Electronic Supplementary Information for Dalton Transactions

A lanthanide binding peptide with short chelating side-chains: structural impact of the backbone coordination

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Supporting information

Contents:

1. Peptide Synthesis and Purification

Abbreviations: Ac_2O, acetic anhydride; DMF, *N*,*N*-dimethylformamide; DIEA, *N*,*N*-diisopropylethylamine; DTT, dithiothreitol; Et_2O, diethyl ether; Fmoc, 9-fluorenylmethyloxycarbonyl; PyBOP, (benzotriazole-1-yloxy)tris(pyrrolidino)phosphonium hexafluorophosphate; TFA, trifluoroacetic acid; TIS, triisopropylsilane; TNBS, 2,4,6-trinitrobenzenesulfonic acid.

P[1] was assembled manually by solid-phase peptide synthesis on Rink Amide MBHA resin (substitution 0.59 mmol g^{-1}, 153 mg) using Fmoc chemistry. The synthesis was started by an initial deprotection of the commercially resin-bound Fmoc with DMF/piperidine (v/v = 4/1). Couplings were performed with Nα-Fmoc-protected amino acids (2 eq), PyBOP (2 eq), and DIEA (6 eq) in DMF for 30 minutes. In the case of Fmoc-$Ada_1(^tBu)_2$-OH the coupling reaction was monitored by a TNBS test.[1] For incomplete reaction, a second coupling with Fmoc-$Ada_1(^tBu)_2$-OH (0.5 eq) PyBOP (1 eq), and DIEA (4 eq) was performed. After each coupling, the resin was treated with DMF/pyridine/Ac_2O (v/v/v = 7/2/1) to acetylate unreacted amino groups (2×2 minutes). Fmoc deprotection was achieved with DMF/piperidine (v/v = 4/1) (3×3 minutes). The yield of each peptide coupling was monitored by UV-vis spectroscopy (ε_{300}=7800 L mol^{-1} cm^{-1} for the piperidine adduct dibenzofulvene). After the final Fmoc deprotection, the peptide was acetylated as described above. The peptide was cleaved from the resin and the side-chain protections were removed by treatment with a cleavage cocktail consisting of 200 mg DTT dissolved in 20 mL TFA/TIS/H_2O (v/v/v = 92/4/4). After 2.5 h of stirring, the solution was evaporated to yield a yellow oil, which was triturated several times in Et_2O to yield a white powder. This solid was analysed RP-HPLC [Merck Purospher® STAR endcapped, 4.6×250 mm, 5µm C18 particles, solvent A=H_2O/TFA (v/v=99.925/0.075), solvent B=CH_3CN/H_2O/TFA (v/v/v=90/10/0.1), elution gradient: from 10% A 90% B to 40% A 60% B in 15 minutes, flow rate 1 mL min^{-1}, UV monitoring at 280 nm]. HPLC analysis indicated that the solid consists essentially of one product eluting at $t_{R=}$10.8 minutes, which was identified by ES-MS as **P[1]**. A minor product (<5%) eluting at 14.2 minutes was identified as a tBu adduct of **P[1]**. The solid residue was dissolved in water/acetonitrile (v/v = 9/1) and easily purified by reversed-phase high-performance liquid chromatography [RP-HPLC, Merck Purospher®, 250×40 mm, 10 µm C18 particles, solvent A=H_2O/TFA (v/v=99.925/0.075), solvent B=CH_3CN/H_2O/TFA (v/v/v=90/10/0.1), elution gradient: from 10% A 90% B to 40% A 60% B in 15 minutes, flow rate 75 mL min^{-1}] to yield

the desired peptide as a white powder. **P[1]**: Ac-WAda$_1$PGAda$_1$G-NH_2, Yield of the on-resin synthesis (UV): 76% Isolated mass: 44.5 mg (isolated yield assuming that the solid is **P[1]**.2TFA: 45%). (+)ES-MS: 861.2 $(M+H)^+$; (–)ES-MS: 859.2 $(M-H)^-$; RP-HPLC: t_r=10.8 min, >95% purity (NMR).

2. Experimental details of physico-chemical experiments

Preparation of aqueous solutions: 5 mM metal solutions, used as stock solutions for luminescence, CD and ES-MS experiments were prepared from the corresponding chloride salts ($LaCl_3.7H_2O$, $EuCl_3.6H_2O$, $TbCl_3.6H_2O$) in pure H_2O. Their precise concentration was obtained by titration with a 5 mM volumetric ethylenediaminetetraacetic acid (Fisher Chemicals) in the presence of a colorimetric indicator. For NMR in deuterated water, a solution of anhydrous $Lu(OTf)_3$ in pure D_2O was prepared and titrated similarly. Peptide solutions were prepared freshly before use and the precise peptide concentration was determined by recording a UV spectrum (ε_{280}=5690 L mol^{-1} cm^{-1}) owing to the presence of a Trp residue. Hepes buffer was prepared by dissolving solid 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid in H_2O (or D_2O) and by adjusting the pH (or pD) to 7.0 with KOH (or NaOD).

Circular Dichroism: CD spectra were recorded at 25 °C on a Applied Photophysics Chirascan Spectrometer in a 1 cm path cell. The pH was adjusted to 7 with KOH. The spectra were obtained from 320 to 190 nm with a 1 nm data interval, a time constant of 2 s and a band width of 1 nm, with 3 scans. CD spectra are reported in ellipticity per α-amino acid residue.

Luminescence: spectra were recorded on a LS50B spectrofluorimeter connected to a computer equipped with FLWILAB 2.0. The measurements were performed at 298 K. Trp fluorescence titrations were performed with 280 nm excitation (excitation slit: 3.0 nm). Emission slit was adjusted to avoid signal saturation during the titration. Tb phosphorescence spectra were recorded upon Trp excitation (280 nm) after a 0.05 ms delay and with a 1.0 ms gate time. Excitation slit was 10.0 nm and emission slit was adjusted to avoid signal saturation. A 430 nm cut-off filter was used.

Longer delays and gate times were used for the competition experiment between **P^1** and **P^2** (3 and 5 ms, respectively). Conditional stability constants were extracted from the spectral data using SPECFIT.[2]

Lifetime measurements were performed on peptide samples containing 0.5 equiv. $TbCl_3$ to ensure exclusive contribution from the monometallic complex. Emission intensities at the most intense Tb^{3+} emission band were recorded after excitation at 280 nm with a first delay of 0.05 ms, a delay increment of 0.05 ms and a number of measurements adjusted to have the final delay > $4\times\tau$.

Mass spectrometry of EuP^1 : Europium was chosen as a representative lanthanide ion because of its characteristic isotopic signature (^{151}Eu 47.8%, ^{153}Eu 52.2%). A 50 µM **P^1** solution was prepared in a 20 mM pH=7 ammonium acetate buffer. $EuCl_3$ was added to this solution. Mass spectra were recorded on a LXQ type THERMO SCIENTIFIC spectrometer equipped with a electrospray ionization (ESI) source and a linear trap detector. Solutions were injected in the spectrometer at a 5 µL min^{-1} flow rate. Ionization voltage and capillary temperature were about 2 kV and 250 °C, respectively.

Peptide NMR: NMR experiments for apopeptide and complex characterization were recorded on a 500 MHz Bruker Avance spectrometer equipped with a BBI probe with a triple-axis gradient field. ^{1}H NMR spectra were recorded with 12 ppm windows and 32 K data points in the time domain. ^{1}H NMR spectra were recorded in H_2O/D_2O (v/v = 9/1) using Watergate or presaturation solvent suppression. TOCSY experiments were performed using a MLEV-17 spin-lock sequence with a mixing time of 70 ms. Spectra were acquired in phase-sensitive mode with TPPI for quadrature detection in the indirect dimension, using 2048×512 matrices over a 6000 Hz spectral width. The 2D NOESY NMR spectrum for structure determination was recorded on a Varian Vnmrs 800 MHz spectrometer equipped with a ^{1}H/^{2}H/^{15}N/^{13}C cryogenic probe. A mixing time of 150 ms was used

3. CD titration supplementary figure

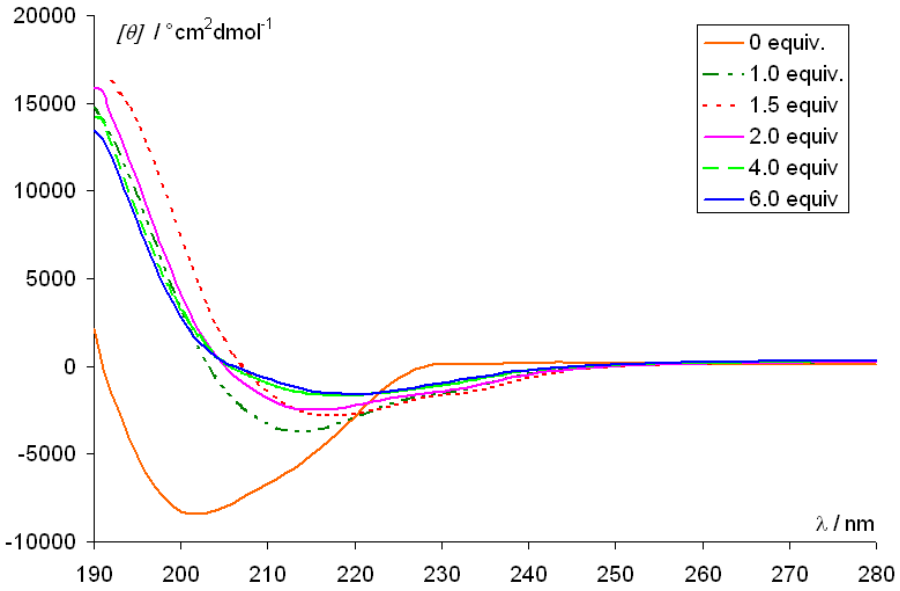

Fig S1. CD titration of **P**[1] with $TbCl_3$ after one Tb equiv.

4. Tb Phosphorescence lifetimes

The luminescence lifetimes of Tb^{3+} in Tb**P**[1] in H_2O and D_2O solutions were measured in order to obtain the hydration number q_{Tb} of these complexed ion by Parker *et al.*[3] To avoid underestimation of luminescence lifetimes in D_2O because peptides are accompanied by H_2O hydration molecules in the solid state, τ_{D2O} was determined as the extrapolated limit of the luminescent decay rates in solutions of increasing D_2O molar fractions tending to an H_2O-free solution (fig S2).

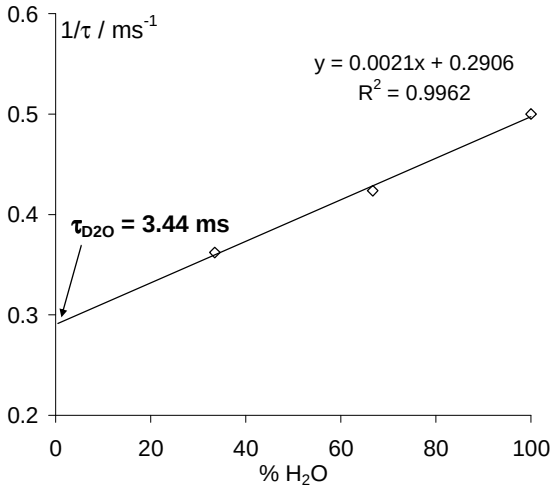

Fig S2. Plot of 1/τ *vs.* % H_2O for the Tb^{3+} complex of P[1]. Conditions: HEPES buffer, pH=7, peptide concentration 17.32 µM, 0.5 Tb^{3+} equiv.

5. Tb luminescence: competition with P^1 and P^2

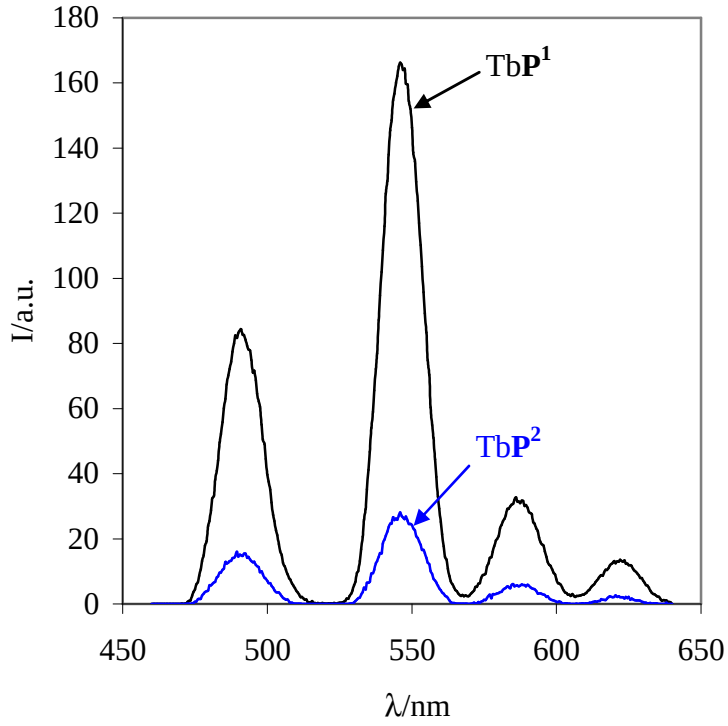

Fig S3. Tb-centred emission spectra of the two complexes Tb**P**^{1} and Tb**P**^{2} (10 µM) in HEPES buffer (10 mM, pH 7). Delay and gate times are 3 and 5 ms, respectively.

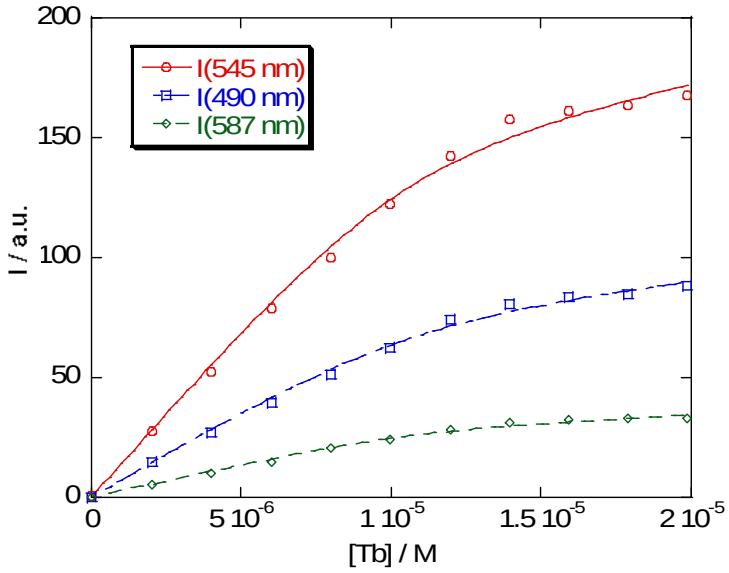

Fig S4. Build-up of the Tb-centred emission at 545, 490 and 587 nm during the titration of an equimolar mixture of **P**^{1} and **P**^{2} (10 µM) with $TbCl_3$ in HEPES buffer (10 mM, pH 7). The lines represent calculated data with $\log\beta_{11}^{pH7}$ = 9.1 and 10.3 for Tb**P**^{2} and Tb**P**^{1} respectively.

6. Supplementary NMR figures and tables

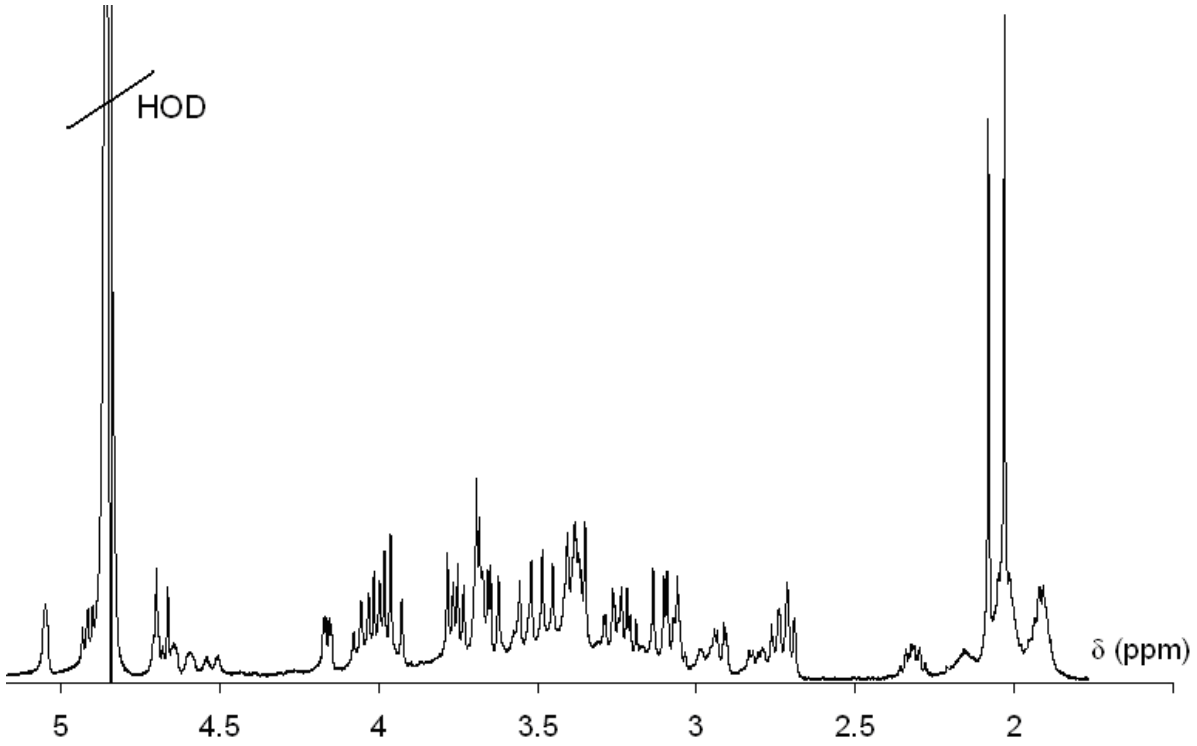

Figure S5. 500 MHz ^{1}H NMR spectrum of Lu**P^{1}** in D_2O at 298K the presence of two conformers may be most easily visualised by the splitting of the acetyl peak around δ=2 ppm. Residual HOD signal was attenuated by presaturation

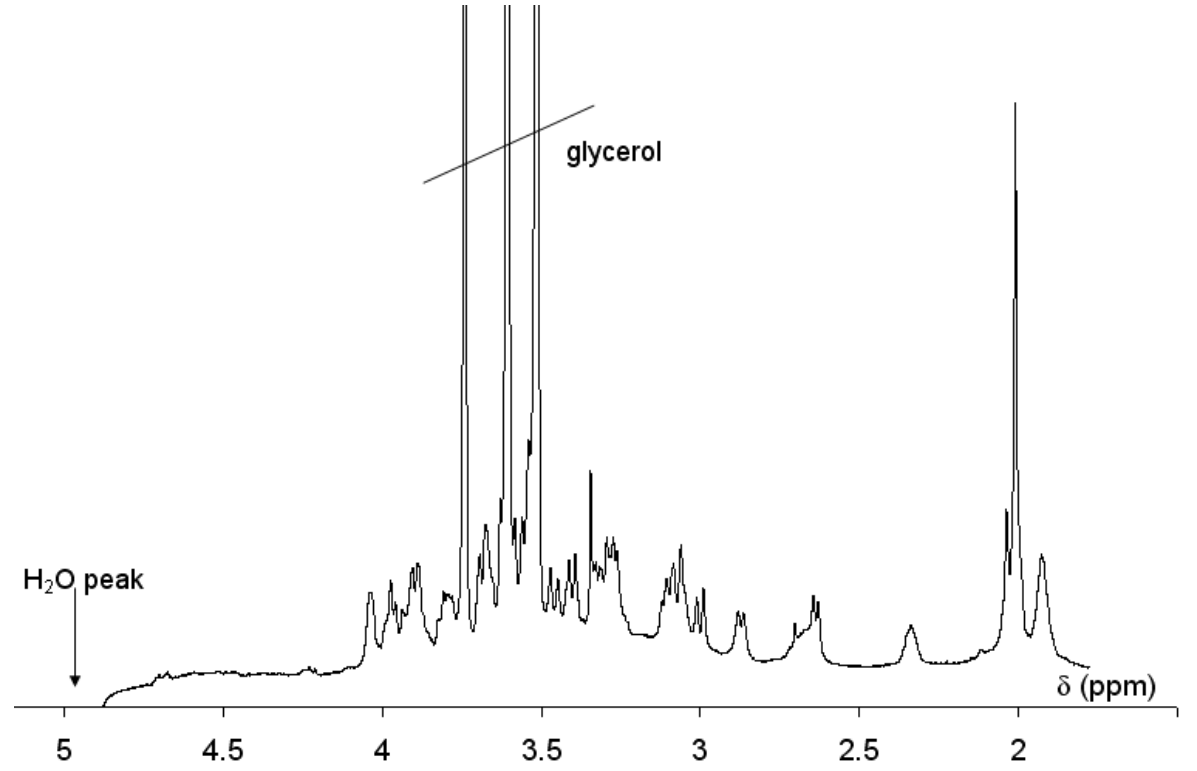

Figure S6. 800 MHz WATERGATE ^{1}H NMR spectrum of Lu**P^{1}** in H_2O/D_2O/glycerol-d_8 (v/v/v 9:1:2) at 273 K.

Table S1 ^{1}H NMR (500 MHz) chemical shifts (δ / ppm) for **P^1** 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
Trp1	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$2	8.40	4.75	3.43, 2.97	
Pro3		4.14	Hβ: 2.09, 1.89	Hγ: 1.89, 1.79, Hδ: 3.34, 2.89
Gly4	8.47	3.99, 3.93		
Ada$_1$5	8.62	4.85	3.76, 3.50	
Gly6	8.53	3.89 (2H)		$CONH_2$: 7.46, 7.07 CH_2COOH: 3.94 (m, 4H), 3.84 (s, 4H)

Table S2 ^{1}H NMR (800 MHz) chemical shifts (δ / ppm) for Lu**P^1** in H_2O/D_2O/glycerol-d_8 9/1/2 v/v/v at 273K, 3.71mM, pH 7.0. Signals assigned by COSY, TOCSY and NOESY 2D experiments

Residue	NH	Hα	Hβ	Others
Trp1	8.63	4.91	3.30(pro-*R*), 3.10(pro-*S*)	Hε$_1$: 10.21, Hε$_3$: 7.63, Hζ$_2$: 7.46, Hη$_2$: 7.24, Hδ$_1$: 7.17, Hζ$_3$: 7.08 Ac: 2.01
Ada$_1$2	9.75	4.99	2.87(pro-*R*); 2.70(pro-*S*);	C*H*$_2$COOLu: [(3.29, 3.00) pro-*R* CH_2]; [(3.80, 3.07) pro-*S* CH_2]
Pro3		4.04	2.33(pro-*S*), 1.93(pro-*R*)	Hγ: 1.93 (2H), Hδ: 3.66(pro-*S*), 3.05(pro-*R*)
Gly4	8.93	4.70(pro-*S*), 3.57(pro-*R*)		
Ada$_1$5	9.22	3.52	3.99(pro-*R*); 2.64(pro-*S*)	C*H*$_2$COOLu: [(3.46, 3.29) pro-*R* CH_2]; [(3.55, 3.40) pro-*S* CH_2]
Gly6	10.15	3.90(pro-*S*); 3.69(pro-*R*)		$CONH_2$: 7.24, 7.14

7. NMR structure calculation

NMR solution structures were obtained with CNSsolve[4] version 1.1 following standard refinement protocols and using "protein-allhdg" forcefield for natural amino acids. For unnatural amino acid side-chains and for the lutetium coordination sphere custom topology and parameter files were generated. The parameters were chosen as following. Parameters for Cα, Cβ, and bound hydrogens were set identical as for Cα and Cε of L-lysine. Parameters for bond lengths of Nγ, Cδs and Cεs were adapted from glycine parameters. Parameters for Lu–N and Lu–O distances, as well as Cβ–Nγ–Cδ, Cε–Oζ1–Lu, and Nγ–Cδ–Cε angles, and Cδ–Cε–Oζ–Lu and Nγ–Cδ –Cε–Oζ torsion angles were obtained from high resolution X-ray data referenced in the Cambridge Structural Database (CSD) of Lu^{3+} complexes containing the *N*-alkyl aminodiacetate moiety. The structure search and visualisation were performed with CSD ConQuest 1.10.[5] 3 relevant structures were found in the database and the average structural parameters were extracted with CSD Vista 2.1. CSD references: ICAGOJ ; LARLEW ; LIRPUY. These structures are the only published examples of octadentate Lu complexes containing the fragment of interest in a nonbridging geometry. Average parameters (standard deviation) (units: Å, °): Lu-N 2.547 (0.063); Lu-O 2.251 (0.03), C-N-C 109.504 (0.99); C-O-Lu 123.73 (4.92); N-C-C 113.03 (1.383). C-C-O-Lu improper was found to be close to 0° and set to the latter value in calculations. Similarly, for Cε–Oζ1 and Cε–Oζ2 (distances between metal bound and metal unbound oxygen atoms in Ada_1 side chain, respectively) average parameters deriving from the same set of structures were used: Cε-Oζ1 1.285 (0.026); Cε-Oζ2 1.235 (0.018). As for the other ions already implemented in CNSsolve, Lu^{3+} nonbonded radius was adapted from crystallographic data considering a coordination number of 8.[6] For calculations performed with constrained CO-Lu distances, a distance of 2.3 Å was taken as for the CO-Tb bond observed in the structure of LnLBTs.[7]

Upper and lower limits for the 118 distance constraints were set to ±15% of the H-H distances obtained by integration of 2D-NOESY spectra (300 ms). r^{-6} averaging was chosen for the NOE restraints. Several unoverlapped geminal NOE cross-peaks (taken as 1.8 Å) as well as Pro Hα/Hβ1 (2.3 Å) were used as references for distances calibration. Pseudo-atom corrections were applied to methyl and overlapping or non-stereospecifically assigned methylenes.[8] Dynamics were performed with a hot phase at 2000 K with 5000 0.003 ps steps followed by a slow cooling phase with 5000 0.005 ps steps with 5 K cooling per step. Final minimizations were performed with the Powell algorithm (200 steps, 100 cycles). An ensemble of 25 structures was generated for each condition (unconstrained and constrained

binding of carbonyl groups). For the unconstrained calculation as well as for that with a constrained $Ada_1 2$ CO-Lu bond, structural convergence was obtained for 13/25 and 11/25 structures, respectively. None of the latter structures displayed NOE violations greater than 0.4 Å. For the lowest energy structure, NOE RMSD was 0.101 Å. Some of the C-O-Lu angles and N-Lu bonds showed "violations" $\leq 10°$ and <0.1 Å. These "violations" are chemically insignificant and reflect merely the scarcity of structural data for similar Lu complexes used to determine structural parameters. The 10 lowest-energy structures were visualized and aligned (backbone atoms) using PyMol v. 0.99 (DeLano Scientific LLC.).[9]

Table S3 Correlations obtained from 800 MHz 2D NOESY data (150 ms mixing time) The lower and upper distance limits, used for CNSsolve calculations were taken as ± 15%. Atoms labelled as in CNSSolve. Trp1 Hβ1: pro-*S* , Trp1 Hβ2: pro-*R*; $Ada_1$2/5 Hβ1: pro-*S* , $Ada_1$2/5 Hβ2: pro-*R*; Pro3 Hβ1: pro-*S*, Pro3 Hβ2: pro-*R*; Pro3 Hδ1: pro-*R*, Pro3 Hδ2: pro-*S*; Gly4/6 Hα1: pro-*S*, Gly4/6 Hα2: pro-*R*; $Ada_1$2 Hδ1#: the two H of the pro-*S* methylene, $Ada_1$2 Hδ2#: pro-*R* methylene; $Ada_1$5 Hδ1 pro-*R* methylene, $Ada_1$5 Hδ2#: pro-*S* methylene.

H1	H2	*d*(H1,H2)
Intraresidue NOEs		
Trp1 Hβ2	Trp1 Hα	2.69
Trp1 Hδ1	Trp1 Hα	3.75
Trp1 Hδ1	Trp1 Hβ1	3.04
Trp1 Hδ1	Trp1 Hβ2	2.8
Trp1 Hε1	Trp1 Hβ1	5.09
Trp1 Hε1	Trp1 Hβ2	4.91
Trp1 Hε3	Trp1 Hβ2	2.84
Trp1 Hε3	Trp1 Hα	2.62
Trp1 NH	Trp1 Hε3	4.09
Trp1 NH	Trp1 Hα	2.73
Trp1 NH	Trp1 Hβ2	2.47
Trp1 NH	Trp1 Hδ1	3.58
Trp1 Hζ3	Trp1 Hα	4.08
Trp1 Hζ3	Trp1 Hβ2	4.5
$Ada_1$2 Hβ2	$Ada_1$2 Hδ2#	2.59
$Ada_1$2 Hδ1#	$Ada_1$2 Hβ1	2.95
$Ada_1$2 Hδ1#	$Ada_1$2 Hβ2	2.5
$Ada_1$2 NH	$Ada_1$2 Hδ2#	4.5
$Ada_1$2 NH	$Ada_1$2 Hβ1	4.01
$Ada_1$2 NH	$Ada_1$2 Hβ2	3.46
$Ada_1$2 NH	$Ada_1$2 Hδ1#	2.49
Gly4 NH	Gly4 Hα1	2.93
Gly4 NH	Gly4 Hα2	2.53
$Ada_1$5 Hα	$Ada_1$5 NH	2.2
$Ada_1$5 Hδ1#	$Ada_1$5 Hβ1	2.54
$Ada_1$5 Hδ1#	$Ada_1$5 Hβ2	2.74
$Ada_1$5 Hδ2#	$Ada_1$5 Hβ1	2.6
$Ada_1$5 Hδ2#	$Ada_1$5 Hβ2	2.46
$Ada_1$5 Hδ2#	$Ada_1$5 Hδ1#	2.23
$Ada_1$5 NH	$Ada_1$5 Hβ1	2.84
$Ada_1$5 NH	$Ada_1$5 Hα	2.13
$Ada_1$5 NH	$Ada_1$5 Hβ2	3.2
$Ada_1$5 NH	$Ada_1$5 Hδ1#	4.21
$Ada_1$5 NH	$Ada_1$5 Hδ1#	3.2
Gly6 NH	Gly6 Hα1	2.59
Gly6 NH	$Ada_1$5 Hβ2	4
Gly6 NH	Gly6 Hα2	2.37

H1	H2	*d*(H1,H2)
Sequential NOEs		
Trp1 Hβ1	Ac Hα#	5.11
Trp1 Hβ2	Ac Hα#	5.05
Trp1 Hε3	Ac Hα#	6.3
Trp1 NH	Ac Hα#	2.88
$Ada_1$2 Hα	Pro3 Hδ1	2.93
$Ada_1$2 Hα	Pro3 Hδ2	2.34
$Ada_1$2 Hδ1#	Trp1 Hα	3.41
$Ada_1$2 NH	Trp1 Hβ1	3.65
$Ada_1$2 NH	Trp1 NH	3.32
$Ada_1$2 NH	Pro3 Hα	5.05
$Ada_1$2 NH	Trp1 Hδ1	4.5
$Ada_1$2 NH	Trp1 Hε3	3.92
$Ada_1$2 NH	Pro3 Hδ2	4.08
$Ada_1$2 NH	Pro3 Hα	5.36
Pro3 Hδ2	$Ada_1$2 Hβ1	3.65
Pro3 Hδ2	$Ada_1$2 Hβ2	3.48
Pro3 Hγ#	Gly4 NH	4.21
Gly4 Hα2	Pro3 Hβ2	3.92
Gly4 NH	Pro3 Hδ2	5.5
Gly4 NH	Pro3 Hα	2.32
Gly4 NH	$Ada_1$5 Hβ1	4.25
Gly4 NH	Pro3 Hβ1	3.34
Gly4 NH	Pro3 Hβ2	2.96
$Ada_1$5 Hδ1#	Gly4 NH	4.3
$Ada_1$5 NH	Gly4 Hα2	2.95
$Ada_1$5 NH	Gly4 Hα1	3.4
$Ada_1$5 NH	Gly4 NH	2.63
$Ada_1$5 NH	Gly6 Hα2	4.54
$Ada_1$5 NH	Gly6 Hα1	4.54
Gly6 NH	$Ada_1$5 NH	2.9
Gly6 NH	$Ada_1$5 Hβ1	4.24
Gly6 NH	$Ada_1$5 Hα	3.53
Gly6 NH	$CONH_2$ H1	2.93
Gly6 NH	$CONH_2$ H2	3.89
$CONH_2$ H1	Gly6 Hα1	3.12
$CONH_2$ H1	Gly6 Hα2	3.23
$CONH_2$ H2	Gly6 Hα1	3.53
$CONH_2$ H2	Gly6 Hα2	3.66

H1	H2	d(H1,H2)
Medium range /long range NOEs		
Trp1 Hδ1	Pro3 Hα	4.43
Trp1 Hδ1	Pro3 Hβ1	4.31
Trp1 Hδ1	Pro3 Hγ1	3.59
Trp1 Hδ1	Pro3 Hδ1	3.16
Trp1 Hδ1	Pro3 Hδ2	3.92
Trp1 Hδ1	Pro3 Hβ1	4.31
Trp1 Hε1	Pro3 Hδ2	4.54
Trp1 Hε1	Pro3 Hα	3.8
Trp1 Hε1	Pro3 Hβ1	3.18
Trp1 Hε1	Pro3 Hβ2	4.04
Trp1 Hε1	Pro3 Hδ1	3.73
Trp1 Hε1	Pro3 Hγ1	3.44
Trp1 Hε3	Pro3 Hδ1	3.95
Trp1 Hε3	$Ada_1$5 Hδ1#	3.92
Trp1 Hη2	Pro3 Hβ2	5.5
Trp1 Hη2	Pro3 Hβ1	5.24
Trp1 Hη2	Pro3 Hα	4.5
Trp1 Hη2	$Ada_1$5 Hβ1	4.78
Trp1 Hη2	$Ada_1$5 Hβ2	4.31
Trp1 Hη2	$Ada_1$5 Hδ1#	3.29
Trp1 Hζ2	Pro3 Hδ1	4.36
Trp1 Hζ2	Pro3 Hγ1	5.14
Trp1 Hζ2	Pro3 Hβ2	4.5
Trp1 Hζ2	Pro3 Hβ1	3.37
Trp1 Hζ2	Pro3 Hα	3.5
Trp1 Hζ3	Pro3 Hγ1	6.6
Trp1 Hζ3	$Ada_1$5 Hβ1	4.78
Trp1 Hζ3	Pro3 Hα	4.58
Trp1 Hζ3	$Ada_1$5 Hβ2	4.5
Trp1 Hζ3	$Ada_1$5 Hδ1#	3.3
$Ada_1$2 NH	Ac Hα#	5
Pro3 Hδ1	Trp1 NH	5.05
$Ada_1$5 Hδ1#	Trp1 Hζ2	4.9
$Ada_1$5 NH	Pro3 Hα	3.23
$Ada_1$5 NH	Pro3 Hβ2	4.91
$Ada_1$5 Hα	$CONH_2$ H1	3.86
$Ada_1$5 NH	$CONH_2$ H1	4.41
$Ada_1$5 NH	Pro3 Hβ1	4.64
Gly6 NH	Gly4 Hα1	3.68
Gly6 NH	Gly4 NH	4.95
$CONH_2$ H1	$Ada_1$5 Hβ1	4.18
$CONH_2$ H1	$Ada_1$5 Hβ2	3.68
$CONH_2$ H1	Gly4 Hα1	4.04
$CONH_2$ H2	Gly4 Hα1	4.37

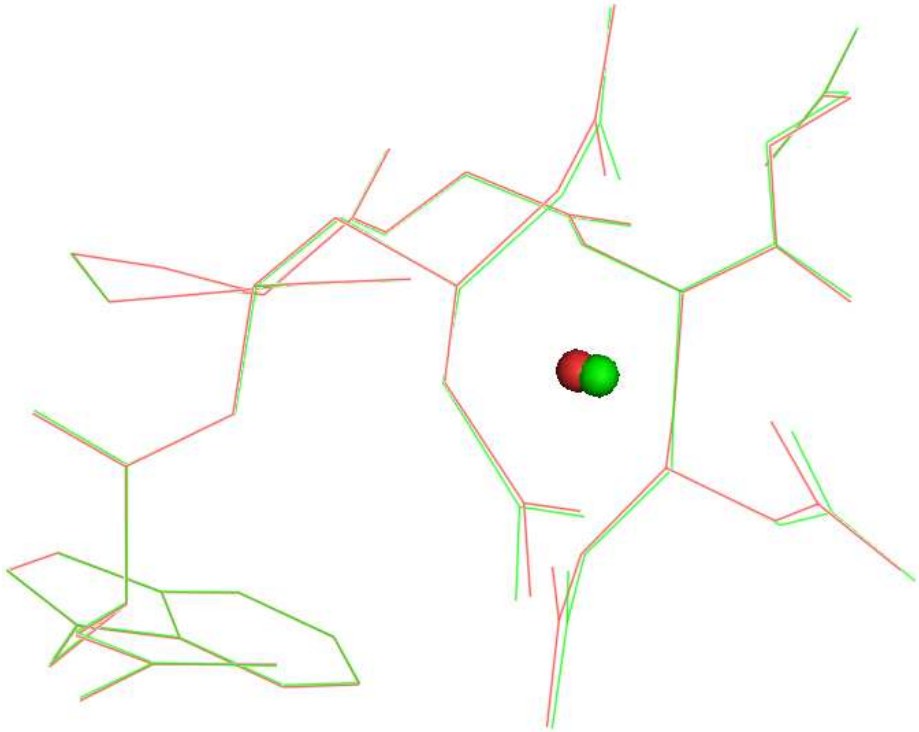

Fig S7. Superimposition of the lowest energy structures obtained with a $Ada_1$2 CO-Lu distance constrained to 2.3 Å (red) and unconstrained (green).

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