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**Electronic Supporting Information**

# Amido/alkoxy-aryl-aryl-picolinate push-pull antennas for long-wavelength sensitization of $Eu^{3+}$ luminescence

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## Content

## Abbreviations

Boc: *tert*-butyloxycarbonyl; DCM: dichloromethane; DIEA: *N*,*N*-diisopropyl-ethylamine; DMAP: *N*,*N*-dimethylaminopyridine; dppf: 1,1'-*bis*(diphenylphosphino)ferrocene; ESI: electrospray ionization; Fmoc: 9-fluorenyl-methoxycarbonyl; HCTU: *O*-(1*H*-6-chlorobenzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; HEPES: 2-(4-(2-hydroxyethyl)piperazin-1-yl)ethanesulfonic acid; HRMS: high resolution mass spectrometry; LRMS: low resolution mass spectrometry; MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide ; PyBOP: (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophos-phate; SPPS: solid-phase peptide synthesis; TEA: triethylamine.

## Materials and methods

**Reagents and solvents:** N-α-Fmoc-protected amino acids for peptide synthesis, HCTU and PyBOP coupling reagent and NovaPEG Rink Amide resin were purchased from Novabiochem or Iris Biotech. Other reagents for peptide synthesis, solvents, buffers and metal salts were purchased from Sigma-Aldrich. All buffer or metal solutions for spectroscopic measurements were prepared with ultrapure water produced by a Millipore Milli-Q purification system (purified to 18.2 MΩ.cm). Buffer solutions were treated with Chelex 100 resin (Bio-Rad) to remove trace metal ions.

**HPLC purifications:** Analytical HPLC/LRMS analyses were performed on an Agilent Infinity 1260 II system equipped with a 6125 MS (ESI) detector using a Waters XBridge BEH130 C18 (2.5 µm, 75 mm × 4.6 mm). Preparative HPLC separations were performed on a VWR LaPrepΣ system using Waters XBridge Peptide BEH130 C18 (5 µm, 150 mm × 19 mm) or Waters XBridge Peptide BEH130 C18 (5 µm, 150 mm × 10 mm) columns at flow rates of 14 or 6 mL/min, respectively. The mobile phase consisted of a gradient of solvent A (0.1 % TFA in $H_2O$) and B (0.1 % TFA in MeCN/$H_2O$ 9:1). For analytical separations, Method B consisted of 5% B during 1 min followed by a 5 to 100 % B gradient in 13 min at 1 mL/min. The eluate was monitored by electronic absorption at 214, 280 and 331 nm as well as by LRMS (ESI+) detection.

**NMR spectroscopy:** $^{1}H$, $^{13}C$ and DEPT NMR spectra were recorded at 400 MHz on a Varian Avance III 400 spectrometer at 298 K unless specified. Coupling constants ($J$) are measured in hertz and the chemical shift ($\delta$) are measured in ppm. All chemical shifts for $^{1}H$ and $^{13}C$ spectra were referenced to the residual solvent peak ($CDCl_3$ $\delta_H$ = 7.26 ppm and $\delta_C$ = 77.2 ppm; $CD_3OD$ $\delta_H$ = 3.31 ppm and $\delta_C$ = 49.0 ppm; $(CD_3)_2CO$ $\delta_H$ = 2.05 ppm and $\delta_C$ = 29.8 ppm; $(CD_3)_2SO$ [D6]-DMSO $\delta_H$ = 2.50 ppm and $\delta_C$ = 39.5 ppm. The following abbreviations are for peak multiplicities: s (singlet), d (doublet), t (triplet), m (multiplet), and br (broad peak(s)).

**Mass spectrometry:** LRMS(ESI) analyses were performed on a Thermo Scientific LXQ spectrometer. HRMS (ESI) were performed on a Thermo Scientific LTQ Orbitrap XL spectrometer with electrospray ionization.

**Optical spectroscopy:** UV-Vis absorption spectra were recorded on a Perkin-Elmer Lambda 35 spectrophotometer or on a Varian Cary 50 spectrometer, both equipped with a thermo-regulated cell holder. Luminescence spectra were measured on a Varian Cary Eclipse spectrometer equipped with a thermo-regulated cell holder or on a modular Fluorolog FL3-22 spectrometer from Horiba-Jobin Yvon-Spex equipped with a double-grating excitation monochromator and an iHR320 imaging spectrometer coupled to Hamamatsu R928P and Hamamatsu R5509 photomultipliers for visible and NIR detection, respectively. Emission spectra were corrected for wavelength-dependent detector response. Time-gated $Ln^{3+}$ luminescence spectra were acquired with 100 µs time delay and 2 ms gate time on the Varian Cary Eclipse spectrometer. $Ln^{3+}$ luminescence lifetimes were measured using the Varian Cary Eclipse spectrometer.

## Synthesis

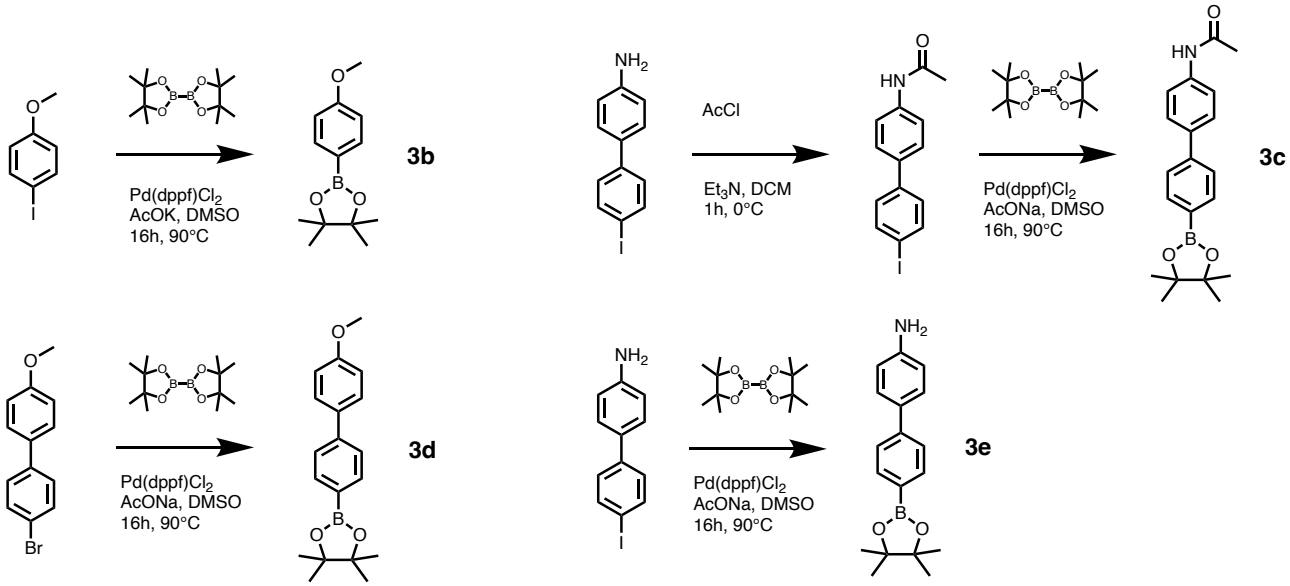


**Figure S1:** Synthetic pathways for **3b-e**.

**2-(4-methoxyphenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane** (**3b**) was prepared according to literature.[1,2]

***N*-(4'-iodo-[1,1'-biphenyl]-4-yl)acetamide:** 4'-iodo-[1,1'-biphenyl]-4-amine[3] (405 mg, 1.37 mmol) was dissolved in DCM (5 mL) before addition of TEA (248 µL, 1.78 mmol) and acetyl chloride (126 µL, 1.78 mmol) at 0 °C and stirred for 1 h. Solvents were removed under reduced pressure. The residue was dissolved in AcOEt (100 mL) and washed with HCl 1M (3 x 40 mL) and brine. The organic layer was dried over $Na_2SO_4$, filtered and solvents were removed under reduced pressure before purification by silica gel chromatography (DCM then DCM/MeOH 9:1) to give the title compound as a slightly orange solid (249 mg, 0.74 mmol, 38 % yield). $^1$H NMR (400 MHz, $(CD_3)_2SO$): $\delta$ = 10.03 (s, 1H), 7.78 (d, $J$ = 8.5 Hz, 2H), 7.66 (d, $J$ = 8.7 Hz, 2H), 7.60 (d, $J$ = 8.7 Hz, 2H), 7.44 (d, $J$ = 8.5 Hz, 2H), 2.06 (s, 3H) ppm; $^{13}$C{$^1$H} NMR (100 MHz, $(CD_3)_2SO$): $\delta$ = 168.4, 139.2, 139.1, 137.6, 133.4, 128.4, 126.7, 119.3, 93.0, 24.0 ppm; LRMS (ESI+): monoisotopic *m/z* = 338.0 (+) / calculated *m/z* = 338.0 $[M+H]^+$ for M = $C_{14}H_{12}INO$.

***N*-(4'-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-[1,1'-biphenyl]-4-yl)acetamide (3c):** Under argon atmosphere, *N*-(4'-iodo-[1,1'-biphenyl]-4-yl)acetamide (160 mg, 0.48 mmol), bis(pinacolato)diboron (170 mg, 0.67 mmol), potassium acetate (167 mg, 1.70 mmol) and $Pd(dppf)Cl_2$ (18.9 mg, 0.03 mmol) were dissolved in anhydrous DMSO (3 mL). The solution was stirred at 90 °C for 16 h. The solution was cooled to room temperature, filtered over celite and washed with AcOEt. The organic layer was washed with water then brine, dried over $Na_2SO_4$, filtered and concentrated under reduced pressure before purification by silica gel column chromatography (DCM/MeOH 95:5) to give **3c** as a yellowish solid (135 mg, 0.40 mmol, 78%). $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ = 7.86 (d, $J$ = 8.4 Hz, 2H), 7.59-7.56 (m, 6H), 2.20 (s, 3H), 1.36 (s, 12H) ppm; $^{13}$C{$^1$H} NMR (100 MHz, $CDCl_3$): $\delta$ = 168.6, 143.2, 137.6, 136.9, 135.4, 127.8, 126.2, 120.3, 83.9, 25.0, 24.7 ppm; LRMS (ESI+): monoisotopic *m/z* = 338.3 (2+) / calculated *m/z* = 338.2 $[M+2H]^{2+}$ for $C_{20}H_{22}BNO_3$. Spectroscopic data corresponded to literature.[4]

**2-(4'-methoxy-[1,1'-biphenyl]-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (3d):** Under argon atmosphere, 4-bromo-4'-methoxy-1,1'-biphenyl[5] (250 mg, 0.95 mmol), bis(pinacolato)diboron (313 mg, 1.23 mmol), potassium acetate (307 mg, 3.13 mmol) and $Pd(dppf)Cl_2$ (34 mg, 0.05 mmol) were dissolved in anhydrous DMSO (3 mL). The solution was stirred at 90 °C for 19 h. The solution was cooled to room temperature, filtered over celite and washed with AcOEt. The

organic layer was washed with water then brine, dried over $Na_2SO_4$, filtered and concentrated under reduced pressure before purification by silica gel column chromatography (Cyclohexane/AcOEt 5:1) to give **3d** (212 mg, 0.68 mmol, 72 %) as a white solid. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ = 7.86 (d, $J$ = 8.1 Hz, 2H), 7.57 (d, $J$ = 8.1, 2H), 7.56 (d, $J$ = 8.8 Hz, 2H), 7.04-6.95 (d, $J$ = 8.8 Hz, 2H), 3.85 (s, 3H), 1.36 (s, 12H). Spectroscopic data corresponded to literature.[6,7]

**4'-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-[1,1'-biphenyl]-4-amine (3e):** Under argon atmosphere, 4'-iodo-[1,1'-biphenyl]-4-amine[3] (500 mg, 1.69 mmol), bis(pinacolato)diboron (559 mg, 2.2 mmol), potassium acetate (548 mg, 5.59 mmol) and $Pd^{II}(dppf)Cl_2$ (62.0 mg, 0.08 mmol) were dissolved in anhydrous DMSO (11 mL). The solution was stirred at 90°C for 16h. The solution was cooled to room temperature and filtered over celite and washed with AcOEt. The organic layer was washed with saturated $NaHCO_3$ and brine, dried over $Na_2SO_4$, filtered and concentrated under reduced pressure before purification by silica gel column chromatography (DCM:MeOH 95:5) to give **3e** (440 mg, 1.49 mmol, 88 %) as a yellowish solid. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ = 7.84 (d, $J$ = 8.1 Hz, 2H), 7.56 (d, $J$ = 8.1 Hz, 2H), 7.44 (d, $J$ = 8.5 Hz, 2H), 6.76 (d, $J$ = 8.5 Hz, 2H), 3.75 (br s, 2H), 1.35 (s, 12H) ppm; $^{13}$C{$^1$H} NMR (100 MHz, $CDCl_3$): $\delta$ = 146.1, 143.8, 135.2, 131.3, 128.1, 125.6, 115.3, 83.7, 24.9 ppm. Spectroscopic data corresponded to literature.[8]

**4-(4-methoxyphenyl)-6-((4,7,10-tris(2-(*tert*-butoxy)-2-oxoethyl)-1,4,7,10-tetraazacyclododecan-1-yl)methyl)picolinic acid (L-Ar-OMe(*t*Bu)$_3$):** Compound **3b** (65 mg, 0.28 mmol) and compound **1**[9] (158 mg, 0.20 mmol) were dissolved in anhydrous DMF (5 mL). Cesium fluoride (152 mg, 1.0 mmol) and polymer-bound $Pd(PPh_3)_4$ (52 mg, 0.02 mmol) were added. The reaction was stirred at 90 °C for 24 h. The reaction mixture was cooled to room temperature, filtered over Celite and AcOEt was added. The organic phase was washed with a saturated $NaHCO_3$ and brine, dried over $Na_2SO_4$, filtered and concentrated under reduced pressure. The resulting yellow oil was dissolved in EtOH (20 mL) and NaOH 6 M (4 mL) was added dropwise. The solution was stirred for 10 min before dropwise addition of HCl 6 M to adjust pH to 7. EtOH was evaporated under reduced pressure and the aqueous solution was extracted with DCM. The organic layers were dried over $Na_2SO_4$, filtered and evaporated under reduced pressure. Purification by RP-HPLC followed by lyophilization gave **L-Ar-OMe(*t*Bu)$_3$** as a white solid (75 mg, 0.067 mmol, 34 % calculated based on the formula **L-Ar-OMe(*t*Bu)$_3$·3TFA**). HPLC (anal.): $t_R$ = 11.1 min (method B); $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ = 8.27 (s, 1H), 7.83 (s, 1H), 7.63 (d, $J$ = 8.7 Hz, 2H), 6.97 (d, $J$ = 8.7 Hz, 2H), 4.59 (br s, 2H), 3.93 (br s, 2H), 3.84 (s, 3H), 3.78-2.95 (br m, 20H), 1.44 (s, 9H), 1.32 (s, 18H) ppm; $^{13}$C{$^1$H} NMR (100 MHz, $CDCl_3$): $\delta$ = 169.3, 166.5, 166.0, 161.5, 161.1 (q, $J$ = 37 Hz, TFA), 151.2, 148.6, 128.6, 128.2, 124.4, 122.1, 116.4 (q, $J$ = 291 Hz, TFA), 114.9, 84.6, 83.2, 57.7, 55.5, 55.2, 54.5, 51.1, 50.8, 49.4, 49.0, 27.9 ppm; LRMS (ESI+): monoisotopic *m/z* = 778.4 (+)/ calculated *m/z* = 778.4 $[M+Na]^+$ for M = $C_{40}H_{61}N_5O_9$; HRMS (ESI+): monoisotopic *m/z* = 756.4545 (+) / calculated *m/z* = 756.4542 $[M+H]^+$ for M = $C_{40}H_{61}N_5O_9$.

**4-(4'-acetamido-[1,1'-biphenyl]-4-yl)-6-((4,7,10-tris(2-(*tert*-butoxy)-2-oxoethyl)-1,4,7,10-tetraazacyclododecan-1-yl)methyl)picolinic acid (L-Ar-Ar-NHAc(*t*Bu)$_3$):** Compound **3c** (101 mg, 0.30 mmol) and compound **1**[9] (170 mg, 0.22 mmol), CsF (163 mg, 1.07 mmol) and polymer-bound $Pd(PPh_3)_4$ (52 mg, 0.02 mmol) were dissolved in anhydrous DMF (5 mL) and stirred at 90 °C under argon atmosphere for 24 h. The reaction mixture was cooled to room temperature, filtered on celite and AcOEt was added. The resulting organic phase was washed with saturated $NaHCO_3$ and brine, dried over $Na_2SO_4$, filtered and evaporated under reduced pressure. The resulting yellow oil was dissolved in EtOH (20 mL) and NaOH 6M (4 mL) was added dropwise. The solution was stirred for 10 min before dropwise addition of HCl 6M to adjust the pH to 7. Solvents were evaporated under reduced pressure and the residue was taken up in MeCN. After filtration to remove non soluble materials, purification by RP-HPLC and lyophilization gave **L-Ar-Ar-NHAc(*t*Bu)$_3$** as a yellowish fluffy solid (116 mg, 0.097 mmol, 44 % yield based on the formula **L-Ar-Ar-NHAc(*t*Bu)$_3$·3TFA**). HPLC (anal.): $t_R$ = 10.2 min (method B); $^1$H NMR (400 MHz, $CD_3OD$): $\delta$ = 8.42 (s, 1H), 8.02 (s, 1H), 7.83 (d, $J$ = 8.4 Hz, 2H), 7.77 (d, $J$ = 8.4 Hz, 2H), 7.64 (m, 4H), 4.67 (br s, 2H), 4.40-2.80 (m, 22H), 2.12 (s, 3H), 1.44 (s, 9H), 1.28 (s, 18H) ppm;

$^{13}C\{^{1}H\}$ NMR (100 MHz, $CD_3OD$): $\delta$ = 171.7, 167.3, 162.5 (q, $J$ = 36 Hz, TFA), 151.7, 149.8, 143.6, 140.0, 136.5, 136.2, 128.8, 128.6, 128.3, 126.3, 123.6, 121.5, 118.0 (q, $J$ = 291 Hz, TFA), 85.7, 84.0, 58.4, 56.0, 55.1, 52.4, 50.7, 28.3, 23.9 ppm; LRMS (ESI+): monoisotopic *m/z* = 859.5 (+)/ calculated *m/z* = 859.5 $[M+H]^+$ for M = $C_{47}H_{66}N_6O_9$; HRMS (ESI+): monoisotopic *m/z* = 859.4963 (+) / calculated *m/z* = 859.4964 $[M+H]^+$ for M = $C_{47}H_{66}N_6O_9$.

**4-(4'-methoxy-[1,1'-biphenyl]-4-yl)-6-((4,7,10-tris(2-(*tert*-butoxy)-2-oxoethyl)-1,4,7,10-tetraazacyclododecan-1-yl)methyl)picolinic acid (L-Ar-Ar-OMe(*t*Bu)$_3$):** Compound **3c** (87 mg, 0.28 mmol) and compound **1**[9] (158 mg, 0.20 mmol), CsF (152 mg, 1.0 mmol) and polymer-bound $Pd(PPh_3)_4$ (52 mg, 0.02 mmol) were dissolved in anhydrous DMF (5 mL) and stirred at 90 °C under argon atmosphere for 24 h. The reaction mixture was cooled to room temperature, filtered on celite and AcOEt was added. The resulting organic phase was washed with saturated $NaHCO_3$ and brine, dried over $Na_2SO_4$, filtered and evaporated under reduced pressure. The resulting yellow oil was dissolved in EtOH (20 mL) and NaOH 6M (4 mL) was added dropwise. The solution was stirred for 10 min before dropwise addition of HCl 6 M to adjust the pH to 7. EtOH was evaporated under reduced pressure and the aqueous suspension was extracted with DCM. The organic layers were combined, dried over $Na_2SO_4$, filtered and concentrated under reduced pressure before purification by RP-HPLC and lyophilization to give **L-Ar-Ar-OMe(*t*Bu)$_3$** as a white solid (90 mg, 0.077 mmol, 39 % yield based on the formula **L-Ar-Ar-OMe(*t*Bu)$_3$·3TFA**). HPLC (anal.): $t_R$ = 12.1 min (method B); $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ = 8.31 (s, 1H), 7.88 (s, 1H), 7.69 (d, $J$ = 8.2, 2H), 7.61 (d, $J$ = 8.2, 2H), 7.53 (d, $J$ = 8.6, 2H), 6.97 (d, $J$ = 8.6, 2H), 4.60 (s, 2H), 3.84 (s, 3H), 3.74-3.00 (m, 22H), 1.43 (s, 9H), 1.32 (s, 18H) ppm; $^{13}C\{^{1}H\}$ NMR (100 MHz, $CDCl_3$): $\delta$ = 169.2 166.0, 161.2 (q, $J$ = 37 Hz, TFA), 159.8, 151.1, 148.8, 142.6, 134.2, 132.2, 128.2, 128.0, 127.6, 127.5, 124.7, 122.5, 116.4 (q, $J$ = 291 Hz, TFA), 114.6, 84.5, 83.3, 61.1, 57.7, 55.5, 55.2, 54.5, 51.1, 50.9, 49.5, 49.1, 28.0 ppm; LRMS (ESI+): monoisotopic *m/z* = 832.4 (+)/ calculated *m/z* = 832.5 $[M+H]^+$ for M = $C_{46}H_{65}N_5O_9$; HRMS (ESI+): monoisotopic *m/z* = 832.4857 (+) / calculated *m/z* = 832.4855 $[M+H]^+$ for M = $C_{46}H_{65}N_5O_9$.

**trimethyl 2,2',2''-(10-((4-(4'-amino-[1,1'-biphenyl]-4-yl)-6-(methoxycarbonyl)pyridin-2-yl)methyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetate (L-Ar-Ar-NH$_2$(Me)$_4$):** Compound **3e** (300 mg, 0.39 mmol), compound **2**·NaBr[10] (150 mg, 0.19 mmol), CsF (297 mg, 1.95 mmol) and polymer-bound $Pd(PPh_3)_4$ (97 mg, 0.04 mmol) were dissolved in anhydrous DMF (9 mL) and stirred at 90°C under an argon atmosphere for 16 h. The reaction mixture was cooled to room temperature, filtered on celite and concentrated under reduced pressure before purification by RP-HPLC. After lyophilization, **L-Ar-Ar-NH$_2$(Me)$_4$** was obtained as a yellow solid (195 mg, 0.17 mmol, 42 % yield based on the formula **L-Ar-Ar-NH$_2$(Me)$_4$·4TFA**). HPLC (anal.): $t_R$ = 7.2 min (method B); ); $^1$H NMR (400 MHz, $(CD_3)_2SO$): $\delta$ = 8.37 (s, 1H), 8.18 (br s, 1H), 7.95 (d, $J$ = 8.6 Hz, 2H), 7.79 (d, $J$ = 8.6 Hz, 2H), 7.55 (d, $J$ = 8.6 Hz, 2H), 6.80 (d, $J$ = 8.6 Hz, 2H) ), 4.74-2.73 (br m, 36H) ppm; $^{13}C\{^{1}H\}$ NMR (100 MHz, $CD_3OD$): $\delta$ = 173.6, 169.1, 166.7, 162.6 (q, $J$ = 37 Hz, TFA), 154.8, 152.3, 151.9, 149.9, 142.9, 141.1, 136.9, 131.5, 129.8, 129.2, 129.0, 126.9, 124.3, 123.6, 118.1 (q, $J$ = 292 Hz, TFA), 61.1, 58.8, 55.4, 53.7, 53.6, 53.4, 52.7, 50.6 ppm; HRMS (ESI+): monoisotopic *m/z* = 705.3604 (+) / calculated *m/z* = 705.3606 $[M+H]^+$ for M = $C_{37}H_{48}N_6O_8$.

**4-((4'-(2-(methoxycarbonyl)-6-((4,7,10-tris(2-methoxy-2-oxoethyl)-1,4,7,10-tetraazacyclododecan-1-yl)methyl)pyridin-4-yl)-[1,1'-biphenyl]-4-yl)amino)-4-oxobutanoic acid (L-Ar-Ar-NHSuc(Me)$_4$)**: **L-Ar-Ar-NH$_2$(Me)$_4$·4TFA** (20 mg, 17.2 µmol), succinic anhydride (3.4 mg, 34 µmol), DIEA (30 µl, 172 µmol) and DMAP (0.3 mg, 1 µmol) were dissolved in DMF (0.8 mL) and stirred at room temperature for 24 h. Solvents were removed under reduced pressure and the crude was purified by RP HPLC and lyophilized to give **L-Ar-Ar-NHSuc(Me)$_4$** as a white fluffy solid (18.2 mg, 15.9 µmol, 92 % yield based on the formula **L-Ar-Ar-NHSuc(Me)$_4$·3TFA**). HPLC (anal.): $t_R$ = 8.3 min (method B); $^1$H NMR (400 MHz, $(CD_3)_2SO$): $\delta$ = 9.58 (s, 1H), 8.45 (d, $J$ = 1.5 Hz, 1H), 8.24 (d, $J$ = 1.5 Hz, 1H), 7.98 (d, $J$ = 8.5 Hz, 2H), 7.86 (d, $J$ = 8.5 Hz, 2H), 7.83 (d, $J$ = 8.8 Hz, 2H), 7.69 (d, $J$ = 8.8 Hz, 2H), 4.83 (br s, 1H), 4.03 (s, 4H), 3.79 (s, 4H), 3.70 (s, 3H), 3.67 (s, 9H), 3.32 (br t, 4H), 3.27-3.04 (m, 8H), 2.72 (m, 4H) ppm; $^{13}C\{^{1}H\}$ NMR (100 MHz, $(CD_3)_2SO$): $\delta$ = 174.1, 171.9, 171.1, 171.0, 165.8, 151.0, 149.7, 143.0, 140.5, 135.5, 135.0, 128.5, 128.1, 128.0, 126.5,

122.7, 120.4, 120.3, 58.2, 55.3, 55.0, 53.2, 52.6, 52.0, 51.0, 49.6, 32.2 ppm; HRMS (ESI+): monoisotopic *m/z* = 805.3767 (+) / calculated *m/z* = 805.3767 $[M+H]^+$ for M = $C_{41}H_{52}N_6O_{11}$.

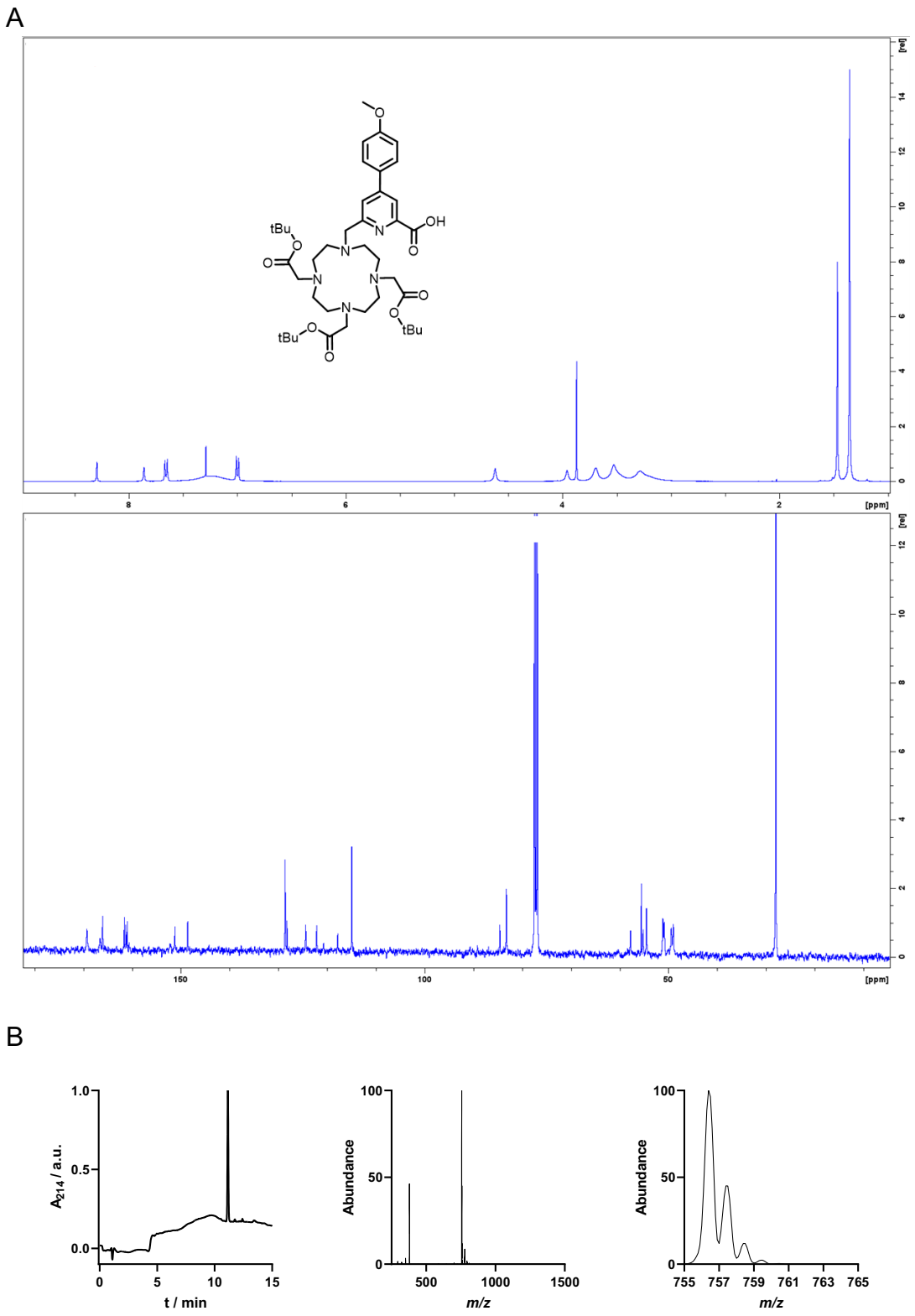


**Figure S2:** (A) $^1$H NMR and $^{13}$C NMR spectra and (B) LCMS analysis of L-Ar-OMe(*t*Bu)$_3$.

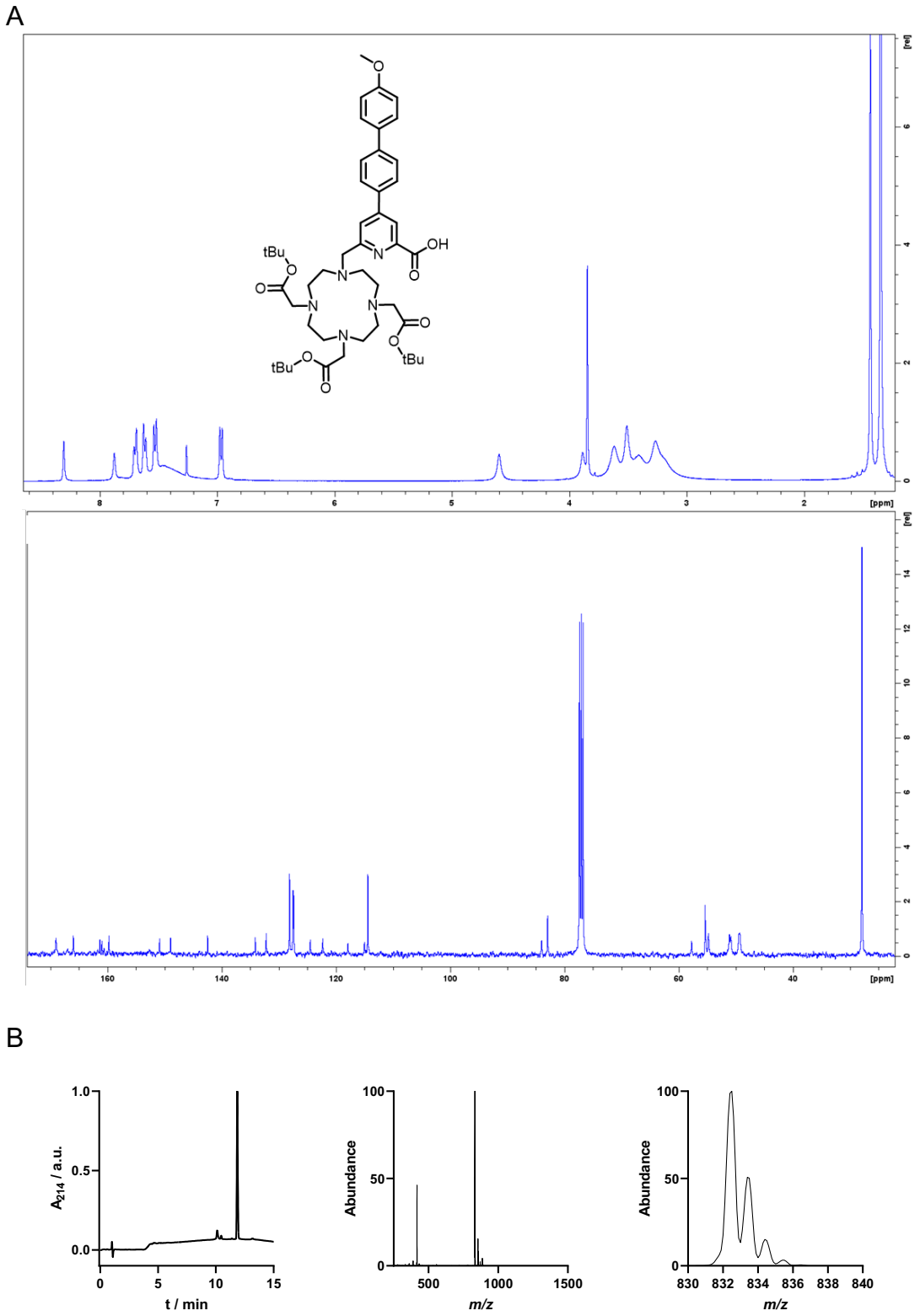


**Figure S3:** (A) $^{1}$H NMR and $^{13}$C NMR spectra and (B) LCMS analysis of L-Ar-Ar-OMe(*t*Bu)$_3$.

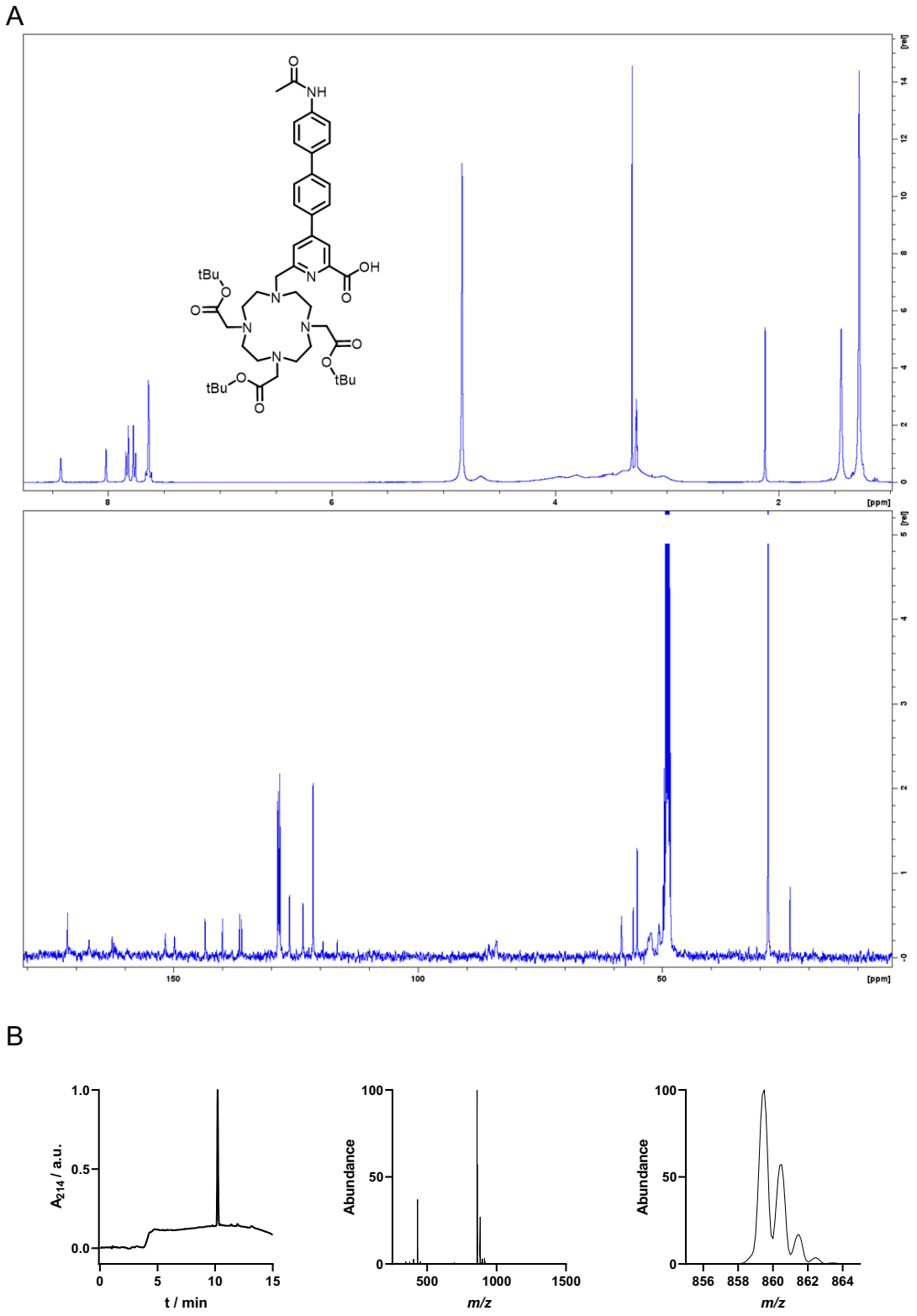


**Figure S4:** (A) $^{1}$H NMR and $^{13}$C NMR spectra and (B) LCMS analysis of L-Ar-Ar-NHAc(*t*Bu)$_3$.

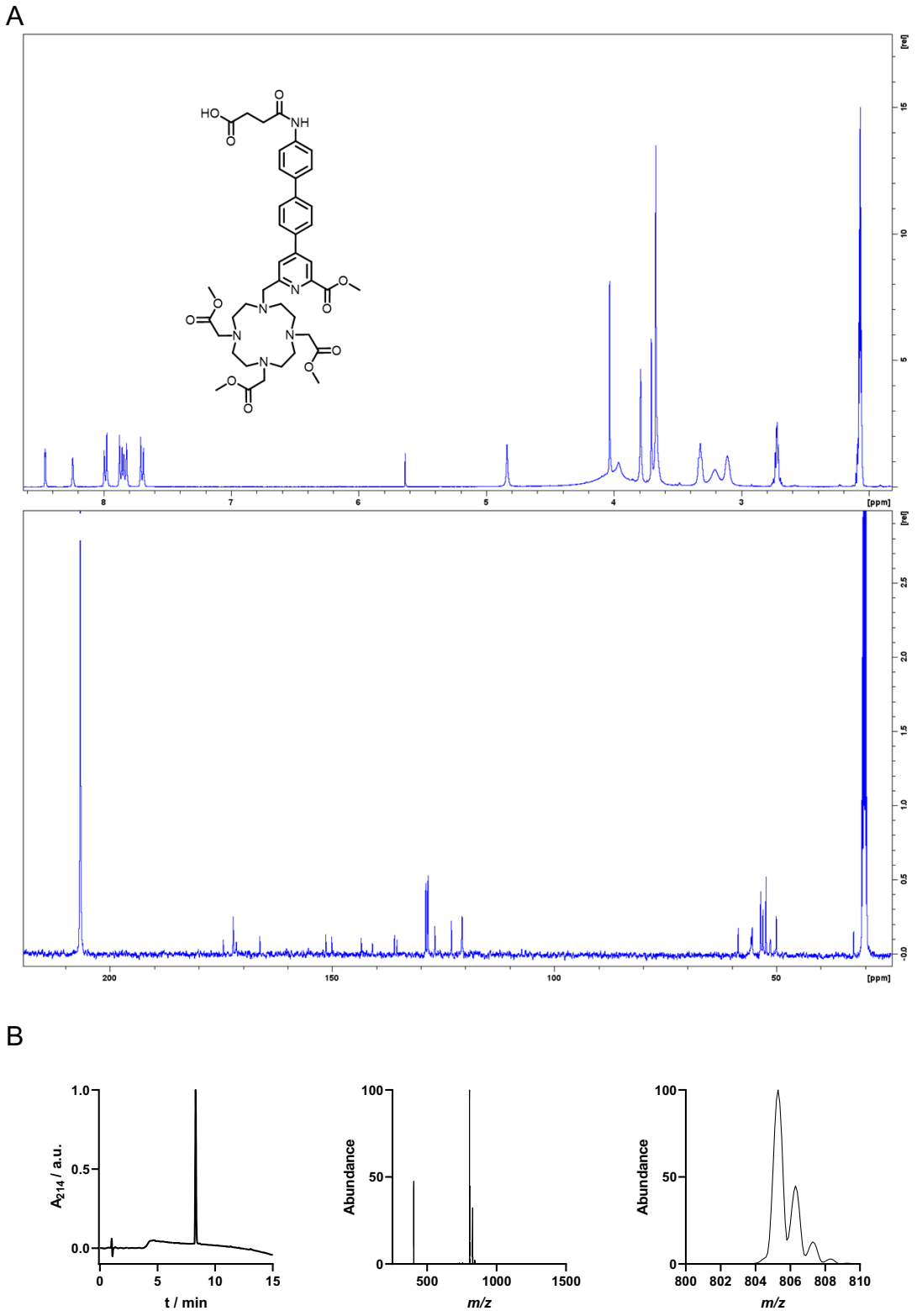


**Figure S5:** (A) $^{1}$H NMR and $^{13}$C NMR spectra and (B) LCMS analysis of L-Ar-Ar-NHSuc(Me)$_4$.

## Peptide sequences

**mTAT[L-R]:** Ac-K(L-R)RKKRRQRRRG-$NH_2$
**mTAT[Eu·L-R]:** Ac-K(Eu·L-R)RKKRRQRRRG-$NH_2$
**mTAT[Gd·L-R]:** Ac-K(Gd·L-R)RKKRRQRRRG-$NH_2$
**mTAT[SucNH-Ar-Ar-L(Me)$_4$]:** Ac-K(SucNH-Ar-Ar-L(Me)$_4$)RKKRRQRRRG-$NH_2$
**mTAT[SucNH-Ar-Ar-L]:** Ac-K(SucNH-Ar-Ar-L)RKKRRQRRRG-$NH_2$
**mTAT[SucNH-Ar-Ar-L·Eu]:** Ac-K(SucNH-Ar-Ar-L·Eu)RKKRRQRRRG-$NH_2$
**CTAT[L-Ar-Ar-NHAc]:** CK(L-Ar-Ar-NHAc)RKKRRQRRRG-$NH_2$
**dTAT[Eu·L-Ar-Ar-NHAc]: (**CK(L-Ar-Ar-NHAc)RKKRRQRRRG-$NH_2$) (disulfide bridge)
**CTAT[SucNH-Ar-Ar-L(Me)$_4$]:** CK(SucNH-Ar-Ar-L(Me)$_4$)RKKRRQRRRG-$NH_2$
**dTAT[SucNH-Ar-Ar-L·Eu]: (**CK(SucNH-Ar-Ar-L·Eu)RKKRRQRRRG-$NH_2$)$_2$ (disulfide bridge)

## Conjugate synthesis

Conjugates mTAT[L-X] with X = Ar-OMe, Ar-Ar-OMe and Ar-Ar-NHAc and their $Eu^{3+}$ or $Gd^{3+}$ complexes were synthesized following the procedure already described for mTAT[L-Ar-NHAc] and mTAT[Eu·L-Ar-NHAc].[9] dTAT[L-Ar-Ar-NHAc] was obtained following the procedure reported for dTAT[L-Ar-NHAc].[9] The synthetic pathways are shown in Figure S6.

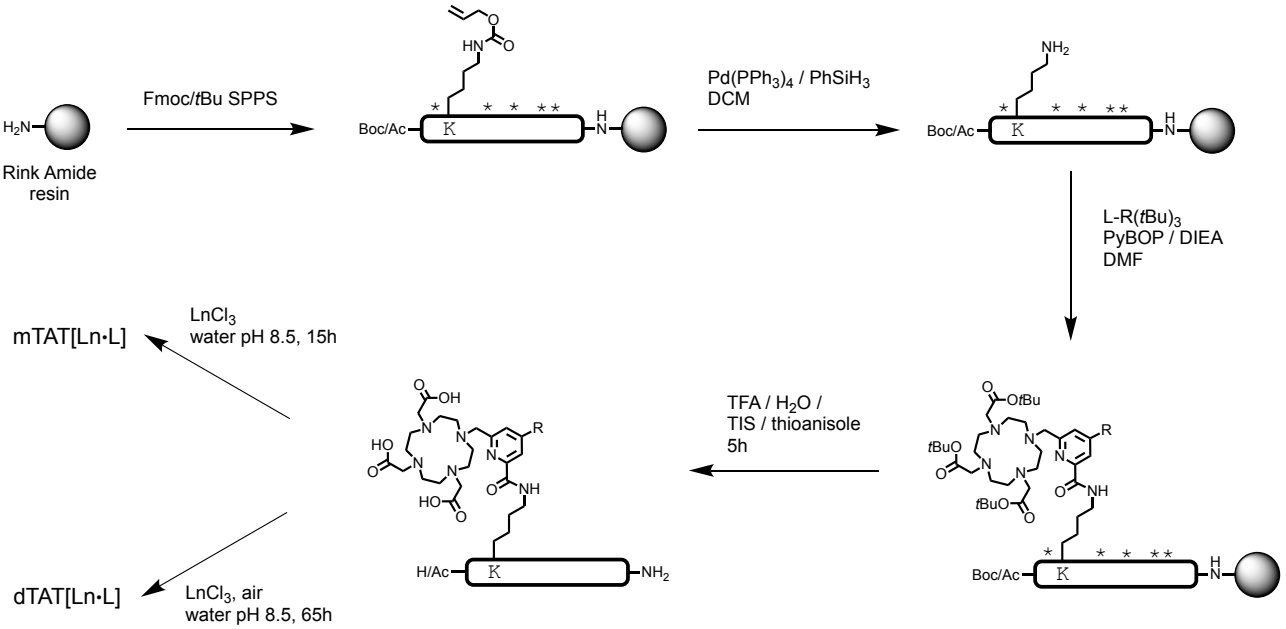


**Figure S6:** Preparation of conjugates mTAT[Ln·L-Ar-NHAc], mTAT[Ln·L-Ar-Ar-NHAc], mTAT[Ln·L-Ar-OMe] and mTAT[Ln·L-Ar-Ar-OMe]. * denote standard side-chain protecting groups used in Fmoc/*t*Bu SPPS.

Conjugates mTAT[SucNH-Ar-Ar-L] and its complexes were obtained in a similar way except that the intermediate mTAT[SucNH-Ar-Ar-L(Me)$_4$] was hydrolyzed with NaOH 2 M for 15 min prior to metalation with the $LnCl_3$ salt, according to a procedure described previously.[10] dTAT[SucNH-Ar-Ar-L·Eu] was obtained in a similar procedure, the intermediate CTAT[SucNH-Ar-Ar-L(Me)$_4$] being hydrolyzed with NaOH 2 M for 15 min prior to metalation with the $EuCl_3$ and disulfide formation. The synthetic pathways are shown in Figure S7.

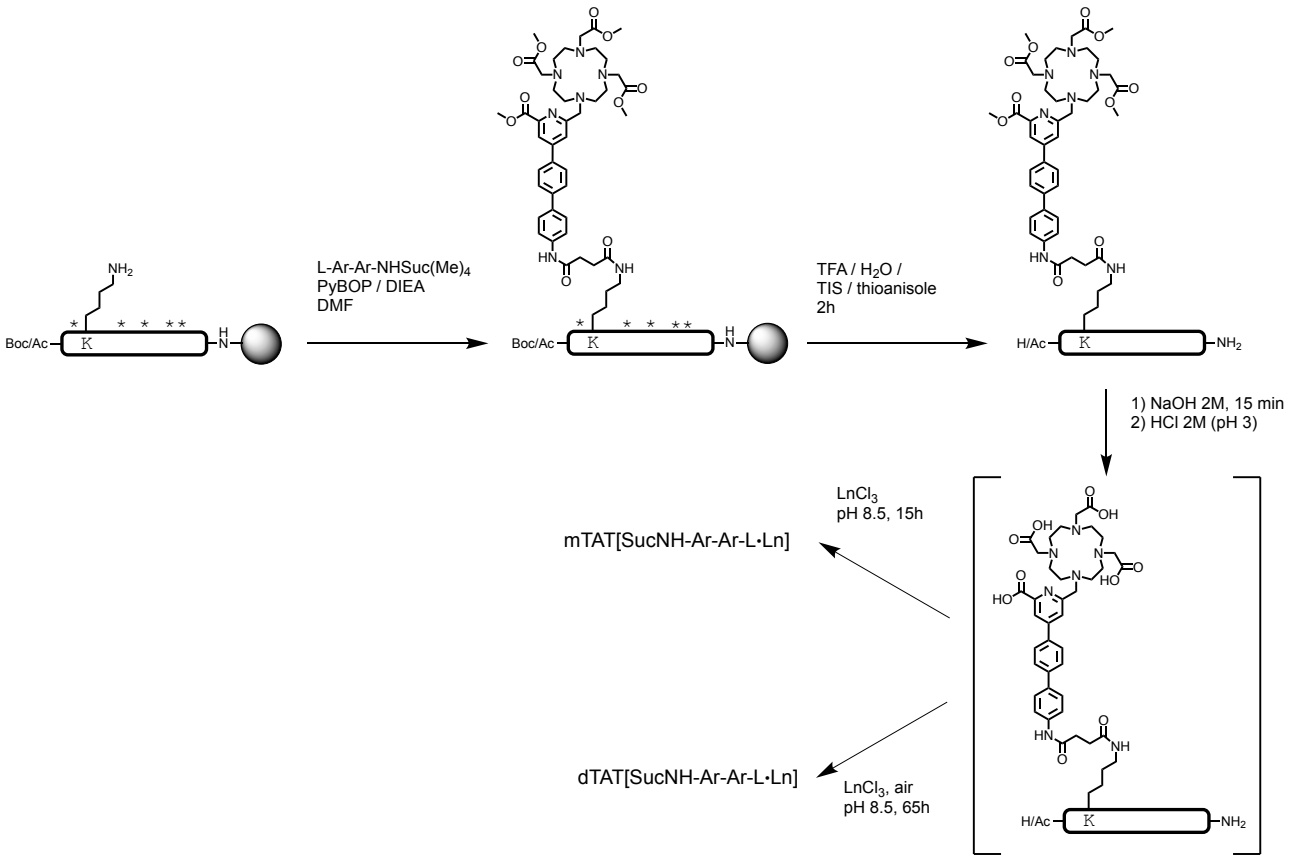


**Figure S7:** Preparation of conjugates mTAT[Ln·L-Ar-NHAc], mTAT[Ln·L-Ar-Ar-NHAc], mTAT[Ln·L-Ar-OMe] and mTAT[Ln·L-Ar-Ar-OMe]. * denote standard side-chain protecting groups used in Fmoc/*t*Bu SPPS.

**mTAT[L-Ar-OMe]:** HPLC (anal.): $t_R$ = 6.2 min (method B); LRMS (ESI+): average *m/z* = 712.9 (3+), 534.9 (4+), 428.2 (5+), 357.0 (6+) / calculated av. *m/z* = 712.8 $[M+3H]^{3+}$, 534.9 $[M+4H]^{4+}$, 428.1 $[M+5H]^{5+}$, 356.9 $[M+6H]^{6+}$ for M = $C_{91}H_{159}N_{39}O_{21}$); deconvoluted mass found = 2136.8 / expected mass = 2135.5 (average isotopic composition).
**mTAT[Eu·L-Ar-OMe]:** HPLC (anal.): $t_R$ = 6.20 min (method B); average *m/z* = 762.6 (3+), 572.4 (4+), 458.2 (5+), 381.8 (6+) / calculated av. *m/z* = 762.5 $[M+3H]^{3+}$, 572.1 $[M+4H]^{4+}$, 457.9 $[M+5H]^{5+}$, 381.7 $[M+6H]^{6+}$ for M = $C_{91}H_{156}N_{39}O_{21}Eu$); deconvoluted mass found = 2286.5 / expected mass = 2284.4 (average isotopic composition).
**mTAT[Gd·L-Ar-OMe]:** HPLC (anal.): $t_R$ = 6.2 min (method B); LRMS (ESI+): average *m/z* = 764.1 (3+), 573.3 (4+), 458.9 (5+), 382.6 (6+) / calculated av. *m/z* = 764.2 $[M+3H]^{3+}$, 573.4 $[M+4H]^{4+}$, 459.0 $[M+5H]^{5+}$, 382.6 $[M+6H]^{6+}$ for M = $C_{91}H_{156}N_{39}O_{21}Gd$); deconvoluted mass found = 2291.1 / expected mass = 2289.7 (average isotopic composition).

**mTAT[L-Ar-Ar-OMe]:** HPLC (anal.): $t_R$ = 7.3 min (method B); LRMS (ESI+): average *m/z* = 554.0 (4+), 443.4 (5+), 369.7 (6+), 317.0 (7+) / calculated av. *m/z* =553.9 $[M+4H]^{4+}$, 443.3 $[M+5H]^{5+}$, 369.6 $[M+6H]^{6+}$ , 316.9 $[M+7H]^{7+}$ for M = $C_{97}H_{163}N_{39}O_{21}$); deconvoluted mass found = 2213.2 / expected mass = 2211.6 (average isotopic composition).
**mTAT[Eu·L-Ar-Ar-OMe]:** HPLC (anal.): $t_R$ = 7.4 min (method B); LRMS (ESI+): average *m/z* = 788.2 (3+), 591.5 (4+), 473.4 (5+), 394.5 (6+) / calculated av. *m/z* =787.8 $[M+3H]^{3+}$, 591.1 $[M+4H]^{4+}$, 473.1 $[M+5H]^{5+}$, 394.4 $[M+6H]^{6+}$ for M = $C_{97}H_{160}N_{39}O_{21}Eu$); deconvoluted mass found = 2361.6 / expected mass = 2361.5 (average isotopic composition).
**mTAT[Gd·L-Ar-Ar-OMe]:** HPLC (anal.): $t_R$ = 7.4 min (method B); LRMS (ESI+): average *m/z* = 789.7 (3+), 592.6 (4+), 474.3 (5+), 395.3 (6+) / calculated av. *m/z* = 789.6 $[M+3H]^{3+}$, 592.5 $[M+4H]^{4+}$, 474.2 $[M+5H]^{5+}$, 395.3 $[M+6H]^{6+}$ for M = $C_{97}H_{160}N_{39}O_{21}Gd$); deconvoluted mass found = 2366.5 / expected mass = 2365.8 (average isotopic composition).

**mTAT(L-Ar-Ar-NHAc)** HPLC (anal.): $t_R$ = 6.3 min (method B); LRMS (ESI+): average *m/z* = 747.2 (3+), 560.7 (4+), 448.8 (5+), 374.1 (6+) / calculated av. *m/z* =747.5 $[M+3H]^{3+}$, 560.9 $[M+4H]^{4+}$, 448.9 $[M+5H]^{5+}$, 374.3 $[M+6H]^{6+}$ for M = $C_{98}H_{165}N_{40}O_{21}$); deconvoluted mass found = 2239.1 / expected mass = 2239.6 (average isotopic composition).
**mTAT[Eu·L-Ar-Ar-NHAc]** HPLC (anal.): $t_R$ = 6.3 min (method B); LRMS (ESI+): average *m/z* = 796.8 (3+), 597.9 (4+), 478.5 (5+) / calculated av. *m/z* = 797.2 $[M+3H]^{3+}$, 598.1 $[M+4H]^{4+}$, 478.7 $[M+5H]^{5+}$ for M = $C_{98}H_{162}N_{40}O_{21}Eu$); deconvoluted mass found = 2388.9 / expected mass = 2388.6 (average isotopic composition).
**mTAT[Gd·L-Ar-Ar-NHAc)]** HPLC (anal.): $t_R$ = 6.3 min (method B); LRMS (ESI+): average *m/z* = 798.5 (3+), 599.2 (4+), 479.5 (5+), 399.8 (6+) / calculated av. *m/z* = 799.0 $[M+3H]^{3+}$, 599.5 $[M+4H]^{4+}$, 479.8 $[M+5H]^{5+}$, 400.0 $[M+6H]^{6+}$ for M = $C_{98}H_{162}N_{40}O_{21}Gd$); deconvoluted mass found = 2393.4 / expected mass = 2393.8 (average isotopic composition).

**mTAT[SucNH-Ar-Ar-L(Me)$_4$]:** HPLC (anal.): $t_R$ = 7.1 min (method B); LRMS (ESI+): average *m/z* = 785.0 (3+), 589.1 (4+), 471.4 (5+), 393.1 (6+) / calculated av. *m/z* = 785.3 $[M+3H]^{3+}$, 589.2 $[M+4H]^{4+}$, 471.6 $[M+5H]^{5+}$, 393.1 $[M+6H]^{6+}$ for M = $C_{104}H_{174}N_{40}O_{23}$); deconvoluted mass found = 2352.4 / expected mass = 2352.7 (average isotopic composition).
**mTAT[SucNH-Ar-Ar-L]:** HPLC (anal.): $t_R$ = 6.0 min (method B); LRMS (ESI+): average *m/z* = 1149.8 (2+), 766.6 (3+), 575.2 (4+), 460.5 (5+) / calculated av. *m/z* = 1149.3 $[M+2H]^{2+}$, 766.5 $[M+3H]^{3+}$, 575.2 $[M+4H]^{4+}$, 460.3 $[M+5H]^{5+}$, for M = $C_{100}H_{166}N_{40}O_{23}$); deconvoluted mass found = 2297.0 / expected mass = 2296.6 (average isotopic composition).
**mTAT[SucNH-Ar-Ar-L·Eu]:** HPLC (anal.): $t_R$ = 6.0 min (method B); LRMS (ESI+): average *m/z* = 816.4 (3+), 612.4 (4+), 490.1 (5+), 408.7 (6+) / calculated av. *m/z* = 816.2 $[M+3H]^{3+}$, 612.4 $[M+4H]^{4+}$, 490.1 $[M+5H]^{5+}$, 408.6 $[M+6H]^{6+}$ for M = $C_{100}H_{163}N_{40}O_{23}Eu$); deconvoluted mass found = 2352.4 / expected mass = 2352.7 (average isotopic composition)

**CTAT[L-Ar-Ar-NHAc]:** HPLC (anal.): $t_R$ = 6.2 min (method B); LRMS (ESI+): average *m/z* = 767.5 (3+), 575.8 (4+) / calculated av. *m/z* = 767.6 $[M+3H]^{3+}$, 575.9 $[M+4H]^{4+}$ for M = $C_{99}H_{167}N_{41}O_{21}S$); deconvoluted mass found = 2300.5 / expected mass = 2299.7 (average isotopic composition).
**dTAT[Eu·L-Ar-Ar-NHAc]:** HPLC (anal.): $t_R$ = 6.6 min (method B); LRMS (ESI+): average *m/z* = 816.7 (6+), 700.5 (7+), 612.9 (8+), / calculated av. *m/z* = 816.9 $[M+6H]^{6+}$, 700.3 $[M+7H]^{7+}$, 612.9 $[M+8H]^{8+}$, for M = $C_{198}H_{326}Eu_2N_{82}O_{42}S_2$); deconvoluted mass found = 4896.4 / expected mass = 4895.3 (average isotopic composition).

**CTAT[SucNH-Ar-Ar-L(Me)$_4$]:** HPLC (anal.): $t_R$ = 7.0 min (method B); LRMS (ESI+): average *m/z* = 805.5 (3+), 604.4 (4+), 483.7 (5+), 403.3 (6+)/ calculated av. *m/z* = 805.6 $[M+3H]^{3+}$, 604.5 $[M+4H]^{4+}$, 483.8 $[M+5H]^{5+}$, 403.3 $[M+6H]^{6+}$ for M = $C_{105}H_{177}N_{41}O_{23}S$); deconvoluted mass found = 2413.6 / expected mass = 2413.8 (average isotopic composition).
**dTAT[(SucNH-Ar-Ar-L·Eu)]** HPLC (anal.): $t_R$ = 6.2 min (method B); LRMS (ESI+): average *m/z* =716.8 (7+), 627.2 (8+), 557.8 (9+) / calculated av. *m/z* = 716.8 $[M+7H]^{7+}$, 627.4 $[M+8H]^{8+}$, 557.8 $[M+9H]^{9+}$, for M =$C_{202}H_{330}Eu_2N_{82}O_{46}S_2$); deconvoluted mass found = 5010.8 / expected mass = 5011.3 (average isotopic composition).

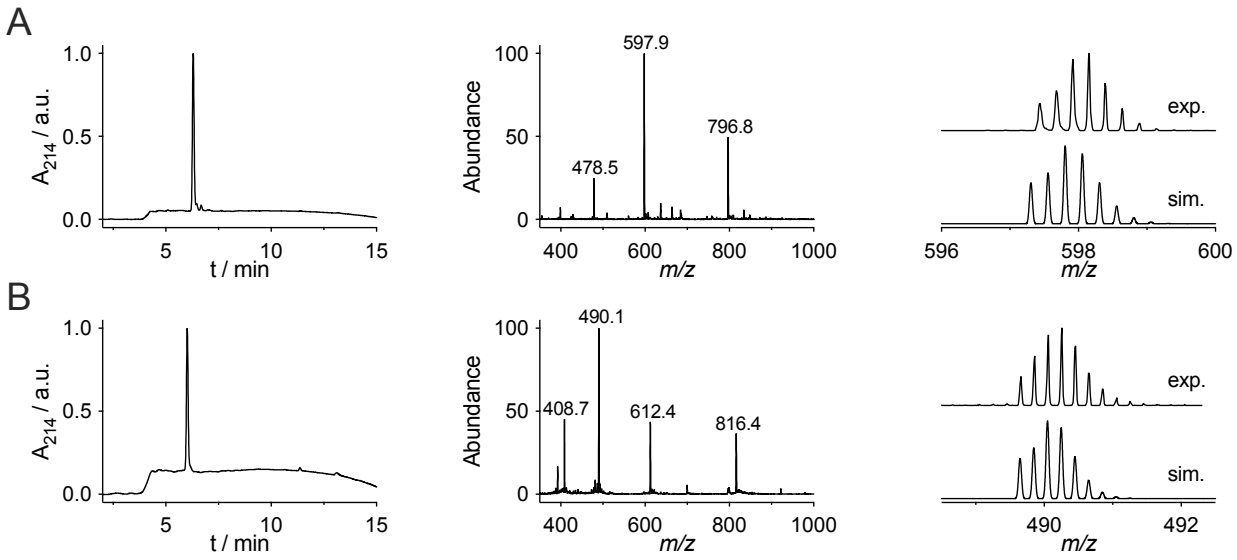


**Figure S8:** Typical examples of HPLC chromatograms (*left*) and LRMS (ESI+) spectra (*middle*: full MS spectrum; *right*: experimental and simulated isotopic pattern) obtained for (A) mTAT[Eu·L-Ar-Ar-NHAc] and (B) mTAT[SucNH-Ar-Ar-L·Eu].

## 1P spectroscopy

**Absorption, excitation and emission spectra:** The determination of molar absorption coefficients ε were performed as previously described[9,10] by titrating a solution of the Ln-free probe in HEPES buffer pH 7.4 with a solution of known concentration of $EuCl_3$. Absorption, excitation and emission spectra were recorded from solution of the conjugates dissolved in PBS at a concentration of *ca.* 10µM.

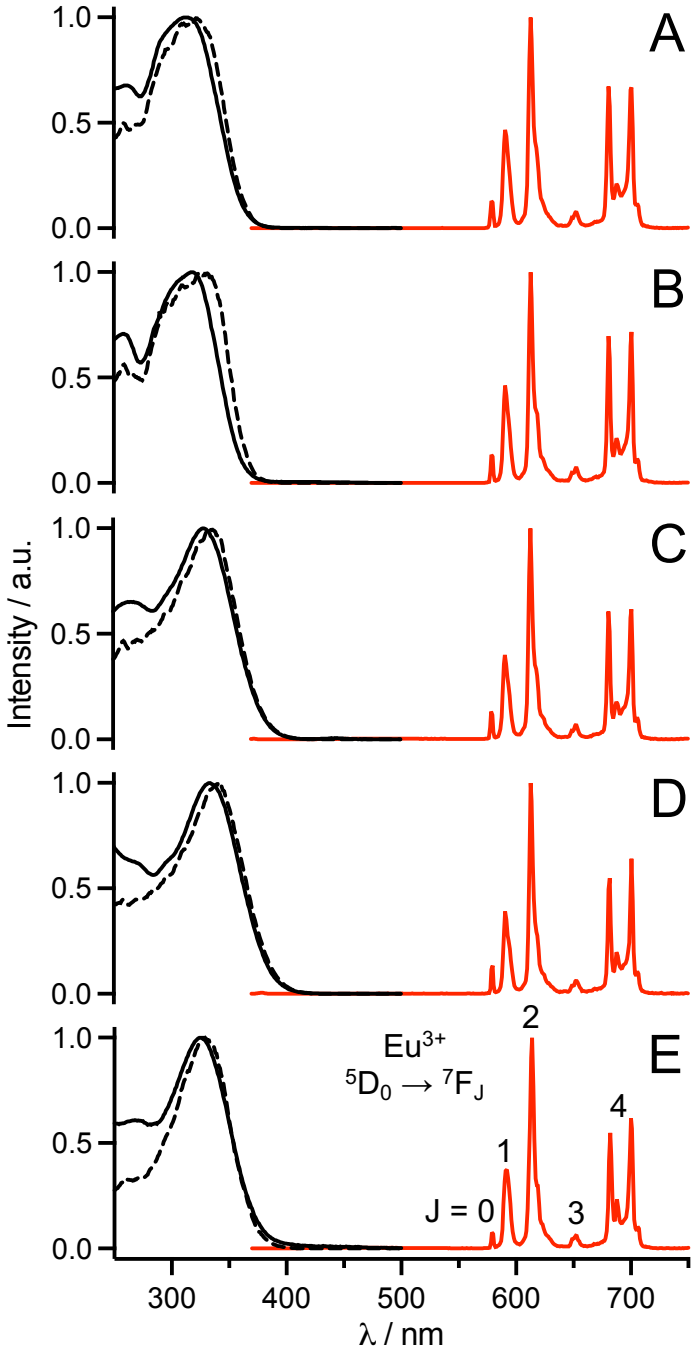


**Figure S9:** Normalized absorption (black solid line), excitation (black dashed line; $\lambda_{em}$ = 615 nm) and emission (red solid line; $\lambda_{ex}$ = 315 mn for A and B and 330 nm for C, D and E) spectra of (A) mTAT[Eu·L-Ar-NHAc], (B) mTAT[Eu·L-Ar-OMe], (C) mTAT[Eu·L-Ar-Ar-NHAc], (D) mTAT[Eu·L-Ar-Ar-OMe] and (E) mTAT[SucNH-Ar-Ar-L·Eu] in PBS.

**Luminescence decay:** $Ln^{3+}$ luminescence decays were measured for each compound in aerated and de-oxygenated PBS, prepared in $H_2O$, in aerated PBS prepared in $D_2O$ and lifetimes, $\tau_{Ln}$, were determined by mono-exponential fit (or bi-exponential fit when required). They are indicated in Table 1. Examples of decay curves and their fits are given in Figure S10. Th hydration number $q$ was determined using Parker's equation $q = 1.2 \times (1/\tau(H_2O) - 1/\tau(D_2O) - c))$, with $\tau$ in ms and $c = 0.325$ or 0.25, for DO3Apicolinamide and DO3Apicolinate ligands, respectively.[11] Values of $q$ are given in Table S1.

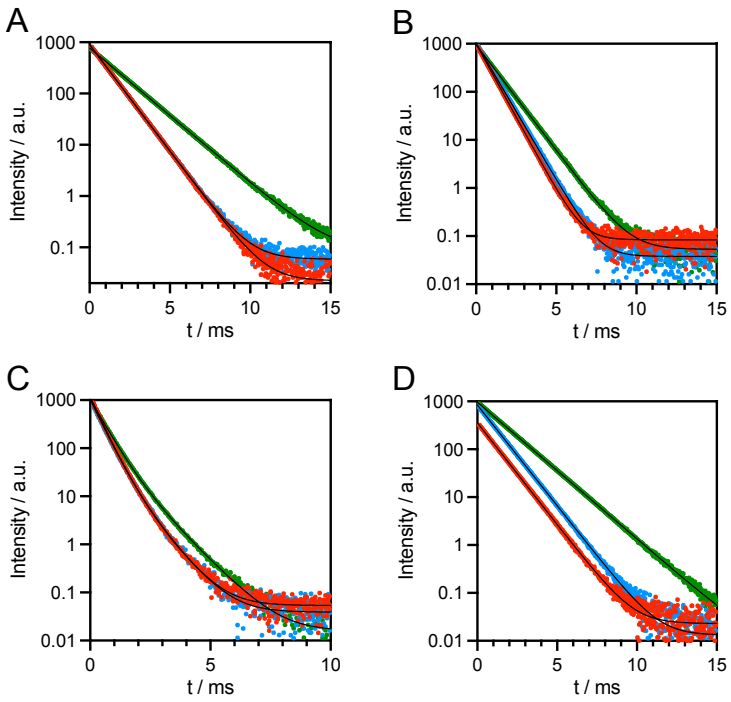


**Figure S10.** Luminescence decays and their mono- or bi-exponential fits (black line) measured for (A) mTAT[Eu·L-Ar-OMe], (B) mTAT[Eu·L-Ar-Ar-NHAc], (C) mTAT[Eu·L-Ar-Ar-OMe] and (D) mTAT[SucNH-Ar-Ar-L·Eu] in PBS (red: $H_2O$ solution, aerated; blue: $H_2O$ solution, de-oxygenated; green: $D_2O$ solution, aerated).

**Table S1.** Hydration number determined using Parker's equation.

| Compound | $q$ (± 0.2) |
|---|---|
| mTAT[Eu·L-Ar-NHAc] | 0.0[9] |
| mTAT[Eu·L-Ar-OMe] | 0.0 |
| mTAT[Eu·L-Ar-Ar-NHAc] | 0.1 |
| mTAT[Eu·L-Ar-Ar-OMe] | 0.1 |
| mTAT[SucNH-Ar-Ar-L·Eu] | 0.1 / –0.2[a] |

[a] Values calculated from the short and long lifetime components, respectively.

**Quantum yields measurements:** Quantum yields were determined using a Fluorolog FL3-22 spectrophotometer by a relative method with quinine sulphate in 0.5 M $H_2SO_4$ as a reference compound ($\Phi = 0.545$)[12,13] using solutions of various concentrations having absorption below 0.1 at the excitation wavelength. The excitation wavelength was the same for the sample compound (S) and the reference. To determine the quantum yields of the sample compound, the following equation was used:

$$\Phi_S = \frac{A_S}{I_S} \times \frac{I_{ref}}{A_{ref}} \times \frac{{n_S}^2}{{n_{ref}}^2} \times \Phi_{ref} \quad (1)$$

where $A$ is the absorbance at the excitation wavelength, $I$ the integrated emission intensity and $n$ the refractive index.

Estimated experimental error for the quantum yield determination is ~10 %.

**Determination of energy of $S_1$ and $T_1$ excited states:** The energy of the $S_1$ state was determined using the cut-off of the absorption band. For the energy of $T_1$ excited state, a solution of the Gd-loaded peptide in PBS/glycerol 9:1 (v/v) was prepared and frozen at 77 K. A time-gated (delay = 100 µs) emission spectrum was recorded to monitor the antenna phosphorescence emission. The energy of the $T_1$ state was estimated using the wavelength at half-maximum on the onset of the phosphorescence spectrum.

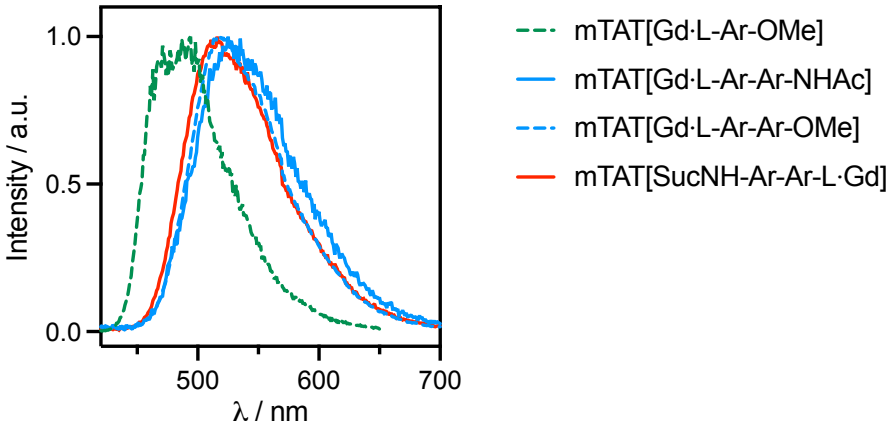


**Figure S11.** Time-gated (100 µs delay) emission spectrum of mTAT[Gd·L-Ar-OMe], mTAT[Gd·L-Ar-Ar-NHAc], mTAT[Gd·L-Ar-Ar-OMe] and mTAT[SucNH-Ar-Ar-L·Eu] in PBS/glycerol 9:1 (v/v) at 77 K (frozen solutions).

## 2P spectroscopy

**Determination of 2P cross-sections $\sigma_{2P}$:** Two-photon excitation spectra and two-photon cross-sections were obtained by two-photon excited fluorescence measurements of diluted PBS solutions of the compounds (*ca.* 10 µM, the exact concentration was determined from absorption spectrum) using a femtosecond Ti:sapphire laser (Coherent Chameleon Ultra II, 80 MHz, 140 fs) in the range 690–990 nm. The excitation beam (2.6 mm diameter) was focused with a 75 mm focal length lens to the sample. The up-converted fluorescence was collected at right-angle using a 30 mm focal length doublet lens. After filtering the scattered excitation beam by low-pass filters, the florescence was coupled to a fiber optic spectrometer (Avantes Hero). The sample was contained in a 1×1 cm quartz cell and continuously stirred with a magnetic stirrer to avoid thermal effects and photodegradation. After verifying that the emission intensity exhibited quadratic power dependence for each sample, the incident power was adjusted to 35 mW to characterize the two-photon absorption spectra. Calibration of the 2P absorption spectra was performed at each excitation wavelength by comparison with that of fluorescein (10 µM, pH 11) as reference compound.[14] 2P absorption spectra are provided in Figure S12.

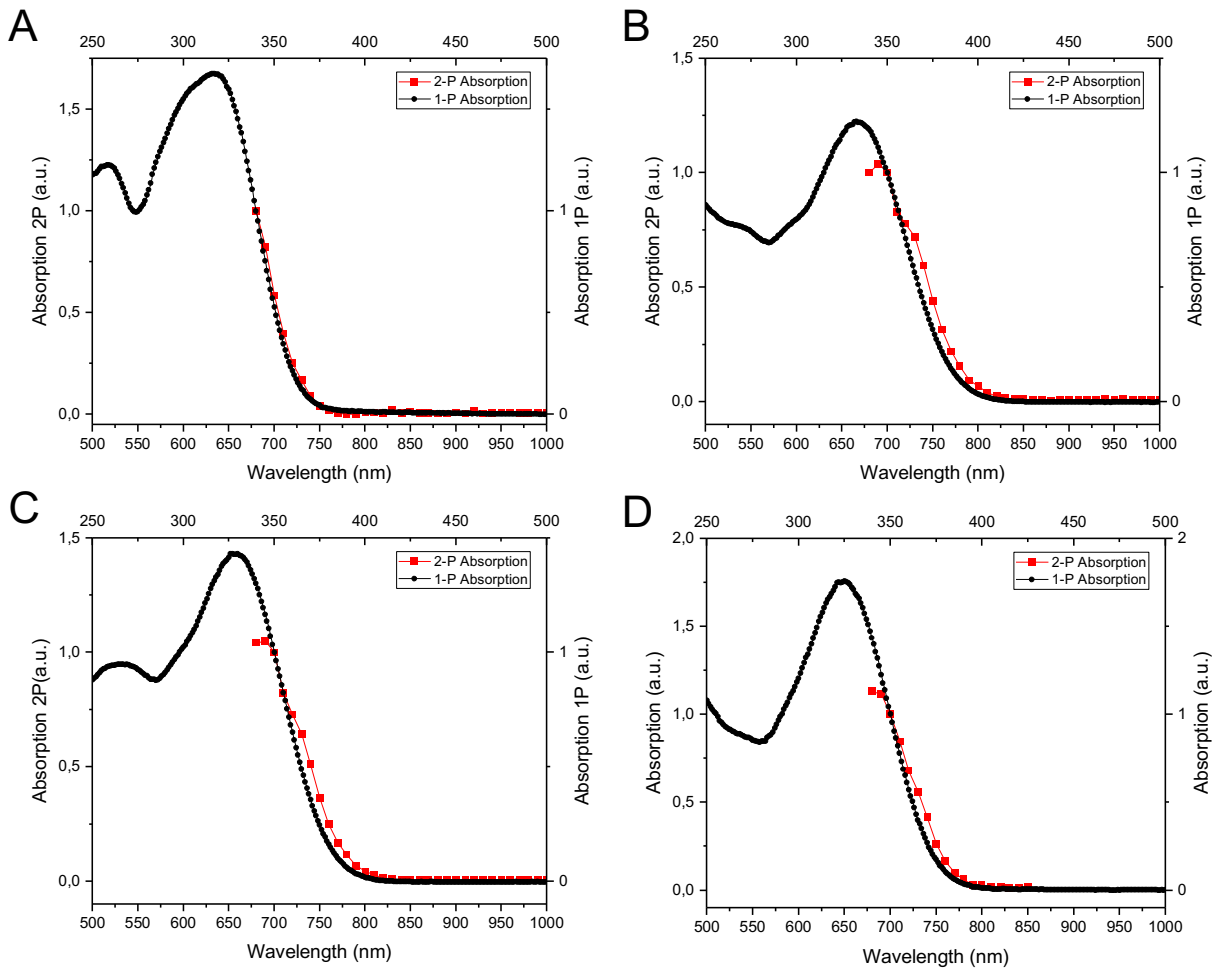


**Figure S12.** Superimposition of 2P absorption spectra (lower abscissa for wavelength, red) and 1P absorption spectra (upper abscissa, black) for (A) mTAT[Eu·L-Ar-OMe], (B) mTAT[Eu·L-Ar-Ar-OMe], (C) mTAT[Eu·L-Ar-Ar-NHAc] and (D) mTAT[SucNH-Ar-Ar-L·Eu] in PBS.

## 2P microscopy

**Cell culture:** HeLa cells were grown in RPMI medium supplemented with 10% fetal bovine serum (v/v) at 37°C in a 5% $CO_2$ humidified atmosphere.

**MTT proliferation assay:** Inhibition of cell proliferation by conjugates was measured by a MTT assay. HeLa cells were seeded into 96-well plates ($3\times10^3$ cells per well) in 100 µL of culture medium (RPMI with 10% of fetal calf serum). After 24 h, cells were washed with EBSS (Earle's Balances Salt Solution) two times, then treated with the compound dissolved in RPMI medium (without phenol red and without serum) during 1 h (9 wells for each different concentration). A control with culture medium only was prepared also. Following incubation of the cells with the samples, the solution was discarded, the cells were washed twice with EBSS and fresh culture medium was added to the wells. After 48 h at 37 °C, plates were centrifugated for 5 min at 400 g. The medium was then discarded and replaced with fresh culture medium containing MTT (0.5 mg/mL, Euromedex, Mundolsheim, France). After 3 h at 37 °C, 100 µL of solubilizing solution (10% Triton-X100, 0.1 M HCl, in isopropanol) were added in each well. Plates were incubated at room temperature under shaking until solubilization of water insoluble purple formazan crystals. Absorbance was then measured on an ELISA reader (Tecan, Männedorf, Switzerland) at a test wavelength of 570 nm and a reference wavelength of 650 nm. Absorbance obtained by cells in control medium was rated as 100 % cell survival. Each data point is the average of two

or three independent experiments. Data were then fitted using GraphPad Prism to determine IC50 values.

**Peptides delivery in live cells:** HeLa cells were seeded at $3\times10^4$ cells/well onto an 8-chamber Labtek-I coverglass system. After 24 h, cells were washed three times with PBS. Cells were incubated with the probe in RPMI medium (without phenol red and without serum) at 37°C for 1 h. Then, cells were washed two times with EBSS before adding RPMI medium supplemented with fetal bovine serum (200 µL) for observation under microscope.

**Confocal microscopy:** Confocal 2P experiments were performed by using an LSM-DuoScan-Confocor3 NLO microscope (Carl Zeiss) composed of a LSM710 confocal module and an inverted motorized stand (AxioObserver) equipped with an on-stage cell incubator. Excitation was provided by a Ti:Sapphire femtosecond laser (Chameleon, Ultra II, Coherent) featuring chirp precompensation. The C-apochromat 40×/1.2 water-immersion objective was used throughout experiments. The pinhole was open during 2P acquisition in descanned detection mode. The spectral PMT detector (Quasar) or avalanche photodiodes were used to register the emission signal in each pixel of the confocal image. Temporal Sampling Lifetime Imaging Microscopy (TSLIM) was used to record luminescence lifetime decay in cells (single pulsed excitation with the 7.56 µs temporal resolution).[15]

**Image analysis:** Fiji/ImageJ was used to analyze images and perform linear unmixing of autofluorescence and $Ln^{3+}$ emission, using the Stowers ImageJ plugins developed by Jay Unruh at the Stowers Institute for Medical Research.

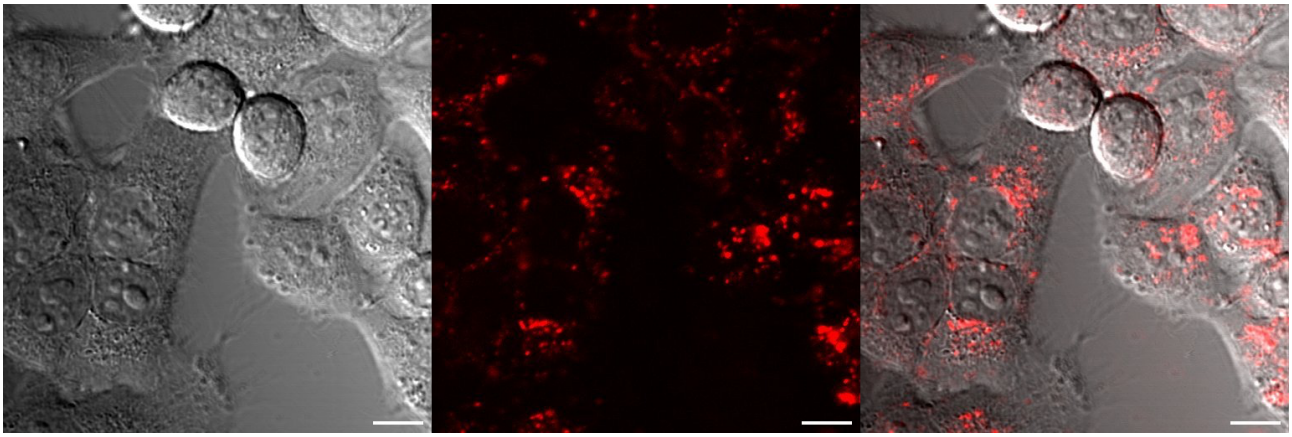

**Figure S13.** 2PM imaging ($\lambda_{ex}$ = 720 nm) of HeLa cells incubated 1 h with dTAT[SucNH-Ar-Ar-L·Eu] (2 µM) in RPMI medium. *Left:* DIC; *Middle*: luminescence recorded using a 420-690 nm bp filter and APD detection; *Right:* merge. Scale bars correspond to 10 µm.

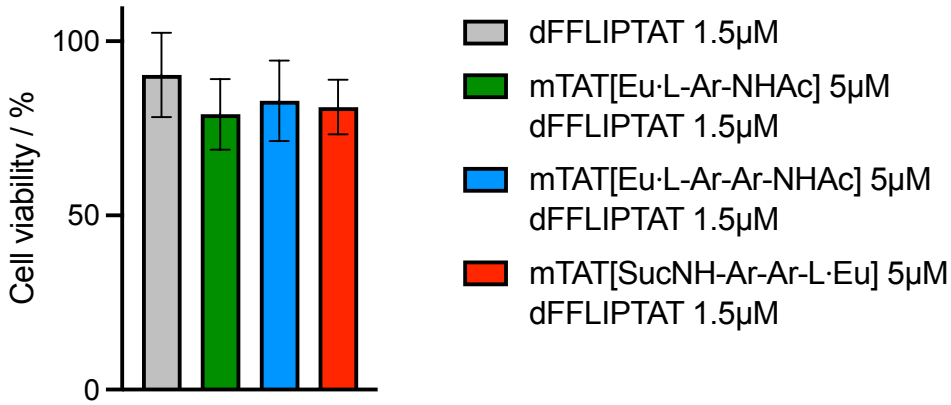


**Figure S14.** MTT proliferation assay with HeLa cells incubated 1 h with 1.5 µM dFFLIPTAT alone (grey) or dFFLIPTAT (1.5 µM) + mTAT-based probe (5 µM). The incubation step was performed in RPMI without serum for 1 h, the proliferation step was performed in RPMI with 10 % fetal calf serum for 48 h.

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