Supporting Information

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Stable Europium(III) Complexes with Short Linkers for Site-Specific Labeling of Biomolecules

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Estimation of the labeling efficiency of BSA:
The coupling reaction of 14-Eu with BSA results in almost complete labeling of all available cysteine residues. It is known that on average every second BSA molecule possesses a reactive sulfhydryl-group.\cite{1} The extinction coefficient of free 14-Eu at 335 nm was determined to be 11900 m\(^{-1}\) cm\(^{-1}\). The formal extinction coefficient of labeled BSA at 280 nm is composed of the extinction coefficient of two BSA molecules (\(2 \times 44300 \text{ m}^{-1} \text{ cm}^{-1}\))\cite{2} and the absorbance of one 14-Eu at 280 nm (8200 m\(^{-1}\) cm\(^{-1}\)), which sums up to 96800 m\(^{-1}\) cm\(^{-1}\). This theoretical extinction coefficient is valid for 100% labeling of the available free 0.5 thiol groups per BSA molecule; it is shown in Table S1. In this maximally labeled complex the ratio of \(\varepsilon(335 \text{ nm})/\varepsilon(280 \text{ nm})\) has the value 0.123. In the actual sample of 14-Eu-labeled BSA, the ratio of \(\varepsilon(335 \text{ nm})/\varepsilon(280 \text{ nm})\) was 0.113, which means that approx. 91% of the available thiol groups of BSA were labeled with 14-Eu.

Table S1: Extinction of the 14-Eu labeled BSA at 335 and 280 nm was used to estimate the labeling efficiency.

|          | \(\varepsilon(335 \text{ nm})\) [M\(^{-1}\) cm\(^{-1}\)] | \(\varepsilon(280 \text{ nm})\) [M\(^{-1}\) cm\(^{-1}\)] | \(\varepsilon(335 \text{ nm})/\varepsilon(280 \text{ nm})\) | Labeling efficiency [%] |
|----------|------------------------------------------------|------------------------------------------------|------------------------------------------------|------------------------|
| Theory   | 11900                                          | 96800                                          | 0.123                                          | 100                    |
| Measured | 11900                                          | 105800                                         | 0.113                                          | 91                     |
Determination of the luminescence quantum yield:
The quantum yields were calculated with the following formula:[3]

$$\phi_D = \phi_S \cdot \frac{E_D \cdot A_S}{A_D \cdot E_S} \cdot \left(\frac{n_D}{n_S}\right)^2$$

$\phi_D$ is the quantum yield of the sample dye, $\phi_S$ is the quantum yield of the reference standard, $E_D/A_D$ is the slope of emission intensity area vs. the absorption of the sample dye at 335 nm and $E_S/A_S$ is the corresponding slope of the reference standard, $n_D = 1.334$ is the refractive index of HBS 7.3 buffer used to dissolve the sample dye and $n_S = 1.3638$ is the refractive index of ethanol used to dissolve the reference standard.

**Table S2:** Determination of the luminescence quantum yield by relative method with rhodamine 6G as standard.[3,4]

| Compound     | Absorbance A at 335 nm [A.U.] | Emission area $E$ [A.U.] | Slope $E/A$ | Quantum yield $\phi$ [%] |
|--------------|-------------------------------|---------------------------|-------------|--------------------------|
| Rhodamine 6G | 0.0081                        | 8.09 \cdot 10^7           |             |                          |
|              | 0.0060                        | 6.71 \cdot 10^7           |             |                          |
|              | 0.0037                        | 4.82 \cdot 10^7           | 6.97 \cdot 10^9 | 94.0\pm3.8              |
|              | 0.0016                        | 3.28 \cdot 10^7           |             |                          |
|              | 1.28 \cdot 10^{-4}           | 2.72 \cdot 10^7           |             |                          |
| 14-Eu        | 0.060                         | 1.60 \cdot 10^8           |             |                          |
|              | 0.045                         | 1.25 \cdot 10^8           |             |                          |
|              | 0.040                         | 1.09 \cdot 10^8           |             |                          |
|              | 0.030                         | 8.50 \cdot 10^7           | 2.50 \cdot 10^9 | 32.3\pm2.0              |
|              | 0.022                         | 6.84 \cdot 10^7           |             |                          |
|              | 0.016                         | 5.23 \cdot 10^7           |             |                          |
|              | 0.0079                        | 2.92 \cdot 10^7           |             |                          |
|              | 0.0070                        | 2.50 \cdot 10^7           |             |                          |
| 14-Tb        | 0.053                         | 7.09 \cdot 10^7           |             |                          |
|              | 0.038                         | 5.10 \cdot 10^7           |             |                          |
|              | 0.027                         | 3.70 \cdot 10^7           |             |                          |
|              | 0.023                         | 2.90 \cdot 10^7           | 1.42 \cdot 10^9 | 18.4\pm2.4              |
|              | 0.018                         | 2.10 \cdot 10^7           |             |                          |
|              | 0.016                         | 1.75 \cdot 10^7           |             |                          |
|              | 0.013                         | 1.47 \cdot 10^7           |             |                          |
| 14-Eu labeled BSA | 0.032                     | 8.25 \cdot 10^7           |             |                          |
|              | 0.024                         | 6.55 \cdot 10^7           |             |                          |
|              | 0.015                         | 4.44 \cdot 10^7           | 2.24 \cdot 10^9 | 28.8\pm2.3              |
|              | 0.0095                        | 3.43 \cdot 10^7           |             |                          |
|              | 0.0069                        | 2.62 \cdot 10^7           |             |                          |
|              | 0.0033                        | 1.67 \cdot 10^7           |             |                          |
Figure S1: Measurements of luminescence quantum yield by relative method, using rhodamine 6G as standard.$^{[3,4]}$

**Determination of the number of water molecules in the inner coordination-sphere:**

Figure S2: Lifetime measurement of 14-Eu-labeled BSA in HBS 7.3 buffer (dark blue solid line) and in deuterated HBS 7.3 buffer (red solid line). Essentially the same lifetimes were observed for 14-Eu in water (blue dashed line) and in deuterated water (pink dashed line).
Eu-complex stability in presence of competitors:
In Figure S3 the luminescence intensity of 17-Eu (3 µM) in HBS 7.3 was monitored in the presence of competitors for more than 24 h. The luminescence intensity was not influenced upon addition of 2 mM EDTA, 2 mM EGTA, 2 mM EGTA-Ca-buffer, 10 µM zinc(II) or 200 µM zinc(II). This compares well with the observations that neither EDTA nor EGTA can extract the Eu-ion from the chelate, and that zinc(II) or calcium(II) are not able to cause transmetalation. Control experiments were performed with 3 µM EuCl₃ in the identical HBS 7.3 buffer and 2 mM EDTA or 2 mM EGTA, in order to ensure that neither Eu-EDTA, Eu-EGTA nor any other buffer contents could cause luminescence, which would lead to misinterpretation of the data.

Figure S3: The luminescence intensity of 17-Eu (3 µM) and 17-Eu in presence of 2 mM EDTA, 2 mM EGTA, 2 mM Ca-EGTA buffer, 10 µM Zn(II) and 200 µM Zn(II) was monitored for more than 24 h. As control experiment the luminescence intensity of 3 µM europium(III) chloride in HBS 7.3 buffer with 2 mM EDTA or 2 mM EGTA was monitored, in order to control if luminescence from buffer components can be observed.
Influence of nucleotides on the luminescence properties of 14-Eu labeled BSA:
The effect of nucleotide present in the solution was strongly dependent on the wavelength used to excite the Eu-complex. For measurements in a fluorimeter cuvette, the samples were excited at 335 nm (slit 10 nm). Hereby the nucleotides (or a common impurity of the nucleotides) caused a significant 335 nm absorption at a nucleotide concentration of approx. 25 mM (see Figure S6). When monitoring the luminescence intensity of 14-Eu labeled BSA incubated with nucleotide solutions over a period of 10 min, the intensity was not changed (see Figure S4). Figure S4B displays the luminescence intensity corrected for the dilution which had been caused by the addition of the nucleotide stock solution. In this case a prominent filter-effect was observed for GTP only. This experiment allows the conclusion, that the nucleotides do not perturb the emission of the Eu-complex when corrected for the “filter effect” (Figure 5B and Figure S6).

Figure S4: Stability of 14-Eu labeled BSA in the presence of different nucleotides or adenosine. The luminescence intensity was monitored at 1 min intervals. A) The emission intensities as measured is shown. B) The data from panel A were corrected for the dilution caused by the addition of the 100 mM stock solution (the stock solution of adenosine was 10 mM). The reduced emission intensity of 14-Eu in presence of 19 mM GTP in panel B is the consequence of much higher absorbance of GTP or a common impurity of GTP at 335 nm, as shown in Figure S6.
Figure S5: Lifetimes of 14-Eu–labeled BSA in presence of nucleotides or competitive chelators like EDTA and EGTA. The measurements were performed with 3 µM europium label in HBS 7.3 buffer (except for the test in phosphate buffer). All time courses exhibited a mono-exponential decay and were fitted to the equation \( I = I_0 + I_1 \cdot e^{(-t/\tau)} \) which takes into account a constant background (resulting from background light and detector noise). The insert illustrates the nearly parallel nature of the curves at levels which are much higher than the background, reflecting essentially equal lifetimes.

Figure S6: Extinction coefficient determined from approx. 10 mM nucleotide or adenosine solutions in HBS 7.3 buffer. The window between 330 and 340 nm was used for 14/17-Eu excitation in the stability tests.
Material and methods

Lifetime measurements: The time-resolved measurements were performed on a home-build setup based on a Nikon Diaphot 300 (Tokyo, Japan). Excitation was performed with a 266 nm Nd:YAG laser from VM-TIM LS-2132UTF (Jena, Germany). Monochromaticity was ensured with two FGUV11 UV-bandpass filters (Thorlabs, Dachau, Germany) and the laser-beam was redirected into the microscope body with two PF10-03-F01 mirrors (Thorlabs). The beam was directed with a z266rdc dichroic mirror Chroma (Bellows Falls, USA) through a Partec (Sysmex, Kobe, Japan) 40x1.25 glycerol immersion quartz objective, to focus the excitation light from the bottom onto the glass slide carrying the sample-solution. The emitted light was collected by the objective, passed through the dichroic mirror and an HQ465lp longpass filter (Chroma). The emitted light is focused onto the detector by a LA1805-A lens (Thorlabs) with focal length of 30 mm. A Perkin Elmer SPCM-AQRH gate d single photon avalanche diode (Waltham, USA) which was controlled by a PicoQuant DSN 101 power supply with integrated counter (Berlin, Germany) was used for photon detection. The system was triggered and decay curves were recorded by a PicoQuant NanoHARP 250. The trigger signal was looped for 30 µs before gating the detector-photodiode, in order to prevent detection of short lived components. The measurements were performed with a droplet of sample solution (typically 50 – 100 µL with a lanthanide complex concentration of approx. 3 µm) on a glass slide. The obtained time courses were the sum of 600 single measurements, recorded in 0.1 s intervals. The total time per single measurement was 30 ms with 7325 data points detected at 4.096 µs intervals. The decay curves were fitted with OriginPro 9.1 (OriginLab Corporation, USA) by applying a statistically weighted least square fit of an exponential decay function (\(I = I_0 + \sum_i I_i e^{-(t/\tau_i)}\)) with \(i\) (the number of different decay times; ranging from 1 - 2). The resulting decay constants \(\tau\) represent the lifetimes measured for the lanthanide complex in the sample solution. \(I_0\) represents the time-independent background light.

Synthesis of 2,2':6',2''-terpyridine 1,1''-di-N-oxide (1): 2,2':6',2''-terpyridine (30 mg, 125 µmol) was placed in a round bottom flask and DCM (2.25 mL) was added to dissolve the solid. While stirring mCPBA (162.3 mg, 77 w/w %, 724 µmol, 5.8 equiv.) was added. The solution was stirred for 24 h at room temperature. The resulting mixture was extracted four times with 10% Na\(_2\)CO\(_3\) (750 µL). The combined organic phases were dried with Na\(_2\)SO\(_4\) and concentrated to dryness under reduced pressure. The crude solid was washed with acetonitrile, filtered and redissolved in DCM. The solvent was evaporated under reduced pressure to obtain product 1 (15.6 mg, 58.8 µmol, 47%). \(^1\)H NMR (300 MHz, CDCl\(_3\), 25°C, TMS): \(\delta=8.94\) (d, \(3J(H,H)=8.0\) Hz, 2H, CH of 3’ and 5’), 8.34 (d, \(3J(H,H)=5.9\) Hz, 2H, CH of 6 and 6’), 8.20 (dd, \(3J(H,H)=7.9\) Hz, \(4J(H,H)=2.1\) Hz, 2H, CH of 3 and 3’), 7.98 (t, \(3J(H,H)=8.0\) Hz, 1H, CH of 4’), 7.38 (dt, \(3J(H,H)=7.7\) Hz, \(4J(H,H)=1.1\) Hz, 2H, CH of 5 and 5’), 7.30 (dt, \(3J(H,H)=6.9\) Hz, \(4J(H,H)=2.0\) Hz, 2H, CH of 4 and 4’); HRMS (ESI+): m/z (%): 266.0927 [M+H]\(^+\) (100), 288.0744 [M+Na]\(^+\) (15), 304.0483 [M+K]\(^+\) (1) (C\(_{15}\)H\(_{11}\)N\(_3\)O\(_2\) requires 266.0924 [M+H]\(^+\), 288.0743 [M+Na]\(^+\), 304.0483 [M+K]\(^+\]).

Synthesis of 2,2':6',2''-terpyridine-6,6''-dicarbonitrile (2): 1 (15.6 mg, 58.8 µmol) was dissolved in DCM (706 µL) and trimethylsilyl cyanide (77 µL, 588 µmol, 10 equiv.) was added. The solution was stirred for 20 min at room temperature and subsequently PhCOCl (28 µL, 235 µmol, 4 equiv.) was added dropwise during a time period of 20 min. The mixture was stirred overnight at room temperature. The
reaction mixture was concentrated to half of the volume, 10% K₂CO₃ (1.4 mL) was added and the suspension was stirred for 1 h at r.t. The resulting precipitate was filtered and washed with water and cold DCM. The remaining solid was dried in vacuum to obtain 2 (11.7 mg, 41.3 µmol, 70%). ¹H NMR (300 MHz, CDCl₃, 25°C, TMS): 8=7.75 (dd, 3J(H,H)=7.6 Hz, 4J(H,H)=1.0 Hz, 2H, CH of 5 and 5’), 8.01 (t, 3J(H,H)=7.9 Hz, 2H, CH of 4 and 4’), 8.05 (t, 3J(H,H)=7.9 Hz, 1H, CH of 4’), 8.58 (d, 3J(H,H)=7.9 Hz, 2H, CH of 3’ and 5’), 8.82 (dd, 3J(H,H)=8.1 Hz, 4J(H,H)=1.0 Hz, 2H, CH of 3 and 3’). ¹³C NMR (75 MHz, CDCl₃, 25°C, TMS): 8=117.4 (2C, C₆-CN and C₆-NCN), 122.8 (2C, CH of 3+3’’ or 5+5’’ or 3’+5’’), 124.3 (2C, CH of 3+3’’ or 5+5’’ or 3’+5’’), 128.5 (2C, CH of 3+3’’ or 5+5’’ or 3’+5’’), 133.5 (2C, C of 6’), 147.8 (2C, COOCH₃), 153.7 (2C, C of 2+2’ or 2’+5’), HRMS (ESI+): m/z (%) 350.1135 [M+H]+ (100), 306.0750 [M+Na]+(20) (C₁₇H₁₀N₅ requires 306.0750 [M+Na]+). Synthesis of dimethyl 2,2’:6’,2’’-terpyridine-6,6’’-dicarboxylate (3): 2 (145 mg, 512 µmol) was dissolved in water (2.3 mL), acetic acid (2.3 mL), and conc. sulfuric acid (512 µL). The mixture was heated to 90 – 100°C and stirred for 24 h. Afterwards the solution was poured into ice-water (20.5 mL) and the precipitate was filtered off. The solid was washed with cold water and acetonitrile. The carboxylic acid was dried 10 – 100 Pa. MeOH (16 mL) was cooled in an ice bath and thionyl chloride (390 µL) was added under argon atmosphere. The mixture was stirred for 15 min at room temperature and was then added to the solid carboxylic acid (170 mg, 529 µmol). The mixture was heated to reflux for 18 h and subsequently the solvent was removed under reduced pressure. The solid was dissolved in CHCl₃ (27 mL) and washed three times with 5% NaHCO₃ (13 mL). The organic phase was dried with Na₂SO₄ and the solvent was removed in vacuum. The crude product was recrystallized from toluene (28 mL) to obtain 3 (126 mg, 445 µmol, 84%). ¹H NMR (300 MHz, CDCl₃, 25°C, TMS): 8=4.06 (s, 6H, COOCH₃), 8.02 (t, 3J(H,H)=7.8 Hz, 3H, CH of 4, 4’ and 4’’), 8.18 (dd, 3J(H,H)=7.7 Hz, 4J(H,H)=1.1 Hz, 2H, CH of 5 and 5’’), 8.63 (d, 3J(H,H)=7.8 Hz, 2H, CH of 3’ and 5’’), 8.81 (dd, 3J(H,H)=7.9 Hz, 4J(H,H)=1.1 Hz, 2H, CH of 3 and 3’’); ¹³C NMR (75 MHz, CDCl₃, 25°C, TMS): 8=53.0 (2C, COOCH₃), 122.3 (2C, CH of 3+3’’ or 5+5’’ or 3’+5’’), 124.5 (2C, CH of 3+3’’ or 5+5’’ or 3’+5’’), 125.3 (2C, CH of 3+3’’ or 5+5’’ or 3’+5’’), 125.4 (2C, CH of 3+3’’ or 5+5’’ or 3’+5’’), 138.0 (2C, CH of 4+4’’), 138.4 (2C, C of 6+6’’), 147.8 (2C, C of 6+6’’), 154.7 (2C, C of 2+2’’ or 2’+5’’), 156.5 (2C, C of 2+2’’ or 2’+5’’), 166.0 (2C, COOCH₃); HRMS (ESI+): m/z (%): 350.1141 [M+H]+ (100), 372.0955 [M+Na]+ (20), 388.0695 [M+K]+ (2), (C₁₉H₁₅N₅O₄ requires 350.1135 [M+H]+, 372.0955 [M+Na]+, 388.0694 [M+K]+). Synthesis of 6,6’’-dihydroxymethyl-2,2’:6’,2’’-terpyridine (4): 3 (120 mg, 344 µmol) was dissolved in dry EtOH (10 mL) and sodium borohydride (77 mg, 1.96 mmol, 5.7 equiv.) was added under argon atmosphere. The mixture was stirred for 3 h at r.t. and subsequently heated to reflux for 1 h. The organic solvent was removed under reduced pressure, sat. NaHCO₃ (4.9 mL) was added and the mixture was shortly heated to boiling. After cooling to r.t. the precipitate was filtered off and washed with water to obtain 4 (87 mg, 297 µmol, 86%). ¹H NMR (300 MHz, [D₆] DMSO, 25°C, TMS): 8=4.69 (s, 4H, CH₂-OH), 5.57 (vbrs, 2H, CH₂-OH), 7.58 (d, 3J(H,H)=7.6 Hz, 2H, CH of 5 and 5’’), 8.01 (t, 3J(H,H)=7.8 Hz, 2H, CH of 4 and 4’’), 8.08 (t, 3J(H,H)=7.8 Hz, 1H, CH of 4’), 8.43 (d, 3J(H,H)=7.8 Hz, 2H, CH of 3’ and 5’), 8.49 (d, 3J(H,H)=7.6 Hz, 2H, CH of 3 and 3’’); ¹³C NMR (75 MHz, [D₆] DMSO, 25°C, TMS): 8=79.2 (2C, CH₂-OH), 118.8 (2C, CH of 3+3’’ or 5+5’’ or 3’+5’’), 120.6 (2C, CH of 3+3’’ or 5+5’’ or 3’+5’’), 120.7 (2C, CH of 3+3’’ or 5+5’’ or 3’+5’’), 137.8 (2C,
CH of 4+4'''), 138.4 (1 C, CH of 4'), 154.1 (2 C, C of 6+6''), 154.9 (2 C, C of 2+2'' or 2'+5'), 161.7 (2 C, C of 2+2'' or 2'+5'); HRMS (ESI+): m/z (%): 294.1232 [M+H]^+ (45), 316.1050 [M+Na]^+ (100), (C_{17}H_{15}N_{3}O_{2} requires 294.1237 [M+H]^+, 316.1056 [M+Na]^+).

Synthesis of 6,6''-dibromomethyl-2,2'-6',2''-terpyridine (5): 4 (137 mg, 467 μmol) and lithium bromide (44.6 mg, 46.7 μmol, 1.1 equiv.) were dissolved in dry DMF (5 mL) in an argon atmosphere. Phosphorus tribromide (213 μL, 2.27 mmol, 4.9 equiv.) was slowly added from a Hamilton™ syringe to the rigorously stirred mixture. Reaction controls were performed by TLC (silica gel; CHCl_3:MeOH = 8:2, R = 0.73). After complete reaction (approx. 4 h) the mixture was frozen in liquid nitrogen, attached to a liquid nitrogen-cooled cold-trap, and DMF was evaporated at 10 – 100 Pa. The residue was dissolved in CHCl_3 (22 mL). The organic phase was washed twice with 5% NaHCO_3 (13 mL). The combined aqueous phase was re-extracted with CHCl_3 (13 mL) and finally the combined organic phases were washed with brine. The organic solution was dried with Na_2SO_4 and the solvent was removed under reduced pressure to obtain 5 (167 mg, 399 μmol, 86%). ^1H NMR (300 MHz, CDCl_3, 25°C, TMS): δ = 4.66 (s, 4H, CH_2-Br), 7.49 (dd, 3J(H, H) = 7.7 Hz, 4J(H, H) = 0.8 Hz, 2H, CH of 5 and 5''), 7.86 (t, 3J(H, H) = 7.8 Hz, 2H, CH of 4 and 4''), 7.96 (t, 3J(H, H) = 7.8 Hz, 1H, CH of 4'), 8.52 (dd, 3J(H, H) = 7.8 Hz, 2H, CH of 3 and 5'), 8.53 (dd, 3J(H, H) = 7.9 Hz, 4J(H, H) = 0.8 Hz, 2H, CH of 3 and 3''); ^13C NMR (75 MHz, CDCl_3, 25°C, TMS): δ = 34.3 (2 C, CH_2-Br), 120.5 (2 C, CH of 3+3'' or 5+5'' or 3'+5'), 121.6 (2 C, CH of 3+3'' or 5+5'' or 3'+5''), 123.6 (2 C, CH of 3+3'' or 5+5'' or 3'+5'), 138.0 (2 C, CH of 4+4''), 138.1 (2 C, CH of 4'), 151.1 (2 C, C of 6+6''), 156.1 (2 C, C of 2+2'' or 2'+5'), 160.6 (2 C, C of 2+2'' or 2'+5'); HRMS (ESI+): m/z (%): 417.9539 [M+H]^+ (50), 419.9517 [M+H]^+ (100), 421.9496 [M+H]^+ (50), (C_{17}H_{13}N_{3}Br_2 requires 417.9549 [M+H]^+ (50), 419.9529 [M+H]^+ (100), 421.9508 [M+H]^+ (50)).

Synthesis of Z-Dab(Boc)-OH: Z-Dab(Boc)-OH-DCHA (100 mg, 187 μmol) was suspended in EtOAc (1.5 mL) and, while stirring, 1 M H_3PO_4 (1.0 mL, 0.94 mmol, 5 equiv.) was added dropwise at r.t. The phases were separated and washed thrice with 1 M H_3PO_4 (1 mL), once with water, and twice with brine. The organic solution was dried with Na_2SO_4 and concentrated in vacuum to obtain Z-Dab(Boc)-OH (62.1 mg, 176 μmol, 94%) ^1H NMR (300 MHz, CDCl_3, 25°C, TMS): δ = 1.46 (s, 9H, C(CH_3)_3), 1.94-2.44 (m, 2H, β-CH_2), 3.07-3.51 (m, 2H, γ-CH_2), 4.41 (t, 3J(H, H) = 6.36 Hz, 1H, α-CH), 5.11 (s, 2H, CH_2-CaH_5), 5.77 (d, 3J(H, H) = 6.81 Hz, 1H, NH), 6.77 (brs, 1H, NH), 7.31-7.38 (m, 5H, aromatic CH of C_6H_5); HRMS (ESI+): m/z (%): 353.1710 [M+H]^+ (100), 370.1973 [M+Na]^+ (20), 375.1526 [M+Na]^+ (10), 391.1266 [M+K]^+ (2), 705.3339 [2M+H]^+ (40), 727.3156 [2M+Na]^+ (20), (C_{17}H_{24}N,O_6 requires 353.1707 [M+H]^+, 370.1973 [M+Na]^+, 375.1527 [M+Na]^+, 391.1266 [M+K]^+, 705.3342 [2M+H]^+, 727.3161 [2M+Na]^+).

Synthesis of tert-butyl 2-(benzyl(2-hydroxyethyl)amino)acetate (8): N-Benzylethanolamine (2.50 mL, 16.5 mmol) was placed in a round bottom flask, DIPEA (2.13 mL, 16.5 mmol) and dry DMF (20 mL) were added. The solution was stirred at 0°C and tert-butyl bromoacetate (2.44 mL, 16.5 mmol) was added dropwise. The mixture was allowed to warm up to room temperature and was stirred over night at r.t. The solvent was removed under reduced pressure and the residue was dissolved in DCM (250 mL). The organic solution was washed four times with water (100 mL), dried with Na_2SO_4 and concentrated in vacuum to obtain 8 (4.40 g, 16.5 mmol, 100%). ^1H NMR (300 MHz, CDCl_3, 25°C, TMS): δ = 1.45 (s, 9H, C(CH_3)_3), 2.85 (t, 3J(H, H) = 5.2 Hz, 2H, N-CH_2-CH_2-OH), 3.22 (s, 2H, N-CH_2-COOC(CH_3)_3), 3.26
(brs, 1H, OH), 3.58 (t, $^3$J(H,H)=5.1 Hz, 2H, N-CH$_2$-CH$_2$-OH), 3.81 (s, 2H, CH$_2$-C$_6$H$_5$), 7.24-7.33 (m, 5H, C$_6$H$_5$); $^{13}$C NMR (75 MHz, CDCl$_3$, 25°C, TMS): $\delta$=28.1 (3 C, C(CH$_3$)$_3$), 55.5 (1 C, N-CH$_2$-CH$_2$-OH), 56.7 (1 C, N-CH$_2$-CH$_2$-OH), 58.7 (1 C, CH$_2$-COOC$_4$H$_9$), 59.1 (1 C, CH$_2$-C$_6$H$_5$), 81.3 (1 C, C(CH$_3$)$_3$), 127.3 (1 C, para-CH of C$_6$H$_5$), 128.4 (2 C, meta-CH of C$_6$H$_5$), 128.9 (2 C, ortho-CH of C$_6$H$_5$), 138.6 (1 C, ipso-C of C$_6$H$_5$), 171.2 (1 C, COOC$_4$H$_9$); HRMS (ESI+): m/z (%): 266.1756 [M+Na]$^+$ (100), 288.1573 [M+Na]$^+$ (10), (C$_{15}$H$_{23}$N$_3$O$_3$ requires 266.1751 [M+Na]$^+$, 288.1570 [M+Na]$^+$).

**Synthesis of tert-butyl 2-(benzyl(2-bromoethyl)amino)acetate (9):** 8 (750 mg, 2.83 mmol) and triphenylphosphine (871 mg, 3.32 mmol, 1.18 equiv.) were combined and dried three times by addition and evaporation of dry toluene. To remove residual toluene, dry DCM was added and evaporated three times. The mixture was immediately dissolved in dry DCM (9.5 mL under argon atmosphere). N-Bromosuccinimide (587 mg, 3.30 mmol, 1.17 equiv., dried in vacuum at 10 – 100 Pa) was added in portions over a period of 1 h at 0°C. The mixture was stirred for another 0.5 h at 0°C and subsequently the solution was allowed to warm up to r.t. Reaction controls were performed by TLC (silica gel; CHCl$_3$:MeOH:acetic acid = 9:1:0.1, $R_f$=0.87). After 4 h of stirring at r.t. the solvent was removed under reduced pressure. The crude product was purified by silica gel chromatography (gradient elution starting with CHCl$_3$:n-heptane = 2:3 and ending with CHCl$_3$:MeOH = 9:1) to obtain 9 (418 mg, 1.27 mmol, 45%). $^1$H NMR (300 MHz, CDCl$_3$, 25°C, TMS): $\delta$=11.47 (s, 9H, C(CH$_3$)$_3$), 3.13 (t, $^3$J(H,H)=7.2 Hz, 2H, N-CH$_2$-CH$_2$-Br), 3.30 (s, 2H, N-CH$_2$-COOC$_4$H$_9$), 3.37 (t, $^3$J(H,H)=7.3 Hz, 2H, N-CH$_2$-CH$_2$-Br), 3.87 (s, 2H, N-CH$_2$-C$_6$H$_5$), 7.22-7.37 (m, 5H, CH$_2$-C$_6$H$_5$); $^{13}$C NMR (75 MHz, CDCl$_3$, 25°C, TMS): $\delta$=28.3 (3 C, C(CH$_3$)$_3$), 30.7 (1 C, N-CH$_2$-CH$_2$-Br), 55.4 (1 C, N-CH$_2$-COOC$_4$H$_9$), 56.0 (1 C, N-CH$_2$-CH$_2$-Br), 58.2 (1 C, N-CH$_2$-C$_6$H$_5$), 81.3 (1 C, C(CH$_3$)$_3$), 127.4 (1 C, para-CH of C$_6$H$_5$), 128.5 (2 C, meta-CH of C$_6$H$_5$), 128.9 (2 C, ortho-CH of C$_6$H$_5$), 138.9 (1 C, ipso-C of C$_6$H$_5$), 170.7 (1 C, COOC$_4$H$_9$); HRMS (ESI+): m/z (%): 248.1645 [M-Br]$^+$ (100), 328.0908 [M+H]$^+$ (80), 330.0886 [M+H]$^+$ (80), (C$_{15}$H$_{23}$N$_3$O$_3$Br requires 248.1645 [M-Br]$^+$, 328.0907 [M+H]$^+$, 330.0886 [M+H]$^+$).

**References**

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[3] A. M. Brouwer, *Pure Appl. Chem.* 2011, 83.
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NMR-, mass- and UV/Vis-spectra of the synthesized compounds:

Figure S7: $^1$H-NMR of compound 1.

Figure S8: High resolution mass-spectrum of compound 1.
Figure S9: $^1$H-NMR of compound 2.

Figure S10: $^{13}$C-NMR of compound 2.
**Figure S11:** High resolution mass-spectrum of compound 2.

**Figure S12:** $^1$H-NMR of compound 3.
**Figure S13:** $^{13}$C-NMR of compound 3.

**Figure S14:** High resolution mass-spectrum of compound 3.
Figure S15: $^1$H-NMR of compound 4.

Figure S16: $^{13}$C-NMR of compound 4.
**Figure S17:** High resolution mass-spectrum of compound 4.

**Figure S18:** $^1$H-NMR of compound 5.
Figure S19: $^{13}$C-NMR of compound 5.

Figure S20: High resolution mass-spectrum of compound 5.
Figure S21: $^1$H-NMR of Z-Dab(Boc)-OH.

Figure S22: High resolution mass-spectrum of compound Z-Dab(Boc)-OH.
Figure S23: $^1$H-NMR of compound 6.

Figure S24: $^{13}$C-NMR of compound 6.
Figure S25: HSQC-NMR of compound 6.

Figure S26: High resolution mass-spectrum of compound 6.
Figure S27: $^1$H-NMR of compound 7.

Figure S28: $^{13}$C-NMR of compound 7.
Figure S29: High resolution mass-spectrum of compound 7.

Figure S30: $^1$H-NMR of compound 8.
Figure S31: $^{13}$C-NMR of compound 8.

Figure S32: High resolution mass-spectrum of compound 8.
Figure S33: $^1$H-NMR of compound 9.

Figure S34: $^{13}$C-NMR of compound 9.
Figure S35: High resolution mass-spectrum of compound 9.

Figure S36: $^1$H-NMR of compound 10.
Figure S37: $^{13}$C-NMR of compound 10.

FF_08 #8-21 RT: 0.10-0.30 AV: 14 NL: 1.29E8
T: FTMS + p ESI Full ms [100.00-2000.00]

Figure S38: High resolution mass-spectrum of compound 10.
Figure S39: $^1$H-NMR of compound 11.

Figure S40: $^{13}$C-NMR of compound 11.
Figure S41: High resolution mass-spectrum of compound 11.

Figure S42: $^1$H-NMR of compound 12.
Figure S43: $^{13}$C-NMR of compound 12.

FF_10_2 #8-16 RT: 0.10-0.22 AV: 9 NL: 1.03E8
T: FTMS + p ESI Full ms [100.00-2000.00]

Figure S44: High resolution mass-spectrum of compound 12.
Figure S45: $^1$H-NMR of compound 13.

Figure S46: $^{13}$C-NMR of compound 13.
**Figure S47:** High resolution mass-spectrum of compound 13.

**Figure S48:** $^1$H-NMR of compound 13-Eu.
Figure S49: $^{13}$C-NMR (APT) of compound 13-Eu.

Figure S50: HSQC-NMR of compound 13-Eu.
**Figure S51:** High resolution mass-spectrum of compound 13-Eu.

**Figure S52:** Representation of 13-Eu with denotation for NMR-signal assignment.
Table S3: NMR-signal assignment.

| Sign. | \(^1\text{H-NMR (700 MHz, CD\textsubscript{3}OD)} \) \(\delta / \text{ppm} \) | \(^1\text{H-discrition} \) | \(^{13}\text{C-NMR (176 MHz, CD\textsubscript{3}OD)} \) \(\delta / \text{ppm} \) | notes |
|-------|-------------------------------------------------|------------------|------------------------------------------|-------|
| 49.20 | [d]                                             |                  |                                          |       |
| 35.45 | [d]                                             |                  |                                          |       |
| 29.15 | [d]                                             |                  |                                          |       |
| 22.85 | [d]                                             |                  |                                          |       |
| \( A_x \) | 20.08 \((1\text{H, s, CH of A}_1 \text{ or A}_2 \text{ or A}_3)\) | 125.4            | [a]                                      |       |
|       | 18.23                                            |                  |                                          |       |
| \( A_x \) | 16.89 \((1\text{H, s, CH of A}_1 \text{ or A}_2 \text{ or A}_3)\) | 164.9            | [a]                                      |       |
| \( A_x \) | 14.93 \((1\text{H, s, CH of A}_1 \text{ or A}_2 \text{ or A}_3)\) | 119.1            | [a]                                      |       |
| \( B_x \) | 14.37 \((1\text{H, s, CH of B}_1 \text{ or B}_2 \text{ or B}_3)\) | 107.3            | [a]                                      |       |
| \([1]\) | 11.18 \((1\text{H, s, CH}_2 \text{ of } 1 \text{ or } 1')\) | 111.8            | [b]                                      |       |
| \( \gamma \) | 10.54 \((1\text{H, s, CH}_2 \text{ of } \gamma)\) | 7.1              | [c]                                      |       |
| \( C_x \) | 7.27 \((1\text{H, s, CH of C}_1 \text{ or C}_2 \text{ or C}_3)\) | 93.1             | [a]                                      |       |
| \( B_x \) | 6.99 \((1\text{H, s, CH of B}_1 \text{ or B}_2 \text{ or B}_3)\) | 147.7            | [a]                                      |       |
| \(1'\) | 5.60 \((1\text{H, s, CH}_2 \text{ of } 1 \text{ or } 1')\) | 111.8            | [b]                                      |       |
| \( \gamma \) | 3.75 \((1\text{H, s, CH}_2 \text{ of } \gamma)\) | 7.1              | [c]                                      |       |
| \( C_x \) | 3.42 \((1\text{H, s, CH of C}_1 \text{ or C}_2 \text{ or C}_3)\) | 141.4            | [a]                                      |       |
| \( \sigma \) | 3.30 \((1\text{H, s, CH of } \sigma)\) | 48.5             | [c]                                      |       |
| \( \beta \) | 2.66 \((1\text{H, s, CH}_2 \text{ of } \beta)\) | 35.4             | [c]                                      |       |
| \( \beta \) | 0.93 \((1\text{H, s, CH}_2 \text{ of } \beta)\) | 35.4             | [c]                                      |       |
| \(2\) | 0.74 \((1\text{H, s, CH}_2 \text{ of } 2 \text{ or } 2')\) | 62.3             | [b]                                      |       |
| \( B_x \) | 0.08 \((1\text{H, s, CH of B}_1 \text{ or B}_2 \text{ or B}_3)\) | 98.2             | [a]                                      |       |
| \(3\) | 0.08 \((1\text{H, s, CH of } 3 \text{ or } 3')\) | 71.6             | [b]                                      |       |
| \( C_x \) | -0.90 \((1\text{H, s, CH of C}_1 \text{ or C}_2 \text{ or C}_3)\) | 90.7             | [a]                                      |       |
| \(3'\) | -7.81 \((1\text{H, s, CH}_2 \text{ of } 3 \text{ or } 3')\) | 71.6             | [b]                                      |       |
|       | -14.01                                           |                  |                                          | [d]   |
| \(2'\) | -15.07 \((1\text{H, s, CH}_2 \text{ of } 2 \text{ or } 2')\) | 62.3             | [b]                                      |       |
| \(4\) | -17.86 \((1\text{H, s, CH}_2 \text{ of } 4 \text{ or } 4')\) | 42.6             | [b]                                      |       |
|       | -18.68                                           |                  |                                          | [d]   |
| \(4'\) | -21.20 \((1\text{H, s, CH}_2 \text{ of } 4 \text{ or } 4')\) | 42.6             | [b]                                      |       |
|       |                                                  |                  |                                          |       |
|       |                                                  |                  |                                          |       |
|       |                                                  |                  |                                          |       |

[a] The aromatic signals originates from the terpyridine unit, but it cannot be distinguished between the different pyridine rings. With a moderately COSY respectively TOCSY it was possible to assign each three protons which are belonging to the same pyridine ring. [b] The numbered signals are CH\(_2\)-groupes which could not be distinguished from each other (They were continuously numbered without further relation to the chemical structure). Due to the paramagnetism of the europium, each proton of one CH\(_2\)-group has a different shift and the signals with the same number (distinguished by an apostrophe) belong to the same CH\(_2\)-group. [c] With a COSY and TOCSY experiment the signals \(\alpha\), \(\beta\) and \(\gamma\) could be assigned. [d] Most of these signals are quaternary carbons, but some of them are CH\(_2\)-groups which are exposed to an outstanding high paramagnetism and therefore the relaxation is too fast to detect any C-H or H-H correlations (e.g. in HSQC or COSY).
Figure S53: UV-VIS spectrum of compound 13-Eu in water.

Figure S54: High resolution mass-spectrum of compound 14-Eu.
Figure S55: Mass spectrum of compound 13-Tb.

Figure S56: UV-VIS spectrum of compound 13-Tb in water.
Figure S57: High resolution mass-spectrum of compound 14-Tb.

Figure S58: $^1$H-NMR of compound 15.
Figure S59: $^1$H-NMR of compound 16.

Figure S60: HSQC-NMR of compound 16.
Figure S61: Mass spectrum of compound 16 (positive mode).

Figure S62: Mass spectrum of compound 16 (negative mode).

Figure S63: $^1$H-NMR of compound 17.
Figure S64: HSQC-NMR of compound 17.

Figure S65: Mass spectrum of compound 17.
Figure S66: High resolution mass-spectrum of compound 17-Eu.

Figure S67: UV-VIS spectrum of compound Eu-17 in HBS 7.3 buffer.
Figure S68: Emission spectrum of compound Eu-17 in HBS 7.3 buffer, excitation at 335 nm.

Figure S69: Size-exclusion chromatogram of 14-Eu-labeled BSA, detecting at 280 and 330 nm absorbance. Fractions 9 – 12 containing the labeled BSA were collected, while the other fractions were discarded.