Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

A Polyamine-Modified Near-Infrared Fluorescent Probe for Selective Staining of Live Cancer Cells

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Additional Experimental Data

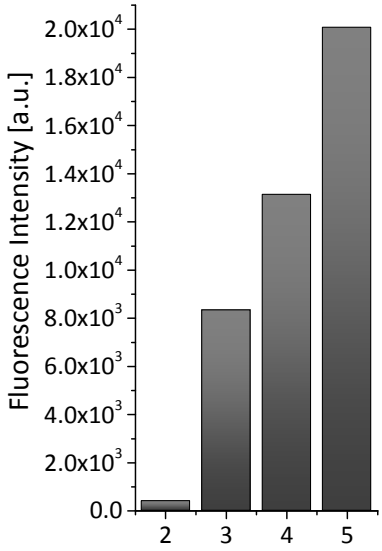


Fig. S1 - Preliminary flow cytometry experiments with dyes **2**, **3**, **4** and **5**. HeLa cells were incubated with 10 μM dye solutions in FCS-free RPMI 1640 medium for 5 min at 37 °C.

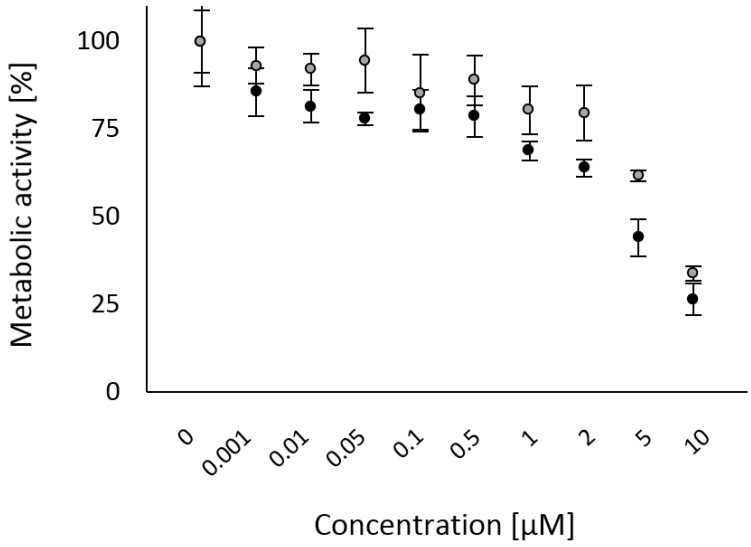


Fig. S2 - Metabolic activity of HeLa cells upon treatment with various concentrations of **5** (black) and **8** (grey) for 24 h.

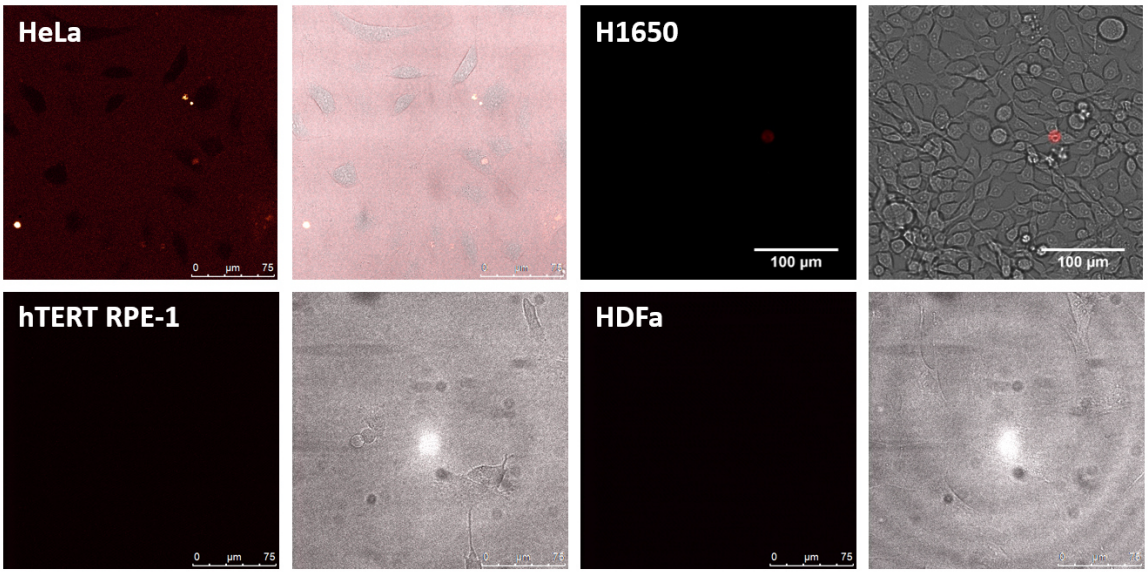


Fig. S3 - Representative microscopy images (fluorescence and overlay with transmission images) of live cancer (HeLa, H1650) and non-cancer cell lines (hTERT RPE-1, HDFa) after incubation with 10 μM of dye **6** in FCS-free RPMI 1640 medium for 5 minutes at 37 °C. Images of HeLa, hTERT RPE-1, and HDFa cells were taken on a confocal microscope (Leica); for H1650 cells, a fluorescence microscope (Olympus) was used.

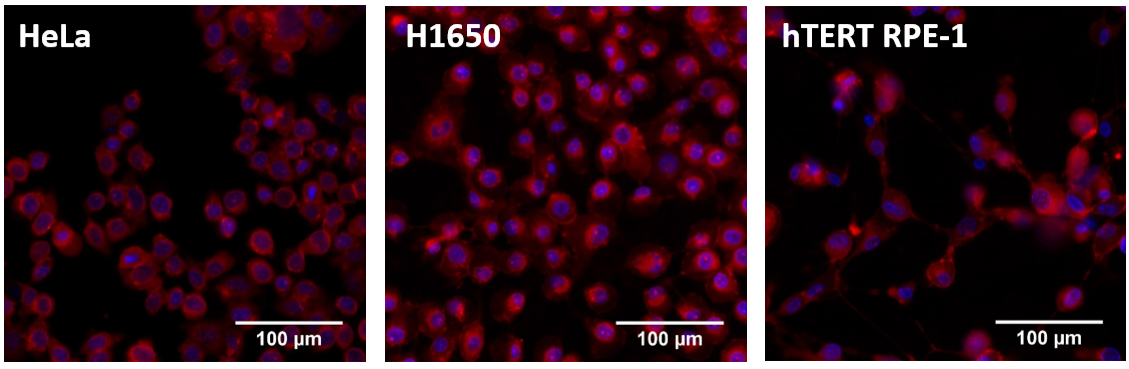


Fig. S4 - Representative fluorescence microscopy images of live cancer HeLa (left), H1650 (middle) and non-cancer hTERT RPE-1 (right) cells after incubation with 10 μM of dye **8** in FCS-free RPMI 1640 medium for 5 minutes at 37 °C. Fluorescence emission of **8** is shown in red; nuclei were co-stained with Hoechst 33342 (blue).

Instruments and Methods

Air- and moisture sensitive substances were handled under a dry, inert argon atmosphere using standard Schlenk-techniques in glassware that was flame-dried prior to use. Argon was used without further drying, anhydrous solvents were obtained commercially or dried using standard techniques and stored under argon over molecular sieves. All temperature sensitive compounds were stored at 4 °C or -20 °C. Dye stock solutions were prepared in anhydrous DMSO and stored at -20 °C in the dark.
In order to improve comprehensibility, simplified names were used for some synthesized compounds rather than using exact IUPAC names. Atom numbering was done for NMR-assignments only and is not always based on the compound's name.

Chemicals used for synthesis were obtained from Sigma-Aldrich, Fluka, Riedel-de-Haën, Merck, Bachem, Roth or bought at the central store of the chemical institutes at the University of Heidelberg. All substances with 98 % purity or higher were used without further purification.

TLCs were performed on Polygram Sil G/UV254 or Alugram PR18 W/UV245 plates (Macherey-Nagel). Spots were made visible with UV light or by staining with iodine, ninhydrine or ammonium molybdate. For flash chromatography, silica gel (40 - 63 μm, 60 Å pore size) by Sigma-Aldrich was used. R_f-values were determined in the solvent system used for flash chromatography unless otherwise specified.

Nuclear magnetic resonance spectra were recorded on Bruker DRX 200 (200 MHz), Bruker Avance II (400 MHz) or Bruker Avance III (600 MHz) spectrometers. Chemical shifts δ are given in ppm and coupling constants J in Hz. All spectra were calibrated using the residual ^{1}H- or ^{13}C-signals of the deuterated solvents.[1] All spectra were recorded at 298 K unless otherwise specified. The following abbreviations were used to describe the multiplicities of the signals: s (singlet), d (doublet), t (triplet), q (quartet), qn (quintet), m (multiplet), br. (broad). Signals were assigned using DEPT, COSY, HSQC and HMBC spectra.

Mass spectra were recorded on a Bruker ApexQe hybrid 9.4 T FT-ICR (HR-ESI), a JEOL JMS-700 magnetic sector (EI and HR-EI) or a Bruker Microflex (MALDI-TOF; used primarily for the identification of HPLC peaks).

Preparative and semi-preparative HPLC-purifications were performed on Shimadzu HPLC systems at 20 °C. All samples were filtered through C18 silica (Waters) prior to HPLC-purification.

The C18 columns named in line with the synthetic protocols were used as stationary phases, deionized H_2O with 0.1 % TFA and MeCN served as eluents unless otherwise specified. Products were collected manually and identified by MALDI-TOF mass spectrometry.

Analytical HPLCs for all derivatives were performed on a Shimadzu HPLC system.

HPLC Gradients

Gradient A		Gradient B	
Time	**% MeCN**	**Time**	**% MeCN**
0	0	**0**	0
5	0	**60**	50
15	12		
30	24		
50	40		

UV/Vis Spectroscopic Analysis and Molar Extinction Coefficients

UV/Vis-Spectra were recorded on a Cary 100 Bio by Varian using 3 mL PMMA cuvettes by Sigma-Aldrich. Stock solutions of the dyes for the determination of molar extinction coefficients were prepared in from weighted samples in DMSO or in DMSO-*d6* with 50 mM 2-propanol as standard using NMR-spectroscopy to determine the concentration of these stock solutions. The molar extinction coefficient was obtained using Beer's law at 0.1 μM to 0.5 μM dilutions in aqueous PBS-buffer.

Fluorescence Measurements and Quantum Yields

Fluorescence measurements were performed on a Cary Eclipse spectrophotometer by Varian. Unless otherwise specified, the temperature was set to 20 °C using a Cary temperature controller. 3 mL PMMA cuvettes by Sigma-Aldrich were used for all measurements. The relative fluorescence quantum yield was determined using indocyanine green as reference following equation (1)[2]

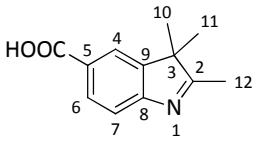
$$\Phi_x = \Phi_{ref} \cdot \left(\frac{A_{ref}}{A_x}\right) \cdot \left(\frac{F_x}{F_{ref}}\right) \cdot \left(\frac{n_x}{n_{ref}}\right)^2 \quad (1)$$

where Φ_x is the quantum yield of the new compound, Φ_{ref} is the quantum yield of the reference substance in DMSO, A_{ref} is the absorbance at the fluorescence excitation wavelength (705 nm) of the reference solution while A_x is the absorbance at 705 nm of the solution of the new compound, n is the refractive index of the solvents used for the measurement (DMSO for indocyanine green and PBS (phosphate-buffered saline) for the new compounds) and F_x and F_{ref} are the areas under the curves of the fluorescence emission spectra for the new compounds and the reference solutions. For these measurements, 705 nm light was used for fluorescence excitation.

Synthetic Procedures

5-Carboxy-2,3,3-trimethyl-3*H*-indole (I)

This is a known compound which was synthesized following a procedure by Terpetschnig *et al*.[3] The work-up was modified.



$C_{12}H_{13}NO_2$
203.24 g/mol

Hydrazinobenzoic acid (4.56 g, 30.0 mmol, 1.0 eq.), sodium acetate (5.00 g, 60.0 mmol, 2.0 eq.) and 3-methyl-butan-2-one (4.60 mL, 3.70 g, 43.5 mmol, 1.5 eq.) were dissolved in glacial acetic acid (30 mL) and stirred at room temperature for 1 hour. Subsequently, the reaction mixture was heated to 130 °C and stirred for 5 hours. After cooling to room temperature, the solvent was removed under reduced pressure. The crude product was dissolved in CH_2Cl_2, the organic phase was washed with water (3 x 100 mL) and dried using anhydrous $MgSO_4$. After removing the solvent under reduced pressure, **I** was obtained as pale brown solid (3.70 g, 18.2 mmol, 61%).

^{1}H NMR (600.13 MHz, $CDCl_3$)

δ = 1.38 (s, 6H, H-10/H-11), 2.40 (s, 3H, H-12), 7.69 (d, $^3J_{H\text{-}H}$ = 8.04 Hz, 1H, H-1), 8.07 (d, $^4J_{H\text{-}H}$ = 1.50 Hz, 1H, H-4), 8.15 (dd, $^3J_{H\text{-}H}$ = 8.05 Hz, $^4J_{H\text{-}H}$ = 1.50 Hz, 1H, H-6).

^{13}C {^{1}H} NMR (150.92 MHz, $CDCl_3$)

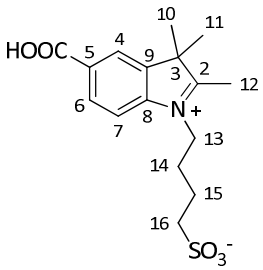
δ = 15.6 (1C, CH_3, C-12), 23.0 (2C, CH_3, C-10/C-11), 54.0 (1C, C_q, C-3), 119.7 (1C, CH, C-7), 123.4 (1C, CH, C-6), 127.2 (1C, C_q, C-5), 131.0 (1C, CH, C-4), 145.5 (1C, C_q, C-9), 157.0 (1C, C_q, C-8), 171.3 (1C, C_q, C-2), 192.9 (1C, C_q, COOH).

MS (HR-EI$^+$)

m/z = 203.0936 $[M+H]^+$, calculated for $C_{12}H_{13}O_2N^+$: 203.0941.

5-Carboxy-1-(4-sulfobutyl)-2,3,3-trimethyl-*3H*-indolium betaine (II)

This is a known compound which was synthesized following a procedure by Terpetschnig *et al*.[3]



$C_{16}H_{21}NO_5S$
339.41 g/mol

I (1.22 g, 6.00 mmol, 1.0 eq.) and 1,4-butanesultone (3.21 mL, 4.30 g, 35.0 mmol, 5.8 eq.) were suspended in 20 mL of 1,2-dichlorobenzene and stirred at 180 °C for 8 hours. After cooling to room temperature, the precipitate was collected by filtration and washed with acetone. The solid material was dried *in vacuo* to yield **II** as red powder (1.85 g, 5.45 mmol, 91%).

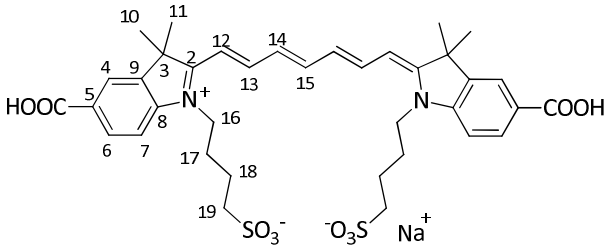
^{1}H NMR (399.89 MHz, d_6-DMSO)
δ = 1.56 (s, 6H, H-10/H-11), 1.72 - 1.74 (m, 2H, H-15), 1.95 - 1.97 (m, 2H, H-14), 2.49 (m, 2H, H-16 (superimposed by DMSO)), 2.88 (s, 3H, H-12), 4.49 - 4.51 (m, 2H, H-13), 8.15 (br. s, 2H, H-4/H-6), 8.37 (s, 1H, H-7).

^{13}C {^{1}H} NMR (150.92 MHz, d_6-DMSO)
δ = 14.2 (1C, CH_3, C-12), 21.7 (2C, CH_3, C-10/C-11), 22.1 (1C, CH_2, C-15), 25.9 (1C, CH_2, C-14), 47.5 (1C, CH_2, C-16), 50.1 (1C, CH_2, C-13), 54.4 (1C, C_q, C-3), 115.8 (1C, CH, C-7), 124.3 (1C, CH, C-6), 130.4 (1C, CH, C-4), 131.5 (1C, C_q, C-5), 142.2 (1C, C_q, C-9), 144.3 (1C, C_q, C-8), 166.4 (1C, C_q, C-2), 199.6 (1C, C_q, COOH).

MS (HR-ESI$^-$)
m/z = 338.1061 $[M-H]^-$, calculated for $C_{16}H_{20}NO_5S^-$: 338.1067.

Bis-1,1‘-(4-sulfobutyl)indotricarbocyanine-5,5‘-dicarboxylic acid, sodium salt (1)

This is a known compound which was synthesized following a procedure by Pavlik *et al*.[4]



$C_{37}H_{43}N_2NaO_{10}S_2$
762.86 g/mol

II (220 mg, 648 μmol, 2.1 eq.), glutaconaldehyde dianyl monohydrochloride (88.0 mg, 309 μmol, 1.0 eq.), and sodium acetate (90.0 mg, 1.10 mmol, 3.6 eq.) were suspended in acetic anhydride (3.3 mL) and acetic acid (2 mL). The reaction mixture was heated to 120 °C for 45 min. After cooling to room temperature, the solution was poured into dry Et_2O (11 mL). The dark green solid was collected, dried *in vacuo* and suspended in a mixture of 2-propanol/water 4/1 (4 mL). After eight hours at 4 °C, the product was collected by filtration, washed with cold 2-propanol, and dried *in vacuo***. 1** was obtained as dark green solid (201 mg, 263 μmol, 85%).

^{1}H NMR (399.89 MHz, d_6-DMSO)

δ = 1.65 (s, 12H, H-10/H-11), 1.70 - 1.82 (m, 8H, H-17/H-18), 2.54 - 2.64 (m, 4H, H-19), 4.04 - 4.17 (m, 4H, H-16), 6.52 (d, $^3J_{H\text{-}H}$ = 13.50 Hz, 2H, H-12), 6.63 (t, $^3J_{H\text{-}H}$ = 12.63 Hz, 2H, H-14), 7.48 (d, $^3J_{H\text{-}H}$ = 8.45 Hz, 2H, H-7), 7.77 - 7.88 (m, 1H, H-15), 7.93 (t, $^3J_{H\text{-}H}$ = 13.50 Hz, 2H, H-13), 7.97 (d, $^3J_{H\text{-}H}$ = 8.45 Hz, 2H, H-6), 8.07 (s, 2H, H-4).

^{13}C {^{1}H} NMR (100.56 MHz, d_6-DMSO)

δ = 22.3 (2C, CH_2, C-17), 26.1 (2C, CH_2, C-18), 27.2 (4H, CH_3, C-10/C-11), 43.8 (2C, CH_2, C-16), 48.5 (2C, C_q, C-3), 50.8 (2H, CH_2, C-19), 105.3 (2C, CH, C-12), 111.0 (2C, CH, C-7), 123.3 (2C, CH, C-4), 126.7 (2C, C_q, C-5), 127.1 (2C, CH, C-14), 130.8 (2C, CH, C-6), 141.2 (2C, C_q, C-9), 146.0 (2C, C_q, C-8), 152.3 (2C, CH, C-13), 157.4* (1C, CH, C-15), 167.1 (2C, C_q, C-2).

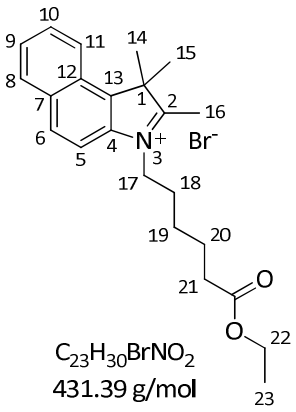
* Not observed in 1D spectra. Signals were detected via indirect excitation (HSQC).

MS (HR-ESI$^-$)

m/z = 739.2379 $[M\text{-}Na]^-$, calculated for $C_{73}H_{43}N_2O_{10}S_2^-$: 739.2365
369.1147 $[M\text{-}Na\text{-}H]^{2-}$ calculated for $C_{73}H_{42}N_2O_{10}S_2^{2-}$: 369.1145.

3-(6-Ethoxy-6-oxohexyl)-1,1,2-trimethyl-1*H*-benz[*e*]indol-3-ium bromide (III)

This compound was synthesized on the basis of the procedure for 3-(5-Ethoxy-5-oxopentyl)-1,1,2-trimethyl-1*H*-benz[*e*]indol-3-ium bromide by Mizrahi *et al*.[5]



To a solution of 1,1,2-Trimethyl-1*H*-benz[e]indole (1.00 g, 4.80 mmol, 1.0 eq.) in MeCN (20 mL), ethyl-6-bromohexanoate (1.78 mL, 2.23 g, 10.0 mmol, 2.1 eq.) and potassium iodide (85.0 mg, 0.50 mmol, 0.01 eq.) were added. The mixture was stirred at 80 °C for four days during which a color change from golden to red to blue was observed. Subsequently, the solvent was evaporated and the residue was triturated with Et_2O. The solid material was collected by filtration and washed with Et_2O. After drying *in vacuo*, **III** was afforded as pale blue solid (1.90 g, 4.40 mmol, 92%) which turns dark blue when exposed to air since the material is very hygroscopic.

^{1}H NMR (600.13 MHz, $CDCl_3$)

δ = 1.19 (t, $^3J_{H\text{-}H}$ = 7.13 Hz, 3H, H-23), 1.51 - 1.57 (m, 2H, H-19), 1.66 - 1.71 (m, 2H, H-20), 1.85 (s, 6H, H-14/H-15), 2.02 (q, $^3J_{H\text{-}H}$ = 7.73 Hz, 2H, H-18), 2.30 (t, $^3J_{H\text{-}H}$ = 7.13 Hz, 2H, H-21), 3.23 (s, 3H, H-16), 4.05 (q, 2H, $^3J_{H\text{-}H}$ = 7.13 Hz, H-22), 4.87 (t, $^3J_{H\text{-}H}$ = 7.73 Hz, 2H, H-17), 7.63 - 7.66 (m, 1H, H-9), 7.70 - 7.73 (m, 1H, H-10), 7.79 (d, $^3J_{H\text{-}H}$ = 8.90 Hz, 1H, H-5), 8.03 (d, $^3J_{H\text{-}H}$ = 8.23 Hz, 1H, H-8), 8.05 - 8.10 (m, 2H, H-6/H-11).

^{13}C {^{1}H} NMR (150.92 MHz, $CDCl_3$)

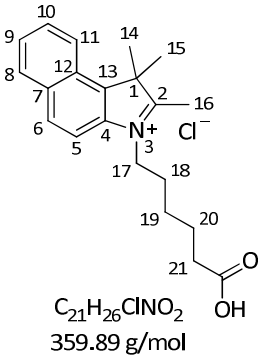
δ = 14.3 (1C, CH_3, C-23), 16.2 (1C, CH_3, C-16), 22.7 (2C, CH_2, C-14/C15), 24.4 (1C, CH_2, C-20), 26.2 (1C, CH_2, C-19), 28.1 (1C, CH_2, C-18), 33.7 (1C, CH_2, C-21), 49.7 (1C, CH_2, C-17), 56.0 (1C, C_q, C-1), 60.5 (1C, CH_2, C-22), 112.6 (1C, CH, C-5), 122.8 (1C, CH, C-6 or C-11), 127.7 (1C, CH, C-9), 127.9 (1C, C_q), 128.7 (1C, CH, C-10), 130.2 (1C, CH, C-8), 131.6 (1C, CH, C-6 or C-11), 133.8 (1C, C_q), 137.2 (1C, C_q), 138.3 (1C, C_q), 173.3 (1C, C_q, O-C=O), 195.8 (1C, C_q, C-2).

MS (HR-ESI$^+$)

m/z = 352.2270 $[M\text{-}Br]^+$, calculated for $C_{23}H_{30}NO_2^+$: 352.2271.

3-(4-Carboxyhexyl)-1,1,2-trimethyl-1*H*-benz[*e*]indol-3-ium chloride (IV)

This compound was synthesized on the basis of the procedure for 3-(4-Carboxybutyl)-1,1,2-trimethyl-1*H*-benz[*e*]indol-3-ium chloride by Mizrahi *et al*.[5]



$C_{21}H_{26}ClNO_2$
359.89 g/mol

III (1.90 g, 4.40 mmol) was suspended in 5 N aqueous HCl and stirred at 90 °C overnight. The yellow solution was filtered and the solvent was evaporated. **IV** was obtained as golden sticky material (1.17 g, 3.26 mmol, 74%).

^{1}H NMR (600.13 MHz, CD_3OD)

δ = 1.46 - 1.59 (m, 2H, H-19), 1.62 - 1.71 (m, 2H, H-20), 1.80 (s, 6H, H-14/H-15), 1.96 - 2.04 (m, 2H, H-18), 2.27 - 2.36 (m, 2H, H-21), 3.56 (s, 3H, H-16), 4.61 (t, $^3J_{H\text{-}H}$ = 6.90 Hz, 2H, H-17), 7.63 - 7.68 (m, 1H, H-9), 7.73 - 7.78 (m, 1H, H-10), 7.99 (d, $^3J_{H\text{-}H}$ = 8.50 Hz, 1H, H-5), 8.10 (d, $^3J_{H\text{-}H}$ = 8.50 Hz, 1H, H-8), 8.20 (d, $^3J_{H\text{-}H}$ = 8.50 Hz, 1H, H-6), 8.28 (d, $^3J_{H\text{-}H}$ = 8.50 Hz, 1H, H-11).

^{13}C {^{1}H} NMR (150.92 MHz, CD_3OD)

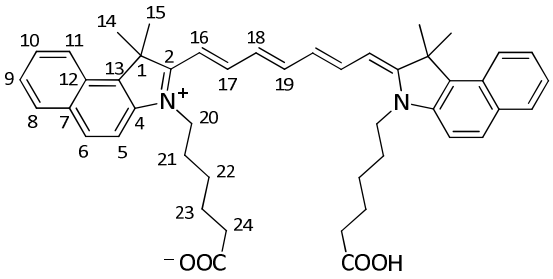
δ = 22.4 (2C, CH_2, C-14/C15), 25.3 (1C, CH_2, C-20), 26.9 (1C, CH_2, C-19), 28.6 (1C, CH_2, C-18), 34.2 (1C, CH_2, C-21), 48.8 (1C, CH_2, C-17), 52.0 (1C, CH_3, C-16), 57.2 (1C, C_q, C-1), 113.9 (1C, CH, C-5), 124.3 (1C, CH, C-11), 128.6 (1C, CH, C-9), 129.0 (1C, C_q), 129.6 (1C, CH, C-10), 130.4 (1C, CH, C-8), 132.3 (1C, CH, C-6), 135.1 (1C, C_q), 138.1 (1C, C_q), 139.7 (1C, C_q), 175.5 (1C, C_q, O-C=O), 197.5 (1C, C_q, C-2).

MS (HR-ESI$^+$)

m/z = 324.1959 $[M\text{-}Cl]^+$, calculated for $C_{21}H_{26}NO_2^+$: 324.1958.

6-(2-((1*E*,3*E*,5*E*,7*Z*)-7-(3-(5-carboxypentyl)-1,1-dimethyl-1*H*-benzo[*e*]indol-2(3*H*)-ylidene)hepta-1,3,5-trien-1-yl)-1,1-dimethyl-1*H*-benzo[*e*]indol-3-ium-3-yl)hexanoate (7)

This compound was synthesized on the basis of the procedure for "Bis-carboxy-ICG" by Mizrahi *et al*.[5]



$C_{47}H_{52}N_2O_4$
708.93 g/mol

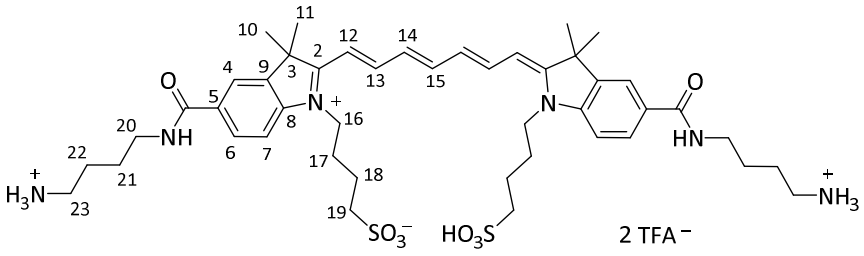
Glutaconaldehyde dianyl monohydrochloride (285 mg, 1.00 mmol, 1.0 eq.), and sodium acetate (213 mg, 2.60 mmol, 2.6 eq.) were added to a solution of **IV** (720 mg, 2.00 mmol, 2.0 eq.) in ethanol (20 mL). The mixture was heated to 90 °C and stirred for 90 minutes. The solvent was evaporated and the residue was resuspended in 2.5 N aqueous HCl and washed with HCl. **7** was obtained as green solid (248 mg, 350 μmol, 35%).

MS (HR-ESI$^+$)
m/z = 709.4003 $[M+H]^+$, calculated for $C_{47}H_{53}N_2O_4^+$: 709.4000.

General Procedure GP1 (Symmetrically Substituted Dyes Based on 1)

Bis-Carboxy dye **1** (15.0 mg**,** 19.6 μmol, 1.0 eq.) was dissolved in anhydrous DMF (2 mL) under argon at 0 °C and DIPEA (4.34 μl, 3.30 mg, 25.5 μmol, 1.3 eq.) and TBTU (13.2 mg, 41.2 μmol, 2.1 eq.) dissolved in anhydrous DMF (1 mL) were added. The reaction mixture was stirred for 30 minutes at this temperature. The corresponding amine was then added and stirring was continued at room temperature for one hour (all derivatives except **5**) or overnight (**5**).

Bis-1,1'-(4-sulfobutyl)indotricarbocyanine-5,5'-bis(4-aminobutyl-1-amide) (2)



$C_{49}H_{66}F_6N_6O_{12}S_2$
1109.21 g/mol

The reaction was carried out as described in general procedure **GP1** using **1** (30.0 mg**,** 39.3 μmol, 1.0 eq.), TBTU (25.5 mg, 86.5 μmol, 2.2 eq.), DIPEA (8.69 μl, 6.60 mg, 52.3 μmol, 1.3 eq.) and *N*-Boc-1,4-butanediamine (22.6 μl, 22.2 mg, 118 μmol, 3.0 eq.) as amine component. Subsequently, the reaction mixture was poured into Et_2O (200 mL), the precipitate was collected by filtration and dried *in vacuo*. 5-6 N HCl in 2-propanol (5 mL) was added and the solution was stirred for 4 hours. The solvent was removed under reduced pressure. Purification by semi-preparative RP HPLC (Latek ProSep C18 (5 μm, 250 mm x 10 mm), deionized H_2O with 0.1% TFA/MeCN, 4 mL/min, gradient A, t_R = 41.6 min) yielded **2** (32.4 mg, 29.2 μmol, 74%) as green amorphous powder.

^{1}H NMR (600.13 MHz, CD_3OD)
δ = 1.65 (s, 12H, H-10/H-11), 1.67 - 1.76 (m, 8H, H-17/H-18), 1.90 - 2.00 (m, 8H, H-21/H-22), 2.88 - 2.94 (m, 4H, H-23), 2.96 - 3.01 (m, 4H, H-19), 3.38 - 3.45 (m, 4H, H-16), 4.09 - 4.22 (m, 4H, H-20), 6.30 - 6.46 (m, 2H, H-12), 6.51 - 6.65 (m, 2H, H-14), 7.37 (d, $^3J_{H\text{-}H}$ = 7.56 Hz, 2H, H-7), 7.54 - 7.65 (m, 1H, H-15), 7.84 - 7.95 (m, 6H, H-4/H-6/H-13).

^{13}C {^{1}H} NMR (150.92 MHz, CD_3OD)
δ = 23.6 (2C, CH_2, C-22), 27.3 (4C, CH_2, C-17/C-18), 27.5 (2C, CH_2, C-21), 28.1 (4H, CH_3, C-10/C-11), 40.4 (2H, CH_2, C-19), 45.0 (2C, CH_2, C-20), 48.4 (2C, CH_2, C-16), 50.1 (2C, C_q, C-3), 51.9 (2C, CH_2, C-23), 106.4 (2C, CH, C-12), 111.8 (2C, CH, C-7), 122.5 (2C, CH, C-4), 128.6 (2C, CH, C-14), 129.8 (2C, CH, C-6), 131.6 (2C, C_q, C-5), 142.6 (2C, C_q, C-9), 146.4 (2C, C_q, C-8), 153.7 (2C, CH, C-13), 157.5 (1C, CH, C-15), 166.1 (2C, C_q, CONH), 173.4 (2C, Cq, C-2).

MS (HR-ESI$^+$)

m/z =	881.4305 $[M\text{-}2TFA\text{-}H]^+$,	calculated for $C_{45}H_{65}N_6O_8S_2^+$:	881.4300
	452.2102 $[M\text{-}2TFA\text{-}H+Na]^{2+}$,	calculated for $C_{45}H_{65}N_6NaO_8S_2^{2+}$:	452.2096
	441.2192 $[M\text{-}2TFA]^{2+}$,	calculated for $C_{45}H_{66}N_6O_8S_2^{2+}$:	441.2187.

UV/Vis (PBS)
$\lambda_{max\ (Abs)}$ [nm] (ε [(l·mol^{-1}·cm^{-1})]) = 755 (286 000).

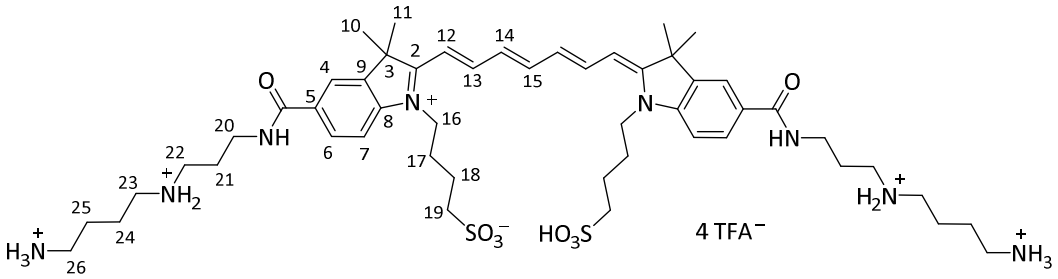
Fluorescence (PBS)

λ_{Ex} [nm] = 705; $\lambda_{max\ (Em)}$ [nm] = 781, ϕ = 0.15.

Analytical HPLC

Latek ProSep C18 (5 μm, 250 mm x 4 mm), deionized H_2O with 0.1% TFA/MeCN, 1 mL/min, gradient A, t_R = 37.8 min, purity (260 nm) = 93%, purity (630 nm) = 96%.

Bis-1,1'-(4-sulfobutyl)indotricarbocyanine-5,5'-bis(8-amino-5-azaoctyl-1-amide) (3)



$C_{59}H_{82}F_{12}N_8O_{16}S_2$

1451.45 g/mol

The reaction was carried out as described in general procedure **GP1** using N^1,N^5-Di-boc-spermidine (31.2 mg, 90.2 μmol, 4.6 eq.) as amine component. Subsequently, the solvent was evaporated, 5-6 N HCl in 2-propanol (5 mL) was added and the solution was stirred for 4 hours. The solvent was removed under reduced pressure. Purification by semi-preparative RP HPLC (Latek ProSep C18 (5 μm, 250 mm x 10 mm) or Macherey-Nagel Nucleosil C18 (5 μm, 250 mm x 10 mm), deionized H_2O with 0.1% TFA/MeCN, 4 mL/min, gradient A, t_R = 35.2 min or t_R = 35.8 min) yielded **3** (10.6 mg, 7.30 μmol, 37%) as green amorphous powder.

^{1}H NMR (600.13 MHz, CD_3OD)

δ = 1.71 (s, 12H, H-10/H-11), 1.78 - 1.88 (m, 8H, H-24/H-25), 1.91 - 2.02 (m, 8H, H-17/H-18), 2.06 (qn, $^3J_{H\text{-}H}$ = 6.50 Hz, 4H, H-21), 2.92 (t, $^3J_{H\text{-}H}$ = 6.75 Hz, 4H, H-19), 3.02 (t, $^3J_{H\text{-}H}$ = 7.14 Hz, 4H, H-26), 3.08 - 3.15 (m, 8H, H-22/H-23), 3.56 (t, $^3J_{H\text{-}H}$ = 6.50 Hz, 4H, H-20), 4.12 - 4.18 (m, 4H, H-16), 6.42 (d, $^3J_{H\text{-}H}$ = 12.61 Hz, 2H, H-12), 6.64 (t, $^3J_{H\text{-}H}$ = 12.15 Hz, 2H, H-14), 7.39 (d, $^3J_{H\text{-}H}$ = 8.33 Hz, 2H, H-7), 7.61 - 7.71 (m, 1H, H-15), 7.95 (d, $^3J_{H\text{-}H}$ = 8.33 Hz, 2H, H-6), 7.98 (s, 2H, H-4) 7.95 - 8.04 (m, 2H, H-13).

^{13}C {^{1}H} NMR (125.15 MHz, CD_3OD)

δ = 23.5 (2C, CH_2, C-17), 24.3 (2C, CH_2, C-25), 25.6 (2C, CH_2, C-24), 27.2 (2C, CH_2, C-18), 27.8 (2C, CH_2, C-21), 28.0 (4C, CH_3, C-10/C-11), 37.6 (2C, CH_2, C-20), 40.0 (2C, CH_2, C-26), 45.0 (2C, CH_2, C-16), 46.5 (2C, CH_2, C-23), 48.2 (2C, CH_2, C-22), 50.2 (2C, C_q, C-3), 51.7 (2C, CH_2, C-19), 106.5* (2C, CH, C-12), 112.0* (2C, CH, C-7), 122.5 (2C, CH, C-4), 128.7* (2C, CH, C-14), 130.0

(2C, CH, C-6), 131.1 (2C, C_q, C-5), 142.6 (2C, C_q, C-9), 146.7 (2C, C_q, C-8), 154.0 (2C, CH, C-13), 158.9* (1C, CH, C-15), 163.0 (2C, C_q, C-2), 170.0 (2C, C_q, CONH).

* Not observed in 1D spectra. Signals were detected via indirect excitation (HSQC).

MS (HR-ESI$^+$)

m/z = 995.5460 $[M\text{-}4TFA\text{-}3H]^+$, calculated for $C_{51}H_{79}N_8O_8S_2^+$: 995.5957
498.2767 $[M\text{-}4TFA\text{-}2H]^{2+}$, calculated for $C_{51}H_{80}N_8O_8S_2^{2+}$: 498.2765
332.5703 $[M\text{-}4TFA\text{-}H]^{3+}$, calculated for $C_{51}H_{81}N_8O_8S_2^{3+}$: 332.5201.

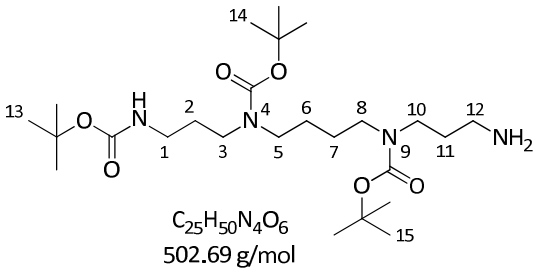
UV/Vis (PBS)
$\lambda_{max\ (Abs)}$ [nm] (ε [($l \cdot mol^{-1} \cdot cm^{-1}$)]) = 755 (260 000).

Fluorescence (PBS)
λ_{Ex} [nm] = 705; $\lambda_{max\ (Em)}$ [nm] = 781, ϕ = 0.15.

Analytical HPLC
Latek ProSep C18 (5 μm, 250 mm x 4 mm), deionized H_2O with 0.1% TFA/MeCN, 1 mL/min, gradient A, t_R = 34.5 min, purity (260 nm) = 96%, purity (630 nm) = 99%.

***N*^{1},*N*^{4},*N*^{9}-Tri-boc-spermine (V)**

This is a known compound which was synthesized following a procedure by Geall and Blagbrough[6] which was slightly modified.



$C_{25}H_{50}N_4O_6$
502.69 g/mol

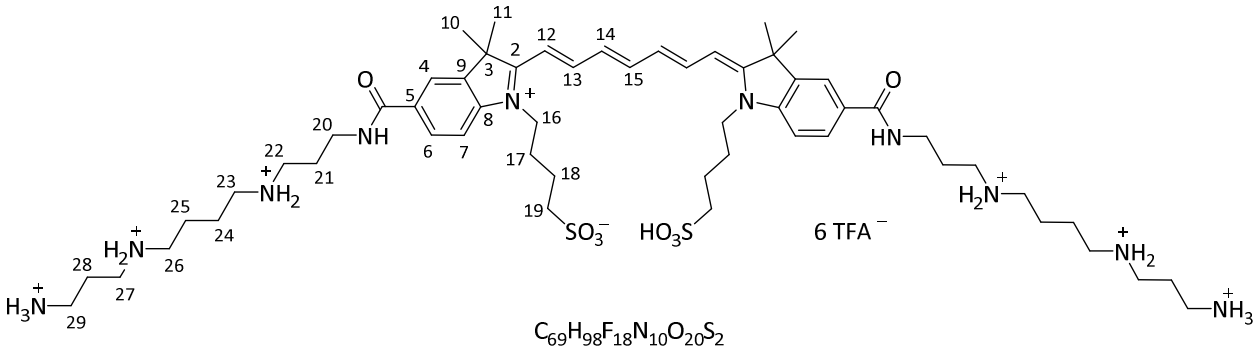
Spermine (4.00 g, 19.8 mmol, 1.0 eq.) was dissolved in MeOH (280 mL) at -78 °C unter argon and ethyl trifluoroacetate (2.35 mL, 2.81 g, 19.8 mmol, 1.0 eq.) dissolved in MeOH (2.5 mL) was added dropwise during one hour. The reaction mixture was allowed to warm to 0 °C and stirred for 30 minutes. Subsequently, di-*tert*-butyl dicarbonate (16.6 mL, 16.9 g, 79.2 mmol, 4.0 eq.) dissolved in MeOH (40 mL) was added dropwise at the same temperature. The reaction mixture was stirred for 18 hours at room temperature. After the reaction was completed (confirmed by TLC and ESI-MS), concentrated aqueous ammonia was added until the pH of the solution reached 11 and the mixture was stirred at 75 °C overnight to remove

the trifluoroacetyl protecting group. The solvent was removed under reduced pressure and the crude product was purified by flash chromatography (SiO_2, h = 25 cm, ø = 3 cm, CH_2Cl_2/MeOH/conc. aq. NH_3 100/10/1, R_f = 0.41 in CH_2Cl_2/MeOH/conc. aq. NH_3 70/10/1). **V** was isolated as pale yellow oil (3.51 g, 6.98 mmol, 35%).

^{1}H NMR (399.89 MHz, CD_2Cl_2)
δ = 1.41 (s, 9H, CH_3), 1.43 (s, 9H, CH_3), 1.44 (s, 9H, CH_3), 1.46 - 1.52 (m, 4H, H-5/H-6), 1.55 - 1.72 (m, 2H, H-11), 1.83 - 2.00 (m, 2H, H-2), 2.87 - 2.99 (m, 2H, H-3), 3.00 - 3.28 (m, 8H, H-5/H-8/H-10/H-12), 3.28 - 3.45 (m, 2H, H-1).

MS (HR-ESI$^+$)
m/z = 503.3799 $[M+H]^+$, calculated for $C_{25}H_{51}N_4O_6^+$: 503.3803.

<u>Bis-1,1'-(4-sulfobutyl)indotricarbocyanine-5,5'-bis(12-amino-4,9-diazadodecyl-1-amide) (4)</u>



$C_{69}H_{98}F_{18}N_{10}O_{20}S_2$
1793.68 g/mol

The reaction was carried out as described in general procedure **GP1** using **V** (45.3 mg, 90.2 μmol, 4.6 eq.) as amine component. Subsequently, the solvent was evaporated, 5-6 N HCl in 2-propanol (5 mL) was added and the solution was stirred for 4 hours. The solvent was removed under reduced pressure. Purification by semi-preparative RP HPLC (Latek ProSep C18 (5 μm, 250 mm x 10 mm) or Macherey-Nagel Nucleosil C18 (5 μm, 250 mm x 10 mm), deionized H_2O with 0.1% TFA/MeCN, 4 mL/min, gradient A, t_R = 33.4 min or t_R = 34.8 min) yielded **4** (11.1 mg, 6.19 μmol, 32%) as green amorphous powder.

^{1}H NMR (600.13 MHz, CD_3OD)
δ = 1.67 (s, 12H, H-10/H-11), 1.78 - 1.88 (m, 8H, H-24/H-25), 1.89 - 1.99 (m, 8H, H-17/H-18), 2.01 (qn, $^3J_{H\text{-}H}$ = 7.00 Hz, 4H, H-28), 2.05 (qn, $^3J_{H\text{-}H}$ = 7.60 Hz, 4H, H-21), 2.91 (t, $^3J_{H\text{-}H}$ = 6.75 Hz, 4H, H-19), 3.02 - 3.12 (m, 16H, H-22/H-23/H-26/H-27), 3.14 (t, $^3J_{H\text{-}H}$ = 7.60 Hz, 4H, H-20), 3.48 - 3.55 (m, 4H, H-29), 4.07 - 4.18 (m, 4H, H-16), 6.38 (d, $^3J_{H\text{-}H}$ = 11.97 Hz, 2H, H-12), 6.55 - 6.65 (m, 2H, H-14), 7.36 (d, $^3J_{H\text{-}H}$ = 8.60 Hz, 2H, H-7), 7.60 - 7.67 (m, 1H, H-15), 7.92 (d,

$^{3}J_{H\text{-}H}$ = 8.60 Hz, 2H, H-6), 7.95 (s, 2H, H-4) 7.91 - 7.96 (m, 2H, H-13 (superimposed by the signals of H-4 and H-6)).

^{13}C {^{1}H} NMR (150.92 MHz, CD_3OD)
δ = 23.5 (2C, CH_2, C-17), 24.2 (2C, CH_2, C-25), 24.3 (2C, CH_2, C-24), 25.3 (2C, CH2, C-21), 27.2 (2C, CH_2, C-18), 27.7 (2C, CH2, C-28), 28.0 (4C, CH_3, C-10/C-11), 37.6 (2C, CH_2, C-22 or C-23 or C-26 or C-27), 37.8 (2C, CH2, C-29), 44.9 (2C, CH_2, C-16), 45.9 (2C, CH_2, C-20), 46.5/48.1/48.2 (6C, CH_2, C-22 and/or C-23 and/or C-26 and/or C-27), 50.2 (2C, C_q, C-3), 51.8 (2C, CH_2, C-19), 106.3 (2C, CH, C-12), 111.8 (2C, CH, C-7), 122.5 (2C, CH, C-4), 128.7 (2C, CH, C-14), 130.0 (2C, CH, C-6), 131.1 (2C, C_q, C-5), 142.6 (2C, C_q, C-9), 146.6 (2C, C_q, C-8), 153.7 (2C, CH, C-13), 158.3* (1C, CH, C-15), 163.2 (2C, C_q, C-2), 169.8 (2C, C_q, CONH).

* Not observed in 1D spectra. Signals were detected via indirect excitation (HSQC).

MS (HR-ESI^{+})

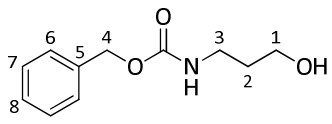
m/z = 1109.6621 $[M\text{-}6TFA\text{-}5H]^{+}$, calculated for $C_{57}H_{93}N_{10}O_8S_2^{+}$: 1109.6614
555.3344 $[M\text{-}6TFA\text{-}4H]^{2+}$, calculated for $C_{57}H_{94}N_{10}O_8S_2^{2+}$: 555.3343
370.5588 $[M\text{-}6TFA\text{-}3H]^{3+}$, calculated for $C_{57}H_{95}N_{10}O_8S_2^{2+}$: 370.5586.

UV/Vis (PBS)
$\lambda_{max\ (Abs)}$ [nm] (ε [($l \cdot mol^{-1} \cdot cm^{-1}$)]) = 755 (264 000).

Fluorescence (PBS)
λ_{Ex} [nm] = 705; $\lambda_{max\ (Em)}$ [nm] = 781; ϕ = 0.10.

Analytical HPLC
Latek ProSep C18 (5 μm, 250 mm x 4 mm), deionized H_2O with 0.1% TFA/MeCN, 1 mL/min, gradient A, t_R = 33.4 min, purity (260 nm) = 96%, purity (630 nm) = 99%.

<u>3-Benzyloxycarbonylaminopropan-1-ol (VI)</u>
This is a known compound which was synthesized following a procedure by Geall and Blagbrough.[6]



$C_{11}H_{15}NO_3$
209.24 g/mol

3-Aminopropan-1-ol (3.03 mL, 3.00 g, 39.9 mmol, 1.0 eq.) was added to 1 M aq. NaOH (44 mL), the solution was cooled to 0 °C and benzyl chloroformate (6.25 mL, 7.51 g, 44.0 mmol, 1.1 eq.) was added dropwise. The solution was allowed to warm to room

temperature and stirred for 1 hour. CH_2Cl_2 (30 mL) was added and stirring was continued for another 3 hours. The solvents were removed under reduced pressure and the crude product was absorbed on celite and purified by flash chromatography (SiO_2, h = 30 cm, ø = 6 cm, CH_2Cl_2/MeOH/conc. aq. NH_3 300/10/1, R_f = 0.25). **VI** was obtained as white solid (7.52 g, 35.9 mmol, 90%).

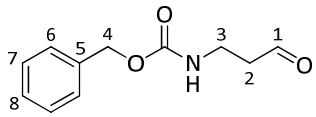
^{1}H NMR (600.13 MHz, $CDCl_3$)
δ = 1.70 (qn, $^3J_{H\text{-}H}$ = 6.00 Hz, 2H, H-2), 3.35 (t, $^3J_{H\text{-}H}$ = 6.00 Hz, 2H, H-3), 3.67 (t, $^3J_{H\text{-}H}$ = 6.00 Hz, 2H, H-1), 5.11 (s, 2H, H-4), 7.28 - 7.39 (m, 5H, H-6/H-7/H-8).

^{13}C {^{1}H} NMR (150.90 MHz, $CDCl_3$)
δ = 32.7 (1C, CH_2, C-2), 37.9 (1C, CH_2, C-3), 59.7 (1C, CH_2, C-1), 67.0 (1C, CH_2, C-4), 128.2 (2C, CH, C-6), 128.3 (1C, CH, C-8), 128.7 (2C, CH, C-7), 136.6 (1C, C_q, C-5), 157.5 (1C, C_q, OCONH).

MS (HR-ESI$^+$)

m/z = 248.0683 $[M+K]^+$, calculated for $C_{11}H_{15}KNO_3^+$: 248.0684
232.0944 $[M+Na]^+$, calculated for $C_{11}H_{15}NNaO_3^+$: 232.0944.

3-Benzyloxycarbonylaminopropanal (VII)
This is a known compound which was synthesized following a procedure by Geall and Blagbrough.[6]



$C_{11}H_{13}NO_3$
207.23 g/mol

Oxalyl chloride (1.57 mL, 2.33 g, 18.4 mmol, 1.1 eq.) was added to anhydrous CH_2Cl_2 (45 mL) under argon at -78 °C. Anhydrous DMSO (2.36 mL, 2.60 g, 33.4 mmol, 2.0 eq.) was added dropwise over 3 minutes and the solution was stirred for 10 minutes. **VI** (3.50 g, 16.7 mmol, 1.0 eq.) dissolved in anhydrous CH_2Cl_2 (10 mL) was added dropwise over 3 minues and the resulting suspension was warmed to approx. -30 °C until it became clear. After cooling to -78 °C again and stirring for 10 minutes, triethylamine (11.6 mL, 83.5 mmol, 5.0 eq.) was added. The solution was allowed to warm to 25 °C and water (60 mL) was added. Two phases formed which were separated. The aqueous phase was extracted with CH_2Cl_2 (2 x 50 mL), the combined organic phases were dried using anhydrous $MgSO_4$, concentrated under reduced pressure and purified by flash chromatography (SiO_2, h = 30 cm, ø = 6 cm, EtOAc, R_f = 0.69). **VII** was obtained as colorless oil (2.82 g, 13.6 mmol, 81%).

^{1}H NMR (399.89 MHz, $CDCl_3$)
δ = 2.73 (t, $^3J_{H\text{-}H}$ = 5.85 Hz, 2H, H-2), 3.48 (q, $^3J_{H\text{-}H}$ = 5.85 Hz, 2H, H-3), 5.08 (s, 2H, H-4), 7.28 - 7.40 (m, 5H, H-6/H-7/H-8), 9.79 (s, 1H, H-1).

^{13}C {^{1}H} NMR (125.75 MHz, $CDCl_3$)

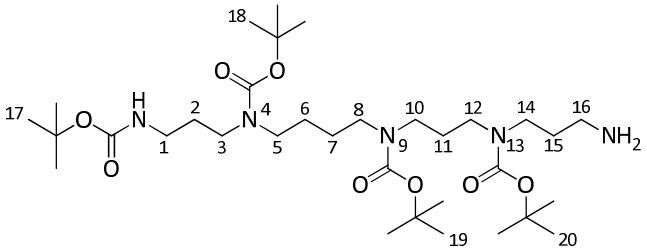
δ = 34.6 (1C, CH_2, C-3), 44.2 (1C, CH_2, C-2), 66.9 (1C, CH_2, C-4), 128.2 (2C, CH, C-6), 128.3 (1C, CH, C-8), 128.7 (2C, CH, C-7), 136.5 (1C, C_q, C-5), 156.4 (1C, C_q, C=O), 201.3 (1C, C_q, C-1).

MS (HR-ESI$^+$)

m/z = 437.1682 $[2M+Na]^+$, calculated for $C_{22}H_{26}N_2NaO_6^+$: 437.1683
246.0526 $[M+K]^+$, calculated for $C_{11}H_{13}KNO_3^+$: 246.0527
230.0788 $[M+Na]^+$, calculated for $C_{11}H_{13}NNaO_3^+$: 230.0785.

***N*^{1},*N*^{4},*N*^{9},*N*^{13}-Tetra-Boc-1,16-diamino-4,9,13-triazahexadecane (VIII)**

This is a known compound which was synthesized following a procedure by Geall and Blagbrough.[6]



$C_{33}H_{65}N_5O_8$
659.90 g/mol

N^1,*N*^{4},*N*^{9}-Tri-boc-spermine **V** (1.15 g, 2.28 mmol, 1.2 eq.) was dissolved in anhydrous MeOH (18 mL) with 4 Å molecular sieves (4.00 g) under argon and **VII** (394 mg, 1.90 mmol, 1.0 eq.), $NaCNBH_3$ (179 mg, 2.85 mmol, 1.5 eq.) and a catalytic amount of glacial acetic acid were added. The reaction mixture was stirred at room temperature for 24 hours. The molecular sieves were removed by filtration and the solvent was removed under reduced pressure. The crude material was purified by flash chromatography (SiO_2, h = 30 cm, ø = 4 cm, CH_2Cl_2/MeOH/conc. aq. NH_3 100/10/1, R_f = 0.4) yielding the intermediate product with one Cbz- and three Boc-protecting groups as yellow oil.

MS (HR-ESI$^+$)

m/z = 694.4748 $[M+H]^+$, calculated for $C_{36}H_{64}N_5O_8^+$: 694.4749.

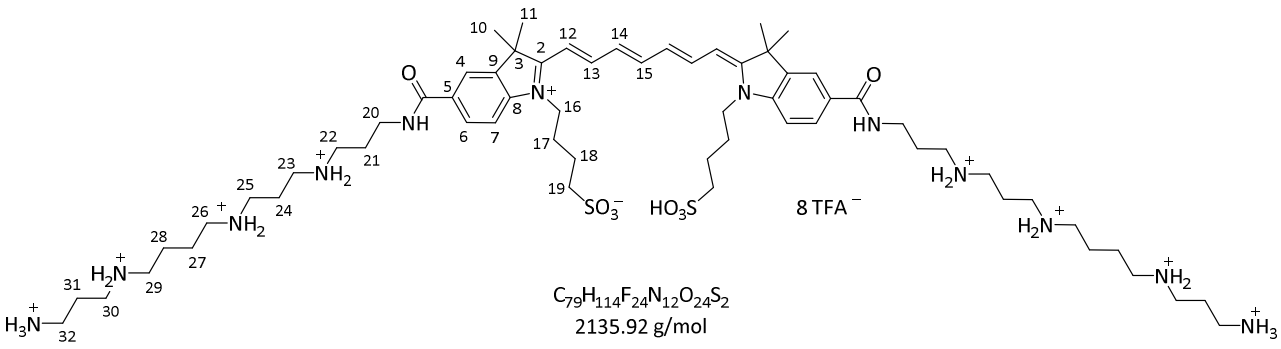
The intermediate product was then dissolved in anhydrous DMF (10 mL) under argon. Di-*tert*-butyl dicarbonate (813 μl, 829 mg, 3.80 mmol, 2.0 eq.) was added dropwise to afford the completely protected polyamine. After two hours of stirring at room temperature, the excess of di-*tert*-butyl dicarbonate was quenched with conc. aq. NH_3 (1 mL), the mixture was stirred for another 30 minutes after which the solvent was removed *in vacuo*. The residue was dissolved in MeOH (11 mL) under nitrogen and Perman's catalyt ($Pd(OH)_2$ on carbon, 20 % loading, 540 mg) was added. The flask was evacuated und flushed with hydrogen (3 x). After stirring for four hours at room temperature under hydrogen, the catalyst was removed by filtration through a pad of celite. The solution was concentrated *in vacuo* and the raw product

was subjected to column chromatography (SiO_2, h = 30 cm, ø = 4 cm, CH_2Cl_2/MeOH/conc. aq. NH_3 100/10/1, R_f = 0.24). The combined fractions were filtered through Na_2SO_4 and the solvent was removed *in vacuo* to yield **VIII** as pale oil (469 mg, 0.71 mmol, 37 %).

^{1}H NMR (600.13 MHz, CD_3OD)
δ = 1.44 - 1.47 (m, 36 H, H-17/H-18/H-19/H-20), 1.51 - 1.55 (m, 4H, H-6/H-7), 1.66 - 1.80 (m, 6H, H-2/H-11/H-15), 2.67 - 2.69 (m, 2H, H-1), 3.03 (t, $^3J_{H\text{-}H}$ = 6.70 Hz, 2H, H-16), 3.19 - 3.29 (m, 12H, H-3/H-5/H-8/H-10/H-12/H-14).

MS (HR-ESI$^+$)
m/z = 660.4899 $[M+H]^+$, calculated for $C_{33}H_{66}N_5O_8^+$: 660.4906.

<u>Bis-1,1'-(4-sulfobutyl)indotricarbocyanine-5,5'-bis(16-amino-4,8,13-triazahexadecyl-1-amide) (5)</u>



The reaction was carried out as described in general procedure **GP1** using **1** (60.0 mg**,** 79.0 μmol, 1.0 eq.), TBTU (52.8 mg, 164 μmol, 2.1 eq.), DIPEA (17.3 μl, 13.2 mg, 102 μmol, 1.3 eq.) and **VIII** (156 mg, 237 μmol, 3.0 eq.) as amine component. Subsequently, the solvent was evaporated, 5-6 N HCl in 2-propanol (5 mL) was added and the solution was stirred for 4 hours. The solvent was removed under reduced pressure. Purification by semi-preparative RP HPLC (Latek ProSep C18 (5 μm, 250 mm x 10 mm) deionized H_2O with 0.1% TFA/MeCN, 4 mL/min, gradient A, t_R = 32.8 min) yielded **5** (86.0 mg, 40.3 μmol, 51%) as green amorphous powder.

^{1}H NMR (600.13 MHz, D_2O)
δ = 1.54 (s, 12H, H-10/H-11), 1.72 - 1.75 (m, 8H, H-27/H-28), 1.80 - 1.83 (m, 4H, H-18), 1.87 - 1.89 (m, 4H, H-17), 1.95 (qn, $^3J_{H\text{-}H}$ = 7.40 Hz, 4H, H-21), 2.03 - 2.12 (m, 8H, H-24/H-31), 2.91 (t, $^3J_{H\text{-}H}$ = 8.50 Hz, 4H, H-19), 3.05 - 3.16 (m, 28H, H-20/H-22/H-23/H-25/H-26/H-29/ H-30), 3.36 - 3.38 (m, 4H, H-32), 3.97 - 4.07 (m, 4H, H-16), 6.07 (d, $^3J_{H\text{-}H}$ = 12.40 Hz, 2H, H-12),

6.33 (t, $^3J_{H\text{-}H}$ = 12.40 Hz, 2H, H-14), 7.27 (d, $^3J_{H\text{-}H}$ = 8.30 Hz, 2H, H-7), 7.29 - 7.38 (m, 1H, H-15), 7.70 (t, $^3J_{H\text{-}H}$ = 12.40 Hz, 2H, H-13), 7.73 (d, $^3J_{H\text{-}H}$ = 8.30 Hz, 2H, H-6), 7.78 (s, 2H, H-4).

^{13}C NMR (150.92 MHz, CD_3OD)
δ = 21.6 (2C, CH_2, C-18), 22.6 (2C, CH_2, C-24 or C-31), 22.7 (4C, CH_2, C-27/C-28), 23.7 (2C, CH_2, C-24 or C-31), 25.5 (2C, CH_2, C-17), 25.7 (2C, CH_2, C-21), 26.8 (4C, CH_3, C-10/C-11), 36.4 (CH_2, C-20 and/or C-22 and/or C-23 and/or C-25 and/or C-26 and/or C-29 and/or C-30), 36.5 (2C, CH_2, C-32), 44.4 (2C, CH_2, C-16), 44.4 (CH_2, C-20 and/or C-22 and/or C-23 and/or C-25 and/or C-26 and/or C-29 and/or C-30), 45.3 (CH_2, C-20 and/or C-22 and/or C-23 and/or C-25 and/or C-26 and/or C-29 and/or C-30), 46.9 (CH_2, C-20 and/or C-22 and/or C-23 and/or C-25 and/or C-26 and/or C-29 and/or C-30), 50.4 (2C, CH_2, C-19), 109.4 (2C, CH, C-7), 121.3 (2C, CH, C-4), 127.4 (2C, CH, C-14), 128.2 (2C, CH, C-6), 152.1 (2C, CH, C-13), 156.4 (1C, CH, C-15).
Quaternary carbon atoms and the signal for C-12 were not observed.

MS (HR-ESI$^+$)

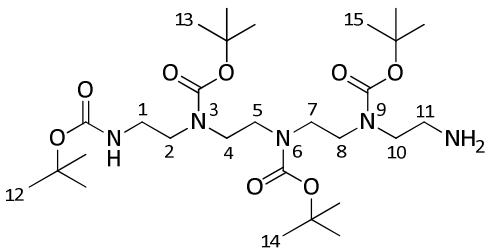
m/z = 1223.7770 $[M\text{-}8TFA\text{-}7H]^+$, calculated for $C_{63}H_{107}N_{12}O_8S_2^+$: 1223.7771
612.3920 $[M\text{-}8TFA\text{-}6H]^{2+}$, calculated for $C_{63}H_{108}N_{12}O_8S_2^{2+}$: 612.3922
408.5971 $[M\text{-}8TFA\text{-}5H]^{3+}$, calculated for $C_{63}H_{109}N_{12}O_8S_2^{3+}$: 408.5972.

UV/Vis (PBS)
$\lambda_{max\ (Abs)}$ [nm] (ε [($l \cdot mol^{-1} \cdot cm^{-1}$)]) = 755 (268 000).

Fluorescence (PBS)
λ_{Ex} [nm] = 755; $\lambda_{max\ (Em)}$ [nm] = 781; ϕ = 0.10.

Analytical HPLC
Latek ProSep C18 (5 μm, 250 mm x 4 mm), deionized H_2O with 0.1% TFA/MeCN, 1 mL/min, gradient A, t_R = 31.9 min, purity (260 nm) = 95%, purity (630 nm) = 98%.

***N*^{1},*N*^{3},*N*^{6},*N*^{9}-Tetra-boc-1,11-diamino-3,6,9-triazaundecane (IX)**



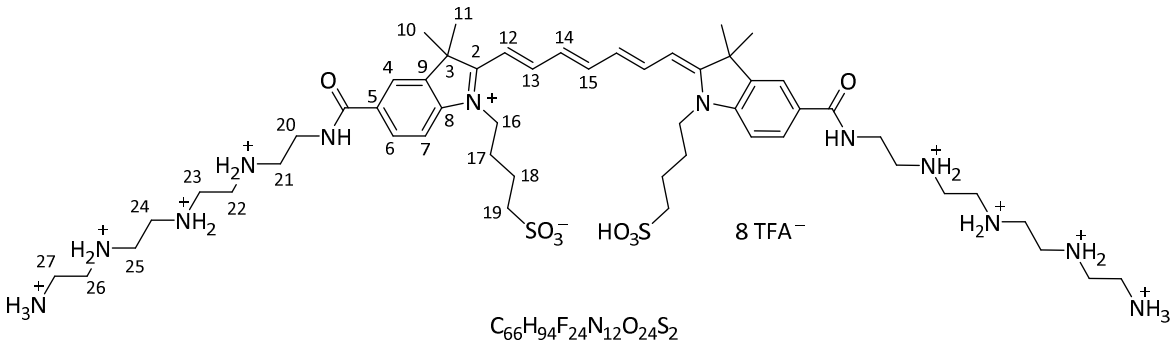
$C_{28}H_{55}N_5O_8$
589.76 g/mol

Tetraethylenepentamine (1.47 mL, 1.44 mg, 7.80 mmol, 1.0 eq.) was dissolved in MeOH (210 mL) at -78 °C unter argon and ethyl trifluoroacetate (0.93 mL, 1.11 g, 7.80 mmol, 1.0 eq.) dissolved in MeOH (2 mL) was added dropwise during one hour. The reaction mixture was stirred for 30 minutes at 0 °C. Subsequently, di-*tert*-butyl dicarbonate (8.97 mL, 9.15 g, 39.0 mmol, 5.0 eq.) dissolved in MeOH (25 mL) was added dropwise at the same temperature. The reaction mixture was stirred for 18 hours at room temperature. After the reaction was completed (confirmed by TLC and ESI-MS), concentrated aqueous ammonia (420 mL) was added and the solution was stirred at 75 °C overnight in order to remove the trifluoroacetyl protecting group. The solvent was removed under reduced pressure and the crude product was purified by flash chromatography (SiO_2, h = 30 cm, ø = 5 cm, CH_2Cl_2/MeOH/conc. aq. NH_3 200/10/1, R_f = 0.43 in CH_2Cl_2/MeOH/conc. aq. NH_3 70/10/1). **IX** was isolated as pale yellow oil (583 mg, 0.99 mmol, 13%).

^{1}H NMR (399.89 MHz, CD_2Cl_2)
δ = 1.40 (s, 9H, H-12), 1.44 (s, 27H, H-12/H-13/H-14), 2.77 (br. s, 2H, NH_2), 3.14 - 3.35 (m, 16H, H-1/H-2/H-4/H-5/H-7/H-8/H-10/H-11).

MS (HR-ESI$^+$)
m/z = 590.4123 $[M+H]^+$, calculated for $C_{28}H_{56}N_5O_8^+$: 590.4123.

Bis-1,1'-(4-sulfobutyl)indotricarbocyanine-5,5'-bis(11-diamino-3,6,9-triazaundecane-1-amide) (6)



$C_{66}H_{94}F_{24}N_{12}O_{24}S_2$
1995.65 g/mol

The reaction was carried out as described in general procedure **GP1** using **1** (60.0 mg**,** 79.0 μmol, 1.0 eq.), TBTU (52.8 mg, 164 μmol, 2.1 eq.), DIPEA (17.3 μl, 13.2 mg, 102 μmol, 1.3 eq.) and **IX** (212 mg, 359 μmol, 4.6 eq.) as amine component. Subsequently, the solvent was evaporated, 5-6 N HCl in 2-propanol (5 mL) was added and the solution was stirred for 4 hours. The solvent was removed under reduced pressure. Purification by preparative RP HPLC (Latek ProSep C18 (8 μm, 250 mm x 20 mm), deionized H_2O with 0.1% TFA/MeCN, 8 mL/min, gradient A, t_R = 36.7 min) yielded **6** (53.8 mg, 27.0 μmol, 34%) as green amorphous powder.

^{1}H NMR (600.13 MHz, D_2O)

δ = 1.55 (s, 12H, H-10/11), 1.75 (m, 8H, H-17/18), 2.75 - 2.81 (m, 4H, H-19), 3.32 - 3.39 (m, 8H, H-26/27), 3.43 - 3.47 (m, 4H, H-21), 3.47 - 3.51 (m, 16H, H-22/23/24/25), 3.61 - 3.67 (m, 4H, H-20), 3.94 (m, 4H, H-16), 6.04 (d, $^{3}J_{H\text{-}H}$ = 12.58 Hz, 2H, H-12), 6.31 (t, $^{3}J_{H\text{-}H}$ = 12.58 Hz, 2H, H-14), 7.24 (d, $^{3}J_{H\text{-}H}$ = 8.26 Hz, 2H, H-7), 7.29 - 7.39 (m, 1H, H-15), 7.70 (t, $^{3}J_{H\text{-}H}$ = 12.58 Hz, 2H, H-13), 7.77 (d, $^{3}J_{H\text{-}H}$ = 8.26 Hz, 2H, H-6), 7.82 (s, 2H, H-4).

^{13}C {^{1}H} NMR (150.90 MHz, D_2O)

δ = 21.5 (2C, CH_2, C-17), 25.5 (2C, CH_2, C-18), 26.8 (4C, CH_3, C-10/11), 35.4 (2C, CH_2, C-26), 36.2 (2C, CH_2, C-20), 43.5 (2C, CH_2, C-16), 43.6 (6C, CH_2, C-22/C-23/C-24), 43.6 (2C, CH_2, C-25), 44.6 (2C, CH_2, C-21), 48.2 (2C, CH_2, C-27), 48.6 (2C, CH_2, C-3), 50.2 (2C, CH_2, C-19), 104.9 (2C, CH, C-12), 110.5 (2C, CH, C-7), 115.3 (2C, C_q, C-9), 117.3 (2C, C_q, C-8), 121.3 (2C, CH, C-4), 127.3* (2C, CH, C-14), 128.6 (2C, CH, C-6), 141.3 (2C, C_q, C-3), 145.3 (2C, C_q, C-5), 152.4 (2C, CH, C-13), 157.0* (1C, CH, C-15), 162.8 (2C, C_q, C-2), 169.9 (2C, C_q, CONH).

* Not observed in 1D spectra. Signals were detected via indirect excitation (HSQC).

MS (HR-ESI^{+})

m/z = 542.3146 $[M\text{-}8TFA\text{-}6H]^{2+}$, calculated for $C_{53}H_{88}N_{12}O_8S_2^{2+}$: 542.3140

361.8789 $[M\text{-}8TFA\text{-}5H]^{3+}$, calculated for $C_{53}H_{89}N_{12}O_8S_2^{3+}$: 361.8784.

UV/Vis (PBS)

$\lambda_{max\ (Abs)}$ [nm] (ε [($l \cdot mol^{-1} \cdot cm^{-1}$)]) = 755 (268 000).

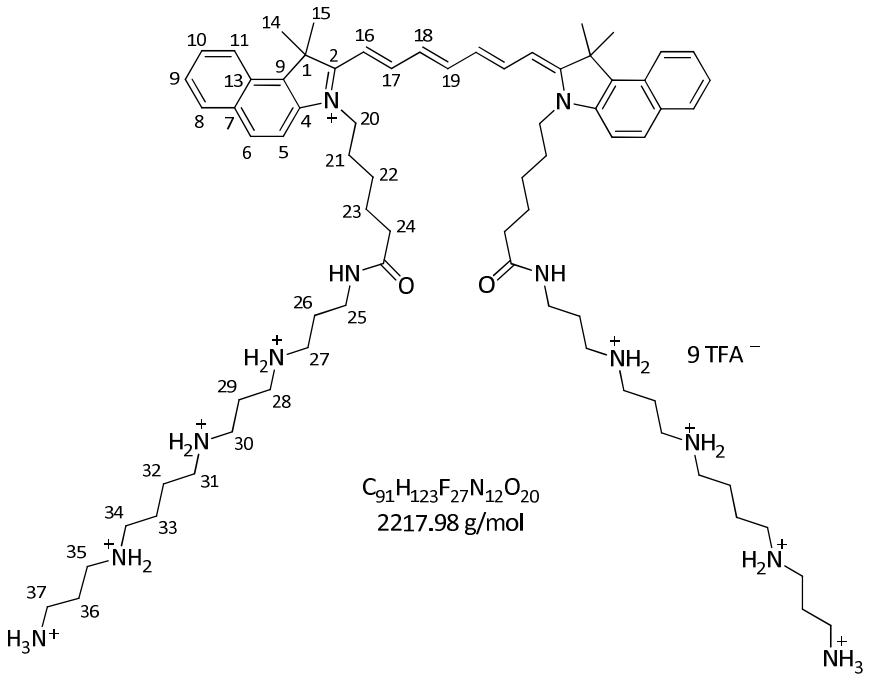
Fluorescence (PBS)

λ_{Ex} [nm] = 705; $\lambda_{max\ (Em)}$ [nm] = 781; ϕ = 0.11.

Analytical HPLC

Latek ProSep C18 (8 μm, 250 mm x 4 mm), deionized H_2O with 0.1% TFA/MeCN, 1 mL/min, gradient A, t_R = 36.8 min, purity (260 nm) = 97%, purity (630 nm) = 98%.

3-(5-(16-amino-4,8,13-triazahexadecyl-1-amido)pentyl)-2-((1*E*,3*E*,5*E*,7*Z*)-7-(3-(5-(16-amino-4,8,13-triazahexadecyl-1-amido)pentyl)-1,1-dimethyl-1*H*-benzo[*e*]indol-2(3*H*)-ylidene)hepta-1,3,5-trien-1-yl)-1,1-dimethyl-1*H*-benzo[*e*]indol-3-ium (8)



The reaction was carried out as described in general procedure **GP1** using **7** (80.0 mg**,** 113 μmol, 1.0 eq.), TBTU (76.2 mg, 273 μmol, 2.1 eq.), DIPEA (46.4 μl, 35.3 mg, 273 μmol, 2.1 eq.) and **VIII** (187 mg, 283 μmol, 2.5 eq.) as amine component. Subsequently, the solvent was evaporated and the residue was purified by flash chromatography (SiO_2, h = 10 cm, ø = 1 cm, CH_2Cl_2/MeOH 20/1). The grass green fractions were combined, the solvent was evaporated, 5-6 N HCl in 2-propanol (3 mL) was added to the residue and the solution was stirred for 4 hours. The solvent was removed under reduced pressure. Purification by semi-preparative RP HPLC (Latek ProSep C18 (5 μm, 250 mm x 10 mm), deionized H_2O with 0.1% TFA/MeCN, 4 mL/min, gradient B, t_R = 46.2 min) yielded **8** (7.41 mg, 3.34 μmol, 3%) as green amorphous powder.

^{1}H NMR (600.13 MHz, CD_3OD)

δ = 1.47 - 1.52 (m, 4H, H-22), 1.68 - 1.76 (m, 12H, H-23/H-32/H-33), 1.81 (qn, $^3J_{H\text{-}H}$ = 6.70 Hz, 4H, H-36), 1.88 (qn, $^3J_{H\text{-}H}$ = 7.56 Hz, 4H, H-21), 2.00 (s, 12H, H-14/H-15), 1.98 - 2.05 (m, 8H, H-26/H-29), 2.24 (t, $^3J_{H\text{-}H}$ = 7.56 Hz, 4H, H-24), 2.90 (t, $^3J_{H\text{-}H}$ = 7.37 Hz, 4H, H-35), 2.94 (m, 8H, H-31/H-34), 3.00 - 3.05 (m, 16H, H-25/H-27/H-28/H-30), 3.22 (t, $^3J_{H\text{-}H}$ = 6.70 Hz, 4H, H-37), 4.22 (t, $^3J_{H\text{-}H}$ = 7.56 Hz, 4H, H-20), 6.33 (d, $^3J_{H\text{-}H}$ = 13.48 Hz, 2H, H-16), 6.60 (t, $^3J_{H\text{-}H}$ = 12.60 Hz, 2H, H-18), 7.45 (t, $^3J_{H\text{-}H}$ = 7.30 Hz, 2H, H-8), 7.57 (d, $^3J_{H\text{-}H}$ = 8.89 Hz, 2H, H-11), 7.62 - 7.66 (m, 3H, H-6/H-19), 7.98 - 8.02 (m, 4H, H-9/H-10), 8.06 (t, $^3J_{H\text{-}H}$ = 13.00 Hz, 2H, H-17), 8.24 (d, $^3J_{H\text{-}H}$ = 8.89 Hz, 2H, H-5).

MS (HR-ESI$^+$)

m/z = 596.4682 [M-9TFA-7H]$^{2+}$, calculated for $C_{73}H_{116}N_{12}O_2^{2+}$: 596.4667
397.9810 [M-9TFA-6H]$^{3+}$, calculated for $C_{73}H_{117}N_{12}O_2^{3+}$: 397.9802.

UV/Vis (PBS)

$\lambda_{max\ (Abs)}$ [nm] (ε [(l·mol^{-1}·cm^{-1})]) = 781 (115 000).

Fluorescence (PBS)

λ_{Ex} [nm] = 705; $\lambda_{max\ (Em)}$ [nm] = 805; ϕ = 0.022.

Analytical HPLC

Latek ProSep C18 (5 μm, 250 mm x 4 mm), deionized H_2O with 0.1% TFA/MeCN, 1 mL/min, gradient B, t_R = 48.4 min, purity (260 nm) = 94%, purity (630 nm) = 98%.

Cell Experiments

Cell culture

For cell experiments, the following cell lines were used:

Human cervical cancer cell line (HeLa T-REX stable LAPtag) was a kind gift from B. Cerikan (ZMBH, Heidelberg). The cells were cultivated in DMEM (Gibco) supplemented with 10% FCS (Biochrom) and 1% penicillin/streptomycin (Biochrom).

Human lung adenocarcinoma cell line (H1650) was obtained from the German Cancer Research Center (DKFZ, Heidelberg) and cultivated in DMEM (Lonza) supplemented with 10% FCS (Biochrom), 1% penicillin/streptomycin (Biochrom), 1% L-glutamine (Gibco).

Human dermal fibroblasts (HDFa) were obtained from Life Technologies and cultivated in DMEM (Lonza) supplemented with 10% FCS (Biochrom), 1% penicillin/streptomycin (Biochrom), 1% L-glutamine (Gibco).

Human retinal pigment epithelial cell line (hTERT RPE-1) was a kind gift from B. Cerikan (ZMBH, Heidelberg). The cells were cultivated in DMEM F-12 (Gibco) supplemented with 10% FCS (Biochrom), 1% penicillin/streptomycin (Biochrom).

All cell lines were cultivated in a sterile incubator at 37 °C with 95% humidity and 5% CO_2.

Cytotoxicity/Cell viability assay

HeLa cells (5000 cells per well) were seeded in 96-well plates 24 h before treatment. The cells were then incubated with different concentrations of the dyes (in quadruplicates) for 24 hours. The metabolic activity was determined using CellTiter-Glo (Promega); the assay was performed following the manufacturer's instructions. The IC_{50} concentration was defined as the compound concentration required to reduce cell survival to 50%.

Flow Cytometry

Flow cytometry experiments were performed on a C6 cytometer (Accuri). HeLa and hTERT RPE-1 cells were seeded in 24-well plates 24 h prior to the measurement. Solutions of the selected dyes were prepared in 10 μM concentrations in either RPMI-1640 medium without FCS or pooled human normal plasma in sodium citrate (PrecisionBioLogic). After removal of the growth medium, cells were washed with PBS and incubated with the dye solutions for 5 min at 37 °C. Cells were then washed with PBS (Gibco) and detached from the well-plate with trypsin (Biochrom).

In order to block the Polyamine transport system (PTS), HeLa cells were seeded on 24-well plates and treated with 100 μM of benzylviologen (Sigma-Aldrich) for 24 h. The cells were then incubated with 10 μM of dye 5 in RPMI-1640 for 5 min at 37°C, washed with PBS and detached from the well-plate with trypsin.

All flow-cytometry measurements include 10000 events in the gated area that was chosen as the area of healthy cells using a blank sample. Fluorescence excitation was at 640 nm, emission was detected using a 780/60 nm filter. The mean fluorescence intensity of this channel was plotted.

Confocal Microscopy

Cells were seeded in 8-well glass slides (ibidi GmbH) (20.000 cells per well) 24 h before the measurement. For staining, the cells were incubated with 10 μM of the mentioned dyes in RPMI 1640 without FCS for 5-7 minutes at 37 °C. Subsequently, the cells were washed with PBS twice and images were taken on a Leica TCS SP5 X confocal microscope.

Images were recorded at 100 Hz or 10 Hz and a resolution of 512x512 pixels. Excitation: pulsed white-light laser (80 MHz, SuperK Extreme, Koheras) set to 670 nm; intensity 70 %; detection: photomultipliers, 680 - 800 nm.

Fluorescence Microscopy

Cells were seeded in 8-well glass slides (ibidi GmbH) (20.000 cells per well) 24 h before the measurement. Hoechst 33342 (Sigma-Aldrich) staining was done according to manufacturer's instructions. Following nuclei staining, the cells were incubated with 10 μM of the mentioned dyes in RPMI 1640 without FCS for 5-7 minutes at 37 °C.

For the cell mixture, HeLa T-REX stable LAPtag cells were induced by 1 μg/mL Doxycycline for 24 h in DMEM in order to express green fluorescent protein (GFP). The cells were then mixed in a 1:1 ratio with the HDFa cell line in DMEM, and seeded on glass slides (ibidi GmbH). Hoechst 33342 (Sigma-Aldrich) staining was done according to manufacturer's instructions. Following nuclei staining, the cells were incubated with 10 μM of the mentioned dyes in RPMI 1640 without FCS for 5-7 minutes at 37 °C.

Subsequently, the cells were washed with PBS twice and images were taken on an Olympus IX81 ScanˆR automated inverted microscope (Olympus Biosystems) controlled by ScanˆR acquisition software. A 20× 0.4 NA air objective (UPlanSApo; Olympus Biosystems), 150 W Hg/Xe mixed gas arc burner, Chroma filters for DAPI and eGFP, and a Chroma Cy7 filter (49007) were used.

Image Analysis

Confocal microscopy images were extracted using Leica LAS AF Lite software; all other images were extracted with ImageJ.

Statistical Analysis

Two-tailed student's t-test was used for statistical analysis. The required level of significance was defined to be 5 % ($p \leq 0.05$).

Spectra

Analytical Data for Bis-1,1'-(4-sulfobutyl)indotricarbocyanine-5,5'-bis(4-aminobutyl-1-amide) (2)

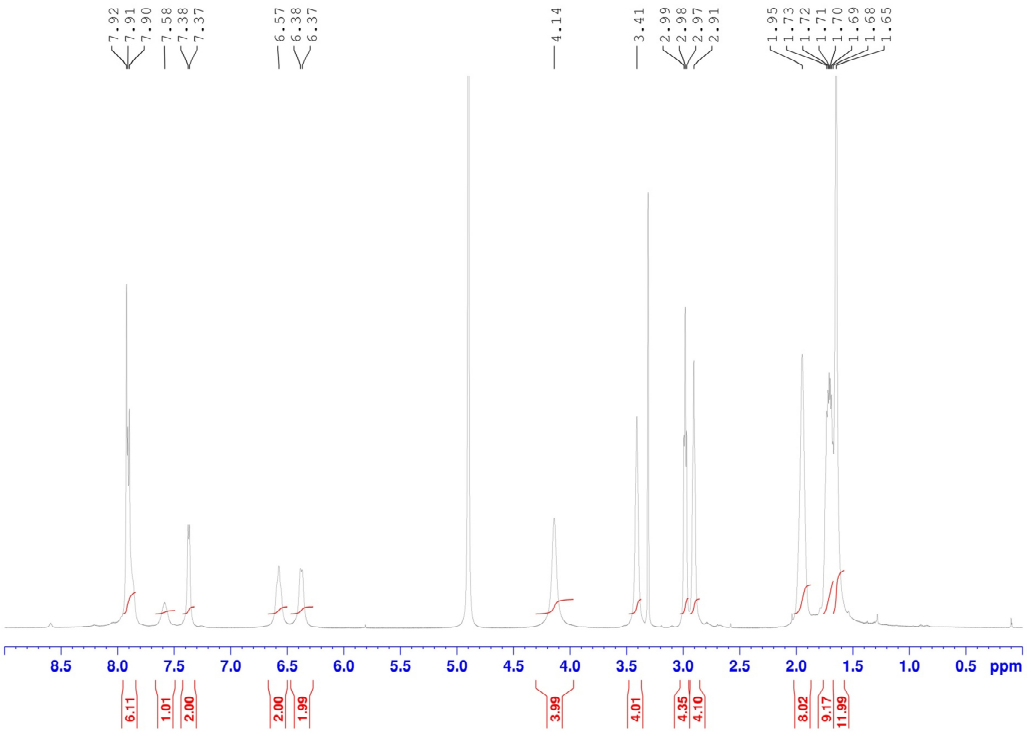


Fig. S5 - ^{1}H NMR of **2**, 600.13 MHz, CD_3OD.

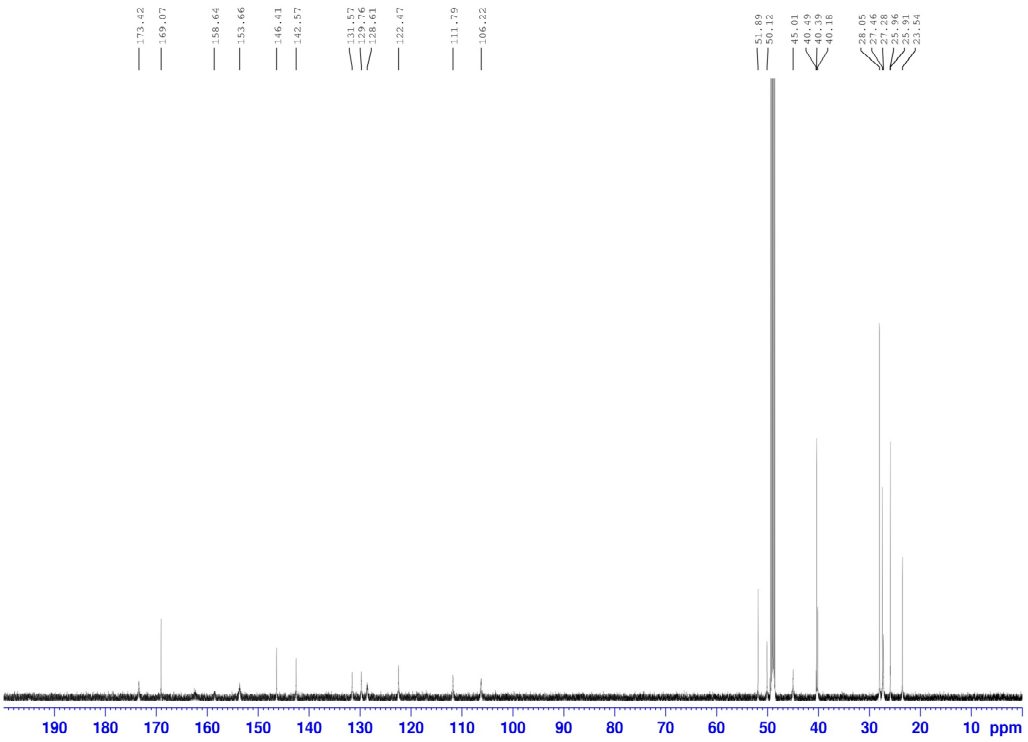


Fig. S6 - ^{13}C {^{1}H} NMR of **2**, 150.92 MHz, CD_3OD.

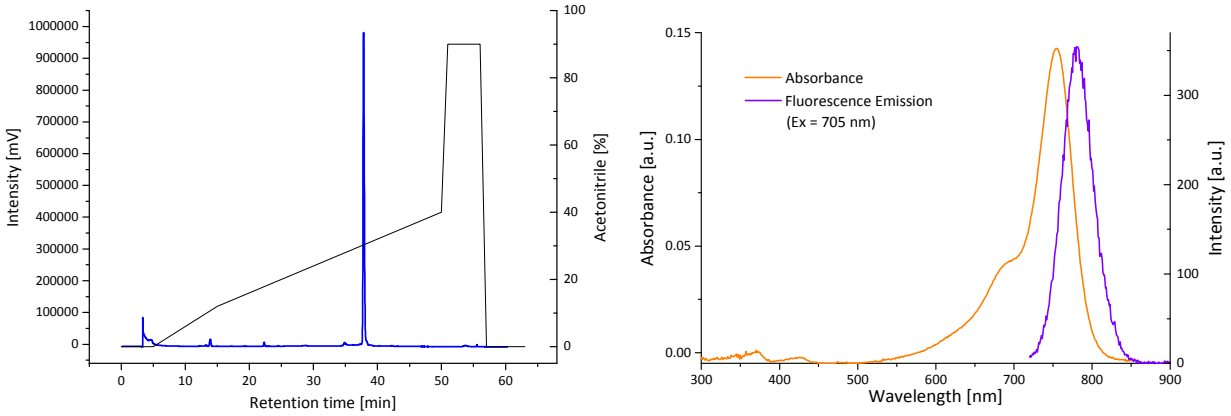


Fig. S7 - **Left:** Analytical HPLC of **2** (Latek ProSep C18 (5 μm, 250 mm x 4 mm), deionized H_2O with 0.1% TFA/MeCN, 1 mL/min, gradient A, t_R = 37.8 min). **Right:** UV/Vis absorbance and fluorescence emission spectra of **2** (0.5 μM solution in PBS; $\lambda_{max,Abs}$ = 755 nm, $\lambda_{max,Em}$ = 781 nm).

<u>Analytical Data for Bis-1,1'-(4-sulfobutyl)indotricarbocyanine-5,5'-bis(8-amino-5-azaoctyl-1-amide) (3)</u>

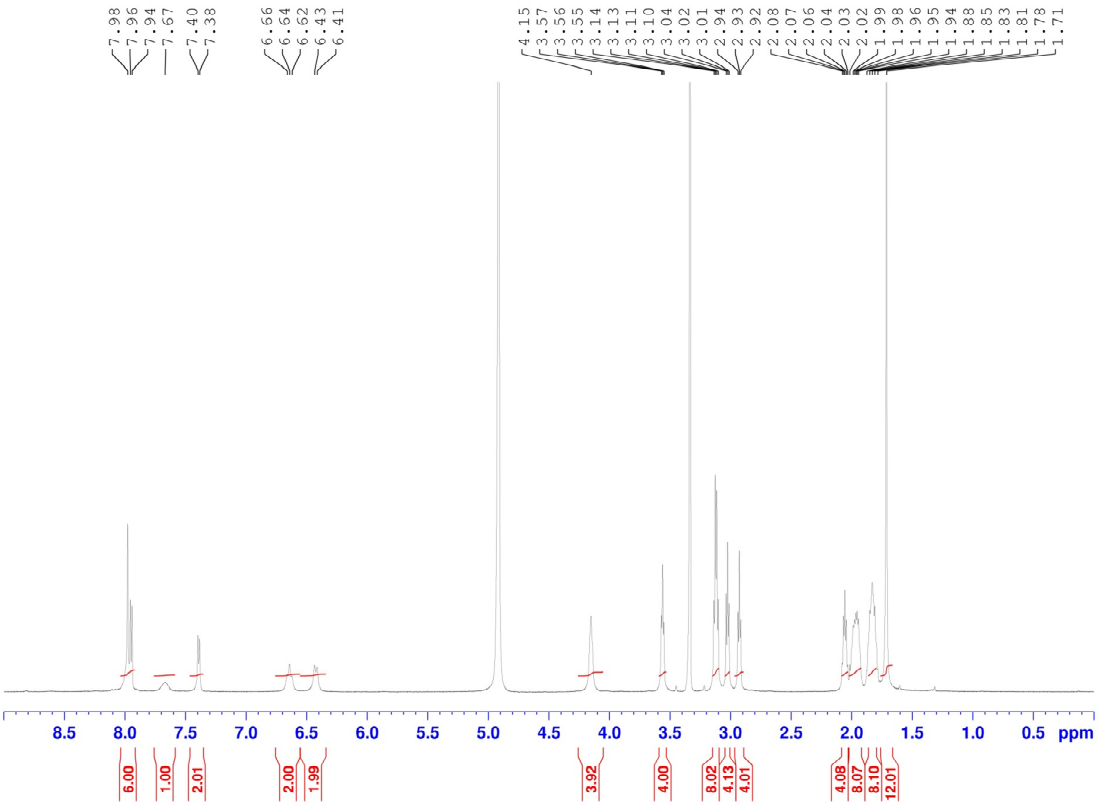


Fig. S8 - ^{1}H NMR of **3**, 600.13 MHz, CD_3OD.

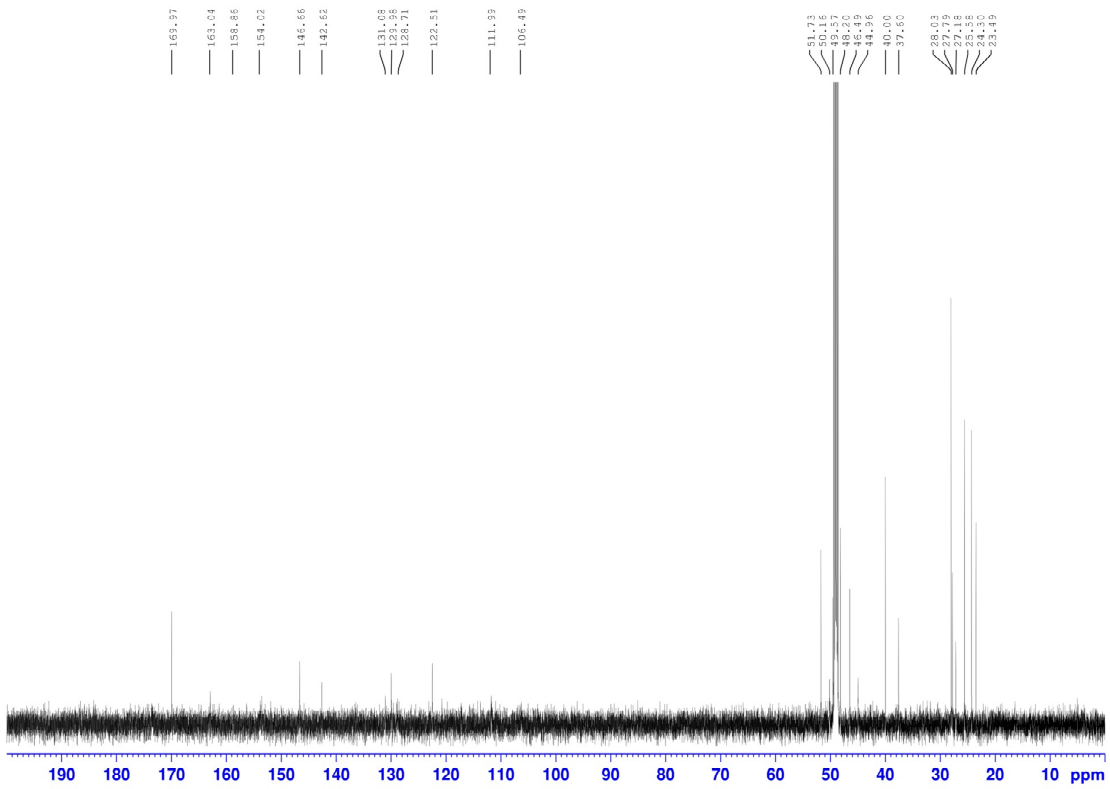


Fig. S9 - ^{13}C {^{1}H} NMR of **3**, 150.92 MHz, CD_3OD.

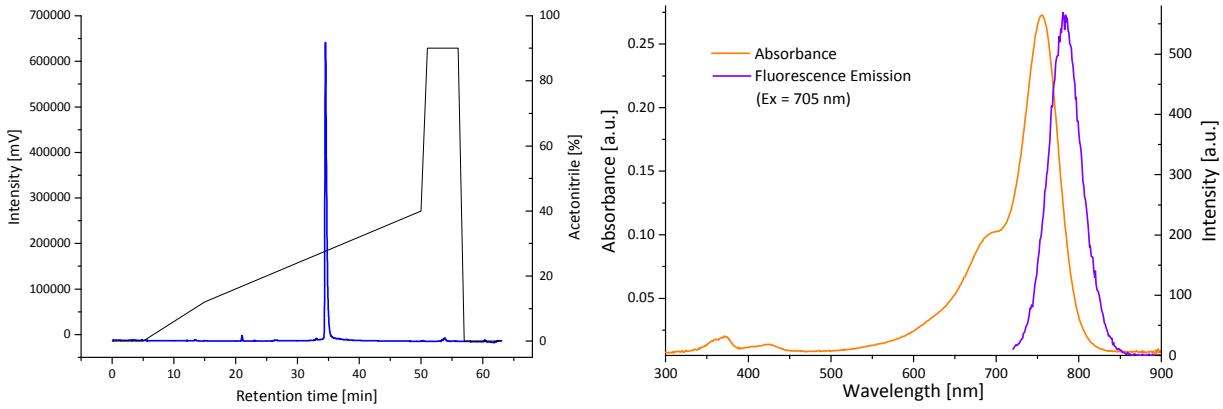


Fig. S10 - **Left:** Analytical HPLC of **3** (Latek ProSep C18 (5 μm, 250 mm x 4 mm), deionized H_2O with 0.1% TFA/MeCN, 1 mL/min, gradient A, t_R = 34.5 min). **Right:** UV/Vis absorbance and fluorescence emission spectra of **3** (1 μM solution in PBS; $\lambda_{max,Abs}$ = 755 nm, $\lambda_{max,Em}$ = 781 nm).

Analytical Data for Bis-1,1'-(4-sulfobutyl)indotricarbocyanine-5,5'-bis(12-amino-4,9-diazadodecyl-1-amide) (4)

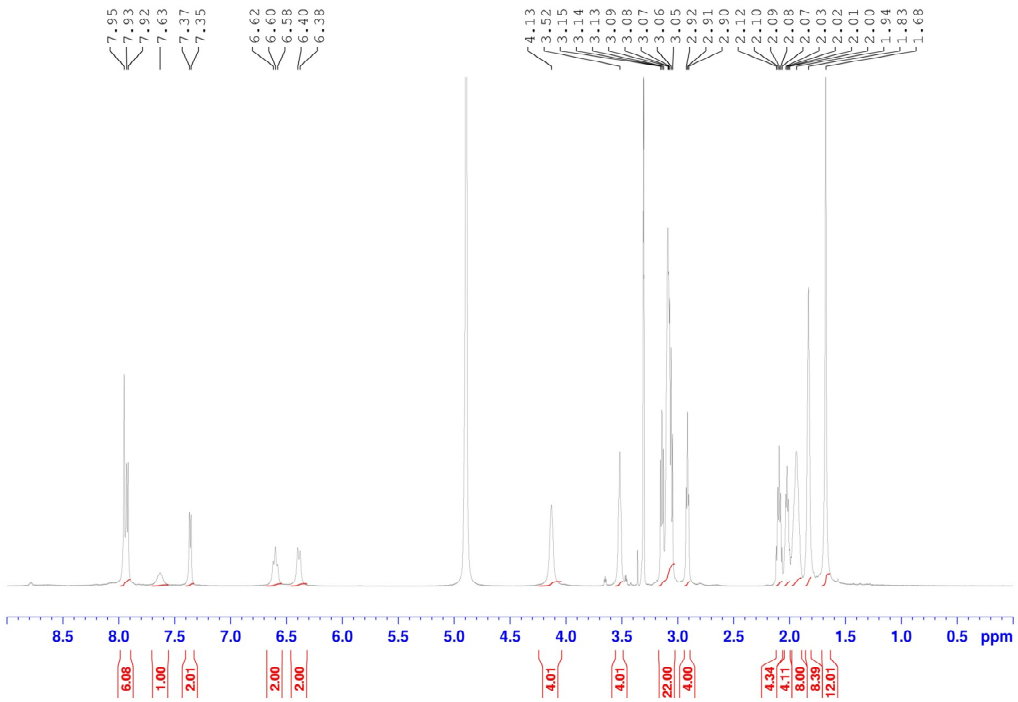


Fig. S11 - ^{1}H NMR of **4**, 600.13 MHz, CD_3OD.

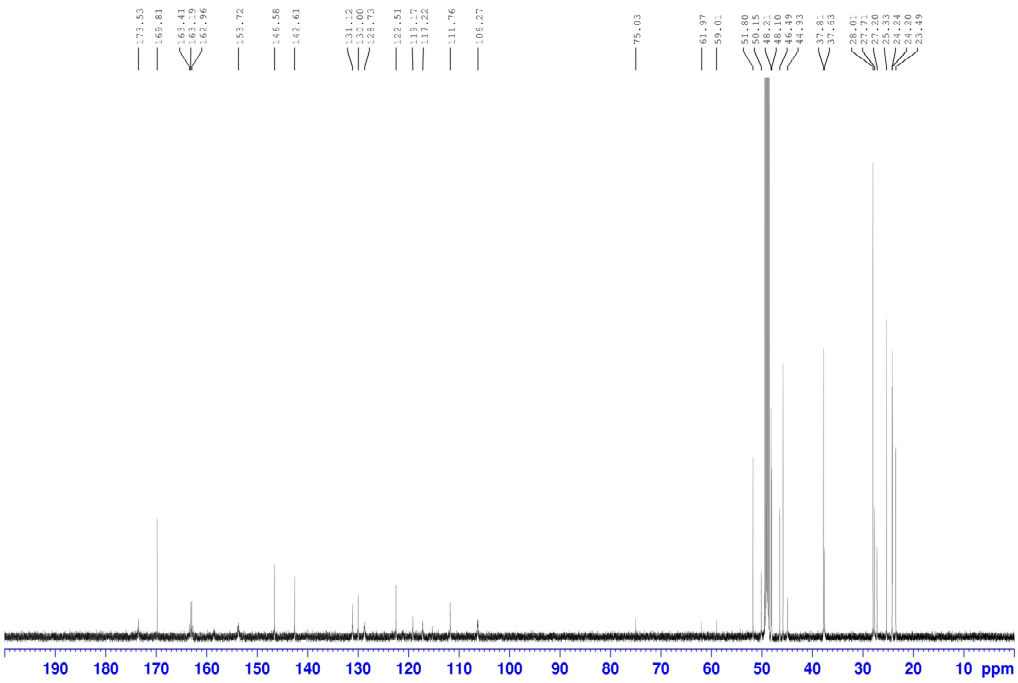


Fig. S12 - ^{13}C NMR of **4**, 150.92 MHz, CD_3OD.

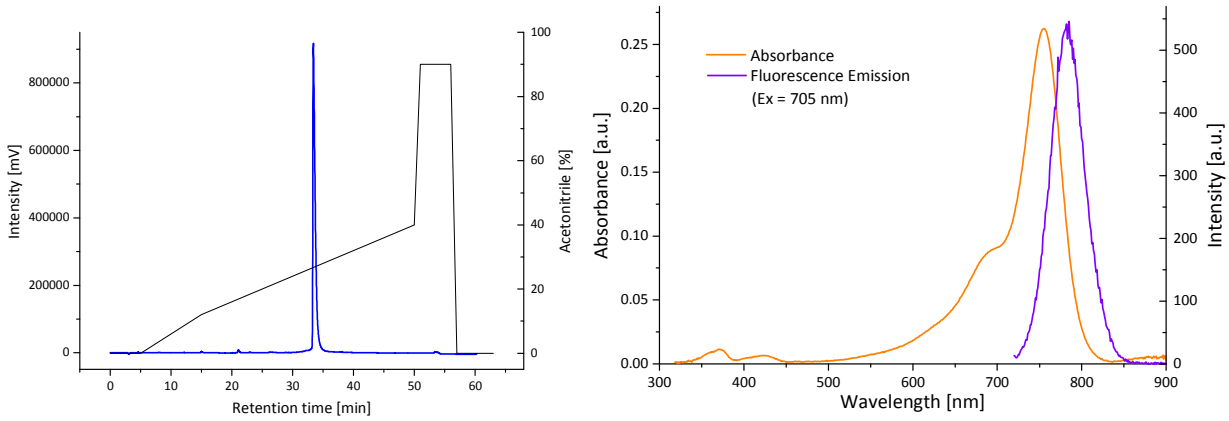


Fig. S13 - **Left:** Analytical HPLC of **4** (Latek ProSep C18 (5 μm, 250 mm x 4 mm), deionized H_2O with 0.1% TFA/MeCN, 1 mL/min, gradient A, t_R = 33.4 min). **Right:** UV/Vis absorbance and fluorescence emission spectra of **4** (1 μM solution in PBS; $\lambda_{max,Abs}$ = 755 nm, $\lambda_{max,Em}$ = 781 nm).

Analytical Data for Bis-1,1'-(4-sulfobutyl)indotricarbocyanine-5,5'-bis(16-amino-4,8,13-triazahexadecyl-1-amide) (5)

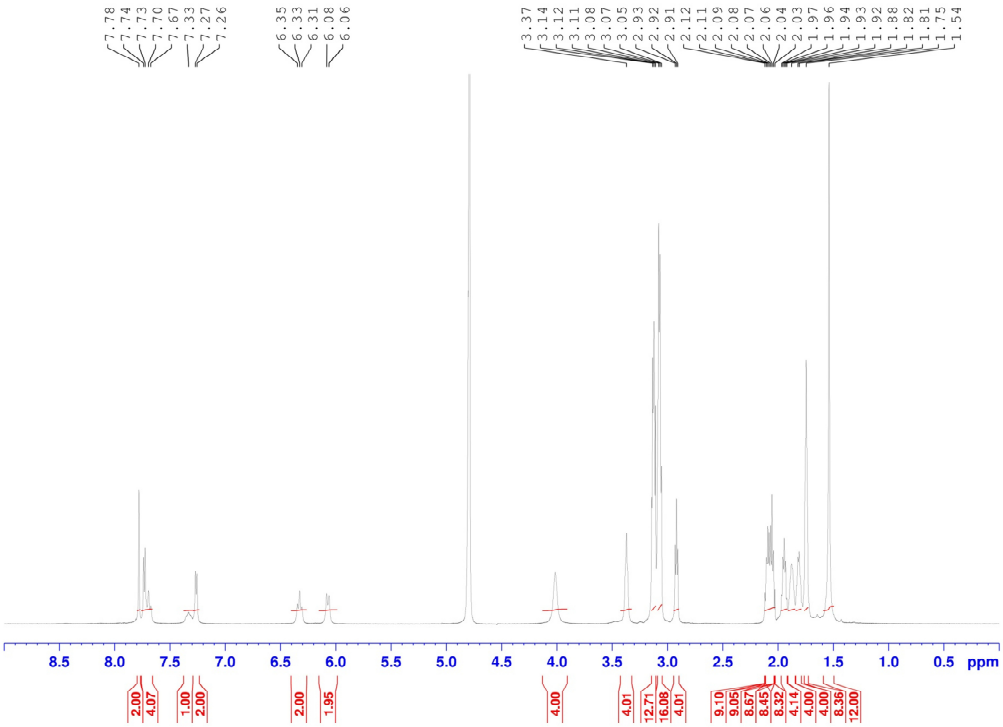


Fig. S14 - ^{1}H NMR of **5**, 600.13 MHz, D_2O.

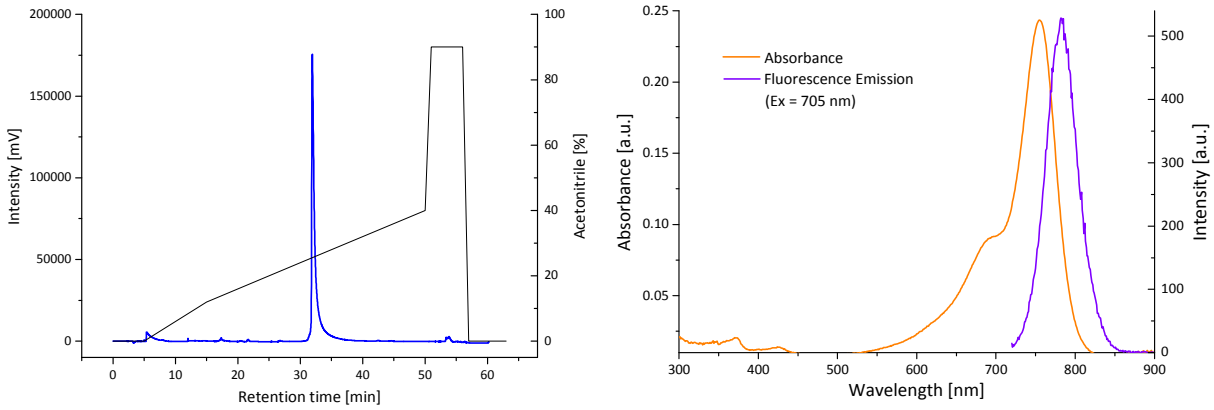


Fig. S15 - **Left:** Analytical HPLC of **5** (Latek ProSep C18 (5 μm, 250 mm x 4 mm), deionized H_2O with 0.1% TFA/MeCN, 1 mL/min, gradient A, t_R = 31.9 min). **Right:** UV/Vis absorbance and fluorescence emission spectra of **5** (1 μM solution in PBS; $\lambda_{max,Abs}$ = 755 nm, $\lambda_{max,Em}$ = 781 nm).

<u>Analytical Data for Bis-1,1'-(4-sulfobutyl)indotricarbocyanine-5,5'-bis(11-diamino-3,6,9-triazaundecane-1-amide) (6)</u>

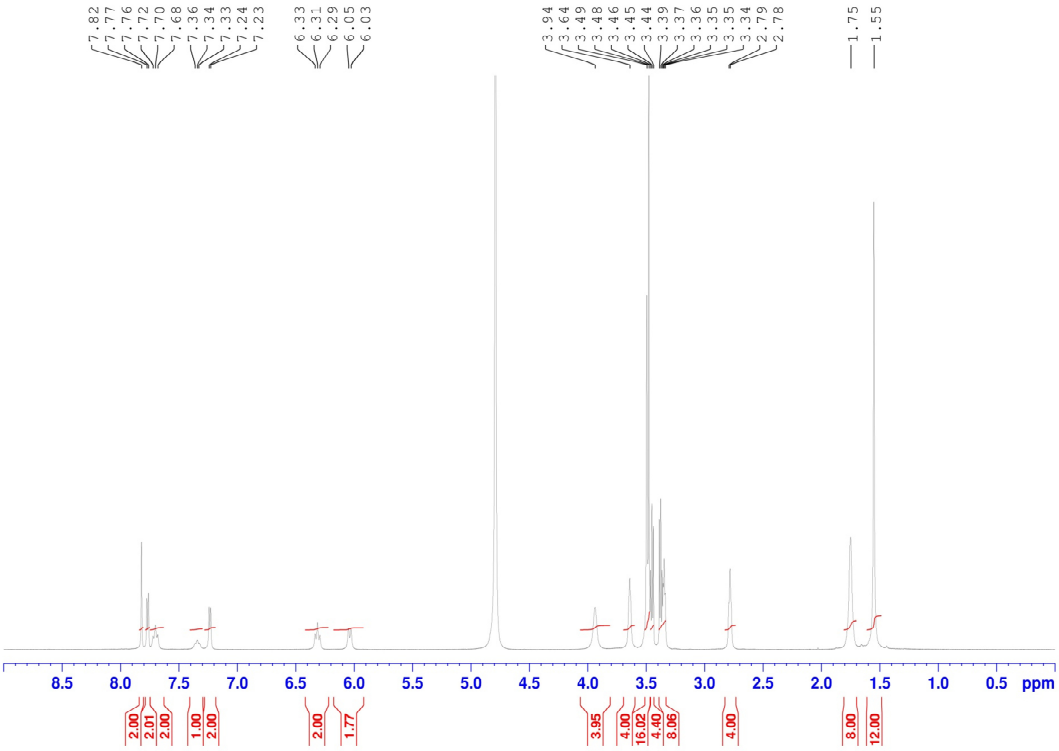


Fig. S16 - ^{1}H NMR of **6**, 600.13 MHz, D_2O.

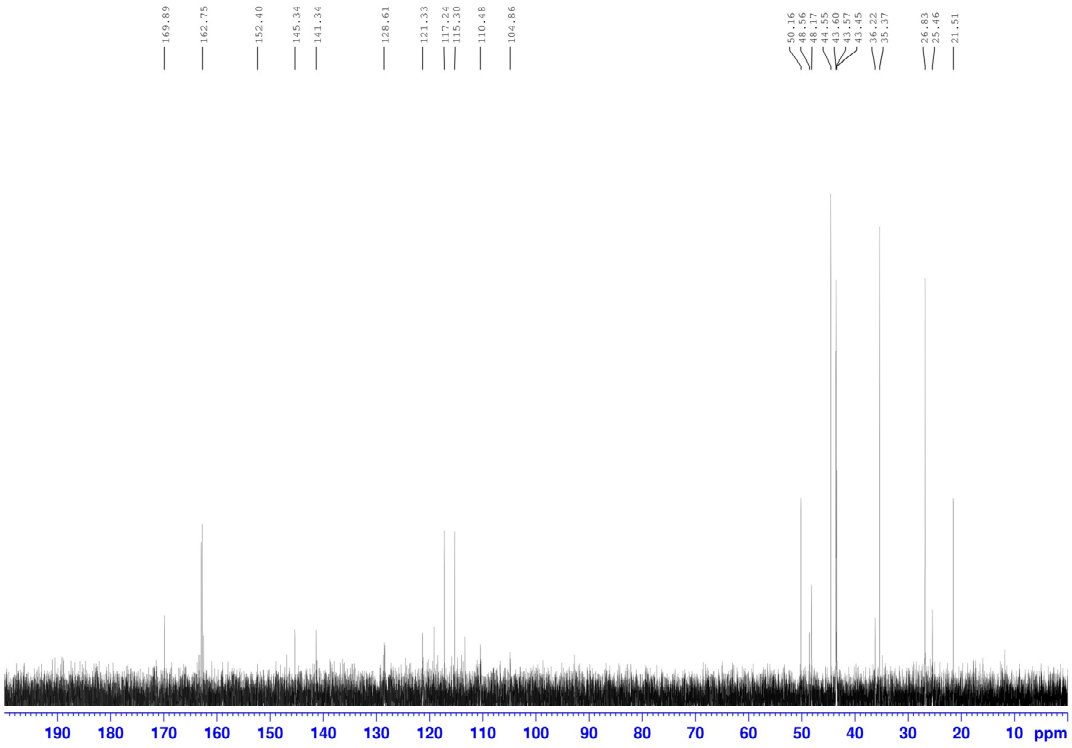


Fig. S17 - ^{13}C {^{1}H} NMR of **6**, 150.92 MHz, D_2O.

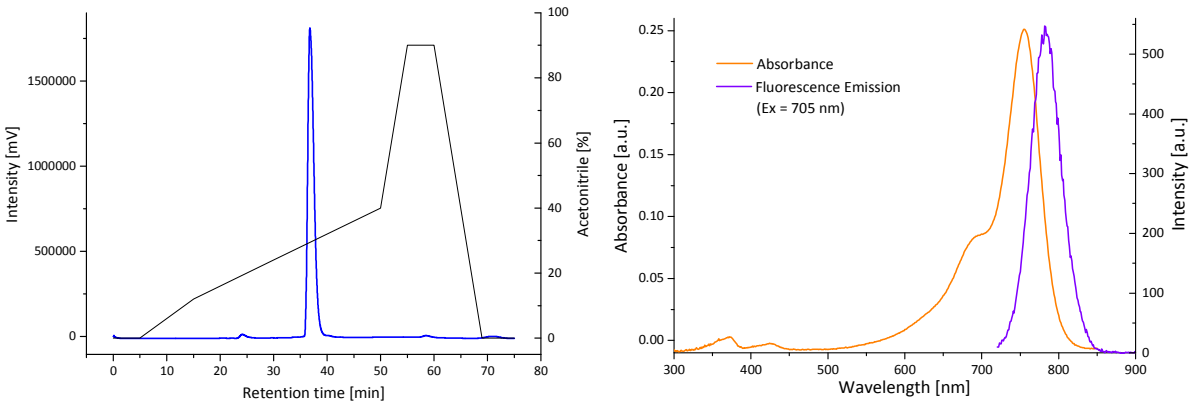


Fig. S18 - **Left:** Analytical HPLC of **6** (Latek ProSep C18 (8 μm, 250 mm x 4 mm), deionized H_2O with 0.1% TFA/MeCN, 1 mL/min, gradient A, t_R = 36.8 min). **Right:** UV/Vis absorbance and fluorescence emission spectra of **6** (1 μM solution in PBS; $\lambda_{max,Abs}$ = 755 nm, $\lambda_{max,Em}$ = 781 nm).

Analytical Data for 3-(5-(16-amino-4,8,13-triazahexadecyl-1-amido)pentyl)-2-((1*E*,3*E*,5*E*,7*Z*)-7-(3-(5-(16-amino-4,8,13-triazahexadecyl-1-amido)pentyl)-1,1-dimethyl-1*H*-benzo[*e*]indol-2(3*H*)-ylidene)hepta-1,3,5-trien-1-yl)-1,1-dimethyl-1*H*-benzo[*e*]indol-3-ium (8)

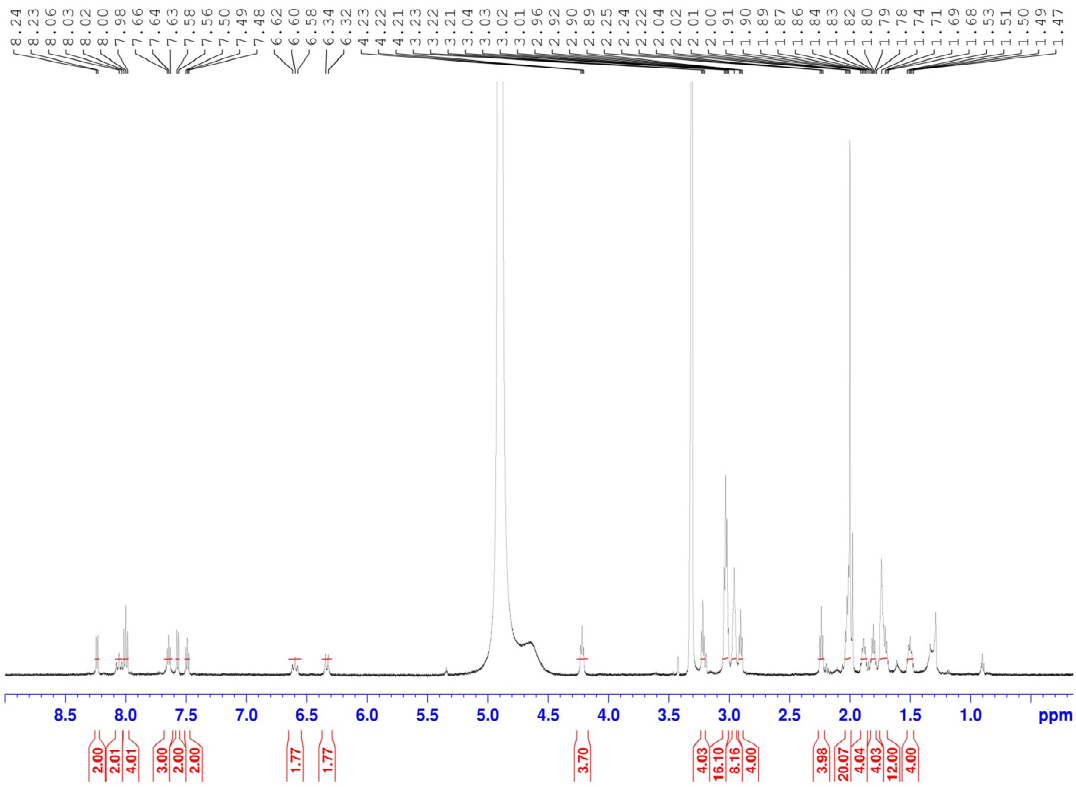


Fig. S19 - ^{1}H NMR of **8**, 600.13 MHz, CD_3OD.

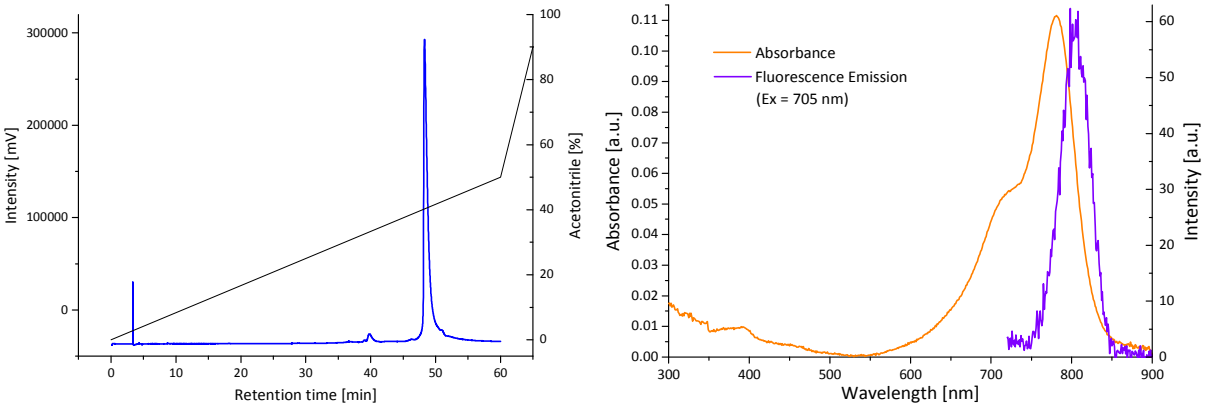


Fig. S20 - **Left:** Analytical HPLC of **8** (Latek ProSep C18 (5 μm, 250 mm x 4 mm), deionized H_2O with 0.1% TFA/MeCN, 1 mL/min, gradient B, t_R = 48.4 min). **Right:** UV/Vis absorbance and fluorescence emission spectra of **8** (1 μM solution in PBS; $\lambda_{max,Abs}$ = 781 nm, $\lambda_{max,Em}$ = 805 nm).

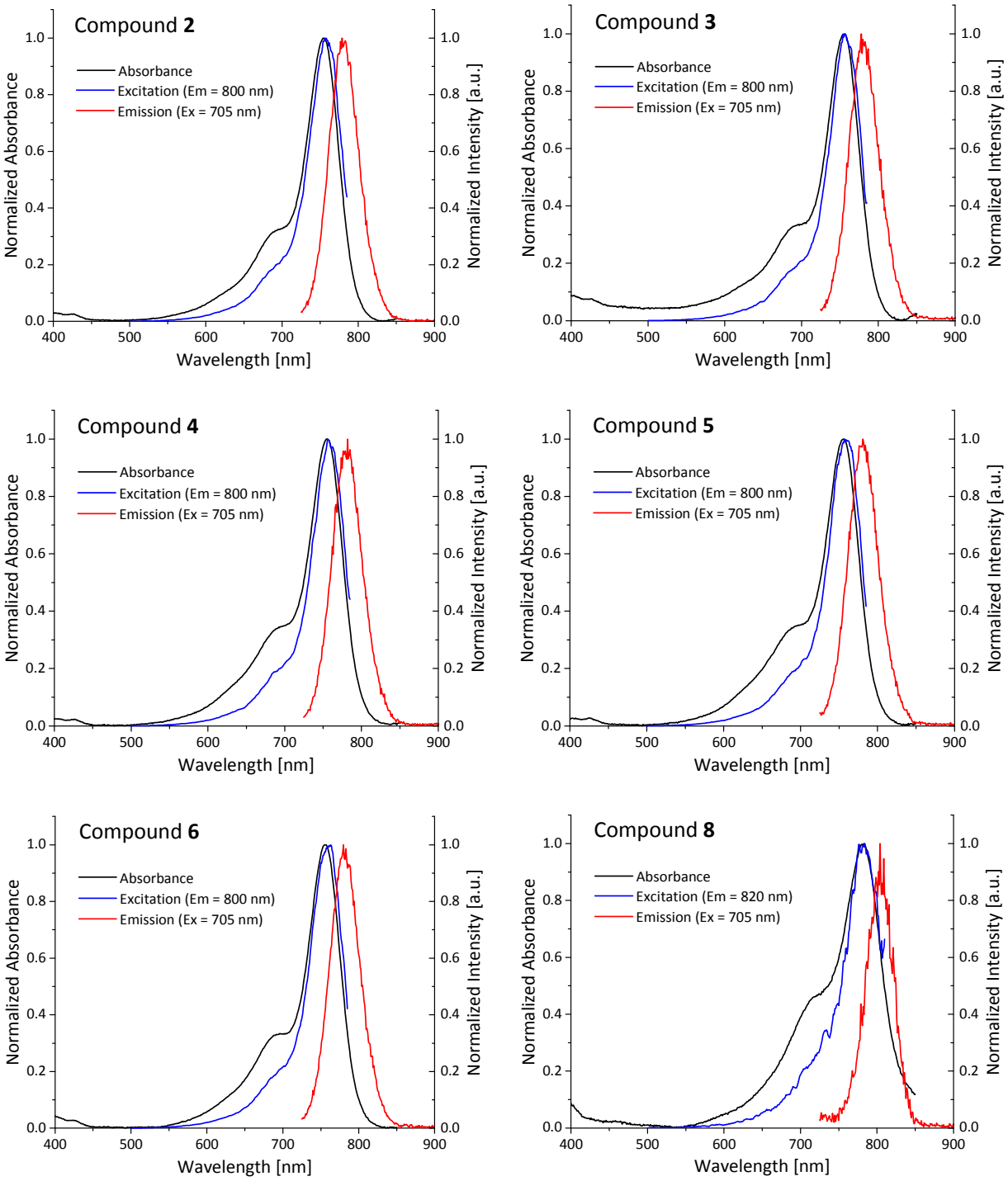


Fig. S21 - Normalized UV/Vis absorbance, fluorescence excitation and fluorescence emission spectra for all polyamine-dye conjugates as 0.5 μM solutions in PBS. All emission spectra were recorded for an excitation wavelength of 705 nm. Compounds **2** - **6**: Excitation spectra were recorded for an emission wavelength of 800 nm; for compound **8** the emission wavelength was set to 820 nm. Fluorescence excitation spectra do not show a perfect match with the respective absorption curves, therefore the presence of a small amount of aggregates, e.g. H-type dimers, cannot be ruled out.

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