Electronic Supplementary Material (ESI) for Chemical Science.

Supporting Information

Long-lived lanthanide emission via a pH-sensitive and switchable LRET complex

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Reagents

All commercially available reagents were bought from Sigma Aldrich or Fisher Scientific and used without further purification. Deuterated solvents were bought from Goss Scientific.

Chromatography

Thin-layer chromatography was conducted using pre-coated Merck Kieselgel 60, F254 plates. Column chromatography was performed using silica gel and laboratory grade solvents, under mild pressure or using a Biotage automated column with Biotage SNAP KP-Sil cartridges of adequate sizes (10 g, 25 g or 50 g). Size exclusion chromatography was performed using Sephadex G-10. Dialysis was performed using a Float-A-Lyser system with a 1000 Da molecular weight cut-off cellulose membrane against distilled water.

Spectroscopy

NMR spectra were recorded on a Bruker AMX-400 spectrometer at room temperature. Coupling constants are quoted in Hz, chemical shifts in parts per million (ppm) and multiplicities are abbreviated as: s = singlet, d = doublet, t = triplet, m = multiplet where substantial overlap of resonances or complex coupling of signals did not allow for precise assignment. Broad signals were marked as br. Spectra were analysed using MestReNova software. Electrospray Time-of-Flight and high-resolution mass spectra were obtained using a Waters LCT Premier by the mass spectrometry facilities of Imperial College London, Department of Chemistry. MALDI-ToF spectra were obtained on a Micromass MALDI Micro MX spectrometer.

UV-vis absorption spectra were recorded on a Perkin Elmer 650 spectrometer and fluorescence excitation and emission spectra were obtained using a Varian Cary Eclipse spectrophotometer at 0.1 mM concentrations. Time-gated emission spectra were recorded with a 0.2 ms delay (350 nm excitation). Lanthanide-based lifetimes were measured using a Varian Cary Eclipse spectrophotometer in the phosphorescence mode in methanol/water mixtures. The integrated intensity was recorded after excitation of the samples at 350 nm. The gate time was fixed at 0.1 ms with both excitation and emission slits set at 20 nm for lifetime measurements.

A bi-exponential fit for luminescence decay was achieved using the in-built OriginPro 8 curve fitting function according to equation 2:

$$y = y_0 + A_1 \exp\left(\frac{x - x_0}{\tau_1}\right) + A_2 \exp\left(\frac{x - x_0}{\tau_2}\right) \quad \textit{(Eq S1)}$$

Values for A1 and A2 determined as 1.49 and 1.82 for pH 3 and 0.77 and 2.77 for pH 7 respectively.

For pH measurements a Jenway model 3510 pH/mV/temperature meter was used calibrated against pH 10.00, pH 7.00 and pH 4.00 buffers.

$$k_{ET} = \frac{1}{\tau_{DA}} - \frac{1}{\tau_D} \#(Eq\ S2)$$

The energy transfer rate was calculated as 740 s^{-1}, as shown in equation S2:

$$k_{ET} = \left(\frac{R_0}{R}\right)^6 \frac{1}{\tau_D} \#(Eq\ S3)$$

Synthetic procedures

Compound **1**[1]**,** rhodamine-B diethylene triamine[2] and Quin C1[3,4] were prepared as described in previous reports.

*Compound **5** and **5a***

Rhodamine B diethylene triamine (0.150 g, 0.28 mmol) and triethylamine (60 µL, 0.56 mmol) were dissolved in dichloromethane (10 mL) and cooled to 0°C. chloroacetyl chloride (0.026 g, 0.23 mmol) in dichloromethane (2 mL) was added dropwise. After 3 hours stirring at room temperature, the mixture was washed with water and dried over magnesium sulphate. Both **5** and the mono-adduct (**5a**) were isolated from the crude mixture by flash column chromatography as deep pink/purple solids (**5**: 0.080 g, 58 % and **5a**: 0.037 g, 24 %).

5a: ^{1}H-NMR (400 MHz, $CDCl_3$), δ_H (ppm): 1.17 (12H, t, $^3J_{HH}$ = 7.1 Hz), 2.86 (2H, br, m), 3.16 (2H, br, m), 3.34 (10H, br, overlaid signals q, $^3J_{HH}$ = 7.0 Hz), 3.38 (2H, br, m), 4.05 (2H, s) 6.19-6.51 (6H, m), 7.10 (1H, m), 7.49 (2H, m), 7.87 (1H, m). HRMS *m/z*: calculated: 604.3054, found: 604.3064 for $\{M+H\}^+$

5: ^{1}H-NMR (400 MHz, $CDCl_3$), δ_H (ppm): 1.16 (12H, t, $^3J_{HH}$ = 7.1 Hz), 3.03-3.41 (16H, br), 3.78 (2H, s), 3.90 (2H, s), 6.15-6.48 (6H, m), 7.12 (1H, m), 7.48 (2H, m), 7.91 (1H, m). HRMS *m/z*: calculated: 680.2770, found: 680.2777 for $\{M+H\}^+$

Further structural assignment was assessed using correlations detected by NOESY. In the doubly- substituted species, correlations between the amide hydrogen and one of the -CH_2-Cl singlet resonances (at 3.90 ppm) were observed, as seen in Figure S1. At the same time, a cross-peak between the second singlet at 3.78 ppm and the proton environments assigned to

H_b and H_c established that the two groups had substituted onto distinct amine functionalities, with the lower chemical shift singlet indicating attachment to the central amine. The ^{1}H-NMR spectrum of isolated **5a** contained only one singlet -CH_2-Cl signal at 4.05 ppm, indicating one species attached to the terminal amine was present.

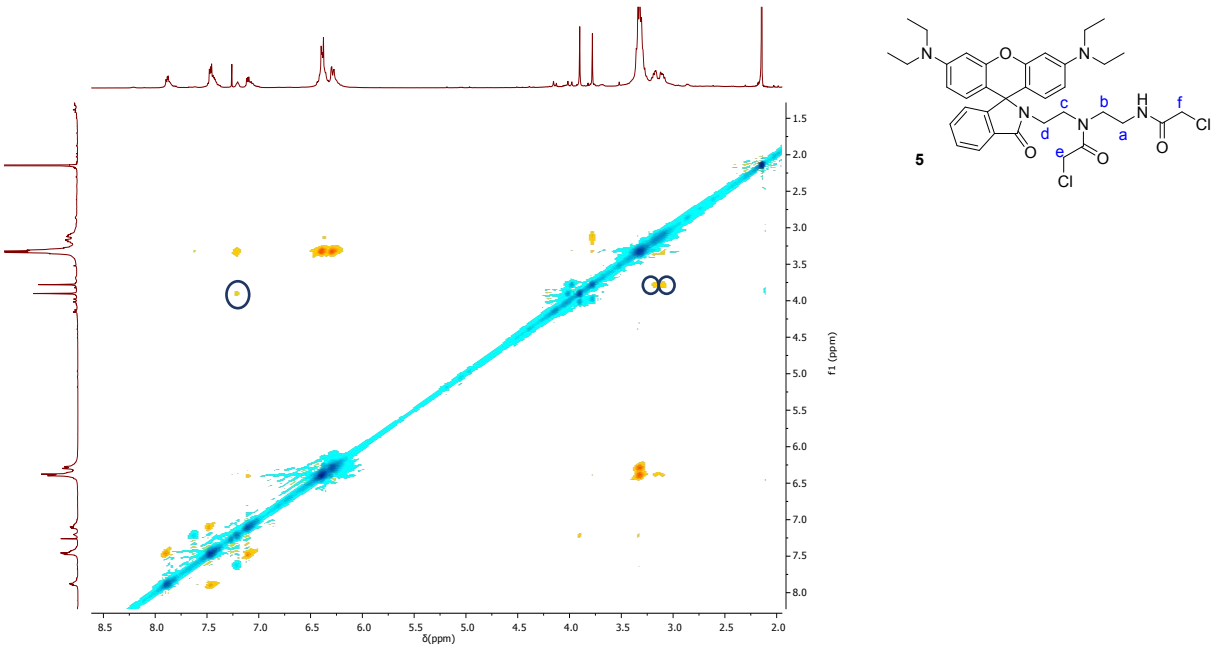

***Figure S1**: NOESY spectrum and molecular structure of **41** in $CDCl_3$ run at 400 MHz and 298 K. Relevant cross-peaks that enabled structural assignment are circled.*

*Compound **6***

Tert-butyl DO3A (0.108 g, 0.21 mmol) and potassium carbonate (0.122 g, 0.88 mmol) were suspended in acetonitrile (15 mL) and stirred for about an hour. Subsequently, **5** (0.200 g, 0.29 mmol) was added and the reaction mixture was heated at reflux overnight. Inorganic salts were filtered off and the crude mixture was purified by silica automated column chromatography eluting with a methanol/dichloromethane gradient (5 % - 50 %). **6** was isolated as a pale pink solid (**6**: 0.019 g, 79 %).

6: ^{1}H-NMR (400 MHz, $CDCl_3$), δ_H (ppm): 1.05- 3.51 (81 H, broad, overlapping resonances), 3.99 (2H, s), 6.20-6.47 (6H, m), 7.07 (1H, m), 7.45 (2H, m), 7.86 (1H, m).

^{13}C-NMR (400 MHz, $CDCl_3$) δ_H (ppm): 12.8, 28.0, 28.3, 42.8, 44.5, 45.1, 46.0, 47.6, 49.4, 51.5, 54.5, 55.9, 58.3, 65.0, 81.8, 97.9, 105.1, 108.5, 122.9, 124.1, 128.4, 128.9, 149.1, 153.2, 153.6, 166.6, 167.9, 169.8, 170.6, 172.8

HRMS *m/z*: calculated: 1158.6734, found: 1158.6766 for $\{M+H\}^+$ (~60 % relative intensity) and 1180.6617 $\{M+Na\}^+$ (100 % relative intensity)

*Compound **7***

Quin C1[3] (0.033 g, 0.07 mmol) and *N*,*N*-diisopropylethylamine (30 µL, 0.17 mmol) were stirred in acetonitrile (6 mL) for half an hour before addition of **6** (0.060 g, 0.05 mmol). The reaction mixture was heated at 65 °C for 48 hours. Inorganic salts were removed by filtration. The crude mixture was separated by silica column chromatography eluting in a methanol/ dichloromethane gradient (2 % - 50 %) to afford a pale pink solid (0.044 g, 54 %).

^{1}H-NMR (400 MHz, d_6-acetone), δ_H (ppm): 0.80- 3.94 (> 87H, br, partly obscured by solvents), 4.06 (2H, t, $^3J_{HH}$ = 6.4 Hz), 6.04-7.99 (22H, br).

HRMS *m/z*: calculated: 1567.8969, found: 1567.8879 for $\{M+H\}^+$ (~50 % relative intensity)

*Compound **8***

Compound **7** (0.010 g, 0.01 mmol) was stirred at room temperature in concentrated hydrochloric acid (1 mL) for two hours before dropwise addition of an aqueous sodium hydroxide (1 M) until the pH value was adjusted to 10. The aqueous phase was extracted using dichloromethane (3 x 5 mL), the organic fractions were combined and dried over magnesium sulphate to yield an orange solid. (0.005 g, 60 %)

^{1}H-NMR (400 MHz, d_6-acetone), δ_H (ppm): 0.72- 3.61 (> 61H, br, partly obscured by solvents), 3.91(3H, s), 4.05 (2H, t, $^3J_{HH}$ = 6.4 Hz), 6.04-7.99 (22H, br).

HRMS *m/z*: calculated: 1396.7570, found: 1396.8394 for $\{M+H\}^+$ (~30 % relative intensity)

General synthetic procedure for preparation of lanthanide complexes

The lanthanide trichloride hexahydrate salt (1.5 equivalents) was added to the macrocyclic ligand system (1 equivalent) in a small amount of water and stirred at pH ~5.5 at room temperature overnight. Subsequently, the aqueous pH was raised to 12 and the appearing lanthanide precipitate was removed by centrifugation followed by filtration. Next, the pH of the filtrate was adjusted to pH ~7, solvents were removed in vacuo and the compound was dissolved in water and purified by dialysis using a 500-1000 Dalton cut-off membrane.

Compound **2**: HRMS: calculated: 1146.3875, found: 1146.3888 for $\{M+H\}^+$

Compound **3**: HRMS: calculated: 1171.6428, found: 1171.6399 for $\{M+H\}^+$

Compound **9**: MALDI-ToF *m/z*: 1588.9 $\{M+MeOH+H\}^+$

References

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