Displacement assay detection by a dimeric lanthanide luminescent ternary Tb(III)-cyclen complex: High selectivity for of phosphate and nitrate anions

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Electronic Supporting Information

General Experimental Details: All chemicals were purchased from Sigma-Aldrich Ireland Ltd., Acros Organics, and TCI Ltd., and were used without further purification, unless otherwise stated. Deuterated solvents for NMR analysis were all obtained from Apollo Scientific. Dry solvents were prepared in accordance with standard procedures described by Vogel, with distillation prior to each use.[1] Chromatographic columns were performed manually using either silica gel 60 (230-240 mesh ASTM) or aluminium oxide (activated, neutral, Brockman I STD grade, 150 mesh). Chromatographic columns were also run on a Teldyne Isco Combiflash Companion automatic machine using pre-packed silica or alumina columns. Thin-layer chromatography (TLC) was conducted using both Merck Kiesegel 60 F_{254} silica plates and Polygram Alox N/UV_{254} aluminium oxide plates, and observed by UV light or developed in an iodine chamber. Melting Points were determined using an Electrothermal IA900 digital melting point apparatus. NMR spectra were recorded using either a Bruker Spectrospin DPX-400 instrument, operating at 400.13 MHz for ^{1}H NMR and 100.6 MHz for ^{13}C NMR, or a Bruker AV-600 instrument, operating at 600.1 MHz for ^{1}H NMR and 150.2 MHz for ^{13}C NMR. All NMR spectra were measured at 293 K. Chemical shifts are expressed in parts per million (ppm or δ) relative to the non-deuterated solvent peak and, for ^{1}H NMR spectra, are reported alongside the number of protons, splitting pattern, coupling constant where applicable, and proton assignment (in that order). Multiplicities are abbreviated as follows; singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), and broad (br). Electro mass spectra were recorded using a Mass Lynx NT V 3.4 on a Waters 600 controller connected to a 996 photodiode array detector, with HPLC grade carried solvents. Accurate molecular weights were determined by a peak-matching method, using leucine enkephaline (H-Tyr-Gly-Gly-Phe-Leu-OH) as the standard reference (*m/z* = 556.2771); all

accurate mass were reported within ± 5 ppm of the expected mass. Infrared spectra were recorded on a Perkin Elmer Spectrum One FT-IR spectrometer fitted with a universal ATR sampling accessory.

UV-vis absorption and luminescence spectroscopy: Using a 1.0 cm path length quartz cell, UV-vis absorption and luminescence spectra were recorded using a Varian CARY 50 and a Varian Cary Eclipse spectrophotometer, respectively. The solvents utilised were of either HPLC or spectrophotometric grade. The concentrations of the ligands and complexes analysed were the same for both UV-vis and luminescence titrations. All measurements were performed at 298 K, with this maintained using a thermostated unit block. Baseline correction measurements were employed for all UV-vis spectra, where the blank was a sample of the solvent system in which the titration was performed. The parameters used for the UV-vis absorption and luminescence titration studies reported are listed in Table S1.

Table S1. Luminescence settings for titrations involving ligand **2** and **Eu.1/Tb.1** (including anion displacement assays).

Mode: Phosphorescence	**Excitation:** 279 nm	**Scan:** 570 – 720 nm (Eu) 450 – 700 nm (Tb)
Exc. Slit: 20 nm	**Em. Slit:** 1.5 nm	**Voltage:** Variable
Flash Count: 1	**Gate Time:** 5 ms	**Delay Time:** 0.1 ms
Averaging Time: 0.1 s	**Total Decay Time:** 0.02 s	**Data Interval:** 1 nm

Luminescence lifetime measurements: Phosphorescence lifetimes of the Eu($^5D_0$) excited state were measured in methanol and deuterated methanol with a Varian Carey Eclipse Fluorimeter in time-resolved mode at 298 K. All lifetime values were obtained from an average of six independent measurements, each recorded with a different gate time ranging from 0.020 – 0.045 ms. The fluorimeter settings for the lifetime measurements are shown below in Table S2.

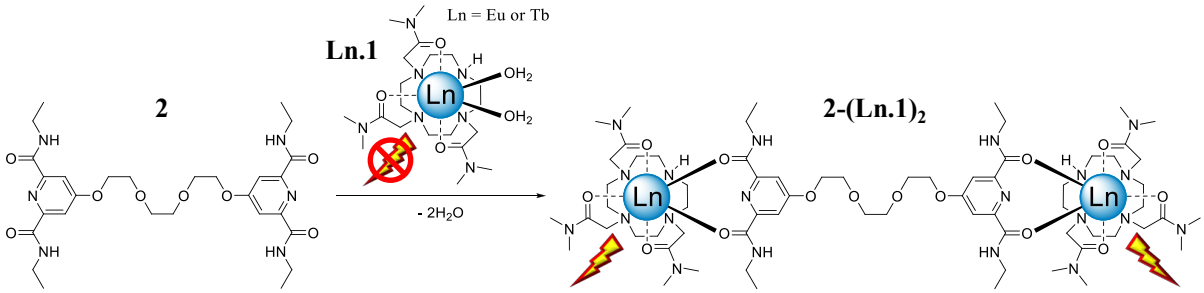
Table S2. Luminescence settings for the lifetime measurements of **2-(Eu.1)$_2$** in methanol and deuterated methanol.

Excitation: 281 nm	**Emission:** 616 nm	**Delay Time:** 0.01 ms
Exc. Slit: 20 nm	**Em. Slit:** 20 nm	**Gate Time:** Variable
Flash Count: 1	**Total Decay Time:** 20 ms	**Delay Time:** 0.1 ms

The number of water molecules directly coordinated to the Eu(III) centre (q value) was determined according to the equation developed by Parker *et al.*;

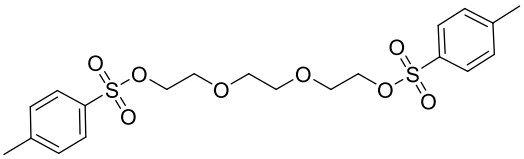
$$q^{Eu(III)} = 2[(1/\tau_{O\text{-}H} - 1/\tau_{O\text{-}D}) - 0.25 - 0.075x] \pm 0.5 \qquad \textbf{Equation 1}$$

where $\tau_{O\text{-}H}$ is the lifetime in methanol, $\tau_{O\text{-}D}$ is the lifetime in deuterated methanol, and x is the number of N-H oscillators directly bound to the metal.



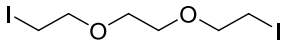
Scheme S1. Schematic illustration of the formation of the ternary assembly **2-(Ln.1)$_2$** (where Ln = Eu or Tb), accompanied by the *switching on* of the metal-centred luminescence.

Di-(tosyl-ethoxy)ethane (4)[2]



Triethylene glycol (4.84 g, 4.31 mL, 32.23 mmol, 1.00 eq.) in THF (50 mL) was added to a solution of NaOH (4.12 g, 103.03 mmol, 3.20 eq.) in H_2O (30 mL) at 0 °C. 4-toluenesulfonyl chloride (14.75 g, 77.37 mmol, 2.40 eq.) was added to the solution and the mixture was allowed to warm to room temperature. The reaction was then left stirring for 72 h, after which time, the THF was removed under reduced pressure to give a white solid. This was dissolved in $CHCl_3$ before washing twice with 1M NaOH and once with H_2O. The organic phase was dried over $MgSO_4$ and the solvent evaporated. Recrystallisation of the crude product from MeOH gave **4** as a white crystalline solid (9.31 g, 20.30 mmol, 63% yield). m.p. 82 – 84 °C; HRMS (*m*/*z*) (ES^+) Calculated for $C_{20}H_{26}O_8S_2Na$ *m*/*z* = 481.0967 $[M + Na]^+$. Found *m*/*z* = 481.0962; ^1H NMR (400 MHz, $CDCl_3$) δ_H: 7.79 (4H, d, *J* = 8.1 Hz, Ts-H), 7.34 (4H, d, *J* = 8.1 Hz, Ts-H), 4.14 (4H, t, *J* = 4.8 Hz, CH_2), 3.65 (4H, t, *J* = 4.8 Hz, CH_2), 3.53 (4H, s, CH_2), 2.45 (6H, s, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C: 145.00, 133,17, 129.99, 128.11, 70.86, 69.34, 68.91, 21.79; IR υ_{max} (cm^{-1}): 2897, 1596, 1457, 1352, 1261, 1175, 1132, 1071, 982, 911, 850, 815, 779, 664, 584, 553.

Di-(iodo-ethoxy)ethane (5)[2]



Compound **4** (5.02 g, 10.95 mmol, 1.00 eq.) and NaI (4.92 g, 32.82 mmol, 3.00 eq.) were refluxed in acetone (50 mL) for 16 h. The white precipitate which formed was removed by suction filtration and the solvent was removed under reduced pressure. The residue was then dissolved in $CHCl_3$ and washed twice with H_2O, brine and dried over $MgSO_4$. The solvent was removed under reduced pressure to give **5** as a reddish brown oil (3.56 g, 9.62 mmol, 88% yield); HRMS (*m*/*z*) (ES^+) Calculated for $C_6H_{12}O_2I_2Na$ *m*/*z* = 392.8825 $[M + Na]^+$. Found *m*/*z* = 392.8822; ^1H NMR (400 MHz, $CDCl_3$) δ_H: 3.76 (4H, t, *J* = 7.0 Hz, CH_2), 3.66 (4H, s, CH_2), 3.26 (4H, t, *J* = 7.0 Hz, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C: 72.10, 70.30, 3.06; IR υ_{max} (cm^{-1}): 2864, 1722, 1460, 1412, 1350, 1262, 1188, 1168, 1089.

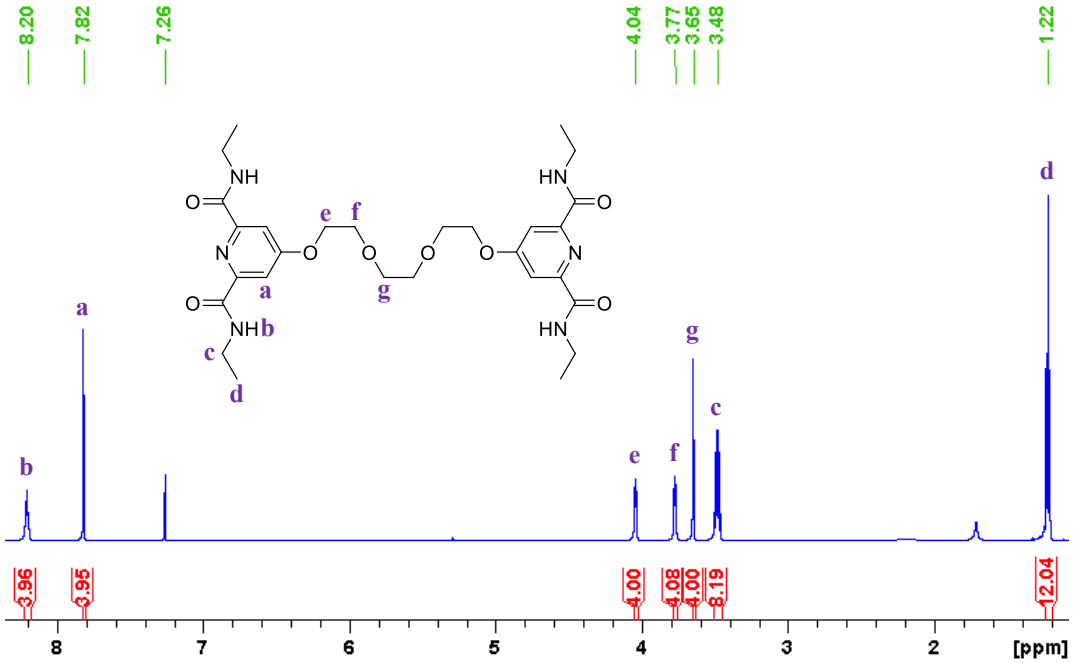


Figure S1. ^{1}H NMR (600 MHz, $CDCl_3$) spectrum of ligand **2**.

Experimental and data fitting details for ternary self-assembly titrations: Photophysical titrations of ligand **2** with complex **Ln.1** (where Ln = Eu or Tb) were performed in CH_3OH. In each case, a stock solution (3 mL; *ca*. 1×10^{-5} M) of ligand **2** was prepared and titrated against known aliquots of a standard solution of either **Eu.1** or **Tb.1**. After each addition, UV-vis absorption and phosphorescence spectra were recorded, allowing 5 min every time for equilibration. Fitting of the phosphorescence spectral changes was later accomplished, using the non-linear least squares regression program SPECFIT® by conforming to the set of equilibria equations shown below (**Equation 2** – **4**), used to define the self-assembly process.

$$\mathbf{2} + \mathbf{Ln.1} \overset{K_{1:1}}{\rightleftharpoons} \mathbf{2\text{-}Ln.1} \qquad \textbf{Equation 2}$$

$$\mathbf{2\text{-}Ln.1} + \mathbf{Ln.1} \overset{K_{1:2}}{\rightleftharpoons} \mathbf{2\text{-}(Ln.1)_2} \qquad \textbf{Equation 3}$$

$$\beta_{1:2} = K_{1:1} \times K_{1:2} \qquad \textbf{Equation 4}$$

Figures:

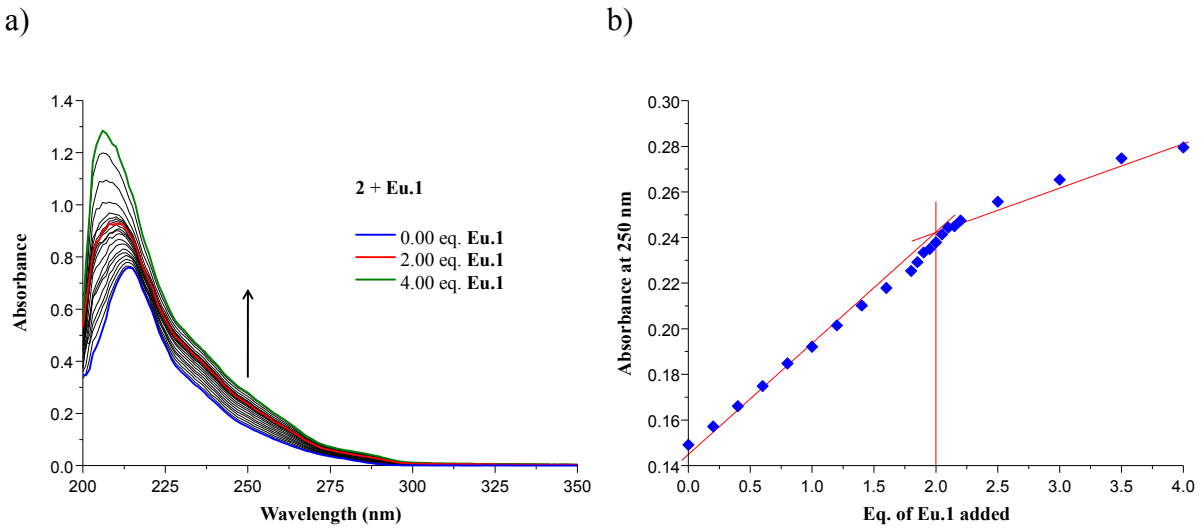


Figure S2. a) Changes in the absorption spectra of **2** (*ca*. 1×10^{-5} M) upon titrating with complex **Eu.1** (0.00 → 4.00 eq.) in MeOH at 298 K. b) Binding isotherm for the titration measured at 250 nm.

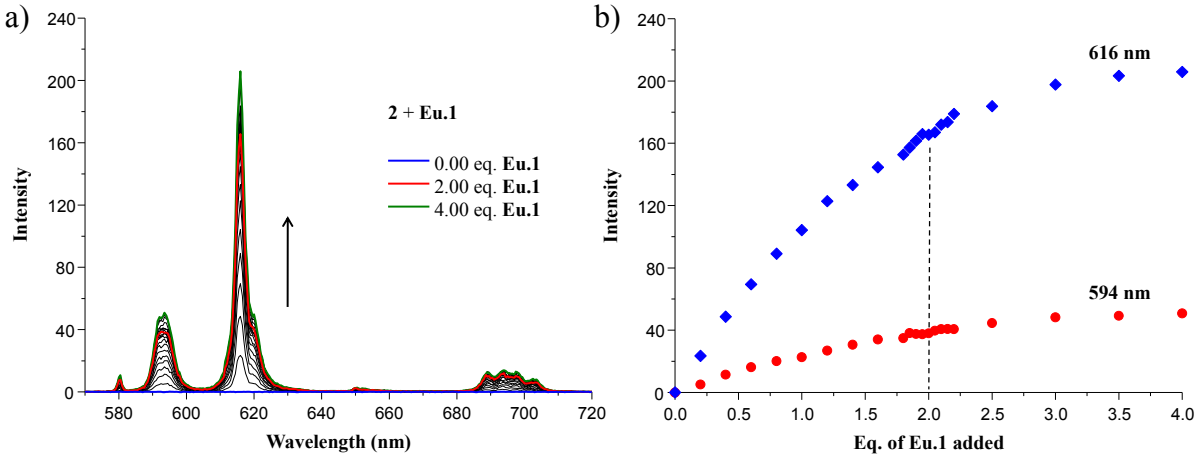


Figure S3. a) Evolution of the phosphorescence emission in the titration of **2** (*ca*. 1×10^{-5} M) with complex **Eu.1** (0.00 → 4.00 eq.) in MeOH at 298 K (λ_{exc} = 279 nm). b) Binding isotherms for the titration measured at 594 nm and 616 nm.

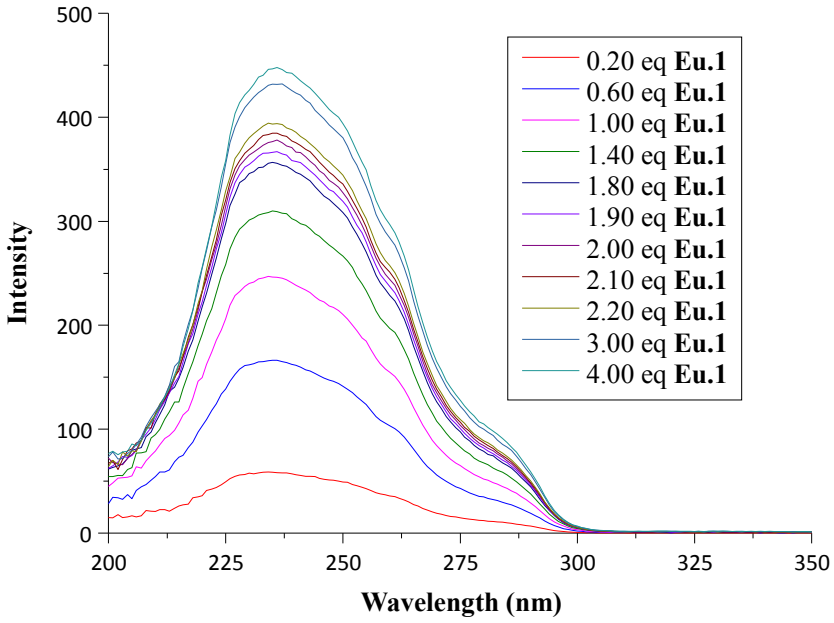


Figure S4. Phosphorescence excitation spectra measured at 616 nm for the titration of **2** (*ca.* 1×10^{-5} M) with complex **Eu.1** (0.00 → 4.00 eq.) in MeOH at 298 K.

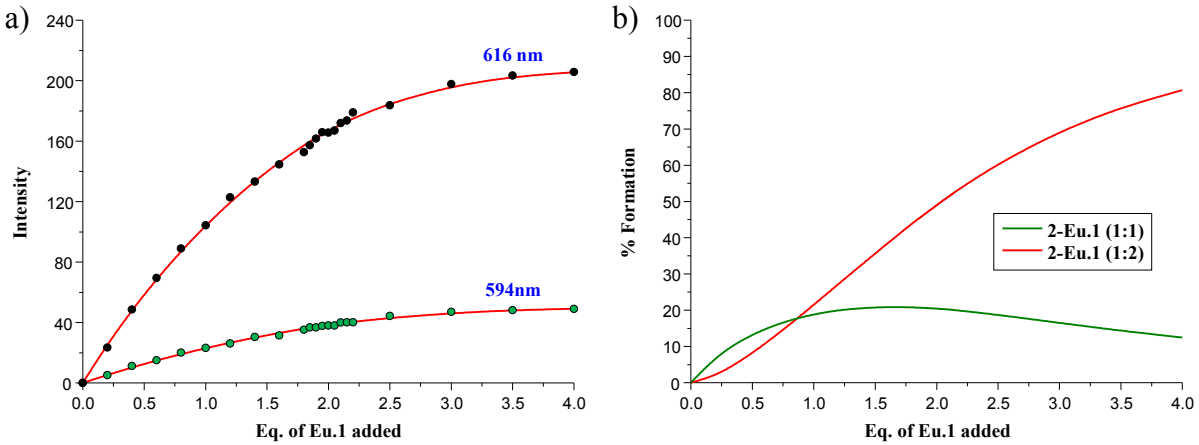


Figure S5. a) Experimental binding isotherms for the phosphorescence titration of **2** (*ca.* 1×10^{-5} M) with complex **Eu.1** in MeOH at 298 K (λ_{exc} = 279 nm) and their corresponding fit by means of SPECFIT. b) Speciation-distribution diagram obtained from the fit.

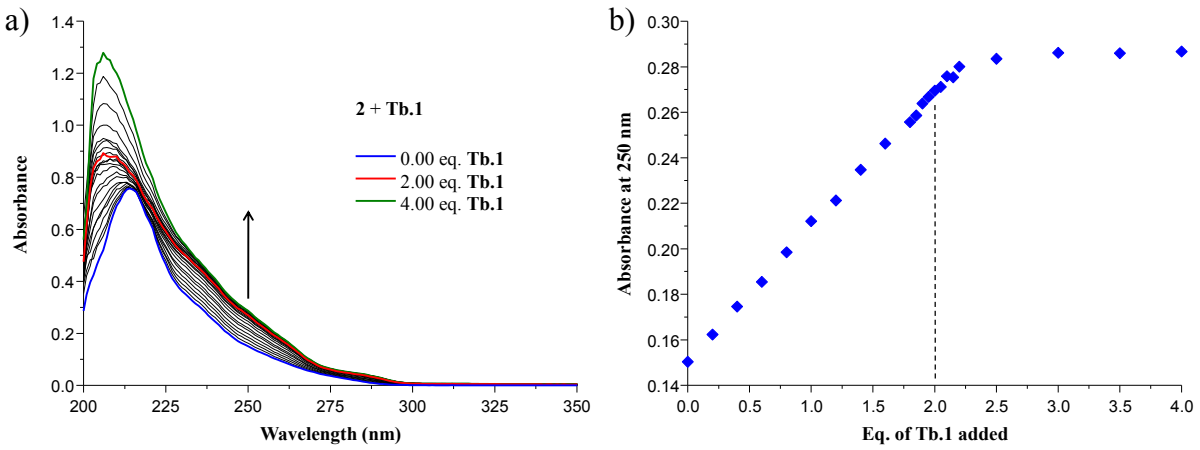


Figure S6. a) Changes in the absorption spectra of **2** (*ca.* 1×10^{-5} M) upon titrating with complex **Tb.1** (0.00 → 4.00 eq.) in MeOH at 298 K. b) Binding isotherm for the titration measured at 250 nm.

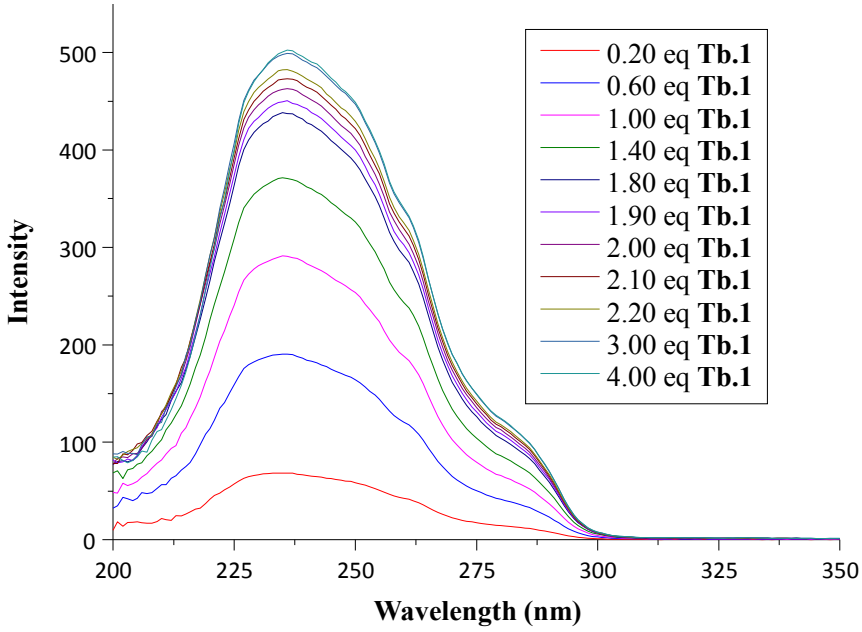


Figure S7. Phosphorescence excitation spectra measured at 545 nm for the titration of **2** (*ca.* 1×10^{-5} M) with complex **Tb.1** (0.00 → 4.00 eq.) in MeOH at 298 K.

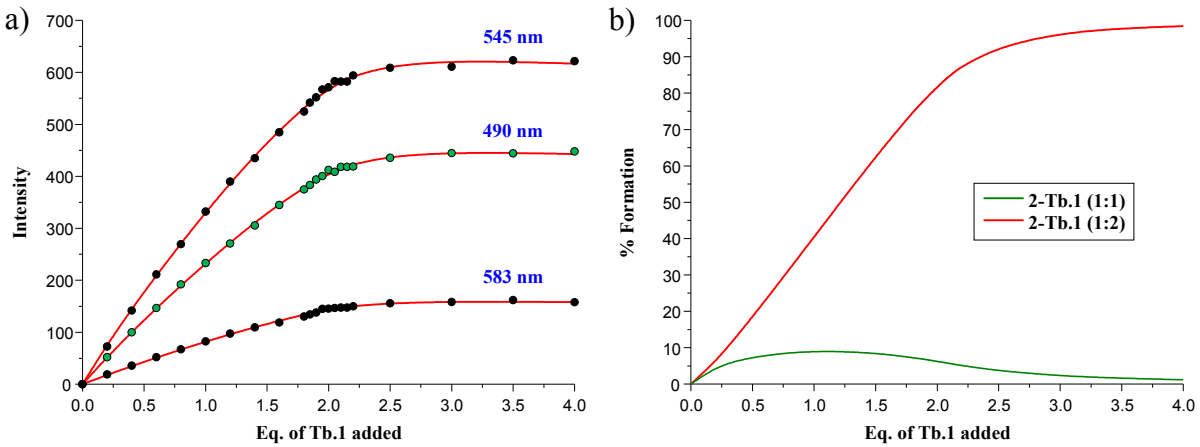


Figure S8. a) Experimental binding isotherms for the phosphorescence titration of **2** (*ca*. 1 × 10^{-5} M) with complex **Tb.1** in MeOH at 298 K (λ_{exc} = 279 nm) and their corresponding fit by means of SPECFIT. b) Speciation-distribution diagram obtained from the fit.

Table S3. Lifetime measurements of **2-(Eu.1)$_2$** in methanol (MeOH and MeOD) with their corresponding rate constants (k) and calculated q value. Each lifetime is an average of six measurements and fit to a mono-exponential decay.

Complex	Solvent	$\tau_{O\text{-}H}$ (ms)	$\tau_{O\text{-}D}$ (ms)	$k_{O\text{-}H}$ (ms^{-1})	$k_{O\text{-}D}$ (ms^{-1})	q (± 0.5)
2-(Eu.1)$_2$	Methanol	0.48 ± 0.01	0.56 ± 0.03	2.08	1.79	− 0.07

Experimental and data fitting details for luminescent displacement assays: Displacement assays were carried out in CH_3OH, corresponding to the titrations probing ternary complex formation. For each assay, the 1:2 (L:C) Tb(III) system was prepared *in situ* by adding 2 eq. of **Tb.1** to a stock solution (3mL; *ca*. 1×10^{-5} M) of ligand **2**. Following self-assembly, dimeric **2-(Tb.1)$_2$** was titrated against known volumes of a standard solution of anion. The anions were used as their tetrabutylammonium (TBA) salts and included; acetate ($CH_3CO_2^-$), chloride (Cl^-), nitrate (NO_3^-), phosphate ($H_2PO_4^-$), and pyrophosphate ($HP_2O_7^{2-}$). Upon each addition, UV-vis absorption and phosphorescence spectra were recorded, giving 5 min for equilibration every time. When possible, fitting of the phosphorescence data was completed using either linear or non-linear (SPECFIT®) regression analysis. Linear fits were made in accordance with the Stern-Volmer equation (**Equation 5**), where $[A^-]$ represents the anion concentration, K is the formation constant, I is the emission intensity, and I_0 is the emission intensity when $[A^-] = 0$.

$$I_0/I = 1 + K[A^-] \qquad \textbf{Equation 5}$$

Note: It is imperative to state that all references to *anion eq.* are made relative to the cell concentration (*ca*. 1×10^{-5} M) of **2**.

Figures:

a) b)

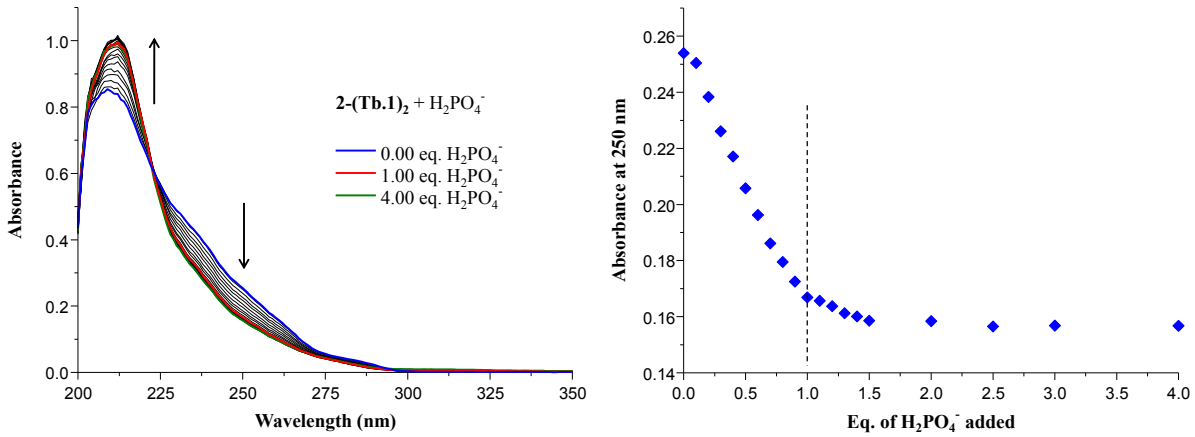


Figure S9. a) Changes in the absorption spectra of **2-(Tb.1)$_2$** ([**2**] = *ca.* 1 × 10^{-5} M) upon titrating with $H_2PO_4^-$ (0.00 → 4.00 eq.) in MeOH at 298 K. b) Binding isotherm for the titration measured at 250 nm.

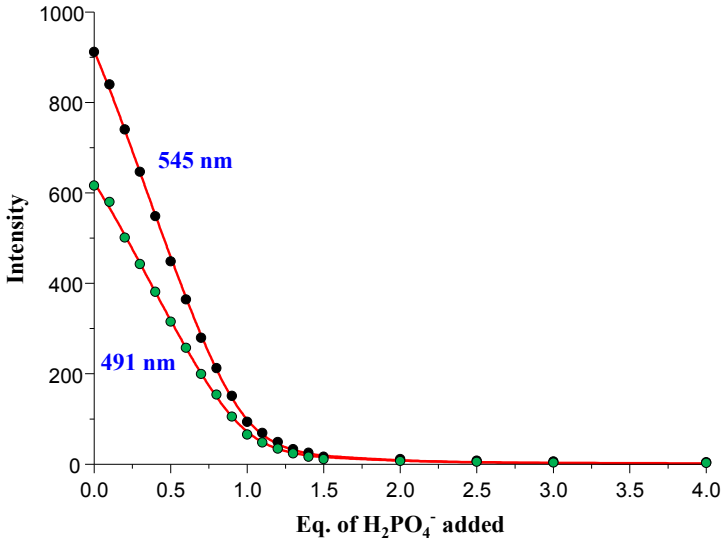


Figure S10. Experimental binding isotherms for the phosphorescence titration of **2-(Tb.1)$_2$** ([**2**] = *ca.* 1 × 10^{-5} M) with $H_2PO_4^-$ in MeOH at 298 K (λ_{exc} = 279 nm) and their corresponding fit by means of SPECFIT.

Table S4. Summary of the binding constants obtained through data fitting for the phosphorescence titration of **2-(Tb.1)$_2$** ([**1**] = *ca.* 1×10^{-5} M) with $H_2PO_4^-$ in MeOH, with percentage composition of the 1:2 and 1:1 (A:C) species at 1 eq. of $H_2PO_4^-$.

Anion	Complex	log $\beta_{1:2}$	log $\beta_{1:1}$	% of 1:2 (A:C) at 1 eq. of $H_2PO_4^-$	% of 1:1 (A:C) at 1 eq. of $H_2PO_4^-$
$H_2PO_4^-$	**2-(Tb.1)$_2$**	13.82 ± 0.04	6.59 ± 0.10	80	6

Figures:

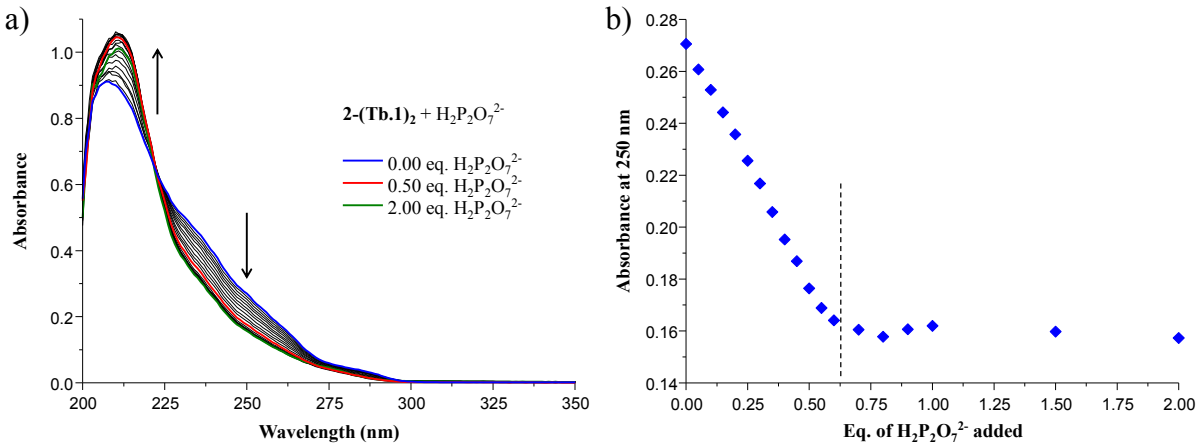


Figure S11. a) Changes in the absorption spectra of **2-(Tb.1)$_2$** ([**2**] = *ca.* 1 × 10^{-5} M) upon titrating with $H_2P_2O_7^{2-}$ (0.00 → 2.00 eq.) in MeOH at 298 K. b) Binding isotherm for the titration measured at 250 nm.

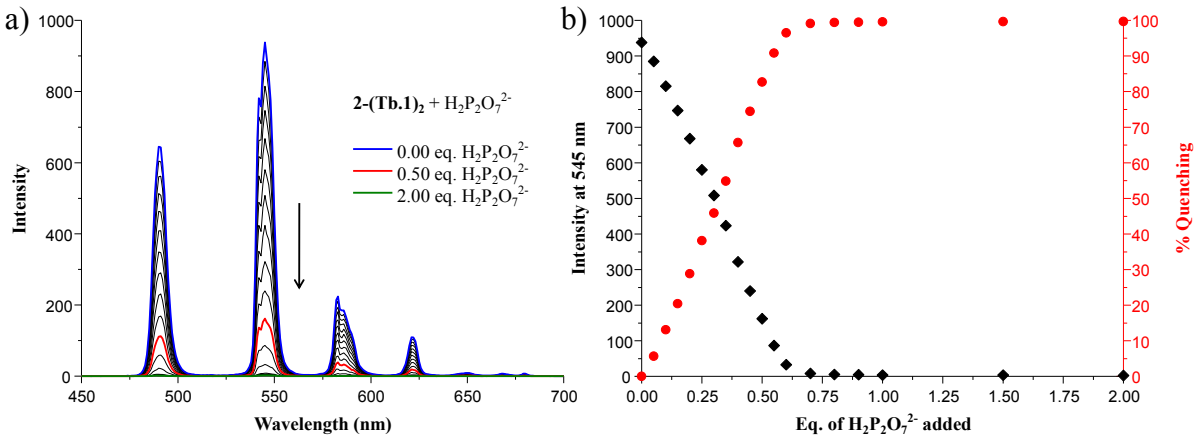


Figure S12. a) Changes in the phosphorescence spectra of **2-(Tb.1)$_2$** ([**2**] = *ca.* 1 × 10^{-5} M) upon titrating with $H_2P_2O_7^{2-}$ (0.00 → 2.00 eq.) in MeOH at 298 K (λ_{exc} = 279 nm). b) Changes in emission intensity (black) and percentage quenching (red) at 545 nm.

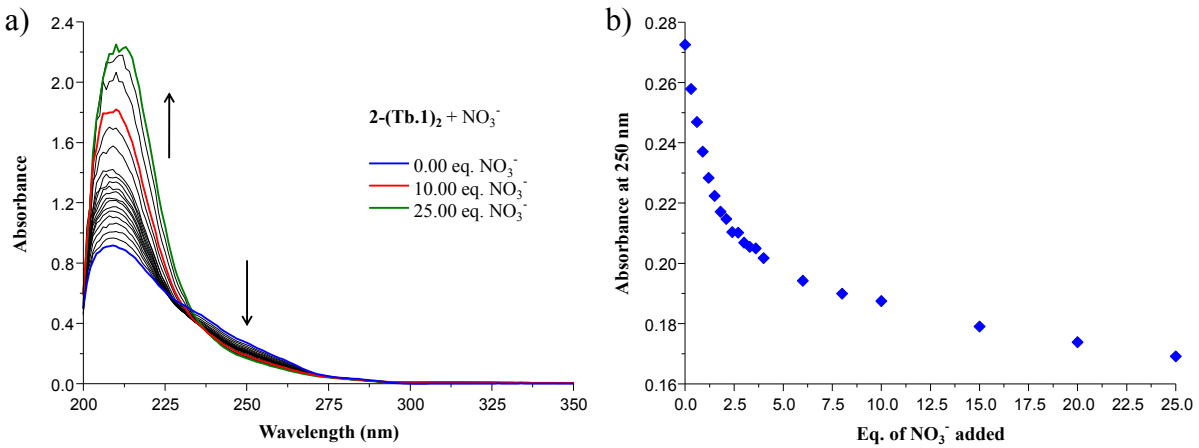


Figure S13. a) Changes in the absorption spectra of **2-(Tb. 1)$_2$** ([**2**] = *ca.* 1 × 10^{-5} M) upon titrating with NO_3^- (0.00 → 25.00 eq.) in MeOH at 298 K. b) Binding isotherm for the titration measured at 250 nm.

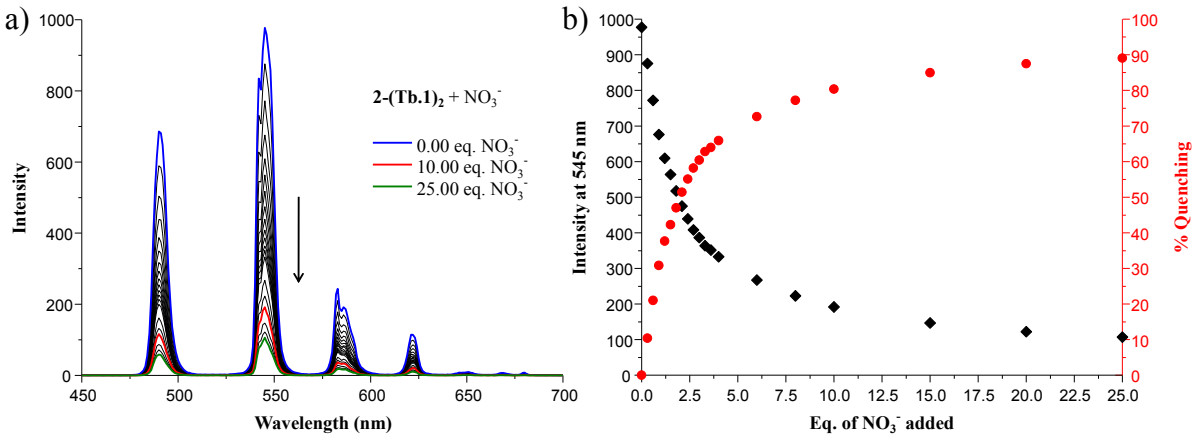


Figure S14. a) Changes in the phosphorescence spectra of **2-(Tb.1)$_2$** ([**2**] = *ca.* 1 × 10^{-5} M) upon titrating with NO_3^- (0.00 → 25.00 eq.) in MeOH at 298 K (λ_{exc} = 279 nm). b) Changes in emission intensity (black) and percentage quenching (red) at 545 nm.

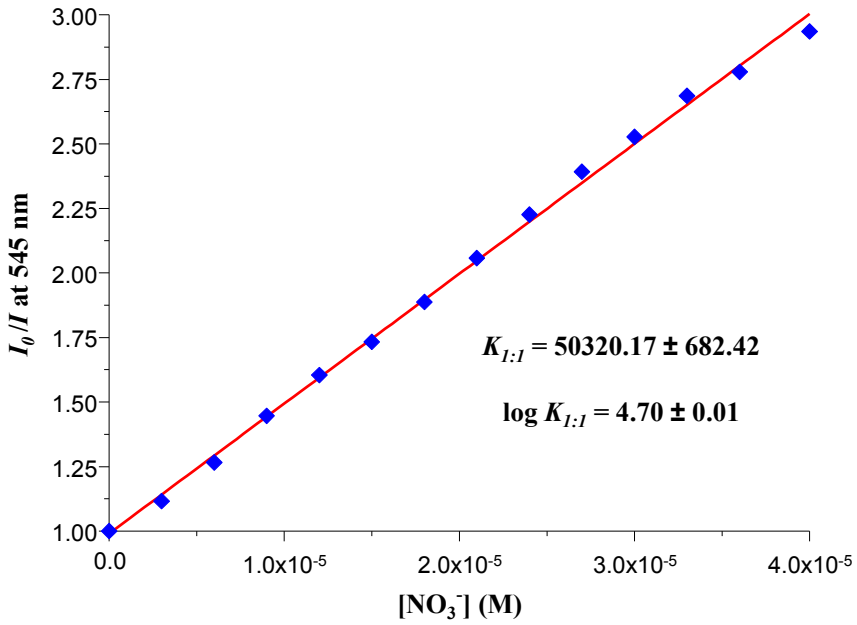


Figure S15. Stern-Volmer linear fit of the changes in emission intensity at 545 nm for the phosphorescence titration of **2-(Tb.1)$_2$** ([**2**] = *ca.* 1 × 10^{-5} M) with NO_3^- (0.00 → 25.00 eq.) in MeOH at 298 K (λ_{exc} = 279 nm).

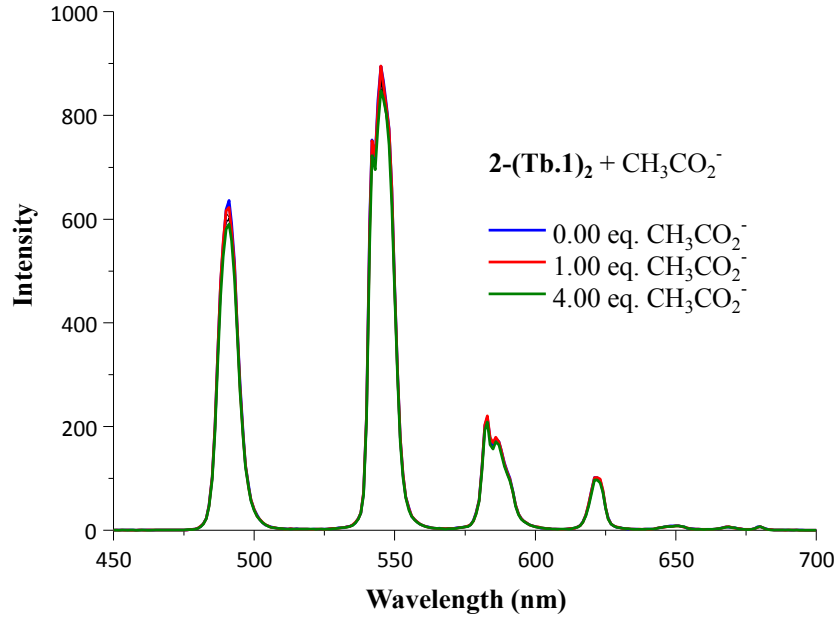


Figure S16. Changes in the phosphorescence spectra of **2-(Tb.1)$_2$** ([**2**] = *ca.* 1 × 10^{-5} M) upon titrating with $CH_3CO_2^-$ (0.00 → 4.00 eq.) in MeOH at 298 K (λ_{exc} = 279 nm).

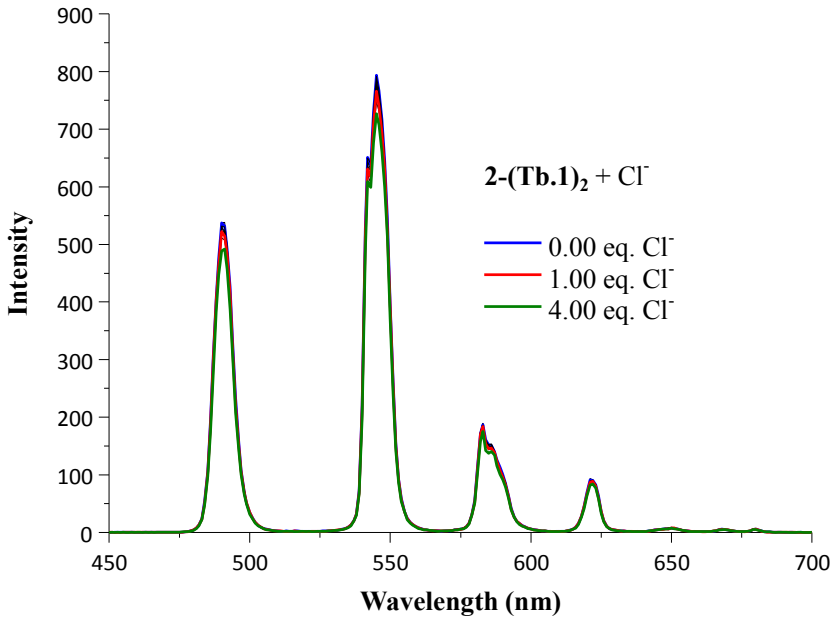


Figure S17. Changes in the phosphorescence spectra of **2-(Tb.1)$_2$** ([**2**] = *ca*. 1 × 10^{-5} M) upon titrating with Cl^- (0.00 → 4.00 eq.) in MeOH at 298 K (λ_{exc} = 279 nm).

(1) Vogel, A. I.; Tatchell, A. R.; Furnis, B. S.; Hannaford, A. J.; Smith, P. W. G. *Vogel's Textbook of Practical Organic Chemistry*; 5th ed.; Prentice Hall: New York, **1996**.

(2) Arnaud, A.; Belleney, J.; Boué, F.; Bouteiller, L.; Carrot, G.; Wintgens, V. *Angew. Chem. Int. Ed.* **2004**, *43*, 1718.

(3) Vandevyver, C. D. B.; Chauvin, A.-S.; Comby, S.; Bünzli, J.-C. G. *Chem. Commun.* **2007**, 1716.