Electronic Supplementary Information

Luminogenic "Clickable" Lanthanide Complexes for Protein Labeling.

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SI 1- Synthesis and characterization of ligands and ABPs

1.1 General remarks

NMR spectra were recorded in Fourier Transform mode with a Bruker AVANCE 400, JEOL-Delta 400 (¹H at 400 MHz, 13C at 100 MHz), or Bruker DPX-500 (¹H at 500 MHz, 13C at 125 MHz) spectrometers, at 298K. Data are reported as chemical shifts (δ) in ppm. Residual solvent signals were used as internal references (¹H, ¹³C). Monitoring of SPAAC reactions were performed using a Bruker AVANCE 500 spectrometer (¹H at 500 MHz). Electrospray (positive mode) high-resolution mass spectra were recorded on a Q-TOF micro spectrometer (Waters), using an internal lock mass (H₃PO₄) and an external lock mass or a LTQ XL (ThermoScientific) spectrometer using external mass calibration. IR spectra were recorded on a Shimadzu Fourier Transform Infrared Spectrophotometer FTIR-8400S. Absorption spectra were recorded with a Perkin-Elmer Lambda 25 spectrometer. RP-HPLC analyses for all peptide derivatives were performed using a VARIAN Pro Star 210 system, equipped with UV detection, using an analytical C18 VYDAC column (300 Å, 4.6 mm × 150 mm, 5 µm, 1 mL/min). The eluent was a linear gradient of water (0.1% TFA) and acetonitrile. The retention time (Rt) of the analytical RP-HPLC is given in minutes with the gradient in percentage of acetonitrile. Semi-preparative RP-HPLC was performed using the same chromatographic system and a preparative C18 VYDAC column (300 Å, 20 mm \times 250 mm, 10 µm, 5 mL/min) in combination with the same solvents. A Christ Alpha 1-4 LSC freeze dryer was used for lyophilisation of the peptides. Ligands $1aH_2$ and $1bH_2$ were synthesised according to literature.¹

1.2 Synthesis of the ligands



a)4–Chloro–2,6–bis[N,N–bis(tert–butoxycarbonylmethyl)aminomethyl] pyridine. To a solution of 4–chloro–2,6–bis(bromomethyl)pyridine (724 mg, 2.4 mmol) in anhydrous acetonitrile (30 mL) was added di–*tert*–butyl iminodiacetate (1.19 g, 4.8 mmol) then sodium carbonate (2.60 g, 24.5 mmol). The resulting suspension was stirred at room temperature for 24 hours then filtered. The solvent was evaporated under reduced pressure and the oily residue was taken up with 30 mL of chloroform. The solution was washed with water (2x15 mL), dried over MgSO₄ then concentrated to yield a yellow viscous oil (1.46 g, 96%). This material was used without further purification. R_f : 0.40 (silica, ethyl acetate/cyclohexane: 1/3). ¹H NMR (400 MHz, DMSO–d₆) δ (ppm): 7.53 (2H, s), 3.89 (4H, s), 3.41 (8H, s), 1.39 (18H, s). ¹³C NMR (100 MHz, DMSO–d₆) δ (ppm): 169.8, 160.8, 143.7, 120.4, 80.1, 58.8, 55.3, 27.6. IR (oil) v (cm⁻¹): 2978, 1740, 1570, 1369, 1219, 1148, 991, 853, 745. HRMS (ES+): calcd for C₃₁H₅₁N₃O₈C1 [M+H]⁺: 628.3365, found 628.3382.

b) 4–Azido–2,6–bis[N,N–bis(tert–butoxycarbonylmethyl)amino methyl]pyridine. To a solution of 4–chloro–2,6–bis[N,N–bis(tert–butoxycarbonylmethyl) aminomethyl]pyridine (1.50 g, 2.4

mmol) in anhydrous DMF (35 mL) was added NaN₃ (1.55 g, 24 mmol) then NBu₄HSO₄ (163 mg, 0.48 mmol). The resulting suspension was heated at 85°C for 8h and further stirred at room temperature during 16 h. Water (60 mL) was added before extraction with tertbutylmethylether (2×60 mL). The combined organic phases were dried over MgSO₄ and concentrated under reduced pressure. Silica gel chromatography (ethyl acetate: cyclohexane v/v 1: 4) afforded a colourless oil (1.51 g, 2.4 mmol, quantitative yield). Rf: 0.35 (ethyl acetate/cyclohexane 1/3). ¹H NMR (400 MHz, CDCl₃) δ (ppm): 7.22 (1H, s), 3.97 (2H, s), 2.04 (4H, s), 1.44 (18H, s). ¹³C NMR (100 MHz, CDCl₃) δ (ppm): 170.4, 161.1, 150.2, 111.2, 81.0, 59.6, 55.8, 28.1. IR (oil) v (cm⁻¹): 2978, 2114, 1740, 1593, 1369, 1223, 1146. c) 4-azido-2,6-bis[N,N-bis(carboxymethyl)aminomethyl] pyridine•4/3 trifluoroacetic acid (*TFA*). To a solution of 4–azido–2,6–bis[*N*,*N*–bis(tert–butoxycarbonylmethyl)aminomethyl] pyridine (120 mg, 0.19 mmol) in dichloromethane (2 mL) was added at 0°C trifluoroacetic acid (8 mL). The reaction mixture was stirred at this temperature for 2h then at room temperature for 20h. After evaporation of the solvent under reduced pressure then with a high vacuum pump, the residue was dissolved in water (20 mL) and lyophilized to furnish a white solid (115 mg, 100%). ¹H NMR (400 MHz, DMSO–d6) δ (ppm): 7.50 (2H, s), 4.16 (4H, s), 3.58 (4H, s). ¹³C NMR (100 MHz, D₂O) δ (ppm): 170.3, 156.1, 151.8, 115.1, 57.4, 55.7, IR (KBr) v (cm⁻¹): 3016, 2550, 2126, 1732, 1605, 1400, 1335, 1200, 895, 721. HRMS (ES+): calcd for $C_{15}H_{17}N_6O_8[M+H]^+$: 409.1108, found 409.1115. See SI 1.3 for the determination of TFA content, which may vary in different batches.

Synthesis of **2b**H₄·1.5 TFA.



a) 4–Azido–2,6–bis[*N*,*N*–bis(*tert*–butoxycarbonylmethyl) amino methyl] pyridine (538 mg, 0.85 mmol, 1.0 eq.) was dissolved in 10 mL of CH₂Cl₂ and the solution was degassed for 10 minutes by bubbling argon. Propargyl alcohol (150 μ L, 3.0 eq.) was added, then [CuCl(SIMes)(4,7-dichloro-1,10-phenan throline)]ⁱⁱ (30 mg, 0.046 mmol, 5 mol–%) and the reaction mixture was stirred for 1h. Concentration in vacuum to half volume followed by filtration over silica with diethyl ether furnished a colourless oil. (460 mg, 0.66 mmol, 78%). ¹H NMR (400 MHz, CDCl₃) δ (ppm): 8.32 (1H, s), 8.09 (2H, s), 4.90 (2H, s), 4.11 (4H, s), 3.50 (8H, s), 1.45 (36H, s). ¹³C NMR (100 MHz, CDCl₃) δ (ppm): 170.5; 161.8; 149.1; 144.7; 119.9; 111.0; 81.2; 59.7; 56.2; 55.9; 28.1. IR (KBr) v (cm⁻¹): 3433, 2978, 2931, 2877, 1732, 1597, 1369, 1222, 1145, 756. HRMS (ES+): calcd for C₃₄H₅₅N₆O₉: 691.4031, found 691.4029.

b) Trifluoroacetic acid (8 mL) was added at 0°C to a solution of 4–(4-hydroxymethyl-1,2,3-1*H*-triazol-1-yl)–2,6–bis[*N*,*N*–bis(tert–butoxycarbonyl methyl)aminomethyl]pyridine (207 mg, 0.3 mmol) in dichloromethane (2 mL). The reaction mixture was stirred at 0°C for 2h then at room temperature for 20h. After evaporation of trifluoroacetic acid, the residue was dissolved in water (20 mL) and lyophilized to afford a white solid (191 mg, quantitative

yield). ¹H NMR (400 MHz, DMSO–d6) δ (ppm): 8.79 (1H, s), 8.20 (2H, s), 4.65 (2H, s), 4.26 (4H, s), 3.72 (8H, s). ¹³C NMR (100 MHz, DMSO–d6) δ (ppm): 172.0; 159.9; 150.0; 144.6; 120.9; 111.6; 58.9; 55.0; 54.6. IR (KBr) v (cm⁻¹): 3097, 3012, 2970, 1736, 1670, 1612, 1408, 1250, 1195, 1141. HRMS (ES+): calcd for C₁₈H₂₁N₆O₉ [M+H]⁺: 465.1370, found 465.1372. *See below for the determination of TFA content.*

1.3 Determination of the molecular weight of **2a**H₄ x TFA and **2b**H₄ x TFA.

Since a variable amount of trifluoroacetic acid was noticed in the $2\mathbf{a}H_4$ and $2\mathbf{b}H_4$ samples after lyophilization, their apparent molecular weights were determined by ¹H NMR using 2,6–di*tert*butyl–4–methylphenol (M = 220.3 g·Mol⁻¹) as internal standard. The molecular weight M was determined using equation (1)

 $M = M_{\text{std}} \times (I_{\text{std}}/I) \times (m/m_{\text{std}}) (1)$

where M, m and I are: the molecular weight, the measured weight and the aromatic proton signal integral, respectively.

Experimental procedure. e.g. for $2aH_4$: 10.2 mg of the standard 2,6–ditertbutyl–4– methylphenol and 26.2 mg of $2aH_4$ were dissolved in 1 mL of DMSO–d₆. The ¹H NMR spectrum was recorded and the aromatic signals integrals measured. The average of three independent experiments afforded an integrals ratio of 0.945 which resulted in an apparent molecular weight of 601±3 g·Mol⁻¹. This corresponded to the presence of 1.67 equivalents of trifluoroacetic acid.

1.4 Synthesis of activity-based probes 5, 6.

We synthesized the ABP 5 using typical Fmoc-Solid Phase Peptide Synthesis (SPPS). Besides the commercially available amino acids Fmoc-Leu-OH and Fmoc-Tyr(O-tBu)-OH synthesis of 5 required another three units: the alkyne modified glutamic acid 4, an epoxide moiety and a tetraethylene glycol spacer. Thus, compound 4 was obtained in a two-step sequence starting from Fmoc-Glu(O-*t*Bu)-OH by an amide coupling reaction with propargyl HBTU/HOBt (HBTU: O-benzotriazole-N,N,N',N'amine in presence of tetramethyluroniumhexafluoro phosphate; HOBt : N-hydroxybenzotriazole), as activating agents and DIPEA (N,N-diisopropyl ethylamine) as tertiary base followed by TFA mediated deprotection of the *tert*-butyl ester. The epoxide unit (ethyl (2R,3R)-trans-2,3epoxysuccinateⁱⁱⁱ) and the ethylene glycol spacer (14-N-Fmoc-amino-3,6,9,12-tetraoxa-1tetradecanoic acid^{iv}) were obtained as previously described.



Synthesis of ABP 5. alkyne-modified glutamic acid 4.

tert-butyl 2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-5-oxo-5-(prop-2-yn-1a) ylamino)pentanoate. To a solution of Fmoc-Glu-OtBu (1.00 g, 2.35 mmol) in anhydrous DMF, under argon, HBTU (872 mg, 2.30 mmol), HOBt (311 mg, 2.30 mmol) and DIPEA (910 mg, 7.06 mmol) were added and allowed to stir for five minutes, at room temperature. Propargyl amine (129.4 mg, 2.35 mmol) was then added and the reaction mixture was stirred at room temperature overnight. DMF was removed under vacuum and the oily residue was dissolved in dichloromethane (20 mL) and washed with water (2 x 20 mL). The organic phase was separated, dried over MgSO₄, and evaporated. The residue was purified by column chromatography (silica gel, pentane/ethyl acetate v/v 1:1, Rf=0.54) to yield a pure, white product (1.01 g, 94 %). ¹H NMR (400 MHz, CDCl₃) δ (ppm): 7.77 (d, J = 7.3 Hz, 2H), 7.60 (d, J = 7.3 Hz, 2H), 7.41 (t, J = 7.3 Hz, 2H), 7.32 (t, J = 7.3 Hz, 2H), 6.20 (brs, 1H), 5.53 (br. d, 1H), 4.52-4.35 (d, J = 6.4 Hz, 2H), 4.22 (overlapped peaks, 2H), 4.04 (dd, J = 4.8, 2.3 Hz, 2H), 2.22 (overlapped peaks, 4H), 1.99 - 1.80 (m, 1H), 1.47 (s, 9H). ¹³C NMR (126 MHz, CDCl₃) δ (ppm): 171.7, 171.0, 156.5, 143.9, 143.6, 141.4, 141.3, 127.8, 127.1, 125.1, 120.1, 82.7, 79.5, 71.6, 67.0, 53.8, 47.2, 32.3, 29.3, 28.0. HRMS (ES+, Orbit Trap) Calcd for $C_{27}H_{31}N_2O_5$ [M+H]⁺: 463.2227, found 463.2210; $C_{27}H_{30}N_2NaO_5$ [M+Na]⁺: 485.2047, found 485.2028; C₂₇H₃₀N₂KO₅ [M+K]⁺: 501.1786, found 501.1765.

b) 2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-5-oxo-5-(prop-2-yn-1-ylamino)pentanoic acid **4.** To a solution of tert-butyl 2-((((9H-fluoren-9-yl)methoxy)-carbonyl)amino)-5-oxo-5-(prop-2-yn-1-ylamino)pentanoate (1g, 2.16 mmol) in DCM (10 mL), trifluoroacetic acid (2 mL) and water (0.5 mL) were added and the reaction was allowed to stir for 3h at room temperature. The solvent was removed under vacuum and the reaction mixture was triturated

with diethyl ether. The precipitate was filtered to yield pure **4** (854 mg, 97 %). ¹H NMR (400 MHz, CDCl₃) δ (ppm): 7.77 (d, J = 7.2 Hz, 2H), 7.61 (d, J = 7.2 Hz, 2H), 7.40 (t, J = 7.2 Hz, 2H), 7. 2 (t, J = 6.9 Hz, 2H), 6.43 (br., 1H), 5.85 (br., 1H), 4.40 (d, J = 6.6 Hz, 2H), 4.35-4.27 (m, 1H), 4.22 (t, J = 6.6 Hz, 1H), 4.04 (s, 2H), 2.50 – 2.13 (m, 4H), 2.06 (m, 1H). ¹³C-NMR (126 MHz, DMSO-d₆) δ (ppm): 173.9, 171.3, 156.4, 144.1, 141.0, 127.9, 127.4, 125.6, 120.4, 81.5, 73.2, 65.9, 53.7, 46.9, 31.7, 28.1, 26.8. HRMS (ES+, Orbit Trap) Calcd for C₂₃H₂₃N₂O₅ [M+H]⁺: 407.1601, found 407.1606; C₄₆H₄₅N₄O₁₀ [2M+H]⁺: 813.3130, found 813.3152; C₆₉H₆₇N₆O₁₅ [3M+H]⁺: 1219.4659, found 1219.4691.

c) Activity Based Probe 5.

Compound 5 was synthesized by standard protocol for Fmoc Solid-Phase Peptide Synthesis using Rink amide MBHA resin (150 mg, loading capacity 0.7 mmol/g). The Fmoc protecting groups were removed by treatment with a 20% piperidine solution in DMF for 10 min twice. The amino acids were sequentially added using four equivalents of the amino acid with respect to the loading capacity and a ratio amino acid/HBTU/HOBt/DIPEA of 1:0.98:0.98:3 in DMF, except for the alkyne-modified glutamic acid and the tetraethyleneglycol-modified amino acid which were used only as 2 equivalents with respect to the resin loading capacity. Ethyl (2R,3R)-trans-2,3-epoxysuccinate (2 equiv) was coupled with DIC/HOBt (2:2 equiv) in DMF. The coupling reactions were quantitative between 1 to 3 hours as checked by qualitative Kaiser Tests. The resin was washed with DMF, MeOH and CH₂Cl₂. The peptide was cleaved from the resin using 1 mL of cleavage cocktail (95% TFA, 2.5% water, 2.5% triisopropylsilane) and allowing to shake for 2 hours. Ice cold ether was used to precipitate the product, which was analyzed by RP-C₁₈-HPLC column using a linear gradient of 20-45% acetonitrile/water(0.1% TFA) for 15 minutes $R_f = 13.50$ min, detection at $\lambda = 230$ nm and 280 nm, and purified on a corresponding preparative $RP-C_{18}$ -HPLC column, using the same gradient. Fractions containing the product were pooled, frozen and lyophilized to dryness to obtain 16.7 mg (17%) of pure peptide 5. The identity of the product was confirmed by HRMS. HRMS (ES+, Orbit Trap) calcd. for $C_{45}H_{70}N_7O_{15}$ [M+H]⁺: 948.4924, found: 948.4941; C₄₅H₆₉N₇NaO₁₅ [M+Na]⁺: 970.4744, found 970.4741; C₄₅H₆₉N₇KO₁₅ [M+K]⁺: 986.4483, found 986.4471.

Synthesis of ABP 6.

600 μ L solution of ligand **1a**H₂ (2.00 mg, 9.71 μ mol) in methanol/water 1:1 and 400 μ L solution of peptide **5** (8.00 mg, 8.45 μ mol) in acetonitrile/water 2:8 were added in a 5 mL flask, under argon. Aqueous solutions of tris(3-hydroxypropyl triazolylmethyl)amine (THPTA) ligand (2.5 μ L, 1mM), CuSO₄ (2.5 μ L, 1mM) and sodium ascorbate (7.5 μ L, 1mM) were sequentially added and the reaction mixture was stirred for 2h, at room temperature. The reaction was monitored by HPLC. The solvents were removed under vacuum and the product was purified by preparative RP-C₁₈-HPLC using the same gradient elution method as for compound **5**, R_t = 14.23 min to obtain **6** in quantitative yield. HRMS (ES+, Orbit Trap) calcd. for C₅₂H₇₄N₁₁O₁₉ [M+H]⁺: 1156.5157, found: 1156.5151; C₅₂H₇₃N₁₁NaO₁₉ [M+Na]⁺: 1178.4976, found: 1178.4971; C₅₂H₇₃N₁₁KO₁₉ [M+K]⁺: 1194.4711, found: 1194.4711.



1.5 RP-HPLC chromatograms of 5 and 6

Figure S1. RP-C18-HPLC chromatograms of ABPs **5** (top) and **6** (bottom). UV detection at 230 nm. Linear gradient of 20-45% acetonitrile/water (0.1% TFA) for 15 minutes.

SI 2 - NMR study of yttrium(III) complexes

As a preliminary experiment, the SPAAC reaction between the diamagnetic yttrium(III) complex of **2a** and BCN was evaluated by ¹H NMR (Figure below). A fast and quantitative formation of the expected triazole occurred as inferred from the spectral modification – *e.g.* the shift of the pyridinyl protons (6.95 *vs* 7.43 ppm).





Figure S2. ¹H NMR spectra (400 MHz, D_2O : DMSO–d₆ v/v 1 : 1, 298K) of [Y(2a)]⁻, (top) and [Y(2c)]⁻, (bottom), 10⁻²M. * signal due to SPAAC of minor *endo* isomer of BCN.

a) 6.0 mg of $2aH_4$ (10⁻⁵ mol, 1.0 eq.) and 4.2 mg of sodium carbonate (4×10⁻⁵ mol, 4.0 eq.) were dissolved in 500 µL D₂O. 3.3 mg of YCl₃.6H₂O (1.1×10⁻⁵ mol, 1.1 eq.) were added, followed by 500 µL DMSO-*d*₆.

¹H and ¹H, ¹³C HSCQ NMR spectra were recorded. The AB system between 3.4 and 3.1 ppm is typical for methylene protons embedded in a chelate cycle.

b) Starting from the above solution, 1.7 mg of bicyclo[6.1.0]non-4-yn-9-ylmethanol (BCN) (1.13 mmol, 1.13 eq.) were added. The sample was vigorously shaken to dissolve BCN and the reaction was allowed to proceed for 5 minutes.

¹H, ¹H, ¹H–COSY ¹H, ¹³C–HSCQ spectra were recorded. The spectra show a total disappearance of signals associated to the starting material.



Figure S3. ¹H NMR spectrum of $[Y(2a)]^-$ recorded at 298 K. Chemical shifts referenced to DMSO- d_6 signal.



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Figure S4. ¹H NMR spectrum of $[Y(2c)]^-$ recorded at 298 K. Chemical shifts referenced to DMSO-*d*₆ signal. Less intense signals belong to excess unreacted cyclooctyne reagent and to $[Y(2c)]^-$ complex bearing *endo* cyclopropanol groups (commercial BCN containing a small amount of *endo*– species).



Figure S5. ¹H, ¹H–COSY spectrum of $[Y(2c)]^{-}$.



Figure S6. ¹H, ¹³C–HSCQ spectrum of $[Y(2c)]^-$.

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Table S2. NMR assignments of $[Y(2c)]^{-}$ (COSY, HSQC).



position(s)	¹ H NMR (δ / ppm) ¹³ C NMR (δ / ppm)			
h	3.30-3.13 (m, 2H)	66.6		
g	0.57 (m, 1H)	27.9		
f	0.90 (m, 2H)	22.5		
0	2.25(m, 2H),	27.2		
e	1.33 (m, 2H),	27.5		
	2.94 (ddd, 2H, ${}^{3}J = 4.0$ Hz,	25.5		
	$^{3}J = 7.5$ Hz, $^{2}J = 15.5$ Hz),			
	2.75 (ddd, 2H, ${}^{3}J = 3.9$ Hz,			
	$^{3}J = 8.9$ Hz, $^{2}J = 16.2$ Hz)			
d				
u	2.83 (ddd, 2H, ${}^{3}J = 3.3$ Hz,	23.7		
	$^{3}J = 6.4$ Hz, $^{2}J = 16.7$ Hz)			
	2.63 (m, 2H)			
С	7.43 (s, 2H)	119.0		
b	3.93 (s, 2H)	63.1		
a	$3.39 (d, {}^2J = 16.5 Hz),$	63.3		
C.	$3.25 (d, {}^{3}J = 16.5 Hz)*$	00.0		
*overlapped signals				

SI 3- Luminescence measurements

3.1 Instrumental parameters

Luminescence spectra were recorded in the phosphorescence mode with a Cary Eclipse fluorimeter. The spectra were recorded in 1 cm (or 0.2 cm for quantum yield determination) luminescence cells. The following parameters were used: gate time 1 ms, delay: 0.1 ms, total decay time 20.0 ms. The slits were set to 5/2.5 nm (excitation/emission) for complexes with 1a, 1b and 1c ligands and to 5/5 nm for the lanthanide(III) complexes with 2a, 2b and 2c ligands. The lifetimes were determined by recording the luminescence intensity at the maximum emission wavelength while incrementing the delay by 0.02 ms steps with a number of measurements adjusted to have the final delay > $3 \times \tau$.

Kinetic measurements were performed by recording each minute the luminescence intensity using optimum excitation and emission wavelengths of $[Ln(2c)]^{-}$ complexes. The solutions for luminescence measurements were prepared immediately before use as described below.

3.2 Preparation of lanthanide(III) complexes solutions

Lanthanide stock solutions

Lanthanide(III) salts solutions, used as stock solutions for luminescence experiments, were prepared from the corresponding chloride salts (EuCl₃ \cdot 6H₂O, TbCl₃ \cdot 6H₂O) in distilled H₂O. Their exact concentration was obtained by volumetric titration with a solution of EDTA (5 mM, Fisher Chemicals) in acetate buffer 0.1 M, pH = 4.5, using xylenol orange as colorimetric indicator.

Preparation of $[Ln(1b)_3]^{3-}$ solutions

 10^{-4} M [Ln(1b)₃]³⁻ solution was prepared by adding to a 100 mL volumetric flask 10^{-5} mol of EuCl₃ or TbCl₃ (1 mL of 10^{-2} M stock solution), 6×10^{-5} mol **1b**H₂ and Tris-HCl buffer 0.1 M, pH = 7.5 quantum satis.

Preparation of $[Ln(1a)_3]^{3-}$ and $[Ln(1c)_3]^{3-}$ solutions $[Ln(1a)_3]^{3-}$: 10⁻⁵ mol of LnCl₃ (0.1 mL of a 10⁻¹ M stock solution), 1aH₂·H₂O·HCl (15.6) 6×10^{-5} mol, 6.0 eq.), 12.7 mg (1.2×10⁻⁴ mol, 12.0 eq.) of sodium carbonate and 500 µL H₂O were mixed and stirred in a centrifugal vial. Then, the resulting clear solution (concentration 10^{-2} M) was diluted in 0.1 M Tris-HCl buffer for luminescence measurements.

 $[Ln(1c)_3]^{3-}$: 2.64×10⁻⁵ mol of BCN (4.0 mg, 1.1 eq. per azide function) were dissolved in 200 μ L DMSO. This was added to 200 μ L of a [Ln(1a)₃]³⁻ solution prepared as above in a centrifugal vial. After ten minutes, the solution was diluted in 0.1 M Tris-HCl buffer for luminescence measurements.

Preparation of $[Ln(2b)]^{-}$ solutions

Having determined the apparent molecular weight for a given batch of 2bH₄, we prepared a stock solution. Then, the appropriate volumes of Ln^{III} salts and **2b**H₄ stock solutions as well as Tris-HCl buffer (0.1 M, pH = 7.5) were added to prepare 3 mL solution, which was used for luminescence measurements.

Preparation of $[Ln(2a)]^{-}$ and $[Ln(2c)]^{-}$ solutions

A stock solution of $2aH_4$ was prepared. 10^{-5} mol of EuCl₃ or TbCl₃ (0.1 mL of a 10^{-1} M stock solution), $2aH_4$ (7.2 mg, 1.2×10^{-5} mol, 1.2 eq.), 42.4 mg (4×10^{-4} mol, 4.0 eq.) of sodium carbonate and 500 µL H₂O were mixed and stirred in a centrifugal vial. Then, the resulting clear solution was diluted in 0.1 M Tris-HCl buffer for luminescence measurements (total volume: 3 mL).

 1.32×10^{-5} mol of BCN (2.0 mg, 1.1 eq. per azide function) were dissolved in 500 µL DMSO. This was added to 500 µL [Ln(2a)]⁻ solution prepared as above. After ten minutes, the solution was diluted in 0.1 M Tris-HCl buffer for luminescence measurements (total volume: 3 mL).

3.3 Additional luminescence spectra



Figure S7. $\mathbf{Ln^{III}} = \mathbf{Tb^{III}}$ (see Figure 2 in the paper for $\mathbf{Ln^{III}} = \mathbf{Eu^{III}}$). Overlay of the excitation and emission spectra for $[\mathbf{Ln}(\mathbf{1a})_3]^{3-}$, $[\mathbf{Ln}(\mathbf{1b})_3]^{3-}$ and $[\mathbf{Ln}(\mathbf{1c})_3]^{3-}$ complexes $(\mathbf{Ln^{III}} = \mathbf{Eu^{III}}, \mathbf{Tb^{III}})$. $[\mathbf{Ln^{III}}] = 10^{-4} \text{ mol } \mathbf{L}^{-1} \ [\mathbf{1a}]_{tot}/[\mathbf{Ln^{III}}]_{tot} = \ [\mathbf{1b}]_{tot}/[\mathbf{Ln^{III}}]_{tot} = \ [\mathbf{1c}]_{tot}/[\mathbf{Ln^{III}}]_{tot} = 6$. Tris-HCl buffer 0.1 M, pH = 7.5.



Figure S8. $\mathbf{Ln^{III}} = \mathbf{Eu^{III}}$ (see Figure 2 in the paper for $\mathbf{Ln^{III}} = \mathbf{Tb^{III}}$). Overlay of the excitation and emission spectra for $[\mathbf{Ln(2a)}]^-$, $[\mathbf{Ln(2b)}]^-$ and $[\mathbf{Ln(2c)}]^-$ complexes $[\mathbf{Ln}] = 10^{-4} \text{ mol } \mathbf{L^{-1}}$ $[\mathbf{2a}]_{tot}/[\mathbf{Ln^{III}}]_{tot} = [\mathbf{2b}]_{tot}/[\mathbf{Ln^{III}}]_{tot} = [\mathbf{2c}]_{tot}/[\mathbf{Ln^{III}}]_{tot} = 1.2$. Tris-HCl buffer 0.1 M, pH = 7.5.

3.4 Speciation for for Ln-dpa systems.

The speciation diagram presented in figure S9 was computed using published^v stability constants for the europium-dipicolinate system. This diagram shows that for $[Ln] = 10^{-4}$ mol and L^{-1} and [dpa]/[Ln] = 6, the trischelated species $[Ln(dpa)_3]^{3-}$ is virtually the only one present in solution. The modification induced by the triazole functionalization is expected to have only a minimal effect on the speciation in solution. This was verified by the observation of strictly monoexponential decays for all $[Ln(1a,b,c)]^{3-}$ complexes with [1a,b,c]/[Ln] = 6. Hence, the ratios *R* of luminescence intensity described in this paper refer to the trischelated complexes and are to the best confidence unaffected by differential speciation effects, *ie*. presence of mixture of bischelated and trischelated species.



Figure S9. Speciation diagram obtained using HySS 2009 software^{vi} with stability constants reported by Bünzli et al.with $[Eu^{III}] = 10^{-4}M$.

3.5 Luminescence Decays

All decays, presented in Figures S10-S21 (recorded on the same samples as the spectra shown in Figures 1, S7 and S8) are perfectly monoexponential as shown by linear $\ln(I) = f(t)$ plots. The slope is $-1/\tau$.















Figure S15. Luminescence decay for $[Tb(1c)_3]^{3-}$



Figure S16. Luminescence decay for [Eu(2a)]⁻







Figure S18. Luminescence decay for $[Eu(2c)]^{-}$





3.6 Determination of the metal-bound water molecules number for $[Ln(2a,b,c)(H_2O)_q]$ and $[Ln(1a,b,c)_3(H_2O)_q]$ complexes

The lanthanide(III) complexes solutions (containing 1.2 equiv. of **2a** or **2b** ligand or 6.0 equiv. of 1a or 1b) were prepared at $10^{-3} \text{ mol} \cdot \text{L}^{-1}$ in 1 M Tris-HCl and further diluted to $10^{-4} \text{ mol} \cdot \text{L}^{-1}$ using variable proportions of H₂O and D₂O. In the case of **2c**, the complexes were obtained *in situ* starting from [Ln(**2a**)]⁻ as described in SI3.2 and diluted similarly. The plot of $1/\tau$ versus H₂O percentage is linear with a y-intercept equal to $1/\tau$. Sample data for [Tb(**2b**)]⁻ and [Eu(**2b**)]⁻ is shown in figures S22 and S23.

The empirical Beeby-Parker formulas were then used to determine the lanthanide-bound water molecules number. vii

For europium(III) : $q_{\text{H2O}} = 1,2 \times (1/\tau_{\text{H2O}} - 1/\tau_{\text{D2O}} - 0,25)$

For terbium(III) : $q_{\text{H2O}} = 5 \times (1/\tau_{\text{H2O}} - 1/\tau_{\text{D2O}} - 0,06)$







3.7 Quantum yield determination

Solutions of the europium-dpa, europium-**1a** and europium-**1b** at 7.5 10^{-5} mol·L⁻¹ and terbium-dpa, terbium-**1a** and terbium-**1b** at 6.5 10^{-5} mol·L⁻¹ were prepared in Tris-HCl buffer 0.1 M, pH = 7.5 using 250 mL volumetric flasks by weighing the appropriate amount of ligand and adding lanthanide stock solutions to obtain a 1:3 lanthanide/ligand stoichiometry. In this case, a mixture of bischelated and trischelated species is present in solution. 500 µl of each of these solutions was transferred to a fluorescence semi-micro cuvette (500 µL, 0.2 cm optical path length).

Solutions of lanthanide complexes of **2a** and **2b** were prepared directly in the same cuvette by adding the appropriate volumes of lanthanide, ligand stock solutions as well as Tris-HCl buffer 0.1 M, pH = 7.5 to obtain $1.0 \cdot 10^{-4} \text{ mol} \cdot \text{L}^{-1}$ solutions of $[\text{Ln}(2a)]^{-1}$ or $[\text{Ln}(2b)]^{-1}$ complexes (1:1 lanthanide/ligand stoichiometry).

For all these solutions, UV absorption spectra and emission spectra using $\lambda_{exc} = 279$ nm were recorded (figures below).



Figure S24. Electronic absorption spectra of europium(III)+ligand solutions used for quantum yield determination. Absorbance at 279 nm is highlighted.

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Figure S25. Electronic absorption spectra of terbium(III)+ligand solutions used for quantum yield determination. Absorbance at 279 nm is highlighted.



Figure S26. Emission spectra of europium (III)+ligand solutions used for quantum yields determination.



Figure S27. Emission spectra of terbium (III)+ligand solutions used for quantum yields determination.

Each quantum yield (Q) was determined by applying equation $(2)^{viii}$

 $Q = Q_{ref} \times Area / Area_{ref} \times A_{279(ref)} / A_{279}$

(2)

Where:

-Area and Area_{ref} are the sums of emission intensity over the whole spectrum for the species of interest and reference, respectively.

-A $_{279}$ and A $_{279(ref)}$ are the absorbances at 279 nm for the species of interest and reference, respectively

- Q_{ref} is the known quantum yield for the dpa complex of europium(III) or terbium(III)

	dpa (ref)	1a	1b	2a	2b		
Eu							
Em. Spectrum	833.9	12.66	848.9	8.91	97.8		
Area							
A ₂₇₉	0.176	0.287	0.437	0.204	0.315		
Q	0.24	0.0022	0.098	0.0022	0.016		
Tb							
Em. Spectrum	17848	9.96	7553	229.8	3087		
Area							
A ₂₇₉	0.164	0.249	0.307	0.208	0.238		
Q	0.22	0.0001	0.050	0.0022	0.026		

Table S3. Data used for quantum yields determination.



3.8 Kinetic survey of the formation of $[Tb(2c)]^{-}$

Figure S28. SPAAC reaction between $[Tb(2a)]^-$ and BCN (C=10⁻⁴M) in Tris-HCl buffer (pH = 7.5); Right: yield versus time; Left: plot for second–order rate constant

SI 4- Preparative BSA labeling

4.1 BSA labeling procedure with BCN

1.0 mg of activated BCN (Synafix, BCN–POE₃–NH–C(O)CH₂CH₂CH₂C(O)OSu, 1.87 μ M) was dissolved in 0.5 mL of DMF. Bovine Serum Albumin (BSA) (5.0 mg) was dissolved in 2.0 mL of borate buffer (pH = 7.9). The BCN solution was added dropwise to the protein at 0°C over one hour (if precipitation occurred during the addition, a few drops of DMF and borate buffer were added). The reaction was stirred overnight at room temperature then dialyzed (membrane cut–off 10kDa) against water and lyophilized to dryness to afford 5.8 mg of labeled protein.

4.2 MALDI-TOF-MS analysis

MALDI–TOF mass spectrum of BCN-BSA conjugate show the molecular ion peak at m/z= 74.1 kDa for the modified BSA corresponding to a mass increase of 7.5 kDa (as compared to BSA: 66.5 kDa). This corresponded to the addition of 18 cyclooctynes per BSA.



Figure S29. MALDI-TOF mass spectrum of BCN-BSA conjugate showing: a monocharged species at m/z = 74.1 kDa, a doubly charged species at m/z = 37.3 kDa and a triply charged species at m/z=24.8 kDa.

SI 5 Papain labeling

5.1 General procedure for lanthanide complexation of peptide 6 and papain-6 conjugate. 36 μ L solution of **6** (2 mM) in HEPES buffer (50 mM, pH=7, containing 5% DMSO) was treated with 7.2 μ L of terbium(III) nitrate (10 mM) or europium(III) chloride (10 mM). The reaction was adjusted with buffer (6.8 μ L) to a final volume of 50 μ L and kept at room temperature for 1 h. The complexes were used in reactions with papain without isolation. A similar protocol was followed for lanthanide complexation of papain-6 conjugate with typical reactions involving 10 μ L papain-6 conjugate (150 μ M total protein concentration, and approximate 50 μ M papain-6 conjugate concentration) in HEPES buffer (50 mM, pH=7) and 1 μ L terbium(III) nitrate (1.5 mM) or europium(III) chloride (1.5 mM) in HEPES (50 mM pH=7). The reactions were analysed by SDS-PAGE (12.5 % gel).

5.2 General procedure for the reaction between papain and peptide 5, peptide 6 or lanthanide complexes of 6. 25 μ L Papain (250 μ M total protein concentration, 75 μ M active papain concentration) was added to 11 μ L reaction buffer (HEPES 50 mM, DTT (dithiothreitol) 5 mM, pH=6.8) and incubated for 30 min. at room temperature Peptide 5 (4 μ L, 1.8 mM) in DMSO : water (v/v) 1 : 4 was added and the reaction was kept at room temperature until no residual activity of papain was observed by UV-Vis measurements, using p-Glu-Phe-Leu-p-nitroanilide as chromogenic substrate (2 h). The reaction was filtered through a Microcon® YM-10 centrifugal filter (10 kDa MWCO) by centrifugation (14 000 rpm for 4 min) followed by three time washing with double distilled H₂O. The resulting reaction mixture was analyzed by SDS-PAGE (12.5 % gel).

A similar protocol was followed for the reactions of papain with peptide **6** as well as with terbium(III) or europium(III) complexes of **6** using 25 μ L papain (250 μ M total concentration, active papain concentration 75 μ M) and 4 μ L peptide **6** (1.8 mM) or 5 μ L of lanthanide complexes of **6** (1.44 mM), in DMSO : water (v/v) 1 : 4, in a total reaction volume of 40 μ L.

5.3 General procedure for the click reaction between ligand 1aH_2 (or complexes [Ln(1a)_3]^3) and the alkyne-modified papain (papain-5 conjugate). 20 µL papain-5 conjugate (160 µM total protein concentration and approximately 48 µM papain-5 conjugate concentration) and 1 µL ligand 1aH_2 (3.2 mM) was added to 26.5 µL HEPES buffer (50 mM, pH=7). Aqueous solutions of CuSO₄ (1 µL, 3.2 mM), THPTA ligand (1 µL, 3.2 mM) and sodium ascorbate (1 µL, 9.6 mM) were then added. The reaction was allowed to rest at room temperature for 1 h, filtered through a Microcon® YM-10 centrifugal filter (10 kDa MWCO) by centrifugation (14 000 rpm for 4 min) followed by three times washing with ddH₂O and analysis by SDS-PAGE (12.5 % gel).

A similar protocol was followed for the reaction between complexes $[Ln(1a)_3]^{3-}$ and papain-5 conjugate, with typical reactions involving 20 µL Papain-5 conjugate (160 µM total protein concentration and approximate 48 µM papain-5 conjugate concentration) and 1 µL solution of $[Ln(1a)_3]^{3-}$ complexes (3.2 mM) in a total reaction volume of 50 µL.

5.4 Sensitivity of papain detection

We performed labeling of papain (30% active, as determined by E-64 active-site titration^{ix}) increasing concentrations (from 61.2 ng/ μ L to 612 ng/ μ L total protein concentration) with ABP **6**. Since the ABPs are known to react only with active protein, ^xthe amount of protein that can be theoretically labeled range from 18.4 ng/ μ L to 1.84 μ g/ μ L. Reaction products were resolved on SDS-PAGE and visualised under UV light (Figure **S29**, top) or Coomassie blue staining (Figure **S29**, bottom). The results show that papain could be efficiently detected

down to $0.184 \ \mu g$ active protein. Noteworthy, these results were obtained by simple nakedeye visualisation under UV light.



Figure S30. Sensitivity of **6** for papain detection. Top: Visualization under UV light (254 nm) after staining with 5 μ M Tb^{III} in HEPES buffer (50 mM), pH=7. Bottom: Coomassie blue staining. Lanes as follow 1) papain (0.184 μ g active protein)+**6**, 2) papain (0.368 μ g active protein)+**6** 3) papain (0.552 μ g active protein)+**6**, 4) papain (0.736 μ g active protein)+**6**, 5) papain (0.92 μ g active protein)+**6**, 6) papain (1.104 μ g active protein)+**6**, 7) papain (1.472 μ g active protein)+**6**, 8) papain (1.84 μ g active protein)+**6**.

5.5 Labeling of papain in *E. Coli* **BL21 cell lysates.** The same experiments as above (reaction of active papain with terbium(III) complex of **6** and click reaction of papain-**5** conjugate with $[Tb(1a)_3]^{3-}$) were performed in presence of constant amounts of *E. Coli* BL21 cell lysates in reaction mixtures.

a) 10 μ L papain (30 μ M, 45 μ M and 60 μ M respectively, total protein concentration), previously activated by 30 min. incubation in HEPES buffer (50 mM, pH=7) containing 5 mM DTT, was added to 20 μ L *E. Coli* lysate. The reaction mixture was treated with 2 μ L terbium(III) complex of **6** (173 μ M, 260 μ M and 390 μ M, respectively) and the reaction was adjusted with buffer (18 μ L) to a final volume of 50 μ L. After 1 hour incubation at room temperature the reactions were analyzed by SDS-PAGE (12.5 % gel).

b) 10 μ L papain-5 conjugate (30 μ M, 45 μ M and 60 μ M respectively, total protein concentration) was added to 20 μ L *E. Coli* lysate and treated with 1 μ L of $[Tb(1a)_3]^{3-}$ (300 μ M, 450 μ M and 600 μ M respectively), 1 μ L CuSO₄ (300 μ M), 1 μ L THPTA ligand (300 μ M) and 1 μ L sodium ascorbate (1.5 mM) as described above. The reaction mixture was adjusted with buffer (16 μ L) to a final volume of 50 μ L and kept at room temperature for 1 h, then analyzed by SDS-PAGE (12.5 % gel).

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