Heterometallic *bis*-Lanthanide Complexes: Polarity-Dependent, Ratiometric Luminescence.

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Supporting Information

I. Synthetic methods

General

Fmoc amino acids, Rink Amide AM resin, and all coupling reagents used were purchased from NovaBiochem. 2-chlorotritylchloride resin was from Matrix Innovations, Inc. (Quebec, Canada). All other chemicals were purchased from Sigma-Aldrich, Acros, or Strem and were used as received. Solvents were purified by being passed through a column of alumina under an argon atmosphere and used without further purification. Preparative reverse phase (RP) HPLC was performed on a Grace Vydac C18 Protein & Peptide column (cat. # 2018TP1022) with a Waters 600 Controller; fractions were detected with a Waters 2487 Dual λ Absorbance Detector (218 and 254 nm) and data was analyzed using OpenLynx software.

Abbreviations

DOTA = 1,4,7,11-tetrazacyclododecane-N,N',N",N'"-tetraacetic acid; Dpr = 2,3-diaminopropionic acid; β-Ala = 3-aminopropionic acid; DTPA = diethylenetriamine pentaacetic acid; Trp = tryptophan; DMF = N,N-dimethylformamide; TBTU = 2-(1H-Benzotriazole-1-yl)-1,1,3,3-tetramethylaminium tetrafluoroborate; HOBt = N-hydroxybenzotriazole; Fmoc = fluorenylmethoxycarbonyl-; Boc = *t*-butoxycarbonyl-; Mtt = mono-methyltrityl-; DIEA = N,N-diisopropylethylamine; TFA = trifluoroacetic acid; TIS = triisopropylsilane; TEAA = triethylammonium acetate; MALDI-TOF = matrix-assisted laser desorption ionization / time of flight;

Synthesis

DOTA-Dpr(DTPA)-TrpNH$_2$ (5).
Rink Amide AM resin (807 mg, 0.57 mmol) was swelled/deprotected in 20% piperidine/DMF (30mL) for 30 min, and then washed alternately with DMF/CH_2Cl_2 (3x). The resin was suspended in a DMF solution containing four equivalents each of TBTU, HOBt, and Fmoc-L-Trp(Boc)-OH; eight equivalents of DIEA was added, and the resin was agitated for 90 min on a wrist-action shaker. This two-step procedure was repeated for the deprotection of Fmoc-Trp(Boc)-N(H)-RINK and the subsequent coupling of Fmoc-Dpr(Mtt)-OH, and then for the deprotection of Fmoc-Dpr(Mtt)-N(H)-Trp(Boc)-N(H)-RINK and subsequent coupling of DOTA(*t*-Bu)$_3$.[1] After extensive washing with CH_2Cl_2 to remove DMF, deprotection of the Dpr side chain (Mtt) was accomplished using TFA (1%) and (*i*-Pr)$_3$SiH (TIS) (5%) in CH_2Cl_2 for 4 cycles of 2 min each, until the color of the filtered solution was no longer yellow; the resin was washed extensively with CH_2Cl_2, then with 20% piperidine/DMF (to neutralized the TFA salt), then alternately with DMF/CH_2Cl_2. Subsequent coupling of DTPA(*t*-Bu)$_4$[2] was carried out as described above using TBTU/HOBt/DIEA activation. The resin was washed as usual, and then washed with MeOH and Et_2O and dried under high vacuum for 12 h to remove residual solvent. The peptide was cleaved from the solid support and globally deprotected by stirring the dry resin with trifluoroacetic acid (TFA) containing 2.5% H_2O and 2.5% TIS at ambient temperature for 4h, after which time the solvent was removed under reduced pressure and cold diethyl ether was added to precipitate the crude peptide. The crude suspension was extracted with a minimum volume of H_2O, and purified by preparative HPLC, yielding a white solid after lyophilization of the appropriate fractions (61mg, 10% overall). Calculated for $C_{44}H_{66}N_{12}O_{18}$: 1050.46, observed (*FAB-MS*): 1051.69 (MH^+),1073.67 (MNa^+). A RP-HPLC chromatogram of the crude and purified ligand **5** is shown below. Note that the small impurity at 25.3 min is carried through to the next step, and removed in the subseqent purification (Figure S1).

(1) DOTA(*t*-Bu)$_3$ was prepared on 2-chlorotritylchloride resin by a known procedure: Oliver, M.; Jorgensen, M. R.; Miller, A. D. *Synlett*, **2004**, *3*, 453 – 456.
(2) DTPA(*t*-Bu)$_4$ was prepared in solution by a known procedure: Arano *et al. J. Med. Chem.* **1996**, *39*(18), 3451 – 3460.

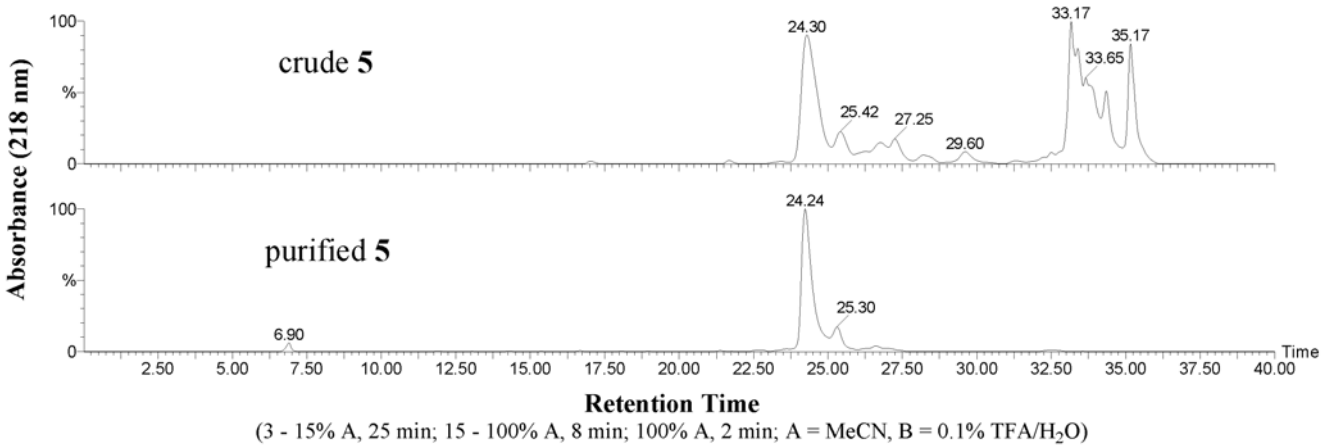

Figure S1. RP-HPLC chromatograms of **5** before and after purification.

Tb:DOTA-Dpr(DTPA)-TrpNH$_2$ (6).
To ligand **5** (25 mg, 0.024 mmol, 1.0 eq.) in 100 mM TEAA was added $TbCl_3 \cdot 6H_2O$ (42 mg, 0.113 mmol, 4.7 eq.) and the solution was stirred for 12hrs. The solution was purified by RP-HPLC using a linear gradient of acetonitrile (A) and 0.1% (v/v)TFA/H_2O(B) (3-20% A over 20min, 20-100% A over 10min). A RP-HPLC chromatogram of the crude and purified complex **6** is shown in Figure S2. The major peak was collected, lyophilized, and identified by FAB-MS to be the mono-Tb adduct (*m/z* = 1207.2) with a small amount of the bis-Tb adduct (*m/z* = 1362.3) (Figure S3). The bis-Tb adduct may result from fragment coupling in the mass spectrometer, since no HPLC peak corresponding to this species is observed, and since subsequent complexation with Eu^{3+} shows no such peak in the mass spectrum (see below).

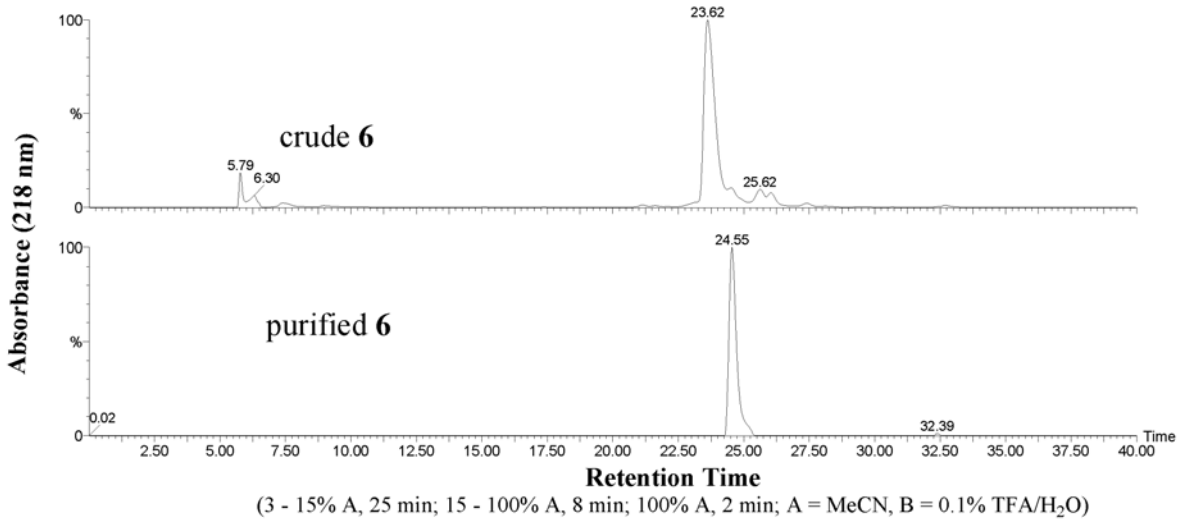

Figure S2. RP-HPLC chromatograms of **6** before and after purification.

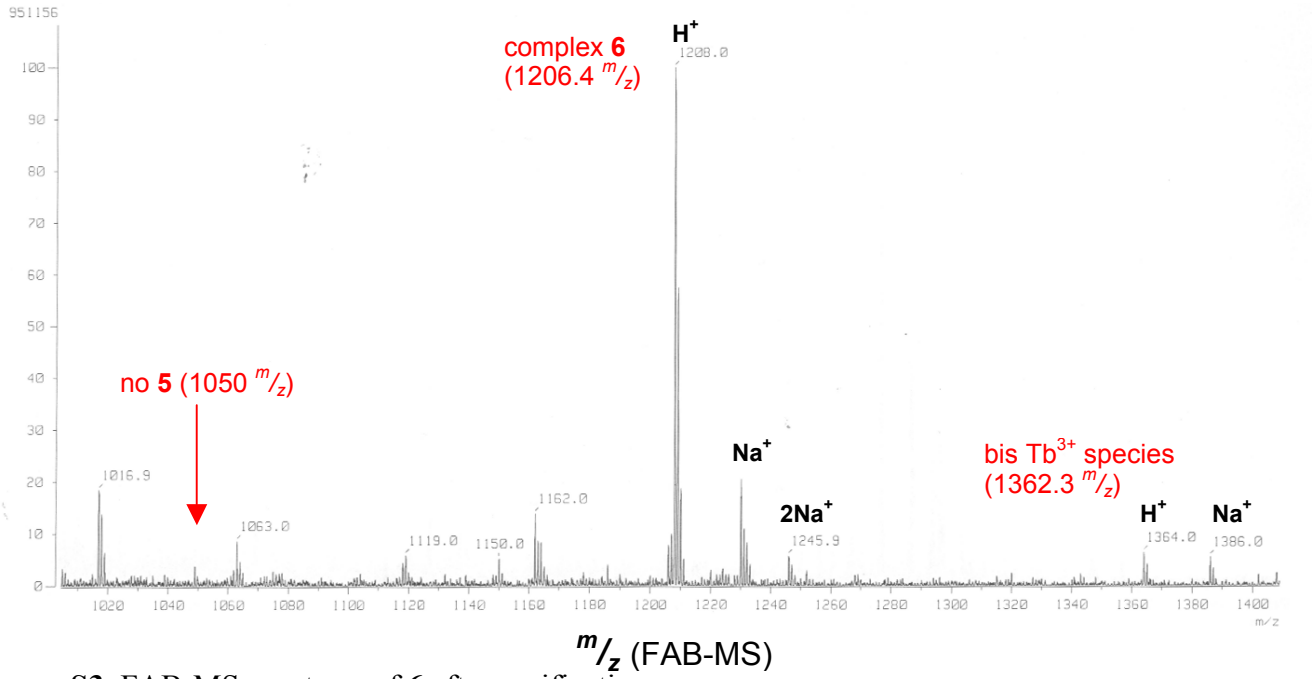

Figure S3. FAB-MS spectrum of **6** after purification.

Tb:DOTA-Dpr(Eu:DTPA)-TrpNH$_2$ (7).
To complex **6** (0.1 to 5 mM in H_2O or TEAA buffer) was added an equimolar amount of $EuCl_3$ (5 to 50 mM in H_2O). The complexation took place almost instanteously as judged by luminescence measurements and confirmed by MS; the mass spectrum showed none of the free ligand ($1050^m/_z$), or the mono-Tb^{3+} complex **6** ($1206^m/_z$), and gave an isotopic pattern consistent with Tb and Eu (Figure S4).

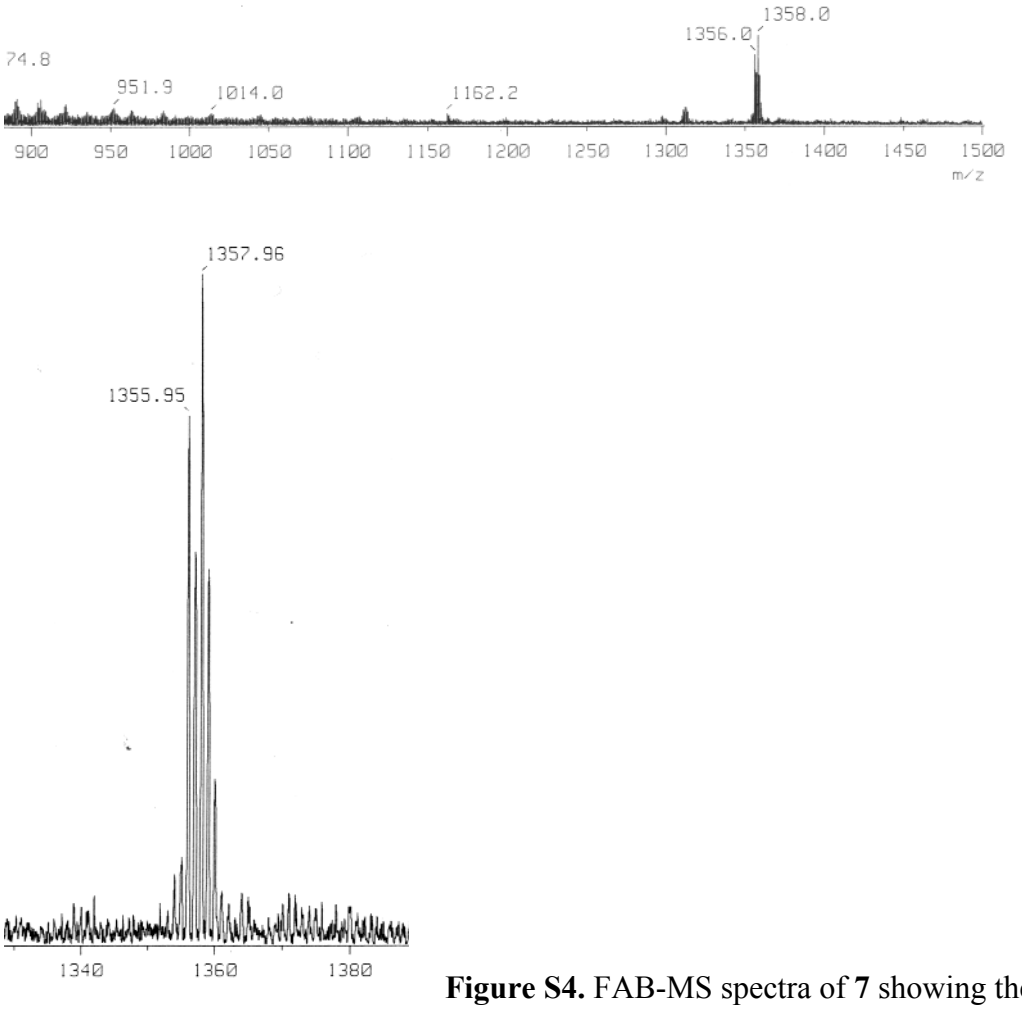

Figure S4. FAB-MS spectra of **7** showing the absence of **5** and **6**, and the appropriate isotopic pattern for Tb / Eu.

Tb:DOTA-Dpr(Gd:DTPA)-TrpNH$_2$ (8).

Bis complex **8** was prepared as described for the preparation of **7** and showed similar MS data (Figure S5). The luminescence spectrum of **8** was identical to **6**.

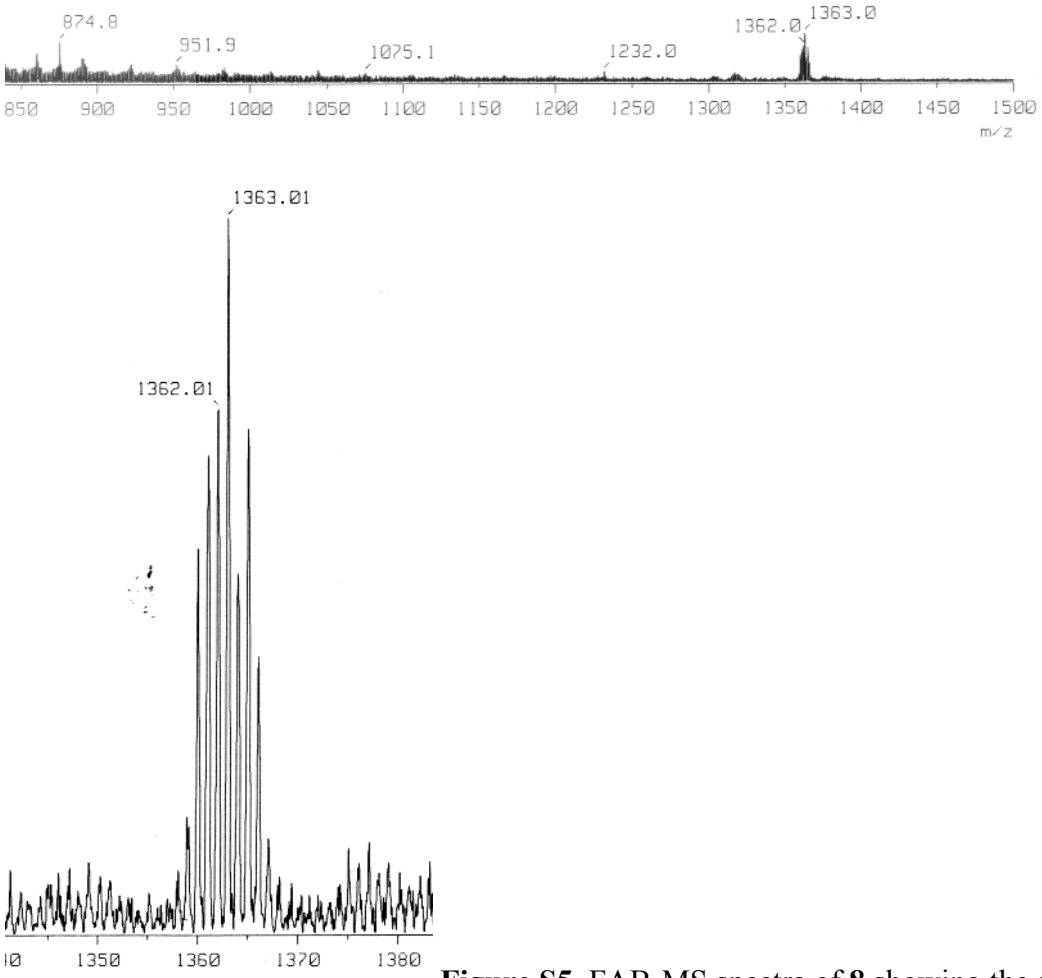

Figure S5. FAB-MS spectra of **8** showing the absence of **5** and **6**, and the appropriate isotopic pattern for Tb / Gd.

II. Photophysical Data

General

Steady state emission/excitation measurements were taken on a Jobin Yvon Fluorolog fluorescence spectrofluorometer. Samples in de-ionized water or spectrophotometrically pure MeOH, EtOH, *n*-PrOH, *i*-PrOH, or *t*-BuOH (50 μM)[3] were excited at 280 nm. For broad spectrum (350 – 650 nm) experiments (Figure 2, main text), excitation and emission slit widths were both 4nm, with HV = 750. For narrow spectrum resolution (Figure 3, main text; Figures S9 below), excitation slit width was 7 nm and emission slit width was 2 nm, with HV = 900. Scattered second order excitation light (560 nm) was avoided using a 380 nm long pass filter.[4]

Eu(III) Luminescence Sensitized by Tryptophan

As noted in the main text, the nature of the ratiometric Tb^{3+}/Eu^{3+} emission profile of compound **7** in various solvents can either be attributed to energy transfer from Tb^{3+} to Eu^{3+}, the efficiency of which is increased as the solvent polarity is decreased, or sensitization of Eu^{3+} by tryptophan, a phenomenon which is not readily observed in water, but which may occur in other solvents. The inter-metal energy transfer is precedented (see main text); to test the plausibility of the second hypothesis, we synthesized Eu:DTPA-β-Ala-TrpNH$_2$ (Figure S6, compound **9**) by standard methods analogous to those described in section I using Fmoc-β-Ala-OH in place of Fmoc-Dpr(Mtt)-OH. After purification by preparative RP-HPLC, the compound gave satisfactory FAB-MS data (expected: 649.3 $^{m}/_{z}$, measured: 650.5 $^{m}/_{z}$ (MH^+), 672.5 $^{m}/_{z}$ (MNa^+)). This compound should have the same Eu^{3+}/Trp distance as compound **7**, but has no Tb:DOTA chelate. The luminescence properties of compound **9** in the same series of alcohol solvents are shown in Figure S7.

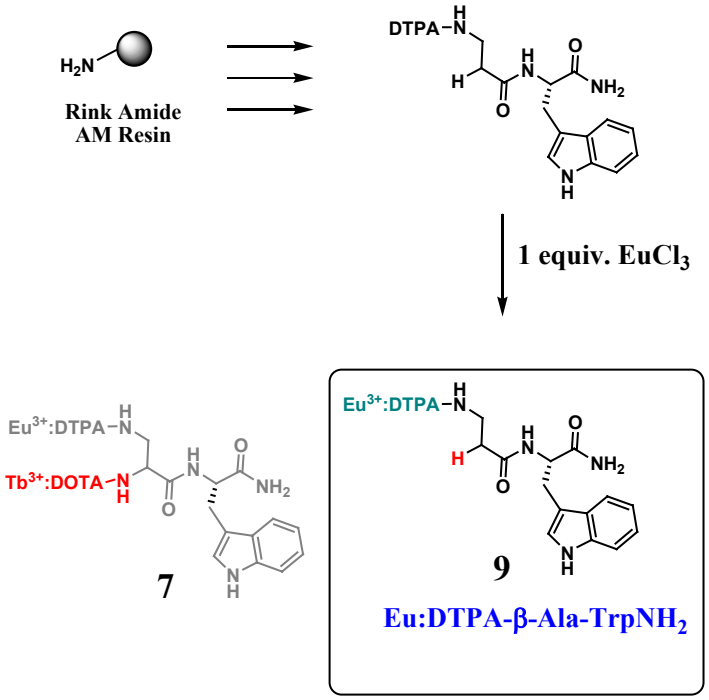

Figure S6. Control compound **9**: Eu:DTPA-β-Ala-TrpNH$_2$.

(3) Samples were prepared by dilution of 5mM aqueous stock solutions (5 μL) in alcohol (495 μL) and contain ~1% H_2O.
(4) As a result of this filter, tryptophan emission is truncated such that the maximum is shifted to 390nm.

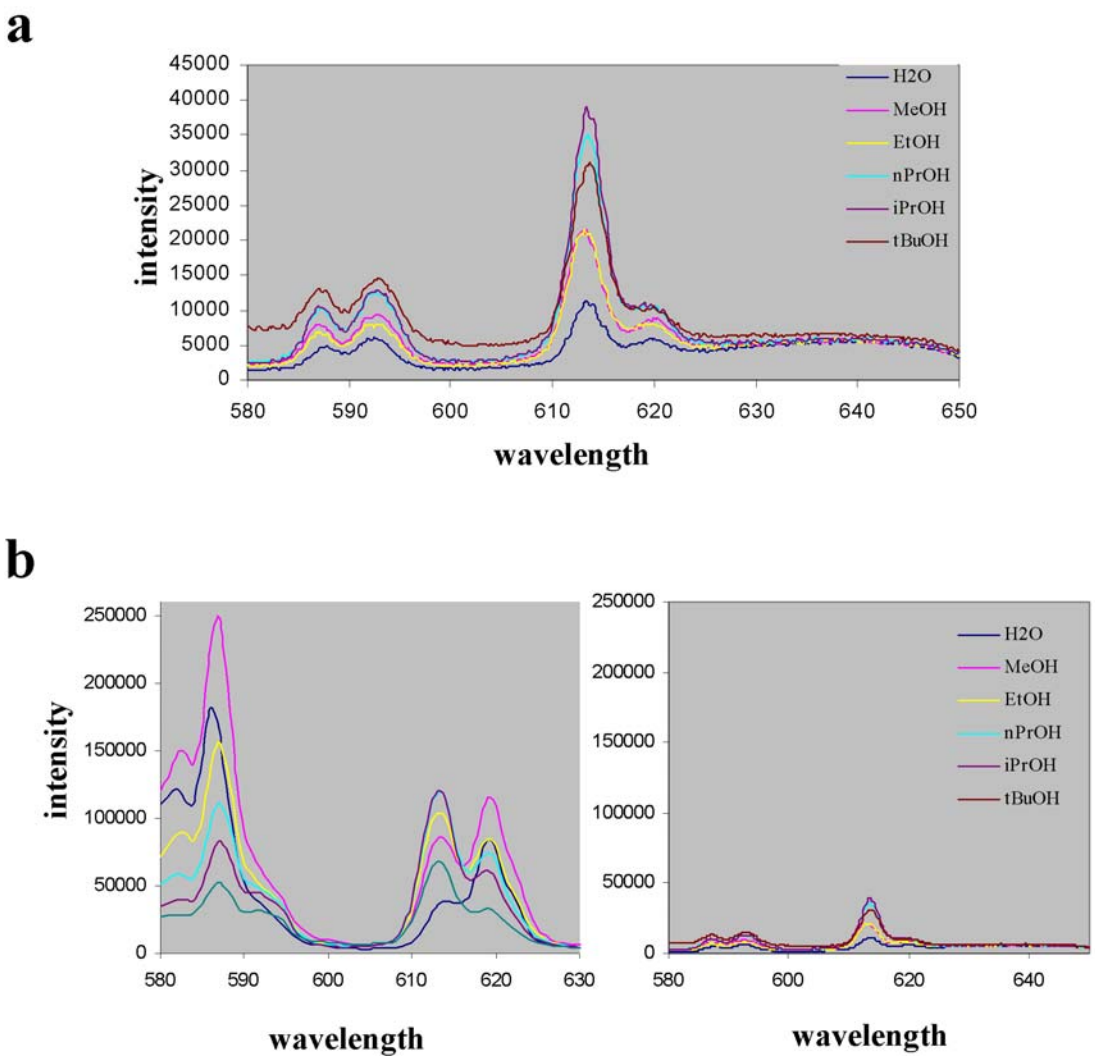

Figure S7. Emission spectra of **9** in various solvents. (a) 50 μM, 25°C, 280 nm excitation. (b) The same spectrum rescaled (right panel) for comparison to compound **7** (left panel).

Althought the same trend is observed for this complex, the magnitude of the luminescence for compound **9** is weaker than that observed for compound **7**. Given that energy transfer efficiency varies with $1/r^6$ where r is the distance between Trp and Eu^{3+}, it is most likely that Trp→Eu^{3+} sensitization accounts for *some*, but not *all*, of the observed Eu^{3+} luminescence. The rest is mostly likely due to Tb^{3+}→Eu^{3+} energy transfer.

Direct Excitation of Eu(III)

The contribution of unsensitized Eu^{3+} luminescence (*i.e.* direct excitation of Eu^{3+}) was measured under the same conditions used to measure compound **7** (Figure 2b, main text) and Eu:DOTA-Trp-SerNH_2 (Figure S7, above) by exciting $EuCl_3$ (50 μM) or $[Eu:DTPA]^{-1}$ (50 μM) at 280 nm in various solvents. Under these conditions, Eu^{3+} emission was negligible. The peak shape was ambiguous because of the low signal, but a conservative estimate of the contribution of unsensitized Eu^{3+} emission to the emission observed for compound **7** would be 1 – 2%.