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Supporting Information

Masked Rhodamine Dyes of Five Principal Colors Revealed by Photolysis of a 2-Diazo-1-Indanone Caging Group: Synthesis, Photophysics, and Light Microscopy Applications

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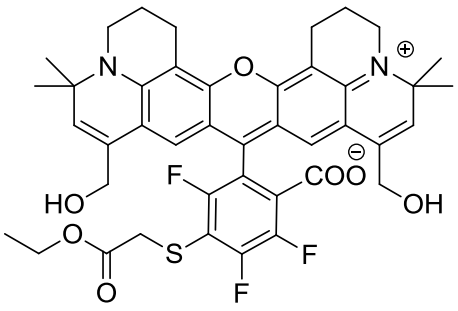
General remarks

UV-visible absorption spectra were recorded on a Varian Cary 4000 UV-Vis spectrophotometer, and fluorescence spectra on a Varian Cary Eclipse fluorescence spectrophotometer. The MICROTOF spectrometer equipped with ESI ion source Apollo and direct injector with LC autosampler Agilent RR 1200 was used for obtaining high resolution mass spectra (ESI-HRMS). ESI-HRMS were obtained also on APEX IV spectrometer (Bruker). HPLC system (Knauer): Smartline pump 1000 (2×), UV detector 2500, column thermostat 4000 (25 °C), mixing chamber, injection valve with 20 and 100 µL loop for the analytical and preparative columns, respectively; 6-port-3-channel switching valve; analytical column: Eurospher-100 C18, 5 µm, 250×4 mm, 1.1 mL/min; solvent A: water + 0.1 % v/v trifluoroacetic acid (TFA); solvent B: CH_3CN + 0.1 % v/v TFA; detection at 254 nm or as specified. Reactions were carried out upon magnetic stirring in Schlenk flasks equipped with septa or reflux condensers with bubble-counters under argon using a standard manifold with vacuum and argon lines. Analytical TLC was performed on ready-to-use plates (Merck KGaA, Darmstadt, Germany) with regular silica gel 60 (F_{254}) and UV and visual detection (unless specified otherwise). Preparative column chromatography performed on regular silica gel with the particle size 40-63 µm, unless otherwise stated.

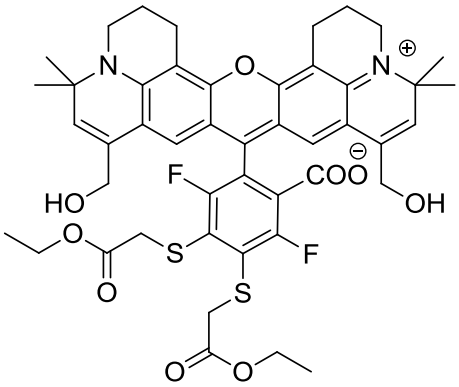
Preparation of mammalian cell samples for confocal and STED microscopy. Staining and sample preparation were carried out according to the standard protocols, described by *C. A. Wurm* and co-workers.[1,2] Primary human dermal fibroblasts, HeLa cells or other cells were seeded on cover slips one day before the experiment. After fixation with formaldehyde (4%, room temp., 5 min) or cold methanol (-20 °C/ 5 min), extraction in 0.5 % Triton X 100 in phosphate buffer with sodium chloride (PBS) and blocking in 5% bovine serum albumin in PBS, the cells were incubated with a mouse monoclonal antibody targeting Nup153 (Abcam, Cambridge, UK), or a mouse monoclonal antibody targeting Tubulin (Sigma-Aldrich). The detection of these primary antibodies was performed using secondary antibodies (Dianova, Hamburg, Germany) custom labelled with the novel dyes described here. They are commercially available as Cage 500, Cage 532, Cage 552, Cage 590 and Cage 635 dyes from Abberior GmbH (Göttingen, Germany) which offers maleimides, *N*-hydroxysuccinimidyl esters, azides and other reactive derivatives of these dyes. Finally, the samples were mounted in Mowiol containing DABCO.

Synthesis

Scheme 2



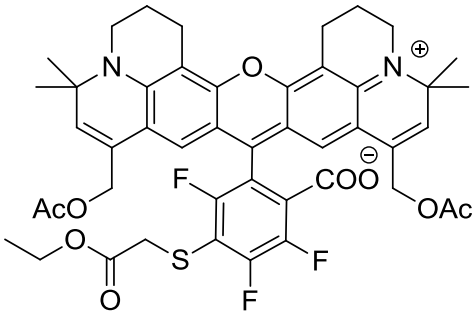
1f-SCH_2CO_2Et,F,H



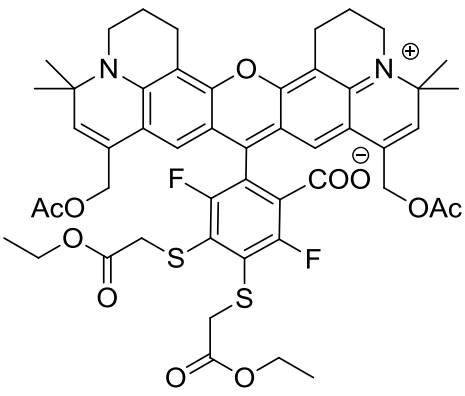
1f-SCH_2CO_2Et,SCH_2CO_2Et,H

Dyes 1f-SCH_2CO_2Et,F,H and 1f-SCH_2CO_2Et,SCH_2CO_2Et,H. Compound **1f**-F,F,H ($C_{38}H_{34}F_4N_2O_5$, M = 647.7, 80 mg, 0.12 mmol) was dissolved in dry DMF (6 mL) in a Schlenk flask (under nitrogen or argon), and the solution was cooled down to -12…-15°C in an ice-salt bath. Then neat ethyl thioglycolate (50 μL, 0.46 mmol) was added with stirring followed by Et_3N (75 μL, 0.54 mmol). The course of the reaction was monitored by HPLC; A/B: 50/50 -100/0 in 25 min, detection at 635 nm; starting material: t_R = 10.1 min, compound **1f**-SCH_2CO_2Et,F,H: t_R = 11.4 min, compound **1f**-SCH_2CO_2Et,SCH_2CO_2Et,H: t_R = 12.7 min. The homogeneous reaction mixture was stirred for several hours at -12…-15°C, until the content of the starting material became less than 5% (HPLC area). Additional portions of ethyl thioglycolate (25 μL, 0.23 mmol) and Et_3N (40 μL, 0.29 mmol) could speed-up the substitution; they may be necessary for completion of the reaction. The reaction was "quenched" by addition of glacial acetic acid (0.4 mL); DMF and excess of AcOH were evaporated in vacuo (ca. 1 mbar) into a trap cooled in an acetone – dry ice bath, an the residue was dissolved in dichloromethane (100 mL). The organic solution was washed with water and brine, dried and evaporated in vacuo. The dark blue mixture of compounds **1f**-SCH_2CO_2Et,F,H and **1f**-SCH_2CO_2Et,SCH_2CO_2Et,H in ratio of ca. 78:22 was used in the next step without further purification. For analyses, the unseparable mixture of these compounds was isolated by column chromatography on SiO_2 ($MeCN/CH_2Cl_2/H_2O$ = 10/1/1). Compound **1f**-SCH_2CO_2Et,F,H ($C_{42}H_{41}F_3N_2O_7S$). ^1H NMR (300 MHz, $CDCl_3$): δ = 1.23 (t, 3 H, $CH_3(CH_2)$, $^3J_{H,H}$ = 7.1 Hz), 1.45/1.48 (2×s, Σ 12 H, CH_3), 2.02 (m_c, 4 H, CH_2), 2.93 (t, $^3J_{H,H}$ = 6.5 Hz, 4 H, CH_2), 3.50 (m_c, 4 H, NCH_2), 3.55 (s, 2 H, SCH_2), 4.14 (q, 2 H, $(CH_3)CH_2$, $^3J_{H,H}$ = 7.1 Hz), 4.23 (A-part of AB-system, 2 H, $^2J_{H,H}$ = 13.0 Hz, OCH_2), 4.34 (B-part of AB-system, 2 H, $^2J_{H,H}$ = 13.0 Hz, OCH_2), 4.45 (br. s, 2 H, OH), 5.68 (s, 2 H, CH=), 7.06 (s, 2 H, $H^{ar.}$) ppm. ^{19}F NMR ($CDCl_3$, 282.4 MHz) δ = -108.8 (d, $J_{F,F}$ = 14.7 Hz), -123.2 (d, $^3J_{F,F}$ = 25.4 Hz),

-139.9 (dd, $^3J_{F,F}$ = 25.1, $J_{F,F}$ = 14.7 Hz) ppm. ESI-MS, positive mode: *m/z* (rel. int., %) = 775 (50) [*M*+H], 797 (100) [*M*+Na]$^+$; HRMS ($C_{42}H_{41}F_3N_2O_7S$+Na): 797.2481 (found *M*+Na), 797.2479 (calc.). Compound **1f**-SCH_2CO_2Et,SCH_2CO_2Et,H ($C_{46}H_{48}F_2N_2O_9S_2$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.20/1.24 (t×2, 3 H, $CH_3(CH_2)$, $^3J_{H,H}$ = 7.2 Hz), 1.45/1.48 (2×s, Σ 12 H, CH_3), 2.02 (m_c, 4 H, CH_2), 2.93 (t, $^3J_{H,H}$ = 6.5 Hz, 4 H, CH_2), 3.50 (m_c, 4 H, NCH_2), 3.61/3.75 (s×2, 2 H, SCH_2), 4.12/4.16 (q×2, 2 H, $(CH_3)CH_2$, $^3J_{H,H}$ = 7.1 Hz), 4.23 (A-part of AB-system, 2 H, $^2J_{H,H}$ = 13.0 Hz, OCH_2), 4.34 (B-part of AB-system, 2 H, $^2J_{H,H}$ = 13.0 Hz, OCH_2), 4.45 (br. s, 2 H, OH), 5.66 (s, 2 H, CH=), 7.11 (s, 2 H, $H^{ar.}$) ppm. ^{19}F NMR ($CDCl_3$, 282.4 MHz) δ = -106.7 (d, $J_{F,F}$ = 16.2 Hz), -107.4 (m) ppm. ESI-MS, positive mode: *m/z* (rel. int., %) = 875 [*M*+H], 897 [*M*+Na]$^+$; HRMS ($C_{42}H_{41}F_3N_2O_7S$+Na): 897.2660 (found *M*+Na), 897.2662 (calc.).



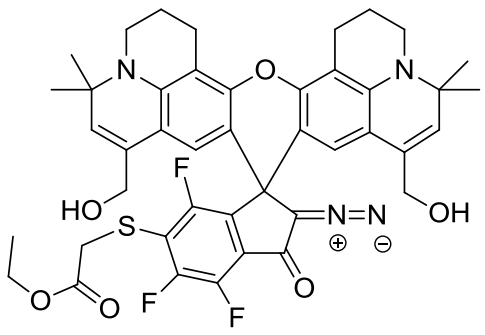
1f-SCH_2CO_2Et,F,Ac



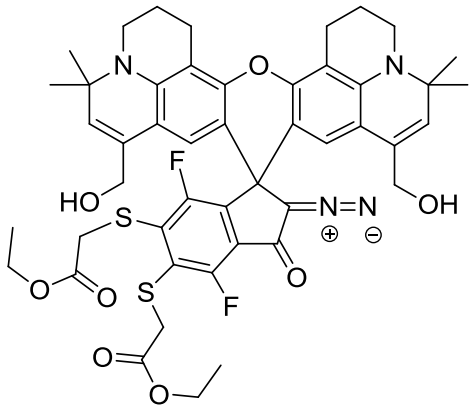
1f-SCH_2CO_2Et,SCH_2CO_2Et,Ac

Acetates 1f-SCH_2CO_2Et,F,Ac and 1f-SCH_2CO_2Et,SCH_2CO_2Et,Ac. The dark blue mixture of compounds **1f**-SCH_2CO_2Et,F,H and **1f**-SCH_2CO_2Et,SCH_2CO_2Et,H in ratio of ca. 78:22 (see above) was dissolved in dry pyridine (2 mL), and acetic anhydride (0.5 mL) was added at 0°C. After stirring overnight at room temperature, the reaction mixture was concentrated in vacuo, the residue was dissolved in dichloromethane (100 mL), washed with 0.1 M aq. HCl (50 mL), saturated aq. $NaHCO_3$ solution (10 mL), dried, and the solvent was evaporated in vacuo. The residue (dark blue oil) was applied on SiO_2 (200 cm^3), and the inseparable mixture of acetates **1f**-SCH_2CO_2Et,F,Ac (t_R = 16.6 min; HPLC conditions are given above) and **1f**-SCH_2CO_2Et,SCH_2CO_2Et,Ac (t_R = 17.6 min) in ratio 76/21 was eluted with a mixture of acetonitrile, water and dichloromethane (10/1/1) as a dark blue band. Yield – 55 mg (55%). Absorption (ε) / emission maxima: 282 nm (33000), 620 nm (56000) / 654 nm (water). Compound **1f**-SCH_2CO_2Et,F,Ac ($C_{46}H_{45}F_3N_2O_9S$). ^{1}H NMR (300 MHz, CD_2Cl_2): δ = 1.23 (t, 3 H, $CH_3(CH_2)$, $^3J_{H,H}$ = 7.1 Hz), 1.47/1.49 (2×s, Σ 12 H, CH_3), 1.98 (s, 6 H, Ac), 2.03 (m_c, 4 H, CH_2), 2.95 (m, 4 H, CH_2), 3.49 (m_c, 4 H, NCH_2), 3.64 (s, 2 H, SCH_2), 4.08 (q, 2 H, $(CH_3)CH_2$, $^3J_{H,H}$ = 7.1 Hz), 4.68 (A-part of AB-system, 2 H, $^2J_{H,H}$ = 12.5 Hz,

OCH_2), 4.87 (B-part of AB-system, 2 H, $^2J_{H,H}$ = 12.6 Hz, OCH_2), 5.65 (s, 2 H, CH=), 6.78 (s, 2 H, $H^{ar.}$) ppm. ^{19}F NMR ($CDCl_3$, 282.4 MHz) δ = -111.7 (m), -125.2 (d, $^3J_{F,F}$ = 25.0 Hz), -142.8 (m) ppm. ESI-MS, positive mode: *m/z* (rel. int., %) = 859 (60) [*M*+H], 881 (100) $[M+Na]^+$; HRMS ($C_{46}H_{45}F_3N_2O_9S$+Na): 881.2702 (found *M*+Na), 881.2690 (calc.). Compound **1f**-SCH_2CO_2Et,SCH_2CO_2Et,Ac ($C_{50}H_{52}F_2N_2O_{11}S_2$). ^1H NMR (300 MHz, CD_2Cl_2): δ = 1.12/1.26 (t×2, 3 H, $CH_3(CH_2)$, $^3J_{H,H}$ = 7.1 Hz), 1.43/1.45 (2×s, Σ 12 H, CH_3), 1.96 (s, 6 H, Ac), 2.02 (m_c, 4 H, CH_2), 2.93 (t, $^3J_{H,H}$ = 6.5 Hz, 4 H, CH_2), 3.44 (m_c, 4 H, NCH_2), 3.68/3.87 (s×2, 2 H, SCH_2), 4.00/4.17 (q×2, 2 H, $(CH_3)CH_2$, $^3J_{H,H}$ = 7.1 Hz), 4.64 (A-part of AB-system, 2 H, $^2J_{H,H}$ = 12.4 Hz, OCH_2), 4.82 (B-part of AB-system, 2 H, $^2J_{H,H}$ = 12.9 Hz, OCH_2), 5.57 (s, 2 H, CH=), 6.64 (s, 2 H, $H^{ar.}$) ppm. ESI-MS, positive mode: *m/z* = 959 [*M*+H], 981 $[M+Na]^+$; HRMS ($C_{50}H_{52}F_2N_2O_{11}S_2$+Na): 981.2885 (found *M*+Na), 981.2873 (calc.).



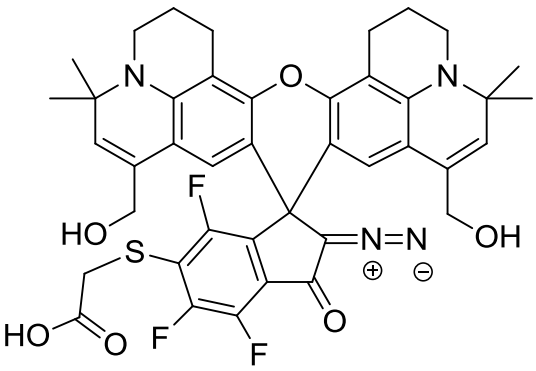
2f-SCH_2CO_2Et,F



2f-SCH_2CO_2Et,SCH_2CO_2Et

Diazoketones 2f-SCH_2CO_2Et,F and 2f-SCH_2CO_2Et,SCH_2CO_2Et. After evaporation of the solvents and drying in vacuo (0.1 mbar), the residue was dissolved under argon in dry CH_2Cl_2 (5 mL), and distilled oxalyl chloride (0.5 mL) was added dropwise at 0°C, followed by 1 drop of dry DMF. The reaction mixture was stirred for 2 h at room temperature, one more drop of DMF was added, and stirring was continued for 1 h more. All volatile materials were evaporated in vacuo into a trap cooled with dry ice – acetone mixture, and the residue was flushed with argon. Then it was dissolved in dry CH_2Cl_2 (8 mL), cooled down to 0°C, and the solution of diazomethane in ether (0.2 M; 5 mL) was added to the dark-blue reaction mixture. Vigorous evolution of nitrogen was observed during addition of the first portions of diazomethane solution. The reaction mixture was stirred overnight at 0°C and 2 h – at room temperature. The color of the solution turned to be dark-green. The solvents (CH_2Cl_2 and ether) were evaporated in vacuo from the reaction flask, and the residue (dark-green oil) was dissolved in CH_2Cl_2 (1-2 mL) and applied onto a column with SiO_2 (100 mL). Compounds **2f**-SCH_2CO_2Et,F (higher R_f) and **2f**-SCH_2CO_2Et,SCH_2CO_2Et (lower R_f) were easily separated by elution with hexane – ethyl

acetate mixture (3/2) and isolated as yellow-green solids after evaporation of the solvents from the pooled homogeneous fractions and triturating of the residues with hexane – ether mixture. **2f**-SCH_2CO_2Et,F ($C_{47}H_{45}F_3N_4O_8S$): 15 mg (34%, t_R = 25.7 min, HPLC conditions are given above). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 0.92 (t, 3 H, $CH_3(CH_2)$, $^3J_{H,H}$ = 7.1 Hz), 1.28/1.31 (2×s, Σ 12 H, CH_3), 1.82 (s, 6 H, Ac), 1.88 (m_c, 4 H, CH_2), 2.78 (m_c, 4 H, CH_2), 3.26 (m_c, 4 H, NCH_2, overlaps with H_2O-peak), 3.75 (s, 2 H, SCH_2), 3.79 (q, 2 H, $(CH_3)CH_2$, $^3J_{H,H}$ = 7.1 Hz), 4.67 (s, 4 H, OCH_2), 5.52 (s, 2 H, CH=), 6.44 (s, 2 H, $H^{ar.}$) ppm. ^{19}F NMR ($CDCl_3$, 282.4 MHz) δ = -116.1 (J = 19.5 Hz), -128.6 (d, $^3J_{F,F}$ = 23.2 Hz), -145.9 (dd, J = 20.3 and 23.5 Hz) ppm. ESI-MS, positive mode: m/z = 905 $[M+Na]^+$; HRMS ($C_{47}H_{45}F_3N_4O_8S$ +Na): 905.2801 (found M+Na), 905.20802 (calc.); **2f**-SCH_2CO_2Et,SCH_2CO_2Et ($C_{51}H_{52}F_2N_4O_{10}S_2$): 5 mg (33%, t_R = 25.8 min, HPLC conditions are given above). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 0.88/1.05 (t×2, 3 H, $CH_3(CH_2)$, $^3J_{H,H}$ = 7.1 and 7.0 Hz), 1.25/1.30 (2×s, Σ 12 H, CH_3), 1.81 (s, 6 H, Ac), 1.87 (m_c, 4 H, CH_2), 2.76 (m_c, 4 H, CH_2), 3.30 (m, 4 H, NCH_2, overlaps with H_2O-peak in DMSO-d_6), 3.68 (q, 2 H, $(CH_3)CH_2$, $^3J_{H,H}$ = 7.1 Hz), 3.74/3.78 (s×2, 4 H, SCH_2), 4.01 (q×2, 2 H, $(CH_3)CH_2$, $^3J_{H,H}$ = 7.1 Hz), 4.63 (s, 4 H, OCH_2), 5.50 (s, 2 H, CH=), 6.41 (s, 2 H, $H^{ar.}$) ppm. ^{19}F NMR ($CDCl_3$, 282.4 MHz) δ = -106.8 (d, $J_{F,F}$ = 20.9 Hz), -111.6 (d, $J_{F,F}$ = 20.9 Hz) ppm. ESI-MS, positive mode: m/z (rel. int., %) = 1005 (100) $[M+Na]^+$; HRMS ($C_{51}H_{52}F_2N_4O_{10}S_2$+Na): 1005.2984 (found M+Na), 1005.2985 (calc.).

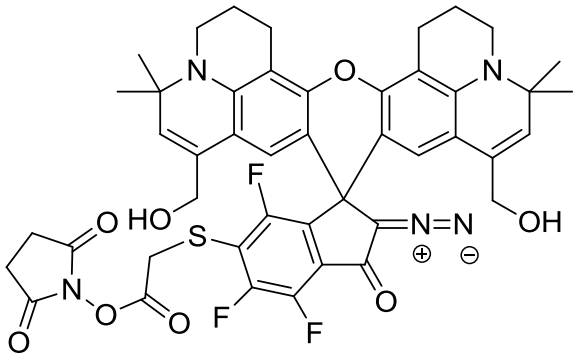


2f-H

Carboxylic acid 2f-H. Ester **2f**-SCH_2CO_2Et,F (12 mg, 0.014 mmol) was dissolved in THF (7 mL), water was added (2 mL), and the mixture was cooled to +7°C in the dark. Then 1 M aq. NaOH (1.4 mL) was added, and the reaction mixture was stirred overnight at +5…+10°C (in the dark, "cold" room). THF was evaporated from the reaction mixture in vacuo, the residue was cooled to 0°C and acidified carefully to pH = 2 – 3 with 5% aq. citric acid. After that, the mixture was extracted with CH_2Cl_2 (2 × 15 mL), and the green organic solutions were dried over Na_2SO_4. The residue obtained after evaporation of the solvent was subjected to chromatography on regular SiO_2 (50 mL) with $CHCl_3$/MeOH/H_2O mixture (75/25/3 – 65/35/5) and afforded the title compound ($C_{41}H_{37}F_3N_4O_6S$, M =770,2) as a yellow solid (9 mg, 85%). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 1.22/1.37 (2×s, Σ 12 H, CH_3), 1.83–1.91 (m, 4 H, CH_2), 2.79 (t, $^3J_{H,H}$ = 6.1 Hz, 4 H, CH_2), 3.22–3.32 (overlapped with H_2O-

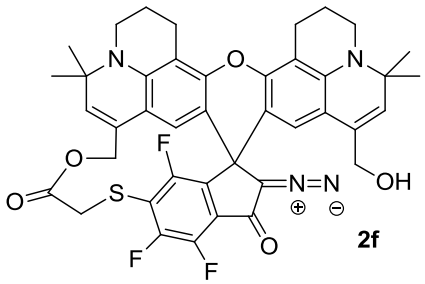
peak in the solvent, m, 4 H, NCH_2), 3.57 (s, 2 H, CH_2), 4.00 (s, 4 H, OCH_2), 5.39 (s, 2 H), 6.53 (s, 2 H) ppm. ESI-MS, positive mode: *m/z* (rel. int., %) = 793 (100) $[M+Na]^+$; HRMS ($C_{41}H_{37}F_3N_4O_6S$): 769.2342 (found *M*–H), 769.2313 (calc.).

*Reactions of carboxylic acid **2f**-H with N-hydroxysuccinimide.*



2f-NHS

***N*-Hydroxysuccinimidyl ester 2f-NHS**. Acid **2f**-H (2.6 mg, 3.4 μmol of light green powder) was dissolved in dry CH_2Cl_2 (1 mL), *N*-hydroxysuccinimide (4 mg, 35 μmol) was added, and to this mixture a solution of *N,N'*-dicyclohexyl carbodiimide (DCC, 80 mg in 1 mL) was added at 0…+5°C in portions of 10 μL (0.8 mg DCC, 3.9 μmol). After addition of each portion and stirring for several hours at 0…+5°C, the reaction mixture was analyzed by HPLC: CH_2Cl_2 was evaporated from an aliquote of 1 – 2 μL, the residue was dissolved in MeCN (+0.1% TFA; solvent A) and injected into a HPLC column; A/B (MeCN/H_2O): 50/50 - 100/0 in 25 min (flow 1.2 mL/min), detection at 254 nm; **2f**-H: t_R = 15.4 min, compound **2f**-NHS: t_R = 16.6 min, compound **2f**: t_R = 20.8 min. Addition of four 10 μL aliquotes of DCC solution (total 15.6 μmol of DCC) at 0°C in 2 days produced 90% conversion to **2f**-NHS and 6% impurity with t_R = 18.9 min (probably, *N*-acyl urea which was formed from **2f**-H and DCC). No macrocyclic lactone **2f** was detected. However, compound **2f**-NHS decomposed in the course of chromatography on regular SiO_2 (in ethyl acetate), though it was stable on TLC and gave a single spot with R_f = 0.5. After isolation by preparative HPLC and liophylization, a blue foam was obtained (much of the fluorescent product), while the content of **2f**-NHS was only 75% (HPLC area). ESI-MS, positive mode: *m/z* (rel. int., %) = 868 (100) $[M+H]^+$; HRMS ($C_{45}H_{40}F_3N_5O_8S$): 868.2601 (found *M*+H), 868.2622 (calc.).

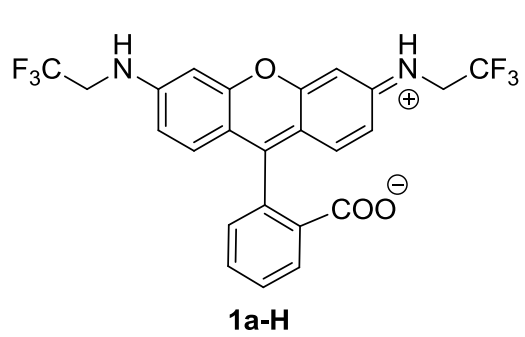


2f

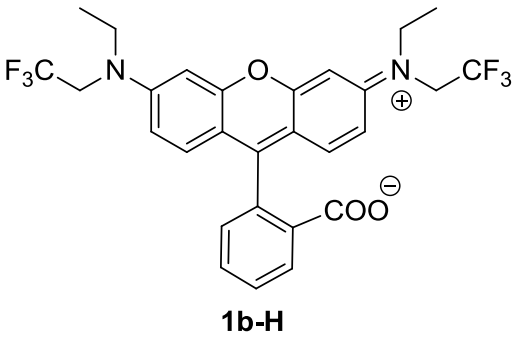
Macrocyclic lactone 2f was obtained as a product of intermolecular cyclization in the course of the reaction of acid **2f**-H with *N*-hydroxysuccinamide and *O*-(7-azabenzotriazol-1-yl)-*N,N,N′,N′*-tetramethyluronium hexafluorophosphate (HATU) in acetonitrile with Et_3N as base. Acid **2f**-H (5.2 mg, 6.8 μmol of green powder) was suspended in dry acetonitrile (5 mL), *N*-hydroxysuccinimide (8 mg, 70 μmol) was added followed by Et_3N (10 μL, 70 μmol), and to this solution HATU (5 mg, 13 μmol) was added at room temperature. The course of the reaction was monitored by HPLC: A/B (MeCN/H_2O,

+0.1%TFA in both solvents): 50/50 -100/0 in 25 min (flow 1.2 mL/min), detection at 254 nm; **2f**-H: t_R = 15.4 min, compound **2f**-NHS: t_R = 16.6 min, compound **2f**: t_R = 20.8 min. Compound **2f**-NHS was found to be a primary product, but after addition of the first portions of the reagents, its content in the reaction mixture was only 10% (HPLC area after 2 h). After that, another portions of HATU (8 mg, 21 μmol), *N*-hydroxysuccinimide (10 mg, 87 μmol), and Et_3N (20 μL, 0.14 mmol) were added. The maximum content of **2f**-NHS was detected to be 20% (with 75% of **2f**-H and 4% of **2f**), but after that its content decreased to 9% (with 75% of **2f**-H and 14% of **2f**). *These results indicate that under basic conditions macro-lactone **2f** is formed from the intermediate N-hydroxysucinimidyl ester **2f**-NHS with higher velocity than this ester – from acid **2f**-H.* The reaction mixture was stirred overnight at room temperature, and HPLC indicated that it consisted from 73% of **2f** and 2% of **2f**-NHS, while 17% of the starting material (**2f**-H) did not react. HATU (10 mg, 26 μmol) and Et_3N (10 μL, 70 μmol) were added, and, after several hours, the conversion to **2f** was found to be 84% (with 9% of **2f**-NHS and 7% of **2f**-H in the reaction mixture). The solvent was evaporated in vacuo, and the residue was applied onto a column with SiO_2 (40 cm^3), equilibrated with hexane – ethyl acetate mixture (1/3). Compound **2f** was eluted as yellow substance which crystallized into a brick-red solid (3.8 mg, 65%). ^{1}H NMR (DMSO-d_6, 600 MHz): δ = 1.13/1.24/1.32/1.43 (4×s, Σ 12 H, CH_3), 1.76–1.95 (m, 2 H, CH_2), 1.99–2.04 (m, 1 H, CH_2), 2.72 (m_c, 1 H, CH_2), 2.80 (t, $^3J_{H,H}$ = 6.4 Hz, 2 H, NCH_2), 2.87–2.94 (m, 1 H, CH_2), 3.18 (m_c, 1 H, CH_2), 3.26 (m, 3 H, CH_2), 3.32–3.38 (m, 1 H, CH_2), 3.84 (d, J = 15.7 Hz, 1 H, SCH_2), 3.98 (d, J = 15.7 Hz, SCH_2), 3.92–4.02 (m, Σ 3 H, OCH_2), 4.18 (d, J = 12.0 Hz, 1 H, OCH_2), 4.67 (t, J = 5.4 Hz, 1 H, OH), 5.16 (d, J = 11.0 Hz, 1 H, OCH_2), 5.41 (s, 1 H), 5.56 (s, 1 H), 5.79 (s, 1 H), 6.48 (s, 1 H) ppm. ^{19}F NMR ($CDCl_3$, 282.4 MHz) δ = -102.9 (d, $J_{F,F}$ = 19.6 Hz), -120.8 (d, $^3J_{F,F}$ = 24.2 Hz), -142.8 (dd, $^3J_{F,F}$ = 24.2, J = 19.6 Hz) ppm. ESI-MS, positive mode: *m/z* (rel. int., %) = 775 (100) $[M+Na]^+$. HRMS ($C_{41}H_{35}F_3N_4O_5S$): 775.2178 (found *M*+Na), 775.2172 (calc.).

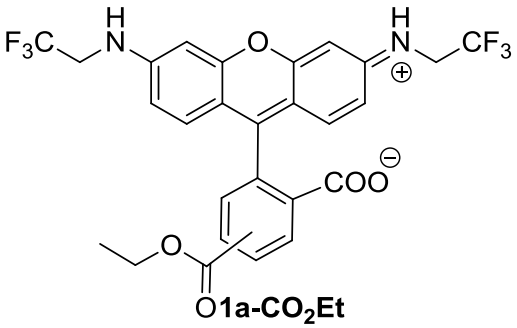
Scheme 3



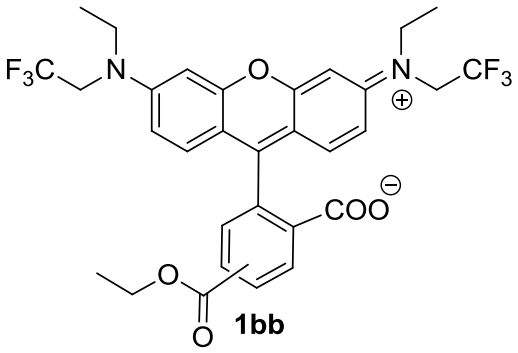
1a-H



1b-H

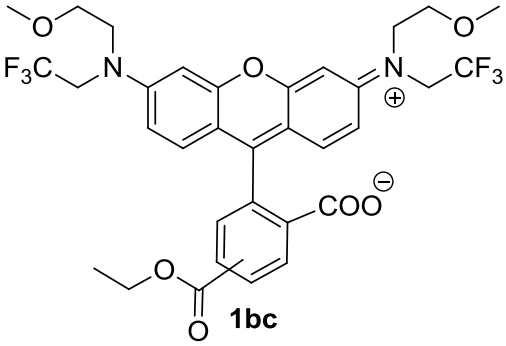


1a-CO_2Et



1bb

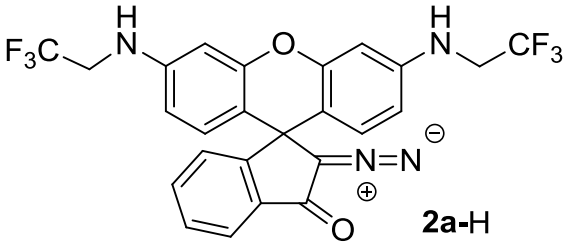
Rhodamine dye **1a**-H was prepared according to the known method.[3] Rhodamine **1b**-H was synthesized as described previously.[4]



1bc

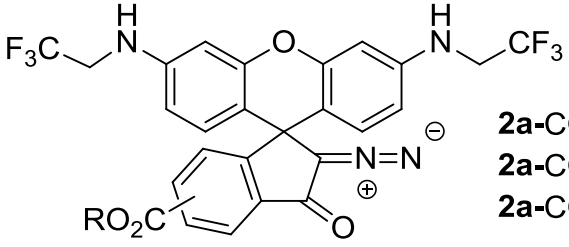
Rhodamines **1a**-CO_2Et, **1bb** and **1bc** were synthesized by heating 4-ethoxycarbonyl phthalic anhydride with 3-[*N*-(2,2,2-trifluoroethyl)amino]phenol (for the preparation of **1a**-CO_2Et), 3-[*N*-ethyl-*N*-(2,2,2-trifluoroethyl)amino]phenol (for the synthesis of **1bb**) and 3-[*N*-(2-methoxyethyl)-*N*-(2,2,2-trifluoroethyl)amino]phenol (for the synthesis of **1bc**) in 1,2-dichlorobenzene, as described.[5] Model compounds **2a**-H and **2b**-H, as well as diazoketones with an ester group (**2a**-CO_2Et, **2bc** and **2d**-CO_2Et) were prepared from the corresponding acid chlorides (obtained, in turn, from rhodamines **1a**-CO_2Et, **1bc**, **7**-H,H,CO_2Et, respectively**)** and diazomethane according to the general method.[5] Saponification of ethyl esters **2a**-CO_2Et, and **2bc** using aq. NaOH gave the corresponding acids **2a**-CO_2H and **2bc**-CO_2H, which were converted into *N*-hydroxysuccinimidyl esters **2a**-CONHS and **2bc**-CONHS according to the standard procedure (TSTU or *N*-hydroxysuccinimide with HATU in MeCN or DMF in the presence of Et_3N at room temperature). The description of the synthesis in some detail is given below. NMR spectra of the single isomers are given for clarity, though in many cases it was not possible to

fully separate the mixtures of 5´- and 6´-carboxy isomers (alkyl esters, free acids and *N*-hydrosuccinimidyl esters).



2a-H

Model diazoketone 2a-H. A dried Schlenk flask was flushed with N_2 and charged with rhodamine **1a**-H (98 mg, 0.19 mmol) and $POCl_3$ (1 mL, 10 mmol). The reaction mixture was stirred at 70–72°C for 2 h. Then $POCl_3$ was distilled off in vacuo (<1 mbar), and the residue was dissolved in dry MeCN (5 mL) under N_2. The solution of CH_2N_2 in Et_2O (5 mL, ~ 0.3 M solution, 1.5 mmol) was added to the reaction mixture at 0 °C with stirring. The reaction mixture was kept at 0 °C for 1.5 h, and then the solvent and excess of CH_2N_2 were removed in vacuo. The distillation flask was cooled with ice water. The title product was isolated by column chromatography on reversed phase [CH_3OH/H_2O (3:1)]; yield - 42 mg (41%) of a pale yellow solid. The prolonged reaction with ethereal solution of CH_2N_2 or the separation on SiO_2 causes the formation of the "dark" product (see Scheme 1 in the main text). HPLC: t_R = 13.0 (area 98.5%), A/B: 50/50 → 0/100 in 25 min, detection at 254 nm. ^{1}H NMR ($CDCl_3$, 300 MHz, ppm): δ = 3.76 (dt, $^3J_{HH}$= 17.4, $^3J_{HF}$= 8.8, 4 H, CH_2CF_3), 4.08 (t, $^3J_{HH}$= 6.9, 2 H, NH), 6.31 (dd, $^3J_{HH}$= 8.6, $^4J_{HH}$= 2.4, 2 H, H-2/H-7), 6.44 (d, $^4J_{HH}$= 2.3, 2 H, H-4/H-5), 6.68 (d, $^3J_{HH}$= 8.6, 2 H, H-1/H-8), 7.09 (d, $^3J_{HH}$= 7.5, 1 H, H-7′), 7.34 – 7.53 (m, 2 H, H-5′ and H-6′), 7.81 (d, $^3J_{HH}$= 7.1, 1 H, H-4′); ^{13}C NMR (DMSO-*d*$_6$, 75.5 MHz, ppm): δ = 43.8 (q, $^2J_{CF}$ = 32, CH_2), 48.5 (C-1′), 74.3 (C=N_2), 98.7 (C-4/5), 109.0 (C-2/7) 110.0 (C-8a/8b), 121.8 (C_6H_4), 125.1 (C_6H_4), 125.7 (q, $^1J_{CF}$= 281, CF_3), 128.1 (C-1/8), 128.7 (C_6H_4), 133.5 (C-2′), 135.1 (C_6H_4), 148.5 (C-3/6), 151.5 (C-4a/4b), 155.8 (C-3′), 185.9 (CO); ESI-MS, positive mode: *m/z* (rel. int., %) = 541 (100) [*M*+Na]$^+$; HRMS ($C_{25}H_{16}F_6N_4O_2$): 541.1061 (found *M*+Na), 541.1070 (calc.).



2a-CO_2Et (R = C_2H_5)
2a-CO_2H (R = H)
2a-CONHS (R = *N*-succinimidyl)

Ethyl ester 2a-CO_2Et. *Rhodamine **1a**-CO_2Et*. A mixture of powdered 4-ethoxycarbonyl phthalic anhydride (trimellitic anhydride ethyl ester, 1.0 g, 4.5 mmol), 3-[*N*-(2,2,2-trifluoroethyl)amino]phenol (700 mg, 3.7 mmol) and 3 mL of 1,2-dichlorobenzene was heated under argon at 180–190°C for 5 min with stirring. Then an additional portion of 3-[*N*-(2,2,2-trifluoroethyl)amino]phenol (700 mg, 3.7 mmol) in 3 mL of 1,2-dichlorobenzene was added to the cooled reaction mixture, and heating was continued at 180–190 °C for 19 h. Then the

solvent was removed in vacuo, and compound **2a**-CO_2Et was isolated (as a mixture of 5- and 6-isomers) from the residue by column chromatography (hexane/EtOAc, 1:2); yield – 680 mg (32%) of bright orange solid. The analytical samples of pure isomers were obtained by additional column chromatography on the SiO_2 (hexane / EtOAc, 1:1). HPLC: t_R = 13.5 min for 5′ isomer; t_R = 13.3 min for 6′-isomer, A/B: 70/30 → 0/100 in 25 min, detection at 532 nm. **1a**-CO_2Et, 5´-isomer: ^{1}H NMR (CD_3CN, 300 MHz, ppm): δ = 1.18 (t, $^3J_{HH}$= 7.1, 3 H, CH_3), 3.83 (qd, $^3J_{HF}$= 9.4, $^3J_{HH}$= 7.1, 4 H, CH_2CF_3), 4.36 (q, $^3J_{HH}$= 7.1, 2 H, OCH_2), 5.23 (t, $^3J_{HH}$= 7.0, 2 H, NH), 6.41 (dd, $^3J_{HH}$= 8.7, $^4J_{HH}$= 2.4, 2 H, H-2/H-7), 6.48 – 6.60 (m, 4 H, H-1/H-8 and H-4/H-5), 7.23 (d, $^3J_{HH}$= 8.0, 1 H, H-7′), 8.22 (dd, $^3J_{HH}$= 8.0, $^4J_{HH}$= 1.5, 1 H, H-6′), 8.43 (dd, $^4J_{HH}$= 1.5, $^5J_{HH}$= 0.7, 1 H, H-4′); ^{13}C NMR (75 MHz, CD_3CN) δ = 169.22 (CO), 165.98 (CO), 157.72, 153.54, 153.52, 150.65, 136.69, 133.47, 129.95, 128.38, 126.6 (q, $^1J_{CF}$ = 293, CF_3), 125.34, 111.15, 108.85, 99.54, 85.13, 62.58 (OCH_2), 45.4 (q, $^2J_{CF}$ = 33.5 Hz, CH_2CF_3), 14.49 (CH_3).

1a-CO_2Et, 6´-isomer: ^{1}H NMR (CD_3CN, 300 MHz, ppm): δ = 1.27 (t, $^3J_{HH}$= 7.1, 3 H, CH_3), 3.87 (qd, $^3J_{HF}$= 9.4, $^3J_{HH}$= 7.1, 4 H, CH_2CF_3), 4.28 (q, $^3J_{HH}$= 7.1, 2 H, OCH_2), 5.24 (t, $^3J_{HH}$= 7.0, 2 H, NH), 6.43 (dd, $^3J_{HH}$= 8.7, $^4J_{HH}$= 2.4, 2 H, H-2/H-7), 6.55 (d, $^3J_{HH}$= 7.7, 2 H, H-1/H-8), 6.57 (d, $^4J_{HH}$= 1.4, 2 H, H-4/H-5), 7.75 (dd, $^4J_{HH}$= 1.4, $^5J_{HH}$= 0.8, 1 H, H-7′), 8.02 (dd, $^3J_{HH}$= 8.0, $^5J_{HH}$= 0.8, 1 H, H-4′), 8.26 (dd, $^3J_{HH}$= 8.0, $^4J_{HH}$= 1.4, 1 H, H-5′); ESI-MS (for isomeric mixture), positive mode: *m/z* (rel. int., %) = 567 (100) $[M+H]^+$, 589 (25) $[M+Na]^+$; HRMS ($C_{27}H_{20}F_6N_2O_5$): 567.1348 (found *M*+H), 567.1349 (calc.).

*Conversion of rhodamine **1a**-CO_2Et* into diazoketone ***2a***-*CO_2Et*. A dried Schlenk flask was flashed with nitrogen and charged with rhodamine ***1a***-*CO_2Et* (5´-isomer, 56 mg, 0.10 mmol) dissolved in CH_2Cl_2 (10 mL). Oxalyl chloride (0.20 mL, 2.3 mmol) was added to the flask, and the reaction solution was stirred at room temperature for 2.5 h. Then one drop of DMF was added, and the solution was stirred for 1 h. The solvent and excess of $(COCl)_2$ were removed in vacuo, the residue was dissolved in dry MeCN (5 mL) under N_2 and cooled with an ice bath. A solution of CH_2N_2 in Et_2O (1.1 mL, 0.3 M solution, 0.33 mmol) was added to the reaction mixture at 0 °C with stirring. The reaction mixture was kept at 0 °C for 1.5 h. Then the solvents and excess of CH_2N_2 were removed in vacuo (*distillation from the flask cooled with an ice bath!*), and the title product was isolated by column chromatography on reversed phase [CH_3OH/H_2O (3:1)]. Yield – 21 mg (36%), HPLC: t_R = 19.3 min (5´-isomer), A/B: 70/30 → 0/100 in 25 min, 254 nm; purity ~ 80% (NMR, HPLC). This material was used in the next (saponification) step without additional purification.

The prolonged reaction with etheral diazomethane or the separation of the reaction mixture on SiO_2 caused the formation of the "dark" by-product (see Scheme 1 in the main text).

2a-CO_2Et, 5′-isomer: ^{1}H NMR ($CDCl_3$, 300 MHz, ppm): δ = 1.37 (t, $^3J_{HH}$= 7.1, 3 H, CH_3), 3.75 (qd, $^3J_{HF}$= 9.4, $^3J_{HH}$= 7.1, 4 H, CH_2CF_3), 4.15 (t, $^3J_{HH}$= 7.0, 2 H, NH), 4.36 (q, $^3J_{HH}$= 7.1, 2 H, OCH_2), 6.31 (dd, $^3J_{HH}$= 8.6, $^4J_{HH}$= 2.5, 2 H, H-2/H-7), 6.45 (d, $^4J_{HH}$= 1.4, 2 H, H-4/H-5), 6.67 (d, $^3J_{HH}$= 7.7, 2 H, H-1/H-8), 7.09 (d, $^3J_{HH}$= 8.0, 1 H, H-7′), 8.12 (dd, $^3J_{HH}$= 8.0, $^4J_{HH}$= 1.5, 1 H, H-6′), 8.43 (d, $^4J_{HH}$= 1.6, 1 H, H-4′); **2a**-CO_2Et, 6′-isomer: ^{1}H NMR (CD_3OD, 300 MHz, ppm): δ = 1.22 (t, $^3J_{HH}$= 7.1, 3 H, CH_3), 3.82 (q, $^3J_{HF}$= 9.4, 4 H, CH_2CF_3), 4.10 (q, $^3J_{HH}$= 7.1, 2 H, OCH_2), 4.80 – 4.90 (m, 2 H, NH), 6.42 (dd, $^3J_{HH}$= 8.6, $^4J_{HH}$= 2.5, 2 H, H-2/H-7), 6.54 (d, $^4J_{HH}$= 1.4, 2 H, H-4/H-5), 6.63 (d, $^3J_{HH}$= 7.7, 2 H, H-1/H-8), 7.62 (d, $^4J_{HH}$= 1.6, 1 H, H-7′), 7.83 (d, $^3J_{HH}$= 8.0, 1 H, H-4′), 8.43 (dd, $^3J_{HH}$= 8.0, $^4J_{HH}$= 1.5, 1 H, H-5′); ESI-MS (**2a**-CO_2Et, mixture of 5′- and 6′-isomers), positive mode: *m/z* (rel. int., %) = 613 (100) $[M+Na]^+$; negative mode: *m/z* (rel. int., %) = 589 (100) $[M–H]^–$; HRMS ($C_{28}H_{20}F_6N_4O_4$): 589.1314 (found *M*–H), 589.1316 (calc.).

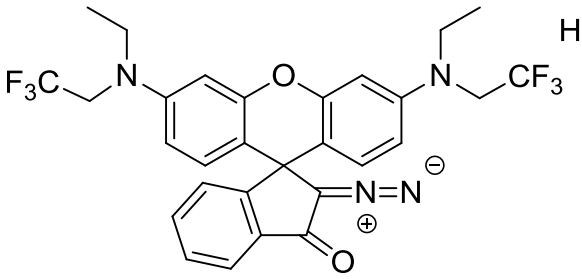
Carboxylic acid 2a-CO_2H. Ester **2a**-CO_2Et (6.5 mg, 11 μmol) was dissolved in EtOH (2 mL), then 1 M aq. NaOH (0.3 mL) was added, and the reaction mixture was kept at room temperature for 14 h. The solvents were removed in vacuo, and the residue was shaken with a mixture of Et_2O (20 mL) and H_2O (10 mL). The aqueous layer was separated, acidified with 1 M aq. $KHSO_4$ to pH=2÷3 and extracted with ether (3×50 mL). The combined organic solutions were washed with brine (50 mL) and dried over Na_2SO_4. The solvent was evaporated in vacuo, and the crude acid was used for further transformations without addition purification. Yield – 5.5 mg (~ 90%). HPLC: t_R = 15.2 min, A/B: 70/30 → 0/100 in 25 min, 254 nm. 6′-carboxy isomer: ^{1}H NMR (CD_3OD, 300 MHz, ppm): δ = 3.82 (q, $^3J_{HF}$= 9.4, 4 H, CH_2CF_3), 4.85–4.96 (br. s, 2 H, NH), 6.42 (dd, $^3J_{HH}$= 8.6, $^4J_{HH}$= 2.5, 2 H, H-2/H-7), 6.54 (d, $^3J_{HH}$= 1.4, 2 H, H-4/H-5), 6.63 (d, $^4J_{HH}$= 7.7, 2 H, H-1/H-8), 7.62 (d, $^4J_{HH}$= 1.6, 1 H, H-7′), 7.86 (d, $^3J_{HH}$= 8.0, 1 H, H-4′), 8.11 (dd, $^3J_{HH}$= 8.0, $^4J_{HH}$= 1.5, 1 H, H-5′); ^{13}C NMR (DMSO-d_6, 126 MHz, ppm): δ = 43.8 (q, $^2J_{CF}$= 32, NCH_2CF_3), 48.6 (C-1′), 75.1 (C=N_2), 98.7 (CH), 108.3(C), 110.0 (CH), 122.2 (CH), 125.5 (q, $^1J_{CF}$= 281, CF_3), 125.3 (CH), 127.9 (CH), 129.4 (CH), 136.5 (C), 136.7 (C), 148.5 (C), 151.3 (C), 155.7 (C), 165.9 (CO_2), 184.7 (CO); ESI-MS (isomeric mixture), positive mode: *m/z* (rel. int., %) = 585 (100) $[M+Na]^+$; negative mode: *m/z* (rel. int., %) = 561 (100) $[M–H]^–$; HRMS ($C_{26}H_{16}F_6N_4O_4$): 561.1004 (found *M*–H), 561.1003 (calc.).

***N*-succinimidyl ester 2a-CONHS.** A dried Schlenk flask was charged with the acid **2a**-CO_2H (5 mg, 1.0 μmol) and TSTU (11 mg, 40 μmol), and dry MeCN (2 mL) was added under N_2. A

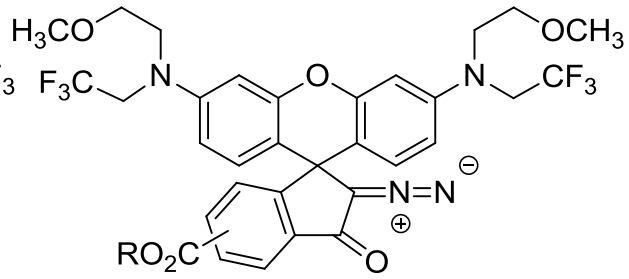
solution of NEt_3 in dry MeCN (0.1 mL of 0.7 M solution, 70 µmol) was added to the reaction mixture at room temperature with stirring. The mixture was stirred at room temperature for 1 h, and then the solvent and excess of NEt_3 were removed in vacuo. The title compound (3.5 mg, 60 %) was isolated by column chromatography on SiO_2 (CH_2Cl_2/MeCN: 8/1). HPLC: t_R = 16.9 min, A/B: 70/30 → 0/100 in 25 min, 254 nm. 5´-isomer: ^{1}H NMR (CD_3OD, 300 MHz, ppm): δ =2.89 (s, 4 H, CH_2), 3.69 – 3.84 (m, 4 H, CH_2CF_3), 4.13 (t, *J* = 7.1 Hz, 2 H, NH), 6.33 (dd, *J* = 8.6, 2.5 Hz, 2 H, H-2/H-7), 6.45 (d, *J* = 2.4 Hz, 2 H, H-4/H-5), 6.66 (d, *J* = 8.6 Hz, 2 H, H-1/H-8), 7.18 (d, *J* = 8.1 Hz, 1 H, H-7′), 8.19 (dd, *J* = 8.1, 1.7 Hz, 1 H, H-6′), 8.59 (d, *J* = 1.6 Hz, 1 H, H-4′). ESI-MS, positive mode: *m/z* (rel. int., %) = 682 (100) $[M+Na]^+$; negative mode: *m/z* (rel. int., %) = 658 (100) $[M–H]^–$; HRMS ($C_{30}H_{19}F_6N_5O_6$): 658.1167 (found *M*–H), 658.1167 (calc.).

*Rhodamine **1bb***. The mixture of 5´- and 6´-ethoxycarbonyl isomers **1bb** was obtained from 3-[*N*-ethyl-*N*-(2,2,2-trifluoroethyl)amino]phenol (552 mg, 2.53 mmol) and 4-ethoxycarbonyl phthalic anhydride (300 mg, 1.4 mmol) as described above for *rhodamine **1a**-CO_2Et*. The dye was isolated by column chromatography on SiO_2 (130 g) with CH_2Cl_2/MeOH (50:1) mixture as an eluent. Yield – 515 mg (65%) of bright orange solid. Two diastereomers could be (partially) separated. HPLC: t_R = 16.4 min (both diastereomers), B/A: 30/70 → 100/0 in 25 min, 254 nm. 5´-isomer: ^{1}H NMR ($CDCl_3$, 300 MHz, ppm): δ = 1.20 (t, $^3J_{HH}$= 8.1, 6 H, CH_3), 1.43 (t, $^3J_{HH}$= 8.1, 2 H, CH_3)**,** 3.49 (q, $^3J_{HH}$= 7.0, 4 H, CH_2N), 3.86 (q, $^3J_{HF}$= 8.5, 4 H, CH_2CF_3), 4.43 (q, $^3J_{HH}$= 7.1, 2 H, OCH_2), 6.42 (dd, $^3J_{HH}$= 8.9, $^4J_{HH}$= 2.7, 2 H, H-2/H-7), 6.57–6.63 (m, 4 H, H-4/H-5 and H-1/H-8), 7.25 (d, $^3J_{HH}$= 8.0, 1 H, H-7′), 8.30 (dd, $^3J_{HH}$= 8.0, $^4J_{HH}$= 1.4, 1 H, H-6′), 8.63 (s, 1 H, H-4′); ^{13}C NMR ($CDCl_3$, 126 MHz, ppm): δ = 11.4 (CH_3), 14.4 (CH_3), 29.7 (C-1′), 45.9 (NCH_2), 52.0 (q, $^2J_{CF}$ = 33, NCH_2CF_3), 61.7 (OCH_2), 99.5 (CH), 107.7(C), 109.1 (CH), 124.3 (CH), 125.1 (q, $^1J_{CF}$ = 282, CF_3), 126.4 (CH), 127.6 (C), 128.9 (CH), 132.3 (C), 135.6 (CH), 149.3 (C), 152.7 (C), 156.3 (C), 165.0 (CO_2), 168.4 (CO_2); 6´-isomer: ^{1}H NMR ($CDCl_3$, 300 MHz, ppm): δ = 1.20 (t, $^3J_{HH}$= 8.1, 6 H, CH_3), 1.32 (t, $^3J_{HH}$= 8.1, 2 H, CH_3), 3.50 (q, $^3J_{HH}$= 7.0, 4 H, CH_2N), 3.85 (q, $^3J_{HF}$= 8.5, 4 H, CH_2CF_3), 4.32 (q, $^3J_{HH}$= 7.1, 2 H, OCH_2), 6.43 (dd, $^3J_{HH}$= 8.9, $^4J_{HH}$= 2.7, 2 H, H-2/H-7), 6.53–6.63 (m, 4 H, H-4/H-5 and H-1/H-8), 7.82 (s, 1 H, H-7′), 8.04 (d, $^3J_{HH}$= 8.0, 1 H, H-4′), 8.26 (dd, $^3J_{HH}$= 8.0, $^4J_{HH}$= 1.4, 1 H, H-5′); ^{13}C NMR ($CDCl_3$, 126 MHz, ppm): δ = 11.4 (CH_3), 14.3 (CH_3), 29.8 (C-1′), 46.0 (NCH_2), 52.1 (q, $^2J_{CF}$= 33, NCH_2CF_3), 61.8 (OCH_2), 99.5 (CH), 108.1(C), 109.3 (CH), 125.1 (q, $^1J_{CF}$= 282, CF_3), 125.2 (CH), 125.5 (CH), 128.5 (C), 129.1 (CH), 130.7 (CH), 130.9 (C), 136.3 (C), 149.5 (C), 153.0 (C), 165.1 (CO_2), 168.4 (CO_2); ESI-MS (mixture

of diastereomers), positive mode: *m/z* (rel. int., %) = 623 (25) $[M+H]^+$, 645 (100) $[M+Na]^+$; HRMS ($C_{31}H_{28}F_6N_2O_5$): 645.1788 (found *M*+Na), 645.1795 (calc.).



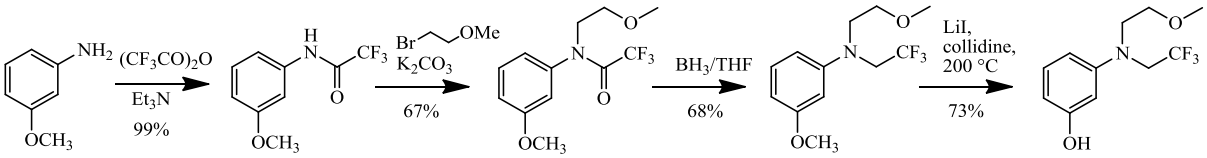
2b-H



2bc (R = C_2H_5)
2bc-CO_2H (R = H)
2bc-CONHS (R = *N*-succinimidyl)

The model caged dye 2b-H was obtained from 100 mg (0.18 mmol) of *N*,*N*′-diethyl-*N*,*N*′-bis(2,2,2-trifluoroethyl)rhodamine (**1b**-H)[4] in 6 mL of 1,2-dichloroethane as described above for diazoketone **2a**-H. Yield – 100 mg (97%) of pale yellow solid. HPLC: t_R = 20.7 min (100%), A/B: 50/50 → 0/100 in 25 min, detection at 254 nm.

^{1}H NMR ($CDCl_3$, 300 MHz, ppm): δ = 1.19 (t, $^3J_{HH}$= 7.0, 6 H, CH_3), 3.47 (q, $^3J_{HH}$= 7.1, 4 H, CH_2CH_3), 3.82 (q, $^3J_{HF}$= 8.9, 4 H, CH_2CF_3), 6.40 (dd, $^3J_{HH}$= 8.8, $^4J_{HH}$= 2.7, 2 H, H-2/H-7), 6.52 (d, $^4J_{HH}$= 2.7, 2 H, H-4/H-5), 6.72 (d, $^3J_{HH}$= 8.8, 2 H, H-1/H-8), 7.06 (d, $^3J_{HH}$= 7.5, $^4J_{HH}$= 0.8, 1 H, H-7′), 7.40 (td, $^3J_{HH}$= 7.4, $^4J_{HH}$= 1.2, 1 H, H-5′ or H-6′), 7.47 (td, $^3J_{HH}$= 7.4, $^4J_{HH}$= 1.5, 1 H, H-6′ or H-5′), 7.79–7.83 (m, 1 H, H-4′); ^{13}C NMR ($CDCl_3$, 75.5 MHz, ppm): δ = 11.5 (CH_3), 45.8 (**CH**$_2$$CH_3$), 48.8 (C-1′), 52.1 (q, $^2J_{CF}$= 32, CH_2CF_3), 76.8 (C=N_2), 100.0 (C-4/5), 109.1 (C-2/7) 110.3 (C-8a/8b), 122.2 (C_6H_4), 125.4 (C_6H_4), 125.3 (q, $^1J_{CF}$= 282, CF_3), 128.4 (C_6H_4), 128.8 (C-1/8), 134.5 (C-2′), 134.6 (C_6H_4), 147.9 (C-3/6), 152.0 (C-4a/4b), 155.7 (C-3′), 186.9 (CO); ESI-MS, positive mode: *m/z* (rel. int., %) = 575 (100) $[M+H]^+$; HRMS ($C_{29}H_{24}F_6N_4O_2$): 597.1693 (found *M*+Na), 597.1696 (calc.).



Diazoketones 2bc, 2bc-CO_2H and 2bc-CONHS. *3-Methoxy-[N-(2-methoxyethyl)-N-trifluoroacetyl]aniline.* A dried Schlenk flask was charged with 3.9 g (18 mmol) of 3-methoxy-(*N*-trifluoroacetyl)aniline[4] in 3 mL DMF and ground powders of K_2CO_3 (4.9 g, 35.5 mmol) and KI (0.3 g, 1.8 mmol). Then 11 g (79 mmol) of 2-bromoethyl methyl ether was added at room temperature with stirring, and the reaction mixture was heated at 90°C for

2.5 h. The solids were filtered off at room temperature and washed with Et_2O. The organic solutions were combined, washed with water (5×10 mL), dried and evaporated in vacuo. The residue was separated over a column with SiO_2 (100 g) using a hexane/EtOAc (4:1) mixture as a mobile phase. Yield – 3.3 g (67%) of the title aniline as yellow oil. ESI-MS, positive mode: *m/z* (rel. int., %) = 300(100) $[M+Na]^+$.

3-Methoxy-[N-(2-methoxyethyl)-N-(2,2,2-trifluoroethyl)]aniline. To the solution of the 3-methoxy-[*N*-(2-methoxyethyl)-*N*-trifluoroacetyl]aniline (5.1 g, 18 mmol) in dry THF (25 mL), BH_3*THF complex (1 M) in THF (37 mL) was added at 0 °C, and the mixture was heated at reflux overnight. Excess of BH_3 was carefully destroyed by adding of MeOH (9 mL) at 0 °C followed by 1 M aq. NaOH (60 mL). After stirring at room temperature for 20 min, the mixture was diluted with ether (100 mL), and the organic layer was separated. The aqueous layer was extracted with ether (3×20 mL); combined organic layers were washed with brine (3×30 mL), dried over Na_2SO_4, and the solvent was evaporated in vacuo. The title aromatic amine was isolated by chromatography on SiO_2 (200 g) with hexane/EtOAc mixture (8:1) and then purified by distillation. Yield –3.3 g (68%) as a clear oil with b.p.= 92°C (0.7 mbar).

^{1}H NMR ($CDCl_3$, 300 MHz, ppm): δ = 3.30 (s, 3 H, OCH_3), 3.52 (t, $^3J_{HH}$= 5.6, 4 H, CH_2CH_2), 3.79 (s, 2 H, OCH_3), 3.89 (t, $^3J_{HF}$= 5.6, 2 H, CH_2CF_3), 6.72−6.85 (m, 2 H, H-2/ H-4 or H-6), 6.92 (dd, $^3J_{HH}$= 8.4, $^4J_{HH}$ = 2.5, 1 H, H-6 or H-4), 7.28 (t, $^3J_{HH}$= 8.1, 1 H, H-5); ^{13}C NMR ($CDCl_3$, 75.5 MHz, ppm): δ = 50.8 (NCH_2), 55.4 (OCH_3), 58.6 (OCH_3), 68.3 (CH_2), 114.2 (Ar), 114.5 (d, $^1J_{CF}$= 288, CF_3), 114.6 (Ar), 120.5 (Ar), 129.8 (Ar), 140.1 (Ar-N), 160.0 (Ar-O). ESI-MS, positive mode: *m/z* (rel. int., %) = 272 (100) $[M+Na]^+$.

3-[N-(2-methoxyethyl)-N-(2,2,2-trifluoroethyl)amino]phenol. In a Schlenk flask flushed with argon, 3-methoxy-[*N*-(2-methoxyethyl)-*N*-(2,2,2-trifluoroethyl)]aniline (2.9 g, 11 mmol) and LiI (4.3 g, 32 mmol) was heated in 2,4,6-trimethylpyridine (10 mL) at 200 °C overnight. Then the reaction mixture was cooled, neutralized with 90 mL of 1 M aq. HCl and extracted with Et_2O (3×100 mL). The combined organic layers were extracted with 1 M aq. NaOH (7×10 mL), and the combined alkaline aqueous solutions were acidified with conc. aq. HCl to pH=3–4 and extracted with Et_2O (3×100 mL) to afford the title compound (2.0 g, 73%) as an oil. ^{1}H NMR ($CDCl_3$, 300 MHz, ppm): δ = 3.32 (s, 3 H, CH_3), 3.56−3.63 (m, 4 H, CH_2), 3.95 (q, $^3J_{HF}$= 5.6, 2 H, CH_2CF_3), 5.04 (s, 1 H, OH), 6.02−6.08 (m, 2 H, H-2/ H-4 or H-6), 6.33 (dd, $^3J_{HH}$= 8.4, $^4J_{HH}$ = 2.5, 1 H, H-6 or H-4), 7.08 (t, $^3J_{HH}$= 8.1, 1 H, H-5); ESI-MS, positive mode: *m/z* (rel. int., %) = 264 (15) $[M+H]^+$, 286 (100) $[M+Na]^+$.

Rhodamine 1bc was obtained as a mixture of two diastereomers from 3-[*N*-(2-methoxyethyl)-*N*-(2,2,2-trifluoroethyl)amino]phenol (1.7 g, 6.8 mmol) and 4-ethoxycarbonyl

phthalic anhydride (0.9 g, 4.1 mmol) as described above for *rhodamine **1a**-CO_2Et*. The dye was isolated by column chromatography (hexane/EtOAc: 1/1), yield – 915 mg (40%) of bright orange solid. HPLC: t_R = 16.0 min for both diastereomers (B/A: 30/70 → 100/0 in 25 min, 532 nm). 5′-isomer: ^{1}H NMR ($CDCl_3$, 300 MHz, ppm): δ = 1.42 (t, $^3J_{HH}$= 8.1, 3 H, CH_2CH_3), 3.31 (s, 6 H, OCH_3), 3.53−3.68 (m, 8 H, NCH_2CH_2O), 4.03 (q, $^3J_{HF}$= 8.8, 4 H, CH_2CF_3), 4.43 (q, $^3J_{HH}$= 7.2, 2 H, OCH_2CH_3), 6.43 (d, $^3J_{HH}$= 8.9, 2 H, H-2/H-7), 6.55–6.69 (m, 4 H, H-4/H-5 and H-1/H-8), 7.25 (d, $^3J_{HH}$= 8.0, 1 H, H-7′), 8.32 (dd, $^3J_{HH}$= 8.0, $^4J_{HH}$= 1.4, 1 H, H-6′), 8.66 (s, 1H, H-4′); ^{13}C NMR ($CDCl_3$, 126 MHz, ppm): δ = 14.4 (CH_3), 29.8 (C-1′), 50.8 (NCH_2), 52.5 (q, $^2J_{CF}$= 33, NCH_2CF_3), 59.2 (OCH_3), 61.7 (OCH_2), 69.9 (OCH_2), 99.5 (CH, C-1/8 or C-4/5), 108.2 (C-9a), 109.3 (CH, C-2/7), 124.5 (CH, C-7′), 125.3 (q, $^1J_{CF}$= 283, CF_3), 126.8 (CH, C-4′), 127.9 (C-7′a), 129.1 (CH, C-4/5 or 1/8), 132.4 (C-3′a), 135.5 (CH, C-6′), 149.6 (C-3/6), 152.9 (C-4a/4b), 156.1 (C-5′), 165.0 (CO_2Et), 168.4 (CO_2); ESI-MS, positive mode: *m/z* (rel. int., %) = 683 (100) [*M*+H]$^+$. 6′-isomer: ^{1}H NMR ($CDCl_3$, 300 MHz, ppm): δ = 1.33 (t, $^3J_{HH}$= 8.1, 3 H, CH_2CH_3), 3.31 (s, 6 H, OCH_3), 3.53−3.68 (m, 8 H, NCH_2CH_2O), 4.03 (q, $^3J_{HF}$= 8.8, 4 H, CH_2CF_3), 4.33 (q, $^3J_{HH}$= 7.2, 2 H, OCH_2CH_3), 6.40–6.48 (m, 2 H, H-2/H-7), 6.55–6.69 (m, 4 H, H-4/H-5 and H-1/H-8), 7.25 (s, 1 H, H-7′), 8.04 (d, $^3J_{HH}$= 8.0, 1 H, H-4′), 8.26 (dd, $^3J_{HH}$= 8.0, $^4J_{HH}$= 1.4, 1 H, H-5′); ^{13}C NMR ($CDCl_3$, 126 MHz, ppm): δ = 14.3 (CH_3), 29.8 (C-1′), 50.8 (NCH_2), 52.6 (q, $^2J_{CF}$= 33, NCH_2CF_3), 59.2 (OCH_3), 61.9 (OCH_2), 69.9 (OCH_2), 99.5 (CH-4/5), 108.3(C-9a), 109.3 (CH-2/7), 125.1 (q, $^1J_{CF}$ = 282, CF_3), 125.2 (CH-4′), 125.5 (CH-7′), 129.1 (CH-1/8), 130.7 (CH-5′), 130.8 (C-6′), 136.3 (C-3′a), 149.5 (C-3/6), 152.2 (C-7′a), 152.9 (C-4a/4b), 165.0 (CO_2Et), 168.3 (CO_2); ESI-MS, positive mode: *m/z* (rel. int., %) = 683 (100) [*M*+H]$^+$, 705 (20) [*M*+Na]$^+$; HRMS ($C_{33}H_{32}F_6N_2O_7$): 683.2193 (found *M*+H), 683.2186 (calc.).

Diazoketone 2bc. A dry Schlenk flask was flushed with argon and charged with rhodamine **1bc** (125 mg, 0.18 mmol) and 1,2-dichloroethane (2.5 mL). $POCl_3$ (0.3 mL, 3.3 mmol) was added to the flask, and the mixture was stirred at 80 °C for 1.5 h. Then the solvent and excess of $POCl_3$ were removed in vacuo (< 1 mbar). The residue was dissolved in CH_2Cl_2 (3 mL) under argon and cooled in an ice bath. A solution of CH_2N_2 in Et_2O (2.2 mL of ca. 0.3 M solution; 0.66 mmol) was added to the reaction mixture at 0 °C with stirring. The reaction mixture was kept at 0 °C for 1.5 h. Then it was diluted with *n*-pentane 5 mL, and the title product was isolated by column chromatography on SiO_2 (*n*-pentane/Et_2O: 2/1 → 0/1). Yield – 118 mg (84 %) of a glass-like foam. HPLC: t_R = 22.8 min, area 93.4% (B/A: 30/70 → 100/0 in 25 min, 254 nm). ^{1}H NMR (5′-isomer, acetone-d_6, 300 MHz, ppm): δ = 1.36 (t, $^3J_{HH}$= 7.1, 3 H, CH_3), 3.29 (s, 6 H, OCH_3), 3.52–3.78 (m, 8 H, NCH_2CH_2O), 4.26 (q, $^3J_{HF}$= 9.3, 4 H,

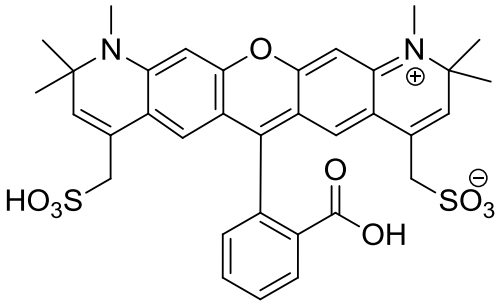
NCH_2CF_3), 4.37 (q, $^3J_{HH}$= 7.1, 2 H, OCH_2), 6.63 (dd, $^3J_{HH}$= 8.8, $^4J_{HH}$= 2.7, 2 H, H-2/H-7), 6.69 (d, $^4J_{HH}$= 2.7, 2 H, H-4/H-5), 6.82 (d, $^3J_{HH}$= 8.8, 2 H, H-1/H-8), 7.19 (dd, $^3J_{HH}$ = 8.1, $^5J_{HH}$ = 0.7, 1 H), 8.17 (dd, $^3J_{HH}$= 8.1, $^4J_{HH}$ = 1.7, 1 H), 8.33 (dd, $^4J_{HH}$= 1.6, $^5J_{HH}$= 0.7, 1 H); ^{13}C NMR (acetone-d_6,126 MHz, ppm): δ = 14.5 (CH_3), 49.6 (C-1′), 51.3 (NCH_2), 52.5 (q, $^2J_{CF}$= 32, NCH_2CF_3), 59.0 (OCH_3), 61.9 (OCH_2), 70.5 (OCH_2), 76.1 ($C=N_2$), 100.8 (CH), 110.15 (C), 110.20 (CH), 123.6 (CH), 126.4 (CH), 126.7 (q, $^1J_{CF}$= 283, CF_3), 129.3 (CH), 131.9 (C), 135.4 (C), 136.0 (CH), 149.2 (C), 152.7 (C), 160.3 (C), 165.5 (CO_2), 185.4 (CO); ^{1}H NMR (6′-isomer) (CD_3OD, 300 MHz, ppm): δ = 1.42 (t, $^3J_{HH}$= 8.1, 3 H, CH_2CH_3), 3.49 (s, 6 H, OCH_3), 3.65−3.82 (m, 8 H, NCH_2CH_2O), 4.17 (q, $^3J_{HF}$= 8.8, 4 H, CH_2CF_3), 4.43 (q, $^3J_{HH}$= 7.2, 2 H, OCH_2CH_3), 6.57 (dd, $^3J_{HH}$= 8.9, $^4J_{HH}$= 2.7, 2 H, H-2/H-7), 6.66 (d, $^4J_{HH}$= 2.6, 2 H, H-4/H-5), 6.83 (d, $^3J_{HH}$= 8.8, 2 H, H-1/H-8), 7.83 (s, 1 H, H-7′), 8.02 (d, $^3J_{HH}$= 8.0, 1 H, H-4′), 8.23 (dd, $^3J_{HH}$= 8.0, $^4J_{HH}$= 1.4, 1 H, H-5′).

Carboxylic acid 2bc-CO_2H. Ethyl ester **2bc** (103 mg, 0.15 mmol) was dissolved in MeOH (1 mL) and THF (0.1 mL) under argon. 1 M aq. NaOH (0.3 mL) was added, and the reaction mixture was kept at 4 °C overnight. Then the reaction mixture was acidified with glacial CH_3COOH to pH=4–5, water (10 mL) was added, and the product was extracted with EtOAc (3×20 mL). The combined organic layers were washed with brine, dried (Na_2SO_4), and the solvent was removed in vacuo. Yield – 68 mg (67%) of amorphous solid. HPLC: t_R = 18.7 min, area 92% (B/A: 30/70 → 100/0 in 25 min, 254 nm); t_R = 12.8 min, (B/A: 50/50 → 100/0 in 25 min, 254 nm). 6′-isomer: ^{1}H NMR ($CDCl_3$, 300 MHz, ppm): δ = 3.33 (br.s, 6 H, OCH_3), 3.55−3.66 (m, 8 H, NCH_2CH_2O), 4.00 (q, $^3J_{HF}$= 8.7, 4 H, CH_2CF_3), 6.40 (dd, $^3J_{HH}$= 8.9, $^4J_{HH}$= 2.7, 2 H, H-2/H-7), 6.53 (d, $^4J_{HH}$= 2.6, 2 H, H-4/H-5), 6.69 (d, $^3J_{HH}$= 8.8, 2 H, H-1/H-8), 7.72 (s, 1 H, H-7′), 7.89 (d, $^3J_{HH}$= 8.0, 1 H, H-4′), 8.11 (dd, $^3J_{HH}$= 8.0, $^4J_{HH}$= 1.4, 1 H, H-5′);
ESI-MS, positive mode: *m/z* (rel. int., %) = 723 (50) $[M+2Na-H]^+$, 701 (100) $[M+Na]^+$. ESI-MS, negative mode: *m/z* (rel. int., %) = 677 (100) $[M-H]^+$; HRMS ($C_{32}H_{28}F_6N_4O_6$): 701.1801 (found *M*+Na), 701.1805 (calc.).

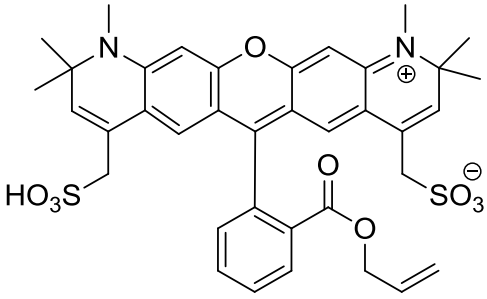
***N*-hydroxysuccinimidyl ester 2bc-CONHS.** A dry Schlenk flask was charged with diazoketone **2bc**-CO_2H (66 mg, 0.09 mmol) and CH_2Cl_2 (1 mL) under argon. *N*-hydroxysuccinimide (40 mg, 0.3 mmol) in 1.2 mL CH_2Cl_2, HATU (60 mg, 0.2 mmol) and NEt_3 (20 μL, 0.3 mmol) were added to the reaction mixture at room temperature with stirring. The mixture was stirred at room temperature for 1 h. Then the solvent and excess of NEt_3 were removed in vacuo. The title product was isolated by column chromatography on SiO_2 (hexane/EtOAc: 1/1→1/2 + 0.1% AcOH). Yield – 41 mg (53%). HPLC: t_R = 15.4 min, area 94% (B/A: 30/70 → 100/0 in 25 min, 254 nm). ESI-MS, positive mode: *m/z* (rel. int., %)

=776 (70) [*M*+H]$^+$, 798 (75) [*M*+Na]$^+$, 814 (100) [*M*+K]$^+$; HRMS ($C_{36}H_{31}F_6N_5O_8$): 776.2147 (found *M*+H), 776.2150 (calc.).

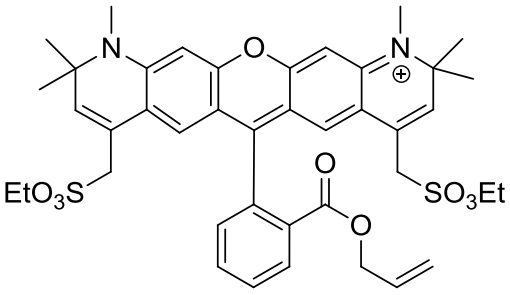
Scheme 4



7-H,SO_3H,H



7-All,SO_3H,H

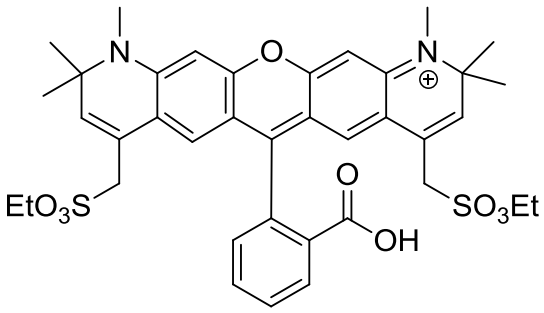


7-All,SO_3Et,H

Allyl ester 7-All,SO_3H,H. Rhodamine **7**-H,SO_3H,H (94 mg, 0.14 mmol) was dissolved in allyl alcohol (3.0 mL, 45 mmol) in a screw-cap tube. Then *p*-TosOH*H_2O (15 mg, 79 μmol) was added, the reaction mixture was purged with argon and stirred at 115 °C for 3 h. Allyl alcohol was evaporated in vacuo, and the product was isolated by chromatography on SiO_2 (50 g) with $CHCl_3$/MeOH/H_2O mixture (65:35:5) as an eluent. Yield – 69 mg (69%) of **7**-All,SO_3H,H as a purple solid. HPLC: t_R = 5.5 min (area 93%), B/A: 30/70 → 100/0 in 25 min, 580 nm. HR-ESI-MS, negative mode: *m/z* = 717.1949 [found *M*–H]$^-$; 717.1946 (calc.).

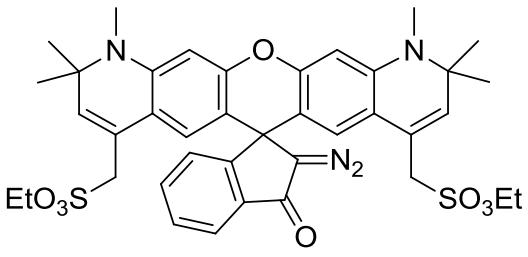
Allyl diethyl disulfonate 7-All,SO_3Et,H. A dry Schlenk flask was flushed with argon and charged with a suspension of rhodamine **7**-All,SO_3H,H (48 mg, 67 μmol) in dry MeCN (0.25 mL). Then the flask was cooled to 0 °C, and 68 mg (0.34 mmol) of Et_3O^+*BF_4^- was quickly added followed by 50 μL (0.3 mmol) of *i*Pr_2NEt. The reaction mixture was stirred for 1 h at room temperature. HPLC: t_R = 16.9 min (area 94%), B/A: 30/70 → 100/0 in 25 min, 580 nm. Quenching of the reaction with glacial AcOH (0.1 mL), followed by evaporation of the solvents and chromatography on SiO_2 ($CHCl_3$/Me, 30:1), afforded the title compound with 78% purity (HPLC; 22% of impurity with t_R = 11.9; presumably, a zwitterionic monoethyl disulfonate was formed). Therefore next transformations on a large scale were performed "in one pot".

Diethyl disulfonate 7-H,SO_3Et,H. Rhodamine **7**-H,SO_3H,H (600 mg, 0.88 mmol) and *p*-TosOH*H_2O (60 mg, 0.32 mmol) were dissolved in allyl alcohol (15.0 mL, 0.23 mol) in a dry Schlenk flask. The reaction mixture was purged with argon and stirred at 115 °C for 1.5 h. Allyl alcohol was evaporated in vacuo, and the resudue was kept overnight under vacuum (0.2



7-H,SO_3Et,H

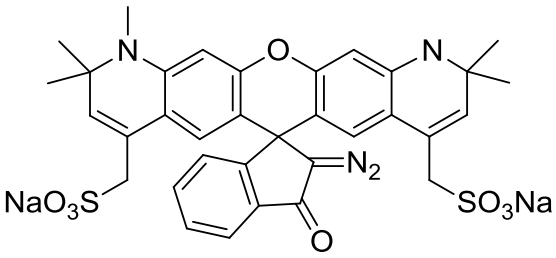
– 0.5 mbar). HPLC indicated that the conversion of **7**-H,SO_3H,H to allyl ester **7**-All,SO_3H,H was 93% (see above). The flask was stopped with a septum, purged with argon, and the solid residue (666 mg) was suspended in dry ACN (50 mL) under argon. $Et_3O^+*BF_4^-$ (712 mg, 3.75 mmol) was quickly added followed by *i*Pr_2NEt (0.70 mL, 4.1 mmol). The reaction mixture became homogeneous, and HPLC indicated that the conversion to **7**-All,SO_3Et,H was more than 88%. Then neat formic acid was added to the reaction mixture (0.31 mL, 8.2 mmol) followed by Et_3N (0.7 mL, 5.0 mmol) and solid $Pd(Ph_3P)_4$ (95 mg, 0.095 mmol). After stirring for 3 h at room temperature, HPLC indicated that all amount of the initially formed allyl ester **7**-All,SO_3Et,H reacted and a new substance with t_R = 14.2 min (area 95%) was formed (B/A: 30/70 → 100/0 in 25 min, 580 nm). The solvent (ca. 2/3 of acetonitrile) was removed in vacuo, the residue was diluted with ca. 2 volumes of water containing 0.05% (v/v) TFA and applied onto a column with RP-C18 silica gel (100 g). Elution with aqueous acetonitrile (1:1) followed by acetonitrile – water mixture (2:1, with 0.1% v/v TFA) afforded the main highly colored fraction containing the title compound. It was collected into ice-cooled flasks, analyzed by TLC, immediately frozen and freeze-dried (liophylized) to afford compound **7**-H,SO_3Et,H (466 mg, 72%) which was stored under argon at –20°C. ESI-MS, positive mode: *m/z* (rel. int., %) = 735 (100) [*M*+H]$^+$; HRMS ($C_{38}H_{42}N_2O_9S_2$): 735.2388 (found *M*+H), 735.2404 (calc.). ESI-MS, negative mode: *m/z* (rel. int., %) = 705 (100) [*M*–Et]$^-$. Diethyl disulfonate **7**-H,SO_3Et,H was found to be unstable at room temperature; it quickly "loosed" one ethyl group (see above ESI-MS in negative mode).



2d-Et,H

Diazoketone 2d-Et,H. Diethyl disulfonate **7**-H,SO_3Et,H (130 mg, 0.18 mmol) was dissolved in dry CH_2Cl_2 (25 mL), 250 µL (2.9 mmol) of $(COCl)_2$ was added dropwise at 0°C, and the reaction mixture was stirred for 2 h at 0°C. During this time (2 h), dry DMF was added twice (each portion 25 µL), followed by $(COCl)_2$ (100 µL). All volatile materials were evaporated in vacuo (<0.5 mbar), the residue was dissolved in CH_2Cl_2 (2 mL), and 6 mL of the diazomethane solution (CH_2N_2 in Et_2O, ca. 0.3 M solution, 1.8 mmol) was added at 0°C. The reaction mixture was stirred in the absence of light at 0°C overnight, evaporated, and the title product was isolated by chromatography on 50 g SiO_2 with CH_2Cl_2/Et_2O (8:1) mixture as an

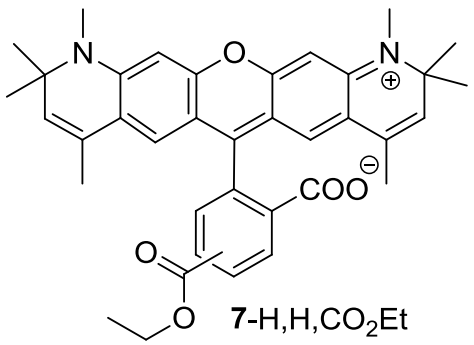
eluent. Separation from the "dark" rearranged compound with t_R = 20.8 min (HPLC, B/A: 30/70 → 100/0 in 25 min, 254 nm) was not full. Yield – 46 mg (34%) of yellow solid. HPLC: t_R = 21.3 min (area 95%), B/A: 30/70 → 100/0 in 25 min, 254 nm. ^{1}H NMR ($CDCl_3$, 600 MHz, ppm): δ = 1.17 (t, $^3J_{HH}$= 7.1, 6 H, CH_2CH_3), 1.32/1.33 (s×2, 12 H, CH_3), 2.81 (s, 6 H, CH_3), AB-system (δ_A=3.81 δ_B= 3.86, J_{AB}= 14.2, 4 H, SCH_2), 3.90–3.99 (m, 4 H, CH_2CH_3) 5.55 (s, 2 H, CH), 6.27 (s, 2 H, CH), 6.54 (s, 2 H, CH), 7.09 (d, $^3J_{HH}$= 7.5, 1 H, H-7′), 7.41 (t, $^3J_{HH}$= 6.9, 1 H, H-5′ or H-6′), 7.48 (dd, $^3J_{HH}$= 11.8, $^4J_{HH}$= 4.4, 1 H, H-5′ or H-6′), 7.81 (d, $^3J_{HH}$= 6.8, 1 H, H-4′); ESI-MS, positive mode: *m/z* (rel. int., %) = 781 (100) $[M+Na]^+$; HRMS ($C_{39}H_{42}N_4O_8S_2$): 781.2365 (found *M*+Na), 781.2336 (calc.).



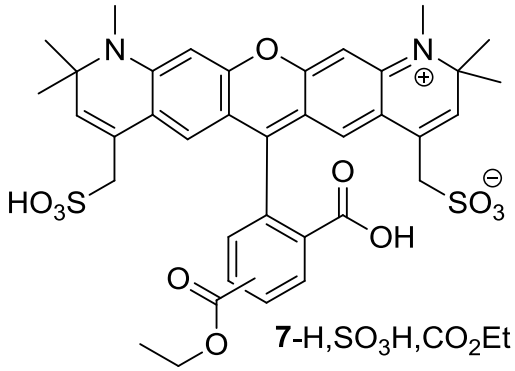
2d-Na,H

Disodium dusulfonate 2d-Na,H. To the suspension of **2d**-Et,H (12 mg, 15 μmol) in EtOH (2 mL), 1 M aq. NaOH (0.5 mL) and 0.5 mL THF were added at 0 °C. The reaction mixture was stirred at 60 °C overnight. The progress of the reaction was monitored by HPLC. t_R = 12.1 min (intermediate with one SO_3Et group), 4.6 min (**2d**-H,H) (B/A: 30/70 → 100/0 in 25 min, 254 nm). The product was isolated by chromatography on 25 g of RP–C18 using MeCN/H_2O (1:4) mixture as an eluent. Yield – 11 mg (98%) of a title compound as voluminous gray-blue powder. HPLC: t_R = 19.6 min (B/A [with 10 mM HCOOH*Et_3N buffer]: 20/80 → 50/50 in 25 min, 254 nm). ^{1}H NMR (D_2O, 300 MHz, ppm): δ = 1.32/1.36 (s×2, 12 H, CH_3), 2.88 (s, 6 H, CH_3), 2.81 (s, 6 H, CH_3), AB-system (δ_A=3.73, δ_B= 3.78, J_{AB}= 14.2, 4 H, SCH_2), 5.73 (s, 2 H, CH), 6.58 (s, 2 H, CH), 6.88 (s, 2 H, CH), 6.96–7.00 (m, 1 H, H-7′), 7.52–7.58 (m, 2 H, H-5′ and H-6′), 7.85–7.88 (m, 1 H, H-4′); ESI-MS, negative mode: *m/z* (rel. int., %) = 350 (100) $[M-2H]^{2-}$; HRMS ($C_{35}H_{34}N_4O_8S_2$): 350.0844 (found *M*–2H), 350.0836 (calc.). UV (water), λ, nm (ε, M^{-1} cm^{-1}): 262 (74200), 289sh (16300), 321 (14200). Photoactivation (uncaging) of a PBS solution (pH 7.4) of **2d**-Na,H by irradiation with a middle pressure mercury lamp through a pyrex filter provided a magenta-colored solution with bright fluorescence; contrast ratio (measured by absorption at 587 nm before and after photoactivation) was found to be ca. 25. UV-VIS (aq. PBS), λ (absorption), nm (ε, M^{-1} cm^{-1}): 587 (43800); λ (emission), nm (ϕ): 609 (0.74; standard dye – Atto 594 in water; ϕ = 0.85).

Rhodamine ethyl ester 7-H,H,CO_2Et was obtained as a mixture of two diastereomers by heating overnight 7-hydroxy-1,2,2,4-tetramethyl-1,2-dihydroquinoline (**5**-Me,H, 500 mg, 2.46 mmol) with trimellitic anhydride ethyl ester (316 mg, 1.43 mmol) in 1,2-dichlorobenzene (3 mL) at 200°C under argon. The solvent was removed in vacuo (oil pump), and the residue

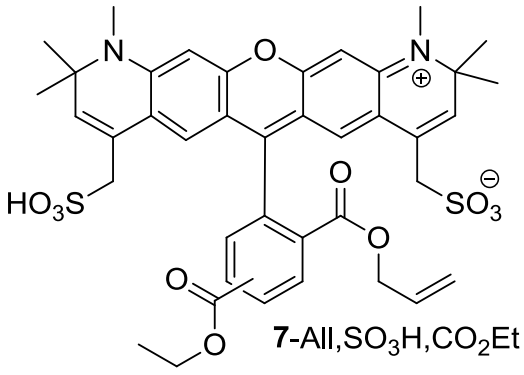


was subjected to chromatography on SiO_2 (120 g) with CH_2Cl_2/MeOH mixture as an eluent. Total yield – 510 mg (69%) of dark purple solid. HPLC: t_R = 20.5 and 20.9 min (two diasteromers), total area 94%, B/A: 30/70 → 100/0 in 25 min, 580 nm. 5′-isomer: ^{1}H NMR ($CDCl_3$, 300 MHz, ppm): δ = 1.26 (s, 6 H, CH_3), 1.28 (s, 6 H, CH_3), 1.42 (t, $^3J_{HH}$= 7.1, 3 H, OCH_2CH_3), 1.62 (d, $^4J_{HH}$= 1.4, 6 H, CH_3), 2.85 (s, 6 H, NCH_3), 4.43 (q, $^3J_{HH}$= 7.2, 2 H, OCH_2CH_3), 5.18 (m, 2 H, CH), 6.31 (s, 2 H, CH), 6.32 (s, 2 H, CH), 7.23 (d, $^3J_{HH}$= 8.0, 1 H, H-7′), 8.27 (dd, $^3J_{HH}$= 8.0, $^4J_{HH}$= 1.5, 1 H, H-6′), 8.72 (dd, $^4J_{HH}$= 1.4, $^5J_{HH}$= 0.7, 1 H, H-4′); 6′-isomer: ^{1}H NMR ($CDCl_3$, 300 MHz, ppm): δ = 1.30 (s, 6 H, CH_3), 1.32 (s, 6 H, CH_3), 1.34 (t, $^3J_{HH}$= 7.1, 3 H, OCH_2CH_3), 1.63 (d, $^4J_{HH}$= 1.4, 6 H, CH_3), 3.31 (s, 6 H, OCH_3), 2.84 (s, 6 H, NCH_3), 4.33 (q, $^3J_{HH}$= 7.2, 2 H, OCH_2), 5.17 (s, 2 H, CH), 6.26 (s, 2 H, CH), 6.28 (s, 2 H, CH), 7.78 (dd, $^4J_{HH}$= 1.4, $^5J_{HH}$= 0.7, 1 H, H-7′), 8.10 (d, $^3J_{HH}$= 8.0, 1 H, H-4′), 8.23 (dd, $^3J_{HH}$= 8.0, $^4J_{HH}$= 1.4, 1 H, H-5′); ESI-MS, positive mode: *m/z* (rel. int., %) = 591 (80) $[M+H]^+$, 613 (100) $[M+Na]^+$; HRMS ($C_{37}H_{38}N_2O_5$): 561.2852 (found *M*+H), 591.2853 (calc.).



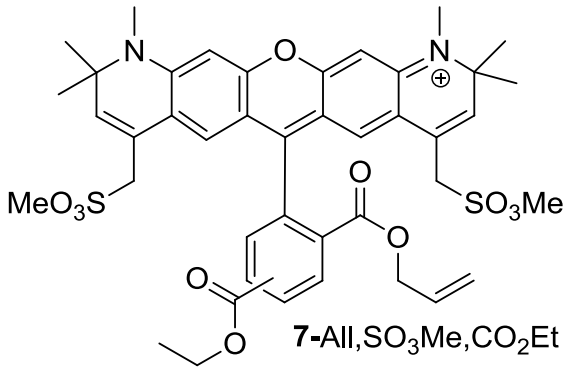
Disulfo rhodamine ethyl ester 7-H,SO_3H,CO_2Et. Rhodamine **7**-H,H,CO_2Et (2.4 g, 4.1 mmol) was placed into a 100 mL flask, cooled to 0°, and 30 mL of 95–97% H_2SO_4 (precooled to 0°C) was added. The reaction mixture was stirred at room temperature for 1–3 d. The double sulfonation reaction was complete after 24 h (HPLC control). The reaction mixture was transferred onto 100 g of frozen dioxane, carefully diluted with cold ether (600 mL), and kept overnight at +4°C. The upper organic layer was decanted, the residue was dissolved in cold water (200 mL) and applied onto a column with RP-C18 silica gel (150 g). (Chromatography column was packed in methanol and then washed with excess of pure water.) Elution with pure water removed sulfuric acid (pH-control), and when pH-value increased ca. to 4, gradual elution with methanol – water mixture (1/4–1/1, v/v) afforded fractions containing: 1) disulfo rhodamine carboxylic acid **7**-H,SO_3H,CO_2H (discarded); 2) disulfo rhodamine ethyl ester **7**-H,SO_3H,CO_2Et (5′-isomer, 0.54 g); 3) mixed fraction (5′- and 6′-isomers, 0.9 g); 4) disulfo rhodamine ethyl ester **7**-H,SO_3H,CO_2Et (6′-isomer, 0.46 g).; 5) 6′-isomer with 12% impurity (0.13 g). Methanol was evaporated in vacuo, and the residues of the pooled fractions were freeze-dried to afford dark purple very light

powders. HPLC: t_R = 14.6 min (5′-isomer) and 15.4 min (6′-isomer), B/A: 20/80 → 50/50 in 25 min, 580 nm. 5´-isomer: ^{1}H NMR (CD_3OD, 300 MHz, ppm): δ = 1.47 (t, $^3J_{HH}$= 7.1, 3 H, OCH_2CH_3), 1.51/1.52 (2×s, ∑12 H, CH_3), 3.15 (s, 6 H, NCH_3), AB-system (δ_A=3.57, δ_B= 3.67, J_{AB}= 14.2, 4 H, SCH_2), 4.47 (q, $^3J_{HH}$= 7.2, 2 H, OCH_2CH_3), 5.86 (br. s, 2 H, CH), 6.79 (br. s, 2 H, CH), 7.14 (br. s, 2 H, CH), 7.52 (d, $^3J_{HH}$= 8.0, 1 H, H-7′), 8.42 (d, $^3J_{HH}$= 7.9, 1 H, H-6′), 8.85 (d, $^4J_{HH}$= 1.3, 1 H, H-4′); ^{13}C NMR (DMSO-d_6, 126 MHz, ppm): δ = 14.0 (CH_3), 28.51 (CH_3), 28.54 (CH_3), 32.8 (NCH_3), 53.3 (C), 59.5 (O_3SCH_2), 61.4 (OCH_2), 94.8 (CH), 112.9 (C), 121.4 (C), 123.0 (CH), 123.4 (C), 130.7 (C), 130.8 (CH), 131.2 (CH), 131.8 (C), 132.3 (CH), 135.6 (CH), 137.0 (C), 152.8 (C), 155.2 (C), 156.7 (C), 164.5 (CO_2), 165.3 (CO_2); 6´-isomer: ^{1}H NMR (CD_3OD, 300 MHz, ppm): 1.37 (t, $^3J_{HH}$= 7.1, 3 H, OCH_2CH_3), 1.51 – 1.52 (2×s, ∑12 H, CH_3), 3.15 (s, 6 H, NCH_3), AB-system (δ_A=3.57 δ_B= 3.67, J_{AB}= 14.2, 4 H, SCH_2), 4.37 (q, $^3J_{HH}$= 7.2, 2 H, CH_2CH_3), 5.86 (d, $^3J_{HH}$= 4.5, 2 H, CH), 6.79 (d, $^3J_{HH}$= 4.8, 2 H, CH), 7.14 (d, $^3J_{HH}$= 10.9, 2 H, CH), 7.97 (d, $^4J_{HH}$= 1.4, 1 H, H-7′), 8.32 – 8.35 (m, 2 H, H-4′/H-5′); ^{13}C NMR (DMSO-d_6, 126 MHz, ppm): δ = 13.9 (CH_3), 28.4 (CH_3), 28.5 (CH_3), 32.8 (NCH_3), 53.3 (C), 59.6 (O_3SCH_2), 61.3 (OCH_2), 95.0 (CH), 113.4 (C), 121.7 (C), 123.3 (CH), 123.6 (C), 131.0 (CH), 131.3 (CH), 131.95 (C), 132.01 (C), 132.7 (CH), 135.8 (CH), 136.8 (C), 155.6 (C), 155.8 (C), 157.2 (C), 164.7 (CO_2), 165.9 (CO_2); ESI-MS (isomeric mixture), negative mode: *m/z* (rel. int., %) = 374 (100) $[M–2H]^{2–}$, 749 (10) $[M–H]^–$; HRMS (isomeric mixture) ($C_{37}H_{38}N_2O_{11}S_2$): 749.1852 (found *M*–H), 749.1844 (calc.).



Allyl ethyl ester 7-All,SO_3H,CO_2Et. Ethyl ester **7**-H,SO_3H,CO_2Et (70 mg, 93 μmol) was dissolved in allyl alcohol (3 mL, 45 mmol) under argon. Then 4 mg (33 μmol) DMAP in 1 mL CH_2Cl_2 and 48 mg (0.2 mmol) DCC in 1 mL CH_2Cl_2 were added, and the reaction mixture was stirred at room temperature overnight. The course of the reaction was monitored by TLC on SiO_2 ($CHCl_3$/MeOH/H_2O = 75/25/3). After completion of the reaction, the solvent and allyl alcohol were evaporated in vacuo, and the title compound was isolated by chromatography on SiO_2 (200 g) with $CHCl_3$/MeOH/H_2O mixture (75:25:3→65:35:5). Evaporation of the fractions with allyl ester **7**-All,SO_3H,CO_2Et afforded the solid material which also contained *N*,*N*′-dicyclohexylurea. The solid was extracted with several portions of hot water, and aqueous solutions were filtered through a glass filter while hot with suction, until the filtrate became

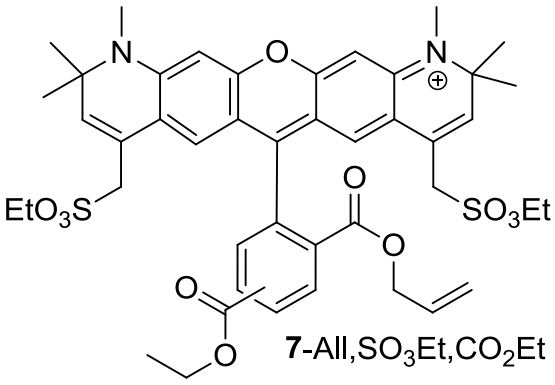
nearly colourless. Lyophilization of the combined aqueous solutions afforded 43 mg (60%) of a title compound as a purple solid. HPLC: t_R = 6.9 min (5′-isomer), t_R = 6.8 min (6′-isomer), B/A: 30/70 → 100/0 in 25 min, 580 nm. 5´-isomer: ^{1}H NMR (CD_3OD, 300 MHz, ppm): δ = 1.45 (t, $^3J_{HH}$= 7.1, 3 H, OCH_2CH_3), 1.52 (s, 12 H, CH_3), 3.15 (s, 6 H, CH_3), AB-system (δ_A=3.54, δ_B= 3.67, J_{AB}= 14.2, 4 H, SCH_2), 4.46 (q, $^3J_{HH}$= 7.1, 2 H, OCH_2CH_3), 4.51 (d, $^3J_{HH}$= 6.0, 2 H, OCH_2CH=), 5.05–5.17 (m, 2 H, CH_2=), 5.62–5.76 (m, 1 H, CH=), 5.87 (s, 2 H, CH), 6.81 (s, 2 H, CH), 7.15 (s, 2 H, CH), 7.52 (d, $^3J_{HH}$= 8.0, 1 H, H-7′), 8.44 (dd, $^3J_{HH}$= 7.9, $^4J_{HH}$= 1.4, 1 H, H-6′), 8.84 (d, $^4J_{HH}$= 1.3, 1 H, H-4′); ^{13}C NMR (DMSO-d_6, 126 MHz, ppm): δ = 14.0 (CH_3), 28.49 (CH_3), 28.51 (CH_3), 32.8 (NCH_3), 53.4 (C), 59.5 (O_3SCH_2), 61.5 (OCH_2), 65.5 (OCH_2), 94.8 (CH), 112.8 (C), 118.2 (CH_2), 121.5 (C), 123.0 (CH), 123.6 (C), 130.2 (C), 130.8 (C), 131.5 (CH), 131.7 (CH), 132.8 (CH), 135.4 (CH), 137.6 (CH), 152.9 (C), 154.6 (C), 156.4 (C), 156.7 (C), 164.5 (CO_2), 165.3 (CO_2); 6´-isomer: ^{1}H NMR (CD_3OD, 300 MHz, ppm): 1.38 (t, $^3J_{HH}$= 7.1, 3 H, OCH_2CH_3), 1.54 (s, 12 H, CH_3), 3.18 (s, 6 H, CH_3), AB-system (δ_A=3.54, δ_B= 3.67, J_{AB}= 14.2, 4 H, SCH_2), 4.39 (q, $^3J_{HH}$= 7.1, 2 H, OCH_2CH_3), 4.48 (d, $^3J_{HH}$= 6.0, 2 H, OCH_2CH=), 5.02–5.17 (m, 2 H CH_2=), 5.57–5.72 (m, 1 H, CH=), 5.87 (s, 2 H, CH), 6.81 (s, 2 H, CH), 7.16 (s, 2 H, CH), 7.97 (d, $^4J_{HH}$= 1.4, 1 H, H-7′), 8.31–8.37 (m, 2 H, H-4′/H-5′); ESI-MS, negative mode: *m/z* (rel. int., %) = 789 (100) [*M*–H]$^-$; HRMS ($C_{40}H_{42}N_2O_{11}S_2$): 789.2152 (found *M*–H), 789.2157 (calc.).



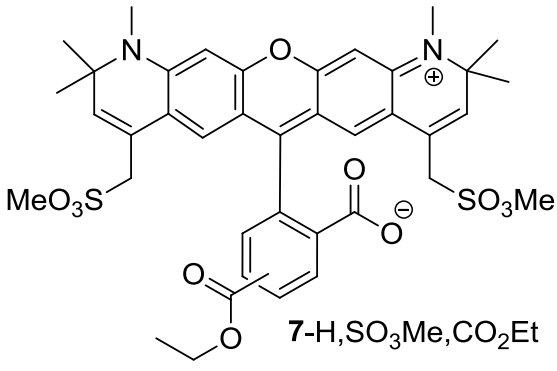
7-All,SO_3Me,CO_2Et

Allyl ethyl ester dimethyl disulfonate 7-All,SO_3Me,CO_2Et. The dry Schlenk flask was charged with 6′-isomer of rhodamine **7-**All,SO_3H,CO_2Et (110 mg, 0.14 mmol), flushed with argon and the 9 mL of dry MeCN was added. Then the flask was cooled to 0 °C, and 310 mg (2.1 mmol) trimethyloxonium tetrafluoroborate was quickly added followed by *i*PrNEt (0.15 mL, 0.90 mmol) in 0.5 mL of dry CH_2Cl_2. The course of the reaction was monitored by HPLC. After stirring for 1.5 h at room temperature, acetonitrile was removed in vacuo, and the product was isolated by chromatography on SiO_2 (200 g) with CH_2Cl_2/MeOH (15:1) mixture. Yield – 90 mg (71%) of a dark purple solid. HPLC: t_R = 16.7 min (6′-isomer), B/A: 30/70 → 100/0 in 25 min, 597 nm. HPLC: t_R = 17.1 min (5′-isomer), B/A: 30/70 → 100/0 in 25 min, 597 nm. 6´-isomer: ^{1}H NMR (CD_3OD, 300 MHz, ppm): δ = 1.36 (t, $^3J_{HH}$= 7.1, 3 H, OCH_2CH_3), 1.54/1.56 (2×s, ∑12 H, CH_3), 3.22 (s, 6 H, NCH_3), 3.74 (s, 6 H, OCH_3), 4.15 (s, 4 H, SCH_2), 4.38 (q, $^3J_{HH}$= 7.1, 2 H, OCH_2CH_3), 4.50 (d, $^3J_{HH}$= 6.0, 2 H, OCH_2CH=), 5.07–5.20 (m, 2 H, CH_2=), 5.60–5.74 (m,

1 H, CH=), 6.03 (s, 2 H, CH), 6.89 (s, 2 H, CH), 6.96 (s, 2 H, CH), 7.97 (d, $^4J_{HH}$= 1.4, 1 H, H-7′), 8.35 (dd, $^3J_{HH}$= 8.0, $^4J_{HH}$= 1.4, 1 H, H-5′), 8.45 (d, $^3J_{HH}$= 8.0, 1 H, H-4′); ESI-MS, positive mode: *m/z* (rel. int., %) = 819 (100) [M^+]; HRMS ($C_{42}H_{47}N_2O_{11}S_2$): 819.2612 (found M^+), 819.2616 (calc.).



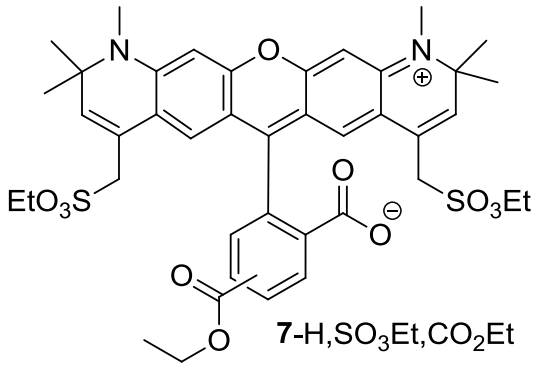
Allyl ethyl ester diethyl disulfonate 7-All,SO_3Et,CO_2Et was obtained from 5′-isomer of **7**-All,SO_3H,CO_2Et (540 mg, 0.64 mmol), Et_3O^+*BF_4^- (540 mg, 2.8 mmol) and *i*Pr_2NEt 0.6 mL (3.6 mmol) as described above for the corresponding dimethyl disulfonate (**7**-All,SO_3Me,CO_2Et). The product was isolated by chromatography on SiO_2 (100 g) with CH_2Cl_2/MeOH (20:1) mixture. Yield – 550 mg (96%) of the dark purple solid. HPLC: t_R = 17.9 min (5′-isomer; B/A: 30/70 → 100/0 in 25 min, 597 nm). 5′-isomer: ^{1}H NMR ($CDCl_3$, 300 MHz, ppm): δ = 1.28 (t, $^3J_{HH}$= 7.1, 6 H, OCH_2CH_3), 1.46 (t, $^3J_{HH}$= 7.1, 3 H, OCH_2CH_3), 1.51/1.52 (2×s, ∑12 H, CH_3), 3.12 (s, 6 H, NCH_3), 3.88 (s, 4 H, SCH_2), 4.12 (q, $^3J_{HH}$= 7.1, 4 H, OCH_2), 4.44 (q, $^3J_{HH}$= 7.1, 2 H, OCH_2), 4.53 (d, $^3J_{HH}$= 6.0, 2 H, OCH_2CH), 5.11–5.24 (m, 2 H, CH_2=), 5.67–5.83 (m, 1 H, CH=), 5.87 (br. s, 2 H, CH), 6.70 (s, 2 H, CH), 6.83 (s, 2 H, CH), 7.43 (d, $^3J_{HH}$= 8.0, 1 H, H-7′), 8.41 (dd, $^3J_{HH}$= 7.9, $^4J_{HH}$= 1.4, 1 H, H-6′), 8.90 (d, $^4J_{HH}$= 1.3, 1 H, H-4′); ESI-MS, positive mode: *m/z* (rel. int., %) = 847 (100) [M^+]; HRMS ($C_{44}H_{51}N_2O_{11}S_2$): 847.2941 (found *M*+), 847.2940 (calc.).



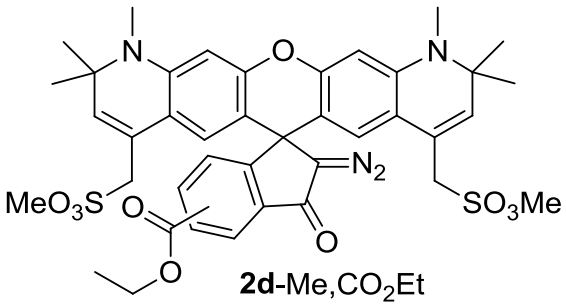
Ethyl ester dimethyl disulfonate 7-H,SO_3Me,CO_2Et. *Experiment 1.* The removal of the allyl protective group was carried out in an argon-flushed Schlenk flask charged with allyl ethyl ester dimethyl disulfonate **7**-All,SO_3Me,CO_2Et (18 mg, 22 μmol) in dry THF (1.1 mL) and Et_3NH(+)*HCOO(-) (0.2 mmol). Then 2 mg (2 μmol) of $(Ph_3P)_4$Pd in THF (dry, 0.25 mL) were added, and the reaction mixture was stirred under argon at room temperature for 2 h. HPLC: t_R = 13.8 min (**7**-H,SO_3Me,CO_2Et**)**, B/A: 30/70 – 100/0 in 25 min, 597 nm. After the reaction was complete (HPLC control), 1 mL of toluene was added, and the solution was concentrated in vacuo to ca. 1.2 mL. The crude product was converted into diazoketone without additional purification; Analytical sample of 6′-isomer was isolated by rapid

chromatography on SiO_2 with CH_2Cl_2/MeOH (20:1→3:1) mixture as an eluent: ^{1}H NMR (CD_3CN, 300 MHz, ppm): δ = 1.32 (t, $^3J_{HH}$= 7.1, 3 H, OCH_2CH_3), 1.49/1.50 (2×s, ∑12 H, CH_3), 3.09 (s, 6 H, NCH_3), 3.68 (s, 6 H, OCH_3), 4.01 (s, 4 H, SCH_2), 4.35 (q, $^3J_{HH}$= 7.1, 2 H, OCH_2CH_3), 5.93 (s, 2 H, CH), 6.80 (s, 2 H, CH), 7.22 (s, 2 H, CH), 7.94 (d, $^4J_{HH}$= 1.4, 1 H, H-7′), 8.29 (dd, $^3J_{HH}$= 8.0, $^4J_{HH}$= 1.4, 1 H, H-5′), 8.35 (d, $^3J_{HH}$= 8.0, 1 H, H-4′); ESI-MS, positive mode: *m/z* (rel. int., %) = 801 (100) $[M+Na]^+$; ESI-MS, negative mode: *m/z* (rel. int., %) = 777 (100) $[M–H]^–$.

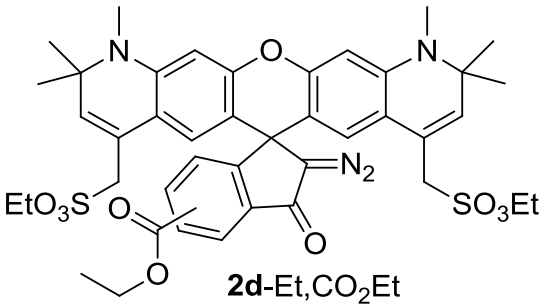
Experiment 2. An argon-flushed Schlenk flask was charged with allyl ethyl ester dimethyl disulfonate **7**-All,SO_3Me,CO_2Et (90 mg, 110 μmol) in dry THF (10 mL) and Et_3NH(+)*HCOO(-) (1 mmol: 1 mL of 1 M solution in THF). Then 21 mg (20 μmol) of $(Ph_3P)_4Pd$ were added, and the reaction mixture was stirred under argon at room temperature. HPLC showed a rapid conversion of the starting material (t_R = 17.1 min) into product (t_R = 14.1 min; B/A: 30/70 – 100/0 in 25 min, 597 nm) which was unstable and in 5 h partially converted into a new substance with t_R = 10.3 min (15% area in HPLC; t_R = 14.1 min: 74% area). The reaction mixture was concentrated in vacuo and applied onto a column with SiO_2 (50 g). Elution with CH_2Cl_2/MeOH (10:1→5:1) mixture separated the title product (t_R = 14.1 min) from the polar compound with t_R= 9.7 min (HPLC on reversed phase; B/A: 30/70 – 100/0 in 25 min, 597 nm) and afforded the sample with ca. 90% content of **7-H**,SO_3Me,CO_2Et. Pure fractions were combined; chlorobenzene was added (2 mL), and the solvents were evaporated in vacuo to the residual volume of ca. 1 mL.



Ethyl ester diethyl disulfonate 7-H,SO_3Et,CO_2Et was obtained from allyl ethyl ester diethyl disulfonate **7**-All,SO_3Et,CO_2Et (5′-isomer, 50 mg, 60 μmol), Et_3NH(+)*HCOO(-) (0.6 mmol) and $(Ph_3P)_4Pd$ (4.8 mg, 4.7 μmol) in dry THF as described above for compound **7**-H,SO_3Me,CO_2Et. After deallylation was complete, the mixture was concentrated to a volume of ca. 1 mL, and the residue was immediately placed on top of a column with 35 g SiO_2. The product was isolated by rapid chromatography with CH_2Cl_2/MeOH (20:1→3:1) mixture as an eluent. The main fraction was diluted with 20 mL toluene and evaporated at room temperature in vacuo. Yield–33 mg (68%) of a dark puple solid. HPLC: t_R = 15.3 min (5′-isomer), area 92%; B/A: 30/70 → 100/0 in 25 min, 254 nm. ESI-MS, positive mode: *m/z* (rel. int., %) = 807 (100) $[M+H]^+$.



Diazoketone 2d-Me,CO_2Et. To the cold (0°C) solution of **7**-H,SO_3Me,CO_2Et in chlorobenzene (1 mL), CH_2Cl_2 (5 mL) and $(COCl)_2$ (0.3 mL) were added at the temperature of an ice bath (evolution of gas was observed). Then the reaction mixture was warmed-up to room temperature. After stirring of the blue solution for 1 h at room temperature, DMF (1 drop) was added, and the reaction mixture was stirred for further 30 min. All volatile materials were removed in vacuo; the residue was kept at p<0.5 mbar, purged with argon, dissolved in dry CH_2Cl_2 (5 mL), and the solution of diazomethane in ether (0.3–0.4 M; 10 mL) was added at 0°C. After keeping the reaction mixture overnight at 0°C in the dark, volatile materials were removed in vacuo, and the residue was applied onto a column with SiO_2 (50 g). Elution with hexane–ethyl acetate (1:1) mixture afforded the title product **2d**-Me,CO_2Et (R_f ~ 0.3). Yield ~ 5 mg of yellow oil (5% over 2 steps). HPLC: t_R = 16.4 min, B/A: 50/50 → 100/0 in 25 min, 254 nm.

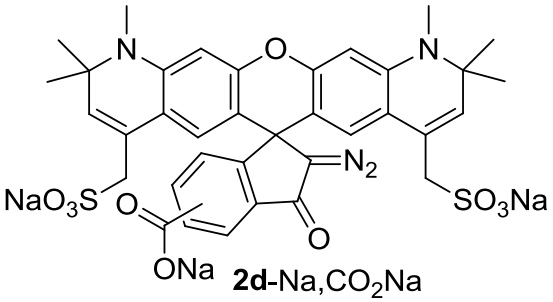


Diazoketone 2d-Et,CO_2Et. Allyl ethyl ester diethyl disulfonate **7**-All,SO_3Et,CO_2Et (116 mg of the mixture of 5´- and 6´-isomers) was obtained from allyl ethyl ester **7**-All,SO_3H,CO_2Et (5´- and 6´-isomers, 104 mg, 131 μmol), Et_3O^+*BF_4^- (130 mg, 0.67 mmol) and iPr$_2$NEt (0.14 mL, 0.86 mmol) in MeCN (2 mL). Rhodamine **7**-All,SO_3H,CO_2Et was placed into a dry Schlenk flasked purged with argon, the solvent was added followed by oxonium salt (as a solid) and a base. The reaction was complete in 1 h at room temperature (TLC). After evaporation of the volatile materials in vacuo, the residue was subjected to chromatography on regular SiO_2 (50 g). Elution with CH_2Cl_2/MeOH (20:1) afforded 116 mg (95%) of **7**-All,SO_3Et,CO_2Et^+*BF_4^- as a dark purple solid. Then **7**-All,SO_3Et,CO_2Et^+*BF_4^- (110 mg, 118 μmol), was dissolved in dry THF (6 mL) under argon, formic acid (48 μL, 59 mg, 1.27 mmol) was added followed by Et_3N (0.19 mL, 130 mg, 1.29 mL) and $(Ph_3P)_4Pd$ (10 mg, 10 μmol, dissolved in dry THF). The reaction was complete in several hours at room temperature (TLC control). The reaction mixture was directly applied onto a prepacked column with silica gel (50 g) equilibrated with CH_2Cl_2–MeOH mixture (10:1). Elution with CH_2Cl_2–MeOH (5:1) afforded magenta-colored fractions (50–100 mL fractions were collected) containing **7**-

H,SO_3Et,CO_2Et (a zwitter-ionic dye). The homogeneous fractions were combined, diluted with toluene (20 mL) and evaporated in vacuo to ca. 10 mL. They contained ethyl ester diethyl disulfonate **7**-H,SO_3Et,CO_2Et which cannot be isolated in a pure and solid state because it decomposed upon complete concentration of the solutions. Probably, one ethyl group migrated from the sulfonic acid residue to the carboxylic acid site, because the rearranged product had the same molecular mass (see above), but its retention time was much shorter than the retention times of **7**-H,SO_3Et,CO_2Et (its 5'- and 6'-isomers have similar retention times of 15.2–15.3 min; B/A: 30/70 → 100/0 in 25 min, 254 nm).

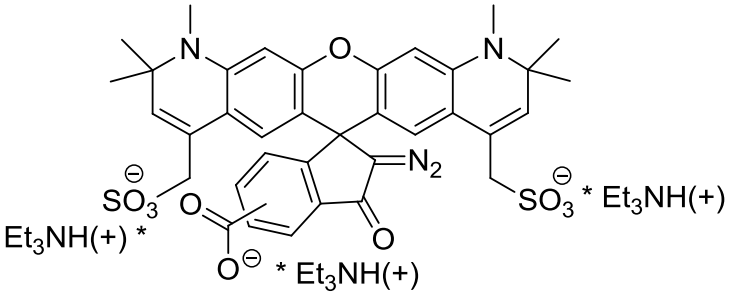
To the cold (0°C) solution of **7**-H,SO_3Et,CO_2Et in toluene (10 mL), $(COCl)_2$ (0.3 mL) was added at the temperature of an ice bath (evolution of gas was observed), and then the reaction mixture was warmed-up to room temperature. After stirring of the violet solution for 1 h at room temperature, DMF (1 drop) was added, and the reaction mixture was stirred for further 30 min. All volatile materials were removed in vacuo; the residue was kept at p<0.5 mbar, purged with argon, dissolved in dry CH_2Cl_2 (5 mL), and the solution of diazomethane in ether (0.3–0.4 M; 5 mL) was added at 0°C. After keeping the reaction mixture overnight at 0°C in the dark, volatile materials were removed in vacuo, and the residue was applied onto a column with SiO_2 (150 g). Elution with hexane–ethyl acetate (1:1) mixture afforded the title product **2d**-Et,CO_2Et as "isomer 1" (14 mg; higher R_f) and "isomer 2" (16 mg, lower R_f). Total yield – 30 mg (27% over 4 steps) of yellow-green solid. Under these separation conditions, it was possible to separate 5'- and 6'-isomers and remove the "dark" product obtained from **2d**-Et,CO_2Et after splitting of N_2 and rearrangement of the carbene intermediate to the seven-membered ring product (see Scheme 1 in the main text and the photolysis results described below for the model compounds). 5′-isomer: HPLC: t_R = 22.3 min, B/A: 30/70 → 100/0 in 25 min, 254 nm. ^{1}H NMR ($CDCl_3$, 300 MHz, ppm): δ = 1.18 (t, $^3J_{HH}$= 7.1, 6 H, CH_2**CH_3**), 1.31/1.34 (2×s, ∑12 H, CH_3), 1.37 (t, $^3J_{HH}$= 7.1, 3 H, OCH_2CH_3), 2.81 (s, 6 H, NCH_3), 3.85 (br. s, 4 H, SCH_2), 3.98 (q, $^3J_{HH}$= 7.1, 4 H, OCH_2), 4.37 (q, $^3J_{HH}$= 7.1, 2 H, OCH_2), 5.55 (br. s, 2 H, CH), 6.29 (s, 2 H, CH), 6.58 (s, 2 H, CH), 7.15 (d, $^3J_{HH}$= 8.1, 1 H, H-7′), 8.14 (dd, $^3J_{HH}$= 7.9, $^4J_{HH}$= 1.6, 1 H, H-6′), 8.46 – 8.47 (m, 1 H, H-4′); ^{13}C NMR (DMSO-d_6, 126 MHz, ppm): δ = 14.5 (CH_3), 15.5 (CH_3), 27.1 (CH_3), 27.6 (CH_3), 31.3 (NCH_3), 50.0 (C-1′), 53.0 (SCH_2), 57.5 (C), 61.8 (OCH_2), 68.1 (SO_3CH_2), 75.4 ($C=N_2$), 99.1 (CH) , 108.1 (C), 117.9 (C), 121.8 (C), 123.6 (CH), 124.4 (CH), 126.3 (CH), 131.4 (C), 135.5 (C), 135.7 (CH), 137.0 (CH), 147.2 (C), 152.8 (C), 160.6 (C), 165.8 (CO_2), 185.5 (CO); ESI-MS, positive mode: *m/z* (rel.

int., %) = 853 (100) [*M*+Na]$^+$; HRMS ($C_{42}H_{46}N_4O_{10}S_2$): 853.2552 (found *M*+Na), 853.2548 (calc.).



2d-Na,CO_2Na

Trisodium salt 2d-Na,CO_2Na. *From diazoketone **2d**-Me,CO_2Et.* **2d**-Me,CO_2Et (5 mg, 2.5 μmol) was dissolved in 1 mL MeOH, and 0.2 mL of 1 M aq. NaOH was added. The reaction mixture was heated at 50°C for 7 h and kept at room temperature for 24 h. After NaOH was neutralized by AcOH (15 μL), the reaction mixture was lyophilized, and the product was isolated on 50 g of RP-C18 with MeCN/H_2O (1:4, +40 mM HCOOH*Et_3N buffer) mixture. HPLC: t_R = 10.6 min (6′-isomer **2d**-H,COOH obtained under acidic conditions from **2d**-Na,CO_2Na), B/A: 20/80→50/50 in 25 min, 254 nm.

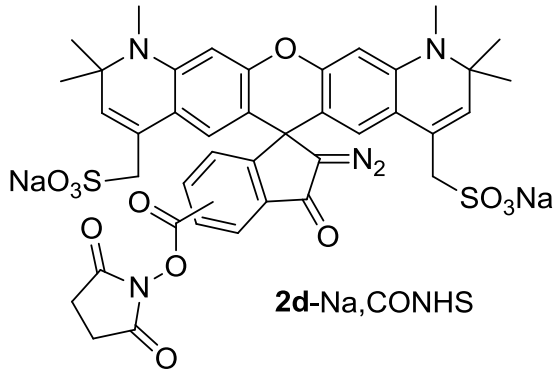


2d-NHEt$_3$,CO_2NHEt$_3$

Triethyl ammonium salt 2d-NHEt$_3$,CO_2NHEt$_3$ *From diazoketone **2d**-Et,CO_2Et via N-methyl-N'-ethylimidazolyl salt **2d**-N-Me-N'-Et-Im(+),CO_2Et.* To a solution of **2d**-Et,CO_2Et (13 mg, 16 μmol) in 5 mL MeCN, four drops of *N*-methylimidazole (2.5 mmol) were added, and the reaction mixture was stirred at 61 °C for 12 h (HPLC control). After the reaction was complete, the solvent and an excess of *N*-methylimidazole were removed in vacuo to afford dark oil. Saponification of the crude product was achieved without addition purification. HPLC: t_R = 6.0 min, B/A: 30/70 → 100/0 in 25 min, 254 nm; t_R = 16.9 min, B/A: 20/80 → 50/50 in 25 min, 254 nm.

The crude salt **2d**-*N*-Me-*N*'-Et-Im(+),CO_2Et was dissolved in EtOH (4 mL) at 0 °C, and 0.4 mL of 1 M aq. NaOH was added. The reaction mixture was kept at room temperature overnight. Glacial acetic acid (50 μL, 0.88 mmol) was added, the reaction mixture was lyophilized, and the product was isolated on 50 g RP-C18 with MeCN/H_2O mixture (1:7→1:5, pH 4÷5, Et_3N*TFA, ca. 30 μM). Direct liophylization of the eluate afforded dark oil (>100 mg). In order to remove the residual buffer (Et_3N*TFA), the dark oil was applied onto RP-C18 (50 g) equilibrated with pure water. Gradient elution with pure water – water/acetonitrile mixture (4/1) *without any buffer* resulted in elution of the fluorescent impurity followed by compound **2d**-NHEt$_3$,CO_2NHEt$_3$ (yellow band). Liophylization of the

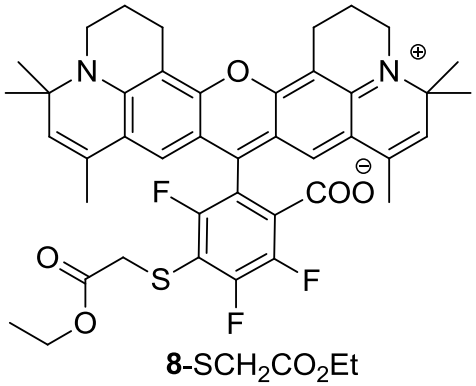
yellow fractions resulted in obtaining of the title triethyl ammonium salt as a very light dark grey-green powder. HPLC: t_R = 12.6 min (area 90%, 5′-isomer **2d**-H,COOH, obtained from Et_3N salt **2d**-$NHEt_3$,CO_2NHEt_3 in the course of HPLC with 0.1% TFA in the eluent), B/A: 20/80 → 50/50 in 25 min, 254 nm. 5´-isomer (Et_3N salt): ^{1}H NMR (D_2O, 300 MHz, ppm): δ = 1.29 (t, $^3J_{HH}$= 7.3, 27 H, 3×Et_3N), 1.32 (s, 12 H, CH_3), 2.77 (s, 6 H, NCH_3), 3.20 (q, $^3J_{HH}$= 7.3, 18 H, 3×Et_3N), 3.67 (d, $^3J_{HH}$= 14.3, 2 H, SCH_2), 3.83 (d, $^3J_{HH}$= 14.3, 2 H, SCH_2), 5.65 (br. s, 2 H, CH), 6.46 (s, 2 H, CH), 6.56 (d, $^3J_{HH}$= 8.0, 1 H, H-7′), 6.92 (s, 2 H, CH), 7.55 (d, $^3J_{HH}$= 8.0, 1 H, H-5′), 8.25 (s, 1H, H-4′); ESI-MS, positive mode: *m/z* (rel. int., %) = 785 (75) [*M*+K]$^+$, 807 (100) [*M*+Na+K–H]$^+$, 813 (35) [*M*+3Na–2H]$^+$, 835 (50) [*M*+4Na–3H]$^+$; HRMS (negative mode; $C_{36}H_{34}N_4O_{10}S_2$): 789.1278 (found *M*–3H+2Na), 789.1282 (calc.).



2d-Na,CONHS

***N*-Hydroxysuccinimidyl ester 2d-Na,CONHS. 2e**-Na,COONa (2 mg, 2,3 μmol) was suspended in 4 mL of anhydrous DMF under argon, and *N*-hydroxysuccinimide (17 mg, 0.15 mmol), HATU (14 mg, 37 μmol), and NEt_3 (10 μL, 72 μmol) were added sequentially to the reaction mixture at room temperature with stirring (HPLC control). The mixture was stirred for 1 h, and then the solvent and excess of NEt_3 were removed in vacuo. The title product was isolated by HPLC. t_R = 13.4 min (6′-isomer), t_R = 12.6 min (6′-isomer), B/A: 20/80 → 50/50 in 25 min, 254 nm. ESI-MS (6´-isomer), negative mode: *m/z* (rel. int., %) = 864 (100) [*M*+Na–2H]$^-$; HRMS ($C_{40}H_{37}N_5O_{12}S_2$): 420.5867 (found *M*–2H), 420.5867 (calc.).

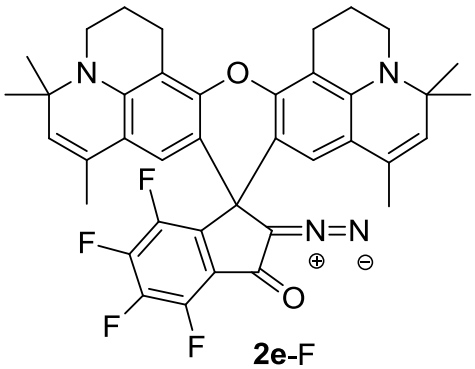
Scheme 5



8-SCH_2CO_2Et

Ester 8-SCH_2CO_2Et: Rhodamine **8**-F (160 mg, 0.25 mmol) was dissolved in dry MeCN (40 mL) in a Schlenk flask under argon, Et_3N (0.14 mL, 0.10 mmol) was added, and the stirred mixture was cooled to –15… –18°C. Then ethyl thioglycolate (0.12 mL, 1.1 mmol) was added dropwise with stirring, and the reaction mixture was stirred at –15… –12°C. After 1 h, HPLC analysis indicated that the reaction was complete; A/B (MeCN/H_2O, +0.1%TFA in both solvents): 80/20; isocratic mode, flow 1.2 mL/min, detection at 630 nm; **8**-F: t_R = 7.7 min, ester **8**-SCH_2CO_2Et: t_R = 7.2 min, disubstituted compound: t_R = 7.9 min. Acetic acid (1 mL) was added, and all volatile

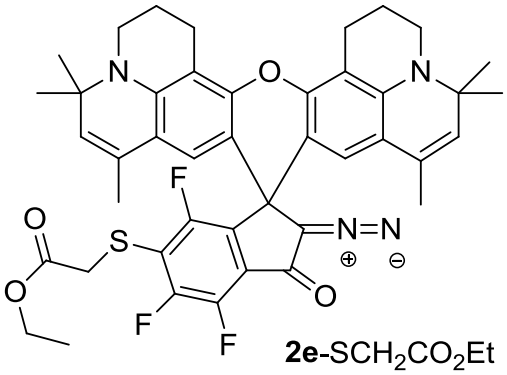
materials were evaporated in vacuo into a flask cooled in dry ice – acetone mixture. The residue was dissolved in DCM (100 mL), and the organic solution was washed with water (50 mL). After drying (Na_2SO_4) and evaporation of the solvent, the title compound was isolated as a dark-blue solid (170 mg, 92%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.19 (t, $^3J_{H,H}$ = 7.1 Hz, 3 H, C<u>H</u>$_3$CH$_2$), 1.44 (s, 12 H, CH_3), 1.86 (d, $^4J_{H,H}$ = 1.2 Hz, 6 H, C<u>H</u>$_3$CCH=), 2.02 (m, 4 H, CH_2), 2.93 (t, $^3J_{H,H}$ = 6.5 Hz, 4 H, CH_2), 3.50 (t, $^3J_{H,H}$ = 5.7 Hz, 4 H, CH_2N), 3.53 (s, 2 H, CH_2S), 4.08 (q, $^3J_{H,H}$ = 7.2 Hz, 2 H, CH$_3$C<u>H</u>$_2$O), 5.38 (q, $^4J_{H,H}$ = 1.2 Hz, 6 H, CH$_3$C<u>H</u>=), 6.86 (s, 2 H, H^{ar}) ppm. ^{19}F NMR ($CDCl_3$, 282.4 MHz) δ = -110.2 (br. s, 1 F), -124.3 (d, $^3J_{F,F}$ = 25 Hz, 1 F), -139.2 (br. s, 1 F) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 14.0 (<u>C</u>H$_3$CH$_2$), 18.6 (<u>C</u>H$_3$CH=), 20.1 (CH$_2$<u>C</u>H$_2$CH$_2$), 20.6 (CH_2Ar), 28.7/28.8 (<u>C</u>H$_3$C), 35.9 (CH_2S), 43.2 (CH_2N), 59.3 (C_qN), 61.6 (CH_2O), 110.0 (C), 114.0 (C), 121.4 (CH), 122.5 (C), 126.6 (C), 131.0 (CH), 150.2 (C), 153.7 (C), 163.4 (COO), 168.4 (<u>C</u>O$_2$Et) ppm. ESI-MS, positive mode: *m/z* (rel. int., %) = 743 (100) [*M*+H]$^+$, 765 (19) [*M*+Na]$^+$. HRMS ($C_{42}H_{41}F_3N_2O_5S$): 743.2757 (found *M*+H), 743.2761 (calc.).



2e-F

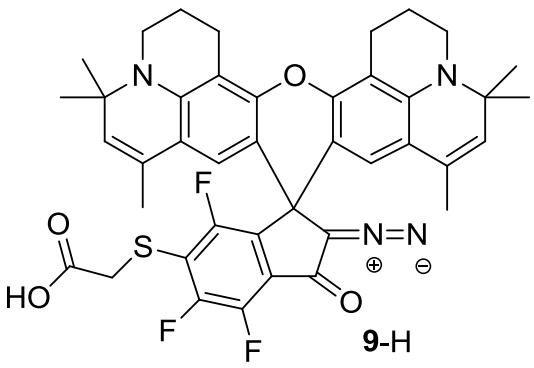
Diazoketone 2e-F: Rhodamine dye **8**-F (100 mg, 0.155 mmol) was dissolved in dry CH_2Cl_2 (40 mL), and $(COCl)_2$ (1.00 mL, 1.50 g, 11.8 mmol) was added to this solution at 0°C followed by DMF (20 µL). The reaction mixture was warmed-up to room temperature, one more drop of DMF (20 µL) was added, and the solution was stirred at room temperature for 2 h. Volatile materials were evaporated in vacuo, and the residue was kept at 0.2 mbar for 30 min. Then it was dissolved in dry CH_2Cl_2 (50 mL), the solution was cooled to 0°C, and 20 mL of cold ~ 0.3 M diazomethane solution (in ether) was added. The reaction mixture was stirred overnight in the dark at 0°C; all volatiles were carefully evaporated in vacuo, the residue was dissolved in minimal amount of CH_2Cl_2 and applied onto a prepared column with SiO_2 (100 mL). Elution with CH_2Cl_2/hexane/EtOAc (20/10/1) gave 46 mg of the title compound as a pure fraction, and 30 mg of impure fraction. The latter was purified once more using hexane/CH_2Cl_2/EtOAc (5:1:0.1) mixture as eluent, and all pure fractions were combined and recrystallized from CH_2Cl_2 – hexane mixture. Yield – 21 mg (20%) of the title product as yellow-green solid. ^{1}H NMR (CD_3COCD_3, 300 MHz): δ = 1.29/1.31 (s×2, 12 H, CH_3), 1.74 (d, $^4J_{H,H}$ = 1.4 Hz, 6 H, C<u>H</u>$_3$CCH=), 1.95 (m, 4 H, CH_2), 2.84 (m, 4 H, CH_2), 3.33 (m, 4 H, CH_2N), 5.21 (q, $^4J_{H,H}$ = 1.4 Hz, 6 H, CH$_3$C<u>H</u>=), 6.58 (s, 2 H, H^{ar}) ppm. ^{19}F NMR (CD_3COCD_3, 282.4 MHz): δ = -139.0 (m, 1 F), -140.2 (m, 1 F), -143.7

(m, 1 F), 151.7 (m, 1 F) ppm. ^{13}C NMR (CD_3COCD_3, 75 MHz): δ = 18.7 ($\underline{C}H_3CH$=), 21.9/22.3 ($CH_2\underline{C}H_2CH_2$ and CH_2Ar), 27.5/27.8 ($\underline{C}H_3C$), 42.1 (CH_2N), 56.9 (C_qN), 104.9 (C), 109.1 (C), 119.8 (C), 120.0 (CH), 127.9 (C), 129.6 (CH), 143.6 (C), 149.7 (C), 210.2 (C=O).



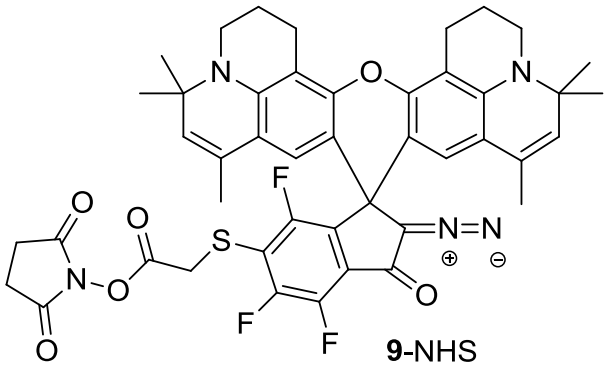
2e-SCH_2CO_2Et

Diazoketone 2e-SCH_2CO_2Et: Rhodamine dye **8**-SCH_2CO_2Et (130 mg, 0.175 mmol) was dissolved in dry CH_2Cl_2 (10 mL), and $(COCl)_2$ (1.00 mL, 1.50 g, 11.8 mmol) was added to this solution at 0°C in five equal portion followed by DMF (0.10 mL). The reaction mixture was warmed-up to room temperature, one more portion of DMF (0.10 mL) was added, and the solution was stirred at room temperature for 2 h. Volatile materials were carefully evaporated in vacuo (1.4 mbar) into a trap cooled with a mixture of dry ice and acetone, and the residue was kept at 0.5 mbar for 30 min. Then it was dissolved in dry CH_2Cl_2 (20 mL), the solution was cooled to 0°C, and 12 mL of cold ~ 0.3 M diazomethane solution (in ether) was added. Strong evolution of gas (N_2) was observed during addition of CH_2N_2. The reaction mixture was stirred overnight in the dark at 0°C; all volatiles were evaporated in vacuo, the residue was dissolved in minimal amount of CH_2Cl_2 and applied onto a prepared wide column with SiO_2 (200 mL). Elution with hexane/EtOAc (1/1) gave 49 mg (37%) of the title compound as brown-green glass-like material. Evaporation of the pooled homogeneous fractions in vacuo produced foam which "collapsed" into a gum, when air was introduced into a flask. HPLC: A/B (MeCN/H_2O, +0.1%TFA in both solvents): 80/20 – 100/0 in 25 min, flow 1.2 mL/min, detection at 254 nm; t_R = 18.9 min (area 93%), impurities with t_R = 13.1 min and t_R = 14.8 min. Trituration of this glass-like foam with methanol gave a pure product as yellow solid. ^{1}H NMR (CD_3OD, 400 MHz): δ = 0.96 (t, $^3J_{H,H}$ = 7.1 Hz, 3 H, $C\underline{H}_3CH_2$), 1.27/1.28 (s×2, 12 H, CH_3), 1.74 (d, $^4J_{H,H}$ = 1.4 Hz, 6 H, $C\underline{H}_3CCH$=), 1.95 (m, 4 H, CH_2), 2.84 (m, 4 H, CH_2), 3.30 (m, CH_2N, overlaps with CHD_2-multiplet of the solvent), 3.54 (s, 2 H, CH_2S), 3.79 (q, $^3J_{H,H}$ = 7.1 Hz, 2 H, $CH_3C\underline{H}_2O$), 5.18 (q, $^4J_{H,H}$ = 1.4 Hz, 6 H, $CH_3C\underline{H}$=), 6.39 (s, 2 H, H^{ar}) ppm. ^{19}F NMR ($CDCl_3$, 377 MHz): δ = -115.7 (d, $^3J_{F,F}$ = 20.3 Hz), -128.6 (d, $^3J_{F,F}$ = 22.0 Hz), -146.7 (t, $^3J_{F,F}$ = 21 Hz) ppm. ESI-MS, positive mode: *m/z* (rel. int., %) = 767 (100) $[M+H]^+$, 789 (80) $[M+Na]^+$. HRMS ($C_{43}H_{41}F_3N_4O_4S$): 767.2870 (found *M*+H), 767.2873 (calc.).



9-H

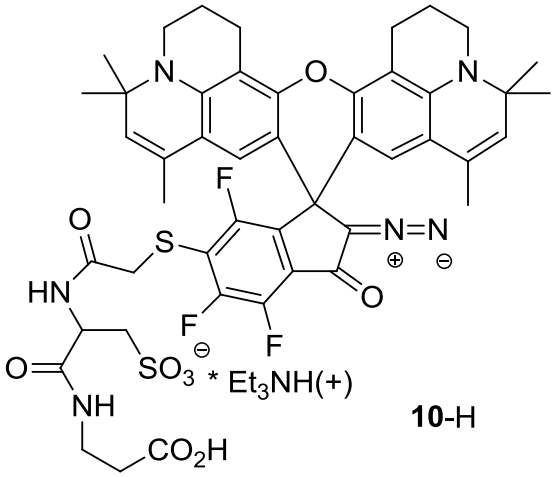
Carboxylic acid 9-H: diazoketone **2e**-SCH_2CO_2Et (49 mg, 64 μmol) was dissolved in THF (5.5 mL), water (0.75 mL) was added, and the solution was cooled to 0°C. Then 1 M aq. NaOH (0.25 mL) was added dropwise at 0°C, and the nearly homogeneous solution was stirred overnight at +5…+7°C. It turned to be darker; HPLC indicated full conversion to a product with t_R = 11.8 min (for HPLC conditions, see preparation of ester **2e**-SCH_2CO_2Et). Glacial AcOH (20 μL) was added to the reaction mixture, and THF was removed in vacuo. The residue was acidified with 5% aq. citric acid to pH=3, and the title compound was extracted with CH_2Cl_2 (2×15 mL). The green–brown organic solution was dried with Na_2SO_4 at 0° in the dark, diluted with hexane (10 mL) and evaporated in vacuo at 15–25°C (bath temp.). Yield – 44 mg (94%) of the title compound as a tan solid. ESI-MS, negative mode: *m/z* (rel. int., %) = 737 (100) $[M–H]^-$. HRMS ($C_{41}H_{37}F_3N_4O_4S$): 737.2424 (found *M*–H), 737.2415 (calc.).



9-NHS

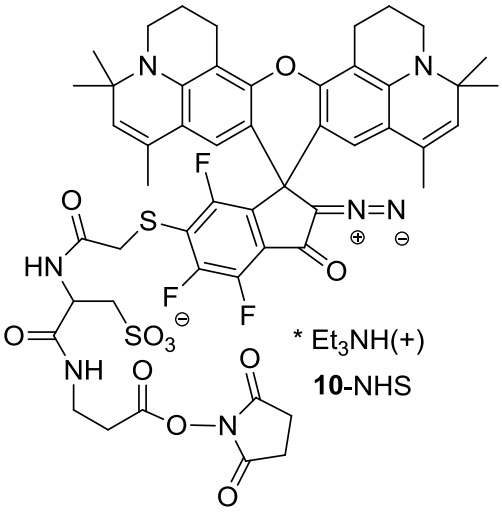
N-Hydroxysuccinimidyl ester 9-NHS and amidocarboxylic acid 10-H: carboxylic acid **9**-H (42 mg, 57 μmol) was dissolved in dry DMF (1 mL), *N*-hydroxysuccinimide (15 mg, 0.13 mmol) was added followed by HATU (38 mg, 0.10 mmol) and Et_3N (20 μL, 0.14 mmol). The mixture was stirred for 2.5 h at room temperature. HPLC analysis indicated that the reaction was nearly complete: t_R = 13.0 min (**9**-NHS; peak area 92%), t_R = 11.9 min (starting material; peak area 4%); for HPLC conditions, see preparation of ester **2e**-SCH_2CO_2Et. Compound **9**-NHS is unstable on regular silica gel: it decomposes partially in the course of chromatography (hexane – ethyl acetate, 1:1). Therefore, it was used without isolation for the preparation of compound **10**-H. For that, "hydrophylisator" ($H_2NCH(CH_2SO_3H)CONH(CH_2)_2CO_2H$ = $C_6H_{12}N_2O_6S$, 60 mg, 0.25 mmol) was added to the reaction mixture, followed by Et_3N (80 μL, 0.56 mmol), and the mixture was stirred at room temperature overnight. HPLC analysis indicated that NHS-ester **9**-NHS was consumed: t_R = 4.2 min (**10**-H; peak area 86%), t_R = 11.9 min (starting material; peak area 4%); for HPLC conditions, see preparation of ester **2e**-SCH_2CO_2Et. DMF was evaporated in high-vacuum (ca. 0.5-1 mbar), the residue was dissolved in eluent ($CHCl_3$/MeOH/H_2O, 65/35/5) and applied onto a column with silica gel (100 mL). Elution of the column followed by pooling together the homogeneous fractions (TLC analysis with UV detection + photoactivation with UV light) afforded compound **10**-H as yellow-green powder (40 mg, 74%). UV (aqueous MeOH): λ_{max}

(nm) = 270 (ε = 5.36×10^4 M^{-1} cm^{-1}), 306 (ε = 1.68×10^4 M^{-1} cm^{-1}). ^{1}H NMR (400 MHz, CD_3OD): δ = 1.26/1.27/1.28/1.29 (4×s, Σ 12 H, CH_3), 1.71/1.72 (d×2, Σ 6 H, C$\underline{H}_3$C=CH, $^4J_{H,H}$ = 1.4 Hz), 1.94 (m_c, 4 H, $CH_2C\underline{H}_2CH_2$), 2.32 (m, 2 H, CH_2COO), 2.82 (m, 4 H, CH_2CO), 3.01 (dd, $^2J_{H,H}$ = 14.4 Hz, $^3J_{H,H}$ = 4.9 Hz, 1 H, C$\underline{H}^AH^BSO_3$), 3.14 (dd, $^2J_{H,H}$ = 14.5 Hz, $^3J_{H,H}$ = 7.1 Hz, 1 H, $CH^A\underline{H}^BSO_3$), 3.34 (m, NCH_2×2+C$\underline{H}_2$NH, overlaps with C$\underline{H}D_2$ in CD_3OD), 3.65 (A-part of AB-system, 1 H, $^2J_{H,H}$ = 14.9 Hz, ArSC$\underline{H}^AH^B$), 3.72 (B-part of AB-system, 1 H, $^2J_{H,H}$ = 14.9 Hz, $ArSCH^A\underline{H}^B$), 4.43 (dd, 1 H, $^3J_{H,H}$ = 7.0 and 4.9 Hz), 5.24 (m, 2 H, CH=), 6.37/6.40 (s×2, Σ 2 H, H$^{ar.}$) ppm. ^{19}F NMR (CD_3OD, 376.4 MHz) δ = -116.4 (d, $J_{F,F}$ = 18.8 Hz), -127.8 (d, $J_{F,F}$ = 21.9 Hz), -146.1 (t, $J_{F,F}$ = 20.8 Hz) ppm. ESI-MS, negative mode: *m/z* (rel. int., %) = 959 (100) [*M*–H]$^-$, HRMS ($C_{47}H_{47}F_3N_6O_9S_2$): 959.2737 (found *M*-H), 959.2725 (calc.).



10-H

Irradiation of the methanolic solution of diazoketone **10**-H with UV-light of the middle pressure mercury lamp with pyrex filter led to a fluorescent product with absorption maximum at 630 nm. Absorption coefficient of the new band (ε = 3.77×10^4 M^{-1} cm^{-1}) was calculated after full conversion of the starting material (HPLC control). HPLC: A/B (MeCN/H_2O, +0.1%TFA in both solvents): 50/50 – 100/0 in 25 min, flow 1.2 mL/min, detection at 254 nm; **10**-H: t_R = 12.4 min (initial area 94%), final product of the photolysis in methanol: t_R = 8.9 min (methyl ester); intermediate: t_R = 16.4 min. Upon completion of the photolysis, the intermediate disappeared, and the content of **10**-H decreased to 3.7% (HPLC area). ESI-MS of the reaction solution after photolysis (negative mode): *m/z* (rel. int., %) = 963 (70) [*M*–H for methyl ester with $C_{48}H_{51}F_3N_4O_{10}S_2$ and M=964], 964 (100) [?], 965 (40; isotopic peak). Emission maximum (λ_{em}) at 648 nm; Φ_{fl} = 0.74 (reference – KK114 dye with Φ_{fl} = 0.53 in water).

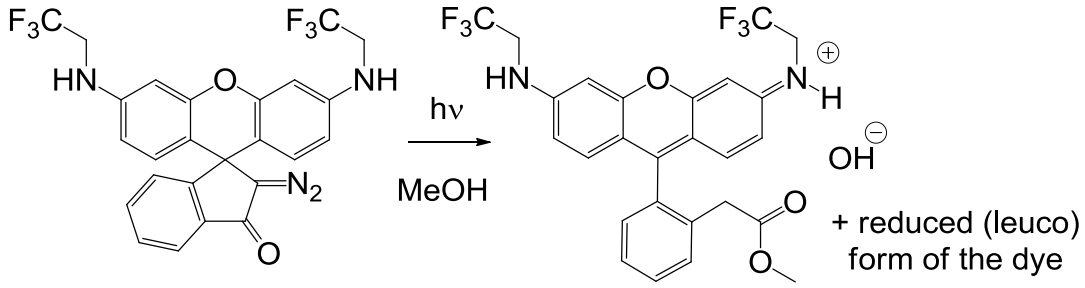


10-NHS

***N*-Hydroxysuccinimidyl ester 10-NHS:** carboxylic acid **10**-H (9.6 mg, 10 µmol) was dissolved in dry DMF (0.5 mL) under argon in a Schlenk flask, *N*-hydroxysuccinimide (12 mg, 0.1 mmol) was added followed by HATU (19 mg, 50 µmol) and Et_3N (10 µL, 70 µmol). The solution was flushed with argon and stirred at room temperature in the dark for 1 h, and then the degree of conversion was controlled by

HPLC. If the conversion of **10**-H to **10**-NHS was not full, 10 µL of Et_3N were added followed by HATU (9 mg). After HPLC indicated the full conversion of the starting acid **10**-H (t_R = 12.4 min) into a product (**10**-NHS) with t_R = 14.2 min (MeCN/H_2O (+0.1% TFA in both solvents): 50/50 – 100/0 in 25 min, flow 1.2 mL/min, detection at 254 nm), DMF and excess of Et_3N were removed in vacuo (0.1 mbar), and the residue was flushed with argon and stored at –20°C. If necessary, compound **10**-NHS can be isolated as green-yellow oil by preparative HPLC followed by careful neutralization of TFA with Et_3N (to pH = 4) and liophylization. Content of **10**-NHS in residual buffer (ca. 1 mg in 30 µL Et_3N*CF_3CO_2H) is determined by measuring absorption at 306 nm ($\varepsilon = 1.68\times10^4$ M^{-1} cm^{-1}). ESI-MS, negative mode: *m/z* (rel. int., %) = 1056 (100) [*M*–H]$^-$, HRMS ($C_{51}H_{50}F_3N_7O_{11}S_2$): 1056.2870 (found *M*–H), 1056.2889 (calc.).

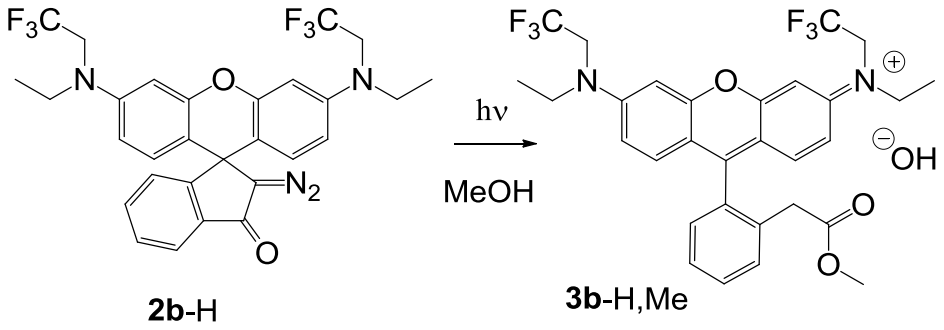
Preparative photolysis experiments according to Scheme 1



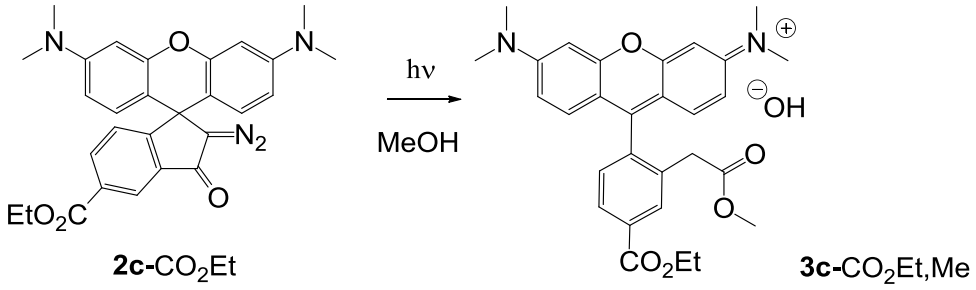
2a-H **3a**-H,Me

*Photolysis of diazoketone **2a**-H.* A solution of compound **2a**-H (10 mg, 0.02 mmol) in methanol (250 mL) was placed into a reactor for photolysis, and argon was bubbled through the solution for 30 min at room temperature with stirring. Then the middle-pressure mercury lamp (150 W) was turned on, and the solution was irradiated through a Pyrex filter at room temperature with stirring under argon for 15 min, until compound **2a**-H fully reacted (HPLC or TLC control). The solvent was removed in vacuo, the residue was dissolved in CH_2Cl_2 (1−2 mL), and the "bright" (fluorescent) product **3a**-H,Me was isolated by column chromatography on regular SiO_2 (MeCN/H_2O/CH_2Cl_2 20:1:1). Yield - 3 mg (29%) of red-orange solid. HPLC: t_R = 6.6 min, A/B: 50/50 → 0/100 in 25 min, detection at 254 nm. UV-VIS (MeOH): λ_{max} = 508 nm ($\varepsilon = 5.08*10^4$), λ_{em} = 533 nm, Φ_{fl} = 0.74. ^{1}H NMR (CD_3CN, 400 MHz, ppm), δ = 3.34 (s, 3 H, CO_2CH_3), 3.40 (s, 2 H, CH_2CO_2), 4.20 (q, J = 8.5, 4 H, NCH_2), 7.04 (dd, $^3J_{H,H}$ = 9.2, $^4J_{H,H}$ =2.3 Hz, 2 H, H-2/7), 7.09 (d, $^4J_{H,H}$ = 2.3 Hz, 2 H, H-4/5), 7.15 1 – 7.21 (m, 3 H, NH, OH), 7.25 (d, $^3J_{H,H}$ = 9.3 Hz, 1 H, H-1/8), 7.29 (d, $^3J_{H,H}$ = 7.0 Hz, 1 H, H^{ar}),

7.55−7.61 (m, 2 H, H^{ar}), 7.64−7.70 (m, 1 H, H^{ar}) ppm. ^{19}F NMR (CD_3CN, 376.4 MHz) δ = -76.7 (s) ppm. ESI-MS, positive mode: *m/z* (rel. int., %) = 523 (100) $[M+H]^+$; ESI-MS, negative mode: *m/z* (rel. int., %) = 521 (100) $[M-H]^-$. $C_{26}H_{20}F_6N_2O_3$, HR-MS (ESI, positive mode): 523.1449 $[M+H]^+$ (found), 523.1456 (calculated).

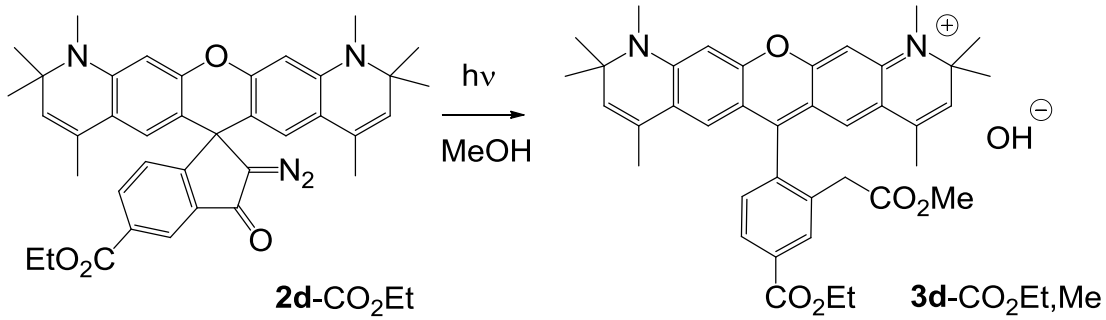


*Photolysis of diazoketone **2b**-H.* A solution of diazoketone **2b**-H (12 mg, 0.021 mmol) in methanol (250 mL) was irradiated at room temperature with stirring under argon for 40 min, until the starting compound was consumed (HPLC or TLC control). Then the solvent was removed in vacuo, the residue was dissolved in CH_2Cl_2 (1−2 mL), and the fluorescent product **3b**-H,Me was isolated by column chromatography on SiO_2 ($MeCN/H_2O/CH_2Cl_2$, 20:1:1→10:1:1). Yield - 4 mg (33%) of red solid; HPLC: t_R = 11.2 min, A/B: 50/50 → 0/100 in 25 min, detection at 254 nm. UV-VIS (MeOH): λ_{max} = 532 nm (ε = 82300), λ_{em} = 555 nm, Φ_{fl} = 0.84. ^{1}H NMR (CD_3CN, 400 MHz, ppm), δ = 1.37 (t, *J* = 7.0, 3 H, CH_3), 3.34 (s, 3 H, CO_2CH_3), 3.51 (s, 2 H, CH_2CO_2), 3.97 (t, *J* = 7.0, 4 H, NCH_2CH_3), 4.75 (q, *J* = 8.9, 4 H, NCH_2CF_3), 7.35−7.41 (m, 4 H), 7.42−7.50 (m, 3 H), 7.59−7.73 (m, 3 H). ESI-MS, positive mode: *m/z* (rel. int., %) = 579 (100) $[M]^+$. $C_{30}H_{29}F_6N_2O_3$(+), HR-MS (ESI, positive mode): 579.2078 $[M]^+$ (found), 579.2077 (calculated).

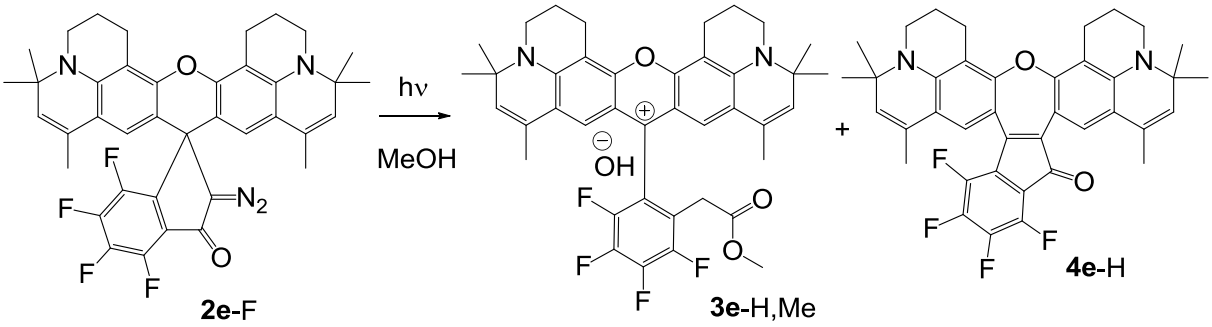


*Photolysis of diazoketone **2c**-CO_2Et.* A solution of diazoketone **2c**-CO_2Et (27 mg, 0.056 mmol, 86/14 mixture of 5'- and 6'-isomers) in MeOH (250 mL) was irradiated for 35 min at room temperature with stirring under argon, until the starting compound fully reacted (TLC or HPLC control). HPLC (**2c**-CO_2Et): t_R = 12.9 and 13.8 min for 6'- and 5'-isomers, respectively,

A/B: 50/50 → 0/100 in 25 min, detection at 254 nm. The solvent was removed in vacuo, the residue was dissolved in CH_2Cl_2, and the "bright" product **2c**-CO_2Et,Me was isolated by column chromatography (CH_2Cl_2/MeOH 5:1, then CH_2Cl_2/MeOH 1:1). Yield - 7 mg (24%) of dark red solid. HPLC: t_R = 7.9 and 8.2 min for 6'- and 5'-isomers, respectively, A/B: 50/50 → 0/100 in 25 min, detection at 254 nm. UV-VIS (MeOH): λ_{max} = 558 nm (ε = 42500), λ_{em} = 584 nm, Φ_{fl} = 0.44. ^{1}H NMR (CD_3CN, 400 MHz, ppm), δ = 1.40 (t, J = 7.1, 3 H, CH_3), 3.25 (s, 12 H, NCH_3), 3.35 (s, 3 H, CO_2CH_3), 3.47 (s, 2 H, CH_2CO_2), 4.42 (t, J = 7.1, 2 H, OCH_2CH_3), 6.84 (d, J = 2.3, 2 H, H-4/5), 6.96 (dd, J = 2.3 and 9.6, 2 H, H-2/7), 7.07 (d, J = 9.5, 2 H, H-1/8), 7.39 (d, J = 7.9, 1 H, H-7'), 8.13 (dd, J = 1.6 and 7.9, 1 H, H-6'), 8.18 (d, J = 1.6, 1 H, H-4'). ESI-MS, positive mode: *m/z* (rel. int., %) = 487 (100) $[M]^+$. $C_{29}H_{31}N_2O_5$(+), HR-MS (ESI, positive mode): 487.2235 $[M]^+$ (found), 487.2227 (calculated).



*Photolysis of diazoketone **2d**-CO_2Et.* A solution of compound **2d**-CO_2Et (14 mg, 0.023 mmol) in MeOH (250 mL) was irradiated at room temperature with stirring under argon for 8 min. TLC indicated that the starting material reacted fully. HPLC (**2d**-CO_2Et): t_R = 21.6 and 22.9 min for two isomers, A/B: 20/80 → 0/100 in 25 min, detection at 254 nm. The solvent was removed in vacuo, and the residue was dissolved in ca. 1 mL of MeCN/H_2O/CH_2Cl_2 mixture (10:1:1). The fluorescent product **3d**-CO_2Et,Me was isolated by column chromatography (MeCN/H_2O/CH_2Cl_2, 10:1:1, then MeCN/H_2O, 4:1). Yield - 12 mg (84%) of the dark violet solid. HPLC (**3d**-CO_2Et,Me): t_R = 6.9 and 7.5 min for two isomers, A/B: 20/80 → 0/100 in 25 min, detection at 254 nm. UV-VIS (MeOH): λ_{max} = 596 nm (ε = 61600), λ_{em} = 619 nm, Φ_{fl} = 0.72. ^{1}H NMR (acetone-d_6, 400 MHz, ppm), δ = 1.42 (t, J = 7.1, 3 H, CH_3), 1.54 (s, 12 H, CH_3), 1.76 (d, J = 1.4, 6 H, CH_3), 3.30 (s, 9 H, NCH_3 and CO_2CH_3), 3.37 (s, 2 H, CH_2CO_2), 4.44 (t, J = 7.1, 2 H, OCH_2), 5.75 (s, 2 H, CH), 6.70 (s, 2 H, H^{ar}), 6.87 (s, 2 H, H^{ar}), 7.55 (d, J = 7.9, 1 H, H-7'), 8.19 (dd, J = 1.7 and 7.9, 1 H, H-6'), 8.26 (d, J = 1.7, 1 H, H-4'). ESI-MS, positive mode: *m/z* (rel. int., %) = 619 (100) $[M]^+$. $C_{39}H_{43}N_2O_5$(+), HR-MS (ESI, positive mode): 619.3169 $[M]^+$ (found), 619.3166 (calculated).



*Photolysis of diazoketone **2e**-F*. A solution of compound ***2e**-F* (18 mg, 0.028 mmol) in MeOH (250 mL) was irradiated at room temperature with stirring under argon for 10 min, until the starting compound fully reacted (HPLC). Then the solvent was removed in vacuo, the residue was dissolved in minimal amount of $MeCN/H_2O/CH_2Cl_2$ (10:1:1), and subjected to chromatography on SiO_2. Elution with $MeCN/H_2O/CH_2Cl_2$ (10:1:1) afforded two products. The first fraction contained the "dark" product **4e**-H. HPLC (A/B: 20/80 → 0/100 in 25 min, detection at 254 nm): t_R = 29.9 min. $C_{39}H_{34}F_4N_2O_2$, HR-MS (ESI, positive mode): 638.2556 $[M]^{+\cdot}$ (found), 638.2551 (calculated). It is typical for all "dark" products **4a**-**4e** that their ESI mass-spectra in a positive mode contain *both* peaks M^+ (it corresponds, in fact, to a cation-radical $M^{+\cdot}$) and $[M+H]^+$ (cation).

The fraction with lower R_f contained the "bright" fluorescent compound **3e**-H,Me; yield - 4 mg (21%) of blue solid. HPLC (A/B: 20/80 → 0/100 in 25 min, detection at 254 nm): t_R = 10.9 min. UV-VIS (MeOH): λ_{max} = 632 nm (ε = 31400), λ_{em} = 651 nm, Φ_{fl} = 0.94. ^{1}H NMR (CD_3CN, 400 MHz, ppm), δ = 1.48 (s, 12 H, CH_3), 1.84 (d, J = 1.5, 6 H, CH_3), 2.00−2.06 (m, 4 H, CH_2), 2.99 (td, J = 6.4, 3.7, 4 H, $ArCH_2$), 3.42 (s, 3 H, CO_2CH_3), 3.44 (d, J = 2.0, 2 H, CH_2CO_2), 3.58−3.64 (m, 4 H, NCH_2), 5.64 (d, J = 1.5, 2 H, CH), 6.57 (s, 2 H, H^{ar}). ESI-MS, positive mode: *m/z* (rel. int., %) = 671 (100) $[M]^+$. $C_{40}H_{39}F_4N_2O_3$(+), HR-MS (ESI, positive mode): 671.2894 $[M]^+$ (found), 671.2891 (calculated).

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[2] C. A. Wurm, D. Neumann, M. A. Lauterbach, B. Harke, A. Egner, S. W. Hell, S. Jakobs, *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 13546–13551.

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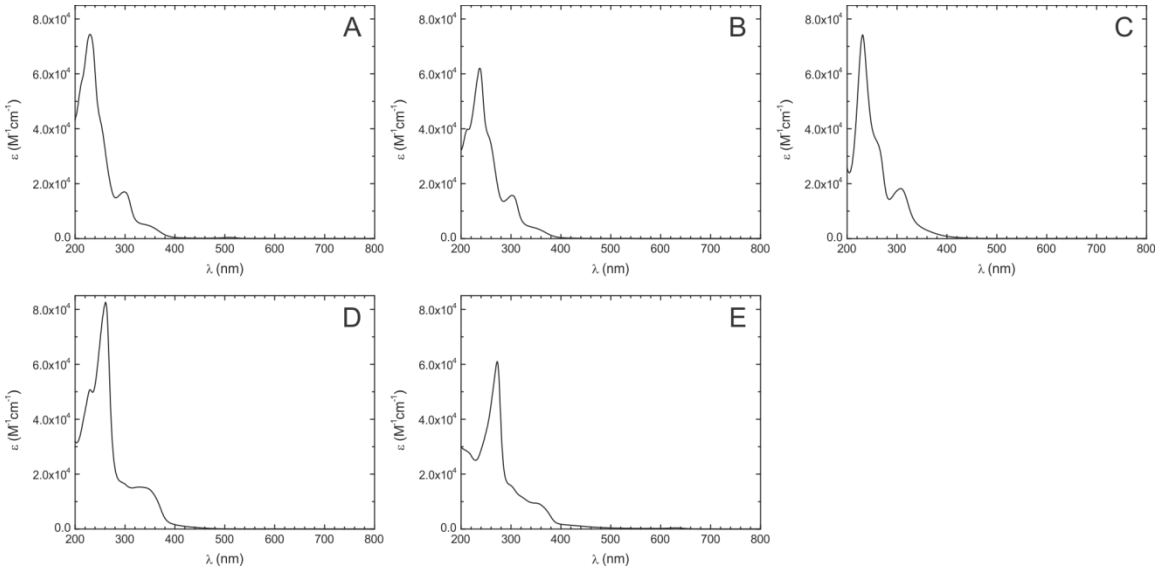
Details of immunofluorescence and STED microscopy. To show the applicability of the caged dyes with 2-diazo-1-indanone group in STED nanoscopy, we labeled the nuclear pore complex protein Nup153 in mammalian cells (Vero cell line) by indirect immunofluorescence. The primary antibody against Nup153 was visualized by a secondary antibody that was coupled with the caged carbopyronine dye KK1012[6] (for structure, see Figure 4 in the main text).

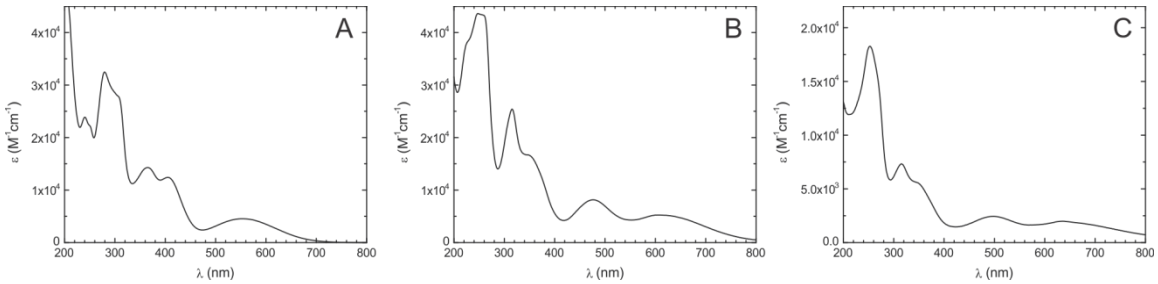
For sub-diffraction imaging, the dye was unmasked by using either bright-field UV-illumination at 375 nm, or applying only 750 nm depletion laser (photoactivation in a two-photon mode). The fluorescence of the dye was excited at 638 nm, depleted at 750 nm and detected in an emission window of 675±60 nm. Image acquisition was performed by stage-scanning in one and beam scanning in the other spatial direction in a region of interest (ROI) of 9×6 μm² and a pixel size of 20 nm. To reduce photobleaching, 200 frames per ROI were recorded in fast-scanning mode with a respective pixel dwell time of 1 μs and subsequently summed up to yield the image.

The confocal reference image of the object stained with the still uncaged dye KK1012 was also obtained (under conditions described above; yet only one frame of the ROI with a pixel dwell time of 150 μs was recorded). It proved that most of the dye residues were not yet photoactived, before STED pulse or UV-light were applied.

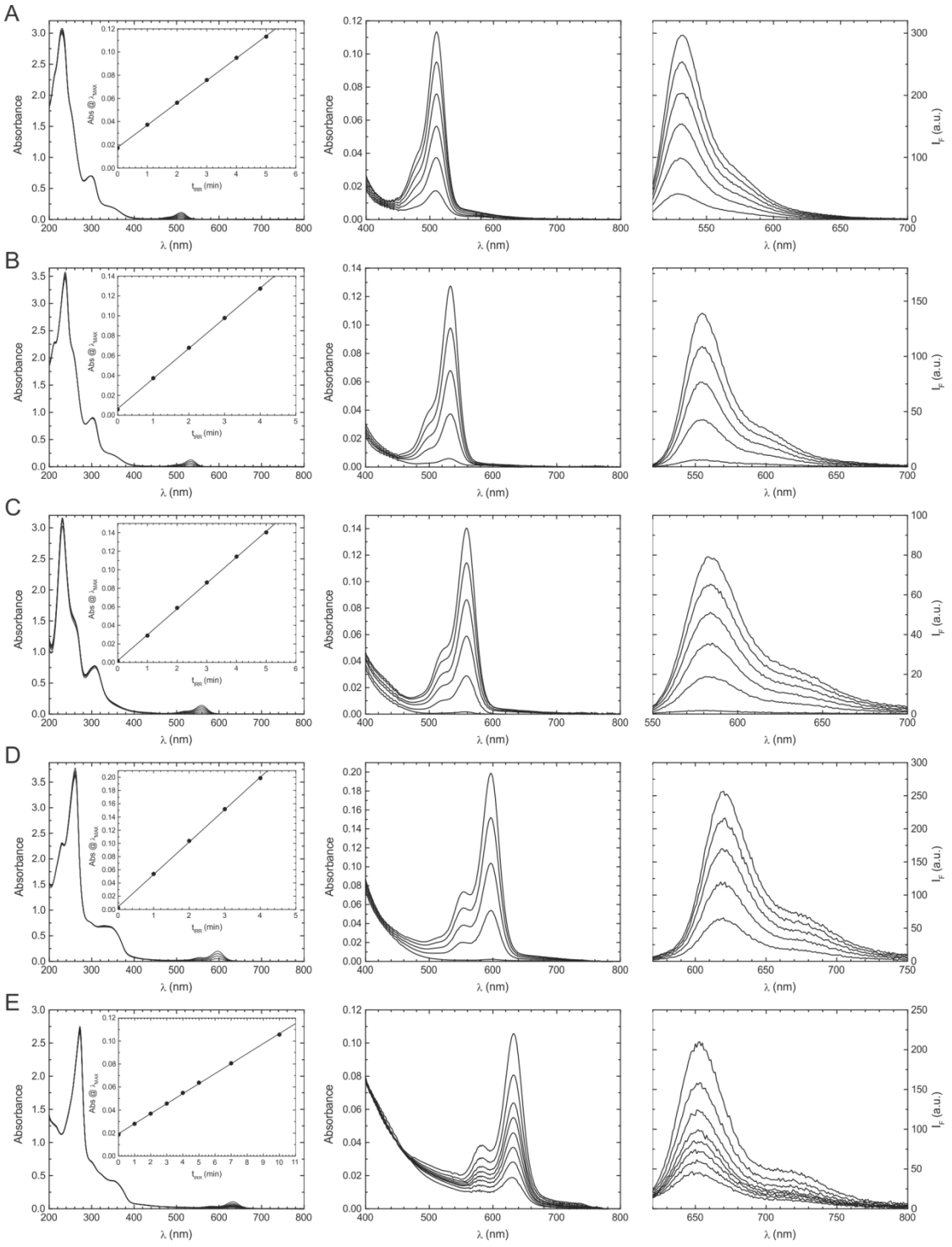
S-1. Absorption spectra of the initial cyclic forms (CF) of the model caged compounds in methanol: **2a**-H (A), **2b**-H (B), **2c**-CO_2Et (C), **2d**-CO_2Et (D), and **2e**-F (E):



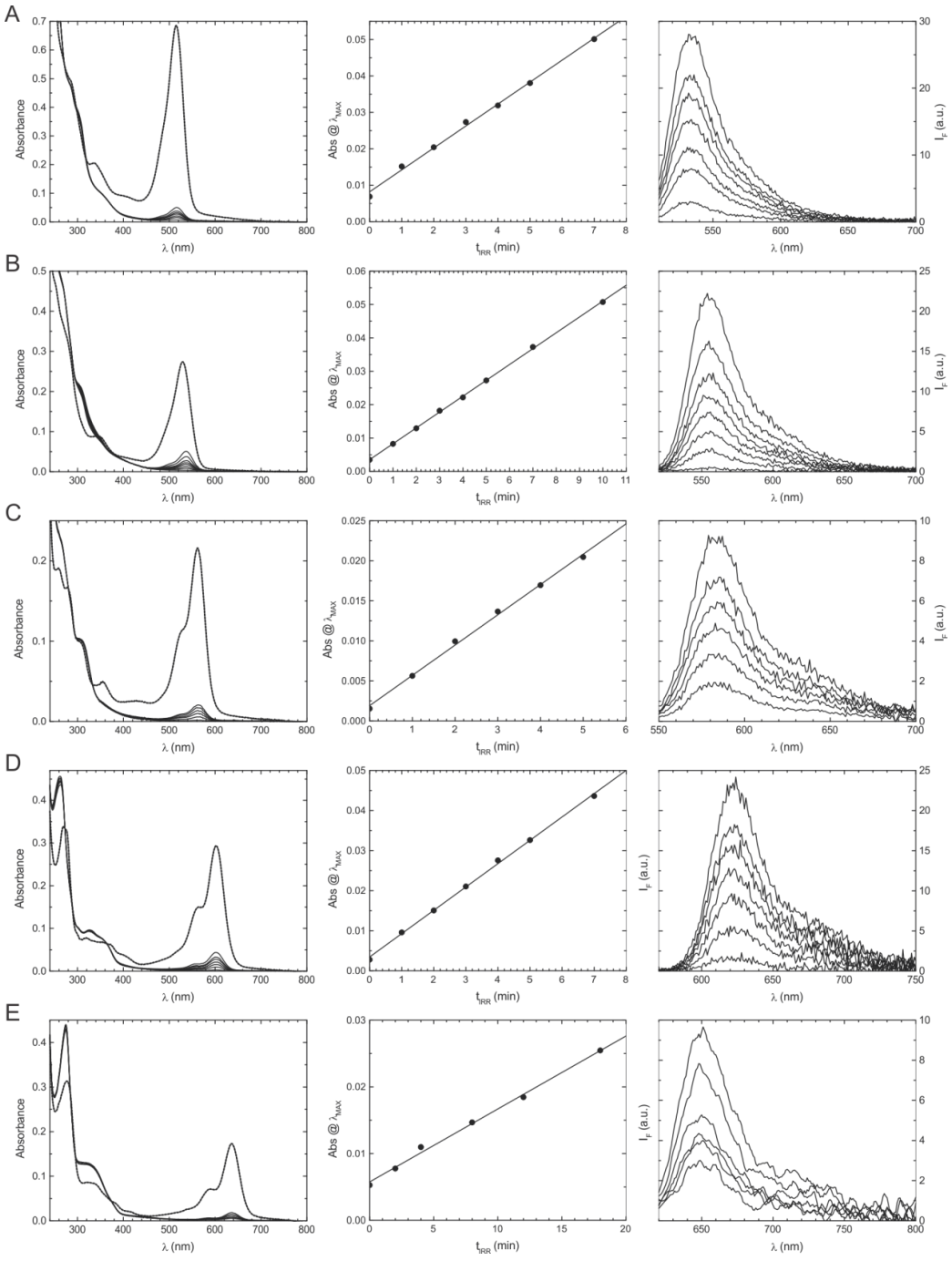
S-2. Absorption spectra of the isolated "dark" products (DP) formed under irradiation of methanolic solutions of compounds **2b**-H (A), **2d**-CO_2Et (B), and **2e**-F (C) with UV light of the middle pressure mercury lamp (150 W) equipped with pyrex filter (>330 nm):



S-3. Irradiation sequences of caged compounds in methanol: Absorption (left and middle column) end emission spectra (right column) recorded in the course of irradiation of compound **2a**-H (A), **2b**-H (B), **2c**-CO_2Et (C), **2d**-CO_2Et (D), and **2e**-F (E). Absorption at the maximum of the fluorescent product (FP; see Table 1 in the main text) as a function of time, along with a linear fit, is plotted in the inset of the first column. The irradiation times attributed to each spectrum are shown in the inset plot.

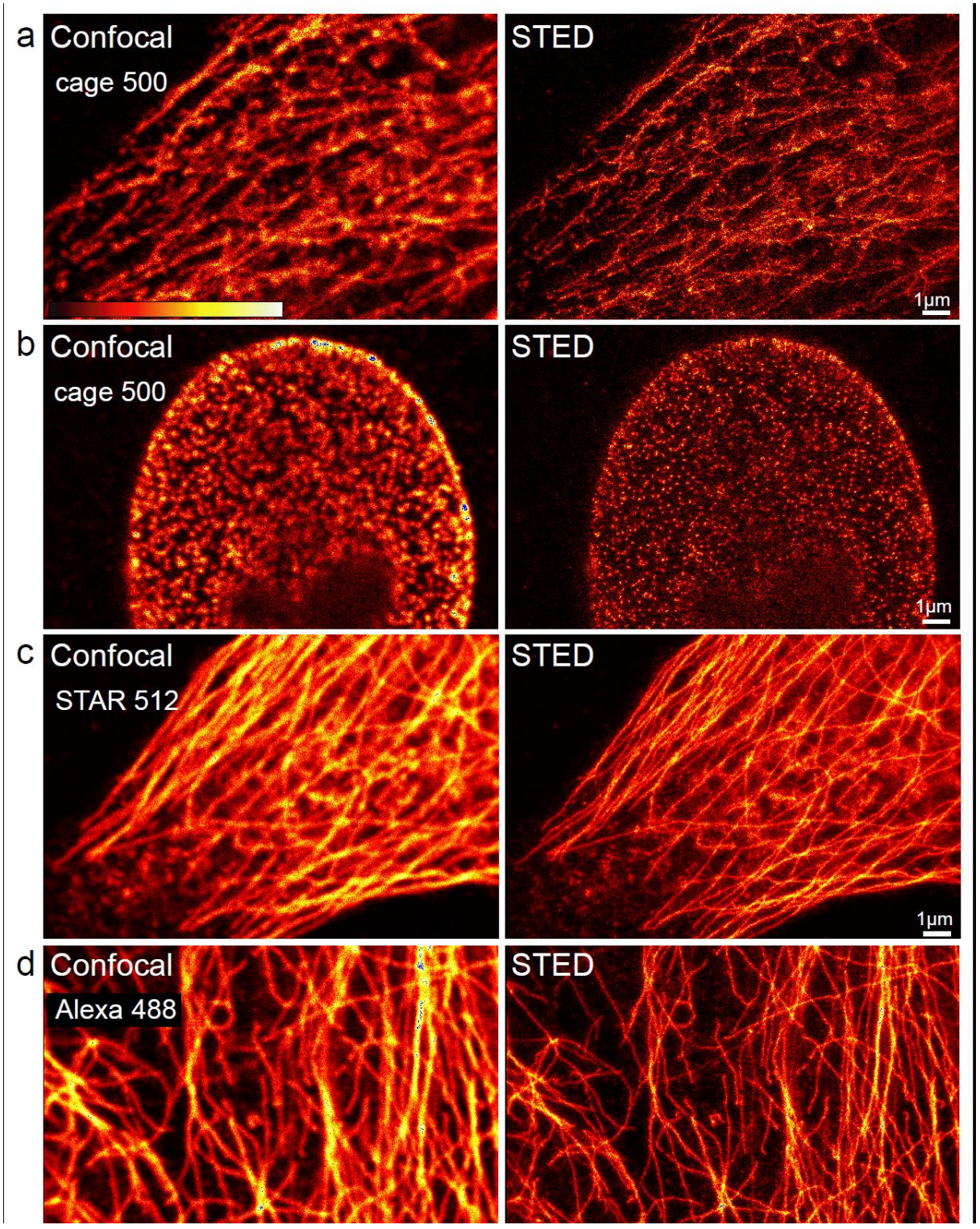


S-4. Irradiation sequences of BSA-conjugates prepared from compounds 2a-CONHS, 2bc-CONHS, 2c-CONHS (Scheme 3), 2d-Na,CONHS (Scheme 4) and 10-NHS (Scheme 5) in aqueous PBS buffer: Absorption spectra (left column), absorption at the maximum of the FP as a function of time (middle column) and emission spectra (right column) obtained during irradiation series of BSA-conjugates prepared from *N*-hydroxysuccinimidyl esters **2a**-CONHS, **2bc**-CONHS, **2c**-CONHS, **2d**-Na,CONHS and **10**-NHS. The irradiation times of each spectrum (left and right columns) are shown in the plot of the central column. The absorption spectra in broken lines (left column) correspond to the fully photoactivated dye solution.



S-5. Example of the cell-permeable caged carbopyronine; unspecific staining of living cells followed by photoactivation. Living Hela cells were unspecifically stained with the caged carbopyronine having two 1,2,3,4-dihydroquinoline fragments. The structure is given; for preparation, see ref. 6 above (=2b in the main text). Incubation for 30 min at 37°C, $c = 10^{-6}$ M in the culture medium; followed by washing and observation in the epifluorescence microscope. *Left:* before uncaging (irradiation with 630/±20 nm light produced no fluorescent image); right: the same area after illumination with 420/±30 nm light was irradiated for 1 ms with 630/±20 nm light, and a bright fluorescent image was observed.

S-6. STED microscopy of mammalian cells using green emitting photoactivable dyes. a) Microtubules and b) nuclear pore complex NUP153 immunolabelled with **2a-CONHS**. After ~ 1min of widefield illumination with 340–380 nm light, the dye was uncaged and became visible. A confocal reference image was recorded with excitation at 490 nm, and then a superresolution STED image was obtained by switching-on and applying the STED beam at 590 nm. With an optical resolution of ~ 85 nm, STED microscopy reveals much more details than the diffraction-limited confocal image with ~ 200 nm resolution. The resolution was estimated from line profiles on the microtubules decorated with primary and secondary antibodies. c) The fluorescent dye Abberior Star 512 is spectrally very similar to the uncaged (photoactivated) dye **2a-COOH** and provides a similar resolution of 85 nm. It performs equally well in confocal and STED microscopy, although the signal-to-noise is expectedly much better. d) For another reference, confocal and STED microscopy images of microtubule immunolabelled with the benchmark Alexa Fluor™ 488 (a standard dye for STED microscopy at the given wavelengths) are also given. Powers of light beams at backfocal planes: 1.3 µW, 43 mW (a, b); 1.6 µW, 43 mW (c) and 0.1 µW, 68 mW (d) for excitation and STED, respectively. Note that the STED power is several orders of magnitude greater that the power of excitation light, so that only very photostable dyes perform well under these harsh illumination conditions.


a
Confocal
cage 500
STED
1µm
b
Confocal
cage 500
STED
1µm
c
Confocal
STAR 512
STED
1µm
d
Confocal
Alexa 488
STED