Electronic Supplementary Material (ESI) for ChemComm.

A turn-on luminescent europium probe for cyanide detection in water

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Supporting Information

General considerations. Unless otherwise stated, all chemicals were purchased from commercial suppliers and used without further purification. Deuterated solvents were obtained from Cambridge Isotope Laboratories (Tewskbury, MA, USA). Distilled water was further purified by a Millipore Simplicity UV system (resistivity 18×10^6 **Ω**). All organic extracts were dried over anhydrous $MgSO_4$ (s). Flash chromatography was performed on Merck Silica Gel. ^{1}H NMR and ^{13}C NMR spectra were recorded at room temperature on Bruker Advance III 400 at 400 MHz and 100 MHz, respectively, at the LeClaire-Dow instrumentation facility of the department of Chemistry of the University of Minnesota. The residual solvent peaks were used as internal references. Data for ^{1}H NMR are recorded as follows: chemical shift (δ, ppm), multiplicity (s, singlet; d, doublet, t, triplet; q, quartet; br, broad; m, multiplet), coupling constant (Hz), integration. Data for ^{13}C NMR are recorded as follows: chemical shift (δ, ppm). Low resolution (LR) and high resolution (HR) electrospray spray ionization time-of-flight mass spectrometry (ESI/TOF-MS) were recorded on a Bruker BioTOF I at the LeClaire-Dow instrumentation facility of the Department of Chemistry of the University of Minnesota. Fourier transform infrared (FTIR) spectra were obtained on a Thermo Scientific Nicolet iS5 Infrared Spectrometer. UV-visible spectra were recorded on a Varian Cary 100 Bio Spectrophotometer. Data was collected over the range of 200 – 800 nm. Luminescence data was acquired on a Varian Cary Eclipse Fluorescence Spectrophotometer using a quartz cell with a path length of 1 cm, with excitation and emission slit widths of 10 nm. Time-gated luminescent spectra were recorded with a time delay of 0.1 ms and a gate time of 5 ms. Luminescence data processing was done on SciDAVis 1.22 and QtiPlot 0.9.8.9 software. pH measurements were taken using a Thermo Orion 3 Benchtop pH meter. High-performance liquid chromatography (HPLC) data was collected on a Varian Prostar Model 210, coupled with an Agilent ZORBAX Eclipse XDB-C18 column, and with a Varian ProStar 335 diode array detector. HPLC measurements were performed with isocratic elution by Milli-Q at a flow rate 1.0 mL min^{-1} unless specified otherwise.

1-(benzyloxy)-6-(2-thioxothiazolidine-3-carbonyl)pyridin-2(1*H*)-one (1). Benzyl-protected 1,2-hydroxypyridinone (1,2-HOPO) was synthesized as previously reported[1] with successful synthesis confirmed by ^{1}H NMR and LR ESI-MS.

***N, N′*-(Azanediylbis(propane-3,1-diyl))bis(1-(benzyloxy)-6-oxo-1,6-dihydropyridine-2-carboxamide) (2).** Triethylamine (101 μL, 0.720 mmol) was added to a solution of the benzyl protected 1,2-HOPO (**1**, 250 mg, 0.72 mmol) and bis(3-aminopropyl)amine) (47 mg, 0.36 mmol) in CH_2Cl_2 (5 mL). The reaction mixture was stirred at room temperature overnight. The solvents were removed under reduced pressure and the crude product was purified by flash chromatography over silica using 86.5% CH_2Cl_2/12.5% CH_3OH/1% NEt_3 as an eluent. The solvents were removed under reduced pressure to yield the amine intermediate **2** as a colourless oil (206 mg, 98%). ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 8.08 (br, 2 H), 7.43-7.40 (m, 4 H), 7.31-7.19 (m, 8H), 6.61 (dd, J_1 = 7 Hz, J_2 = 2 Hz, 2 H), 6.34 (dd, J_1 = 7 Hz, J_2 = 2 Hz, 2 H), 5.18 (s, 4 H), 3.30-3.21 (m, 4 H), 2.34 (t, 4 H, J = 6 Hz), 1.49-1.40 (m, 4 H).^{13}C-NMR (100 MHz, $CDCl_3$): δ = 160.2, 158.6, 143.0, 138.3, 133.3, 130.3, 129.4, 128.6, 123.7, 106.1, 79.3, 47.5, 39.1, 28.4. ESI-HRMS: *m/z* = 586.2665 ($[M+H]^+$), (Calcd. 586.2666).

***N*^{2}-(1-(Benzyloxy)-6-oxo-1,6-dihydropyridine-2-carbonyl)-*N*^{6}-(*tert*-butoxycarbonyl)-*L*-lysine (3).** Triethylamine (201 μL, 1.44 mmol) was added to a solution of the benzyl protected 1,2-HOPO (**1,** 500 mg, 1.44 mmol) and H-Lys(Boc)-OH (355 mg, 1.44 mmol) in CH_3CN (20 mL).

The reaction mixture was stirred at room temperature overnight. The solvents were then removed under reduced pressure and the crude product was purified by flash chromatography over silica using 92.5% CH_2Cl_2/7% CH_3OH/0.5% CH_3CO_2H as an eluent. The solvents were removed under reduced pressure to yield the intermediate **3** as a colourless oil (511 mg, 75%). ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.7 (d, J = 7 Hz, 1 H), 7.44 (b, 2 H), 7.29-7.24 (m, 3 H), 6.73 (d, J = 9 Hz, 1 H), 6.45 (d, J = 6 Hz, 1 H), 5.34 (d, J = 8 Hz, 1 H), 5.21 (d, J = 8 Hz, 1 H), 4.57 (m, 1 H), 2.90 (b, 2 H), 1.83 (b, 1 H), 1.67 (b, 1 H), 1.41-1.27 (m, 13 H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 173.9, 159.9, 159.1, 158.0, 156.3, 142.1, 138.5, 133.1, 130.3, 129.4, 128.5, 124.0, 107.3, 81.0, 79.4, 79.3, 52.9, 41.0, 39.9, 31.2, 29.4, 29.0, 28.4, 22.4. ESI-HRMS: m/z = 472.2087 ($[M-H]^-$), (Calcd. 472.2084).

***tert*-Butyl (*S*)-(5-(1-(benzyloxy)-6-oxo-1,6-dihydropyridine-2-carboxamido)-6-(bis(3-(1-(benzyloxy)-6-oxo-1,6-dihydropyridine-2-carboxamido)propyl)amino)-6-oxohexyl)carbamate (4).** Triethylamine (64 μL, 0.47 mmol) and O-(7-azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (HATU, 178 mg, 0.469 mmol) were added to a solution of the amine intermediate **2** (275 mg, 0.469 mmol) and the acid intermediate **3** (221 mg, 0.469 mmol) in CH_2Cl_2 (10 mL). The reaction mixture was stirred at room temperature for 6 hours. The reaction mixture was then washed with 1 M HCl (*aq*) (3 × 10 mL) and $NaHCO_3$ (10% *aq*) (3 × 10 mL). The organic phase was dried with anhydrous $MgSO_4$ (*s*) and filtered. The solvent was removed under reduced pressure and the crude product was purified by flash chromatography over silica using 93% CH_2Cl_2/7% CH_3OH as an eluent. The solvents were removed under reduced pressure to yield the protected ligand **4** as a colourless foam (150 mg, 30%). ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.54-7.38 (m, 8 H), 7.25-7.20 (m, 6 H), 7.18-7.10 (m, 4 H), 6.61 (d, J = 9 Hz, 1 H), 6.48 (d, J = 9 Hz, 2 H), 6.26 (d, J = 7 Hz, 1 H), 6.16 (d, J = 7 Hz, 2 H), 5.43-5.09 (m, 9 H), 4.65-4.56 (m, 1 H), 3.31-2.61 (m, 10 H), 1.50-1.18 (m, 10 H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 171.4, 161.1, 160.6, 160.3, 158.6, 158.5, 158.3, 156.3, 143.2, 143.1, 141.7, 138.4, 138.3, 137.9, 133.4, 133.3, 130.7, 130.4, 130.1, 129.9, 129.6, 129.5, 129.3, 128.6, 128.6, 128.5, 124.5, 123.7, 123.6, 106.4, 105.3, 104.7, 79.7, 79.4, 79.0, 53.5, 50.4, 49.8, 44.0, 42.4, 39.9, 36.6, 32.2, 29.6, 28.4, 27.5, 26.7, 22.6. ESI-HRMS: m/z = 1063.4581 ($[M+Na]^+$), (Calcd. 1063.4542).

(*S*)-6-(bis(3-(1-hydroxy-6-oxo-1,6-dihydropyridine-2-carboxamido)propyl)amino)-5-(1-hydroxy-6-oxo-1,6-dihydropyridine-2-carboxamido)-6-oxohexan-1-aminium chloride (Lys-HOPO). The protected ligand **4** (116 mg, 0.114 mmol) was dissolved in a 1:1 mixture of HCl (*aq*) (1.0 M) and CH_3CO_2H (6.7 mL). The reaction mixture was stirred at room temperature overnight. The volatiles were removed under reduced pressure. Addition of methanol-diethyl ether solution (1:1, 10 mL) resulted in a precipitate that was filtered and dried in a desiccator, yielding the deprotected ligand **5** as a beige solid (78 mg, 99%). ^{1}H-NMR (400 MHz, CD_3OD): δ = 7.57-7.46 (m, 3 H), 6.85-6.58 (m, 6 H), 5.03-4.97 (m, 1 H), 3.69-3.56 (m, 3 H), 3.55-3.34 (m, 5 H), 3.03-2.91 (m, 2 H), 2.13-2.00 (m, 2 H), 1.95-1.80 (m, 4 H), 1.78 – 1.69 (m, 2 H), 1.62-1.48 (m, 2 H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 173.2, 162.6, 162.3, 161.9, 160.2, 142.6, 142.5, 142.0, 140.1, 139.7, 139.4, 129.8, 129.8, 129.4, 120.7, 120.5, 110.2, 109.6, 109.4, 51.5, 47.1, 47.0, 45.0, 40.8, 40.7, 38.5, 32.9, 29.8, 28.3, 23.9. IR (NaCl pellet, cm^{-1}): ν = 3424, 2079, 1643. ESI-HRMS: m/z = 671.2785 ($[M+H]^+$), (Calcd. 671.2790).

Eu^{III}-Lys-HOPO. The deprotected ligand Lys-HOPO (31 mg, 0.044 mmol) and $EuCl_3 \cdot 6H_2O$ (16 mg, 0.044 mmol) were dissolved in 1:1 mixture of CH_3OH (2.5 mL) and Milli-Q (2.5 mL),

followed by the injection of pyridine (36 µL). The reaction mixture was stirred at 80 ºC for 8 hours. The mixture was cooled down to room temperature and the solvent was removed under reduced pressure. The crude product was triturated with methanol five times (5 × 10 mL), and dissolved in Milli-Q. The suspension was then filtered out, and the water of the filtrate was removed under reduced pressure. The residue was further dried in a desiccator yielding the final Eu^{III} complex as a beige powder (38 mg, 99%). IR (NaCl, cm^{-1}): ν = 3571, 3500, 1647, 1610, 1365, 1289. ESI-LRMS: *m/z* = 819.2 ($[M+M]^{2+}$), (Calcd. 819.2).

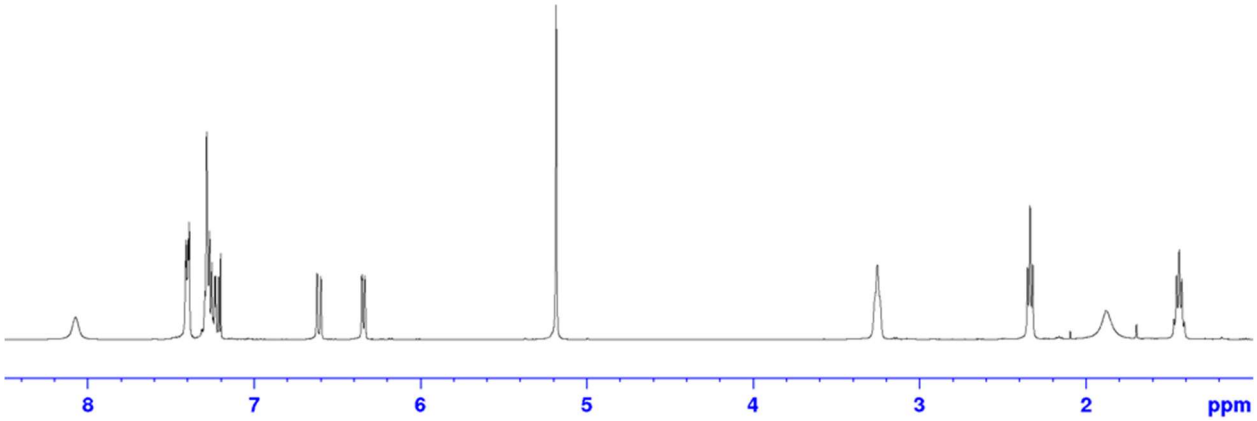

Figure S1. ^{1}H NMR spectrum of *N,N′*-(azanediylbis(propane-3,1-diyl))bis(1-(benzyloxy)-6-oxo-1,6-dihydropyridine-2-carboxamide) (**2**, 400 MHz, $CDCl_3$).

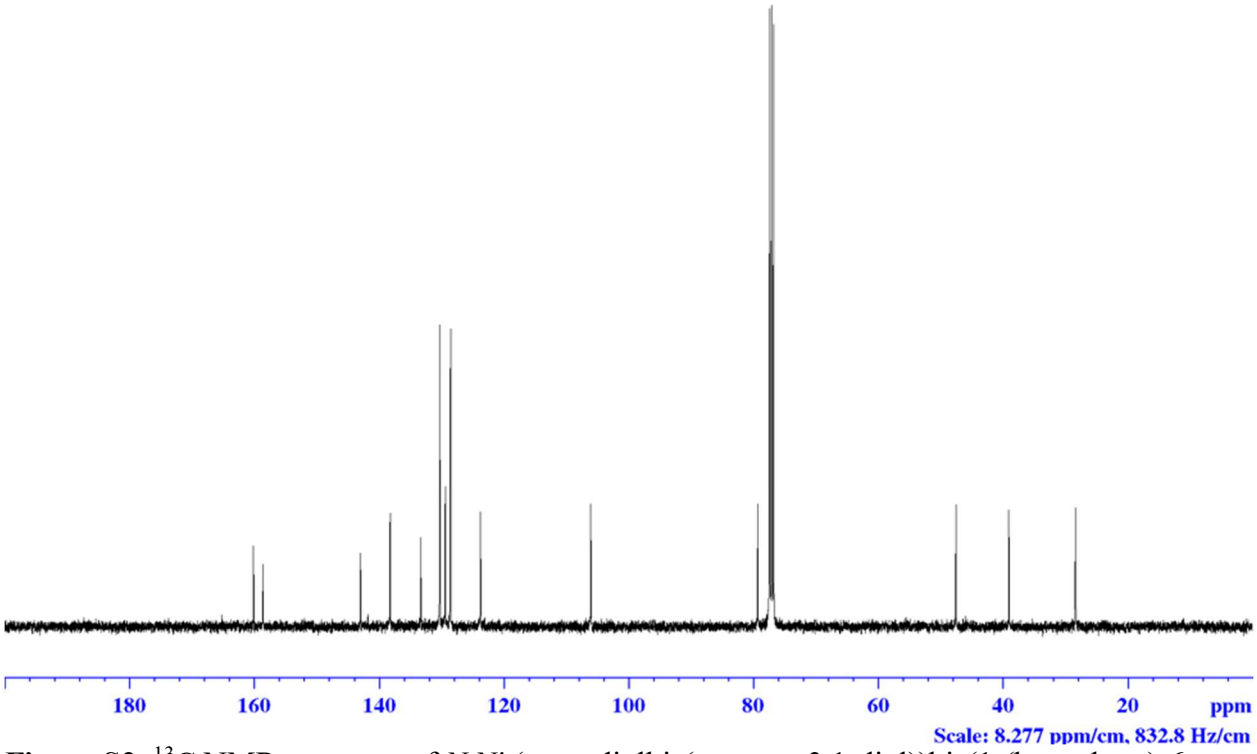

Figure S2. ^{13}C NMR spectrum of *N,N′*-(azanediylbis(propane-3,1-diyl))bis(1-(benzyloxy)-6-oxo-1,6-dihydropyridine-2-carboxamide) (**2**, 100 MHz, $CDCl_3$).

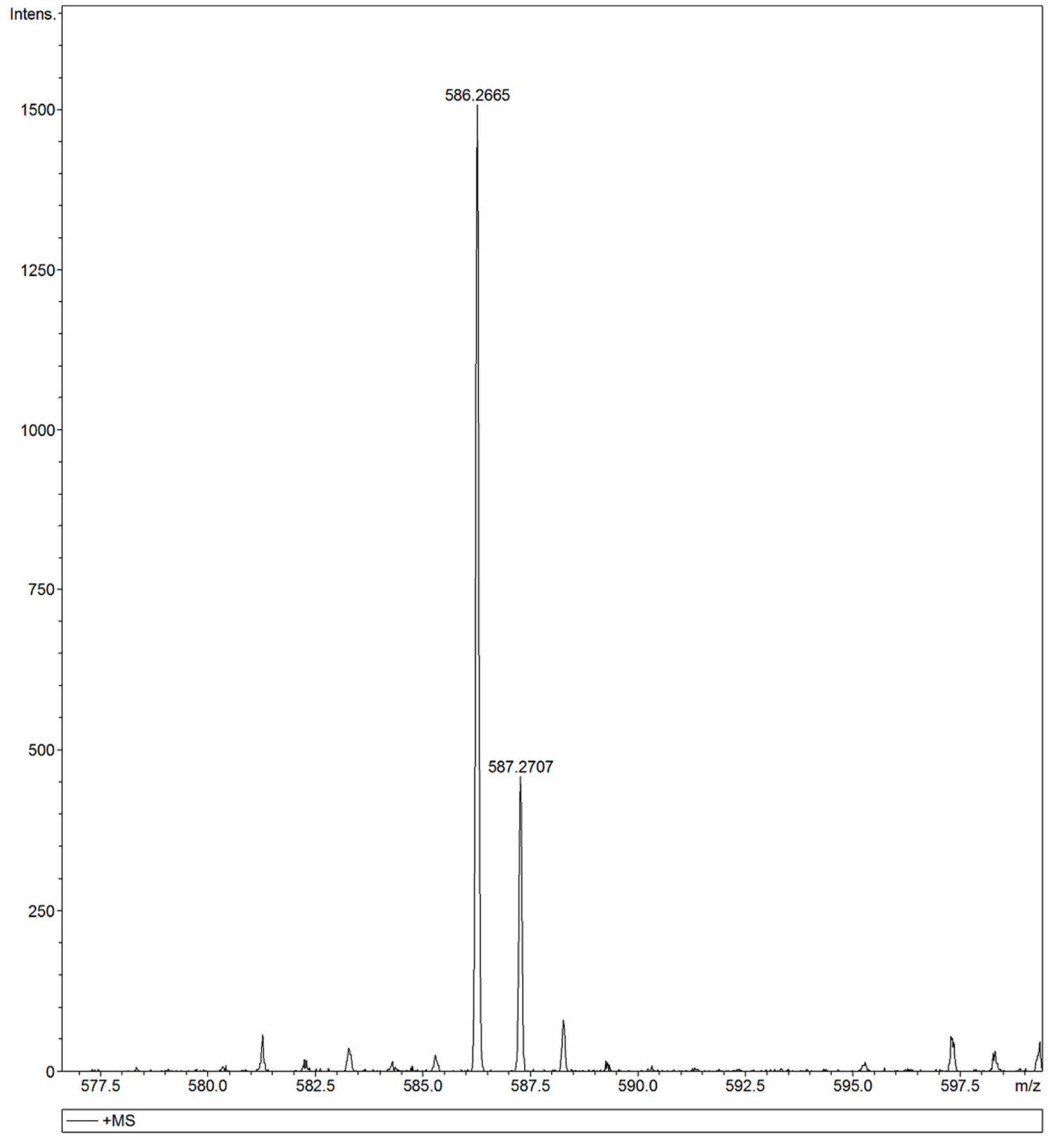

Figure S3. ESI-MS spectrum of *N,N′*-(azanediylbis(propane-3,1-diyl))bis(1-(benzyloxy)-6-oxo-1,6-dihydropyridine-2-carboxamide) (**2**).

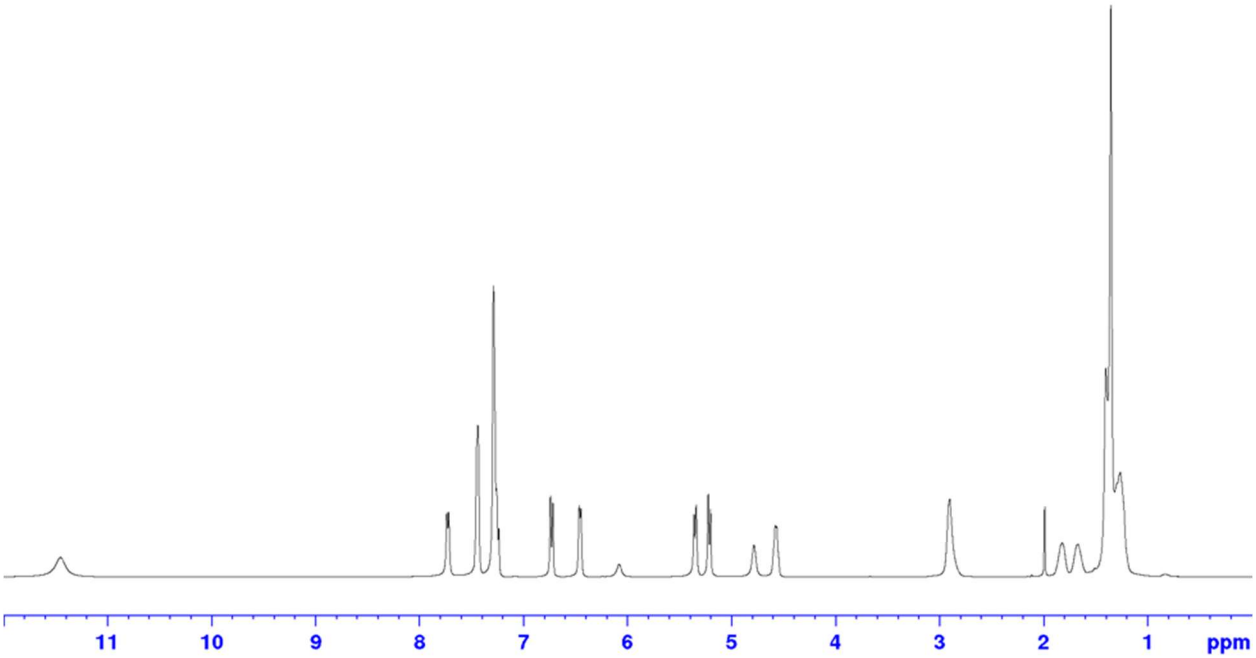

Figure S4. ^{1}H NMR spectrum of N^{2}-(1-(benzyloxy)-6-oxo-1,6-dihydropyridine-2-carbonyl)-N^{6}-(*tert*-butoxycarbonyl)-*L*-lysine (**3**, 400 MHz, $CDCl_3$).

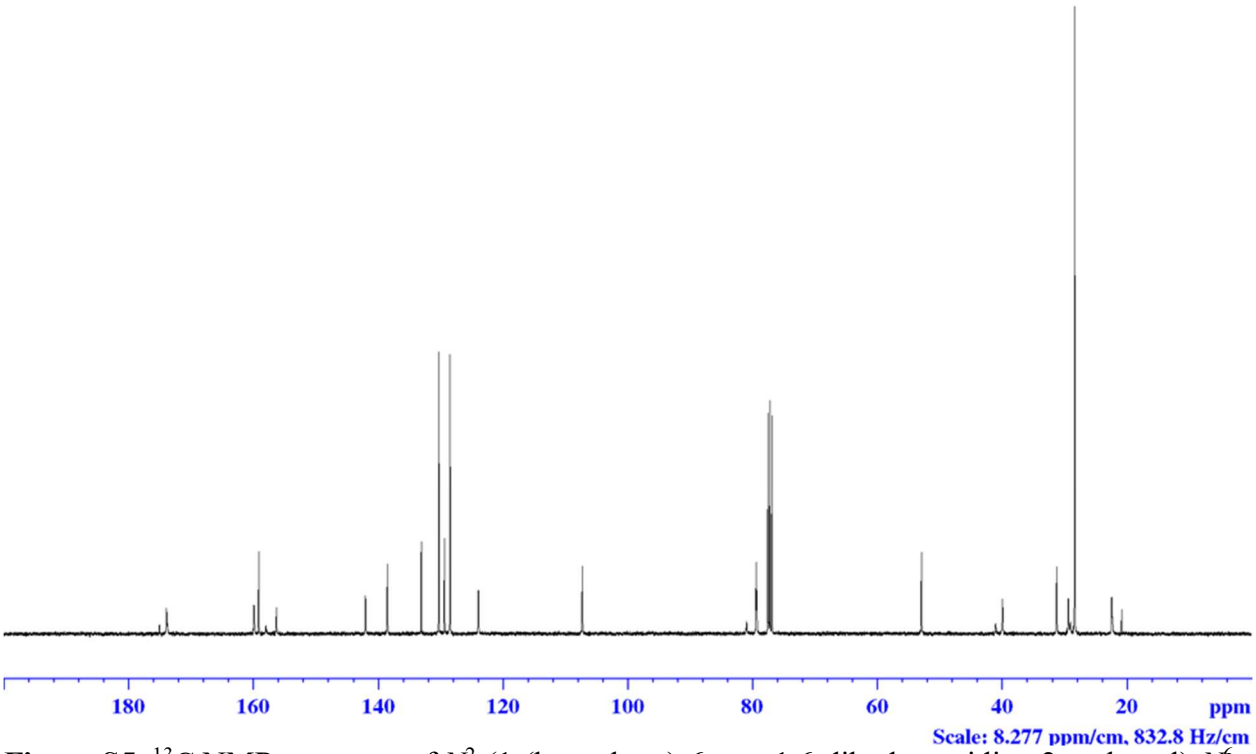

Figure S5. ^{13}C NMR spectrum of N^{2}-(1-(benzyloxy)-6-oxo-1,6-dihydropyridine-2-carbonyl)-N^{6}-(*tert*-butoxycarbonyl)-*L*-lysine (**3**, 100 MHz, $CDCl_3$).

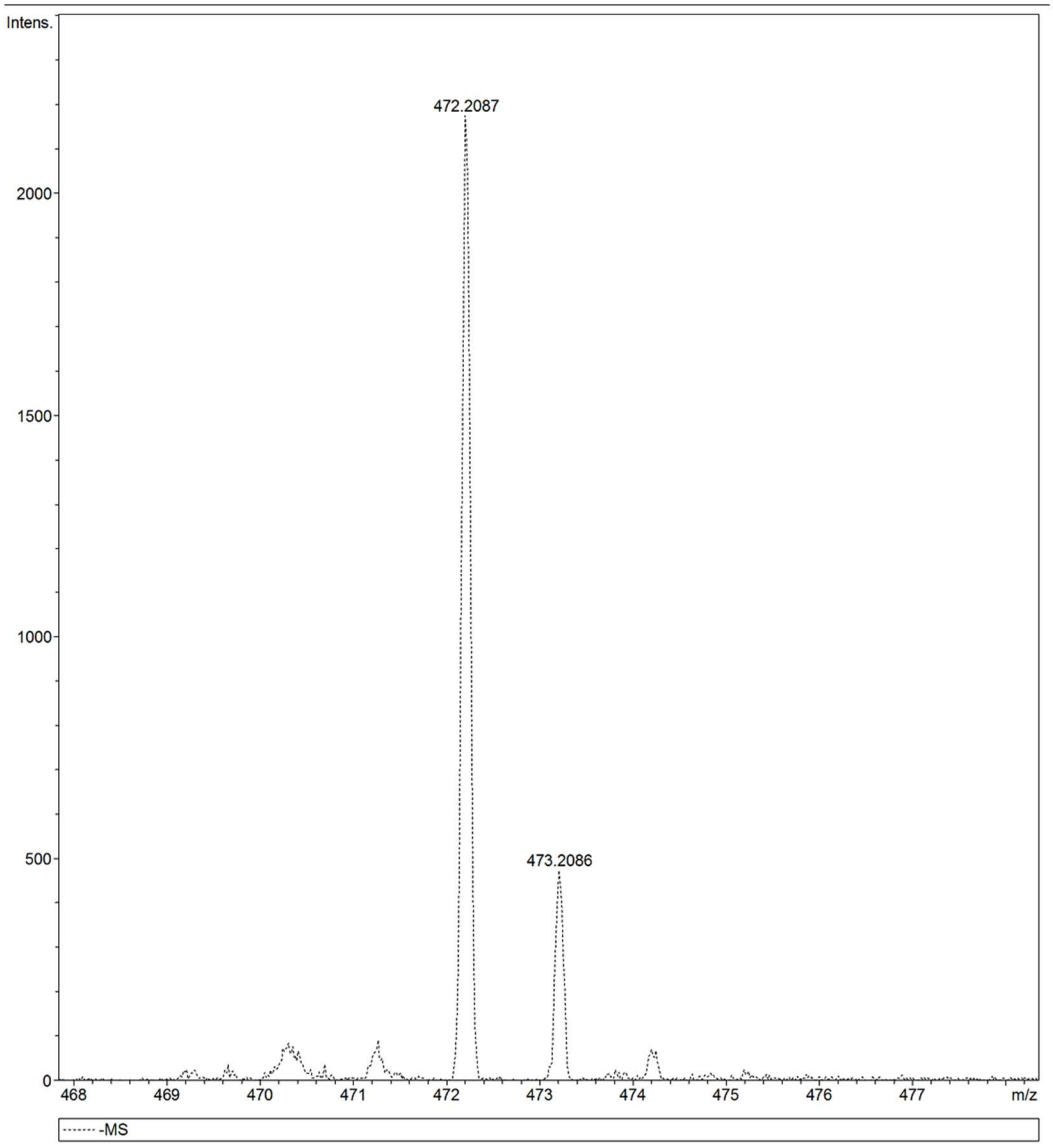

Figure S6. ESI-MS spectrum of N^2-(1-(benzyloxy)-6-oxo-1,6-dihydropyridine-2-carbonyl)-N^6-(*tert*-butoxycarbonyl)-*L*-lysine (**3**).

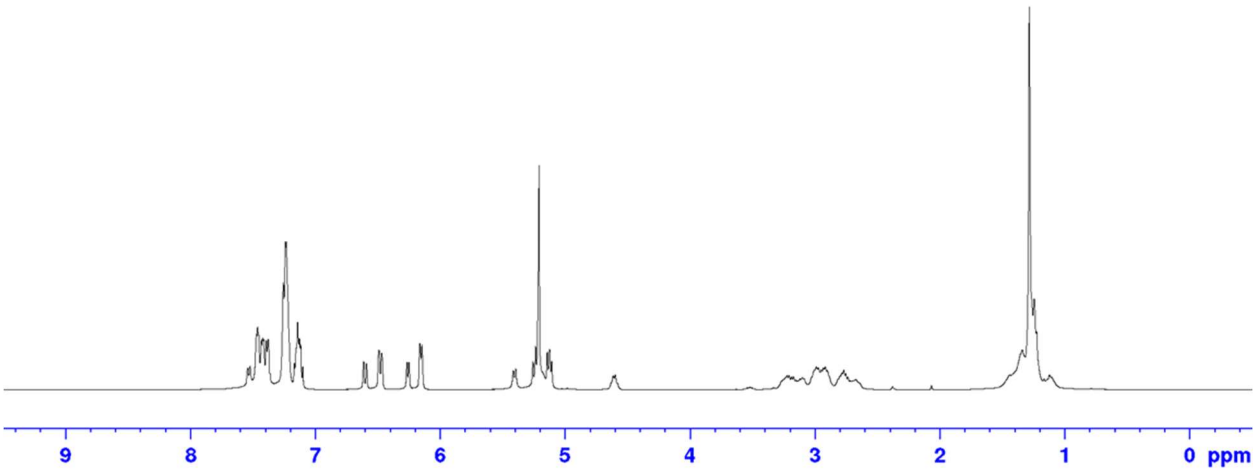

Figure S7. ^{1}H NMR spectrum of *tert*-butyl (*S*)-(5-(1-(benzyloxy)-6-oxo-1,6-dihydropyridine-2-carboxamido)-6-(bis(3-(1-(benzyloxy)-6-oxo-1,6-dihydropyridine-2-carboxamido)propyl)amino)-6-oxohexyl)carbamate (**4**, 400 MHz, $CDCl_3$).

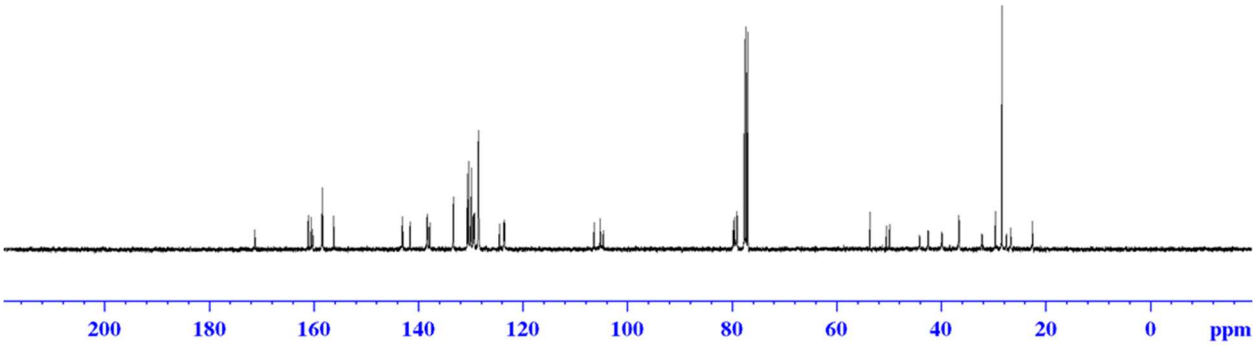

Figure S8. ^{13}C NMR spectrum of *tert*-butyl (*S*)-(5-(1-(benzyloxy)-6-oxo-1,6-dihydropyridine-2-carboxamido)-6-(bis(3-(1-(benzyloxy)-6-oxo-1,6-dihydropyridine-2-carboxamido)propyl)amino)-6-oxohexyl)carbamate (**4**, 100 MHz, $CDCl_3$).

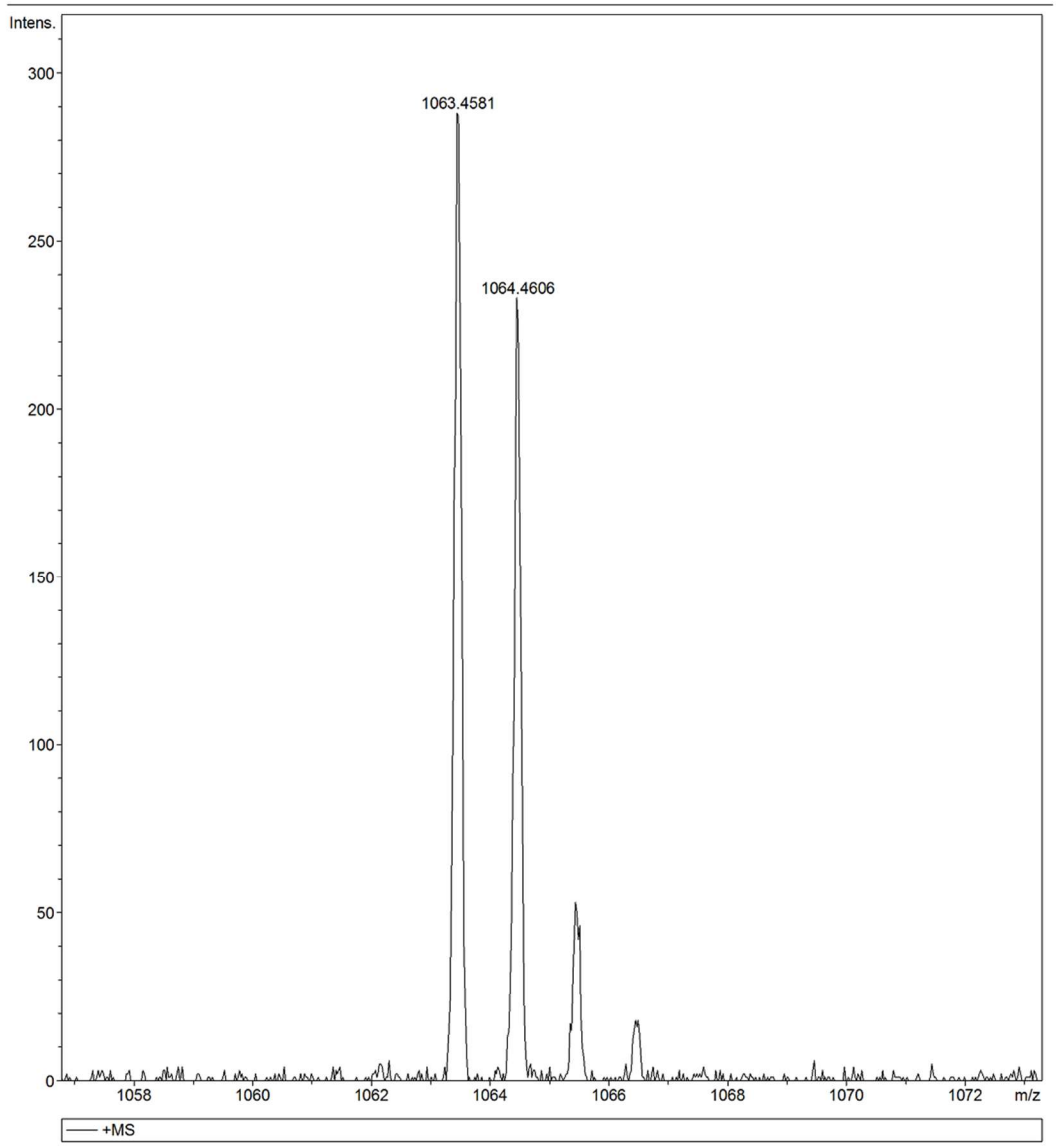

Figure S9. ESI-MS spectrum of *tert*-Butyl (*S*)-(5-(1-(benzyloxy)-6-oxo-1,6-dihydropyridine-2-carboxamido)-6-(bis(3-(1-(benzyloxy)-6-oxo-1,6-dihydropyridine-2-carboxamido)propyl)amino)-6-oxohexyl)carbamate (**4**).

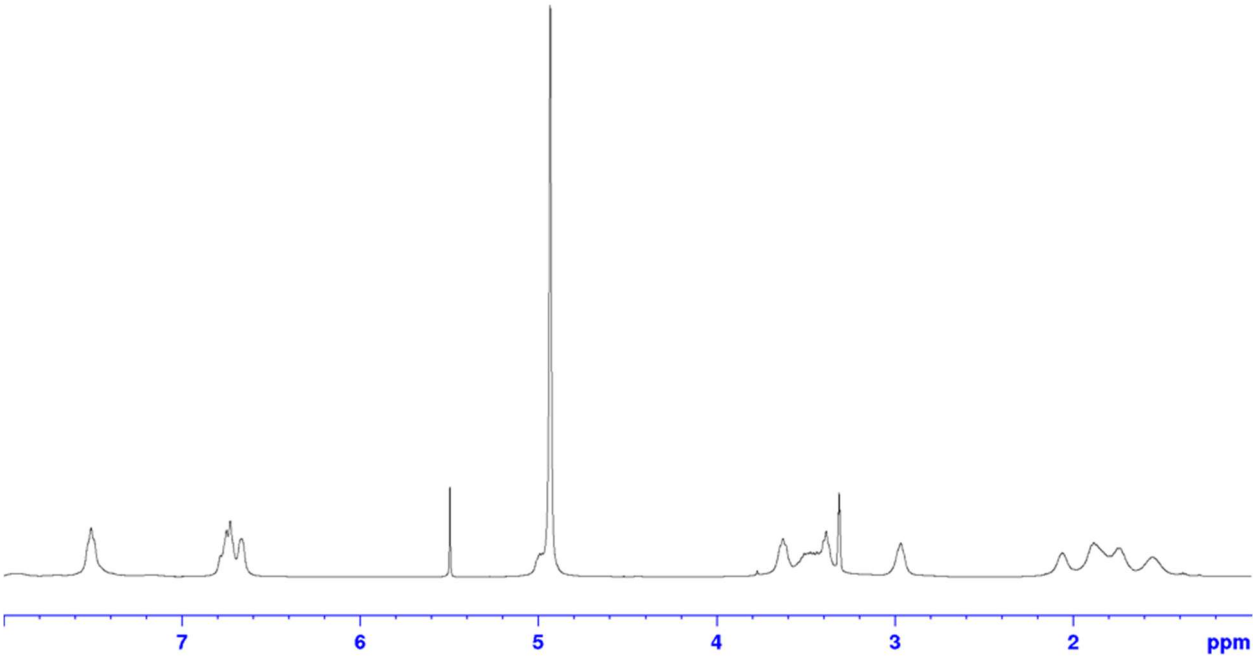

Figure S10. ^{1}H NMR spectrum of Lys-HOPO (400 MHz, CD_3OD).

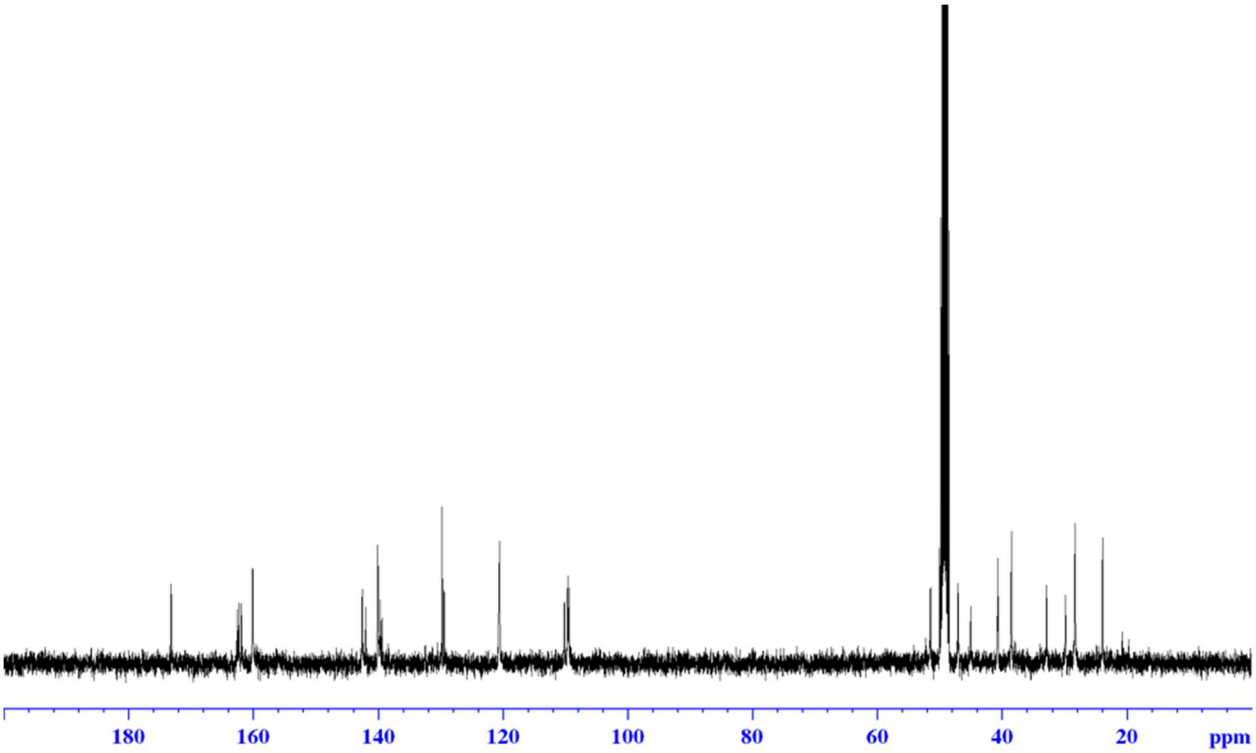

Figure S11. ^{13}C NMR spectrum of Lys-HOPO (100 MHz, CD_3OD).

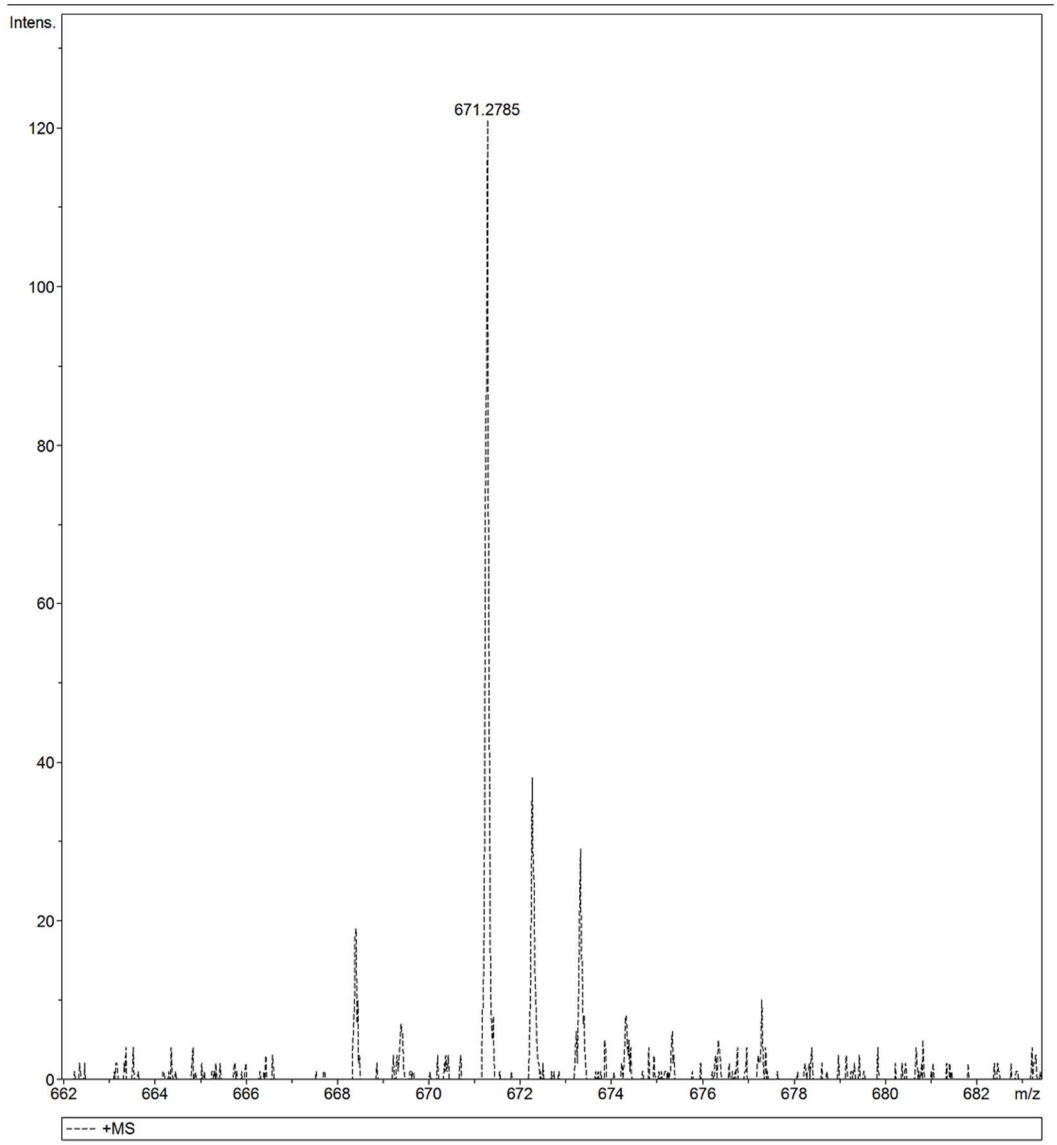

Figure S12. ESI-MS spectrum of Lys-HOPO.

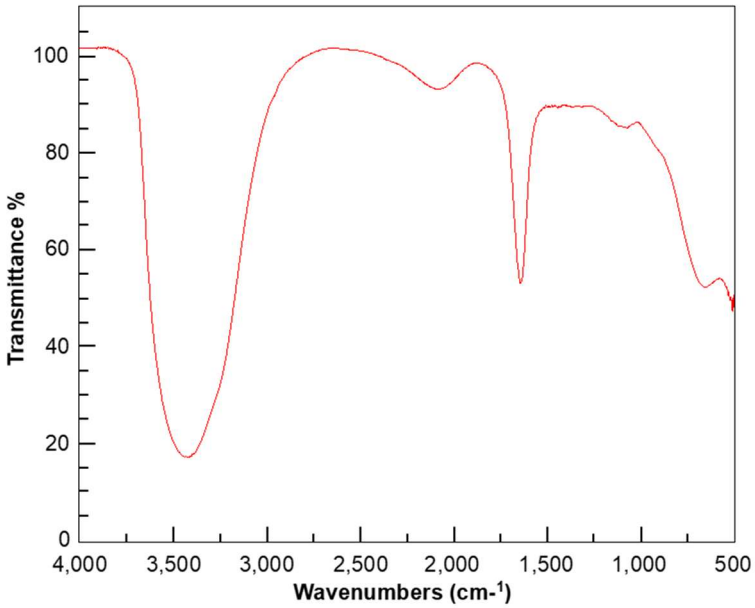

Figure S13. FT-IR spectrum of Lys-HOPO.

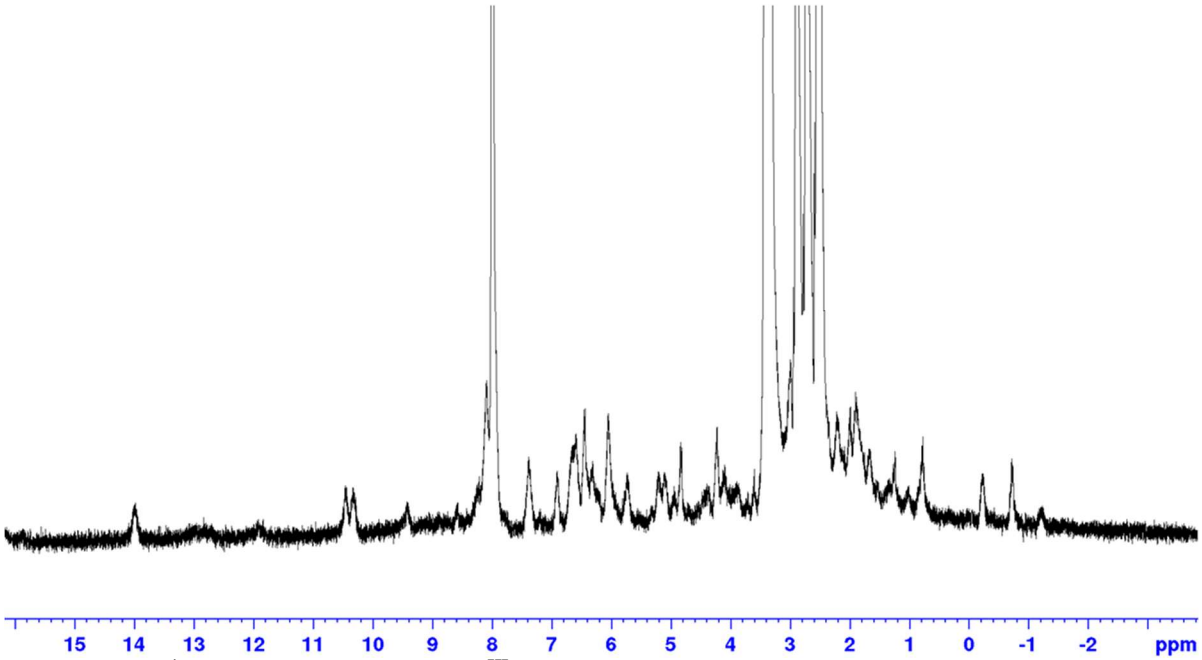

Figure S14. ^{1}H NMR spectrum of Eu^{III}-Lys-HOPO (400 MHz, DMF-d_7/DMSO-d_6 1:1).

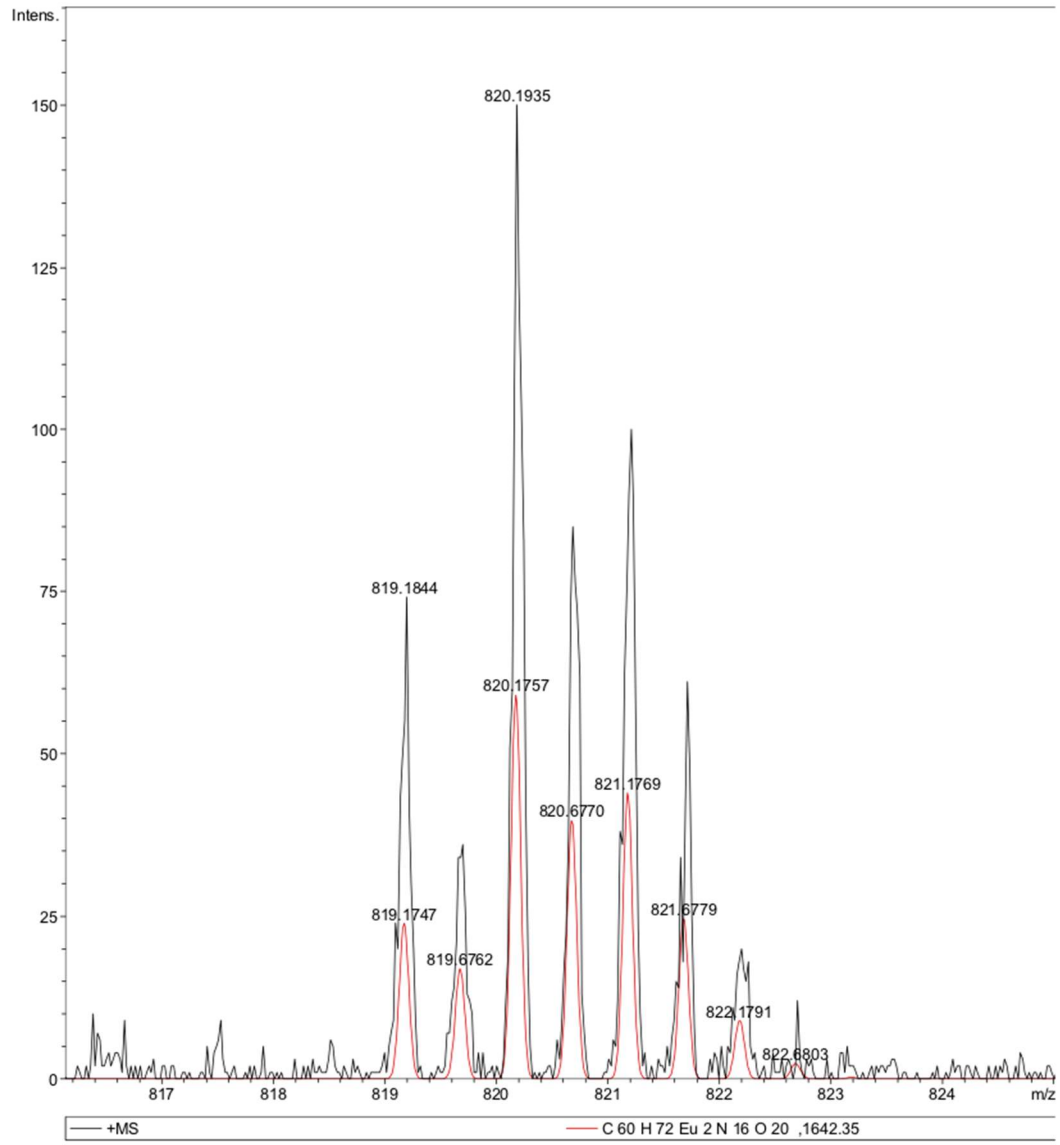

Figure S15. Experimental (black) and calculated (red) ESI-MS spectrum of Eu^{III}-Lys-HOPO.

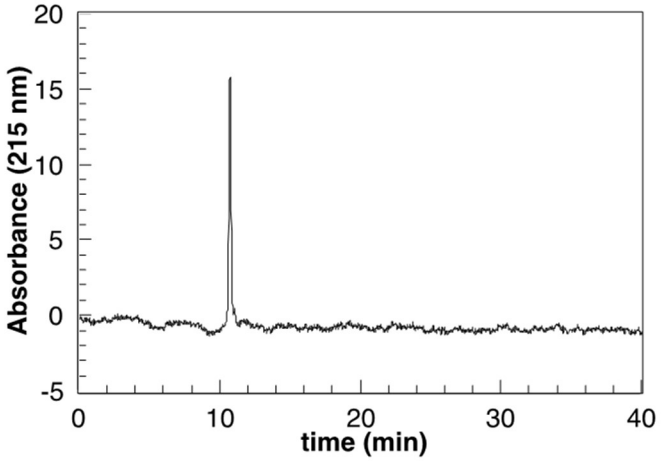

Figure S16. HPLC chromatogram of Eu^{III}-Lys-HOPO.

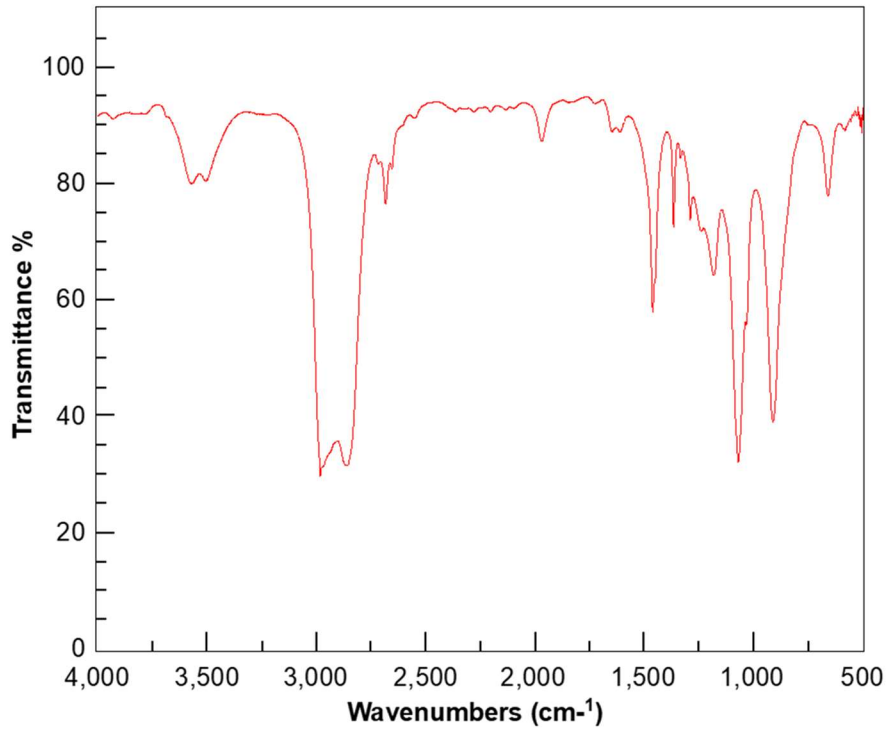

Figure S17. FT-IR spectrum of Eu^{III}-Lys-HOPO. Note the residual THF.

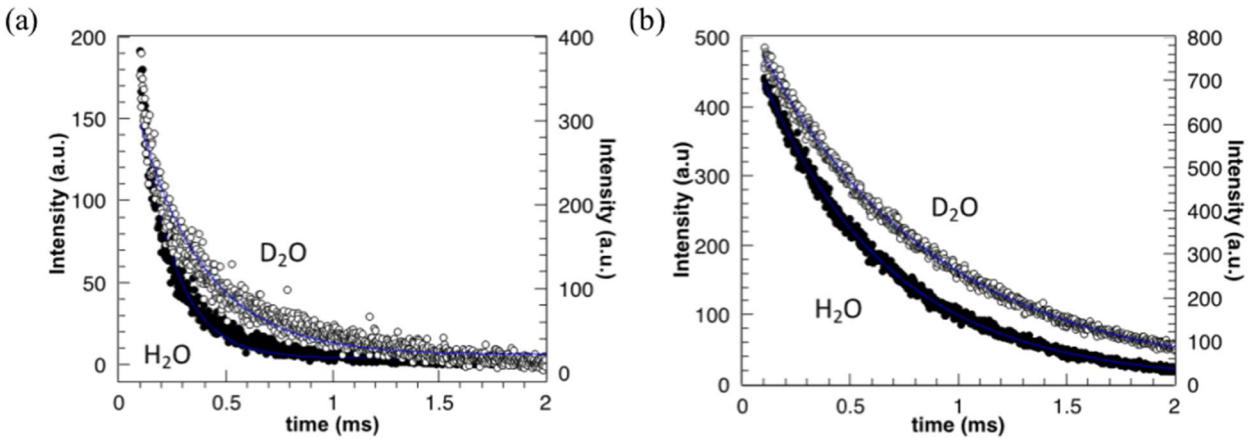

Figure S18. Luminescence lifetime profiles of (a) Eu^{III}-Lys-HOPO in H_2O (filled circle) and D_2O (open circle) and (b) Eu^{III}-Lys-HOPO + 100 eq. CN^- in H_2O (filled circle) and D_2O (open circle). Experimental conditions: [Eu^{III}-Lys-HOPO] = 7.6 μM, pH 9.8, delay time = 0.1 ms, gate time = 0.002 ms, λ_{ex} = 335 nm, λ_{em} = 615 nm, excitation and emission slit widths = 20 nm, voltage = 800 V.

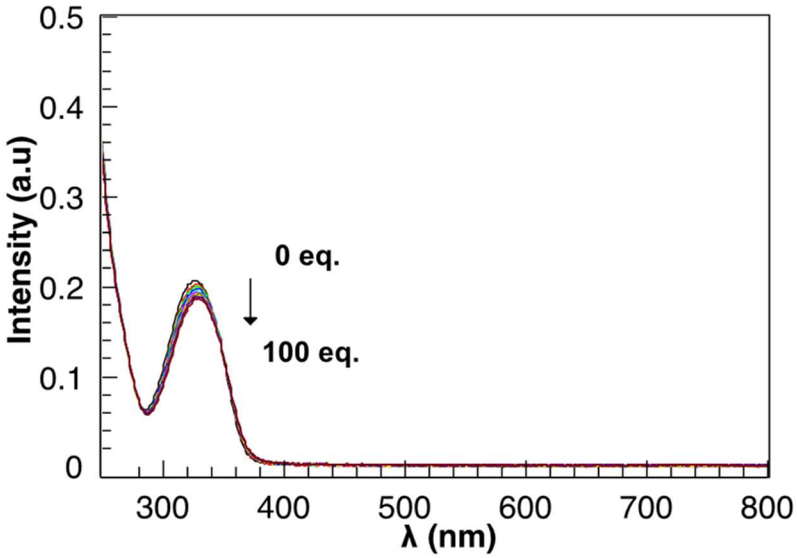

Figure S19. UV-Vis spectrum of Eu^{III}-Lys-HOPO with and without 100 eq. of NaCN. Experimental conditions: [Eu^{III}-Lys-HOPO] = 7.6 μM in water, pH 9.8.

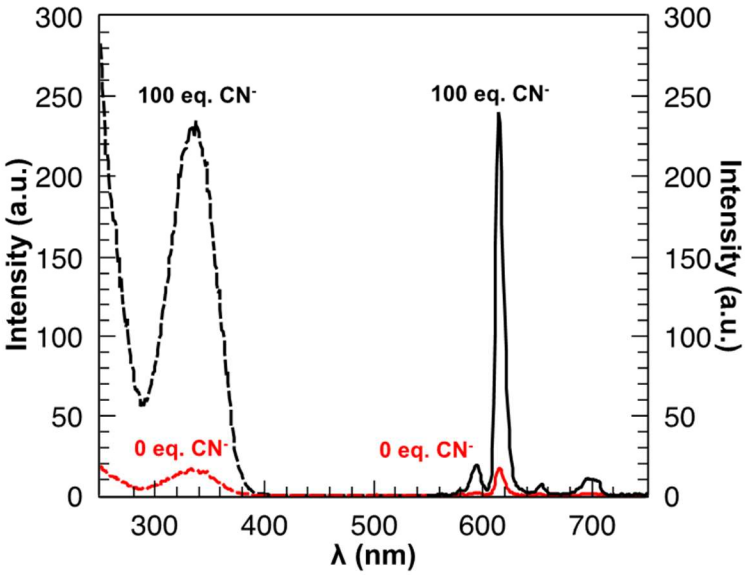

Figure S20. Excitation (dashed lines) and emission (solid lines) profiles of Eu^{III}-Lys-HOPO in the absence (red) and presence (black) of 100 eq. of NaCN. Experimental conditions: [Eu^{III}-Lys-HOPO] = 7.6 μM in water, pH 9.8, delay time = 0.1 ms, gate time = 5 ms, λ_{ex} = 335 nm, excitation and emission slit widths = 20 nm.

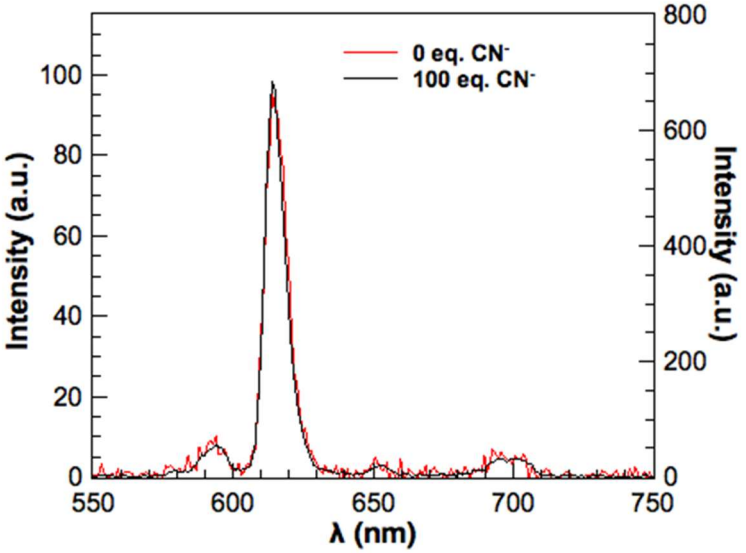

Figure S21. Zoomed in Emission (solid lines) profiles of Eu^{III}-Lys-HOPO in the absence (red) and presence (black) of 100 eq. of NaCN. Experimental conditions: [Eu^{III}-Lys-HOPO] = 7.6 μM in water, pH 9.8, delay time = 0.1 ms, gate time = 5 ms, λ_{ex} = 335 nm, excitation and emission slit widths = 5 nm.

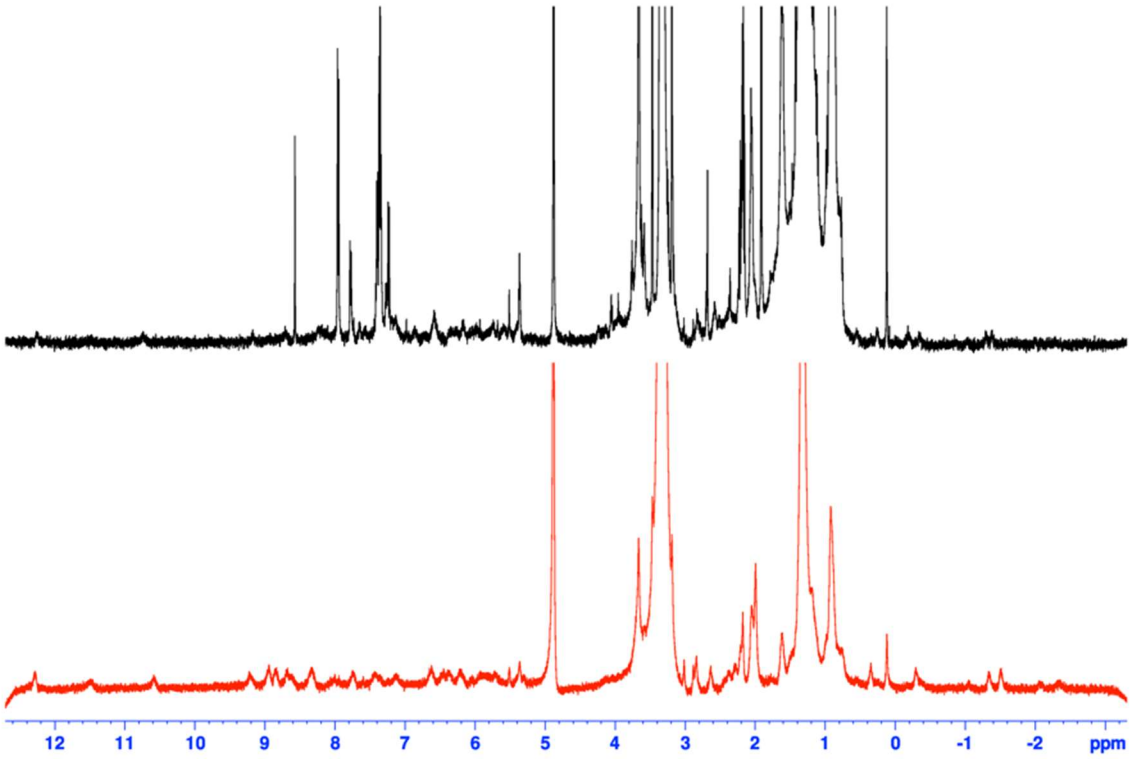

Figure S22. ^{1}H NMR spectra of Eu^{III}-Lys-HOPO in the absence (red) and presence (black) of 100 eq. of NaCN in CD_3OD.

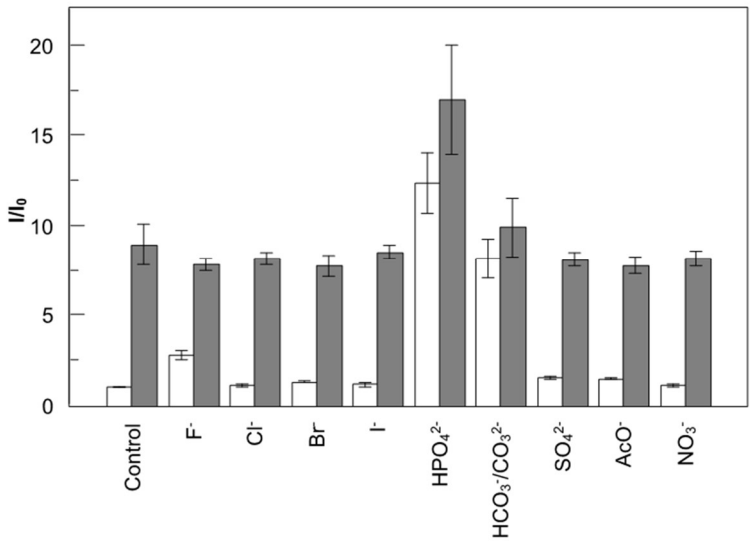

Figure S23. Selectivity of Eu^{III}-Lys-HOPO to various environmentally-relevant anions. White bars represent the time-delayed relative luminescence intensity after addition of 100 eq. of the appropriate anions (NaF, NaCl, NaBr, NaI, NCN, $NaHPO_4$, $NaHCO_3$, Na_2SO_4, NaOAc, and $NaNO_3$) and 100 eq. of $CaCl_2$. Grey bars represent the time-delayed relative luminescence intensity after subsequent addition of 100 eq. of NaCN. Experimental conditions: I=integrated time-delayed

luminescence from 550 nm to 750 nm, I_o=integrated luminescence of Eu^{III}-Lys-HOPO in the absence of any anion, [Eu^{III}-Lys-HOPO] = 16 μM in H_2O, pH 9.8, $\lambda_{excitation}$ = 335 nm, delay time = 0.1 ms, excitation slit widths = 10 nm, emission slit width = 5 nm. Error bars represent s.d., *n* = 3.

Data fitting

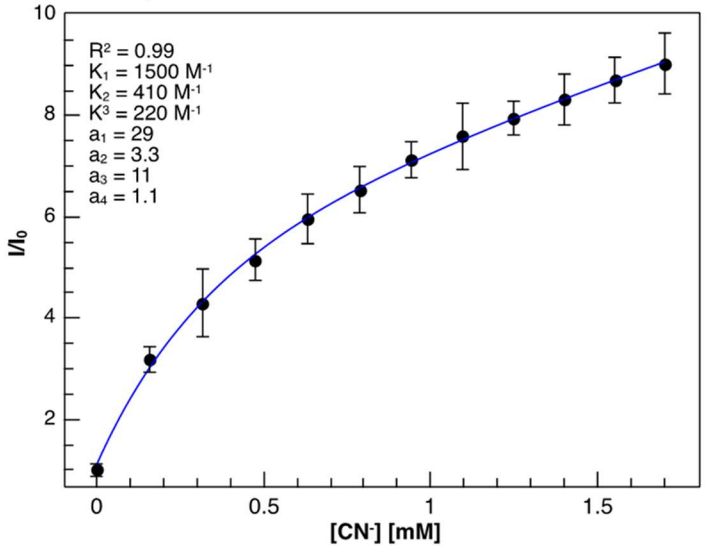

Figure S24. Data fitting of Eu^{III}-Lys-HOPO + NaCN.

Data fitting of Eu^{III}-Lys-HOPO + NaCN uses the following equations:

$$[H] + [G] \rightleftharpoons [HG]$$
$$[HG] + [G] \rightleftharpoons [HG_2]$$
$$[HG_2] + [G] \rightleftharpoons [HG_3]$$

where H denotes the host (Eu^{III}-Lys-HOPO), G the guest (CN^-), and HG_n (n = 1, 2 or 3) the complex. Equilibrium constants K_1, K_2, K_3 are defined as:

$$K_1 = \frac{[HG]}{[H][G]}$$
$$K_2 = \frac{[HG_2]}{[HG][G]}$$
$$K_3 = \frac{[HG_3]}{[HG_2][G]}$$

The luminescence intensity increase can be described as:[2]

$$\frac{F}{F_o} = \frac{k_H[H] + k_{HG}[HG] + k_{HG_2}[HG_2] + k_{HG_3}[HG_3]}{k_{H_o}[H]_o}$$

where k is the proportionality constant for the individual luminescent species.
This equation can be further simplified to

$$\frac{F}{F_o} = \frac{a_3K_1K_2K_3[G]^3 + a_2K_1K_2[G]^2 + a_1K_1[G] + a_0}{1 + K_1K_2K_3[G]^3 + K_1K_2[G]^2 + K_1[G]}$$

where a_n (n = 0, 2, … 4) are constants.
The cumulative or overall constant can be calculated as:

$$[H] + 3[G] \rightleftharpoons [HG_3]$$
$$\beta_{13} = K_1K_2K_3 = 1.39 \times 10^8\ M^{-3}$$

Reference

1. Moore, E. G.; Xu, J.; Jocher, C. J.; Werner, E. J.; Raymond, K. N., *J. Am. Chem. Soc.* **2006,** *128* (33), 10648-10649.
2. Thordarson, P., *Chem. Soc. Rev.* **2011,** *40* (3), 1305-1323.