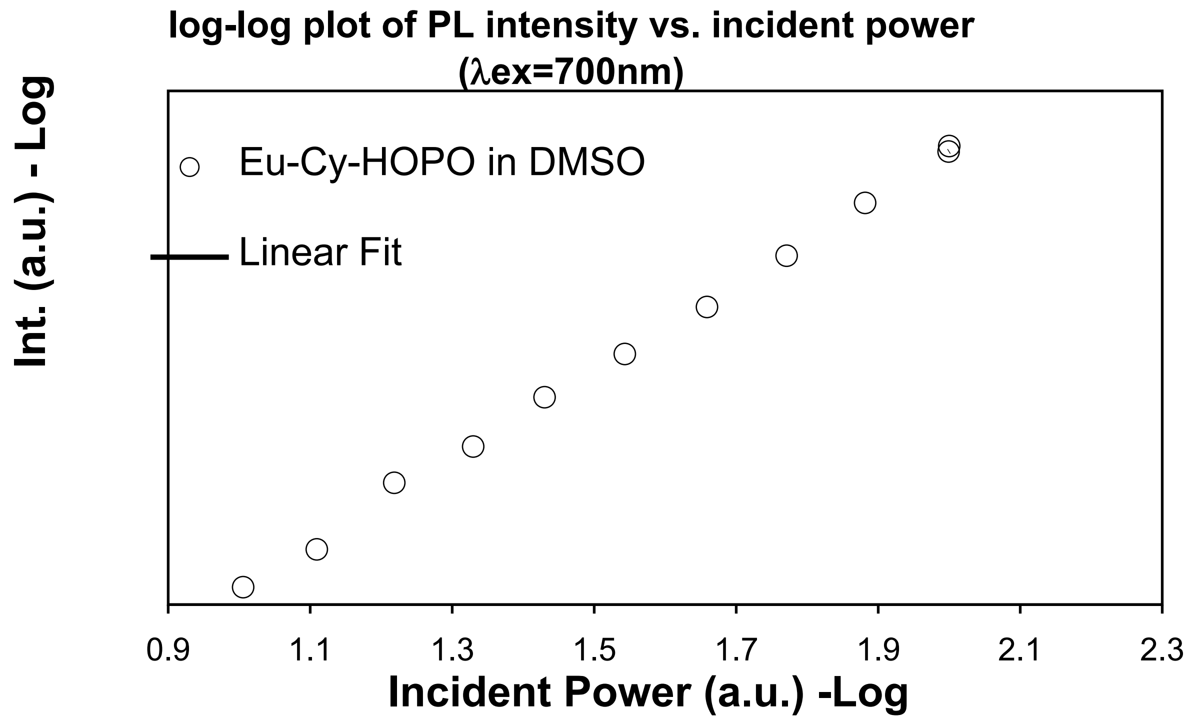


Figure S35. Dependence of luminescence intensity on incident power of **Eu-Cy-HOPO** in DMSO.

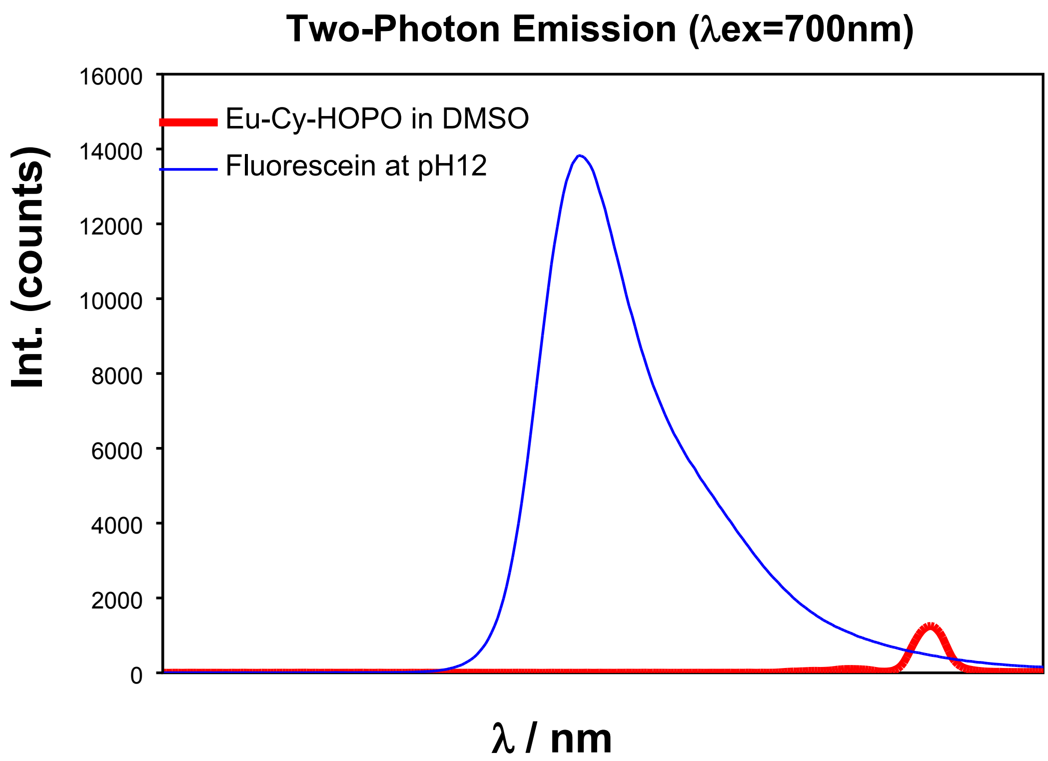


Figure S36. Two-photon excitation emission spectra of Fluorescein at pH 12 and **Eu-Cy-HOPO** in DMSO.

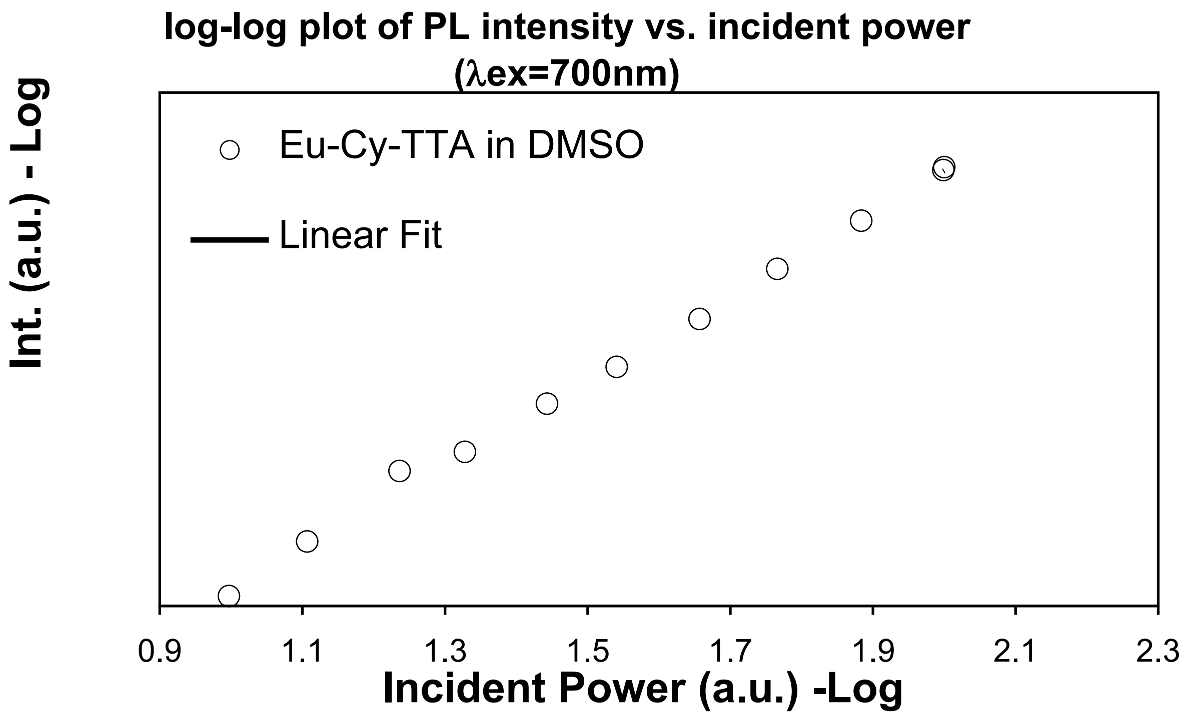


Figure S37. Dependence of luminescence intensity on incident power of **Eu-Cy-TTA** in DMSO.

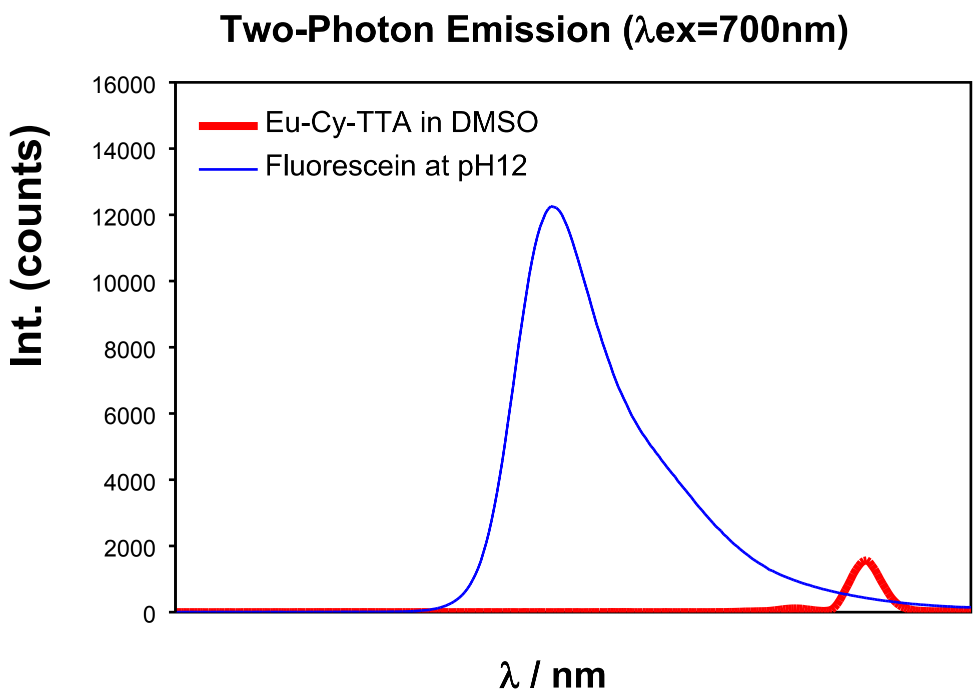


Figure S38. Two-photon excitation emission spectra of Fluorescein at pH 12 and **Eu-Cy-TTA** in DMSO.

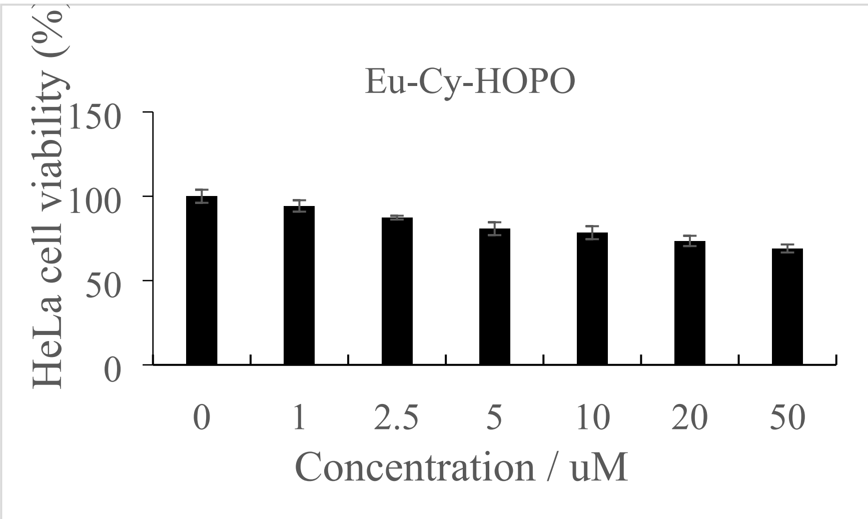


Figure S39. Viability of HeLa cells incubated with **Eu-Cy-HOPO** for 24 hours.

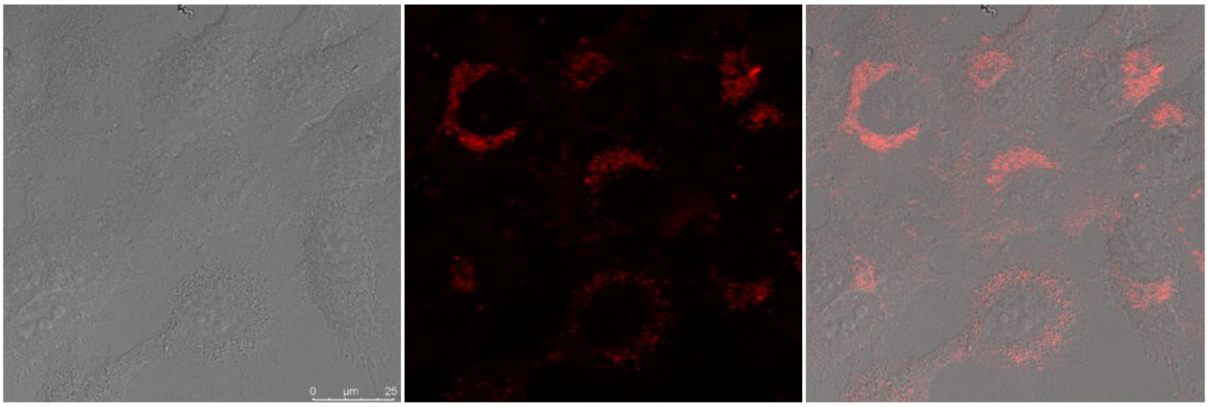


Figure S40. Bright field (left) and fluorescent microscopy image (middle) and overlaid image of **Sm-Cy-HOPO** (40 μM) in HeLa cells after 3 hours of incubation.

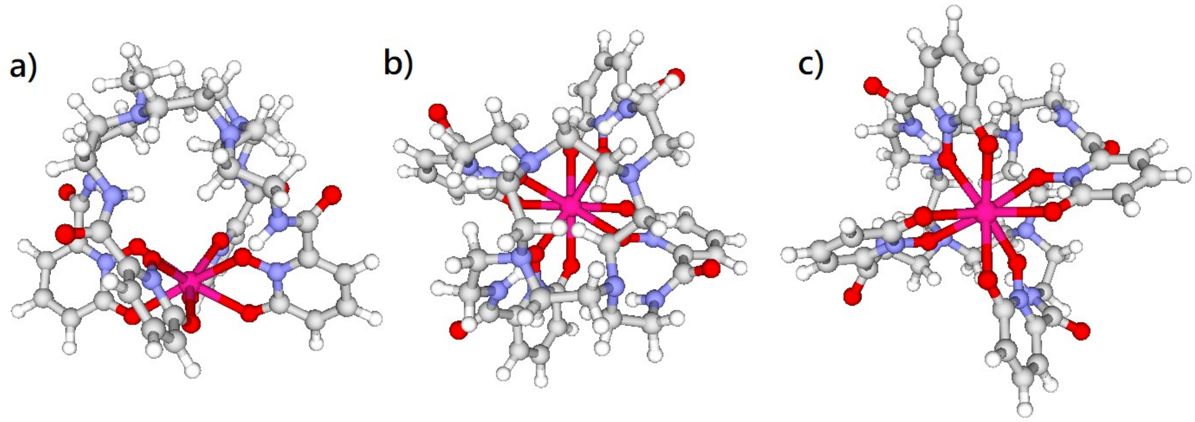


Figure S41. Optimized structure of **Sm-Cy-HOPO**. View from side (a); above cyclen backbone (b); view from below Sm(III) center (c).

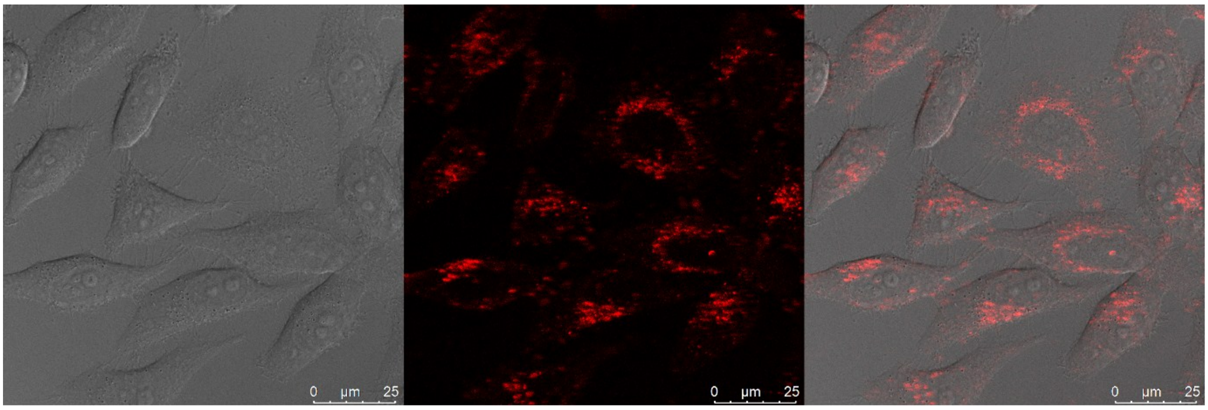


Figure S42. Bright field (left) and multi-photon microscopy image (middle) and overlaid image (right) of **Eu-Cy-HOPO** (4 µM) after 3 hours of incubation (λ_{ex} = 760 nm).