

Femtomolar Ln(III) affinity in a peptide-based ligand containing unnatural chelating amino acids

*Agnieszka Niedźwiecka, Federico Cisnetti, Colette Lebrun, Pascale Delangle**

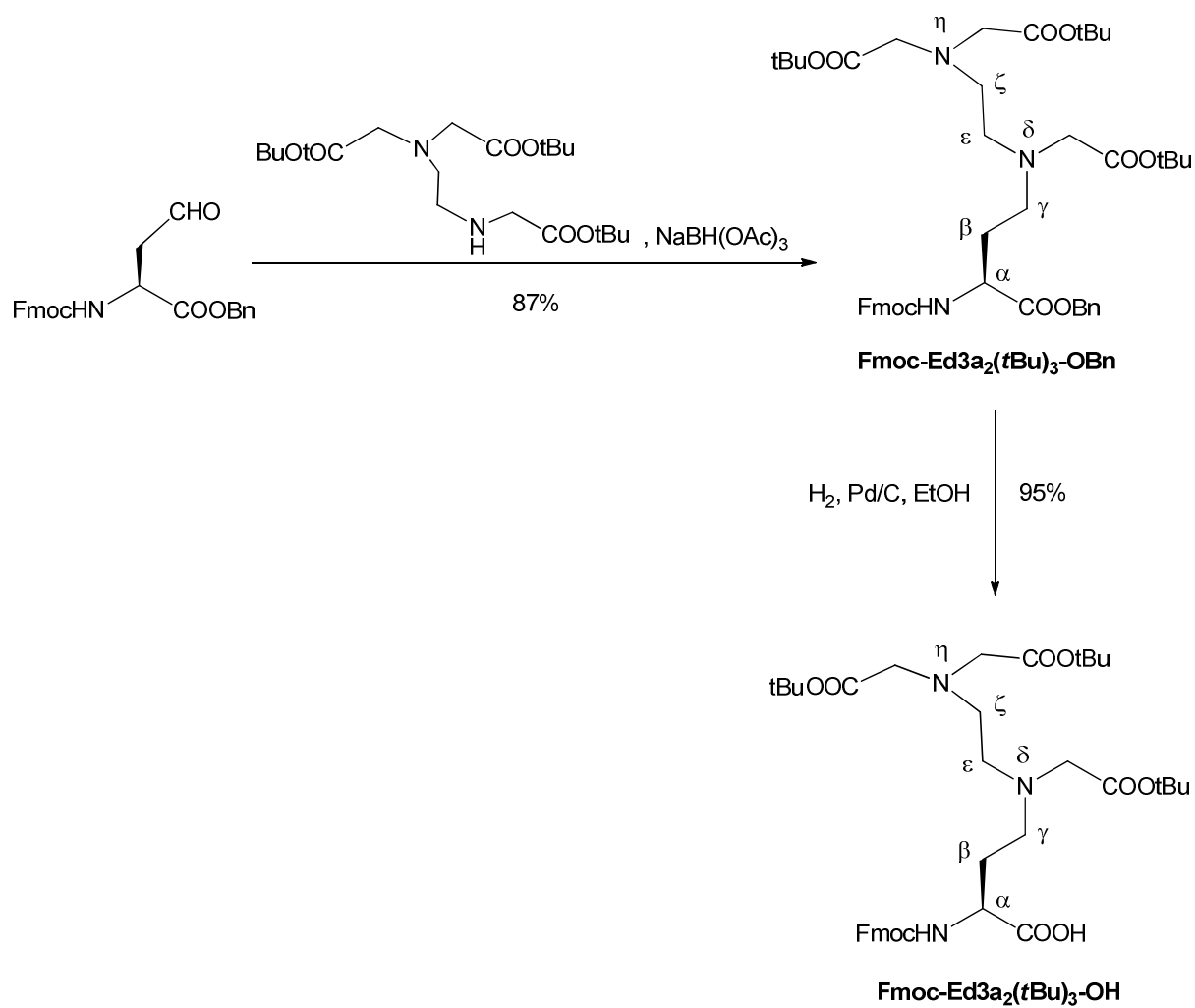
*INAC, Service de Chimie Inorganique et Biologique (UMR_E 3 CEA UJF)
Commissariat à l'Energie Atomique
17 Rue des Martyrs, 38054, Grenoble Cedex, France
Fax: +33 4 3878 5090; Tel: +33 4 3878 9822
E-mail: pascale.delangle@cea.fr*

Supplementary information

Contents:

1. Synthesis of unnatural amino acid Fmoc-Ed3a ₂ (<i>t</i> Bu) ₃ -OH	p S2
2. Supplementary NMR tables	p S3
3. Electrospray Mass Spectrometry (ES-MS) table	P S4
4. CD titration of P^{HD2} with Tb ³⁺	p S5
5. Titrations of P^{HD5} . Formation of polymetallic adducts	p S6
6. Tb-centered luminescence in P^{HD2} titration	p S8
7. Determination of Tb ³⁺ luminescence lifetimes	p S9
8. pH-metric titration	p S10

1. Synthesis of the unnatural amino acid Fmoc-Ed3a₂(tBu)₃-OH.



Scheme S1. Synthesis of Fmoc-protected **Ed3a₂(tBu)₃-OH** amino acid, with atom numbering.

2. Supplementary NMR tables.

Table S1. ^1H NMR (500 MHz) chemical shifts (δ / ppm) for \mathbf{P}^{HD2} in $\text{H}_2\text{O}/\text{D}_2\text{O}$ v/v 9/1 at 298K, 1.88 mM, pH = 2.4. Signals assigned by COSY, TOCSY and ROESY 2D experiments.

Residue	HN	H α	H β	Others
Trp(1)	8.31	4.50	3.24, 3.14	H ϵ_1 : 10.14, H ϵ_3 : 7.54, H ζ_2 : 7.48, H δ_1 : 7.27, H η_2 : 7.23, H ζ_3 : 7.13 Ac: 2.02
Ed3a ₂ (2)	8.48	4.51	2.31, 2.11	H γ : 3.36 (m, 2H), H ϵ , H ζ : 3.35 (s, 4H), N δ CH ₂ COOH: 3.92, 3.82
Pro(3)		4.10	2.20, 1.89	H γ : 1.89 (m, 2H), H δ : 3.41, 3.19
Gly(4)	8.41	3.91(2H)		
Ada ₂ (5)	7.91	4.42	2.05, 1.82	H γ : 3.08, 3.01
Gly(6)	8.48	3.90 (2H)		CONH ₂ : 7.47, 7.04
				Ed3a ₂ (2) N η CH ₂ COOH, Ada ₂ (5) N δ CH ₂ COOH: 3.93 (s, 4H), 3.77 (s, 4H)

Table S2. ^1H NMR (500 MHz) chemical shifts (δ / ppm) for \mathbf{P}^{HD5} in $\text{H}_2\text{O}/\text{D}_2\text{O}$ v/v 9/1 at 298K, 1.43 mM, pH = 2.4. Signals assigned by COSY, TOCSY and ROESY 2D experiments.

Residue	HN	H α	H β	Others
Trp(1)	8.25	4.50	3.28, 3.16	H ϵ_1 : 10.15, H ϵ_3 : 7.54, H ζ_2 : 7.49, H δ_1 : 7.30, H η_2 : 7.23, H ζ_3 : 7.14 Ac: 2.02
Ada ₂ (2)	8.53	4.45	2.33, 2.14	H γ : 3.35 (m, 2H), N δ CH ₂ COOH: 3.86 (s, 4H)
Pro(3)		4.10	2.19, 1.87	H γ : 1.87 (2H), H δ : 3.41, 3.20
Gly(4)	8.40	3.90 (2H)		
Ed3a ₂ (5)	7.88	4.52	1.94, 1.73	H γ : 2.95 (m, 2H), H ϵ , H ζ : 3.73 (s, 4H), N η CH ₂ COOH, N δ CH ₂ COOH: 3.86 (s, 3H), 3.56 (s, 3H)
Gly(6)	8.40	3.90 (2H)		CONH ₂ : 7.47, 7.04

3. Electrospray Mass Spectrometry (ES-MS) table

Table S3. Significant peaks observed in the ES⁺-MS and ES⁻-MS spectra of 60 μ M peptide solution in AcONH₄ buffer (20 mM, pH = 7.0) in the presence of EuCl₃ (0.5, 1.0 and 2.0 equiv.).^a

			ES ⁺ -MS	ES ⁻ -MS			
P^{HD2}	+0.5 Eu ³⁺	1140.3	[P^{HD2} +Eu ³⁺ -2H ⁺] ⁺	988.3	[P^{HD2} -H] ⁻		
		570.8	[P^{HD2} +Eu ³⁺ -H ⁺] ²⁺	493.8	[P^{HD2} -2H] ²⁻		
				1138.2	[P^{HD2} +Eu ³⁺ -4H ⁺] ⁻		
	+1.0 Eu ³⁺			568.7	[P^{HD2} +Eu ³⁺ -5H ⁺] ²⁻		
		1140.3	[P^{HD2} +Eu ³⁺ -2H ⁺] ⁺	1138.2	[P^{HD2} +Eu ³⁺ -4H ⁺] ⁻		
		570.8	[P^{HD2} +Eu ³⁺ -H ⁺] ²⁺	568.7	[P^{HD2} +Eu ³⁺ -5H ⁺] ²⁻		
	+2.0 Eu ³⁺	^b		^b			
	P^{HD5}	+0.5 Eu ³⁺	1140.3	[P^{HD5} +Eu ³⁺ -2H ⁺] ⁺	988.3	[P^{HD5} -H] ⁻	
			570.8	[P^{HD5} +Eu ³⁺ -H ⁺] ²⁺	493.8	[P^{HD5} -2H] ²⁻	
				1138.2	[P^{HD5} +Eu ³⁺ -4H ⁺] ⁻		
+1.0 Eu ³⁺				568.7	[P^{HD5} +Eu ³⁺ -5H ⁺] ²⁻		
		1140.3	[P^{HD5} +Eu ³⁺ -2H ⁺] ⁺	1138.2	[P^{HD5} +Eu ³⁺ -4H ⁺] ⁻		
		570.8	[P^{HD5} +Eu ³⁺ -H ⁺] ²⁺	568.7	[P^{HD5} +Eu ³⁺ -5H ⁺] ²⁻		
+2.0 Eu ³⁺		644.6	[P^{HD5}+2Eu³⁺-4H⁺]²⁺ ^b	378.8	[P^{HD5} +Eu ³⁺ -6H ⁺] ³⁻ ^b		
		835.1	[2(P^{HD5}-4H)+3Eu³⁺+2K⁺]³⁺				
^a Sodium (Δ = +22/z) and potassium (Δ = +38/z) adducts were also detected. ^b The same peaks as 1.0 equiv.							

^a Sodium ($\Delta = +22/z$) and potassium ($\Delta = +38/z$) adducts were also detected. ^b The same peaks as 1.0 equiv.

4. CD titration of P^{HD2} with Tb^{3+}

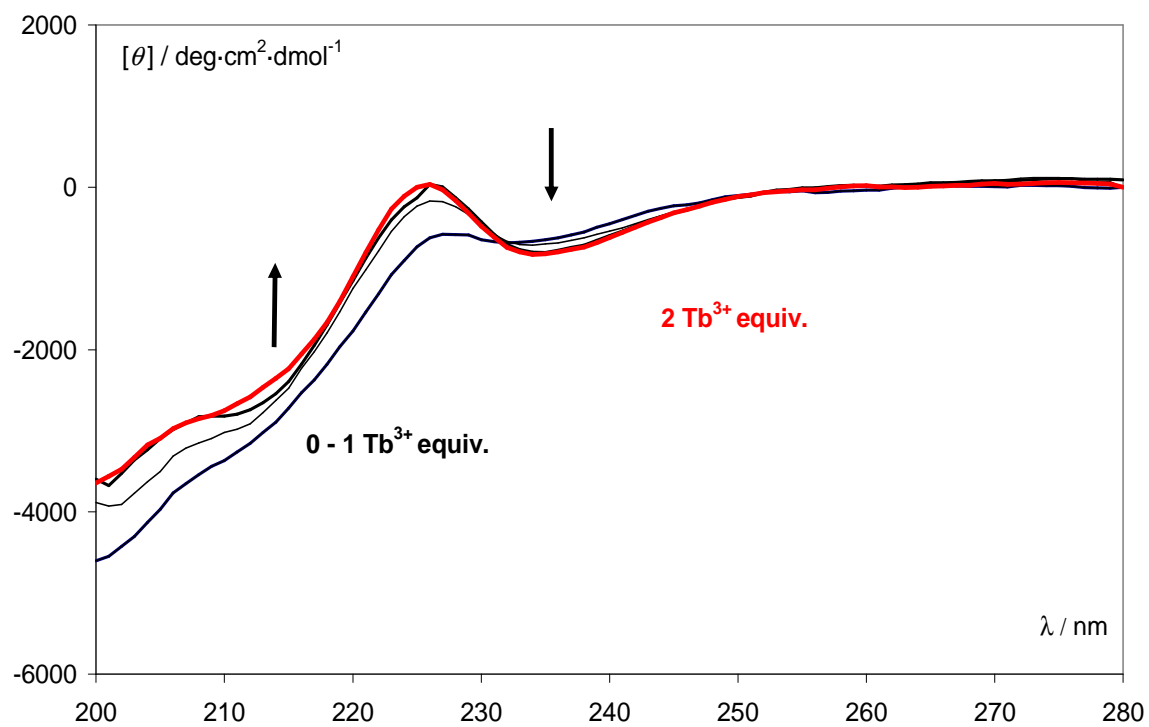


Figure S1. Circular dichroism titration of 20 μM water solutions of P^{HD2} with $Tb(\text{OTf})_3$ at $\text{pH} = 7.0$. Titration with 0.33 until 1 equiv. (black curves) and 2 equiv (red curve).

5. Titrations of \mathbf{P}^{HD5} . Formation of polymetallic adducts

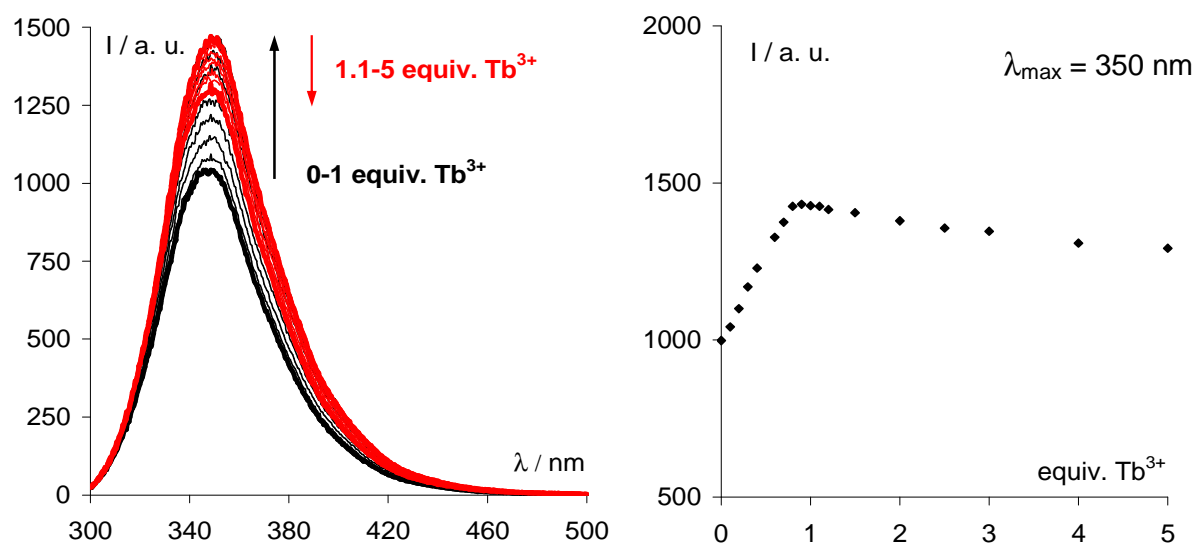


Figure S2. Titrations of \mathbf{P}^{HD5} (45 μM) with TbCl_3 in HEPES buffer (10 mM, pH = 7.0). Evolution of tryptophan fluorescence upon excitation at 280 nm.

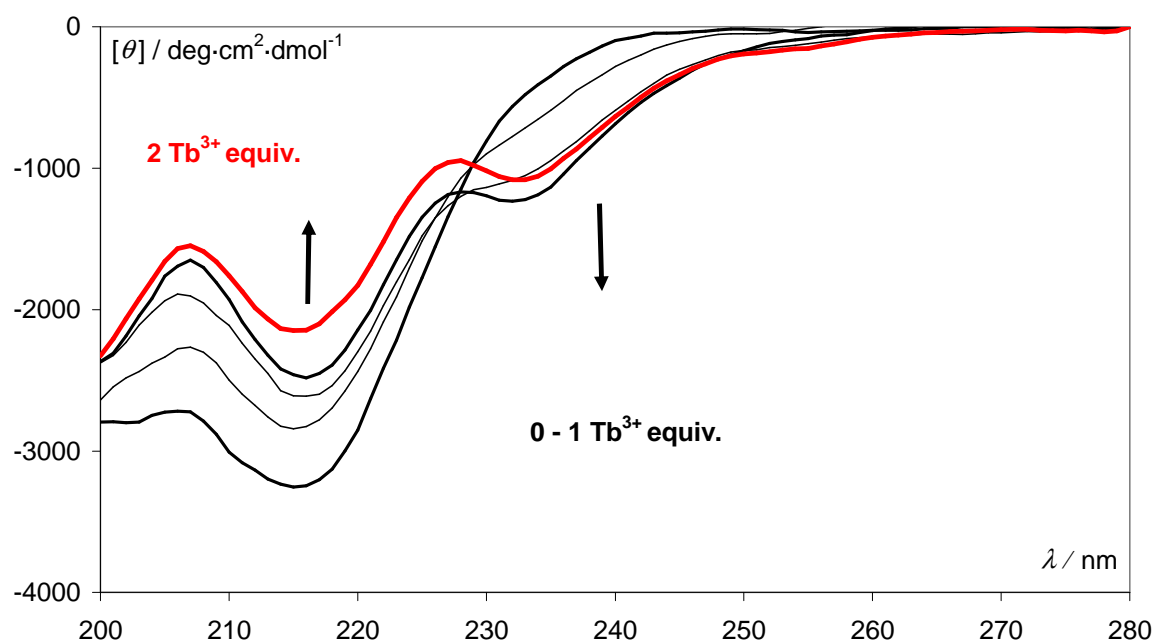


Figure S3. Circular dichroism titration of 20 μM water solutions of \mathbf{P}^{HD5} with $\text{Tb}(\text{OTf})_3$ at pH = 7.0. Titration with 0.33 until 1 equiv. (black curves) and 2 equiv (red curve).

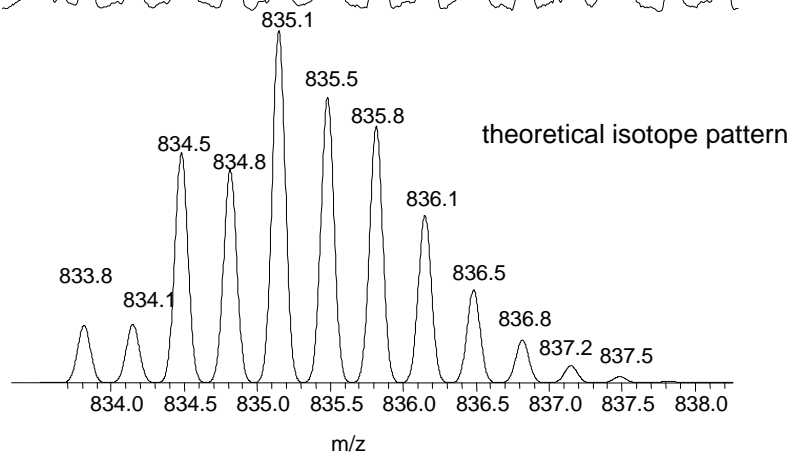
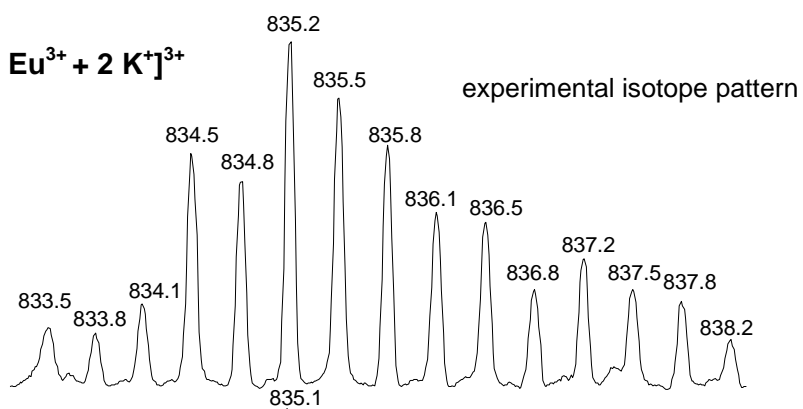
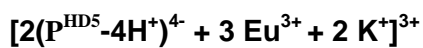
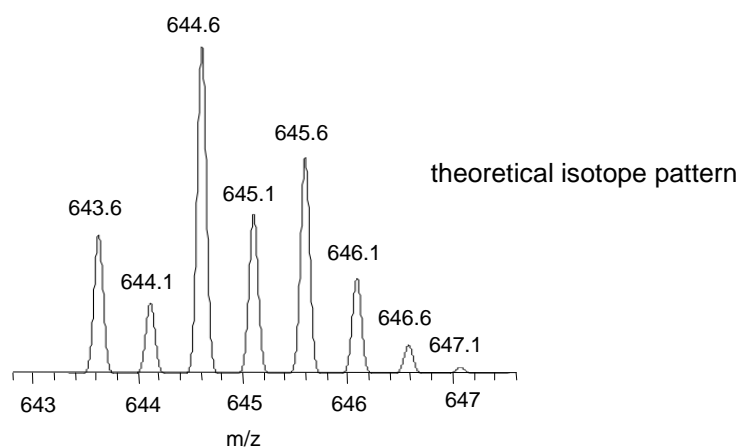
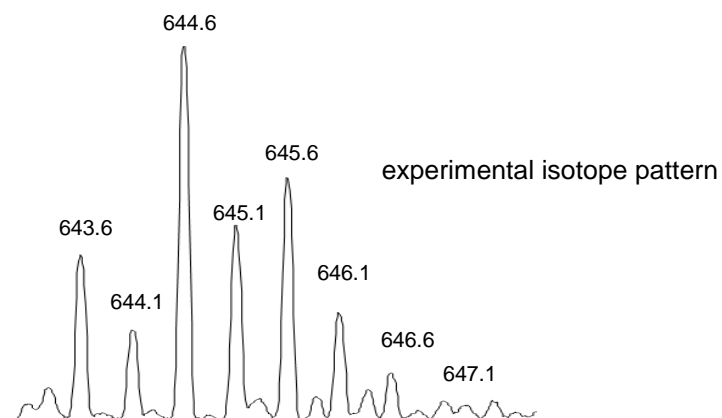


Figure S4. Experimental and theoretical isotope patterns of the polymetallic complexes of P^{HD5} formed in excess of Eu^{3+} , detected by ES^+ -MS.

6. Tb-centered luminescence in P^{HD2} titration

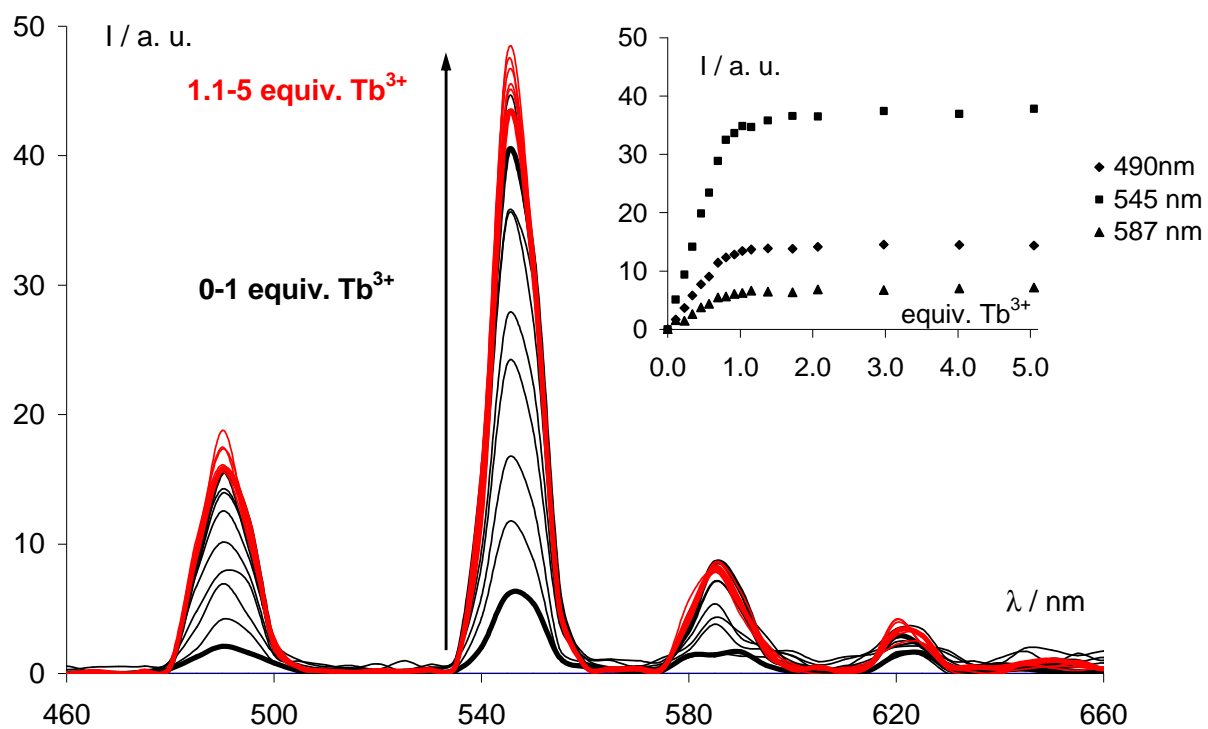


Figure S5. Titrations of P^{HD2} (5 μ M) with $TbCl_3$ in HEPES buffer (10 mM, pH = 7.0). Evolution of Tb-centered luminescence upon excitation at 280 nm (inserts: evolution at λ_{em} = 490, 545 and 587 nm).

7. Determination of Tb^{3+} luminescence lifetimes

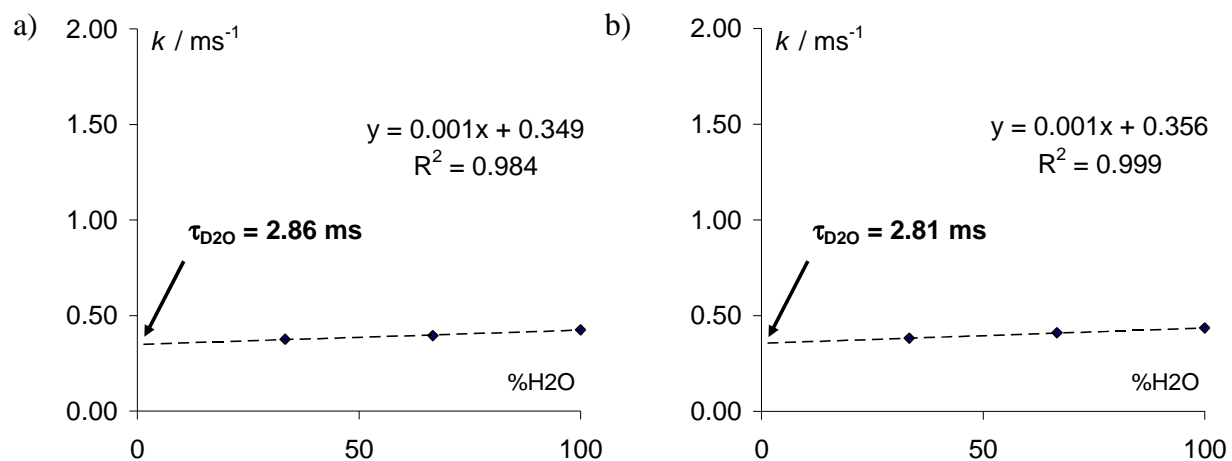


Figure S6. Relationship between the mole fraction of H_2O in $\text{H}_2\text{O}/\text{D}_2\text{O}$ mixtures and the radiative rate constant (k) for $30\ \mu\text{M}$ (a) $\text{TbP}^{\text{HD}2}$ and (b) $\text{TbP}^{\text{HD}5}$ complexes in HEPES buffer (10 mM, 0.1 M KCl, pH = 7.0).

8. pH-metric titration.

Continuous potentiometric titrations with 0.1 M KOH were conducted in 5 mL aqueous solutions of peptide (0.5 mM) with KCl (0.1 M) as background electrolyte. The reverse titrations with 0.1 M HCl were performed after each experiment to check whether equilibration had been achieved. In a typical experiment, 90 points (2 μ L increment) were measured with a 2 min delay between the measurements. Experimental data were refined using the computer program Hyperquad 2000.¹ All equilibrium constants were expressed as concentration rather than activities ratios. The ionic product of water at 25°C and 0.1 M ionic strength was $pK_w = 13.78$.² The initial concentration of the ligand was determined by UV-vis spectroscopy ($\epsilon_{280} = 5690 \text{ L mol}^{-1} \text{ cm}^{-1}$ for tryptophan residues), and proton content was adjusted to fit the observed values (since the peptides are present in the lyophilised solid as a mixture of protonation states, the proton content was reproducible for a given peptide). All values and errors (one standard deviation) reported represent the average of three independent experiments.

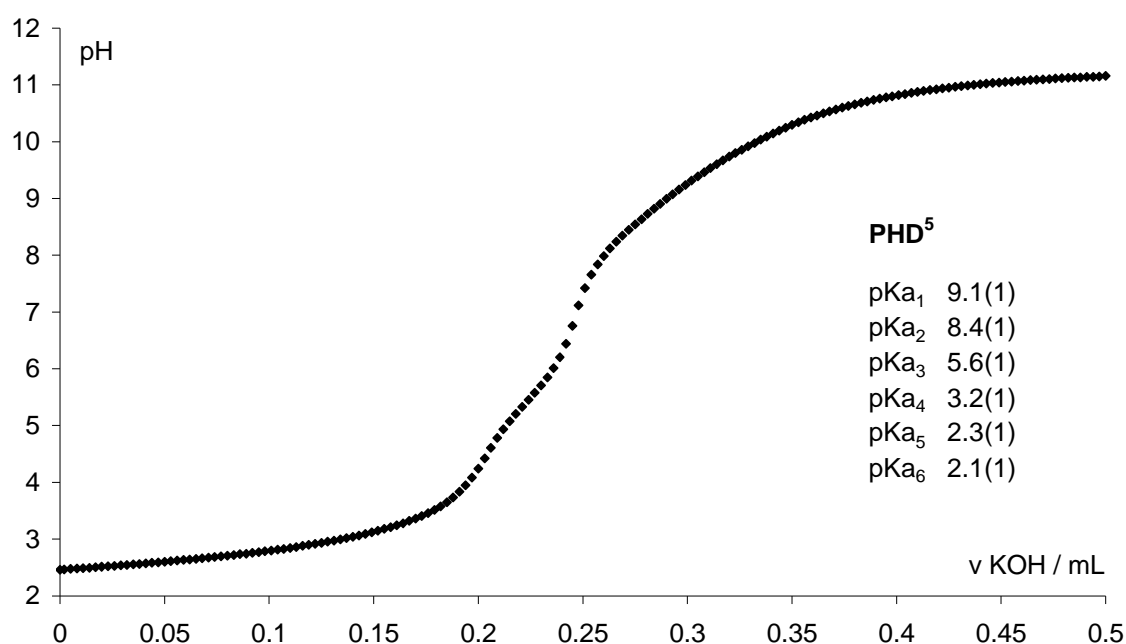


Figure S7. pH-metric titration of **P^{HD5}** (0.731 mM) in 0.1 M KCl with 0.1 M KOH at 298 K. It is expected that **P^{HD5}** and **P^{HD2}** display a similar behavior towards protonation as the **P^{HD}** peptides do not exhibit specific secondary structures that could affect the pK_a values.

¹ P. Gans, A. Sabatini and A. Vacca, *Talanta*, 1996, **43**, 1739-1753.

² R. M. Smith, A. E. Martell and R. J. Motekaitis, NIST Critically Selected Stability Constants of Metal Complexes Database, NIST Standard Reference Database 46, 2001.