Electronic Supplementary Material (ESI) for Dalton Transactions

Lanthanide-binding peptides with two pendant aminodiacetate arms: impact of the sequence on chelation

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Supplementary information

Contents:

1. pH-metric titrations

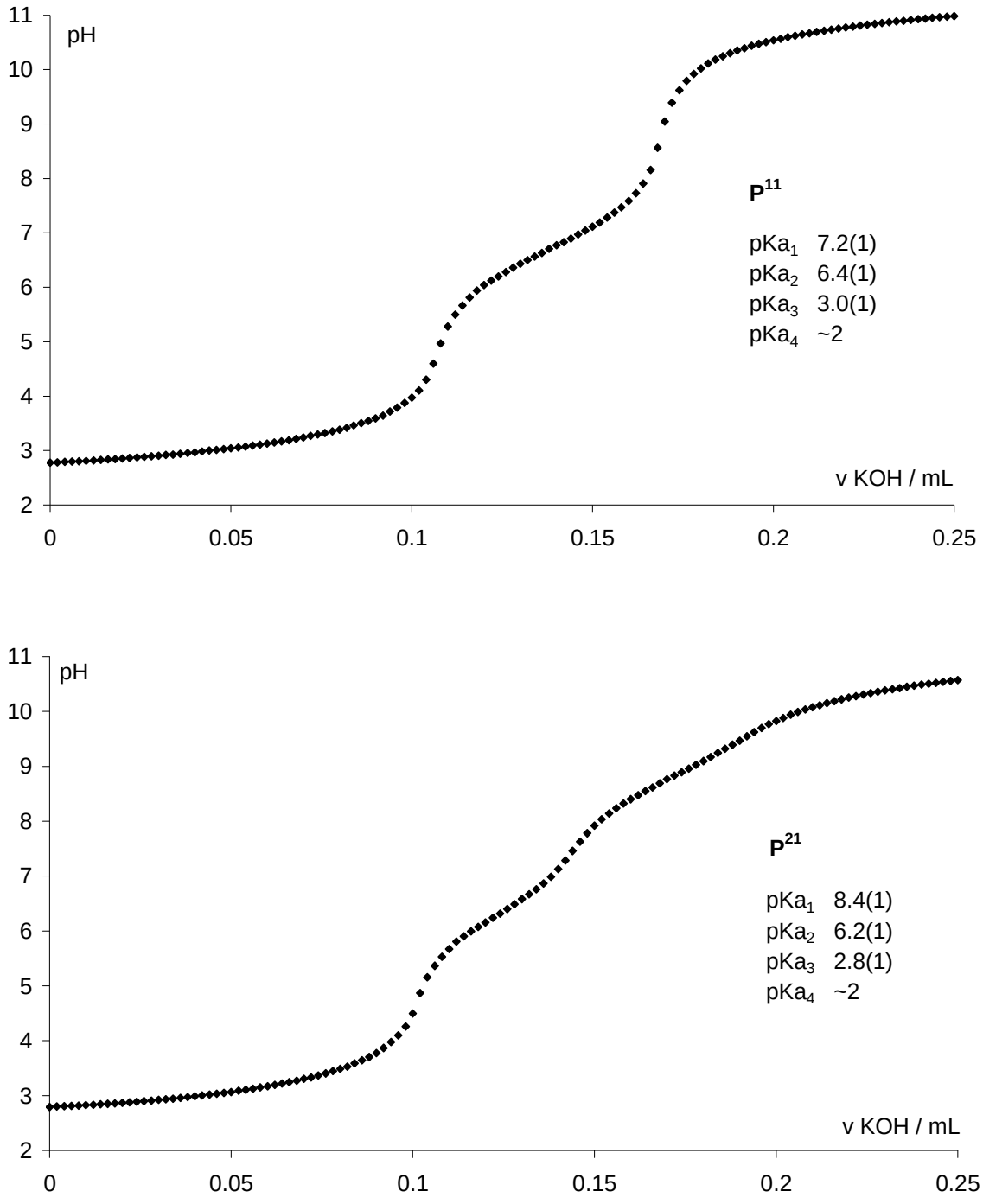

Figure S1. pH-metric titrations of $\mathbf{P^{11}}$ (0.559 mM) and $\mathbf{P^{21}}$ (0.826 mM) in 0.1 M KCl with 0.1 M KOH at 298 K.

2. ES-MS signature of the polymetallic species formed with $\mathbf{P^{21}}$

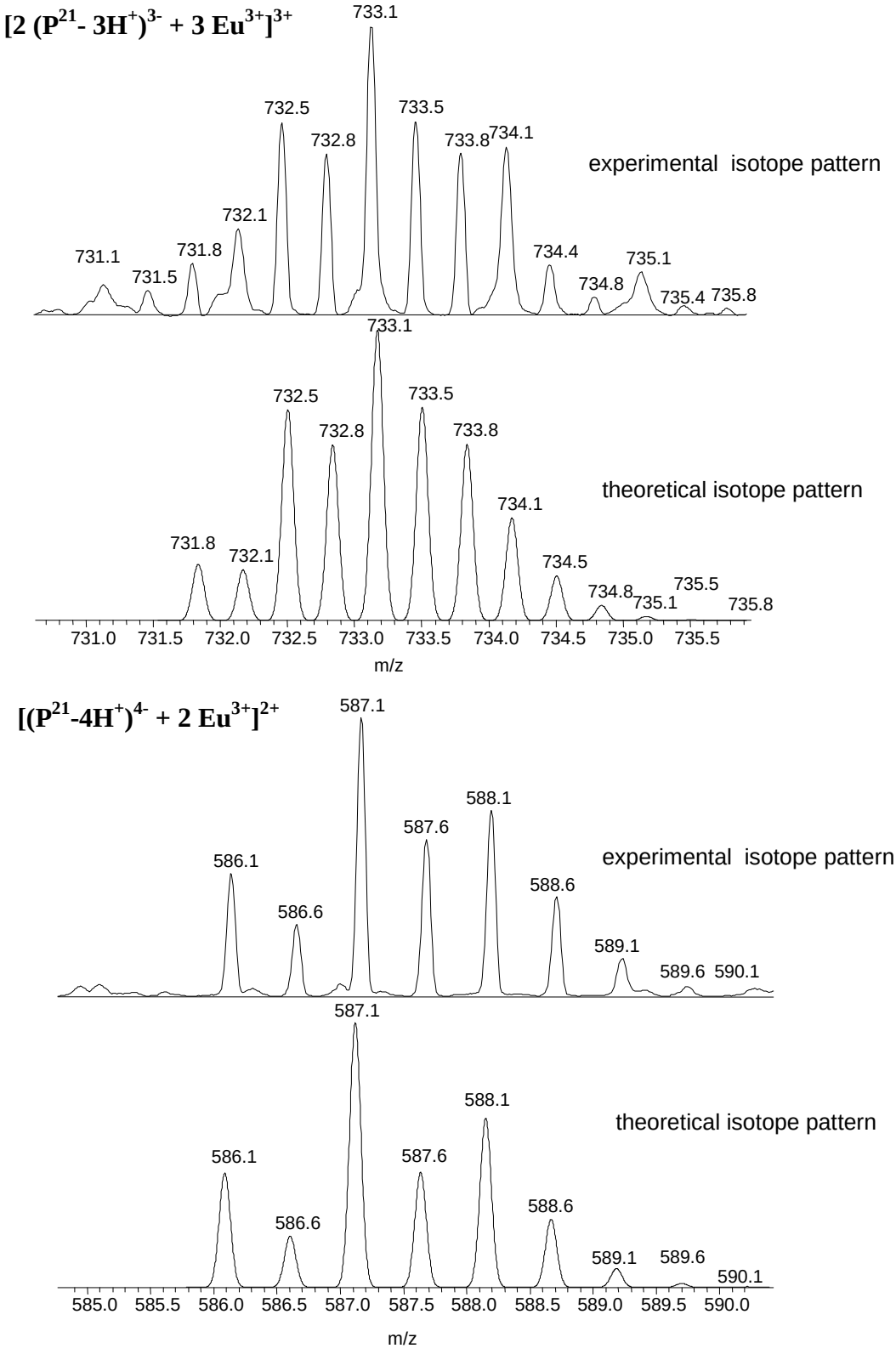

Figure S2. Experimental and theoretical isotope patterns of the polymetallic species formed with Eu^{3+} and $\mathbf{P^{21}}$, detected by ES^{+}-MS.

3. Competition experiments

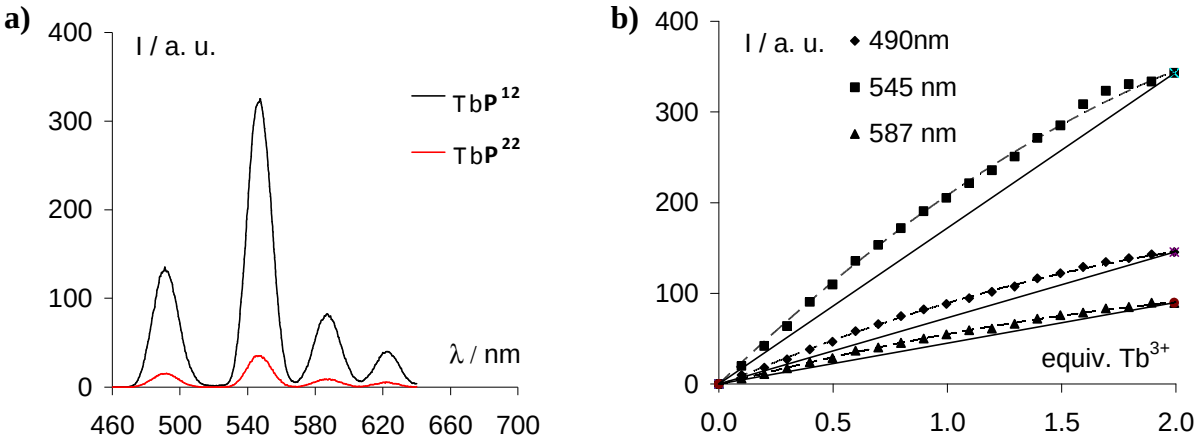

Figure S3. a) Emission spectra of 15 μM Tb**P**^{12} and Tb**P**^{22} in HEPES buffer (10 mM, pH = 7.0). **b)** Evolution of the Tb-centred luminescence during the titration of an equimolar mixture of **P**^{12} and **P**^{22} (15 μM) with $TbCl_3$ in HEPES buffer (10 mM, pH = 7.0). The emission spectra were recorded after 3 ms delay, during 5 ms gate time.

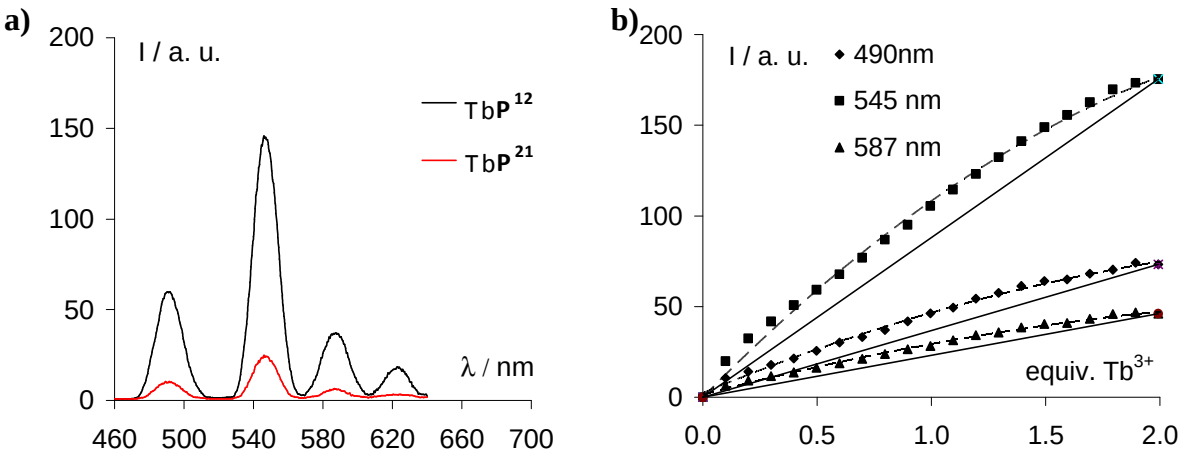

Figure S4. a) Emission spectra of 15 μM Tb**P**^{12} and Tb**P**^{21} in HEPES buffer (10 mM, pH = 7.0). **b)** Evolution of the Tb-centred luminescence during the titration of an equimolar mixture of **P**^{12} and **P**^{21} (15 μM) with $TbCl_3$ in HEPES buffer (10 mM, pH = 7.0). The emission spectra were recorded after 4 ms delay, during 5.5 ms gate time.

4. Supplementary luminescence experiments

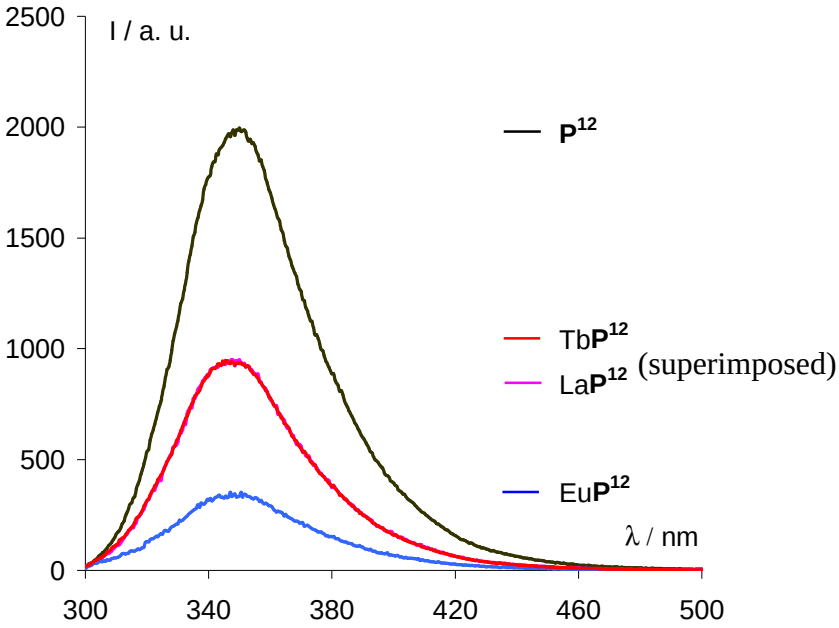

Figure S5. Fluorescence of tryptophan residue in $\mathbf{P^{12}}$ and its Ln complexes upon excitation at 280 nm (40 µM, HEPES buffer 10 mM, pH = 7.0).

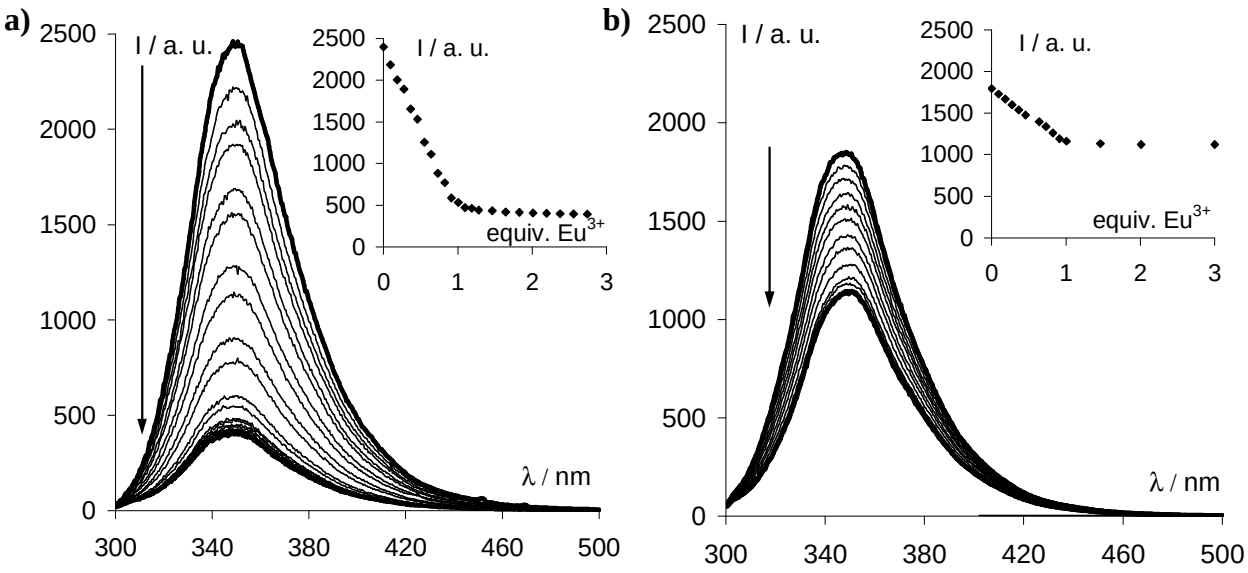

Figure S6. Titration of 51 µM (a) $\mathbf{P^{12}}$ and (b) $\mathbf{P^{22}}$ with $EuCl_3$ in HEPES buffer (10 mM, pH = 7.0). Evolution of the tryptophan fluorescence upon excitation at 280 nm.

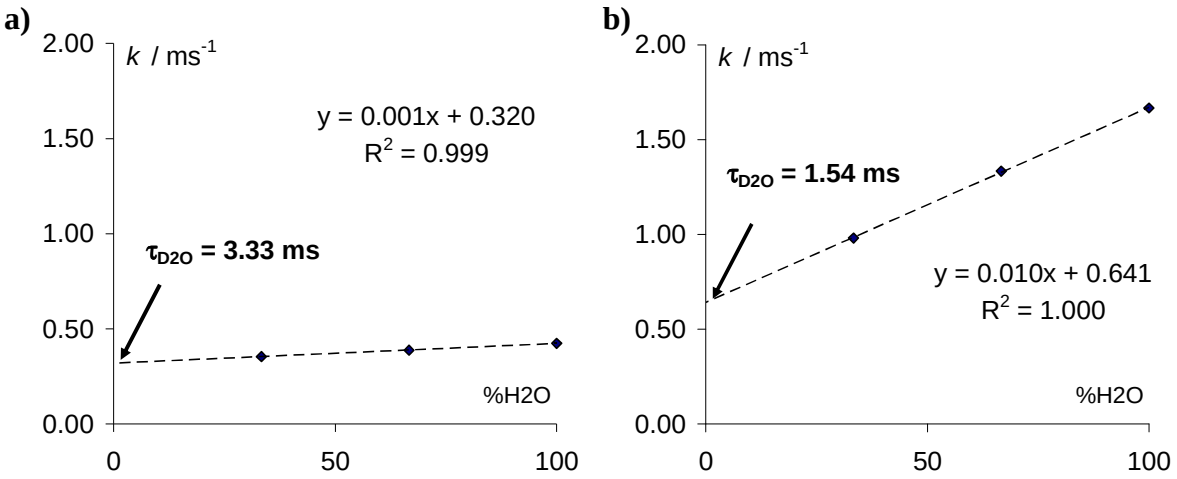

Figure S7. Relationship between the mole fraction of H_2O in H_2O/D_2O mixtures and the radiative rate constant (*k*) for 30 μM (a) Tb**P**^{12} and (b) Eu**P**^{12} complexes in HEPES buffer (10 mM, pH = 7.0).

5. Supplementary NMR tables

Table S1. ^{1}H NMR (500 MHz) chemical shifts (δ / ppm) for **P^{11}** in H_2O/D_2O v/v 9/1 at 298K, 0.99 mM, pH = 2.0. Signals assigned by COSY, TOCSY and ROESY 2D experiments.

Residue	HN	Hα	Hβ	Others
Trp(1)	8.29	4.62	3.25, 3.21	Hε$_1$: 10.18, Hε$_3$: 7.60, Hζ$_2$: 7.49, Hδ$_1$: 7.27, Hη$_2$: 7.24, Hζ$_3$: 7.15 Ac: 2.00
Ada$_1$(2)	8.40	4.75	3.43, 2.97	
Pro(3)		4.14	2.09, 1.89	Hγ: 1.89, 1.79, Hδ: 3.34, 2.89
Gly(4)	8.47	3.99, 3.93		
Ada$_1$(5)	8.62	4.85	3.76, 3.50	
Gly(6)	8.53	3.89 (2H)		CONH$_2$: 7.46, 7.07 CH$_2$COOH: 3.94 (m, 4H), 3.84 (s, 4H)

Table S2. ^{1}H NMR (500 MHz) chemical shifts (δ / ppm) for **P^{12}** in H_2O/D_2O v/v 9/1 at 298K, 2.37 mM, pH = 2.4. Signals assigned by COSY, TOCSY and ROESY 2D experiments.

Residue	HN	Hα	Hβ	Others
Trp(1)	8.30	4.64	3.24 (m, 2H)	Hε$_1$: 10.19, Hε$_3$: 7.63, Hζ$_2$: 7.50, Hδ$_1$: 7.29, Hη$_2$: 7.26, Hζ$_3$: 7.17 Ac: 2.00
Ada$_1$(2)	8.45	4.75	3.44, 2.95	
Pro(3)		4.16	2.08, 1.90	Hγ:1.88, 1.79, Hδ: 3.35, 2.89
Gly(4)	8.50	3.94 (2H)		
Ada$_2$(5)	8.46	4.50	2.33, 2.13	Hγ: 3.36 (m, 2H)
Gly(6)	8.50	3.94 (2H)		CONH$_2$: 7.48, 7.08 CH$_2$COOH: 3.90 (m, 4H), 3.83 (s, 4H)

Table S3. ^{1}H NMR (500 MHz) chemical shifts (δ / ppm) for **P^{21}** in H_2O/D_2O v/v 9/1 at 298K, 1.14 mM, pH = 2.5. Signals assigned by COSY, TOCSY and ROESY 2D experiments.

Residue	HN	Hα	Hβ	Others
Trp(1)	8.26	4.46	3.27, 3.13	Hε$_1$: 10.13, Hε$_3$: 7.51, Hζ$_2$: 7.47, Hδ$_1$: 7.28, Hη$_2$: 7.21, Hζ$_3$: 7.11 Ac: 2.00
Ada$_2$(2)	7.75	4.49	1.94, 1.71	Hγ: 2.99(m, 2H)
Pro(3)		4.07	2.19, 1.87	Hγ: 1.87 (m, 2H), Hδ: 3.37, 3.13
Gly(4)	8.43	3.96		
Ada$_1$(5)	8.67	4.87	3.77, 3.49	
Gly(6)	8.53	3.88		$CONH_2$: 7.45, 7.05 CH_2COOH: 3.89 (s 4H), 3.78 (s, 4H)

Table S4. ^{1}H NMR (500 MHz) chemical shifts (δ / ppm) for Lu**P^{12}** in H_2O/D_2O v/v 9/1 at 298K, 2.78 mM, pH = 7.0. Signals assigned by COSY, TOCSY and ROESY 2D experiments.

Residue	HN	Hα	Hβ	Others
Trp(1)	8.25	4.59	3.30, 3.15	Hε$_1$: 10.18, Hε$_3$: 7.62, Hζ$_2$: 7.51, Hδ$_1$: 7.26, Hη$_2$: 7.24, Hζ$_3$: 7.16 Ac: 2.00
Ada$_1$(2)	8.53	4.26	3.40, 3.00	
Pro(3)		4.34	2.15, 1.95	Hγ:1.95, 1.83, Hδ: 2.70, 2.50
Gly(4)	8.52	4.20, 4.00		
Ada$_2$(5)	8.53	nd*	3.2, 2.8	
Gly(6)	8.52	3.80 (2H)		$CONH_2$: 7.49, 7.10

* under the water signal.

Table S5. ^{1}H NMR (500 MHz) chemical shifts (δ / ppm) for La**P^{21}** in H_2O/D_2O v/v 9/1 at 278K, 4.3 mM, pH = 6.8. Signals assigned by COSY, TOCSY and ROESY 2D experiments.

Residue	HN	Hα	Hβ	Others
Trp(1)	8.46	4.55	3.19 (2H)	Hε$_1$: 10.27, Hε$_3$: 7.56, Hζ$_2$: 7.48, Hδ$_1$: 7.26, Hη$_2$: 7.22, Hζ$_3$: 7.12 Ac: 2.00
Ada$_2$(2)	8.32	4.97	2.09, 1.61	Hγ: 3.19, 3.02
Pro(3)		4.06	2.12, 1.85	Hγ: 1.85 (m, 2H), Hδ: 3.38, 3.13
Gly(4)	8.54	3.87		
Ada$_1$(5)	9.23	4.67	3.84 (2H)	
Gly(6)	8.71	4.12, 3.73		$CONH_2$: 7.44, 7.07