Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Supporting Information

Novel EDTA-Ligands Containing an Integral Perylene Bisimide (PBI) Core as Optical Reporter Units

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1. Synthesis of precursor amine **2**
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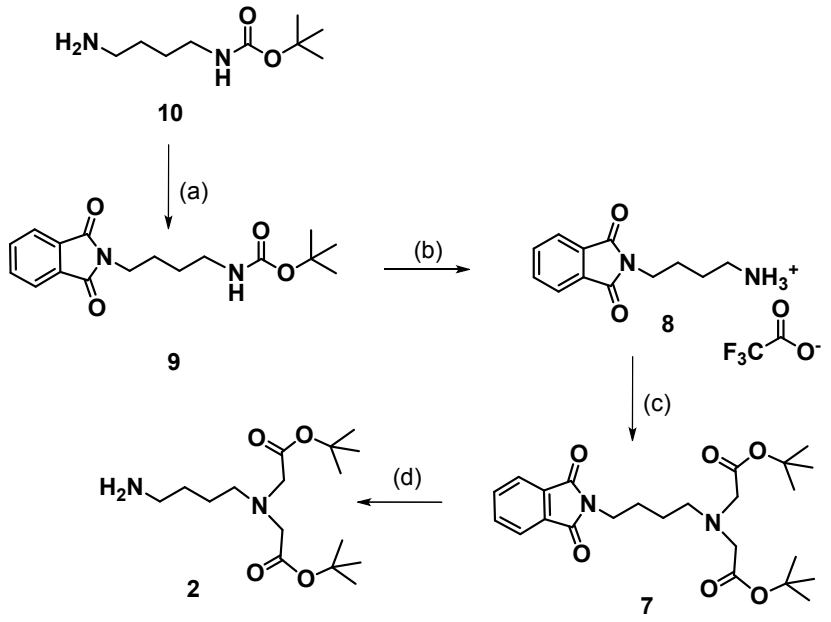
Synthesis of di-tert-butyl 2,2'-((4-(1,3-dioxoisoindolin-2-yl)butyl)azanediyl)diacetate (2)



Scheme S1. Synthesis of precursor amine **2**. (a) Phthalic anhydride, 135°C, 10 min; (b) trifluoroacetic acid, CH_2Cl_2, 4 h, RT; (c) *tert*-butyl 2-bromoacetate, 1,4-dioxan, DIEA, 60°C, 24h; (d) hydrazine hydrate, ethanol, 50°C, 6 h.

Compound **10** was synthesized according to Muller *et al.* [1]

*Synthesis of tert-Butyl 4-(1,3-dioxoisoindolin-2-yl)butylcarbamate (**9**)*:
1.00 g of **10** (5.30 mmol) was reacted with phthalic anhydride (0.80 g, 5.30 mmol) at 135°C for 10 min under solvent free conditions. After cooling the reaction mixture, methanol was added to remove potentially formed side products by filtration. The filtrate was concentrated under vacuum and **9** was purified by column chromatography (SiO_2, dichloromethane/methanol), yielding a white solid; yield 1.22 g (3.85 mmol, 72.6 %); R_f = 0.7 (dichloromethane/methanol 98:2).
^{1}H NMR (300 MHz, $CDCl_3$, 25°C): δ = 1.41 (s, 9H, 3 x CH_3), 1.51 (quintuplet, *J* = 7.6 Hz, 2H, CH_2), 1.69 (quintuplet, *J* = 7.2 Hz, 2H, CH_2), 3.14 (quartet, *J* = 6.4 Hz, 2H, CH_2), 3.69 (t, *J* = 7.2 Hz, 2H, CH_2), 4.58 (bs, 1H, NH of carbmate), 7.70 (dd, J_1 = 3.2 Hz, J_2 = 5.2 Hz, 2H, ArH), 7.83 (dd, J_1 = 2.8 Hz, J_2 = 5.2 Hz, 2H, ArH) ppm.
^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): δ = 25.78 (1 C, CH_2), 27.25 (1 C, CH_2), 28.21 (3 C, CH_3), 37.39 (1 C, CH_2), 39.88 (1 C, CH_2), 78.94 (1 C, quat. C ^{t}Bu), 123.13 (2 C, Ar-CH), 132.02 (2 C, Ar-C), 133.88 (2 C, Ar-CH), 155.92 (2 C, CON), 168.37 (1 C, CO(NH)O) ppm.
MS-ESI(+): *m*/*z* = 318 [M^+], 217 [M^+ - Boc].
IR (ATR): ν = 3371.86, 2978.38, 2934.74, 1701.97, 1679.91, 1527.06, 1398.11, 1361.34, 1310.39, 1271.40, 1247.20, 1170.13, 1051.06, 1012.83, 717.48 cm^{-1}.

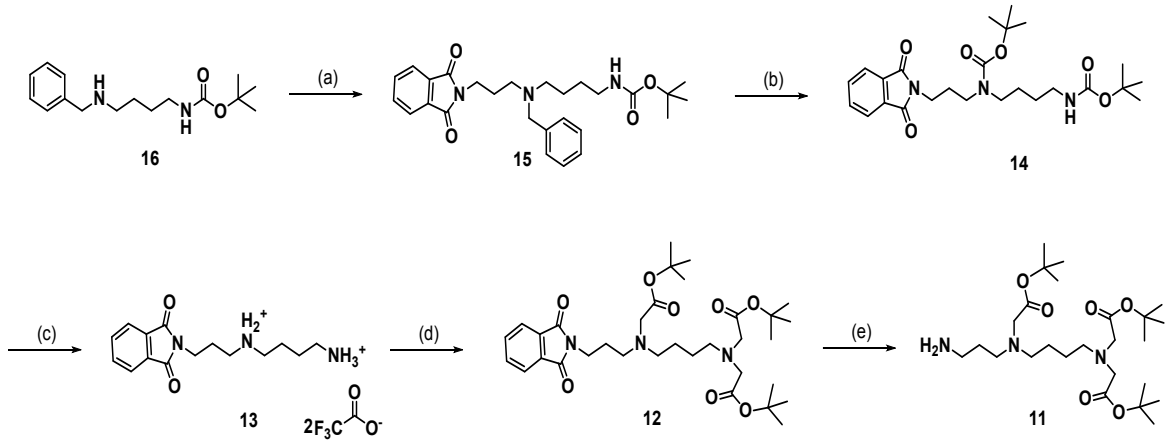
*Synthesis of 2-(4-aminobutyl)isoindoline-1,3-dione (**8**)*:
To a solution of **9** (3.00 g, 9.40 mmol) in 5 mL dicholoromethane 5 mL of trifluoroacetic acid (TFA) was added to cleave the Boc protection group. The reaction mixture was stirred for 4 h at RT. After evaporation of the solvent, the product was precipitated with diethyl ether. After filtration, the product was dried under vacuum yielding compound **8** as TFA salt, white solid, quantitative yield; R_f = 0.35 (dichloromethane/methanol 90:10).

^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): 1.53 (quintuplet, J = 8.8 Hz, 2H, CH_2), 1.62 (quintuplet, J = 6.4 Hz, 2H, CH_2), 2.80 (sextet, J = 6.4 Hz, 2H, $CH_2NH_3^+$, converts to triplet on D_2O exchange), 3.60 (t, J = 6.4 Hz, 2H, CH_2), 7.73 (bs, 3H, NH_3^+ exchanges with D_2O), 7.83-7.89 (m, 4H, ArH) ppm.
^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): δ = 24.39 (1 C, CH_2), 24.94 (1 C, CH_2), 36.75 (1 C, CH_2), 38.34 (1 C, CH_2), 123.05 (2 C, Ar-CH), 131.61 (2 C, Ar-C), 134.47 (2 C, Ar-CH), 168.06 (2 C, CON) ppm.
MS-ESI(+): m/z = 219 [M^+] as free amine.
IR (ATR): ν = 3400.15, 2937.11, 1707.96, 1676.22, 1398.49, 1200.59, 1179.01, 1127.86, 1065.16, 800.49, 721.48, 711.19 cm^{-1}.

*Synthesis of tert-Butyl 2,2'-(4-(1,3-dioxoisoindolin-2-yl)butylazanediyl)diacetate (**7**)*:
To a solution of **8** (1.40 g, 4.20 mmol) in 1,4-dioxane (20 mL), *tert*-butyl 2-bromoacetate (1.43 mL, 8.80 mmol) and diisopropylethyl amine (DIEA) (2.23 mL, 12.65 mmol) was added. The reaction mixture was stirred for 24 h at 60°C. After completion of the reaction (tlc), the reaction mixture was filtered and the solvent was evaporated under vacuum. Purificatio by column chromatography (SiO_2, dichloromethane/methanol) was applied to isolate pure **7** as a liquid, yield 1.54 g (3.45 mmol, 81.9 %); R_f = 0.55 (dichloromethane/methanol 99:1).
^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 1.43 (s, 18H, 6 x CH_3), 1.47-1.54 (m, 2H, CH_2), 1.70 (quintuplet, J = 7.4 Hz, 2H, CH_2), 2.71 (t, J = 7.4 Hz, 2H, CH_2), 3.40 (s, 4H, 2 x NCH_2), 3.69 (t, J = 7.0 Hz, 2H, CH_2), 7.70 (dd, J_1 = 2.8 Hz, J_2 = 5.2 Hz, 2H, ArH), 7.82 (dd, J_1 = 2.8 Hz, J_2 = 5.2 Hz, 2H, ArH) ppm.
^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): δ = 25.18 (1 C, CH_2), 26.07 (1 C, CH_2), 27.99 (6 C, CH_3), 37.66 (1 C, CH_2), 53.49 (1 C, CH_2), 55.75 (2 C, CH_2), 80.76 (2 C, quat. C ^{t}Bu), 123.09 (2 C, Ar-CH), 132.13 (2 C, Ar-C), 133.81 (2 C, Ar-CH), 168.395 (2 C, CON), 170.683 (2 C, COO) ppm.
MS-ESI(+): m/z = 446 [M^+].
IR (ATR): ν = 2976.93, 2934.60, 1771.72, 1708.64, 1394.13, 1366.48, 1218.25, 1145.63, 751.54 cm^{-1}.

*Synthesis of tert-Butyl 2,2'-(4-aminobutylazanediyl)diacetate (**2**)*:
To a solution of **7** (3.40 g, 2.00 mmol) in ethanol (35 mL), hydrazine hydrate (0.92 mL, 4.00 mmol) was added. The reaction mixture was stirred for 6 h at 50°C. The formed precipitate was removed by filtration. The filtrate was concentrated in *vacuo* and the residue was dissolved in dichloromethane and washed with 10% KOH solution. The organic layer was back extracted with brine (50 mL) and dried over Na_2SO_4 and concentrated in vacuum and purified by column chromatography (SiO_2, dichloromethane/methanol/triethylamine) to isolate pure **2** as a liquid, yield 1.63 g (5.16 mmol, 67.8 %); R_f = 0.65 (dichloromethane:methanol:triethylamine 80:15:5).
^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 1.44-1.45 (m, 18H, 6 x CH_3); the broad signals of the Boc group protons superimpose the signals of the putrescine methylene protons (4H, 2 x CH_2), 2.66-2.70 (m, 4H, 2 x CH_2), 3.41 (s, 4H, 2 x NCH_2) ppm.
^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): δ = 25.12 (1 C, CH_2), 27.96 (6 C, CH_3), 30.94 (1 C, CH_2), 41.73 (1 C, CH_2), 53.77 (1 C, CH_2), 55.77 (2 C, CH_2), 80.76 (2 C, quat. C ^{t}Bu), 170.70 (2 C, COO) ppm.
MS-ESI(+): m/z = 316 [M^+].

Synthesis of tert-butyl 2,2'-(4-((3-aminopropyl)(2-tert-butoxy-2-oxoethyl)amino)-butylazanediyl) diacetate (11)



Scheme S2. Synthesis of precursor amine **11**. (a) *N*-(3-bromopropyl)phthalimide, chloroform:acetonitrile (1:1), Na_2CO_3, 80°C, 3 days; (b) 10% Pd/C, H_2, di-tert-butyl dicarbonate, acetic acid (cat.), CH_3OH; (c) trifluoroacetic acid, CH_2Cl_2, 4 h, RT; (d) *tert*-butyl 2-bromoacetate, 1,4-dioxan, DIEA, 60°C, 24 h; (e) hydrazine hydrate, ethanol, 50°C, 6 h.

*Synthesis of tert-butyl 4-(benzylamino)butylcarbamate (**16**)*:
10 (6.00 g, 31.00 mmol) was dissolved in 60 mL of methanol. To this solution benzaldehyde (4.06 g, 38.00 mmol), $MgSO_4$ (7.70 g, 63.00 mmol) and triethylamine (0.97 g, 9.00 mmol) was added and the mixture was stirred at RT overnight. Afterwards, the reaction mixture was cooled to 0°C and $NaBH_4$ (6.00 g, 160.00 mmol) was added over a period of 60 minutes. The reaction mixture was stirred for additional 60 minute at 0°C and then at room temperature for 2 hours. Water (200 mL) was added to quench the reaction and the aqueous layer was extracted with ethyl acetate (3 x 150 mL). The organic layer was dried over Na_2SO_4 and afterwards, the solvent was evaporated under vacuum. The residue was purified by column chromatography (SiO_2, dichloromethane:methanol) yielding **16** as a liquid, yield 6.86 g (21.5 mmol, 67.6 %); R_f = 0.5 dichloromethane:methanol (90:10).
^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 1.41 (s, 9H, 3 x CH_3), 1.51 (quintuplet, *J* = 3.2 Hz, 4H, 2 x CH_2), 1.69 (bs, 1H, NH exchanges with D_2O), 2.62 (bt, *J* = 6.8 Hz, 2H, CH_2), 3.09 (bq, *J* = 5.2 Hz, 2H, CH_2, changes to broad triplet on D_2O exchange), 3.75 (s, 2H, NCH_2), 4.85 (bs, 1H, NH of carbmate), 7.21-7.30 (m, 5H, ArH) ppm.
^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): δ = 27.018 (1 C, CH_2), 27.584 (1 C, CH_2), 28.218 (3 C, CH_3), 40.073 (1 C, CH_2), 48.580 (1 C, CH_2), 53.649 (1 C, CH_2), 78.750 (1 C, quat. C ^{t}Bu), 126.870 (1 C, Ar-CH), 128.063 (2 C, Ar-CH), 128.30 (2 C, Ar-CH), 140.023 (1 C, Ar-C), 155.990 (1 C, CO(NH)O).
MS-ESI(+): *m*/*z* = 279 [M^++H].
IR (ATR): ν = 3338.51, 2975.05, 2930.46, 2862.96, 1690.45, 1514.38, 1452.98, 1390.67, 1364.51, 1270.63, 1248.79, 1167.66, 733.61, 697.56 cm^{-1}.

*Synthesis of tert-butyl 4-(benzyl(3-(1,3-dioxoisoindolin-2-yl)propyl)amino)butylcarbamate (**15**)*:
To a solution of **16** (5.0 g, 18.00 mmol) in acetonitrile:chloroform (CH_3CN:$CHCl_3$) (1:1) (100 mL), *N*-(3-bromopropyl)phthalimide (6.26 g, 23.00 mmol) and Na_2CO_3 (3.8 g, 36.00 mmol) was added. The reaction mixture was stirred for 3 days at 80°C. After completion of the reaction (tlc), the reaction mixture was filtered and the solvent was evaporated under vacuum. The residue was purified by column chromatography (SiO_2, dichloromethane:methanol) yielding **15** as transparent light greenish liquid (slowly become solid when kept in refrigerator), yield 6.83 g (14.7 mmol, 81.7 %); R_f = 0.75 dichloromethane:methanol (97:3).

^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 1.42 (s, 9H, 3 x CH_3); 1.47 (quintuplet, *J* = 3.2 Hz, 4H, 2 x CH_2), 1.82 (quintuplet, *J* = 7.2 Hz, 2H, CH_2), 2.41 (bt, *J* = 6.4 Hz, 2H, CH_2), 2.46 (t, *J* = 6.8 Hz, 2H, CH_2), 3.07 (bq, *J* = 5.6 Hz, 2H, CH_2), 3.52 (s, 2H, NCH_2), 3.68 (t, *J* = 7.6 Hz, 2H, CH_2), 4.78 (bs, 1H, NHCO), 7.16-7.30 (m, 5H, ArH-benzyl), 7.70 (dd, J_1 = 3.2 Hz, J_2 = 5.6 Hz, 2H, ArH), 7.82 (dd, J_1 = 3.2 Hz, J_2 = 5.6 Hz, 2H, ArH) ppm.
^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): δ = 24.285 (1C, CH_2), 25.898 (1C, CH_2), 27.629 (1 C, CH_2), 28.278 (3C, CH_3), 36.251 (1 C, CH_2), 40.327 (1 C, CH_2), 50.988 (1 C, CH_2), 53.203 (1 C, CH_2), 58.454 (1 C, CH_2) 78.750 (1 C, quat. C ^{t}Bu), 123.087 (2 C, Ar-CH), 126.755 (1 C, Ar-CH), 128.089 (1 C, Ar-CH), 128.834 (1 C, Ar-CH), 132.123 (2 C, Ar-C), 133.816 (2 C, Ar-CH), 139.514 (1 C, Ar-C), 156.027 (2 C, CON), 168.384 (1C, CO(NH)O).
MS-ESI(+): *m*/*z* = 466 [M^++H] IR (ATR).
ν = 3389.68, 2944.63, 2867.67, 2799.57, 1703.53, 1685.33, 1522.87, 1390.90, 1365.22, 1270.42, 1243.89, 1172.29, 741.30, 721.08 cm^{-1}.

*Synthesis of tert-butyl 4-((3-(1,3-dioxoisoindolin-2-yl)propyl)(tert-butoxycarbonyl)amino)butyl-carbamate (**14**)*:
To the N_2-flushed solution of **15** (3.00 g, 6.40 mmol) in 30 mL of methanol (dry), 10% Pd/C (0.45 g) was added, followed by the addition of acetic acid (0.25 mL). Di-*tert*-butyldicarbonate (2.26 g, 10.00 mmol) was also added to the above solution. The flask was degassed and saturated with hydrogen and stirred for 24 h at room temperature. The reaction progress was monitored by TLC. After completion of the reaction (tlc), the Pd/C was filtered off (celite), washed with methanol and the combined organic solvents were evaporated under vacuum. The residue was purified by column chromatography (SiO_2, dichloromethane methanol) yielding **14** as liquid, yield 2.60 g (5.47 mmol, 84.9 %); R_f = 0.65 in 2% methanol:dichloromethane.
^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 1.41 [(bs, 18H, CH_3) which splits into two singlets at 1.35 (s, 9H, 3 x CH_3) and 1.38 (s, 9H, 3 x CH_3) when the NMR was taken at -20^{o}C], 1.45-1.47 (m, 2H, CH_2), 1.53 (quintuplet, *J* = 7.4 Hz, 2H, CH_2), 1.89 (quintuplet, J_1 = 7.2 Hz, 2H, CH_2), 3.11 (q, *J* = 6.0 Hz, 2H, CH_2), 3.20-3.26 [(broad, splits into two triplets at 3.16 (t, *J* = 7.2 Hz, 2H, CH_2) and 3.25 (t, *J* = 7.2 Hz, 2H, CH_2) when the NMR was accquired at -20^{o}C], 3.68 (t, *J* = 7.2 Hz, 2H, CH_2), 4.67 (bs, 1H, NH of carbmate), 7.71 (dd, J_1 = 2.8 Hz, J_2 = 5.2 Hz, 2H, ArH), 7.83 (dd, J_1 = 2.8 Hz, J_2 = 5.2 Hz, 2H, ArH) ppm.
^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): δ = 25.502 (1 C, CH_2), 27.214 (1 C, CH_2), 27.633 (1 C, CH_2), 28.225 (6 C, CH_3), 35.680 (1 C, CH_2), 40.061 (1 C, CH_2), 44.437 (1 C, CH_2), 46.713 (1 C, CH_2), 78.872 (1 C, quat. C ^{t}Bu), 79.380 (1 C, quat. C ^{t}Bu), 123.180 (2 C, Ar-CH), 132.054 (2 C, Ar-C), 133.941 (2 C, Ar-CH), 155.430 (1 C, COO), 156.002 (1 C, COO), 168.303 (2 C, CON).
MS-ESI(+): *m*/*z* = 499 (M^+ + Na), 514 (M^+ + K);
IR (ATR): ν = 3367.35, 2974.83, 2932.30, 1772.13, 1707.48, 1687.80, 1394.36, 1364.25, 1247.15, 1166.74, 1029.52, 719.42 cm^{-1}.

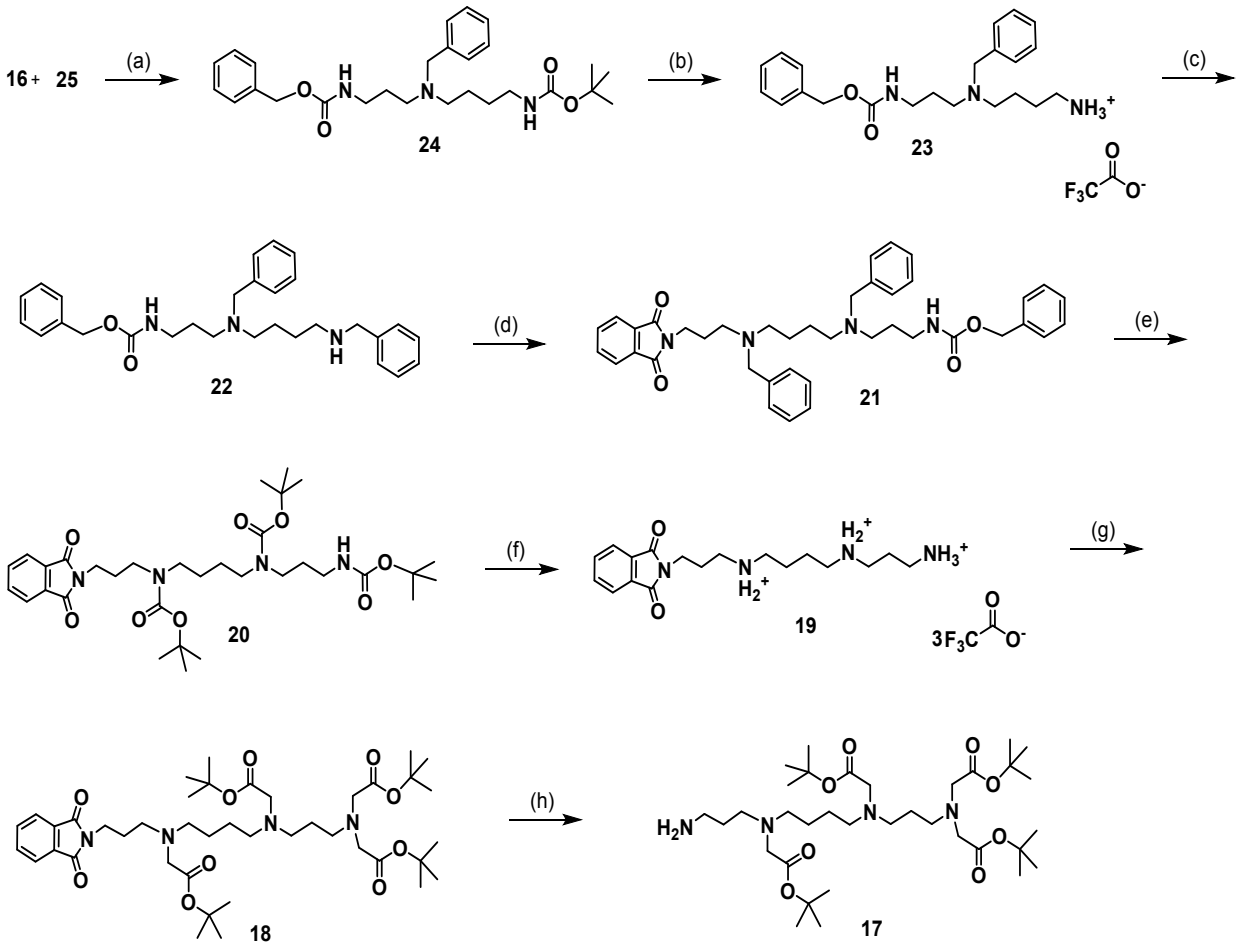
*Synthesis of 2-(3-(4-aminobutylamino)propyl)isoindoline-1,3-dione as TFA salt (**13**)*:
To a solution of **14** (1.00 g, 2.10 mmol) in 2 mL dichloromethane 2 mL of TFA was added to cleave the Boc protection groups. The reaction mixture was stirred for 4 h at RT. After evaporation of the solvent, the residue was treated with diethyl ether (20 mL, 2 times) and the resulting white solid was filtered off and washed with diethyl ether. The solid product was dried under vacuum to yielding **13** as solid, yield quantitative; R_f = 0.37 (dichloromethane:methanol:triethylamine) (7.5:2:0.5) mixture.
^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 1.54-1.61 (m, 4H, 2 x CH_2), 1.93 (quintuplet, *J* = 6.8 Hz, 2H, CH_2), 2.80 (sextet, *J* = 6.2 Hz, 2H, $CH_2NH_3^+$, converts to triplet on D_2O exchange), 2.90 (quintuplet, *J* = 5.6 Hz, 2H, $CH_2NH_2^+$, Converts to triplet on D_2O exchange), 2.96 (quintuplet, *J* = 5.2 Hz, 2H, $CH_2NH_2^+$, Converts to triplet on D_2O exchange), 3.64 (t, *J* = 6.8 Hz, 2H, CH_2), 7.84-7.90 (m, 4H of ArH and 3H of NH_3^+, which exchanges with D_2O), 8.64 (bs, 2H, NH_2^+, which exchanges with D_2O) ppm.

^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): δ = 22.928 (1 C, CH_2), 24.276 (1 C, CH_2), 25.371 (1 C, CH_2), 35.157 (1C, CH_2), 38.509 (1 C, CH_2), 45.042 (1 C, CH_2), 46.566 (1 C, CH_2), 123.724 (2 C, Ar-CH), 132.069 (2 C, Ar-C), 135.183 (2 C, Ar-CH), 168.890 (2 C, CON).
MS-ESI(+): *m*/*z* = 276 [M^+ +1 (free amine)].
IR (ATR): ν = 2946.01, 2854.43, 1710.04, 1667.20, 1616.91, 1431.88, 1395.45, 1361.97, 1195.78, 1178.34, 1126.70, 831.09, 796.37, 718.50 cm^{-1}.

*Synthesis of tert-butyl 2,2'-(4-((2-tert-butoxy-2-oxoethyl)(3-(1,3-dioxoisoindolin-2-yl)propyl)amino) butylazane-diyl)diacetate (**12**)*:
To a solution of **13** (2.5 g, 5.0 mmol) in 1,4-dioxan (65 mL), *tert*-butyl bromoacetate (3.4 g, 2.82 mL, 17.4 mmol) and diisopropylethyl amine (DIEA) (3.2 g, 30.0 mmol) was added. The reaction mixture was stirred for 24h at 60°C. After completion of the reaction (tlc), the reaction mixture was filtered and filtrate was evaporated under vacuum. The residue was purified by column chromatography (SiO_2, dichloromethane:methanol) yielding **12** as a light greenish liquid, yield 1.9 g (3.0 mmol, 62.0 %); R_f = 0.75 dichloromethane:methanol (97:3).
^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 1.42 (s, 9H, 3 x CH_3), 1.44 (bs, 18H, 6 x CH_3 and 2H of CH_2 merge under the singlet), 1.73 (broad quintuplet, 2H, CH_2), 1.80 (quintuplet, *J* = 7.2 Hz, 2H, CH_2), 2.57 (t, *J* = 7.0 Hz, 2H, CH_2), 2.66 (t, *J* = 7.0 Hz, 4H, 2 x CH_2), 3.22 (s, 2H, CH_2), 3.40 (s, 4H, 2 x NCH_2), 3.72 (t, *J* = 7.2 Hz, 2H, CH_2), 7.69 (dd, J_1 = 2.8 Hz, J_2 = 5.2 Hz, 2H, ArH), 7.82 (dd, J_1 = 2.8 Hz, J_2 = 5.2 Hz, 2H, ArH) ppm.
^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): δ = 25.117 (1C, CH_2), 25.591 (1 C, CH_2), 26.527 (1 C, CH_2), 28.018 (9 C, CH_3), 36.144 (1 C, CH_2), 51.550 (1 C, CH_2), 53.662 (1 C, CH_2), 53.952 (1 C, CH_2), 55.197 (1 C, CH_2), 55.738 (2 C, CH_2), 80.591 (1 C, quat. C ^{t}Bu), 80.684 (2 C, quat. C ^{t}Bu), 123.083 (2 C, Ar-CH), 132.196 (2 C, Ar-C), 133.797 (2 C, Ar-CH), 168.367 (2 C, CON), 170.774 (1 C, COO), 170.844 (2 C, COO).
MS-ESI(+): *m*/*z* = 618 [M^++H].
IR (ATR): ν = 2976.69, 2934.86, 1771.96, 1709.98, 1467.22, 1392.99, 1366.37, 1251.52, 1215.52, 1146.15, 1036.89, 843.25, 719.75 cm^{-1}.

*Synthesis of tert-butyl 2,2'-(4-((3-aminopropyl)(2-tert-butoxy-2-oxoethyl)amino)butylazanediyl) diacetate (**11**)*:
To a solution of **12** (2.0 g, 3.2 mmol) in ethanol (30 mL), hydrazine hydrate (0.486 mL, 9.7 mmol) was added and reaction mixture was stirred for 8 h at 50°C. The formed solid precipitate was removed by filtration. The filtrate was concentrated in vacuum and the residue was dissolved in dichloromethane and washed with 10% KOH solution. The organic layer was extracted with brine (50 mL), dried over Na_2SO_4 and concentrated in vacuum yielding a residue which was further purified by column chromatography (SiO_2, dichloromethane:methanol:triethylamine). **10** was isolated as transparent liquid, yield 1.29 g (2.65 mmol, 81.8 %); R_f = 0.65 dichloromethane:methanol:triethylamine (9:0.5:0.5).
^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 1.43 (s, 9H, 3 x CH_3), 1.44 (s, 18H, 6 x CH_3), 1.43-1.48 (m, 4H, 2 x CH_2 signals merge under methyl peaks), 1.57 (quintuplet, *J* = 6.9 Hz, 2H, CH_2), 2.54 (t, *J* = 6.8 Hz, 2H, CH_2), 2.59 (t, *J* = 7.2 Hz, 2H, CH_2), 2.67 (t, *J* = 7.0 Hz, 2H, CH_2), 2.72 (t, *J* = 6.6 Hz, 2H, CH_2), 3.20 (s, 2H, NCH_2), 3.4 (s, 4H, 2 x NCH_2) ppm.
^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): δ = 24.904 (1 C, CH_2), 25.658 (1 C, CH_2), 28.025 (9 C, CH_3), 31.088 (1 C, CH_2), 40.361 (1 C, CH_2), 51.777 (1 C, CH_2), 53.913 (1 C, CH_2), 53.972 (1 C, CH_2), 55.772 (1 C, CH_2), 55.820 (2 C, CH_2), 80.581 (1 C, quat. C ^{t}Bu), 80.719 (2 C, quat. C ^{t}Bu), 170.772 (1 C, COO), 170.985 (2 C, COO) ppm.
MS-ESI(+): *m*/*z* = 488 [M^++H].
IR (ATR): ν = 2977.56, 2852.76, 1730.27, 1456.53, 1392.13, 1367.09, 1254.25, 1218.38, 1146.44, 841.75, 733.85, 701.66 cm^{-1}.

Synthesis of di-tert-butyl 2,2'-((3-((4-((3-aminopropyl)(2-(tert-butoxy)-2-oxoethyl)amino)butyl)(2-(tert-butoxy)-2-oxoethyl)amino)propyl)azanediyl)diacetate (17)



Scheme 3. Synthesis of precursor amine **17**. (a) **16**, *N*-carbobenzyloxy-3-bromopropylamine (**25**), chloroform:acetonitrile (1:1), Na_2CO_3, 70°C, 48 h; (b) trifluoroacetic acid, CH_2Cl_2, 4 h, RT; (c) benzaldehyde, $MgSO_4$, triethylamine, CH_3OH, overnight, then $NaBH_4$ addition at 0°C for 1 h and then RT for 1 h; (d) *N*-(3-bromopropyl)phthalimide, chloroform:acetonitrile (1:1), Na_2CO_3, 80°C, 48 h; (e) 10% Pd/C, H_2, di-*tert*-butyl dicarbonate, acetic acid (cat.), CH_3OH; (f) trifluoroacetic acid, CH_2Cl_2, 4 h, RT; (g) *tert*-butyl 2-bromoacetate, 1,4-dioxan-acetonitrile (8:2), DIEA, 45°C, 24 h; (h) hydrazine hydrate, ethanol, 50°C, 6 h.

*Synthesis of tert-butyl 4-(benzyl(3-(carbobenzyloxyamino)propyl)amino)butylcarbamate (**12**)*:
To a solution of compound **16** (10.00 g, 36.0 mmol) in acetonitrile:chloroform (1:1) mixture (100 mL), *N*-carbobenzyloxy-3-bromopropylamine (11.29 g, 42.0 mmol) and Na_2CO_3 (3.80 g, 36.0 mmol) was added. The reaction mixture was stirred for 48 h at 70°C. After completion of the reaction (tlc), the reaction mixture was filtered and the solvent was concentrated in *vacuo*. The residue was purified through column chromatography (SiO_2, dichloromethane:methanol). **24** was isolated as a liquid, yield 12.3 g (26.3 mmol, 73.2 %); R_f = 0.65 (dichloromethane:methanol, 95:5).
^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 1.43 (s, 9H, 3 x CH_3); 1.45-1.50 (m, 4H, 2 x CH_2), 1.64 (quintuplet, *J* = 6.1 Hz, 2H, CH_2), 2.39 (t, *J* = 6.8 Hz, 2H, CH_2), 2.45 (t, *J* = 6.2 Hz, 2H, CH_2), 3.04 (bq, *J* = 5.6 Hz, 2H, CH_2), 3.20 (q, *J* = 6.0 Hz, 2H, CH_2), 3.49 (s, 2H, NCH_2), 4.61 (bt, 1H, NHCO), 5.08 (s, 2H, OCH_2), 5.77 (bt, 1H, NHCO), 7.20-7.36 (m, 10H, ArH-benzyl) ppm.

^{13}C NMR (75 MHz, $CDCl_3$, 25°C): δ = 24.040 (1 C, CH_2), 26.268 (1 C, CH_2), 27.693 (1 C, CH_2), 28.263 (3 C, CH_3), 40.132 (1 C, CH_2), 40.204 (1 C, CH_2), 52.105 (1 C, CH_2), 53.252 (1 C, CH_2), 58.701 (1 C, CH_2), 66.207 (1 C, CH_2), 78.849 (1 C, quat. C ^{t}Bu), 126.956 (1 C, Ar-CH), 127.904 (1 C, Ar-CH), 127.941 (2 C, Ar-CH), 128.232 (2 C, Ar-CH), 128.406 (2 C, Ar-CH), 128.907 (2 C, Ar-CH), 136.855 (1 C, Ar-C), 139.210 (1 C, Ar-C), 156.006 (1 C, CO(NH)O), 156.352 (1 C, CO(NH)O) ppm.
MS-ESI(+): m/z = 469 [M^+].
IR (ATR): ν = 3337.67, 2933.91, 2865.17, 2802.95, 1689.06, 1514.50, 1453.38, 1364.86, 1247.62, 1167.31, 733.76, 696.73 cm^{-1}.

*Synthesis of benzyl 3-((4-aminobutyl)(benzyl)amino)propylcarbamate as TFA salt (**23**)*:
5 mL of trifluoroacetic acid (TFA) was added to a solution of **24** (4.3 g, 9.2 mmol) in 5 mL dichloromethane. The reaction mixture was stirred for 4h at room temperature. After evaporation of the solvent, the residue was dispersed in diethyl ether to precipitate the product. The product was filtered and dried under vacuum. **23** was isolated as TFA salt, yield 2.9 g (6.0 mmol, 65.8 %); R_f = 0.3 (dichloromethane:methanol 87:13).
^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 1.47-1.52 (m, 4H, 2 x CH_2), 1.62 (quintuplet, J = 6.2 Hz, 2H, CH_2), 2.41 (t, J = 6.6 Hz, 2H, CH_2), 2.44 (t, J = 6.3 Hz, 2H, CH_2), 2.75 (sextet, J = 6.0 Hz, 2H, $CH_2NH_3^+$ converts to triplet on D_2O exchange), 3.18 (q, J = 6.1 Hz, 2H, CH_2), 3.50 (s, 2H, NCH_2), 5.04 (s, 2H, OCH_2), 5.76 (bt, 1H, NHCO), 7.19-7.35 (m, 10H, ArH-benzyl) ppm.
^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): δ = 20.233 (1 C, CH_2), 23.651 (1 C, CH_2), 24.054 (1 C, CH_2), 38.704 (1 C, CH_2), 45.688 (1 C, CH_2), 49.315 (1 C, CH_2), 51.555 (1 C, CH_2), 56.718 (1 C, CH_2), 66.514 (1 C, CH_2), 127.820 (1 C, Ar-CH), 128.077 (1 C, Ar-CH), 128.328 (1 C, Ar-C), 128.519 (2 C, Ar-CH), 129.388 (2 C, Ar-CH), 130.158 (2 C, Ar-CH), 130.878 (2 C, Ar-CH), 136.603 (1 C, Ar-C), 157.162 (1 C, CO(NH)O) ppm.
MS-ESI(+): m/z = 370 [M^+ + 1].
IR (ATR): ν = 3306.30, 2956.68, 1669.28, 1531.22, 1456.17, 1261.20, 1198.55, 1126.48, 1027.59, 833.37, 798.93, 741.42, 720.43, 698.93 cm^{-1}.

*Synthesis of benzyl 3-(benzyl(4-(benzylamino)butyl)amino)propylcarbamate (**22**)*:
Compound **23** (2.5 g, 6.8 mmol) was dissolved in 25 mL of methanol. To this solution, benzaldehyde (0.86 g, 8.1 mmol), $MgSO_4$ (1.63 g, 13.5 mmol) and triethylamine (0.81 g, 8.1 mmol) was added and the reaction mixture was stirred at RT overnight. After cooling to 0°C, $NaBH_4$ (1.53 g, 40.6 mmol) was added over a period of 1 h. The reaction mixture was further stirred at 0°C for 1 h and then at RT for an additional hour. Water (200 mL) was added to quench the reaction and the solution was extracted with ethyl acetate (3 x 150 mL). The combined organic layers were dried over Na_2SO_4 and afterwards, the solvent was concentrated in *vacuo*. The residue was purified through column chromatography (SiO_2, dichloromethane:methanol) to isolate pure **22** as a solid, yield 1.62 g (3.5 mmol, 68.3 %); R_f = 0.55 (dichloromethane:methanol, 90:10).
^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 1.50 (broad quintuplet, 4H, 2 x CH_2), 1.61 (quintuplet, J = 6.2 Hz, 2H, CH_2), 2.37 (bt, 2H, CH_2), 2.43 (t, J = 6.2 Hz, 2H, CH_2), 2.57 (bt, 2H, CH_2), 3.18 (q, J = 5.7 Hz, 2H, CH_2), 3.48 (s, 2H, CH_2N), 3.74 (s, 2H, CH_2N), 5.05 (s, 2H, CH_2O), 5.78 (bt, 1H, NHCO), 7.19-7.33 (m, 15H, ArH-benzyl) ppm.
^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): δ = 24.520 (2 C, CH_2), 26.218 (1 C, CH_2), 27.288 (1 C, CH_2), 40.169 (1 C, CH_2), 48.731 (1 C, CH_2), 52.031 (1 C, CH_2), 53.496 (1 C, CH_2), 58.674 (1 C, CH_2), 66.258 (1 C, CH_2), 127.029 (2 C, Ar-CH), 127.214 (1 C, Ar-CH), 127.940 (2 C, Ar-CH), 128.303 (2 C, Ar-CH), 128.376 (2 C, Ar-CH), 128.455 (2 C, Ar-CH), 128.469 (2 C, Ar-CH), 129.001 (2 C, Ar-CH), 136.924 (2 C, Ar-C), 139.201 (1 C, Ar-C), 156.440 (1 C, CO(NH)O) ppm.
MS-ESI(+): m/z = 460 [M^+ +1].
IR (ATR): ν = 3327.82, 3061.76, 3028.48, 2935.02, 2801.81, 1702.25, 1513.74, 1495.03, 1452.96, 1248.01, 1129.53, 1026.97, 732.80, 695.96 cm^{-1}.

*Synthesis of benzyl 3-(benzyl(4-(benzyl(3-(1,3-dioxoisoindolin-2-yl)propyl)amino)butyl)amino) propylcarbamate (**21**)*:

To a solution of compound **22** (6.0 g, 13.0 mmol) in acetonitrile:chloroform (1:1) (150 mL), N-(3-bromopropyl)phthalimide (4.2 g, 16.0 mmol) and Na_2CO_3 (3.8 g, 13.0 mmol) was added. The reaction mixture was stirred for 3 days at 75°C. After completion of the reaction (tlc), the reaction mixture was filtered and the solvent was concentrated in *vacuo*. The residue was purified by column chromatography (SiO_2, dichloromethane:methanol) yielding **21** as a liquid, yield 4.83 g (7.4 mmol, 57.3 %); R_f = 0.75 (dichloromethane:methanol, 95:5).

^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 1.40-1.47 (m, 4H, 2 x CH_2); 1.59-1.62 (m, 2H, CH_2); 1.78 (quintuplet, J = 7.1 Hz, 2H, CH_2), 2.31-2.37 (m, 4H, 2 x CH_2); 2.41-2.45 (m, 4H, 2 x CH_2); 3.19 (q, J = 5.7 Hz, 2H, CH_2), 3.47 (s, 2H, CH_2N), 3.48 (s, 2H, CH_2N), 3.65 (t, J = 7.6 Hz, 2H, CH_2), 5.04 (s, 2H, CH_2O), 5.84 (bt, 1H, NHCO), 7.15-7.34 (m, 15H, ArH-benzyl); 7.66 (dd, J_1 = 3.2 Hz, J_2 = 5.6 Hz, 2H, ArH), 7.80 (dd, J_1 = 2.8 Hz, J_2 = 5.2 Hz, 2H, ArH) ppm.

^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): δ = 24.441 (1 C, CH_2), 24.689 (1 C, CH_2), 25.958 (1 C, CH_2), 26.224 (1 C, CH_2), 36.302 (1 C, CH_2), 40.240 (1 C, CH_2), 50.999 (1 C, CH_2), 52.101 (1 C, CH_2), 53.344 (1 C, CH_2), 53.602 (1 C, CH_2), 58.419 (1 C, CH_2), 58.725 (1 C, CH_2), 66.178 (1 C, CH_2), 123.094 (2 C, Ar-CH), 126.707 (1 C, Ar-CH), 126.885 (1 C, Ar-CH), 127.872 (1 C, Ar-CH), 127.911 (2 C, Ar-CH), 128.095 (2 C, Ar-CH), 128.224 (2 C, Ar-CH), 128.410 (2 C, Ar-CH), 128.810 (2 C, Ar-CH), 128.934 (1 C, Ar-CH), 132.164 (1 C, Ar-CH), 133.803 (2 C, Ar-CH), 136.953 (1 C, Ar-C), 139.450 (2 C, Ar-C), 139.752 (2 C, Ar-C), 156.404 (1 C, CO(NH)O), 168.405 (2 C, CON) ppm.

MS-ESI(+): m/z = 647 [M^+ + 1].

IR (ATR): ν = 3337.34, 3065.25, 3031.38, 2950.78, 1703.45, 1515.73, 1497.86, 1392.65, 1367.12, 1175.31, 741,54, 732.48, 721.59, 695.02 cm^{-1}.

*Synthesis of tert-butyl 3-((4-((3-(1,3-dioxoisoindolin-2-yl)propyl)(tert-butoxycarbonyl)amino) butyl) (tert-butoxycarbonyl)amino)propylcarbamate (**20**)*:

To the N_2-flushed solution of compound **21** (13.0 g, 20.0 mmol) in 200 mL of methanol (dry), 10% Pd/C (2.0 gm) was added followed by addition of actic acid (4.0 mL). Afterwards, di-*tert*-butyldicarbonate (21.94 g, 100.0 mmol) was added to this solution. The flask was degassed and saturated with hydrogen and stirred for 48 h at room temperature. The degassing and saturation with hydrogen were regularly repeated during this time interval. The reaction progress was monitored by TLC. After completion of the reaction (tlc), the Pd/C was filtered off (celite), washed with methanol and the solvent was concentrated in *vacuo*. The residue was purified by column chromatography (SiO_2, dichloromethane:methanol) to isolate **20** as a liquid, yield 7.2 g (11.4 mmol, 56.6 %); R_f = 0.65 (dichloromethane:methanol, 97:3).

^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 1.39-1.47 [m which shows two large singlets at 1.42 (s, 18H, 6 x CH_3) and 1.43 (s, 9H, 3 x CH_3) along with 4H protons 2 x CH_2), 1.63 (bq, 2H, CH_2), 1.89 (quintuplet, J = 5.4 Hz, 2H, CH_2), 3.08-3.19 (m, 10H, 5 x CH_2), 3.67 (t, J = 5.1 Hz, 2H, CH_2), 5.35 (bs, 1H, NH of carbamate), 7.69-7.71 (m, 2H, ArH), 7.82-7.84 (m, 2H, ArH) ppm.

^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): δ = 24.841 (1 C, CH_2), 25.189 (1 C, CH_2), 26.158 (1 C, CH_2, 26.824 (1 C, CH_2), 28.018 (6 C, CH_3), 28.832 (3 C, CH_3), 37.002 (1 C, CH_2), 40.840 (1 C, CH_2), 51.349 (1 C, CH_2), 52.631 (1 C, CH_2), 53.563 (1 C, CH_2), 53.832 (1 C, CH_2), 78.932 (1 C, quat. C ^{t}Bu), 79.245 (2 C, quat. C ^{t}Bu), 123.344 (2 C, Ar-CH), 132.094 (2 C, Ar-C), 133.911 (2 C, Ar-CH), 155.635 (1 C, COO), 156.052 (2 C, COO), 168.543 (2 C, CON) ppm.

MS-ESI(+): m/z = 633 [M^+], 533 [M^+ - Boc].

IR (ATR): ν = 3357.45, 2976.63, 2942.34, 1778.33, 1709.58, 1690.12, 1398.65, 1369.05, 1251.75, 1169.23, 1035.52, 720.12 cm^{-1}.

*Synthesis of 2-(3-(4-(3-aminopropylamino)butylamino)propyl)isoindoline-1,3-dione as TFA salt (**19**)*:

In order to cleave the Boc protecting groups, 4.5 mL of trifluoroacetic acid was added to a solution of **20** (1.50 g, 2.4 mmol) in 4.5 mL of dichloromethane. The reaction mixture was stirred for 4 h at RT. After evaporation of the solvent, the product was precipitated by addition of diethyl ether. After filtration, the solid product was dried under vacuum yielding **19**, as a white solid, yield quantitaive; R_f = spot remained on base.
^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 1.58 (broad quintuplet, 4H, 2 x CH_2), 1.83-1.95 (m, 4H, 2 x CH_2), 2.83-2.96 (m, 10H, 5 x CH_2), 3.63 (t, *J* = 6.6 Hz, 2H, CH_2), 7.82-7.88 (m, 4H, ArH) ppm.
^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): δ = 22.748 (1 C, CH_2), 22.816 (1 C, CH_2), 23.779 (1 C, CH_2), 25.161 (1 C, CH_2), 34.972 (1 C, CH_2), 36.197 (1 C, CH_2), 44.015 (1 C, CH_2), 44.727 (1 C, CH_2), 46.210 (1 C, CH_2), 46.264 (1 C, CH_2), 123.366 (2 C, Ar-CH), 131.826 (2 C, Ar-C), 134.780 (2 C, Ar-CH), 168.385 (2 C, CON) ppm.
MS-ESI(+): *m*/*z* = 333 [M^+ +1 (free amine)].
IR (ATR): ν = 3036.21, 2946.64, 2857.78, 2554.55, 2494.87, 1776.31, 1711.60, 1665.91, 1612.97, 1431.96, 1396.14, 1362.30, 1196.51, 1175.84, 1126.59, 797.46, 773.51, 719.60 cm^{-1}.

*Synthesis of tert-butyl 2,2'-(3-((2-tert-butoxy-2-oxoethyl)(4-((2-tert-butoxy-2-oxoethyl)(3-(1,3-dioxoisoindolin-2-yl) propyl)amino)butyl)amino)propylazanediyl)diacetate (**18**)*:
To a solution of compound **19** (1.40 g, 2.0 mmol) in dioxane:acetonitrile (8:2) (20 mL), *tert*-butyl bromoacetate (1.5 mL, 9.1 mmol) and diisopropylethylamine (2.90 mL, 16.5 mmol) were added. The reaction mixture was stirred for 24h at 45°C. After completion of the reaction (tlc), the reaction mixture was filtered and the solvent was concentrated in *vacuo*. The residue was purified by column chromatography (SiO_2, dichloromethane:methanol) yielding **18** as a liquid, yield 0.33 g (0.42 mmol, 20.1 %); R_f = 0.8 dichloromethane: methanol, (97:3).
^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 1.42 (s, 18H, 6 x CH_3), 1.43 (s, 18H, 6 x CH_3 These two singlets superimpose on the signals of 4H protons, 2 x CH_2), 1.61 (quintuplet, *J* = 7.3 Hz, 2H, CH_2), 1.80 (quintuplet, *J* = 7.1 Hz, 2H, CH_2), 2.56-2.71 (m, 10H, 5 x CH_2), 3.19 (s, 2H, NCH_2), 3.22 (s, 2H, NCH_2), 3.40 (s, 4H, 2 x NCH_2), 3.72 (t, *J* = 7.4 Hz, 2H, CH_2), 7.69 (dd, J_1 = 3.2 Hz, J_2 = 5.6 Hz, 2H, ArH), 7.82 (dd, J_1 = 3.2 Hz, J_2 = 5.6 Hz, 2H, ArH) ppm.
^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): δ = 25.186 (1 C, CH_2), 25.314 (1 C, CH_2), 26.062 (1 C, CH_2), 26.529 (1 C, CH_2), 28.019 (12 C, CH_3), 36.146 (1 C, CH_2), 51.570 (1 C, CH_2), 51.872 (1 C, CH_2), 52.091 (1 C, CH_2), 53.739 (1 C, CH_2), 54.138 (1 C, CH_2), 55.231 (1 C, CH_2), 55.531 (1 C, CH_2), 55.712, (2 C, CH_2), 80.466 (1 C, quat. C ^{t}Bu), 80.566 (1 C, quat. C ^{t}Bu), 80.664 (2 C, quat. C ^{t}Bu), 123.076 (2 C, Ar-CH), 132.200 (2 C, Ar-C), 133.786 (2 C, Ar-CH), 168.349 (2 C, CON), 170.728 (1 C, COO), 170.823 (1 C, COO), 170.908 (2 C, COO) ppm.
MS-ESI(+): *m*/*z* = 789 [M^+ +1].
IR (ATR): ν = 2977.48, 2944.89, 1711.63, 1392.94, 1366.75, 1254.09, 1216.91, 1146.28, 1037.53, 844.16, 734.94, 721.37, 529.90 cm^{-1}.

*Synthesis of tert-butyl 2,2'-(3-((4-((3-aminopropyl)(2-tert-butoxy-2-oxoethyl)amino)butyl)(2-tert-butoxy-2-oxoethyl)amino)propylazanediyl)diacetate (**17**)*:
To a solution of compound **18** (0.40 g, 0.5 mmol) in ethanol (20 mL), hydrazine hydrate (0.076 mL, 1.5 mmol) was added and the reaction mixture was stirred for 4 h at 45°C. The formed precipitate was filtered off and the filtrate was concentrated in vacuum. The residue was dissolved in ethyl acetate and washed with 10% KOH solution. The organic layer was extracted with brine (50 mL), dried over Na_2SO_4 and concentrated in vacuum to obtained a residue which was further purified by column chromatography (SiO_2, dichloromethane:methanol:triethylamine) yielding **17** as a liquid, yield 0.18 g (0.28 mmol, 55.5 %); R_f = 0.45 (dichloromethane:methanol:triethylamine, 9.5:0.25:0.25).
^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 1.43 (s, 18H, 6 x CH_3), 1.44 (s, 18H, 6 x CH_3 These two singlets superimpose on the signals of 4H protons 2 x CH_2), 1.57-1.66 (m, 4H, 2 x CH_2), 2.46 (bs, 2H,

NH_2), 2.53-2.61 (m, 8H, 4 x CH_2), 2.69 (t, J = 7.4 Hz, 2H, CH_2), 2.79 (t, J = 6.4 Hz, 2H, CH_2), 3.18 (s, 2H, NCH_2), 3.19 (s, 2H, NCH_2), 3.41 (s, 4H, NCH_2) ppm.
^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): δ = 24.877 (1 C, CH_2), 25.239 (1 C, CH_2), 26.021 (1 C, CH_2), 28.007 (12 C, CH_3), 29.730 (1 C, CH_2), 40.240 (1 C, CH_2), 51.879 (1 C, CH_2), 52.003 (1 C, CH_2), 52.083 (1 C, CH_2), 53.962 (1 C, CH_2), 54.074 (1 C, CH_2), 55.668 (1 C, CH_2), 55.730 (1 C, CH_2), 55.862 (2 C, CH_2), 80.524 (1 C, quat. C ^{t}Bu), 80.724 (1 C, quat. C ^{t}Bu), 80.785 (2 C, quat. C ^{t}Bu), 170.738 (1 C, COO), 170.929 (1 C, COO), 171.076 (2 C, COO) ppm.
MS-ESI(+): m/z = 659 [M^+ +1].
IR (ATR): ν = 2975.56, 2934.63, 1727.65, 1456.90, 1391.89, 1366.63, 1251.94, 1130.35, 1246.41, 938.13, 843.93, 749.82, 579.35, 457.19, 433.55 cm^{-1}.

Solubility tests for the characterization of (1a) in solution

Figure S1. Solubility test for **1a.** Legend: B1f → buffer pH = 10 (VWR, H_3BO_3/KCl/NaOH); B2f → buffer pH = 10 (Fisher, K_2CO_3/$K_2B_8O_{13}$/KOH); B3f → buffer pH = 7 (VWR, KH_2PO_4/Na_2HPO_4); Buffer pH = 4 (Fisher, $C_8H_5KO_4$).

Water-induced aggregation of (1a) in DMSO solution

The ^{1}H-NMR spectrum of **1a** in DMSO-d_6 was recorded in presence of TFA and HCl 37% (0.05 mL). As shown in Figure S2, all peaks in the aliphatic region (δ_H 1.6 – 4.2 ppm) remain more or less at the same position in both samples, while the aromatic area (δ_H 8.2 – 8.9 ppm) changes completely. In the presence of TFA, two well resolved doublets are visible and each of them displays a broad undefined band on the side.

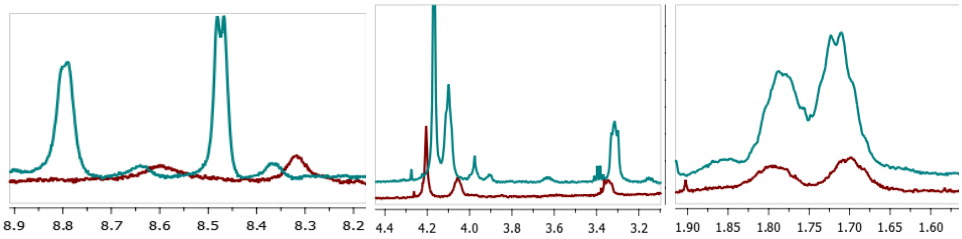


Figure S2. ^{1}H-NMR spectrum of **1a** in DMSO-d_6 with addition of 1 drop of TFA (blue) or HCl (red)

When HCl is added, instead of TFA, just those two broad bands are visible, whereas the two doublets are lost. Since the addition of concentrated HCl adds some water to the sample, it is assumed that the PBI derivative **1a** aggregates more strongly under these conditions. Therefore, the two broad bands are attributed to the aggregated form of **1a**. To further support these preliminary observations, a titration experiment followed by UV/Vis measurements (Figure S3) was carried out.

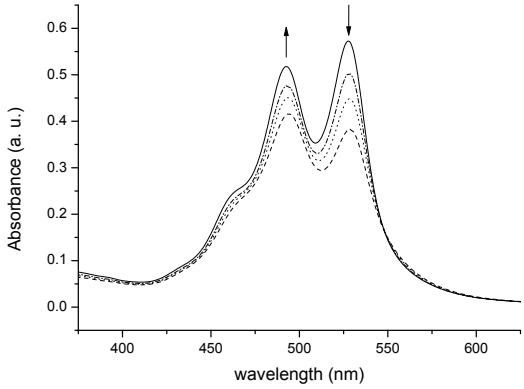


Figure S3. Absorption spectra of PBI **1a** in DMSO upon addition of conc. HCl
(arrows show spectrum modifications upon acid addition)

A solution of PBI **1a** in DMSO was titrated with concentrated HCl (37 %) and the UV/Vis spectra were collected after each addition. As presented in Figure 4, upon addition of HCl the spectrum profile changes significantly: the intensity of the (0,0) peak decreases and at the same time an increase of the intensity of the (0,1) peak is observed. This suggests that aggregation of the perylene bisimide surfactant dye **1a** is taking place.

Complexation experiments for (1a) in water solution

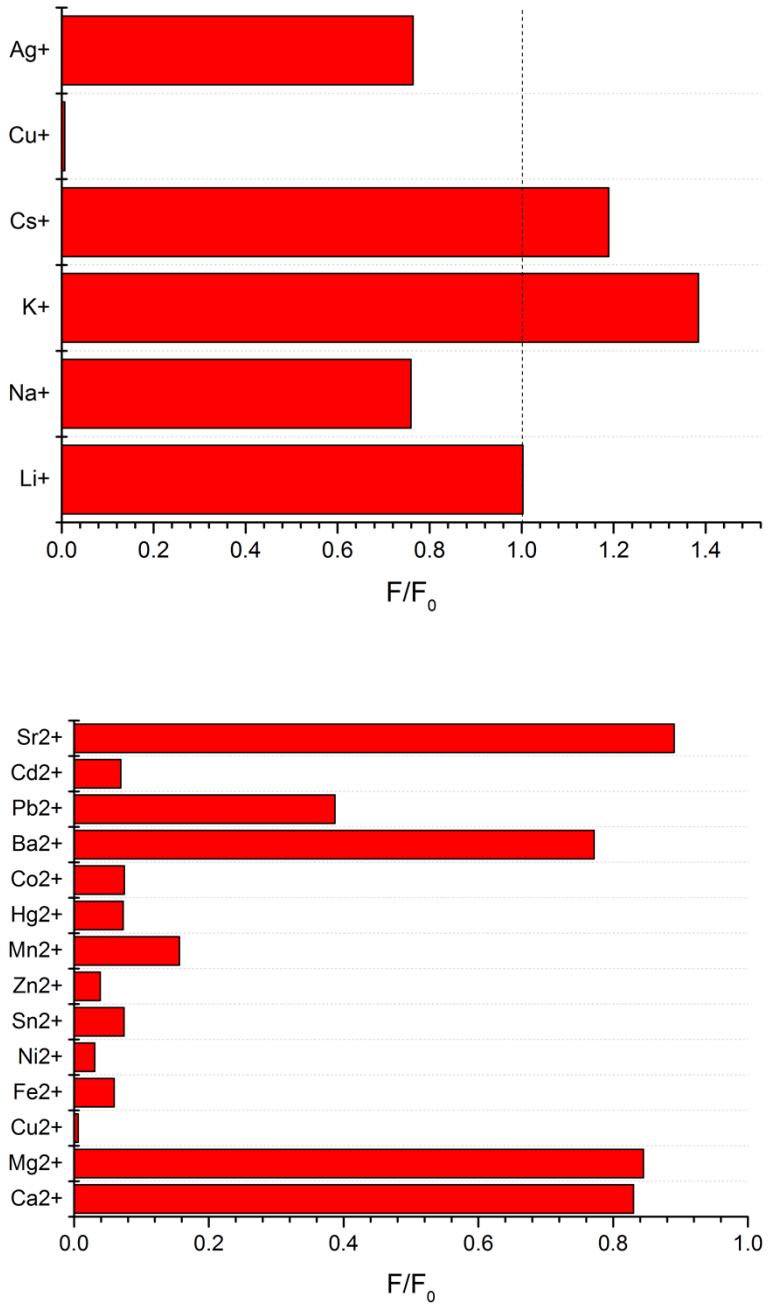


Figure S4. F/F_0 ratio for mono- (top), di- (bottom) valent ions – **1a** complexes in water

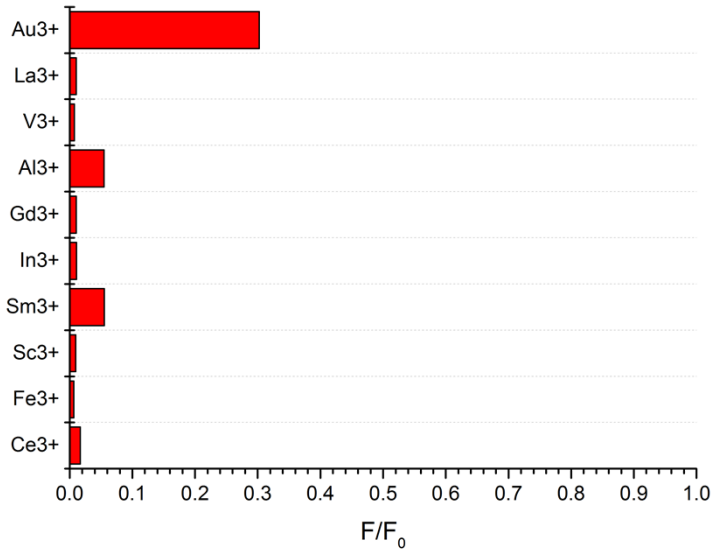


Figure S5. F/F_0 ratio for trivalent ions – **1a** complexes in water

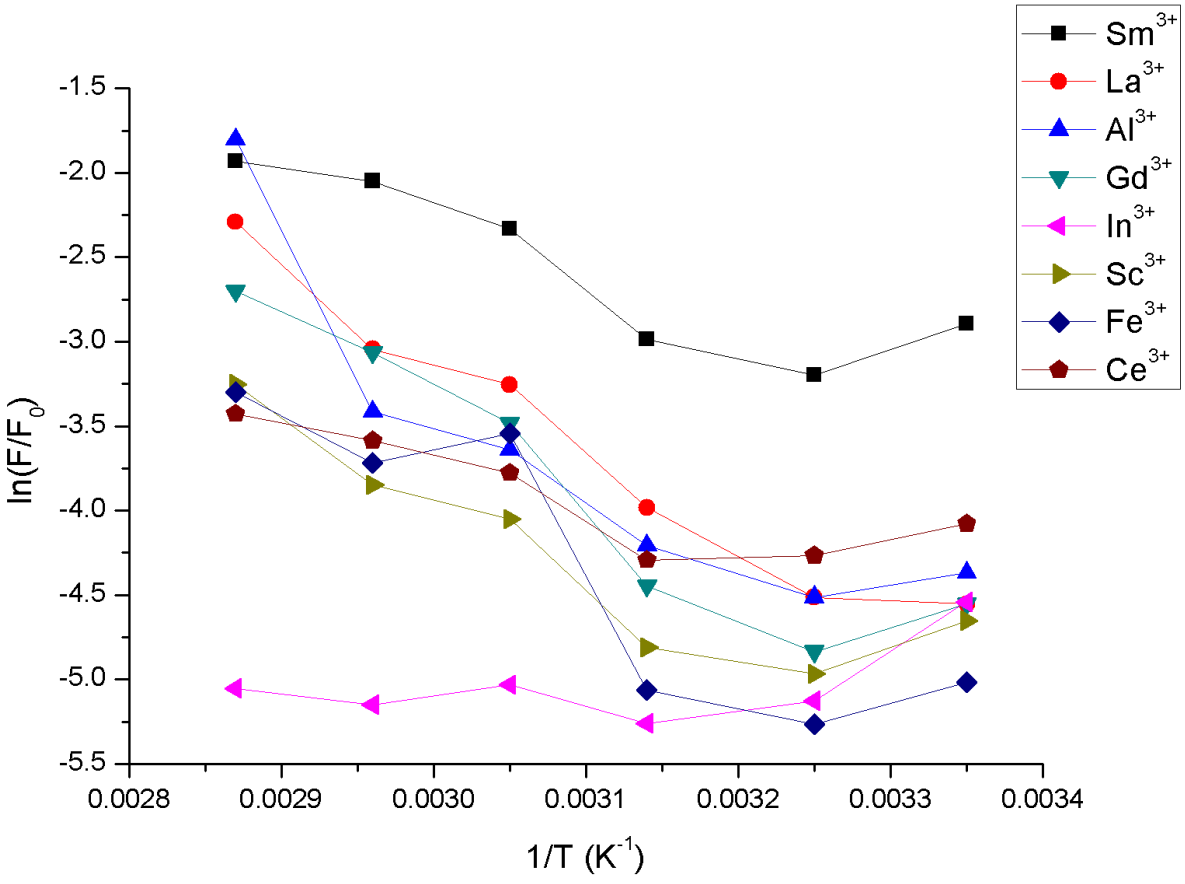


Figure S6. Van't Hoff plots for the stability trivalent ions – **1a** complexes in water by changing the temperature

[1] Muller D., *et al.* J. Org. Chem., **1997**, 62, 411 – 416.