

Triarylmethane Fluorophores Resistant to Oxidative Photobleaching

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Supplementary Methods

General experimental information and synthesis

Thin layer chromatography: Analytical TLC (normal phase) was performed on Merck Millipore ready-to-use aluminum sheets coated with silica gel 60 (F₂₅₄) (Cat. No. 1.05554.0001). Analytical TLC on reversed phase (RP-C₁₈) was performed on Merck Millipore ready-to-use aluminum sheets coated with RP-18 60 (F_{254S}) (Cat. No. 1.05560.0001). Compounds were detected by exposing TLC plates to UV-light (254 or 366 nm), by heating with vanillin stain (6 g vanillin and 1.5 mL conc. H₂SO₄ in 100 mL ethanol), or by heating with 1 N HCl or 1 N NaOH as indicated in the synthetic procedures.

Preparative flash column chromatography: Silica 60 (0.04 – 0.063 mm) for column chromatography was used (Macherey-Nagel, Germany; Cat. No. 815380.5). Reversed phase column chromatography was performed on POLYGOPREP 60-50 C₁₈ (Macherey-Nagel, Cat. No. 711500.1000). Automated separations were performed with an Isolera Spektra One system (Biotage AG, Sweden) using the type of cartridge and solvent gradient indicated.

High-performance liquid chromatography: Analytical HPLC was performed on a Knauer Azura liquid chromatography system with a binary P 6.1L pump (Article No. EPH35, Knauer), UV diode array detector DAD 6.1L (Article No. ADC11, Knauer), an injection valve with a 20 µL loop and two electrical switching valves V 2.1S with 6-port multiposition valve head (Article No. EWA10, Knauer). Analytical columns: Knauer Eurospher II 100-5 C₁₈, 5 µm, 150×4 mm (Article No. 15DE181E2J, Knauer) or Interchim Uptisphere Strategy C₁₈-HQ, 10 µm, 250×4.6 mm (Article No. US10C18HQ-250/P46, Interchim), typical flow rate: 1.2 mL/min, unless stated otherwise.

Preparative HPLC was performed on an Interchim puriFlash 4250 2X preparative HPLC/Flash hybrid system (Article No. 115140, Interchim) with a 5 mL injection loop, a 200-600 nm UV-Vis detector and an integrated ELSD detector (Article No. 1A3640, Interchim). Preparative column: Interchim Uptisphere Strategy C₁₈-HQ, 10 µm, 250×21.2 mm (Article No. US10C18HQ-250/212, Interchim), typical flow rate: 20 mL/min, unless specified otherwise.

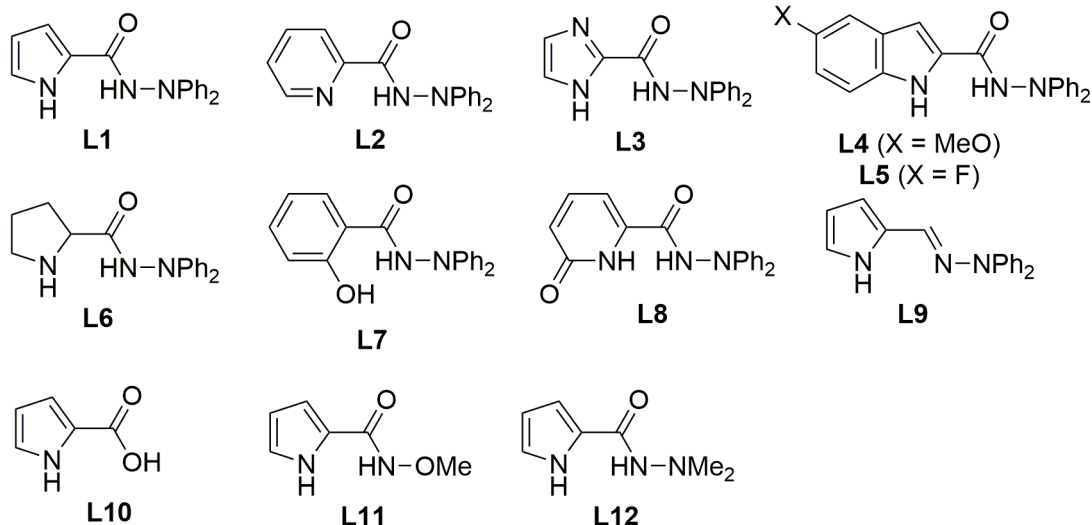
Optical spectroscopy: Absorption spectra were recorded with a Varian Cary 4000 UV-Vis double-beam spectrophotometer (Agilent Technologies, USA). The emission spectra were recorded with a Varian Cary Eclipse fluorescence spectrophotometer (Agilent). The absorption and emission spectra were recorded in quartz cells (optical path length 1 cm). Fluorescence quantum yields (absolute values) were obtained with a Quantaaurus-QY absolute PL quantum yield spectrometer (model C11347-12, Hamamatsu) according to the manufacturer's instructions. Fluorescence lifetimes were measured with a Quantaaurus-Tau fluorescence lifetime spectrometer (model C11367-32, Hamamatsu) according to the manufacturer's instructions. All measurements were performed in air-saturated solvents at ambient temperature.

NMR spectra were recorded at 25 °C with an Agilent 400-MR spectrometer at 400.06 MHz (^1H), 376.40 MHz (^{19}F), and 100.60 MHz (^{13}C) and are reported in ppm. All ^1H spectra are referenced to tetramethylsilane (TMS; $\delta = 0$ ppm) using the signals of added TMS (0.03% v/v) or the residual protons of CHCl_3 (7.26 ppm) in CDCl_3 , CHD_2CN (1.94 ppm) in CD_3CN , CHD_2OD (3.31 ppm) for CD_3OD , $\text{CHD}_2\text{COCD}_3$ (2.05 ppm) for acetone- d_6 , $\text{DMSO-}d_5$ (2.50 ppm) for $\text{DMSO-}d_6$, pyridine- d_4 (8.74 ppm, H-2, H-6) for pyridine- d_5 . In some cases, addition of $\text{CF}_3\text{CO}_2\text{D}$ (1% v/v) was necessary (as indicated in the synthetic procedures). ^{13}C spectra are referenced to TMS ($\delta = 0$ ppm) using the signals of added TMS (0.03% v/v) or the solvent: CDCl_3 (77.16 ppm), CD_3CN (1.32 ppm), CD_3OD (49.00 ppm), $(\text{CD}_3)_2\text{CO}$ (29.84 ppm), $\text{DMSO-}d_6$ (39.52 ppm) or pyridine- d_5 (150.35 ppm, C-2,6). Multiplicities of signals are described as follows: s = singlet, d = doublet, t = triplet, q = quartet, p = pentet, m = multiplet or overlap of non-equivalent resonances; br = broad signal. Coupling constants (J) are given in Hz. For the ^{13}C chemical shifts obtained by indirect detection from HSQC experiments (minimum resolution in F1: $t_1 \geq 192$), only H-coupled C-nuclei are resolved.

ESI-MS were recorded on a Varian 500-MS spectrometer (Agilent). **ESI-HRMS** were recorded on a MICROTOF spectrometer (Bruker) equipped with ESI ion source (Apollo) and direct injector with LC autosampler Agilent RR 1200.

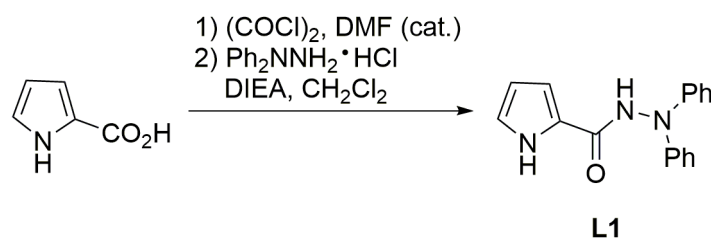
Synthesis and properties of new compounds

Synthesis of ligands



Apart from the preparation of the best-performing ligand **L1**, no efforts were made to optimize the syntheses of ligands **L2-L9**. The compound **L10** is commercially available (e.g., from TCI Europe), and the compounds **L11** [1] and **L12** [2] were prepared according to the published procedures.

L1



Oxalyl chloride (2.7 mL, 32 mmol, 1.6 equiv) was added quickly dropwise to a suspension of pyrrole-2-carboxylic acid (2.22 g, 20 mmol) in dichloromethane (30 mL) and DMF (0.2 mL), placed in a flask equipped with a bubble counter and immersed in an ice-water bath. The ice bath was then replaced with a 20 °C water bath, causing a vigorous reaction, and most of the solids dissolved within 15 min. After 1 h, the mixture was evaporated, chased with dry dichloromethane (2 × 20 mL) and redissolved in a minimal volume of dry dichloromethane.

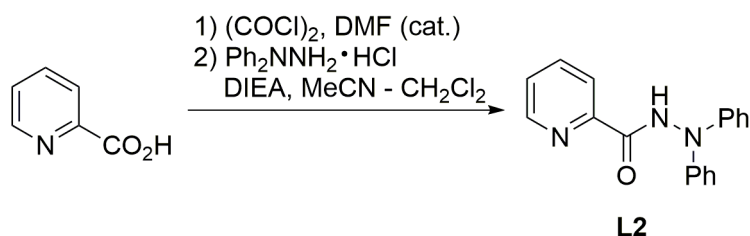
In a separate flask, 1,1-diphenylhydrazine hydrochloride (4.42 g, 20 mmol, 1 equiv) was suspended in dry dichloromethane (30 mL), the flask was flushed with argon, and DIEA (ethyldiisopropylamine; 8.7 mL, 50 mmol, 2.5 equiv) was injected. The resulting clear solution was cooled in ice-water bath, and the solution of pyrrole-2-carboxyl chloride was added

dropwise. The cooling bath was removed, and the reaction mixture was stirred overnight (16 h) at rt under argon. It was then poured into sat. aq. NaHCO₃ (100 mL), extracted with ethyl acetate (3 × 100 mL), the combined organic layers were washed with 0.5 N HCl (3 × 100 mL), water (100 mL), brine and dried over Na₂SO₄. The filtrate was evaporated to dryness and the product was recrystallized from ethyl acetate/hexane to give 2.49 g of **L1** (45%) as white solid (known compound [3]).

¹H NMR (400 MHz, DMSO-d₆): δ 11.62 (br.s, 1H), 10.74 (s, 1H), 7.33 – 7.25 (m, 4H), 7.18 – 7.11 (m, 4H), 7.02 – 6.93 (m, 4H), 6.13 – 6.17 (m, 1H).

¹³C NMR (101 MHz, DMSO-d₆): δ 160.3, 146.1, 129.0, 123.8, 122.6, 122.0, 118.6, 111.0, 109.0.

L2



Oxalyl chloride (0.79 mL, 9 mmol, 3 equiv) was added dropwise to a suspension of pyridine-2-carboxylic acid (369 mg, 3 mmol) in acetonitrile (6 mL) and DMF (30 μL), placed in a flask equipped with a bubble counter and immersed in an ice-water bath. The dark black-brown mixture was then left stirring in a thawing ice bath overnight. Afterwards, the mixture was evaporated, chased with dry toluene (2 × 10 mL) and resuspended in dry dichloromethane – acetonitrile (1:1, 8 mL).

In a separate flask, 1,1-diphenylhydrazine hydrochloride (663 mg, 3 mmol, 1 equiv) was suspended in dry dichloromethane (5 mL), the flask was flushed with argon, and DIEA (2 mL, 12 mmol, 4 equiv) was injected. The resulting clear solution was cooled in ice-water bath, and the suspension of crude pyridine-2-carbonyl chloride hydrochloride was added dropwise. The reaction mixture was stirred in ice-water bath for 1 h and at rt for 1 h (under argon). It was then poured into the mixture of sat. aq. NaHCO₃ (50 mL) and brine (20 mL), extracted with ethyl acetate (4 × 30 mL; filtering off the insoluble black matter); the combined extracts were dried over Na₂SO₄. The product was isolated by flash chromatography on Biotage Isolera system (40 g RediSep Rf cartridge, gradient 10% to 80% EtOAc/hexane); the fractions containing the product were pooled, evaporated and the product was crystallized from ethyl acetate/hexane to give 400 mg of **L2** (46%) as yellowish solid.

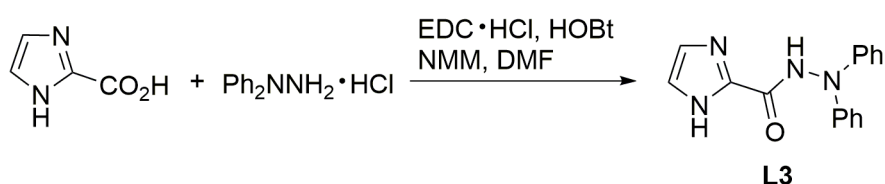
^1H NMR (400 MHz, DMSO- d_6): δ 11.50 (s, 1H), 8.71 (dt, J = 4.7, 1.4 Hz, 1H), 8.07 – 8.01 (m, 2H), 7.67 (td, J = 4.9, 3.7 Hz, 1H), 7.33 – 7.24 (m, 4H), 7.20 – 7.12 (m, 4H), 6.98 (tt, J = 7.3, 1.2 Hz, 2H).

^{13}C NMR (101 MHz, DMSO- d_6): δ 163.7, 149.4, 148.7, 145.7, 137.9, 129.0, 127.1, 122.5, 122.1, 118.9.

MS (ESI): m/z (positive mode, rel. int., %) = 290.1 (44) $[\text{M}+\text{H}]^+$.

HRMS ($\text{C}_{18}\text{H}_{15}\text{N}_3\text{O}$): m/z (positive mode) = 290.1289 (found $[\text{M}+\text{H}]^+$), 290.1288 (calc. $[\text{M}+\text{H}]^+$).

L3



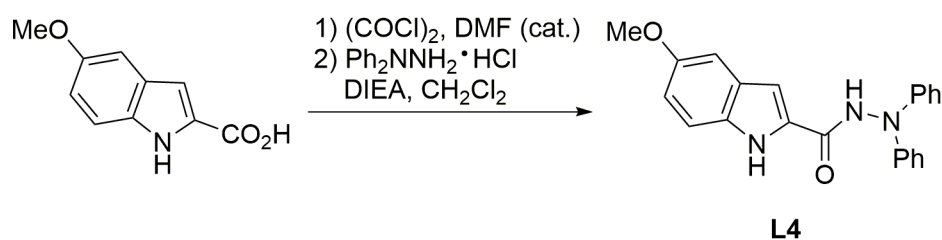
The procedure reported in [4] was followed. Imidazole-2-carboxylic acid (480 mg, 4.29 mmol) and 1,1-diphenylhydrazine hydrochloride (950 mg, 4.3 mmol, 1 equiv) were mixed in dry DMF (5 mL). The resulting suspension was cooled in ice-water bath, followed by addition of HOBT (1-hydroxybenzotriazole hydrate; 581 mg, 4.3 mmol, 1 equiv) and NMM (4-methylmorpholine; 1.42 mL, 12.9 mmol, 3 equiv). To the resulting clear solution, EDC·HCl (*N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride; 3.3 g, 17.2 mmol, 4 equiv) was added portionwise over 5 min. The resulting mixture was stirred in ice-water bath for 1 h and at rt overnight. It was then diluted with brine (150 mL) and extracted with ethyl acetate – 2-propanol (1:1, 3 × 50 mL), the combined extracts were filtered hot through a plug of Na_2SO_4 and evaporated on silica. The product was isolated by flash chromatography on Biotage Isolera system (50 g Biotage SNAP Ultra cartridge, gradient 0% to 100% A:B, A – 10% EtOH/dichloromethane, B – dichloromethane) and crystallized from ethyl acetate/hexane to give 640 mg of **L3** (54%) as colorless needles.

^1H NMR (400 MHz, 1%(v/v) TFA in DMSO- d_6): δ 11.32 (s, 1H), 7.36 (s, 2H), 7.33 – 7.25 (m, 4H), 7.18 – 7.11 (m, 4H), 6.99 (tt, J = 7.3, 1.2 Hz, 2H).

^{13}C NMR (101 MHz, 1%(v/v) TFA in DMSO- d_6): δ 157.3, 145.7, 138.8, 129.1, 124.3, 122.4, 119.0.

MS (ESI): m/z (positive mode, rel. int., %) = 279.1 (100) $[\text{M}+\text{H}]^+$.

HRMS ($\text{C}_{16}\text{H}_{14}\text{N}_4\text{O}$): m/z (positive mode) = 279.1244 (found $[\text{M}+\text{H}]^+$), 279.1240 (calc. $[\text{M}+\text{H}]^+$).

L4

Oxalyl chloride (0.2 mL, 2.29 mmol, 1.7 equiv) was added dropwise to a suspension of 5-methoxyindole-2-carboxylic acid (252 mg, 1.32 mmol) in dichloromethane (5 mL) and DMF (15 μ L), placed in a flask equipped with a bubble counter and immersed in an ice-water bath. The ice bath was then removed and the mixture was stirred at rt for 1.5 h (yellow needles of acyl chloride precipitated). The mixture was then evaporated, chased with dry dichloromethane (2 \times 20 mL) and resuspended in dry dichloromethane (5 mL).

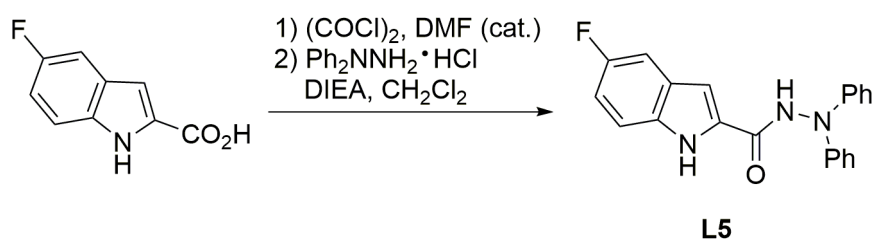
In a separate flask, a solution of 1,1-diphenylhydrazine hydrochloride (291 mg, 1.32 mmol, 1 equiv) and DIEA (0.6 mL, 3.45 mmol, 2.6 equiv) in dry dichloromethane (5 mL) under argon was cooled in ice-water bath, and the suspension of acyl chloride was added with a pipette (the flask was rinsed with 2 mL of dry dichloromethane). The cooling bath was removed, and the reaction mixture was stirred overnight (16 h) at rt under argon. It was then poured into sat. aq. NaHCO₃ (50 mL), extracted with ethyl acetate (50 mL) and dichloromethane – 2-propanol (3:1, 3 \times 50 mL); the combined organic layers were washed with brine and dried over Na₂SO₄, filtered and evaporated on silica. The product was isolated by flash chromatography on Biotage Isolera system (40 g Büchi Sepacore Silica HP cartridge, gradient 10% to 60% EtOAc/hexane); the fractions containing the product were pooled, evaporated and the product was crystallized from ethyl acetate/hexane to give 87 mg of **L4** (18%) as light yellow crystals.

¹H NMR (400 MHz, DMSO-d₆): δ 11.58 (s, 1H), 11.17 (s, 1H), 7.38 – 7.24 (m, 6H), 7.22 – 7.16 (m, 4H), 7.13 (d, J = 2.4 Hz, 1H), 7.00 (tt, J = 7.2, 1.2 Hz, 2H), 6.88 (dd, J = 8.9, 2.4 Hz, 1H), 3.77 (s, 3H).

¹³C NMR (101 MHz, DMSO-d₆): δ 160.8, 153.9, 145.8, 132.1, 129.5, 129.1, 127.3, 122.2, 118.7, 115.2, 113.2, 103.3, 102.0, 55.3.

MS (ESI): m/z (positive mode, rel. int., %) = 358.2 (24) [M+H]⁺.

HRMS (C₂₂H₁₉N₃O₂): m/z (positive mode) = 358.1546 (found [M+H]⁺), 358.1550 (calc. [M+H]⁺).

L5

Oxalyl chloride (0.5 mL, 5.73 mmol, 2 equiv) was added dropwise to a suspension of 5-fluoroindole-2-carboxylic acid (500 mg, 2.79 mmol) in dichloromethane (10 mL) and DMF (30 μ L), placed in a flask equipped with a bubble counter and immersed in an ice-water bath. The ice bath was then removed and the mixture was stirred at rt for 15 min, followed by refluxing for 15 min (bath temperature 60 $^{\circ}$ C). The resulting clear solution was evaporated, chased with dry dichloromethane (2 \times 10 mL) and redissolved in dry dichloromethane (5 mL). In a separate flask, a solution of 1,1-diphenylhydrazine hydrochloride (619 mg, 2.80 mmol, 1 equiv) and DIEA (1.5 mL, 8.63 mmol, 3 equiv) in dry dichloromethane (8 mL) under argon was cooled in ice-water bath, and the suspension of acyl chloride was added with a pipette (the flask was rinsed with 2 mL of dry dichloromethane). The cooling bath was removed, and the reaction mixture was stirred overnight (16 h) at rt under argon. It was then poured into sat. aq. NaHCO_3 (50 mL), extracted with ethyl acetate (50 mL) and dichloromethane – 2-propanol (3:1, 3 \times 40 mL); the combined organic extracts were evaporated on silica without filtration. The product was isolated by flash chromatography on Biotage Isolera system (40 g RediSep Rf cartridge, gradient 10% to 60% EtOAc/hexane); the fractions containing the product were pooled, evaporated and the product was crystallized from ethyl acetate/hexane to give 271 mg of **L5** (28%) as off-white flakes.

^1H NMR (500 MHz, $\text{DMSO-}d_6$): δ 11.86 (s, 1H), 11.28 (s, 1H), 7.49 – 7.42 (m, 2H), 7.35 – 7.28 (m, 5H), 7.22 – 7.16 (m, 4H), 7.09 (td, J = 9.5, 2.5 Hz, 1H), 7.00 (tt, J = 7.3, 1.1 Hz, 2H).

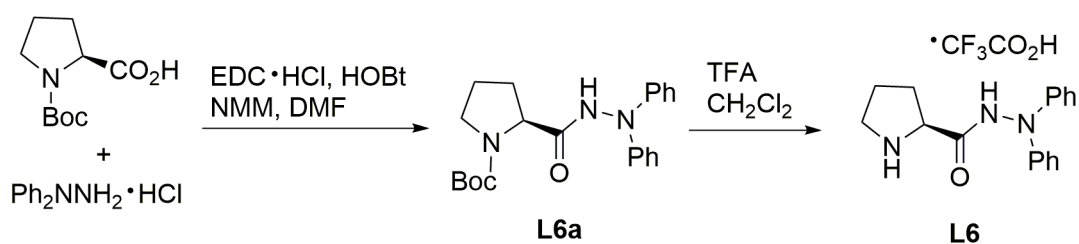
^{19}F NMR (471 MHz, $\text{DMSO-}d_6$): δ -123.49 (td, J = 9.6, 4.6 Hz).

^{13}C NMR (126 MHz, $\text{DMSO-}d_6$): δ 160.6, 158.2, 156.3, 145.8, 133.6, 131.0, 129.1, 127.1 (d, J = 10.5 Hz), 122.3, 118.7, 113.6 (d, J = 9.7 Hz), 112.7 (d, J = 26.6 Hz), 105.9 (d, J = 23.0 Hz), 103.5 (d, J = 5.3 Hz).

MS (ESI): m/z (positive mode, rel. int., %) = 346.1 (58) $[\text{M}+\text{H}]^+$.

HRMS ($\text{C}_{21}\text{H}_{16}\text{N}_3\text{OF}$): m/z (positive mode) = 346.1344 (found $[\text{M}+\text{H}]^+$), 346.1350 (calc. $[\text{M}+\text{H}]^+$).

L6



Boc-Pro-OH (645 mg, 3 mmol) and 1,1-diphenylhydrazine hydrochloride (663 mg, 3 mmol, 1 equiv) were mixed in dry DMF (4 mL); the mixture was cooled in ice-water bath, followed by addition of HOBT (405 mg, 3 mmol, 1 equiv) and NMM (0.99 mL, 9 mmol, 3 equiv). To the resulting clear solution, EDC·HCl (691 mg, 3.6 mmol, 1.2 equiv) was added portionwise over 5 min. The resulting mixture was stirred in ice-water bath for 1 h and at rt overnight. It was then poured into water (100 mL) and extracted with ethyl acetate (3 × 40 mL), the combined extracts were washed with water, brine and dried over Na₂SO₄. The product was isolated by flash chromatography on Biotage Isolera system (40 g RediSep Rf cartridge, gradient 10% to 80% EtOAc/hexane), fractions containing the product were evaporated and dried *in vacuo* to provide the intermediate **L6a** (321 mg, 28%) as light greenish foam, used directly in the next step without further characterization.

MS (ESI): *m/z* (positive mode, rel. int., %) = 404.2 (100) [M+Na]⁺.

HRMS (C₂₂H₂₇N₃O₃): *m/z* (positive mode) = 404.1955 (found [M+Na]⁺), 404.1945 (calc. [M+Na]⁺).

A solution of **L6a** (304 mg, 0.8 mmol) in dichloromethane (3 mL) and TFA (trifluoroacetic acid; 1 mL) was stirred at rt for 3 h, evaporated and chased twice with toluene. The residue was redissolved in dichloromethane with minimal amount of methanol added and evaporated on silica. The product was isolated by flash chromatography on Biotage Isolera system (12 g Büchi Sepacore Silica HP cartridge, gradient 0% to 100% A:B, A – 33% EtOH/dichloromethane, B – dichloromethane) and freeze-dried from aq. dioxane to give 271 mg of **L6** (86%) as off-white solid (trifluoroacetate salt); the retention of absolute configuration was not verified.

¹H NMR (400 MHz, 1%(v/v) TFA in DMSO-d₆): mixture of two rotameric forms in ~ 0.77:0.23 ratio; δ 11.23 (s, 0.77H, major), 10.84 (s, 0.23H, minor), 9.62 (br.s, 0.77H, major), 9.50 (br.s, 0.23H, minor), 8.76 (br.s, 0.77H, major), 8.56 (br.s, 0.23H, minor), 7.45 – 7.35 (m, 4×0.23H, minor), 7.34 – 7.28 (m, 4×0.77H, major), 7.23 – 7.16 (m, 4×0.23H, minor), 7.14 – 7.07 (m, 4×0.77H, major), 7.05 – 6.99 (m, 0.23H + 0.77H, minor+major), 4.58 – 4.47 (m, 0.23H, minor), 4.42 – 4.32 (m, 0.77H, major), 3.30 – 3.19 (m, 2×0.77H, major), 3.23 – 3.11 (m, 2×0.23H, minor), 2.47 – 2.35 (m, 4×0.23H, minor), 2.00 – 1.87 (m, 4×0.77H, major).

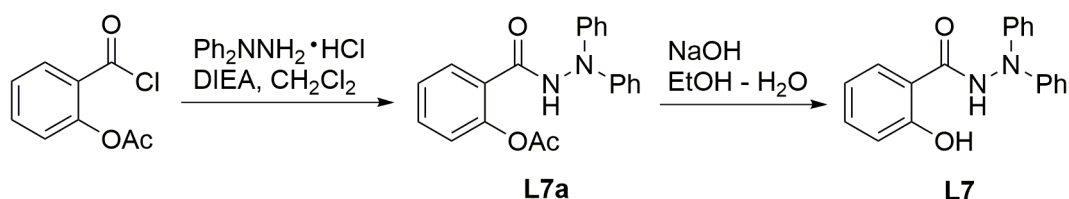
^{19}F NMR (376 MHz, $\text{DMSO-}d_6$): δ -73.63.

^{13}C NMR (101 MHz, $\text{DMSO-}d_6$): mixture of two rotameric forms, only the signals of the major rotamer are listed: δ 167.9, 145.4, 129.2, 122.6, 118.8, 58.0, 45.5, 29.0, 23.5.

MS (ESI): m/z (positive mode, rel. int., %) = 282.1 (100) $[\text{M}+\text{H}]^+$.

HRMS ($\text{C}_{17}\text{H}_{19}\text{N}_3\text{O}$): m/z (positive mode) = 282.1599 (found $[\text{M}+\text{H}]^+$), 282.1601 (calc. $[\text{M}+\text{H}]^+$).

L7



O-Acetylsalicyloyl chloride (597 mg, 3 mmol), dissolved in dry dichloromethane (5 mL), was added to a cooled (ice-water bath) solution of 1,1-diphenylhydrazine hydrochloride (663 mg, 3 mmol, 1 equiv) and DIEA (1.6 mL, 9.2 mmol, ~3 equiv) in dichloromethane (10 mL), and the mixture was left stirring overnight at rt. It was then diluted with sat. aq. NH_4Cl (30 mL) and extracted with dichloromethane (3 \times 20 mL), the combined extracts were washed with brine and dried over Na_2SO_4 . The filtrate was evaporated to give light tan solid of crude **L7a**, which was used directly in the next step.

The entire amount of **L7a** was dissolved in ethanol (20 mL), argon was slowly bubbled through the solution, and the solution of NaOH (800 mg) in water (5 mL) was added. The resulting solution was stirred at rt under argon for 1 h. 1 N HCl (30 mL) was then added, the product was extracted with ethyl acetate (3 \times 30 mL), the combined extracts were washed with brine, dried over Na_2SO_4 , filtered and evaporated on silica. The product was isolated by flash chromatography on Biotage Isolera system (40 g RediSep Rf cartridge, gradient 10% to 60% EtOAc/hexane), fractions containing the product were evaporated and the product was crystallized from ethyl acetate/hexane to give 560 mg of **L7** (61%) as white crystals.

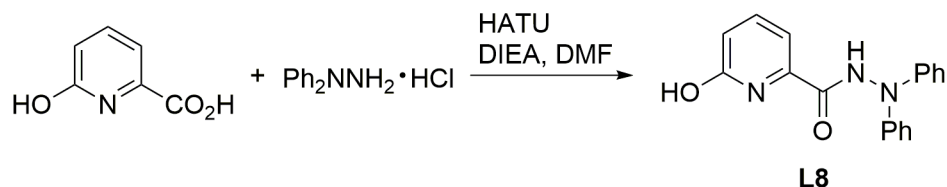
^1H NMR (300 MHz, $\text{DMSO-}d_6$): δ 11.69 (br.s, 1H), 11.20 (s, 1H), 7.90 (dd, J = 7.9, 1.6 Hz, 1H), 7.45 (ddd, J = 8.3, 7.2, 1.7 Hz, 1H), 7.37 – 7.25 (m, 4H), 7.22 – 7.14 (m, 4H), 7.05 – 6.90 (m, 4H).

^{13}C NMR (126 MHz, $\text{DMSO-}d_6$): δ 168.0, 158.9, 145.3, 133.8, 128.8, 128.0, 122.1, 118.8, 118.6, 117.1, 115.0.

MS (ESI): m/z (positive mode, rel. int., %) = 327.1 (100) $[\text{M}+\text{Na}]^+$.

HRMS (C₁₉H₁₆N₂O₂): *m/z* (positive mode) = 327.1100 (found [M+Na]⁺), 327.1104 (calc. [M+Na]⁺).

L8



HATU (1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate; 1.71 g, 4.5 mmol, 1.5 equiv) was added portionwise to a solution of 6-hydroxypyridine-2-carboxylic acid (500 mg, 3.6 mmol, 1.2 equiv), 1,1-diphenylhydrazine hydrochloride (663 mg, 3 mmol) and DIEA (1.6 mL, 9.2 mmol, ~3 equiv) in DMF (5 mL), cooled in ice-water bath. The flask was flushed with argon, and the reaction mixture was stirred at rt overnight. The mixture was then poured into brine (100 mL) and extracted with dichloromethane – 2-propanol (3:1, 3 × 40 mL); the combined extracts were washed with brine, dried over Na₂SO₄, filtered and evaporated on silica. The product was isolated by flash chromatography on Biotage Isolera system (40 g Büchi Sepacore Silica HP cartridge, gradient 40% to 100% EtOAc/dichloromethane, then washed with 10% methanol/EtOAc), fractions containing the product were evaporated and the product was crystallized from ethanol/water to give 479 mg of **L8** (52%) as light yellow crystals.

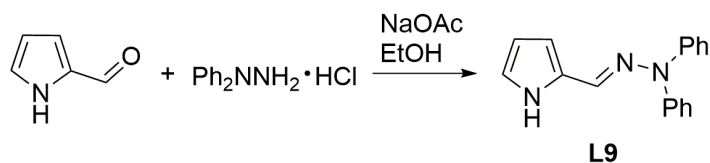
¹H NMR (400 MHz, 1%(v/v) TFA in DMSO-d₆): δ 11.17 (s, 1H), 10.78 (br.s, 1H+TFA), 7.72 (dd, *J* = 8.6, 7.1 Hz, 1H), 7.33 – 7.23 (m, 5H), 7.17 – 7.11 (m, 4H), 6.99 (tt, *J* = 7.3, 1.2 Hz, 2H), 6.79 (dd, *J* = 8.7, 0.8 Hz, 1H).

¹³C NMR (101 MHz, 1%(v/v) TFA in DMSO-d₆): δ 162.6, 162.5, 145.6, 140.4, 129.1, 122.4, 119.0, 117.8 (br), 111.2 (br).

MS (ESI): *m/z* (positive mode, rel. int., %) = 328.1 (55) [M+Na]⁺.

HRMS (C₁₈H₁₅N₃O₂): *m/z* (positive mode) = 328.1052 (found [M+Na]⁺), 328.1056 (calc. [M+Na]⁺).

L9



Pyrrole-2-carboxaldehyde (190 mg, 2 mmol), 1,1-diphenylhydrazine hydrochloride (553 mg, 2.5 mmol, 1.25 equiv) and anhydrous sodium acetate (246 mg, 3 mmol, 1.5 equiv) were mixed

in a 5 mL microwave vial, absolute ethanol (4 mL) was added, the vial was flushed with argon and sealed. It was then stirred under microwave irradiation (Biotage Initiator+; 80 °C, power \leq 250 W, typically around 40 W, with simultaneous air cooling) for 15 min. The reaction mixture was evaporated on silica, and the product was isolated by flash chromatography on Biotage Isolera system (25 g Büchi Sepacore Silica HP cartridge, gradient 0% to 50% EtOAc/hexane), fractions containing the product were evaporated and the product was freeze-dried from dioxane to give 499 mg of **L9** (96%) as light yellow fluffy solid.

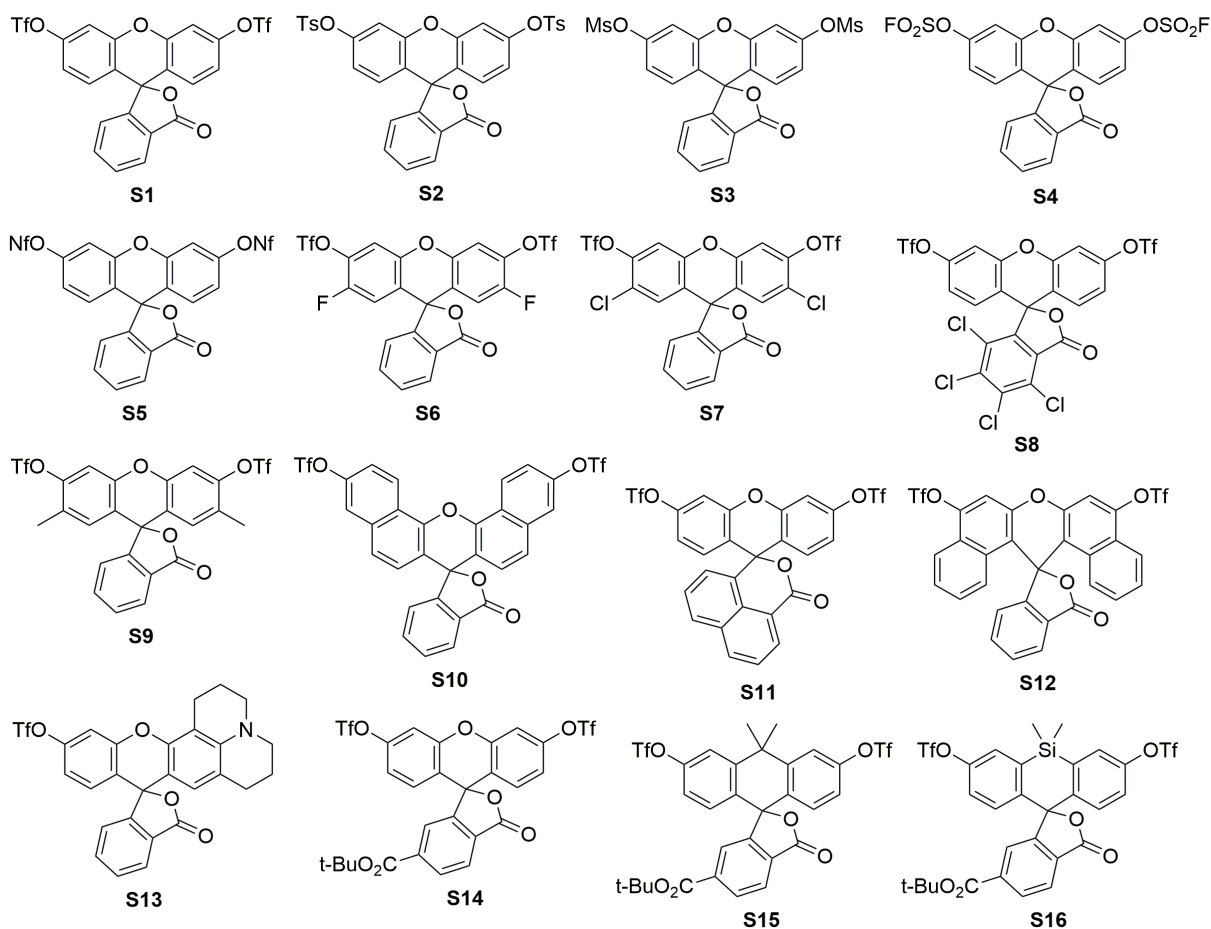
^1H NMR (300 MHz, CDCl_3): δ 9.03 (br.s, 1H), 7.48 – 7.38 (m, 4H), 7.23 – 7.10 (m, 7H), 6.86 (tdd, J = 2.7, 1.4, 0.6 Hz, 1H), 6.21 (dt, J = 3.5, 2.6 Hz, 1H), 6.16 (ddd, J = 3.6, 2.4, 1.4 Hz, 1H).

^{13}C NMR (126 MHz, CDCl_3): δ 143.7, 129.6, 129.3, 129.0, 124.1, 122.4, 119.5, 110.5, 109.5.

MS (ESI): m/z (positive mode, rel. int., %) = 262.1 (100) $[\text{M}+\text{H}]^+$.

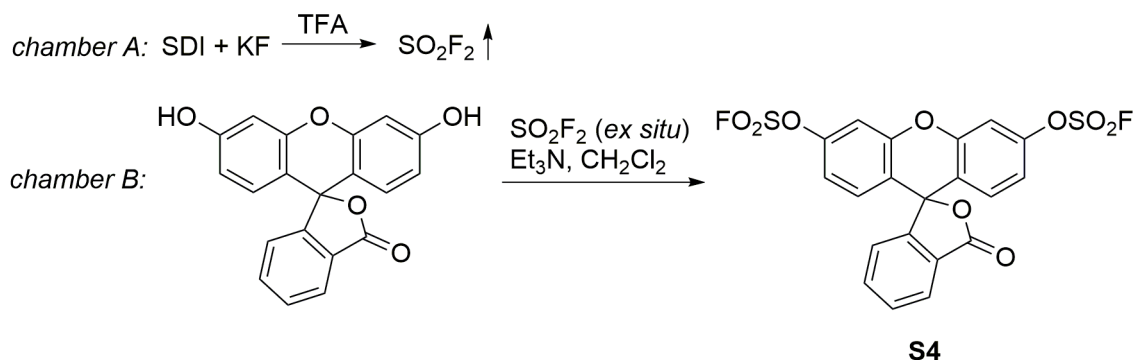
HRMS ($\text{C}_{17}\text{H}_{15}\text{N}_3$): m/z (positive mode) = 262.1335 (found $[\text{M}+\text{H}]^+$), 262.1339 (calc. $[\text{M}+\text{H}]^+$).

Synthesis of the starting aryl sulfonates



The compounds **S1**, **S6**, **S7**, **S10** [5], **S2** [6], **S3** [7], **S13** [8], **S14** [9], **S15** [10] and **S16** [11] were prepared according to the published procedures.

S4



The procedure with *ex situ* generation of the toxic odorless SO_2F_2 gas on a limited scale [12] was followed. In the chamber A of a dried small two-chamber Skrydstrup reactor (total inner volume 20 mL, available from Sigma-Aldrich as “COware gas reactor”, Cat. No. STW1-1EA), equipped with stirring bars in both chambers, SDI (1,1'-sulfonyldiimidazole; 594 mg, 3 mmol,

3 equiv) and potassium fluoride (464 mg, 8 mmol, 8 equiv) were placed. The chamber B of the same reactor was charged with fluorescein (332 mg, 1 mmol), dichloromethane (4 mL) and triethylamine (0.56 mL, 4 mmol, 4 equiv). The reactor was closed, and trifluoroacetic acid (2 mL) was injected into the chamber A through the septum, and the reaction mixture was stirred at rt for 18 h. TLC check (silica/EtOAc–hexane 1:1) showed incomplete conversion: $R_f(\text{product}) = 0.57$, $R_f(\text{mono-fluorosulfonate}) = 0.43$, $R_f(\text{fluorescein}) = 0.23$. The contents of the chamber B was evaporated, the residue was dissolved in dichloromethane (3 mL) and triethylamine (0.56 mL, 4 mmol, +4 equiv), the reactor was recharged as described above and the reaction was repeated. After 18 h at rt, the contents of the chamber B was transferred into water (50 mL) and acetic acid (2 mL), extracted with ethyl acetate (3 × 25 mL), the combined extracts were washed with brine and dried over Na_2SO_4 . The product was isolated by flash chromatography on Biotage Isolera system (12 g Büchi Sepacore Silica HP cartridge, gradient 20% to 100% EtOAc/hexane), fractions containing the product were evaporated and the product was freeze-dried from dioxane to give 378 mg of **S4** (76%) as white solid.

^1H NMR (400 MHz, CDCl_3): δ 8.10 – 8.06 (m, 1H), 7.78 – 7.67 (m, 2H), 7.37 (d, $J = 2.4$ Hz, 1H), 7.21 – 7.17 (m, 1H), 7.11 (dd, $J = 8.8, 2.4$ Hz, 1H), 7.00 (d, $J = 8.8$ Hz, 2H).

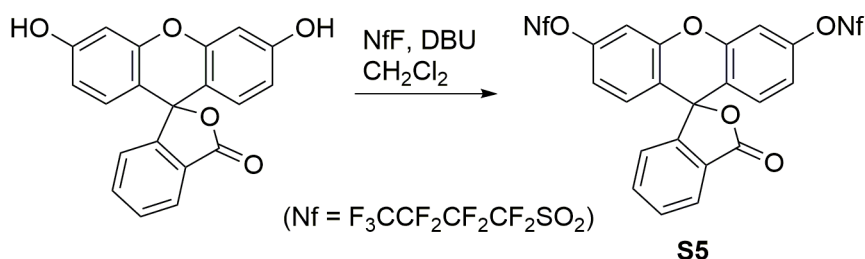
^{19}F NMR (376 MHz, CDCl_3): δ -198.45.

^{13}C NMR (101 MHz, CDCl_3): δ 168.6, 152.3, 151.5, 150.7, 136.0, 130.9, 130.3, 125.9, 125.7, 123.9, 119.8, 117.4 (d, $^4J_{\text{C-F}} = 1.2$ Hz), 110.5 (d, $^4J_{\text{C-F}} = 1.1$ Hz), 80.1.

MS (ESI): m/z (positive mode, rel. int., %) = 519.0 (13) $[\text{M}+\text{Na}]^+$.

HRMS ($\text{C}_{20}\text{H}_{10}\text{F}_2\text{O}_9\text{S}_2$): m/z (positive mode) = 518.9620 (found $[\text{M}+\text{Na}]^+$), 518.9627 (calc. $[\text{M}+\text{Na}]^+$).

S5



A suspension of fluorescein (1 g, 3 mmol) in dichloromethane (30 mL) was cooled in ice-water bath, and DBU (1,8-diazabicyclo[5.4.0]undec-7-ene; 1.8 mL, 12 mmol, 4 equiv) was added. Clear orange-red solution formed. NfF (perfluoro-1-butananesulfonyl fluoride, nonafllyl fluoride; 1.6 mL, 9 mmol, 3 equiv) was added quickly dropwise, and the color of the solution changed to light orange. The cooling bath was removed, and the mixture was stirred at rt for 1.5 h. It was then poured into 0.3 M HCl (150 mL), extracted with dichloromethane (3 × 30 mL), the

combined extracts were washed with brine and dried over Na₂SO₄. The product was isolated by flash chromatography on Biotage Isolera system (40 g RediSep Rf cartridge, gradient 10% to 60% EtOAc/hexane), fractions containing the product were evaporated and the product was freeze-dried from dioxane to give 2.49 g of **S5** (93%) as white solid.

¹H NMR (400 MHz, CDCl₃): δ 8.10 – 8.06 (m, 1H), 7.74 (td, *J* = 7.4, 1.4 Hz, 1H), 7.70 (td, *J* = 7.4, 1.2 Hz, 1H), 7.31 (d, *J* = 2.4 Hz, 2H), 7.21 – 7.17 (m, 1H), 7.04 (dd, *J* = 8.8, 2.5 Hz, 2H), 6.96 (d, *J* = 8.8 Hz, 2H).

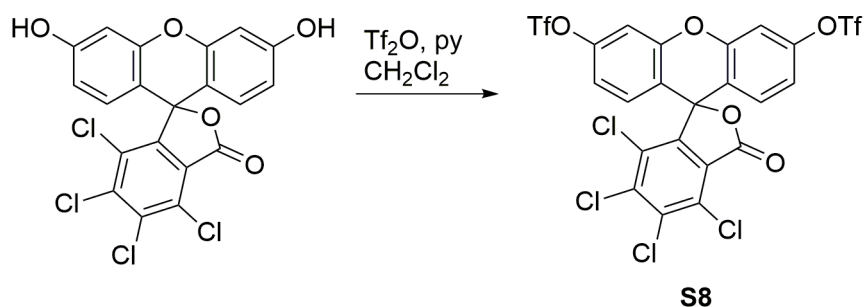
¹⁹F NMR (376 MHz, CDCl₃): δ -80.65 (app.t, *J* = 10.4 Hz, 6F), -108.54 (app.t, *J* = 14.2 Hz, 4F), -120.76 – -120.92 (m, 4F), -125.73 – -125.91 (m, 4F).

¹³C NMR (126 MHz, CDCl₃): δ 168.6, 152.3, 151.5, 150.6, 135.9, 130.8, 130.1, 125.9, 125.8, 123.9, 119.4, 117.8, 110.8, 80.2; the signals of perfluorobutyl tails demonstrated complex splitting and were not resolved.

MS (ESI): *m/z* (positive mode, rel. int., %) = 897.2 (100) [M+H]⁺.

HRMS (C₂₈H₁₀F₁₈O₉S₂): *m/z* (positive mode) = 896.9558 (found [M+H]⁺), 896.9552 (calc. [M+H]⁺).

S8



Trifluoromethanesulfonic anhydride (1.6 mL, 9.5 mmol, 4.1 equiv) was added quickly dropwise to a suspension of 3,4,5,6-tetrachlorofluorescein (1.08 g, 2.3 mmol) and pyridine (1.5 mL, 18.6 mmol, 8.1 equiv) in dry dichloromethane (50 mL), cooled in ice-water bath. The resulting clear light-brown solution was stirred at rt overnight. It was then poured into cold water (100 mL), extracted with dichloromethane (3 × 40 mL), the combined extracts were washed with water, brine and dried over Na₂SO₄. The product was isolated by flash chromatography on Biotage Isolera system (40 g RediSep Rf cartridge, gradient 5% to 50% EtOAc/hexane), fractions containing the product were evaporated and the product was crystallized from dichloromethane/hexane to give 1.22 g of **S8** (72%) as yellowish crystals.

^1H NMR (500 MHz, CDCl_3): δ 7.30 (d, J = 2.4 Hz, 2H), 7.09 (dd, J = 8.8, 2.4 Hz, 2H), 7.04 (d, J = 8.8 Hz, 2H).

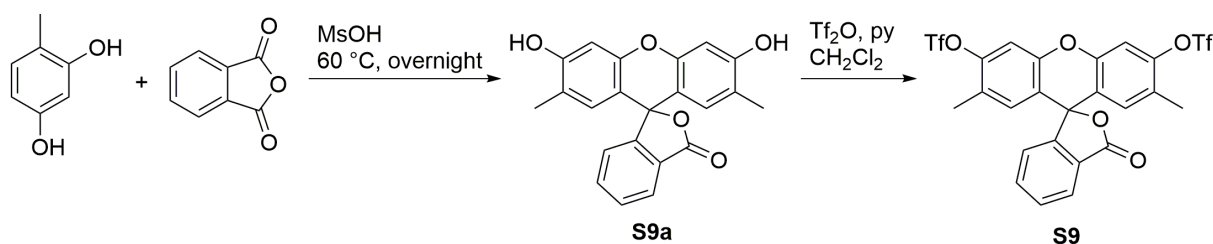
^{19}F NMR (471 MHz, CDCl_3): δ -72.53.

^{13}C NMR (126 MHz, CDCl_3): δ 163.4, 151.6, 150.8, 148.7, 141.4, 137.5, 131.8, 129.2, 128.4, 122.3, 118.8 (q, J = 320.9 Hz), 118.0, 116.0, 110.9, 78.5.

MS (ESI): m/z (positive mode, rel. int., %) = 734.8 (13) $[\text{M}+\text{H}]^+$.

HRMS ($\text{C}_{22}\text{H}_6\text{O}_9\text{S}_2\text{F}_6\text{Cl}_4$): m/z (positive mode) = 732.8184 (found $[\text{M}+\text{H}, 4\times^{35}\text{Cl}]^+$), 732.8184 (calc. $[\text{M}+\text{H}, 4\times^{35}\text{Cl}]^+$).

S9



A solution of 2,4-dihydroxytoluene (744 mg, 6 mmol, 2 equiv; prepared according to literature procedure [13]) and phthalic anhydride (444 mg, 3 mmol) in methanesulfonic acid (10 mL) was stirred at 60 °C overnight (15 h). The reaction mixture was then poured into ice-water (100 mL), the product was filtered off, washed with water and rinsed with diethyl ether/hexane, dried thoroughly on the filter and then at rt in air overnight. The yield of crude 2',7'-dimethylfluorescein **S9a** (orange solid, 1.18 g) was nearly quantitative, and the dry material was used directly in the next step.

Pyridine (2 mL, 24 mmol, 8 equiv) was added quickly dropwise to a suspension of **S9a** (1.08 g, 3 mmol) in dichloromethane (45 mL), cooled in ice-water bath; lots of yellow precipitate formed. Trifluoromethanesulfonic anhydride (2 mL, 12 mmol, 4 equiv) was added quickly dropwise to the stirred suspension, and the solids dissolved. The resulting light-orange solution was allowed to warm up to rt and stirred overnight. It was then poured into cold water (150 mL), extracted with dichloromethane (3 × 50 mL), the combined extracts were washed with water, brine and dried over Na_2SO_4 . The product was isolated by flash chromatography on Biotage Isolera system (40 g RediSep Rf cartridge, gradient 30% to 100% dichloromethane/hexane), fractions containing the product were evaporated and the product was freeze-dried from dioxane to give 959 mg of **S9** (51%) as white solid.

^1H NMR (500 MHz, CDCl_3): δ 8.11 – 8.07 (m, 1H), 7.74 (td, J = 7.5, 1.3 Hz, 1H), 7.70 (td, J = 7.4, 1.1 Hz, 1H), 7.25 (s, 2H), 7.20 – 7.14 (m, 1H), 6.76 (s, 2H), 2.23 (s, 6H).

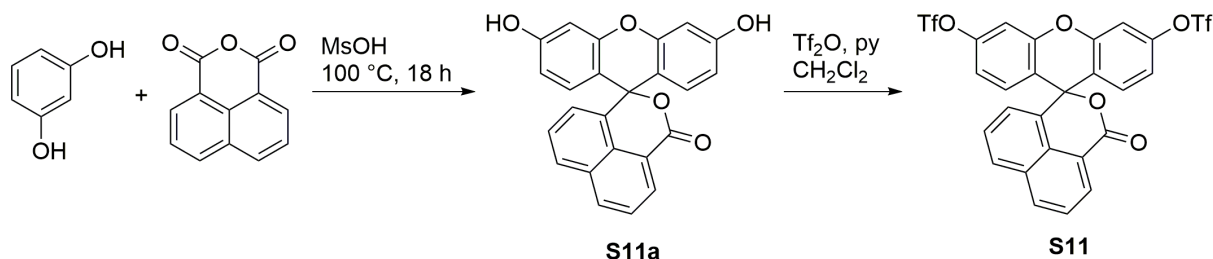
^{19}F NMR (471 MHz, CDCl_3): δ -73.54.

^{13}C NMR (126 MHz, CDCl_3): δ 169.0, 152.8, 149.5, 149.0, 135.9, 130.9, 130.7, 127.3, 125.8, 125.5, 123.9, 119.0, 118.7 (q, $J = 320.3$ Hz), 110.5, 80.5, 16.0.

MS (ESI): m/z (positive mode, rel. int., %) = 625.0 (100) $[\text{M}+\text{H}]^+$.

HRMS ($\text{C}_{24}\text{H}_{14}\text{F}_6\text{O}_9\text{S}_2$): m/z (positive mode) = 625.0056 (found $[\text{M}+\text{H}]^+$), 625.0056 (calc. $[\text{M}+\text{H}]^+$).

S11



A solution of resorcinol (1.1 g, 10 mmol, 2 equiv) and 1,8-naphthalic anhydride (990 mg, 5 mmol) in methanesulfonic acid (3 mL) was stirred at 100 °C for 18 h. The dark red-brown reaction mixture was diluted with water (100 mL), the product was extracted with ethyl acetate (3 × 50 mL), the combined extracts were diluted with methanol to dissolve the brown tarry precipitate, dried over Na_2SO_4 and evaporated on Celite. The product was isolated by flash chromatography on Biotage Isolera system (80 g Macheray-Nagel CHROMABOND Flash RS SiOH cartridge, gradient 30% to 100% ethyl acetate/hexane), fractions containing the product were evaporated and the product was freeze-dried from dioxane to give 420 mg of crude resorcinolnaphthalein **S11a** (15-20% considering purity), which was used in the next step without further purification (the above preparation was repeated twice).

Pyridine (1.2 mL, 14.2 mmol, 8 equiv) was added to a suspension of crude **S11a** (681 mg, 1.8 mmol) in dichloromethane (30 mL), cooled in ice-water bath. Trifluoromethanesulfonic anhydride (1.2 mL, 7.1 mmol, 4 equiv) was added in quickly dropwise. The resulting light-orange solution was stirred at rt for 1 h. It was then poured into ice-water (100 mL), extracted with dichloromethane (3 × 40 mL), the combined extracts were washed with water, brine and dried over Na_2SO_4 . The product was isolated by flash chromatography on Biotage Isolera system (twice: 25 g Macheray-Nagel CHROMABOND Flash RS SiOH cartridge, gradient 5% to 60% ethyl acetate/hexane, followed by 40 g Büchi Reveleris HP, gradient 5% to 60% ethyl acetate/hexane), fractions containing the product were evaporated and the product was freeze-dried from dioxane to give 533 mg of **S11** (46%) as white solid.

^1H NMR (400 MHz, CDCl_3): δ 8.62 (dd, $J = 7.2, 1.2$ Hz, 1H), 8.30 (dd, $J = 8.4, 1.2$ Hz, 1H), 8.00 (dd, $J = 8.3, 1.0$ Hz, 1H), 7.82 (dd, $J = 8.3, 7.2$ Hz, 1H), 7.56 (dd, $J = 8.3, 7.3$ Hz, 1H),

7.29 (d, $J = 2.5$ Hz, 2H), 7.15 (d, $J = 8.8$ Hz, 2H), 7.04 (dd, $J = 7.3, 1.1$ Hz, 1H), 6.98 (dd, $J = 8.8, 2.5$ Hz, 2H).

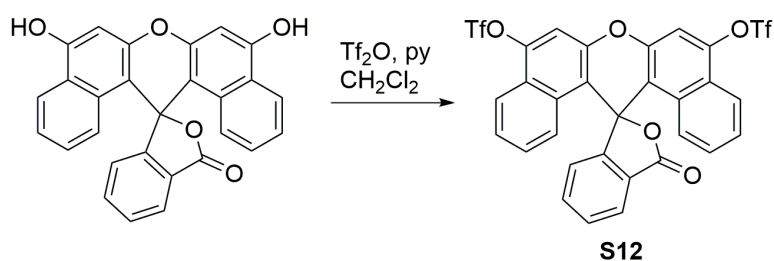
^{19}F NMR (376 MHz, CDCl_3): δ -72.66.

^{13}C NMR (101 MHz, CDCl_3): δ 161.5, 150.04, 150.01, 134.6, 133.9, 132.1, 131.2, 130.8, 128.5, 128.2, 127.7, 127.3, 127.1, 124.0, 119.5, 118.8 (q, $J = 320.7$ Hz), 117.8, 110.6, 80.5.

MS (ESI): m/z (positive mode, rel. int., %) = 669.0 (100) $[\text{M}+\text{Na}]^+$.

HRMS ($\text{C}_{26}\text{H}_{12}\text{F}_6\text{O}_9\text{S}_2$): m/z (positive mode) = 668.9706 (found $[\text{M}+\text{Na}]^+$), 668.9719 (calc. $[\text{M}+\text{Na}]^+$).

S12



Pyridine (0.68 mL, 8.44 mmol, 8.1 equiv) was added to a suspension of 1',2',7',8'-dibenzofluorescein (451 mg, 1.04 mmol; prepared according to literature procedure [14]) in dichloromethane (20 mL), cooled in ice-water bath, and trifluoromethanesulfonic anhydride (0.71 mL, 4.22 mmol, 4.1 equiv) was added quickly dropwise. The resulting orange solution was stirred at rt overnight. It was then poured into 0.1 N HCl (50 mL), extracted with dichloromethane (4 × 40 mL), the combined extracts were washed with brine and dried over Na_2SO_4 . The product was isolated by flash chromatography on Biotage Isolera system (24 g Büchi Reveleris HP, gradient 20% to 80% dichloromethane/hexane), followed by chromatography on neutral alumina (25 g, gradient 10% to 30% ethyl acetate/hexane). Fractions containing the product were evaporated and the product was freeze-dried from dioxane to give 196 mg of **S12** (27%) as white solid.

^1H NMR (400 MHz, CDCl_3): δ 8.22 (dd, $J = 7.5, 1.1$ Hz, 1H), 8.09 (dt, $J = 8.5, 1.6$ Hz, 2H), 7.68 – 7.60 (m, 3H), 7.59 – 7.53 (m, 3H), 7.53 – 7.47 (m, 2H), 7.42 (ddd, $J = 8.7, 7.0, 1.6$ Hz, 2H), 7.23 – 7.18 (m, 1H).

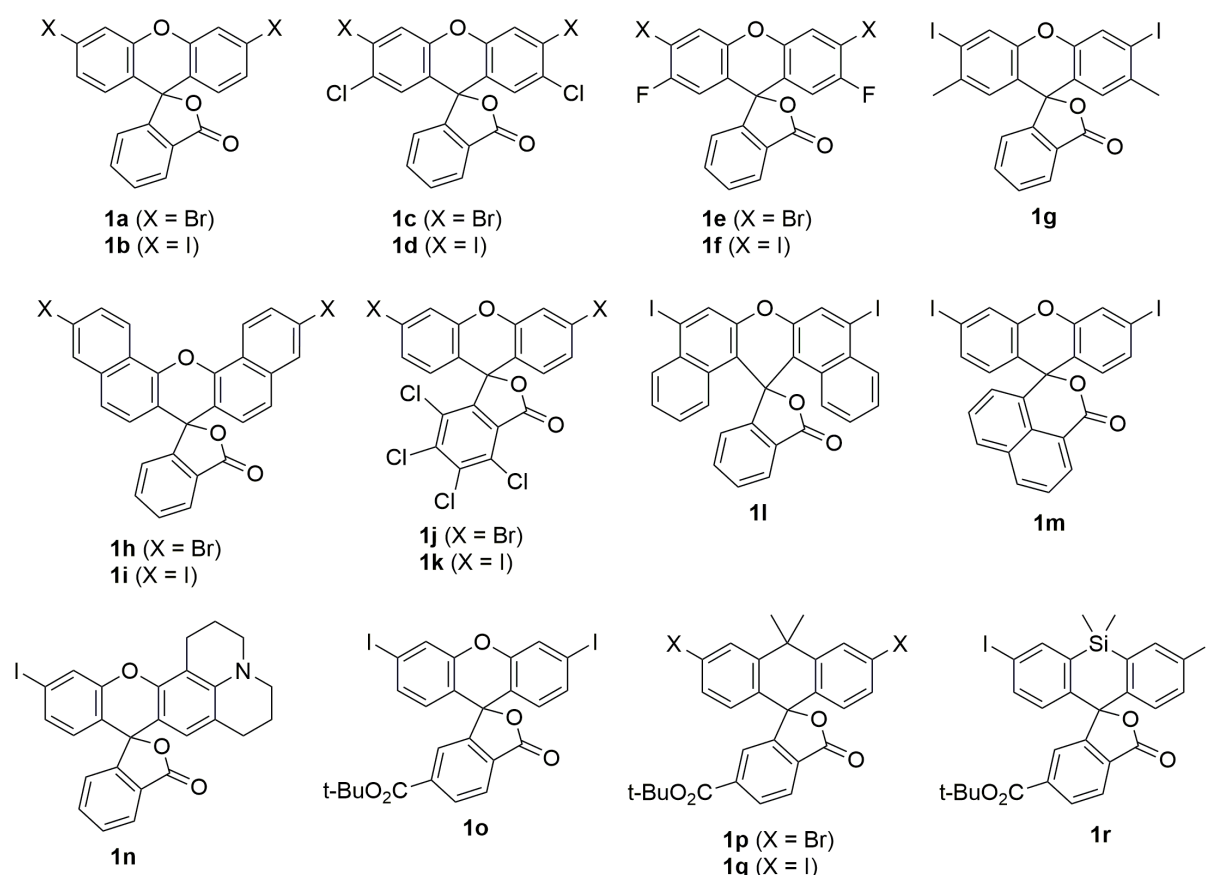
^{19}F NMR (376 MHz, CDCl_3): δ -73.08.

^{13}C NMR (101 MHz, CDCl_3): δ 170.9, 153.9, 147.6, 147.5, 136.1, 132.1, 130.8, 129.5, 128.0, 126.4, 125.00, 124.96, 124.3, 124.1, 122.4, 118.81 (q, $J = 320.5$ Hz), 111.2, 109.9, 84.7.

MS (ESI): m/z (positive mode, rel. int., %) = 697.0 (100) $[\text{M}+\text{H}]^+$.

HRMS ($\text{C}_{30}\text{H}_{14}\text{F}_6\text{O}_9\text{S}_2$): m/z (positive mode) = 697.0048 (found $[\text{M}+\text{H}]^+$), 697.0056 (calc. $[\text{M}+\text{H}]^+$).

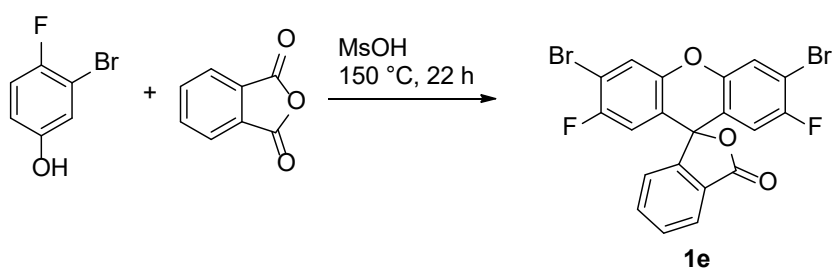
Synthesis of halofluorans and their analogs



3',6'-Dibromofluoran **1a** was prepared according to the published procedure [15] by condensation of 3-bromophenol with phthalic anhydride in strong acid (MsOH, 140 °C, 18 h; 53% yield). A similar but lower yielding (25%) preparation has been reported for 3',6'-diiodofluoran **1b** [16]. All other halofluorans of the present study, according to our literature search, have not been previously accessed. We have prepared several 3',6'-dibromofluorans (**1c**, **1e**, **1j**) via similar acid-mediated condensations (procedure A below) with consistently low yields, which highlights the practical utility of the catalytic method (procedure B).

General procedure for preparation of halofluorans by acid-mediated condensation

The following procedure is representative (procedure A):



3-Bromo-4-fluorophenol (1.91 g, 10 mmol, 2 equiv) and phthalic anhydride (740 mg, 5 mmol) were mixed in a 15 mL pressure tube (Ace Glass, 8648-04), methanesulfonic acid (5 mL) was added, and the tube was flushed with argon and stopped with a septum pierced with a 20G needle. The reaction mixture was stirred at 150 °C (bath temperature) for 22 h. Upon cooling below 100 °C, it was poured into ice-water (200 mL), extracted with chloroform (3 × 50 mL), the combined extracts were washed with 0.1 N NaOH (2 × 100 mL), water (100 mL), brine and dried over Na₂SO₄. The filtrate was evaporated on silica and the product was isolated by flash chromatography on Biotage Isolera system (40 g RediSep Rf cartridge, gradient 40% to 100% dichloromethane/hexane). Fractions containing the product were evaporated and the product was crystallized from dichloromethane/hexane to give 533 mg of **1e** (22%) as off-white crystals.

¹H NMR (300 MHz, CDCl₃): δ 8.10 – 8.02 (m, 1H), 7.76 – 7.65 (m, 2H), 7.55 (d, ⁴J_{H-F} = 5.7 Hz, 1H), 7.19 – 7.11 (m, 1H), 6.57 (d, ³J_{H-F} = 8.3 Hz, 2H).

¹⁹F NMR (282 MHz, CDCl₃): δ -111.96 (dd, *J* = 8.3, 5.7 Hz).

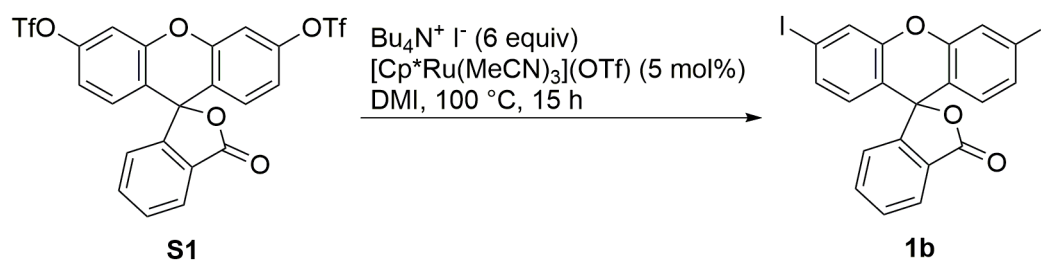
¹³C NMR (126 MHz, CDCl₃): δ 168.3, 155.5 (d, ¹J_{C-F} = 245.0 Hz), 151.9, 147.1 (d, ⁴J_{C-F} = 2.6 Hz), 135.8, 130.8, 125.9, 125.5, 123.6, 122.3, 118.8 (d, ³J_{C-F} = 6.3 Hz), 114.3 (d, ²J_{C-F} = 25.3 Hz), 112.1 (d, ²J_{C-F} = 23.4 Hz), 80.8.

MS (ESI): *m/z* (positive mode, rel. int., %) = 516.9 (100) [M+Na]⁺.

HRMS (C₂₀H₈Br₂F₂O₃): *m/z* (positive mode) = 516.8673 (found [M+Na, 1×⁷⁹Br, 1×⁸¹Br]⁺), 516.8681 (calc. [M+Na, 1×⁷⁹Br, 1×⁸¹Br]⁺).

General procedure for preparation of halofluorans by Ru-catalyzed substitution

The following procedure is representative (procedure B):



The compound **S1** (1.19 g, 2 mmol), tetrabutylammonium iodide (4.43 g, 12 mmol, 6 equiv) and [Cp^{*}Ru(MeCN)₃](OTf) (tris(acetonitrile)pentamethylcyclopentadienylruthenium(II) trifluoromethanesulfonate, Strem Chemicals, Cat. No. 44-7890; 51 mg, 0.10 mmol, 0.05 equiv) were mixed in a dried 20 mL crimp-top vial (a 10-20 mL Biotage microwave vial was used) equipped with a stirring bar, the vial was flushed with argon and sealed. DMI (1,3-dimethyl-2-imidazolidinone, dry over molecular sieves; 8 mL) was injected, and the mixture was degassed on a Schlenk line with argon through a 21G needle. It was then stirred for 15 h at 100 °C (bath temperature), the initial dark violet color changing to dark brown. On cooling, the

reaction mixture was poured into water (400 mL), extracted with chloroform/hexane (3:1, 3 × 80 mL), the combined organic layers were washed with water (2 × 300 mL), brine and dried over Na₂SO₄. The filtrate was evaporated on Celite and the product was isolated by flash chromatography on Biotage Isolera system (80 g RediSep Rf cartridge, gradient 10% to 70% EtOAc/hexane). Fractions containing the product were evaporated and the product was freeze-dried from dioxane to give 1.06 g of **1b** (96%) as off-white solid.

Following the general procedure above, the same compound **1b** can be prepared from **S1** (596 mg, 1 mmol), tetrabutylammonium iodide (2.21 g, 6 mmol, 6 equiv) using a less expensive Ru(III) catalyst [Cp*RuCl₂]_n (dichloro(pentamethylcyclopentadienyl)ruthenium(III) polymer, Strem Chemicals, Cat. No. 44-0440; 15 mg, 0.05 mmol, 0.05 equiv). Yield 532 mg (96%).

Following the general procedure above, the same compound **1b** can be prepared from the fluorosulfonate **S4** (198 mg, 0.4 mmol), tetrabutylammonium iodide (886 mg, 2.4 mmol, 6 equiv) and [Cp*Ru(MeCN)₃](OTf) catalyst (10 mg, 0.02 mmol, 0.05 equiv). Yield 212 mg (91%).

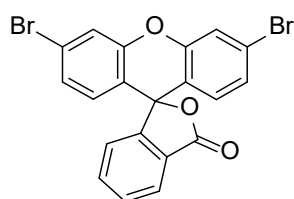
Following the general procedure above, the same compound **1b** can be prepared from the nonaflate **S5** (358 mg, 0.4 mmol), tetrabutylammonium iodide (886 mg, 2.4 mmol, 6 equiv) and [Cp*Ru(MeCN)₃](OTf) catalyst (20 mg, 0.04 mmol, 0.1 equiv), reaction time 23 h. Yield 212 mg (96%).

¹H NMR (300 MHz, CDCl₃): δ 8.06 – 8.01 (m, 1H), 7.71 – 7.60 (m, 2H), 7.68 (d, *J* = 1.6 Hz, 2H), 7.37 (dd, *J* = 8.3, 1.6 Hz, 2H), 7.14 – 7.09 (m, 1H), 6.54 (d, *J* = 8.3 Hz, 2H).

¹³C NMR (126 MHz, CDCl₃): δ 169.0, 152.9, 150.9, 135.5, 133.3, 130.3, 129.2, 126.4, 125.8, 125.5, 123.7, 118.8, 95.7, 81.3.

MS (ESI): *m/z* (positive mode, rel. int., %) = 552.9 (100) [M+H]⁺.

1a



1a

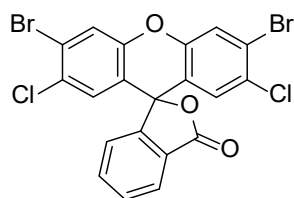
Prepared following the general procedure B from **S1** (120 mg, 0.2 mmol), anhydrous lithium bromide (104 mg, 1.2 mmol, 6 equiv) and [Cp*Ru(MeCN)₃](OTf) catalyst (5 mg, 0.01 mmol, 0.05 equiv). Yield 85 mg (93%), white solid (known compound [15]).

Following the general procedure B, the same compound **1a** can be prepared from **S1** (68 mg, 0.11 mmol), tetrabutylammonium bromide (220 mg, 0.68 mmol, 6 equiv) and [Cp*Ru(MeCN)₃](OTf) catalyst (3 mg, 5.7 μmol, 0.05 equiv). Yield 42 mg (80%).

^1H NMR (400 MHz, CDCl_3): δ 8.08 – 8.00 (m, 1H), 7.71 – 7.61 (m, 2H), 7.49 (d, J = 1.9 Hz, 2H), 7.19 (dd, J = 8.5, 1.9 Hz, 2H), 7.16 – 7.09 (m, 1H), 6.70 (d, J = 8.5 Hz, 2H).

^{13}C NMR (101 MHz, CDCl_3): δ 169.1, 152.9, 151.3, 135.6, 130.4, 129.3, 127.7, 125.9, 125.6, 124.3, 123.8, 120.5, 118.1, 81.2.

1c



1c

Prepared following the general procedure A from 3-bromo-4-chlorophenol (2.07 g, 10 mmol) and phthalic anhydride (740 mg, 5 mmol), reaction time 38 h. Yield 486 mg (18%) of white crystals.

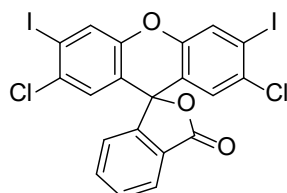
^1H NMR (400 MHz, CDCl_3): δ 8.11 – 8.06 (m, 1H), 7.76 – 7.67 (m, 2H), 7.61 (s, 2H), 7.16 – 7.11 (m, 1H), 6.89 (s, 2H).

^{13}C NMR (101 MHz, CDCl_3): δ 168.6, 152.0, 149.2, 136.0, 130.9, 130.3, 128.8, 126.0, 125.4, 124.9, 123.7, 122.6, 119.5, 80.2.

MS (ESI): m/z (positive mode, rel. int., %) = 548.8 (34) $[\text{M}+\text{Na}]^+$.

HRMS ($\text{C}_{20}\text{H}_8\text{Br}_2\text{Cl}_2\text{O}_3$): m/z (positive mode) = 548.8084 (found $[\text{M}+\text{Na}, 1\times^{79}\text{Br}, 1\times^{81}\text{Br}]^+$), 548.8088 (calc. $[\text{M}+\text{Na}, 1\times^{79}\text{Br}, 1\times^{81}\text{Br}]^+$).

1d



1d

Prepared following the general procedure B from **S7** (259 mg, 0.39 mmol) and tetrabutylammonium iodide (863 mg, 2.34 mmol) with 10 mol% of $[\text{Cp}^*\text{Ru}(\text{MeCN})_3](\text{OTf})$ catalyst (20 mg, 0.039 mmol). Yield 209 mg (86%) of off-white solid.

Following the general procedure B from **S7** (266 mg, 0.4 mmol) and tetrabutylammonium iodide (886 mg, 2.4 mmol, 6 equiv), the same preparation was attempted using 10 mol% of $[\text{Cp}^*\text{Ru}(\eta^6\text{-C}_{10}\text{H}_8)](\text{BF}_4)$ catalyst [17] (18 mg, 0.04 mmol), providing 93 mg of a mixture of **S7**:mono-iodide/mono-triflate:**1d** in 0.32:0.55:0.16 ratio.

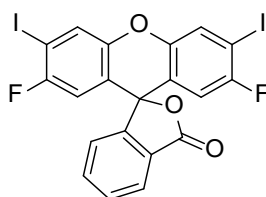
^1H NMR (400 MHz, CDCl_3): δ 8.11 – 8.05 (m, 1H), 7.83 (s, 2H), 7.75 – 7.66 (m, 2H), 7.15 – 7.10 (m, 1H), 6.86 (s, 2H).

^{13}C NMR (101 MHz, pyridine- d_5): δ 169.3, 152.6, 149.6, 136.6, 134.7, 131.4, 129.9, 127.9, 126.4, 126.3, 124.6, 121.6, 103.2, 81.1.

MS (ESI): m/z (positive mode, rel. int., %) = 620.8 (24) $[\text{M}+\text{Na}]^+$.

HRMS ($\text{C}_{20}\text{H}_8\text{O}_3\text{I}_2\text{Cl}_2$): m/z (positive mode) = 620.7990 (found $[\text{M}+\text{Na}]^+$), 620.8013 (calc. $[\text{M}+\text{Na}]^+$).

1f



1f

Prepared following the general procedure B from **S6** (1 g, 1.58 mmol) and tetrabutylammonium iodide (3.5 g, 9.5 mmol) with 5 mol% of $[\text{Cp}^*\text{RuCl}_2]_n$ catalyst (25 mg, 0.08 mmol). Yield 741 mg (80%) of white solid.

^1H NMR (400 MHz, CDCl_3): δ 8.09 – 8.04 (m, 1H), 7.75 – 7.66 (m, 2H), 7.73 (d, $^4J_{\text{H-F}} = 5.1$ Hz, 2H), 7.17 – 7.12 (m, 1H), 6.49 (d, $^3J_{\text{H-F}} = 7.6$ Hz, 2H).

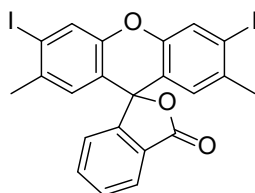
^{19}F NMR (282 MHz, CDCl_3): δ -99.21 (dd, $J = 7.6, 5.1$ Hz).

^{13}C NMR (101 MHz, CDCl_3): δ 168.5, 158.1 (d, $^1J_{\text{C-F}} = 243.2$ Hz), 152.1, 147.2 (d, $^4J_{\text{C-F}} = 2.6$ Hz), 135.9, 130.9, 128.1 (d, $^3J_{\text{C-F}} = 1.9$ Hz), 126.0, 125.5, 123.7, 119.9 (d, $^3J_{\text{C-F}} = 6.6$ Hz), 113.1 (d, $^2J_{\text{C-F}} = 27.3$ Hz), 84.2 (d, $^2J_{\text{C-F}} = 28.2$ Hz), 81.0.

MS (ESI): m/z (positive mode, rel. int., %) = 610.8 (100) $[\text{M}+\text{Na}]^+$.

HRMS ($\text{C}_{20}\text{H}_8\text{O}_3\text{I}_2\text{Cl}_2$): m/z (positive mode) = 610.8417 (found $[\text{M}+\text{Na}]^+$), 610.8423 (calc. $[\text{M}+\text{Na}]^+$).

1g



1g

Prepared following the general procedure B from **S9** (499 mg, 0.8 mmol) and tetrabutylammonium iodide (1.77 g, 4.8 mmol) with 10 mol% of $[\text{Cp}^*\text{Ru}(\text{MeCN})_3](\text{OTf})$ catalyst (40 mg, 0.08 mmol), reaction time 38 h. Yield 438 mg (94%) of light tan solid.

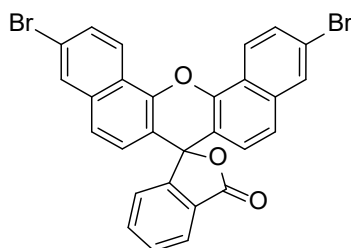
^1H NMR (400 MHz, CDCl_3): δ 8.08 – 8.02 (m, 1H), 7.77 (s, 2H), 7.70 – 7.60 (m, 2H), 7.14 – 7.08 (m, 1H), 6.62 (s, 2H), 2.27 (s, 6H).

^{13}C NMR (101 MHz, CDCl_3): δ 135.5, 130.1, 127.4, 127.3, 125.4, 123.7, 27.2 (indirect detection from a gHSQC experiment, only H-coupled carbons are resolved).

MS (ESI): m/z (positive mode, rel. int., %) = 580.9 (100) $[\text{M}+\text{H}]^+$.

HRMS ($\text{C}_{22}\text{H}_{14}\text{I}_2\text{O}_3$): m/z (positive mode) = 580.9109 (found $[\text{M}+\text{H}]^+$), 580.9105 (calc. $[\text{M}+\text{H}]^+$).

1h



1h

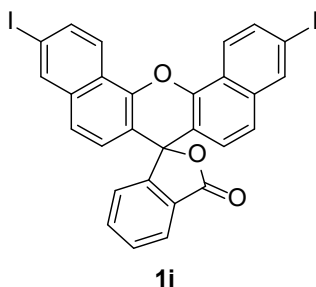
Prepared following the general procedure B from **S10** (139 mg, 0.2 mmol) and tetrabutylammonium bromide (386 mg, 1.2 mmol) with 10 mol% of $[\text{Cp}^*\text{Ru}(\text{MeCN})_3](\text{OTf})$ catalyst (10 mg, 20 μmol). Yield 104 mg (93%) of white solid.

^1H NMR (400 MHz, pyridine- d_5): δ 8.70 (d, J = 8.9 Hz, 2H), 8.33 – 8.27 (m, 1H), 8.15 (d, J = 1.9 Hz, 2H), 7.89 (dd, J = 8.9, 1.9 Hz, 2H), 7.74 – 7.65 (m, 2H), 7.56 (d, J = 8.6 Hz, 2H), 7.44 – 7.38 (m, 1H), 7.09 (d, J = 8.6 Hz, 2H).

^{13}C NMR (101 MHz, pyridine- d_5): δ 170.0, 154.5, 147.1, 136.4, 136.3, 131.1, 131.0, 130.9, 127.2, 126.1, 125.9, 125.12, 125.08, 123.23, 123.18, 114.3, 83.2.

MS (ESI): m/z (positive mode, rel. int., %) = 580.9 (100) $[\text{M}+\text{Na}]^+$.

HRMS ($\text{C}_{28}\text{H}_{14}\text{Br}_2\text{O}_3$): m/z (positive mode) = 580.9172 (found $[\text{M}+\text{H}, 1\times^{79}\text{Br}, 1\times^{81}\text{Br}]^+$), 580.9184 (calc. $[\text{M}+\text{H}, 1\times^{79}\text{Br}, 1\times^{81}\text{Br}]^+$).

1i

Prepared following the general procedure B from **S10** (348 mg, 0.5 mmol) and tetrabutylammonium iodide (1.11 g, 3 mmol) with 10 mol% of $[\text{Cp}^*\text{RuCl}_2]_n$ catalyst (15 mg, 0.05 mmol). Yield 311 mg (95%) of white solid.

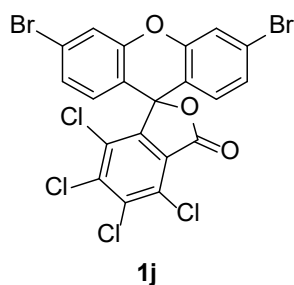
Following the general procedure B, the same compound **1i** can be prepared from **S10** (139 mg, 0.2 mmol), tetrabutylammonium iodide (443 mg, 1.2 mmol, 6 equiv) and 10 mol% of $[\text{Cp}^*\text{Ru}(\text{MeCN})_3](\text{OTf})$ catalyst (10 mg, 20 μmol). Yield 140 mg of white crystals, containing ~0.6 eq dioxane (99% yield).

^1H NMR (400 MHz, pyridine- d_5): δ 8.57 (d, $J = 8.8$ Hz, 2H), 8.40 (d, $J = 1.7$ Hz, 2H), 8.33 – 8.27 (m, 1H), 8.09 (dd, $J = 8.7, 1.7$ Hz, 2H), 7.73 – 7.65 (m, 2H), 7.54 (d, $J = 8.6$ Hz, 2H), 7.43 – 7.37 (m, 1H), 7.08 (d, $J = 8.6$ Hz, 2H).

^{13}C NMR (101 MHz, pyridine- d_5): δ 170.0, 154.5, 147.1, 137.4, 136.6, 136.41, 136.35, 131.1, 127.2, 125.9, 125.7, 125.1, 124.7, 124.0, 123.5, 114.3, 96.0, 83.3.

MS (ESI): m/z (positive mode, rel. int., %) = 652.9 (66) $[\text{M}+\text{H}]^+$.

HRMS ($\text{C}_{28}\text{H}_{14}\text{I}_2\text{O}_3$): m/z (positive mode) = 652.9093 (found $[\text{M}+\text{H}]^+$), 652.9105 (calc. $[\text{M}+\text{H}]^+$).

1j

Prepared following the general procedure A from 3-bromophenol (3.46 g, 20 mmol) and tetrachlorophthalic anhydride (2.86 g, 10 mmol), reaction time 20 h. Yield 720 mg (12%) of white crystals.

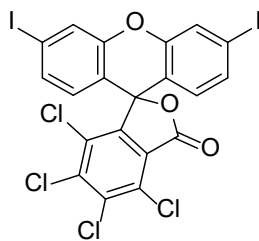
^1H NMR (400 MHz, CDCl_3): δ 7.50 (d, $J = 1.9$ Hz, 2H), 7.24 (dd, $J = 8.5, 1.9$ Hz, 2H), 6.76 (d, $J = 8.5$ Hz, 2H).

^{13}C NMR (101 MHz, CDCl_3): δ 163.8, 151.4, 149.0, 141.0, 137.0, 131.4, 128.44, 128.36, 127.9, 125.1, 122.6, 120.7, 114.7, 79.7.

MS (ESI): m/z (positive mode, rel. int., %) = 618.7 (4) $[\text{M}+\text{Na}]^+$.

HRMS ($\text{C}_{20}\text{H}_6\text{Br}_2\text{Cl}_4\text{O}_3$): m/z (positive mode) = 618.7278 (found $[\text{M}+\text{Na}, 1\times^{79}\text{Br}, 1\times^{81}\text{Br}]^+$), 618.7283 (calc. $[\text{M}+\text{Na}, 1\times^{79}\text{Br}, 1\times^{81}\text{Br}]^+$).

1k



1k

Prepared following the general procedure B from **1k** (731 mg, 1 mmol) and tetrabutylammonium iodide (2.21 g, 6 mmol) with 5 mol% of $[\text{Cp}^*\text{Ru}(\text{MeCN})_3](\text{OTf})$ catalyst (25 mg, 0.05 mmol) at 120 °C (bath temperature), reaction time 16 h. Yield 675 mg (98%) of yellowish solid.

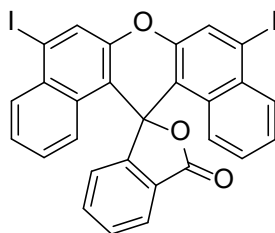
^1H NMR (400 MHz, CDCl_3): δ 7.69 (d, $J = 1.7$ Hz, 2H), 7.43 (dd, $J = 8.4, 1.7$ Hz, 2H), 6.59 (d, $J = 8.4$ Hz, 2H).

^{13}C NMR (101 MHz, CDCl_3): δ 163.8, 151.1, 149.0, 141.0, 136.9, 133.6, 131.4, 128.4, 128.3, 126.6, 122.6, 115.4, 96.5, 79.8.

MS (ESI): m/z (positive mode, rel. int., %) = 690.7 (3) $[\text{M}+\text{H}]^+$.

HRMS ($\text{C}_{20}\text{H}_6\text{O}_3\text{I}_2\text{Cl}_4$): m/z (positive mode) = 690.7186 (found $[\text{M}+\text{H}]^+$), 690.7206 (calc. $[\text{M}+\text{H}]^+$).

1l



1l

Prepared following the general procedure B from **S12** (188 mg, 0.27 mmol) and tetrabutylammonium iodide (598 g, 1.62 mmol) with 5 mol% of $[\text{Cp}^*\text{RuCl}_2]_n$ catalyst (4.2 mg, 13.5 μmol). Yield 145 mg (82%) of white solid.

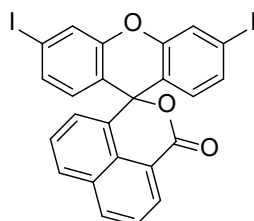
^1H NMR (400 MHz, CDCl_3): δ 8.22 – 8.14 (m, 1H), 8.14 (s, 2H), 8.14 – 8.11 (m, 2H), 7.59 – 7.54 (m, 3H), 7.49 (td, $J = 7.5, 1.2$ Hz, 1H), 7.40 (ddd, $J = 8.3, 6.9, 1.1$ Hz, 2H), 7.31 (ddd, $J = 8.6, 6.9, 1.5$ Hz, 2H), 7.17 – 7.10 (m, 1H).

^{13}C NMR (101 MHz, pyridine- d_5): δ 155.1, 148.6, 136.8, 135.2, 133.4, 131.5, 131.4, 130.4, 129.4, 129.1, 126.8, 125.23, 125.18, 124.7, 111.6, 106.4, 86.4.

MS (ESI): m/z (positive mode, rel. int., %) = 625.9 (100) $[\text{M}+\text{H}]^+$.

HRMS ($\text{C}_{28}\text{H}_{14}\text{I}_2\text{O}_3$): m/z (positive mode) = 652.9092 (found $[\text{M}+\text{H}]^+$), 652.9105 (calc. $[\text{M}+\text{H}]^+$).

1m



1m

Prepared following the general procedure B from **S11** (323 mg, 0.5 mmol) and tetrabutylammonium iodide (1.11 g, 3 mmol) with 10 mol% of $[\text{Cp}^*\text{RuCl}_2]_n$ catalyst (16 mg, 0.05 mmol), reaction time 20 h. Yield 243 mg (81%) of off-white solid (purity 90%).

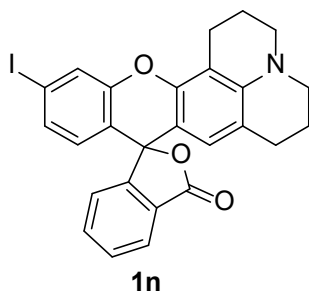
^1H NMR (400 MHz, CDCl_3): δ 8.59 (dd, $J = 7.2, 1.3$ Hz, 1H), 8.25 (dd, $J = 8.3, 1.3$ Hz, 1H), 7.93 (dd, $J = 8.3, 1.1$ Hz, 1H), 7.78 (dd, $J = 8.4, 7.2$ Hz, 1H), 7.68 (d, $J = 1.7$ Hz, 2H), 7.49 (dd, $J = 8.2, 7.3$ Hz, 1H), 7.32 (dd, $J = 8.4, 1.7$ Hz, 2H), 7.00 (dd, $J = 7.3, 1.0$ Hz, 1H), 6.73 (d, $J = 8.4$ Hz, 2H).

^{13}C NMR (101 MHz, CDCl_3): δ 161.9, 149.5, 134.8, 134.4, 133.4, 132.0, 130.4, 128.03, 128.00, 127.6, 127.1, 126.9, 126.2, 124.8, 123.5, 119.9, 117.3, 95.3, 81.4.

MS (ESI): m/z (positive mode, rel. int., %) = 625.2 (100) $[\text{M}+\text{Na}]^+$.

HRMS ($\text{C}_{24}\text{H}_{12}\text{I}_2\text{O}_3$): m/z (positive mode) = 602.8948 (found $[\text{M}+\text{H}]^+$), 602.8949 (calc. $[\text{M}+\text{H}]^+$).

1n



Prepared following the general procedure B from **S13** (163 mg, 0.3 mmol) and tetrabutylammonium iodide (332 mg, 0.9 mmol, 3 equiv) with 5 mol% of $[\text{Cp}^*\text{Ru}(\text{MeCN})_3](\text{OTf})$ catalyst (7.6 mg, 0.015 mmol). Yield 143 mg (91%) of light pink solid.

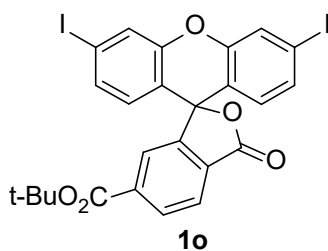
^1H NMR (400 MHz, CDCl_3): δ 8.04 – 7.96 (m, 1H), 7.67 (d, $J = 1.7$ Hz, 1H), 7.67 – 7.57 (m, 2H), 7.28 (dd, $J = 8.3, 1.7$ Hz, 1H), 7.18 – 7.15 (m, 1H), 6.45 (d, $J = 8.3$ Hz, 1H), 3.21 – 3.11 (m, 4H), 2.91 (t, $J = 6.6$ Hz, 2H), 2.61 – 2.44 (m, 2H), 2.07 – 1.97 (m, 2H), 1.92 – 1.81 (m, 2H).

^{13}C NMR (101 MHz, CDCl_3): δ 169.6, 153.1, 152.2, 147.5, 144.8, 135.0, 132.2, 129.7, 129.5, 127.1, 126.3, 125.1, 124.8, 124.1, 119.3, 118.2, 107.3, 104.6, 95.0, 84.2, 50.0, 49.5, 27.5, 21.8, 21.2, 21.1.

MS (ESI): m/z (positive mode, rel. int., %) = 522.1 (100) $[\text{M}+\text{H}]^+$.

HRMS ($\text{C}_{26}\text{H}_{20}\text{NO}_3\text{I}$): m/z (positive mode) = 522.0563 (found $[\text{M}+\text{H}]^+$), 522.0561 (calc. $[\text{M}+\text{H}]^+$).

1o



Prepared following the general procedure B from **S14** [9] (205 mg, 0.3 mmol) and tetrabutylammonium iodide (664 mg, 1.8 mmol) with 5 mol% of $[\text{Cp}^*\text{Ru}(\text{MeCN})_3](\text{OTf})$ catalyst (7.6 mg, 0.015 mmol). Yield 165 mg (86%) of yellowish solid.

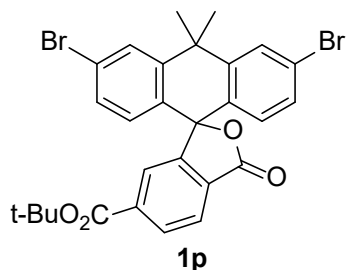
^1H NMR (400 MHz, CDCl_3): δ 8.23 (dd, $J = 8.0, 1.3$ Hz, 1H), 8.06 (dd, $J = 8.0, 0.8$ Hz, 1H), 7.70 (d, $J = 1.6$ Hz, 2H), 7.67 (dd, $J = 1.3, 0.8$ Hz, 1H), 7.38 (dd, $J = 8.4, 1.6$ Hz, 2H), 6.51 (d, $J = 8.4$ Hz, 2H), 1.55 (s, 9H).

^{13}C NMR (101 MHz, CDCl_3): δ 168.3, 163.9, 152.9, 150.9, 138.9, 133.5, 131.5, 129.2, 128.9, 126.6, 125.5, 124.8, 118.2, 96.0, 83.0, 81.7, 28.2.

MS (ESI): m/z (positive mode, rel. int., %) = 674.9 (100) $[\text{M}+\text{Na}]^+$.

HRMS ($C_{25}H_{18}I_2O_5$): m/z (positive mode) = 674.9137 (found $[M+Na]^+$), 674.9136 (calc. $[M+Na]^+$).

1p



Prepared following the general procedure B from **S15** [10] (87 mg, 0.12 mmol) and anhydrous lithium bromide (63 mg, 0.72 mmol) with 5 mol% of $[Cp^*Ru(MeCN)_3](OTf)$ catalyst (3 mg, 0.006 mmol). Yield 48 mg (68%) of white solid.

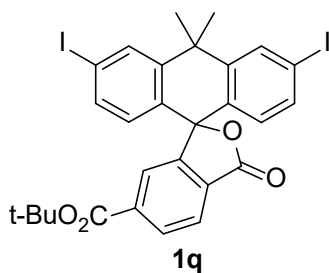
1H NMR (400 MHz, $CDCl_3$): δ 8.19 (dd, $J = 8.0, 1.3$ Hz, 1H), 8.06 (dd, $J = 8.0, 0.7$ Hz, 1H), 7.79 (d, $J = 2.0$ Hz, 2H), 7.53 (dd, $J = 1.3, 0.7$ Hz, 1H), 7.28 (dd, $J = 8.5, 2.0$ Hz, 2H), 6.65 (d, $J = 8.5$ Hz, 2H), 1.87 (s, 3H), 1.78 (s, 3H), 1.53 (s, 9H).

^{13}C NMR (101 MHz, $CDCl_3$): δ 169.4, 163.9, 154.8, 146.5, 138.6, 130.9, 130.6, 130.1, 129.7, 129.4, 128.6, 125.6, 124.5, 124.2, 85.1, 82.8, 38.4, 34.7, 33.7, 28.1.

MS (ESI): m/z (positive mode, rel. int., %) = 607.0 (100) $[M+Na]^+$.

HRMS ($C_{28}H_{24}Br_2O_4$): m/z (positive mode) = 606.9914 (found $[M+Na, 1 \times ^{79}Br, 1 \times ^{81}Br]^+$), 606.9915 (calc. $[M+Na, 1 \times ^{79}Br, 1 \times ^{81}Br]^+$).

1q



Prepared following the general procedure B from **S15** [10] (163 mg, 0.23 mmol) and tetrabutylammonium iodide (498 mg, 1.35 mmol) with 10 mol% of $[Cp^*Ru(MeCN)_3](OTf)$ catalyst (12 mg, 0.023 mmol), reaction time 38 h. Yield 128 mg (84%) of white solid.

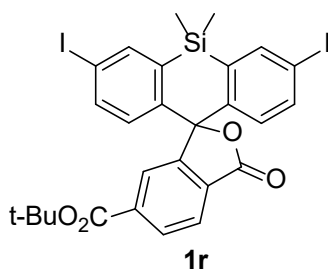
1H NMR (400 MHz, $CDCl_3$): δ 8.18 (dd, $J = 8.0, 1.3$ Hz, 1H), 8.05 (dd, $J = 8.0, 0.8$ Hz, 1H), 7.98 (d, $J = 1.8$ Hz, 2H), 7.52 (dd, $J = 1.3, 0.8$ Hz, 1H), 7.47 (dd, $J = 8.4, 1.8$ Hz, 2H), 6.49 (d, $J = 8.4$ Hz, 2H), 1.86 (s, 3H), 1.77 (s, 3H), 1.53 (s, 9H).

^{13}C NMR (101 MHz, CDCl_3): δ 169.4, 163.9, 154.8, 146.5, 138.5, 136.4, 136.2, 130.9, 130.3, 129.4, 128.6, 125.6, 124.5, 96.2, 85.2, 82.8, 38.0, 34.7, 33.8, 28.1.

MS (ESI): m/z (positive mode, rel. int., %) = 701.0 (100) $[\text{M}+\text{Na}]^+$.

HRMS ($\text{C}_{28}\text{H}_{24}\text{I}_2\text{O}_4$): m/z (positive mode) = 700.9658 (found $[\text{M}+\text{Na}]^+$), 700.9656 (calc. $[\text{M}+\text{Na}]^+$).

1r



Prepared following the general procedure B from **S16** [11] (296 mg, 0.4 mmol) and tetrabutylammonium iodide (886 mg, 2.4 mmol) with 10 mol% of $[\text{Cp}^*\text{Ru}(\text{MeCN})_3](\text{OTf})$ catalyst (20 mg, 0.04 mmol), reaction time 38 h. Yield 221 mg (79%) of white solid.

^1H NMR (400 MHz, CDCl_3): δ 8.18 (dd, $J = 8.0, 1.3$ Hz, 1H), 8.05 (dd, $J = 8.0, 0.8$ Hz, 1H), 7.98 (d, $J = 1.8$ Hz, 2H), 7.52 (dd, $J = 1.3, 0.8$ Hz, 1H), 7.47 (dd, $J = 8.4, 1.8$ Hz, 2H), 6.49 (d, $J = 8.4$ Hz, 2H), 1.86 (s, 3H), 1.77 (s, 3H), 1.53 (s, 9H).

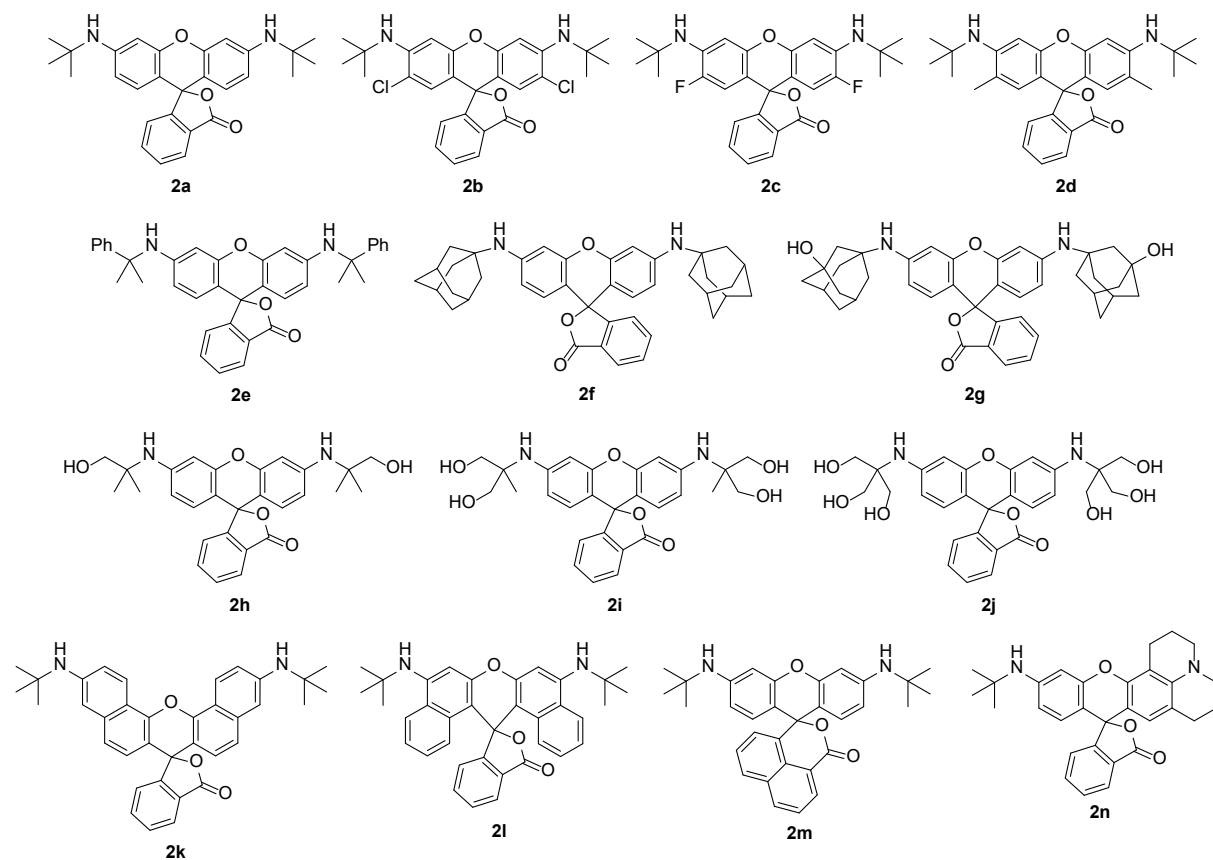
^1H NMR (400 MHz, CDCl_3): δ 8.13 (dd, $J = 8.0, 1.3$ Hz, 1H), 8.01 – 7.97 (m, 3H), 7.75 (dd, $J = 1.3, 0.8$ Hz, 1H), 7.63 (dd, $J = 8.6, 2.0$ Hz, 2H), 6.92 (d, $J = 8.6$ Hz, 2H), 1.54 (s, 9H), 0.76 (s, 3H), 0.63 (s, 3H).

^{13}C NMR (101 MHz, CDCl_3): δ 169.7, 163.8, 154.6, 143.3, 142.9, 139.4, 138.0, 137.1, 130.7, 128.1, 127.2, 126.4, 124.3, 95.9, 89.1, 82.8, 28.2, -0.09, -0.11.

MS (ESI): m/z (positive mode, rel. int., %) = 695.0 (100) $[\text{M}+\text{H}]^+$.

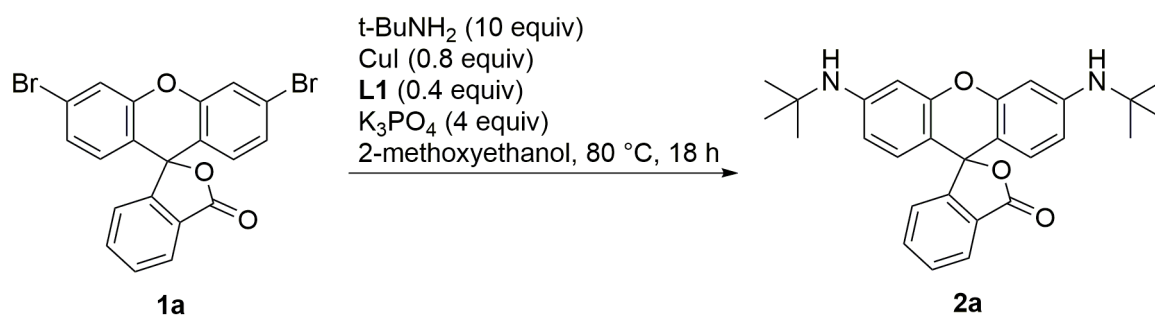
HRMS ($\text{C}_{27}\text{H}_{24}\text{I}_2\text{O}_4\text{Si}$): m/z (positive mode) = 694.9699 (found $[\text{M}+\text{H}]^+$), 694.9606 (calc. $[\text{M}+\text{H}]^+$).

Synthesis of *N*-*tert*-alkylrhodamine dyes by Cu-catalyzed amination of halofluorans



General procedure for preparation of rhodamines by Cu-catalyzed amination of halofluorans

The following procedure is representative (procedure C):



3',6'-Dibromofluoran **1a** (92 mg, 0.2 mmol), copper(I) iodide (31 mg, 0.16 mmol, 0.8 equiv), ligand **L1** (24 mg, 0.08 mmol, 0.4 equiv) and powdered K₃PO₄ (170 mg, 0.8 mmol, 4 equiv) were mixed in a 5 mL crimp-top tube containing a stirring bar (a 2-5 mL Biotage microwave vial was used), 2-methoxyethanol (0.4 mL) was injected, and the mixture was degassed with

argon on a Schlenk line. *tert*-Butylamine (0.2 mL, 1.9 mmol, ~10 equiv) was then injected (alternatively, solid and high-boiling liquid amines are added together with the solid reactants before the degassing step). The vial was immersed in an 80 °C oil bath and the reaction mixture was stirred for 18 h. The deep-red reaction mixture was poured into brine (50 mL), acetic acid was added to pH 4-5, and the mixture was extracted with ethyl acetate (3 × 80 mL). The combined extracts were dried over Na₂SO₄ and the filtrate was evaporated on Celite. The product was isolated by flash chromatography on Biotage Isolera system (12 g Büchi Sepacore Silica HP cartridge, gradient 0% to 100% A/B, A = dichloromethane – ethanol – 25% aq. NH₃ 70:30:2, B = dichloromethane). Fractions containing the product (bright red) were evaporated and the product was freeze-dried from aqueous dioxane to give 67 mg of **2a** (76%) as fluffy red solid.

Following the general procedure C, the same compound **2a** can be prepared from **1b** (110 mg, 0.2 mmol), yield 50 mg (57%). With 20 mol% copper(I) iodide (8 mg, 0.04 mmol) and 20 mol% of **L1** (12 mg, 0.04 mmol) in diethylene glycol, the yield improved to 66%.

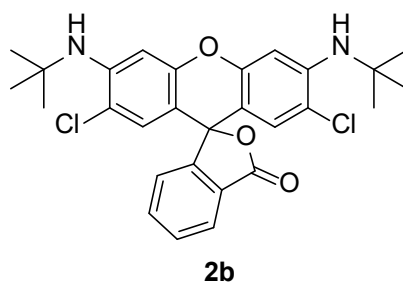
¹H NMR (300 MHz, acetone-*d*₆): δ 7.95 (dt, *J* = 7.5, 1.0 Hz, 1H), 7.78 (td, *J* = 7.4, 1.2 Hz, 1H), 7.69 (td, *J* = 7.4, 1.2 Hz, 1H), 7.27 (dt, *J* = 7.6, 1.0 Hz, 1H), 6.63 – 6.55 (m, 2H), 6.52 – 6.38 (m, 4H), 2.85 br.s (NH + H₂O), 1.40 (s, 18H).

¹³C NMR (101 MHz, pyridine-*d*₅): δ 170.4, 154.1, 151.1, 135.5, 130.3, 129.5, 129.0, 125.6, 125.3, 113.6, 107.7, 100.7, 51.4, 29.9.

MS (ESI): *m/z* (positive mode, rel. int., %) = 443.2 (100) [M+H]⁺.

HRMS (C₂₈H₃₀N₂O₃): *m/z* (positive mode) = 443.2327 (found [M+H]⁺), 443.2329 (calc. [M+H]⁺).

2b



Prepared following the general procedure C from **1d** (124 mg, 0.2 mmol) at 50 °C (bath temperature), reaction time 68 h. Yield 45 mg (44%) of red solid.

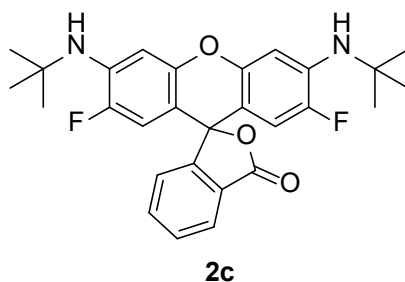
¹H NMR (400 MHz, CDCl₃): δ 8.24 – 8.19 (m, 1H), 7.75 – 7.64 (m, 2H), 7.22 – 7.17 (m, 1H), 6.92 (s, 2H), 6.88 (s, 2H), 5.43 (br.s, 2H, NH), 1.52 (s, 18H).

^{13}C NMR (101 MHz, CDCl_3): δ 167.8, 153.9, 147.8, 133.7, 130.5, 128.9, 128.6, 126.7, 119.4, 110.7, 98.8, 52.8, 29.2.

MS (ESI): m/z (positive mode, rel. int., %) = 511.2 (100) $[\text{M}+\text{H}]^+$.

HRMS ($\text{C}_{28}\text{H}_{28}\text{Cl}_2\text{N}_2\text{O}_3$): m/z (positive mode) = 511.1551 (found $[\text{M}+\text{H}]^+$), 511.1550 (calc. $[\text{M}+\text{H}]^+$).

2c



Prepared following the general procedure C from **1f** (118 mg, 0.2 mmol) at 50 °C (bath temperature), reaction time 63 h. Yield 45 mg (47%) of light pink solid.

^1H NMR (400 MHz, CDCl_3): δ 8.00 (dt, $J = 7.7, 1.1$ Hz, 1H), 7.67 (td, $J = 7.5, 1.3$ Hz, 1H), 7.60 (td, $J = 7.4, 1.1$ Hz, 1H), 7.19 (dt, $J = 7.6, 1.0$ Hz, 1H), 6.72 (d, $^4J_{\text{H-F}} = 7.6$ Hz, 2H), 6.26 (d, $^3J_{\text{H-F}} = 12.1$ Hz, 2H), 4.12 (s, 2H, NH), 1.43 (s, 18H).

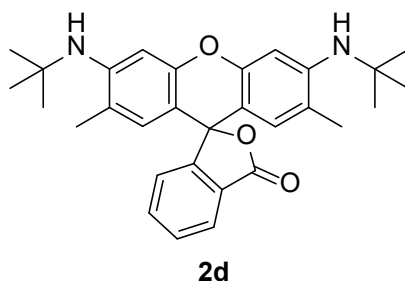
^{19}F NMR (376 MHz, CDCl_3): δ -139.63 (dd, $J = 12.1, 7.6$ Hz).

^{13}C NMR (101 MHz, CDCl_3): δ 169.3, 152.4, 148.7 (d, $^4J_{\text{C-F}} = 1.6$ Hz), 148.4 (d, $^1J_{\text{C-F}} = 235.8$ Hz), 137.6 (d, $^2J_{\text{C-F}} = 12.3$ Hz), 135.0, 129.9, 127.4, 125.1, 124.2, 112.0 (d, $^2J_{\text{C-F}} = 22.4$ Hz), 104.6 (d, $^3J_{\text{C-F}} = 6.9$ Hz), 101.2 (d, $^3J_{\text{C-F}} = 2.6$ Hz), 84.9, 51.3, 29.6.

MS (ESI): m/z (positive mode, rel. int., %) = 479.2 (100) $[\text{M}+\text{H}]^+$.

HRMS ($\text{C}_{28}\text{H}_{28}\text{F}_2\text{N}_2\text{O}_3$): m/z (positive mode) = 479.2139 (found $[\text{M}+\text{H}]^+$), 479.2141 (calc. $[\text{M}+\text{H}]^+$).

2d



Prepared following the general procedure C from **1g** (116 mg, 0.2 mmol), reaction time 40 h. Yield 42 mg (45%) of red solid.

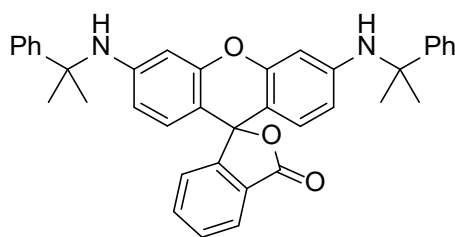
^1H NMR (400 MHz, acetone- d_6): δ 7.94 (dt, J = 7.4, 1.0 Hz, 1H), 7.75 (td, J = 7.5, 1.2 Hz, 1H), 7.68 (td, J = 7.5, 1.2 Hz, 1H), 7.22 (dt, J = 7.6, 1.0 Hz, 1H), 6.67 (s, 2H), 6.37 (d, J = 0.8 Hz, 2H), 4.16 – 4.07 (m, 2H, NH), 1.93 (d, J = 0.8 Hz, 6H), 1.48 (s, 18H).

^{13}C NMR (101 MHz, acetone- d_6): δ 169.8, 154.3, 151.9, 147.7, 135.6, 130.3, 129.3, 128.5, 125.1, 125.0, 120.2, 107.0, 99.6, 51.6, 29.7, 17.4.

MS (ESI): m/z (positive mode, rel. int., %) = 471.3 (100) $[\text{M}+\text{H}]^+$.

HRMS ($\text{C}_{30}\text{H}_{34}\text{N}_2\text{O}_3$): m/z (positive mode) = 471.2647 (found $[\text{M}+\text{H}]^+$), 471.2642 (calc. $[\text{M}+\text{H}]^+$).

2e



2e

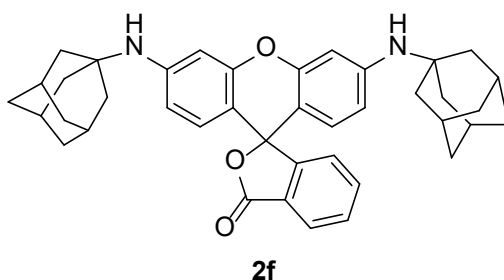
Prepared following the general procedure C from **1a** (124 mg, 0.2 mmol) and 1-methyl-1-phenylethylamine (270 mg, 2 mmol, 10 equiv). Yield 93 mg (82%) of red solid.

^1H NMR (400 MHz, pyridine- d_5): δ 8.22 – 8.14 (m, 1H), 7.77 – 7.73 (m, 1H), 7.57 – 7.50 (m, 4H), 7.45 – 7.38 (m, 1H), 7.34 – 7.25 (m, 4H), 7.21 – 7.14 (m, 2H), 7.06 (dt, J = 7.3, 0.9 Hz, 1H), 6.75 (br.s, 2H, NH), 6.53 (d, J = 8.7 Hz, 2H), 6.43 (dd, J = 8.7, 2.3 Hz, 2H), 6.38 (d, J = 2.2 Hz, 2H), 1.61 (s, 12H).

^{13}C NMR (101 MHz, acetone- d_6): δ 153.8, 153.6, 150.1, 148.1, 135.4, 130.2, 129.5, 129.13, 129.12, 127.3, 127.2, 126.3, 126.0, 125.3, 125.0, 113.0, 107.9, 101.6, 56.4, 32.7, 31.0, 30.5.

MS (ESI): m/z (positive mode, rel. int., %) = 567.3 (100) $[\text{M}+\text{H}]^+$.

HRMS ($\text{C}_{38}\text{H}_{35}\text{N}_2\text{O}_3$): m/z (positive mode) = 567.2630 (found $[\text{M}+\text{H}]^+$), 567.2642 (calc. $[\text{M}+\text{H}]^+$).

2f

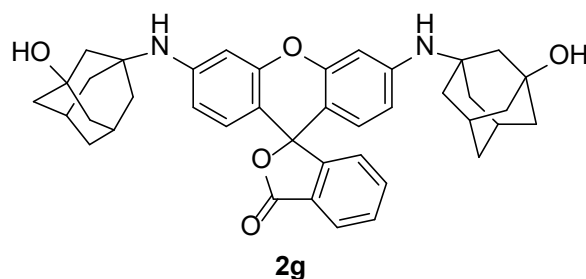
Prepared following the general procedure C from **1a** (92 mg, 0.2 mmol) and 1-adamantylamine (302 mg, 2 mmol, 10 equiv). Yield 81 mg (68%) of pink solid.

^1H NMR (400 MHz, pyridine- d_5): δ 8.48 (d, J = 7.2 Hz, 1H), 7.80 – 7.71 (m, 4H), 7.45 – 7.40 (m, 1H), 7.11 (d, J = 2.2 Hz, 2H), 7.03 (br.d, J = 9.2 Hz, 2H), 6.94 (d, J = 9.0 Hz, 2H), 2.11 (app. br.d, J = 2.9 Hz, 12H), 2.00 (br.s, 6H), 1.58 (app. br.t, J = 2.9 Hz, 12H).

^{13}C NMR (101 MHz, pyridine- d_5): δ 133.9, 130.2, 129.9, 128.7, 127.8, 116.6, 99.4, 42.0, 36.4, 29.9 (indirect detection from a gHSQC experiment, only H-coupled carbons are resolved).

MS (ESI): m/z (positive mode, rel. int., %) = 599.3 (100) $[\text{M}+\text{H}]^+$.

HRMS ($\text{C}_{40}\text{H}_{42}\text{N}_2\text{O}_3$): m/z (positive mode) = 599.3270 (found $[\text{M}+\text{H}]^+$), 599.3268 (calc. $[\text{M}+\text{H}]^+$).

2g

Prepared following the general procedure C from **1b** (110 mg, 0.2 mmol) and 3-aminoadamantan-1-ol (334 mg, 2 mmol, 10 equiv). Yield 120 mg (80%) of red solid (trifluoroacetate salt).

^1H NMR (400 MHz, CD_3OD): δ 8.33 (dd, J = 7.8, 1.4 Hz, 1H), 7.83 (td, J = 7.5, 1.5 Hz, 1H), 7.78 (td, J = 7.6, 1.5 Hz, 1H), 7.38 (dd, J = 7.5, 1.4 Hz, 1H), 7.04 (d, J = 2.2 Hz, 2H), 6.99 (d, J = 9.4 Hz, 2H), 6.90 (br.d, J = 9.4, 2H), 2.35 (br.s, 4H), 2.10 – 1.98 (m, 12H), 1.85 – 1.58 (m, 12H).

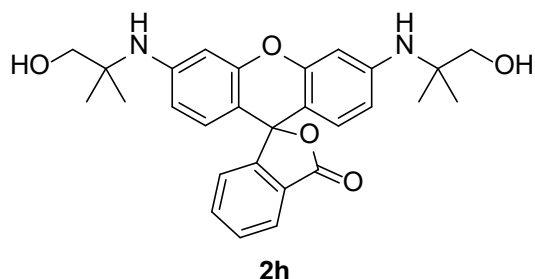
^{19}F NMR (376 MHz, CD_3OD): δ -77.12 (CF_3CO_2^-).

^{13}C NMR (101 MHz, CD_3OD): δ 168.1, 161.4 (br), 159.2, 157.7, 135.2, 133.9, 132.5, 132.1, 131.4, 119.5 (br), 114.9, 98.3 (br), 69.6, 57.2, 44.7, 41.2, 35.8, 32.1.

MS (ESI): m/z (positive mode, rel. int., %) = 631.3 (100) $[M+H]^+$.

HRMS ($C_{40}H_{42}N_2O_5$): m/z (positive mode) = 631.3160 (found $[M+H]^+$), 631.3166 (calc. $[M+H]^+$).

2h



Prepared following the general procedure C from **1b** (110 mg, 0.2 mmol) and 2-amino-2-methyl-1-propanol (178 mg, 2 mmol, 10 equiv). Yield 61 mg (64%) of red solid.

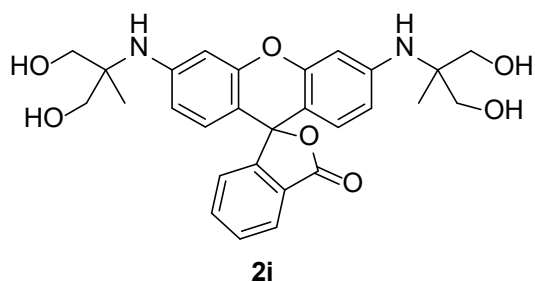
1H NMR (400 MHz, CD_3OD): δ 8.11 – 8.06 (m, 1H), 7.67 – 7.56 (m, 2H), 7.24 – 7.20 (m, 1H), 7.13 (d, J = 9.3 Hz, 2H), 7.00 (d, J = 2.2 Hz, 2H), 6.87 (dd, J = 9.3, 2.2 Hz, 2H), 3.66 (s, 4H + remainder dioxane), 1.46 (s, 12H).

^{13}C NMR (101 MHz, CD_3OD): δ 173.2, 163.1, 159.2, 157.9, 133.9, 131.9, 131.1, 130.74, 130.66, 130.4, 119.1, 115.0, 98.0, 69.3, 57.4, 24.12, 24.08.

MS (ESI): m/z (positive mode, rel. int., %) = 475.2 (100) $[M+H]^+$.

HRMS ($C_{28}H_{30}N_2O_5$): m/z (positive mode) = 475.2226 (found $[M+H]^+$), 475.2227 (calc. $[M+H]^+$).

2i



Prepared following the general procedure C from **1b** (110 mg, 0.2 mmol) and 2-amino-2-methyl-1,3-propanediol (210 mg, 2 mmol, 10 equiv). Yield 54 mg (53%) of red solid.

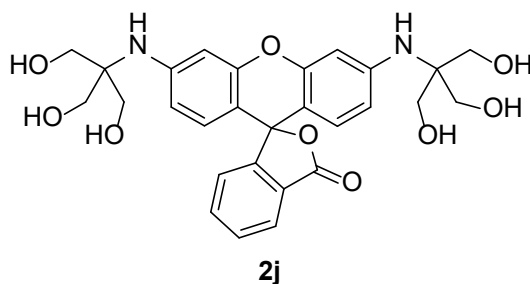
1H NMR (400 MHz, CD_3OD): δ 8.16 – 8.11 (m, 1H), 7.69 – 7.59 (m, 2H), 7.24 – 7.19 (m, 1H), 7.08 (d, J = 9.3 Hz, 1H), 7.04 (d, J = 2.2 Hz, 2H), 6.87 (dd, J = 9.3, 2.3 Hz, 2H), 3.77 – 3.67 (m, 8H), 1.41 (s, 6H).

^{13}C NMR (101 MHz, CD_3OD): δ 172.4, 162.2, 159.2, 158.2, 139.6, 134.3, 131.8, 131.2, 130.9, 130.5, 119.3, 115.1, 98.4, 65.61, 65.59, 61.2, 19.0.

MS (ESI): m/z (positive mode, rel. int., %) = 507.2 (100) $[\text{M}+\text{H}]^+$.

HRMS ($\text{C}_{28}\text{H}_{30}\text{N}_2\text{O}_7$): m/z (positive mode) = 507.2122 (found $[\text{M}+\text{H}]^+$), 507.2126 (calc. $[\text{M}+\text{H}]^+$).

2j



Prepared following the general procedure C from **1b** (110 mg, 0.2 mmol) and tris(hydroxymethyl)aminomethane (242 mg, 2 mmol, 10 equiv). Yield 45 mg (42%) of red solid.

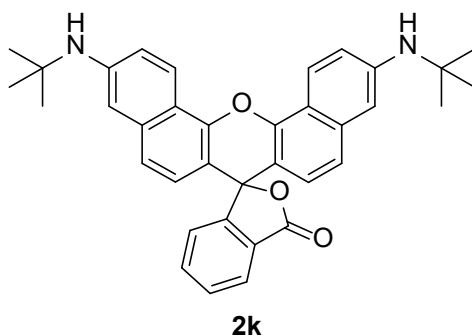
^1H NMR (400 MHz, CD_3OD): δ 8.07 (dd, $J = 7.6, 1.5$ Hz, 1H), 7.66 (td, $J = 7.6, 1.5$ Hz, 1H), 7.61 (td, $J = 7.4, 1.5$ Hz, 1H), 7.21 – 7.09 (m, 5H), 6.94 (dd, $J = 9.3, 2.2$ Hz, 2H), 3.87 (s, 12H).

^{13}C NMR (101 MHz, CD_3OD): δ 173.6, 163.1, 159.3, 158.3, 140.7, 133.5, 131.9, 131.0, 130.9, 130.8, 130.4, 119.4, 115.3, 111.3, 98.5, 64.6, 62.0.

MS (ESI): m/z (positive mode, rel. int., %) = 539.2 (100) $[\text{M}+\text{H}]^+$.

HRMS ($\text{C}_{28}\text{H}_{30}\text{N}_2\text{O}_9$): m/z (positive mode) = 539.2017 (found $[\text{M}+\text{H}]^+$), 539.2024 (calc. $[\text{M}+\text{H}]^+$).

2k



Prepared following the general procedure C from **1h** (112 mg, 0.2 mmol), yield 74 mg (68%) of white solid.

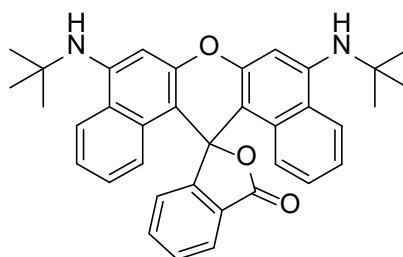
^1H NMR (400 MHz, CDCl_3): δ 8.49 (d, $J = 9.0$ Hz, 2H), 8.11 – 8.05 (m, 1H), 7.65 – 7.56 (m, 2H), 7.26 (d, $J = 8.7$ Hz, 2H), 7.13 – 7.04 (m, 3H), 6.99 (d, $J = 2.3$ Hz, 2H), 6.71 (d, $J = 8.7$ Hz, 2H), 3.97 (br.s, 2H, NH), 1.46 (s, 18H).

^{13}C NMR (101 MHz, CDCl_3): δ 170.1, 154.4, 147.1, 146.3, 136.3, 135.0, 129.6, 126.9, 125.0, 124.5, 124.2, 123.2, 122.2, 120.5, 117.1, 109.1, 108.4, 84.8, 51.7, 29.9.

MS (ESI): m/z (positive mode, rel. int., %) = 543.3 (100) $[\text{M}+\text{H}]^+$.

HRMS ($\text{C}_{36}\text{H}_{34}\text{N}_2\text{O}_3$): m/z (positive mode) = 543.2637 (found $[\text{M}+\text{H}]^+$), 543.2642 (calc. $[\text{M}+\text{H}]^+$).

2l



2l

Prepared following the general [procedure C](#) from **1m** (98 mg, 0.15 mmol), yield 6 mg (7%) of purple solid.

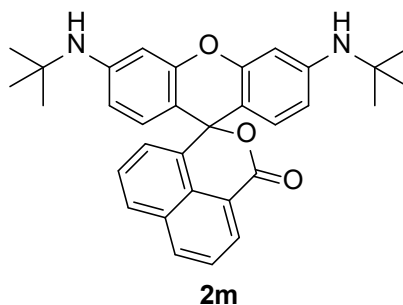
^1H NMR (500 MHz, $\text{DMSO}-d_6$): δ 8.49 (d, $J = 8.4$ Hz, 2H), 8.27 (dd, $J = 7.5, 1.7$ Hz, 1H), 7.97 – 7.88 (m, 2H), 7.77 (br.s, 2H, NH), 7.56 – 7.48 (m, 3H), 7.22 (ddd, $J = 8.4, 7.0, 1.2$ Hz, 2H), 7.16 (s, 2H), 6.80 (d, $J = 8.7$ Hz, 2H), 1.64 (s, 18H).

^{13}C NMR (126 MHz, $\text{DMSO}-d_6$): δ 166.4, 158.2, 152.4, 134.7, 131.9, 130.7, 130.7, 130.1, 129.1, 128.4, 126.3, 124.8, 124.1, 124.0, 109.4, 94.2, 53.4, 28.6.

MS (ESI): m/z (positive mode, rel. int., %) = 543.3 (100) $[\text{M}+\text{H}]^+$.

HRMS ($\text{C}_{36}\text{H}_{34}\text{N}_2\text{O}_3$): m/z (positive mode) = 543.2643 (found $[\text{M}+\text{H}]^+$), 543.2642 (calc. $[\text{M}+\text{H}]^+$).

2m



Prepared following the general procedure C from **1l** (90 mg, 0.15 mmol), yield 34 mg (46%) of light pink solid.

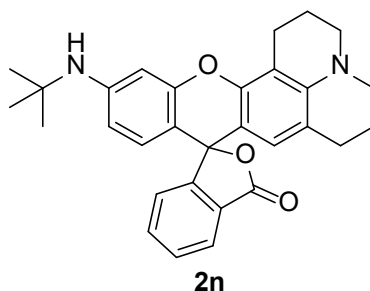
$^1\text{H NMR}$ (400 MHz, CDCl_3): δ 8.54 (dd, $J = 7.2, 1.2$ Hz, 1H), 8.20 (dd, $J = 8.3, 1.2$ Hz, 1H), 7.89 (dd, $J = 8.2, 1.1$ Hz, 1H), 7.72 (dd, $J = 8.3, 7.2$ Hz, 1H), 7.48 (dd, $J = 8.2, 7.3$ Hz, 1H), 7.18 (dd, $J = 7.3, 1.1$ Hz, 1H), 6.63 (d, $J = 8.6$ Hz, 2H), 6.55 (d, $J = 2.4$ Hz, 2H), 6.22 (dd, $J = 8.7, 2.4$ Hz, 2H), 1.37 (s, 18H).

$^{13}\text{C NMR}$ (101 MHz, CDCl_3): δ 163.3, 151.4, 148.5, 136.5, 133.7, 131.9, 129.7, 129.6, 128.6, 128.4, 127.1, 126.9, 126.3, 121.0, 113.1, 112.9, 101.1, 51.5, 29.9.

MS (ESI): m/z (positive mode, rel. int., %) = 493.2 (100) $[\text{M}+\text{H}]^+$.

HRMS ($\text{C}_{32}\text{H}_{32}\text{N}_2\text{O}_3$): m/z (positive mode) = 493.2484 (found $[\text{M}+\text{H}]^+$), 493.2486 (calc. $[\text{M}+\text{H}]^+$).

2n



Prepared following the general procedure C from **1n** (63 mg, 0.12 mmol), yield 37 mg (66%) of purple solid.

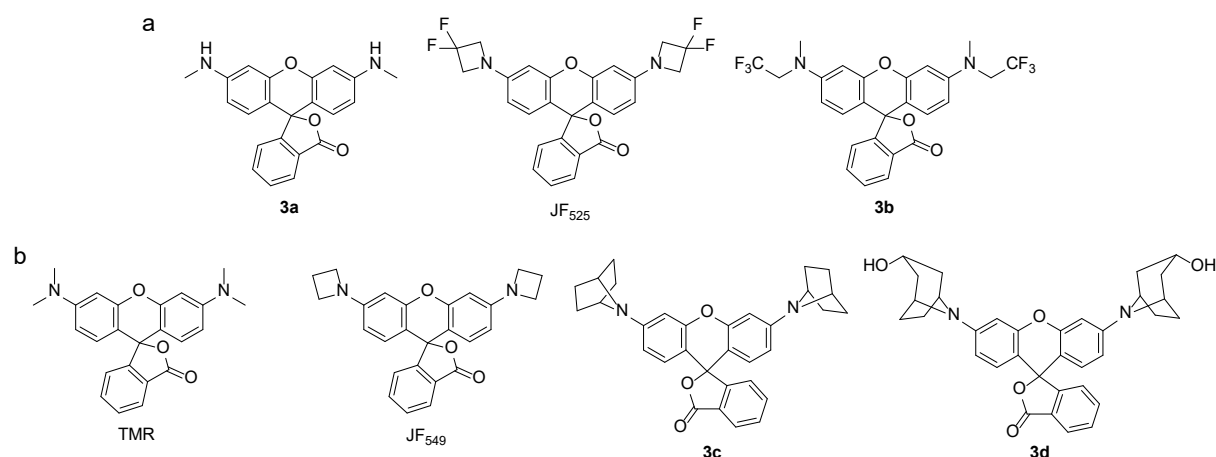
$^1\text{H NMR}$ (400 MHz, $\text{DMSO}-d_6$): δ 7.96 (dt, $J = 7.5, 1.0$ Hz, 1H), 7.76 (td, $J = 7.5, 1.2$ Hz, 1H), 7.68 (td, $J = 7.5, 1.0$ Hz, 1H), 7.24 (dt, $J = 7.7, 0.9$ Hz, 1H), 6.56 (d, $J = 2.3$ Hz, 1H), 6.48 (dd, $J = 8.8, 2.4$ Hz, 1H), 6.35 (d, $J = 8.8$ Hz, 1H), 6.06 (s, 1H), 6.00 (br.s, 1H, NH), 3.23 – 3.09 (m, 4H), 2.87 (t, $J = 6.5$ Hz, 2H), 2.55 – 2.38 (m, 2H), 2.01 – 1.88 (m, 2H), 1.82 – 1.73 (m, 2H), 1.33 (s, 9H).

^{13}C NMR (101 MHz, DMSO- d_6): δ 168.7, 152.5, 150.1, 148.0, 144.8, 134.8, 129.7, 127.9, 127.6, 124.9, 124.7, 124.4, 117.8, 112.4, 106.2, 106.1, 99.1, 50.4, 49.2, 48.8, 29.1, 26.7, 21.1, 20.5.

MS (ESI): m/z (positive mode, rel. int., %) = 467.2 (100) $[\text{M}+\text{H}]^+$.

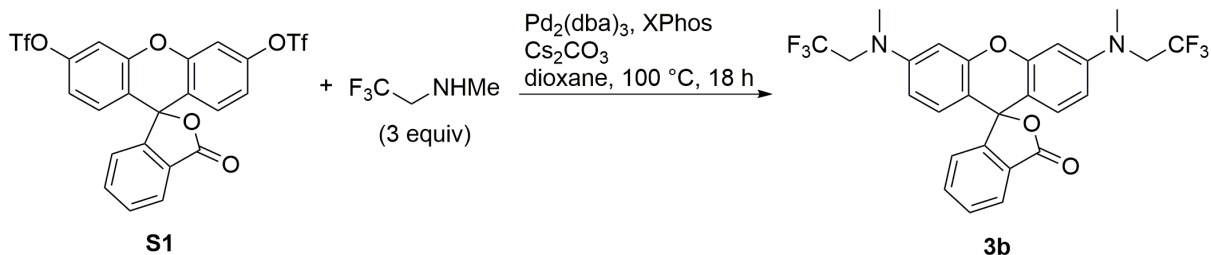
HRMS ($\text{C}_{30}\text{H}_{30}\text{N}_2\text{O}_3$): m/z (positive mode) = 467.2324 (found $[\text{M}+\text{H}]^+$), 467.2329 (calc. $[\text{M}+\text{H}]^+$).

Synthesis of reference dyes



a) Spectral analogs of *N,N'*-dimethylrhodamine **3a** containing either a four-membered azetidine ring (**JF₅₂₅**) or a fluorinated *N*-alkyl substituent (**3b**). b) Spectral analogs of tetramethylrhodamine (**TMR**) containing either a four-membered azetidine ring (**JF₅₄₉**) or an *N*-bridged bicyclic system (**3c**, **3d**). The dyes **3a** [5], **JF₅₂₅** [18] and **JF₅₄₉** [9] were prepared according to the reported procedures.

3b



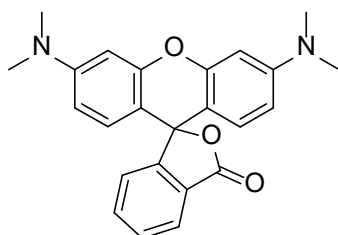
The procedure reported in [5] was followed. The compound **S1** (119 mg, 0.2 mmol), $\text{Pd}_2(\text{dba})_3$ (tris(dibenzylideneacetone)dipalladium(0), Strem Chemicals, Cat. No. 46-3000; 18 mg, 0.02 mmol, 0.1 equiv), XPhos (29 mg, 0.06 mmol, 0.3 equiv) and cesium carbonate (196 mg, 0.6 mmol, 3 equiv) were mixed in a 2 mL crimp-top tube containing a stirring bar (a 0.5-2 mL Biotage microwave vial was used). Dioxane (1.5 mL) was injected, and the mixture was degassed with argon on a Schlenk line. *N*-Methyl-*N*-(2,2,2-trifluoroethyl)amine (60 μL , ~3 equiv) was then injected with a Hamilton syringe, the vial was immersed in a $100\text{ }^\circ\text{C}$ oil bath, and the reaction mixture was stirred for 18 h. The deep-red reaction mixture was poured into brine (50 mL), acetic acid (2 mL) was added, and the mixture was extracted with ethyl acetate ($3 \times 25\text{ mL}$). The combined extracts were dried over Na_2SO_4 and the filtrate was evaporated on Celite. The product was isolated by flash chromatography on Biotage Isolera system (12 g Büchi Sepacore Silica HP cartridge, gradient 25% to 100% ethyl acetate/hexane).

containing the product were evaporated and the product was freeze-dried from dioxane to give 85 mg of **3b** (81%) as fluffy light pink solid (known compound [19]).

^1H NMR (400 MHz, CDCl_3): δ 8.03 – 7.98 (m, 1H), 7.65 (td, $J = 7.4, 1.3$ Hz, 1H), 7.60 (td, $J = 7.4, 1.1$ Hz, 1H), 7.17 (dt, $J = 7.6, 1.1$ Hz, 1H), 6.64 (d, $J = 8.9$ Hz, 2H), 6.61 (d, $J = 2.6$ Hz, 2H), 6.47 (dd, $J = 8.9, 2.6$ Hz, 2H), 3.88 (q, $^3J_{\text{H-F}} = 8.8$ Hz, 3H), 3.09 (s, 6H).

^{19}F NMR (376 MHz, CDCl_3): δ -70.39 (t, $^3J_{\text{F-H}} = 8.8$ Hz).

TMR



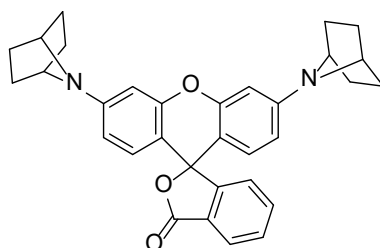
TMR

Prepared following the general procedure C from **1a** (183 mg, 0.4 mmol) and dimethylamine (2 M in THF; 2 mL, 4 mmol, 10 equiv). Yield 97 mg (63%) of purple solid.

^1H NMR (400 MHz, CD_3OD): δ 8.12 – 8.08 (m, 1H), 7.68 – 7.59 (m, 2H), 7.26 (d, $J = 9.5$ Hz, 2H), 7.25 – 7.22 (m, 1H), 6.99 (dd, $J = 9.5, 2.5$ Hz, 2H), 6.88 (d, $J = 2.5$ Hz, 2H), 3.26 (s, 12H).

MS (ESI): m/z (positive mode, rel. int., %) = 387.4 (100) $[\text{M}+\text{H}]^+$.

3c



3c

Prepared following the general procedure C from **1b** (83 mg, 0.15 mmol) and 7-azabicyclo[2.2.1]heptane (Spirochem, Cat. No. SPC-m545; 146 mg, 1.5 mmol, 10 equiv). Yield 44 mg (60%) of pink fluffy solid.

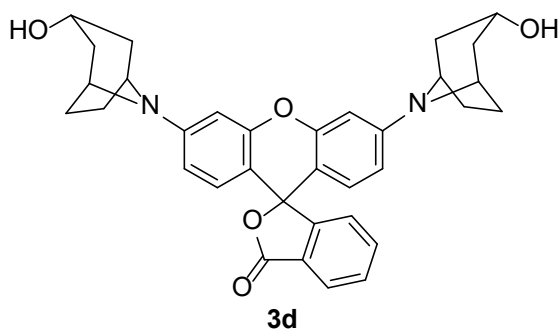
^1H NMR (400 MHz, acetone- d_6): δ 8.22 – 8.18 (m, 1H), 7.86 (td, $J = 7.5, 1.3$ Hz, 1H), 7.80 (td, $J = 7.6, 1.3$ Hz, 1H), 7.40 (dt, $J = 7.6, 0.8$ Hz, 1H), 6.98 – 6.93 (m, 4H), 6.90 (d, $J = 8.8$ Hz, 2H), 4.58 (p, $J = 2.6$ Hz, 4H), 1.91 – 1.76 (m, 8H), 1.65 – 1.57 (m, 8H).

^{13}C NMR (101 MHz, acetone- d_6): δ 167.8, 156.6, 153.9, 134.6, 131.3, 131.0, 130.3, 129.3, 128.6, 115.6, 113.3, 100.6, 58.4, 29.4.

MS (ESI): m/z (positive mode, rel. int., %) = 491.2 (100) $[\text{M}+\text{H}]^+$.

HRMS ($\text{C}_{32}\text{H}_{30}\text{N}_2\text{O}_3$): m/z (positive mode) = 491.2327 (found $[\text{M}+\text{H}]^+$), 491.2329 (calc. $[\text{M}+\text{H}]^+$).

3d



Prepared following the general procedure C from **1b** (110 mg, 0.2 mmol) and nortropine (254 mg, 2 mmol, 10 equiv). Yield 61 mg (55%) of violet solid.

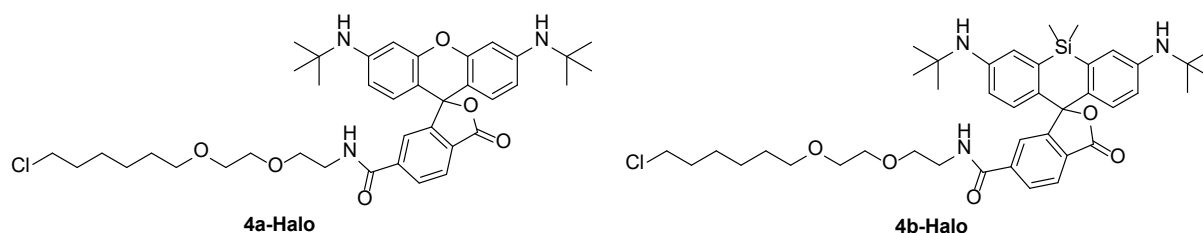
^1H NMR (400 MHz, CD_3OD): δ 8.36 – 8.31 (m, 1H), 7.87 – 7.75 (m, 2H), 7.43 – 7.37 (m, 1H), 7.09 (d, $J = 9.4$ Hz, 2H), 7.02 (dd, $J = 9.4, 2.2$ Hz, 2H), 6.97 (d, $J = 2.2$ Hz, 2H), 4.60 (br.s, 4H), 4.02 (br.t, $J = 4.7$ Hz, 2H), 2.55 – 2.43 m, 4H), 2.09 (m, 8H), 1.94 (br.d, $J = 14.4$ Hz, 4H).

^{13}C NMR (101 MHz, CD_3OD): δ 168.0, 160.5, 159.5, 154.5, 135.4, 133.9, 132.7, 132.5, 132.2, 131.5, 131.4, 116.5, 115.1, 65.5, 56.6, 39.0 (br), 28.5.

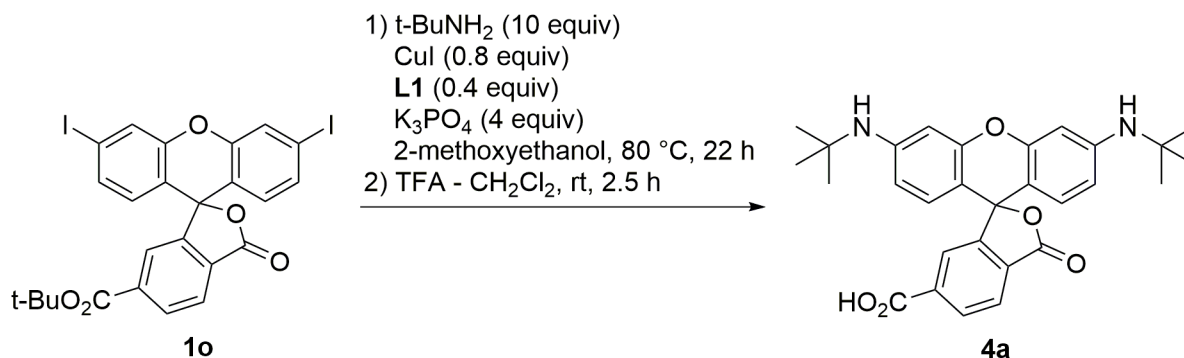
MS (ESI): m/z (positive mode, rel. int., %) = 551.2 (100) $[\text{M}+\text{H}]^+$.

HRMS ($\text{C}_{34}\text{H}_{34}\text{N}_2\text{O}_5$): m/z (positive mode) = 551.2539 (found $[\text{M}+\text{H}]^+$), 551.2540 (calc. $[\text{M}+\text{H}]^+$).

Synthesis of fluorescent HaloTag ligands



4a



Compound **1o** (78 mg, 0.12 mmol), copper(I) iodide (18 mg, 0.096 mmol, 0.8 equiv), ligand **L1** (13 mg, 0.048 mmol, 0.4 equiv) and powdered K₃PO₄ (102 mg, 0.48 mmol, 4 equiv) were mixed in a 5 mL crimp-top tube containing a stirring bar (a 2-5 mL Biotage microwave vial was used), 2-methoxyethanol (0.3 mL) was injected, and the mixture was degassed with argon on a Schlenk line. *tert*-Butylamine (0.13 mL, 1.2 mmol, ~10 equiv) was then injected, the vial was immersed in an 80 °C oil bath, and the reaction mixture was stirred for 22 h. The deep-red reaction mixture was poured into brine (50 mL), acetic acid (2 mL), and the mixture was extracted with ethyl acetate – methanol (5:1, 4 × 50 mL). The combined extracts were dried over Na₂SO₄, filtered and evaporated to dryness. The solid residue was resuspended in dichloromethane (3 mL), trifluoroacetic acid (1 mL) was added, and the intense red mixture was stirred at rt for 2.5 h. The reaction mixture was diluted with toluene, evaporated, chased with toluene (2 × 5 mL), redissolved in acetone and evaporated on Celite. The product was isolated by flash chromatography on Biotage Isolera system (25 g Interchim puriFlash SiHP 30 μm silica cartridge, gradient 0% to 30% ethanol – dichloromethane, then washed with dichloromethane – ethanol – acetic acid 70:30:5). Fractions containing the product (bright red) were evaporated, the residue was dissolved in acetonitrile-water (1:1, with minimal amount of trifluoroacetic acid added to dissolve the solids) and filtered through reversed phase (8 g RP-C₁₈, eluting first with acetonitrile-water 1:1 and then with 0.5% trifluoroacetic acid in acetonitrile). Fractions containing the product (bright red) were pooled, evaporated, and the

product was freeze-dried from aqueous dioxane to give 45 mg of **4a** (62%) as red solid (trifluoroacetate salt).

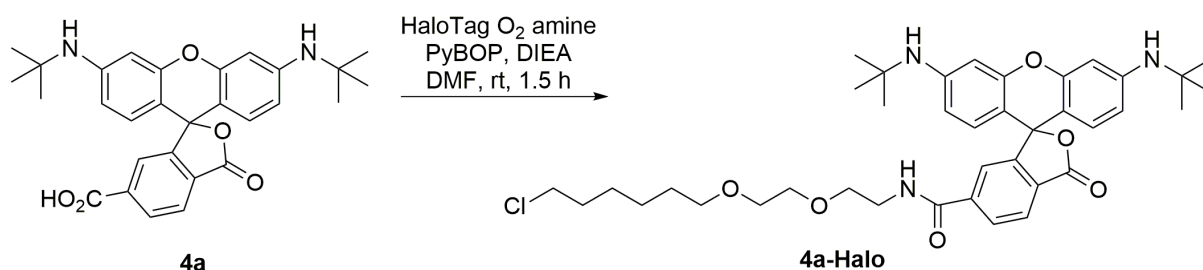
^1H NMR (300 MHz, $\text{DMSO-}d_6$ + 0.1% TFA): δ 8.37 – 8.22 (m, 2H), 7.90 (d, J = 1.1 Hz, 1H), 7.10 – 9.90 (m, 6H), 6.64 (br.s, $\text{NH}+\text{H}_2\text{O}+\text{TFA}$), 1.45 (s, 18H).

^{13}C NMR (126 MHz, $\text{DMSO-}d_6$ + 0.1% TFA): δ 165.8, 165.6, 157.9 (br), 157.6 (br), 157.1 (br), 156.0 (br), 134.4, 134.1, 133.3, 131.2, 130.61, 130.58, 129.9 (br), 112.7, 96.1 (br), 52.4, 28.6.

MS (ESI): m/z (positive mode, rel. int., %) = 487.2 (100) $[\text{M}+\text{H}]^+$.

HRMS ($\text{C}_{29}\text{H}_{30}\text{N}_2\text{O}_5$): m/z (positive mode) = 487.2223 (found $[\text{M}+\text{H}]^+$), 487.2227 (calc. $[\text{M}+\text{H}]^+$).

4a-Halo



To a solution of **4a** (10 mg, 16.6 μmol), HaloTag (O₂) amine [20] (7.5 mg, 33.3 μmol , 2 equiv) and DIEA (30 μL) in DMF (200 μL), a solution of PyBOP (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate; 21 mg, 41.6 μmol , 2.5 equiv) in DMF (100 μL) was added, and the resulting mixture was stirred at rt for 1 h. The solvents were removed *in vacuo*, and the product was isolated by preparative HPLC (gradient 35/65 to 80/20 A:B, A – acetonitrile, B – 0.1% TFA in water) and freeze-dried from dioxane to give 6 mg (52%) of **4a-Halo** as red fluffy solid (trifluoroacetate salt).

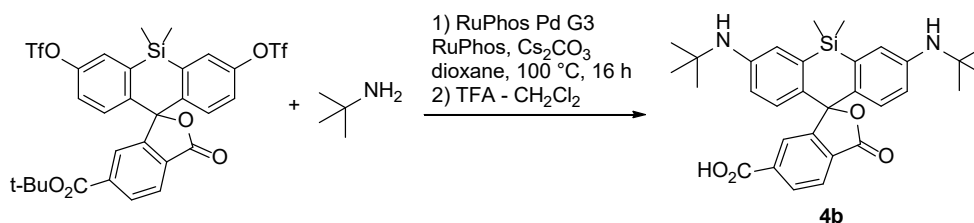
^1H NMR (400 MHz, pyridine- d_5): δ 9.79 (t, J = 5.6 Hz, 1H, CONH), 8.68 (dd, J = 8.1, 1.6 Hz, 1H), 8.52 (d, J = 8.1 Hz, 1H), 8.34 (d, J = 1.5 Hz, 1H), 6.96 (d, J = 2.0 Hz, 2H), 6.88 (d, J = 8.9 Hz, 2H), 6.84 (dd, J = 9.0, 2.1 Hz, 2H), 5.80 (br.s, 2 \times NH + H₂O), 3.90 (q, J = 5.6 Hz, 2H), 3.80 (t, J = 5.6 Hz, 2H), 3.61 (dd, J = 5.9, 3.7 Hz, 2H), 3.51 (dd, J = 5.9, 3.7 Hz, 2H), 3.49 (t, J = 6.7 Hz, 2H), 3.34 (t, J = 6.5 Hz, 2H), 1.66 – 1.56 (m, 2H), 1.52 – 1.44 (m, 2H), 1.44 (s, 18H), 1.37 – 1.21 (m, 4H).

^{13}C NMR (101 MHz, pyridine- d_5): δ 129.9, 129.8, 128.5, 126.4, 116.0, 98.9, 71.2, 70.8, 70.5, 70.3, 45.7, 40.9, 32.9, 29.9, 29.3, 27.0, 25.8 (indirect detection from a gHSQC experiment, only H-coupled carbons are resolved).

MS (ESI): m/z (positive mode, rel. int., %) = 692.2 (100) $[\text{M}+\text{H}]^+$.

HRMS (C₃₉H₅₀ClN₃O₆): *m/z* (positive mode) = 692.3456 (found [M+H]⁺), 692.3461 (calc. [M+H]⁺).

4b



6-(*tert*-Butoxycarbonyl)silafluorescein ditriflate (compound 19c in [11]; 100 mg, 0.135 mmol), RuPhos Pd G3 (23 mg, 0.027 mmol, 0.2 equiv), RuPhos (13 mg, 0.027 mmol, 0.2 equiv) and cesium carbonate (137 mg, 0.41 mmol, 3 equiv) were mixed in a 5 mL crimp-top tube containing a stirring bar (a 2-5 mL Biotage microwave vial was used). Dioxane (0.7 mL) was injected, the mixture was degassed with argon on a Schlenk line, *tert*-butylamine (0.3 mL) was injected and the vial was immersed in a 100 °C oil bath. The reaction mixture was stirred at this temperature for 16 h. The reaction mixture was then poured into brine (40 mL) and extracted with ethyl acetate (3 × 25 mL). The combined extracts were dried over Na₂SO₄ and the filtrate was evaporated on Celite. The product was isolated by flash chromatography on Biotage Isolera system (25 g Interchim Puriflash Silica HP 30µm cartridge, gradient 10% to 40% ethyl acetate/hexane). Fractions containing the product were evaporated and the product was freeze-dried from dioxane to give 30 mg of the *tert*-butyl ester intermediate as light yellow fluffy solid, which was used directly in the next step.

MS (ESI): *m/z* (positive mode, rel. int., %) = 585.4 (100) [M+H]⁺.

HRMS (C₃₅H₄₄N₂O₄Si): *m/z* (positive mode) = 585.3142 (found [M+H]⁺), 585.3143 (calc. [M+H]⁺).

The *tert*-butyl ester was dissolved in dichloromethane (600 µL) and trifluoroacetic acid (200 µL), and the solution was stirred at rt for 3 h. The resulting yellow solution was diluted with toluene, evaporated and chased with toluene (2 × 5 mL). The product was isolated by flash chromatography on Biotage Isolera system (25 g Interchim Puriflash Silica HP 15µm cartridge, gradient 0% to 100% A/B, A = 20% ethanol – dichloromethane, B = dichloromethane) and freeze-dried from dioxane to give 31 mg (36% over 2 steps) of **4b** as blue fluffy solid (trifluoroacetate salt).

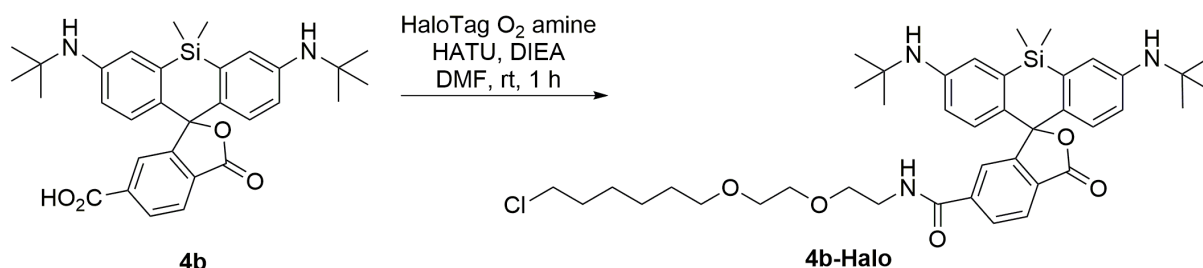
¹H NMR (400 MHz, acetone-*d*₆): δ 8.34 (dd, *J* = 8.0, 1.2 Hz, 1H), 8.21 (br.s, 1H), 8.12 (dd, *J* = 8.0, 0.7 Hz, 1H), 8.02 (d, *J* = 2.4 Hz, 2H), 7.45 (dd, *J* = 8.6, 2.4 Hz, 2H), 7.30 (d, *J* = 8.6 Hz, 2H), 1.42 (s, 18H), 0.75 (s, 3H), 0.69 (s, 3H).

^{13}C NMR (101 MHz, acetone- d_6): δ 131.9, 131.3, 128.2, 127.1, 127.0, 126.7, 26.1, -0.4, -2.1 (indirect detection from a gHSQC experiment, only H-coupled carbons are resolved).

MS (ESI): m/z (positive mode, rel. int., %) = 529.3 (100) $[\text{M}+\text{H}]^+$.

HRMS ($\text{C}_{31}\text{H}_{36}\text{N}_2\text{O}_4\text{Si}$): m/z (positive mode) = 529.2515 (found $[\text{M}+\text{H}]^+$), 529.2517 (calc. $[\text{M}+\text{H}]^+$).

4b-Halo



To a solution of **4b** (8 mg, 15.1 μmol), HaloTag (O₂) amine [20] (6.8 mg, 30.2 μmol , 2 equiv) and DIEA (30 μL) in DMF (200 μL), a solution of HATU (1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxid hexafluorophosphate; 8.9 mg, 23.3 μmol , 1.5 equiv) in DMF (100 μL) was added, and the resulting mixture was stirred at rt for 1 h. Acetic acid (50 μL) was then added, the solvents were removed *in vacuo*, and the product was isolated by preparative HPLC (gradient 30/70 to 60/40 A:B, A – acetonitrile, B – 0.1% TFA in water), fractions containing the product were evaporated and the residue was freeze-dried from dioxane to give 3.5 mg (30%) of **4b-Halo** as light blue fluffy solid.

^1H NMR (400 MHz, pyridine- d_5): δ 9.62 (t, J = 5.6 Hz, 1H, CONH), 8.50 (dd, J = 7.9, 1.4 Hz, 1H), 8.42 (dd, J = 1.4, 0.7 Hz, 1H), 8.26 (dd, J = 7.9, 0.7 Hz, 1H), 7.27 (d, J = 2.7 Hz, 2H), 6.99 (d, J = 8.7 Hz, 2H), 6.75 (dd, J = 8.7, 2.7 Hz, 2H), 5.66 (br.s, 2H, NH), 3.83 (q, J = 5.6 Hz, 2H), 3.74 (t, J = 5.4 Hz, 2H), 3.62 – 3.57 (m, 2H), 3.55 – 3.51 (m, 2H), 3.49 (t, J = 6.7 Hz, 2H), 3.35 (t, J = 6.5 Hz, 2H), 1.68 – 1.56 (m, 2H), 1.55 – 1.43 (m, 2H), 1.38 (s, 18H), 1.36 – 1.18 (m, 4H), 0.56 (s, 3H), 0.55 (s, 3H).

^{13}C NMR (101 MHz, pyridine- d_5): δ 129.1, 128.9, 125.9, 124.4, 121.0, 115.9, 71.2, 70.8, 70.5, 70.2, 45.6, 40.9, 32.9, 30.0, 29.8, 27.0, 25.8, 0.4, -1.5 (indirect detection from a gHSQC experiment, only H-coupled carbons are resolved).

MS (ESI): m/z (positive mode, rel. int., %) = 734.5 (100) $[\text{M}+\text{H}]^+$.

HRMS ($\text{C}_{41}\text{H}_{56}\text{ClN}_3\text{O}_5\text{Si}$): m/z (positive mode) = 734.3746 (found $[\text{M}+\text{H}]^+$), 734.3751 (calc. $[\text{M}+\text{H}]^+$).

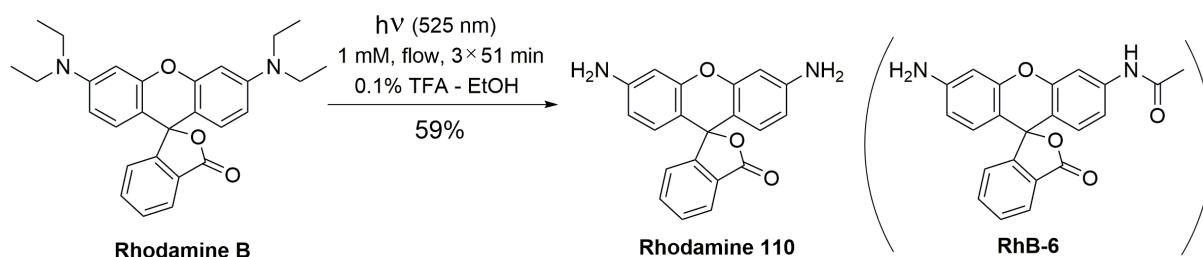
Semipreparative photolysis of rhodamine dyes and identification of photoproducts

Description of the flow photoreactor

A custom-built flow photoreactor (see Figures S2 and S3), inspired by a model described by Jackl et al. [21], was used for semipreparative photolysis of the dyes and for photolysis of the dyes in buffer solutions. The reaction mixture (air saturated) was passed through a glass chip (LTF-V, channel size 1 mm, volume 1.7 mL; Little Things Factory GmbH, Germany) with the help of a syringe pump (NE-1000; New Era Pump Systems Inc., NY, USA) or, for large volumes, a peristaltic pump (NE-9000G; New Era Pump Systems Inc.). Lectro-Cath tubing with Luer-Lok connectors (article 1155.03, length 30 cm, ID 1.0 mm, volume 0.49 mL; Vygon, France) was used to connect the chip with the receiving flask and the pump. The reactor chip was mounted inside a black polycarbonate box (Figure S3,b) and irradiated from the distance of 3.0 cm with a 300 W high power LED array (model SDP300WG64100030010B for 525 nm or SDP300WB3410003003A for 465 nm; SnowDragon Industrial Co., Ltd., Shenzhen, China; see Figure S1 for the emission spectra). The LED array was powered using an HLG-480H-36A (MeanWell Enterprises Co., Ltd., Taiwan) constant voltage/constant current LED driver set at 35V and 9.2A. The LED array was cooled with a 12V be quiet! Dark Rock Pro 3 CPU cooler (article BK019, Listan GmbH &Co., KG, Germany), mounted to the aluminium sink of the LED with a 62×40×10 mm solid copper plate (Figure S3,a) incorporating an NTC thermistor sensor (TH1, "LED"). The backside of the glass chip was attached to the cold side (default radiating surface, 70×70 mm) of CPM-2F thermoelectric (Peltier) module (12Vdc, 6 A, connected at inverse voltage in reactor cooling mode; CUI Inc., OR, USA) with a 90×70×6 mm solid copper plate incorporating another NTC thermistor sensor (TH3, "TEC, cold side"). The hot side (default cooling surface, 44.5×44.5 mm) of the thermoelectric module was fitted with a copper radiator incorporating a third NTC thermistor sensor (TH2, "TEC, hot side"). The reactor box was equipped with a PFB1212UHE-T50F 12V 4A DC fan (minimal airflow 6.44 m³/min; Delta Electronics, Thailand) for efficient air cooling of the TEC module radiator. The temperatures of the LED (typically around 45 °C) and of the TEC radiator were monitored using a generic digital dual thermometer circuit, powered together with the LED cooler and the fan by a 12V 12.5A DC switching power supply (AHM150PS12, XP Power, Singapore). The temperature of the reactor chip (set at 30±1 °C in cooling mode, unless indicated otherwise) was controlled by means of a generic W1209 thermostat, and the polarity of the TEC module could be reversed with the switch SW1 (M2123TCFW02, NKK Switches, Japan) if heating of the flow reactor were desired.

For a semipreparative photolysis, the entire collected photolysate solution was evaporated (bath temperature ≤ 35 °C) and the individual photoproducts were isolated by preparative HPLC (column: Interchim Uptisphere Strategy C18-HQ, 10 μ m, 250 \times 21.2 mm), flow rate: 20 mL/min, gradient acetonitrile/0.1% TFA in water as indicated below for individual experiments. Fractions containing the photoproducts of interest were evaporated (bath temperature ≤ 40 °C), and the residue materials were freeze-dried from dioxane or aqueous dioxane.

Exhaustive photooxidative dealkylation of Rhodamine B



A solution of Rhodamine B (20 mg, 41.8 μ mol) in 0.1% TFA – ethanol (40 mL, ~ 1 mM) was irradiated in the flow photoreactor (525 nm, residence time 51 min), repeating the photolysis on the same solution three times sequentially. After the third pass, the entire photolysate solution was evaporated (bath temperature ≤ 35 °C) and the products were isolated by preparative HPLC (gradient: acetonitrile/0.1% TFA in water 20/80 \rightarrow 50/50 over 25 min). The pure fractions were evaporated and freeze-dried from aqueous dioxane to give 11 mg (59%) of Rhodamine 110. The byproduct (< 1 mg) of the reaction (**RhB-6**) was identified as *N*-acetyl rhodamine 110 (known compound [22]).

Derivatization of JF₅₄₉-derived diethyl acetals with 2,4-dinitrophenylhydrazine

A solution of JF₅₄₉ (12 mg, 29.3 μ mol) in 0.1% TFA – ethanol (24 mL, ~1.2 mM) was irradiated in the flow photoreactor (525 nm, residence time 102 min). 2,4-Dinitrophenylhydrazine (17 mg, 88 μ mol, ~3 equiv) was added to the photolysate, and it was evaporated down to 5 mL. Water (1 mL) and trifluoroacetic acid (20 μ L) were added, and the reaction mixture was stirred at 80 °C for 1 h. The solution was then evaporated (bath temperature \leq 35 °C) and the products were isolated by preparative HPLC (gradient: acetonitrile/0.1% TFA in water 30/70 \rightarrow 80/20 over 25 min). The pure fractions were evaporated and freeze-dried from aqueous dioxane to provide the individual photoproducts.

Photolysis of rhodamine dyes and chemometric analysis of the photobleaching/photobleaching reaction kinetics

Experimental setup

Samples were irradiated in a home-built setup, consisting of a green LED (M530L3, Thorlabs) and a cell holder (Luma 40, Quantum Northwest). The sample solution was stirred at 2500 rpm and thermostated at 20 °C during the whole experiment. Two lenses were used to have the beam approximately collimated within the path length of the 10x10 mm quartz cuvette (119F-10-40, Hellma Analytics). The intensity of the irradiation light was calibrated with a chemical actinometer (ring-opening reaction of Aberchrome 670 in toluene [23]). The absorption spectrum was measured at a right angle with respect to the irradiation source (LED), with a Deuterium/Xenon lamp (DH-2000-BAL, Ocean Optics) as an illumination source and a diode array spectrometer (FLAME-S-UV-VIS-ES, Ocean Optics). Emission was measured using the LED irradiation source and the same spectrometer, in a 90-degree configuration. The reference spectrum for absorbance measurements was recorded with the solvent before starting the irradiation. Because of this, the baseline changes occurring during the experiment, mostly due to instability of the lamp, were not compensated and contributed to the data noise. A custom-written routine (Matlab, MathWorks) controlled the shutter of the lamp, the spectrometer, and the LED power source via TTL signals through a data acquisition system (National Instruments, myDAQ). During the experiment, the LED is switched on for a fixed interval (typically 2 minutes) and then the absorption and emission spectra are recorded. For the latter, the LED is switched on for only 4 milliseconds, and thus the sample irradiation during this period is negligible. The samples were irradiated for a total of 24 hours. However, due to the different reactivity of the samples, fitting of the data to different reaction kinetic schemes in some cases was performed on shorter time scales (e.g., from 0 up to 250-600 min). At the end of the irradiation period (1440 min) and at selected times, the solutions were analyzed by HPLC (Prominence, Shimadzu) equipped with a diode array absorption detector (SPD-M20A) and a fluorescence detector (RF-20A).

Data analysis

In simple cases of fluorophore photobleaching, only the starting compound (SC) absorbs the irradiation light, therefore there exists at least one range of the absorption spectrum where its concentration can be calculated at any time from known molar extinction coefficient $\epsilon(\lambda)$ values. In such a case, the analysis of the bleaching kinetics is simple, and the extraction of the quantum yield (Φ_{bleach}) for the bleaching process is straightforward. Even in the cases where

there is more than one decomposition pathway from the SC, the value calculated is the sum of the quantum yields for all concurrent photobleaching processes ($\Phi_{bleach} = \Sigma(\Phi_i)$). For a single photochemical step, the change in the concentration of the SC over time depends on the irradiation intensity (I_0), the molar extinction coefficient of the SC (ϵ_{SC}) and the photokinetic factor (F), all measured at the irradiation wavelength [24]:

$$\frac{d[SC]}{dt} = -I_0\Phi_{bleach}F\epsilon_{SC}[SC]$$

This equation has to be numerically solved, except in some particular cases such as total absorption, or whenever F is approximately constant (low conversion, or measurement with diluted samples), with Φ_{bleach} as the only unknown. Typically, $[SC]$ is calculated from the spectrophotometric measurements at a single wavelength.

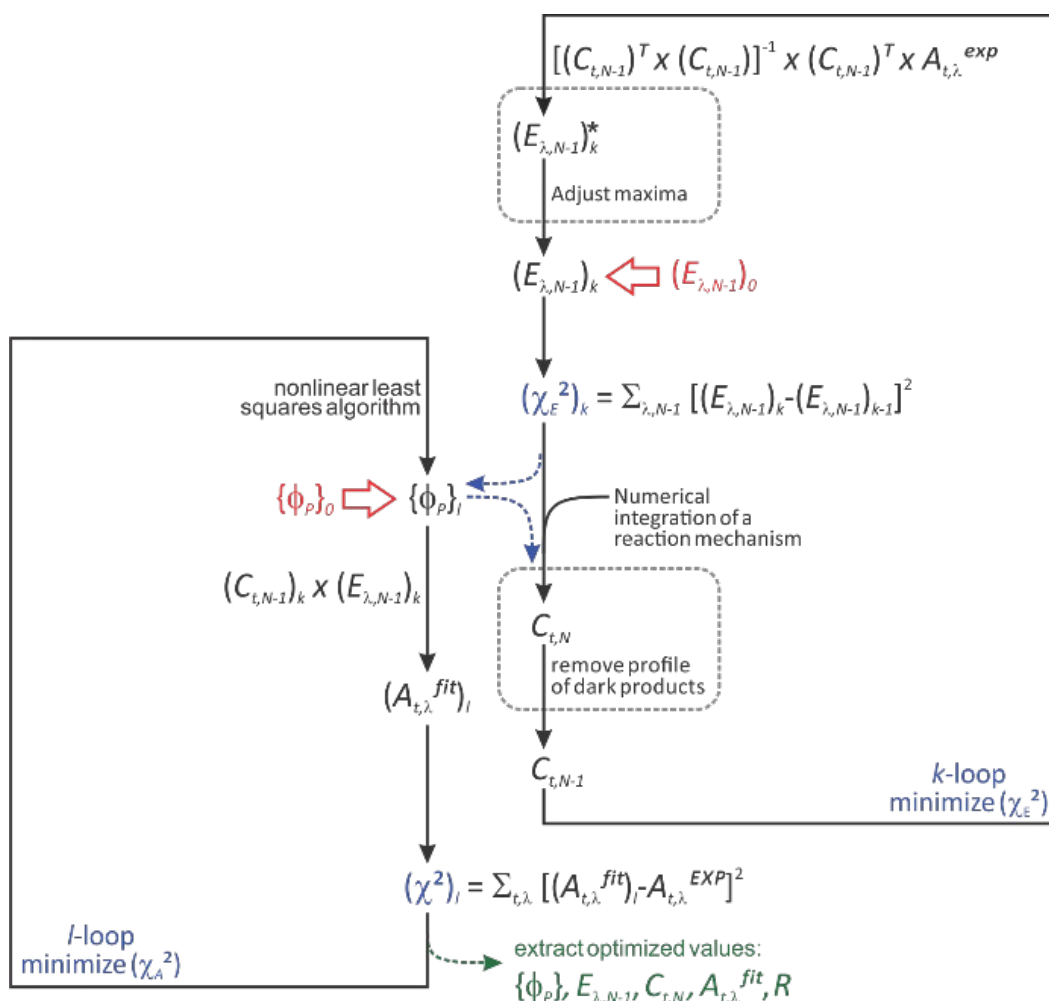
In cases of more complex reaction mechanism, when intermediates and/or products are absorbing the irradiation light, this procedure yields false results and is not applicable. The determination of several quantum yields is necessary, one for each photochemical process (in addition to the reaction rate constants for all thermal processes, if present). Even if the determination of only one quantum yield is desired (i.e., for the decomposition of the SC), the task is becoming complex because multiple reaction products (sometimes with unknown absorption spectra) absorb within the same region. This makes it practically impossible to determine the concentration profile of SC, even with real-time full spectrophotochemical data, unless cumbersome and time-consuming additional quantitative analyses are performed (e.g., by HPLC) during the course of irradiation. However, a method has been recently proposed allowing to calculate the quantum yields of all photochemical processes and the absorption spectra of every involved species in consecutive photochemical processes, provided that only the overall photodegradation mechanism is known [25]. For the best possible solution, several mechanistic hypotheses may be tested, and the simplest one that best describes the data is selected.

In our work, we have applied a slightly modified version (Scheme S1) of this reported method. The main differences are the modifications introduced to account for non-absorbing species (within the measured spectral range) and the calculation of the photokinetic factor(s). In this chemometric approach, the experimental data matrix $A^{EXP}(t, \lambda)$ containing transient absorption measurements during the course of irradiation is decomposed into two smaller matrices: a concentration profiles matrix $C(t, N)$ and an absorption spectra matrix $E(\lambda, N)$, where N is the number of species involved.

$$A^{EXP}(t, \lambda) = C(t, N) \times E(\lambda, N)$$

In a strict sense, the size of the matrices is determined by the number of measurements (size of vector t), the number of channels (size of the wavelengths vector λ) and the number of involved species N , whereby the t and λ vectors must be a column and a row vector, respectively. A unique solution is possible if $C(t, N)$ has a unique dependence on one of the variables (in this case the time due to the stoichiometry of the chemical mechanism), and the response is linear. The complete description of the method used is given in [25] and references therein. Once the appropriate mechanism has been identified, the result of the data analysis is the quantum yields for all photochemical reactions, in addition to the concentration profiles and absorption spectra of all species. To control the validity of the results, the HPLC measurements at selected times help to choose and confirm or discard a mechanism.

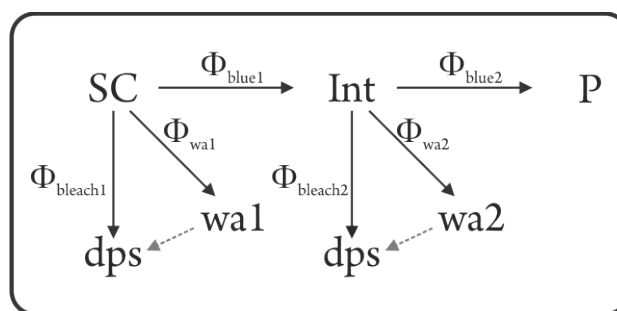
In principle, the same process can be applied to the emission data. However, to fulfil the condition of a linear response of the fluorescence emission, the maximum sample optical density should not exceed 0.05, at which concentrations the absorption data is becoming too noisy (particularly when using a single beam spectrometer). Thus, we have selected such intermediate concentrations that result in satisfactory signal-to-noise ratio of the absorption signal and allow for HPLC measurements, and only absorption data was analytically treated.



Scheme S1. Schematic representation of the iterative fitting procedure used to calculate the quantum yields of the fluorophore photodegradation processes in the presence of dark (non-absorbing) products (steps in grey boxes are added for such cases). The *k*-loop is executed first until convergence of χ_E^2 , and then a single step of the *l*-loop is executed followed by return to the *k*-loop (blue dashed arrows). The process is repeated until convergence of χ_A^2 (green arrow) is reached. The fit requires an initial guess for the reaction quantum yields of *P* photoprocesses involved in the mechanism, and the absorption spectra of the *N* species (red text/arrows). Dark products are accounted for in the differential equations, and their concentration profile is calculated, but not used for the calculation of spectral matrix (*E*) (zero absorption in the observed spectral range). The goodness of the fit is evaluated by visual observation of the residuals ($R = A^{EXP} - A^{fit}$) and the mean squared error value. For the numerical integration of the differential equations of the concentration profiles, the spectral distribution of the irradiation source is taken into consideration.

Mechanistic models of fluorophore photoconversion

The proposed general mechanism involves the following main species (Scheme S2): the starting compound (*SC*), a first-step photobleaching intermediate (*Int*), and the second-step photobleaching product (*P*). Typically, the observed hypsochromic shifts of the absorption maxima are between 5-15 nm for each photobleaching step (*SC*→*Int* and *Int*→*P*) and only slightly depend on the solvent. Thus, the position of the absorption maxima and shifts may differ between absorption recorded during the irradiation (in 0.1%v/v TFA – ethanol) and in the HPLC experiments (in 0.1%v/v TFA in acetonitrile–water gradient). There are also the intermediates present (*wa1*, *wa2* for the first and second photobleaching step, respectively) showing wide absorption spectra that were identified as *N*-acetylated photoproducts (see Figure 1, step v'), and the dark products (*dps*) that do not absorb within the observed range (250 – 800 nm). While thermal or photochemical conversion of *wa1* and *wa2* byproducts into *Int* and *P*, respectively, is probable, at the moment no further evaluation for such processes can be presented.



Scheme S2. The overall mechanism of triarylmethane fluorophore photoconversion (limited to the first two photobleaching steps), based on the experiments of our work. In all following cases, simplified versions of this mechanism were sufficient for fitting of the observed data to a chosen model, at least within the selected timeframe.

The overall mechanism is complex and involves too many variables to obtain the large number of output parameters reliably. Nevertheless, with the help of HPLC analysis at several time points during the course of photolysis, and the batch irradiation experiments described below, it was possible to propose simplified models fitting the experimental data satisfactorily. Thus, the minor photoproducts visible in HPLC traces and kinetics of the corresponding photoprocesses were not taken into account. In some cases, where multiple interdependent photobleaching steps were possible (see photooxidation schemes for the individual fluorophores below), the photoconversion model was simplified by limiting the duration of photolysis until the data can be approximated to an initial two-step photobleaching reaction scheme, as knowing

only the quantum yields of the first step photoprocesses (Φ_{blue1} , $\Phi_{bleach1}$, Φ_{wa1}) is sufficient to evaluate the photostability of a given fluorophore (SC).

As a general result, all tested fluorophores have been separated into two classes (**bluing** and **non-bluing** fluorophores), with some exceptions that have to be discussed separately. In all cases, bleaching to dark products from both SC and *Int* had to be considered. Bleaching from *P* was neglected in all cases, because it was much slower than the other processes (in particular for *N,N'*-disubstituted rhodamines, due to high photostability of Rhodamine 110 product).

Non-bluing fluorophores: neat overall bleaching to dark products: Rhodamine 110, **2a**, **2f**, **2g**, **2m**, **3c** and **3d**. As no photobluing was detected, only one quantum yield value was calculated ($\Phi_{bleach1}$). Nevertheless, the same fitting method was used instead of a single wavelength analysis.

Bluing fluorophores with two-step bluing: Rhodamine 6G, **2h** and **2i**. Two sequential *N*-dealkylations corresponding to the two definite photobluing steps were observed.

Bluing fluorophores with one-step bluing: Rhodamine B, TMR, JF₅₂₅, JF₅₄₉ and **3b**. Because of the high photostability of SC or *Int*, only one-step photobluing (single *N*-dealkylation) was observed.

Bluing fluorophores, bluing with accumulation of *N*-acylated byproducts (*wa1*, *wa2*): **2j**.

Some comments on special cases:

3a: by HPLC analysis of irradiated solution, two photobluing products, and two byproducts with wide absorption spectra (*wa1* and *wa2*) were found. Therefore, the complete mechanism may be approximated to the one in Scheme S2. However, fitting of such a complex multivariable model failed to converge to reasonable values, likely because multiple photoproducts were present at the same time in low concentrations. Therefore, the data at short irradiation times (low conversion) were fitted to a simple clean bleaching model; under these conditions, photobleaching was the prevailing process.

JF₅₄₉: at the end of the irradiation time, there is SC left along with smaller amounts of two blue-shifted products (major **JF₅₄₉-3a** and minor **JF₅₄₉-2**, see Table S13), and it was necessary to add **JF₅₄₉-3a** as a species to the fitting model. The reason for this behavior is the high photostability of azetidine-substituted fluorophores, so that the ring opening photooxidation

steps ($\text{JF}_{549} \rightarrow \text{JF}_{549-2}$, $\text{JF}_{549-2} \rightarrow \text{JF}_{549-3b}$, $\text{JF}_{549-3a} \rightarrow \text{JF}_{549-4}$) are much slower than the *N*-photodealkylation of acyclic substituents ($\text{JF}_{549-2} \rightarrow \text{JF}_{549-3a}$, $\text{JF}_{549-3a} \rightarrow \text{JF}_{549-4}$). This results in accumulation of one of the second-step photobleaching intermediates (JF_{549-3a}), which has no acyclic *N*-substituents. The main photoconversion pathway for JF_{549} dye was the dye bleaching to dark products.

3b: at the end of the irradiation time, most of SC has been consumed and two blue-shifted products, **3b-2** (*Int*) and **3b-3** (*P*), were present, suggesting the predominant photooxidation of methyl (rather than 2,2,2-trifluoroethyl) *N*-substituents. The main photoconversion pathway for **3b** was its bleaching to dark products, and a model with only one photobleaching product fitted satisfactorily.

TMR: the main decomposition path of the first-step bluing product (*Int*) is to the *N*-acylated intermediate *wa2*, which accumulated over time. As the second-step bluing to *P* was hardly observed in HPLC analyses, the decomposition of *wa2* must be slow on the experiment timescale.

JF_{525} : degradation into multiple products visible by HPLC (as well as on attempted semipreparative photolysis, see below).

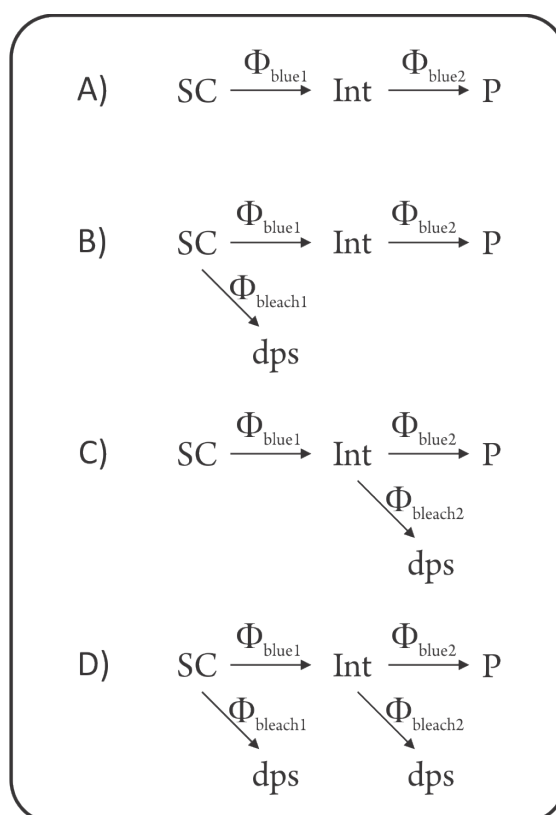
Fitting example for a non-bluing fluorophore (2a)

The absorption and emission decreased monotonically (Figure 4,a, Figure S23), and there was no evidence of the presence of absorbing and emitting products within the observed range from 250 to 800 nm (Figure S24,A). Therefore, the data was fitted to a simple model with only one reaction path from the starting compound to a dark product (Figure S25). The absorption spectrum of the SC was left as a fitting variable; the result obtained was practically identical to the spectrum of the SC, recorded at $t = 0$ min (Figure S25,E).

Fitting example for a fluorophore with two-step photobleaching (2h)

The absorption and emission of compound **2h** demonstrated a hypsochromic shift during irradiation with green light (Figure 4,b; Figure S26,A). By carefully selecting an intermediate wavelength, the presence of an intermediary compound (colored and emissive) is revealed (Figure 5,d,e), and HPLC traces at selected irradiation times are consistent with these observations (Figure S26,B,C). At $t = 1400$ min, only the presence of product *P* was observed (Figure S26,D); further irradiation showed only photobleaching of *P* (data not shown).

Several models of consecutive reactions have been evaluated (Scheme S3), some containing additional bleaching path(s) to dark products (*dps*), non-fluorescent and non-absorbing within the experimental range (250 nm to 800 nm).



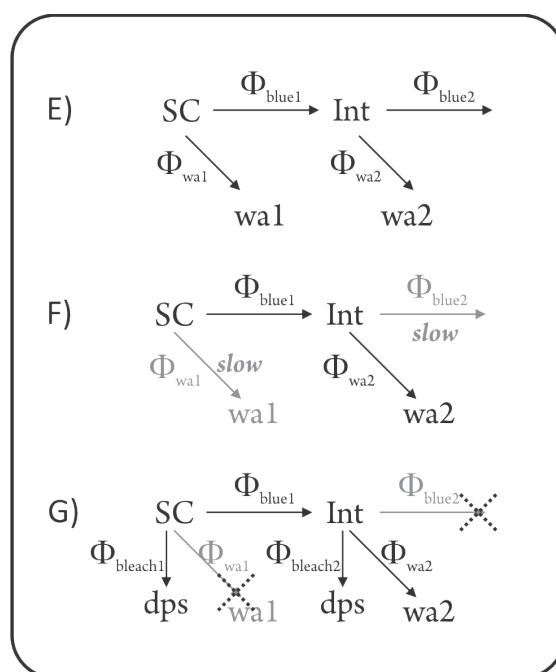
Scheme S3. Kinetic models evaluated for a two-step photobleaching mechanism (with and without photobleaching steps).

In the absence of any photobleaching paths, as in model A, the absorption coefficients of the photobleaching products (*Int* and *P*) decrease sequentially (Figure S27). Despite the goodness of the fit, this was a clear indication of the presence of additional bleaching steps to non-absorbing dark products (*dps*). Hence, further models included the presence of *dps* (with a null absorption spectra in the fitted range). During the initial calculations of the absorption spectra matrix ($E = (C)^T \times A^{EXP}$), any concentration profile for the *dps* species resulted in a non-zero absorption spectrum which tended to correct for experimental errors. Therefore, simple convergence in such models to a reasonable solution was not deemed possible, unless an additional constrain was added. For this reason, we assumed the extinction coefficient at the absorption maximum $\epsilon(\lambda_{\text{max}})$ approximately constant for the starting material and all simple *N*-dealkylation photobleaching products. Models B and C (Scheme S3) included only one of the additional bleaching paths, from *SC* or *Int* respectively. Model B yielded poor fitting, and its convergence

was dependent on the selection of initial parameters, while model C produced better fitting and more robust convergence (Figure S28). As a result, both processes had to be considered, with model D yielding the best fitting (Figure 5,A-C) with comparable photobleaching quantum yields ($\Phi_{\text{bleach1}} = 4.3\text{E-}6$; $\Phi_{\text{bleach2}} = 5.3\text{E-}6$) for both photobleaching steps.

Fitting example for a fluorophore with two-step photobleaching and accumulation of byproducts (2j)

The photodegradation of compound **2j** (Figure S29) represents a two-step photobleaching process with a more complex reaction scheme. At least two other photoproducts, with wider absorption spectra and lower fluorescence emission, are formed (*wa1* and *wa2*, see model E, Scheme S4):



Scheme S4. Kinetic models evaluated for a two-step photobleaching mechanism with accumulation of byproducts (with and without photobleaching steps included).

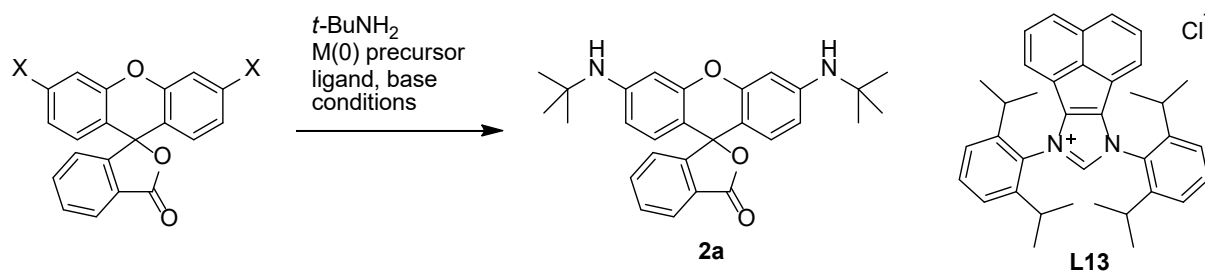
2D HPLC absorption data, recorded at intermediate time points (Figure S30), corroborated the proposed model. The byproducts *wa1* and *wa2* were tentatively identified as unknown *N*-monoacetylated rhodamine derivatives based on the overall photooxidation scheme (Figure 1) and isolation of spectrally similar (Figure S9) *N*-acetyl rhodamine 110 (**RhB-6**) from the Rhodamine B photooxidation reaction mixture. However, *wa1* was accumulated in very low amounts, and thus its formation (Φ_{wa1}) was not included in the overall mechanism. Fitting of

the complete models F or G (Scheme S4, black and gray arrows) proved too complex; however, by limiting the duration of photolysis, both simplified models F and G (with and without the addition of photobleaching; Scheme S4, black arrows only) resulted in robust approximations (Figure S31).

Nonspecific labeling of bovine serum albumin with dye photoproducts

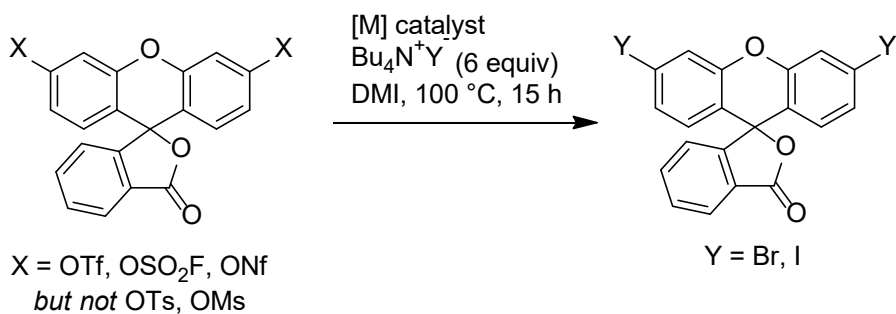
A 10 μ L aliquot of stock solution of a given dye in DMSO (10 mM) was dissolved in 10 mL of phosphate buffer saline pH 7.4 (PBS, Lonza) to give a 10 μ M aqueous buffer solution containing 0.1% (v/v) DMSO. The prepared dye solutions were irradiated in the flow photoreactor (525 nm, residence time 5.1 min), with HPLC control of the photolysates showing ~50% conversion of the starting dye in each case. Bovine serum albumin (BSA, Sigma) was dissolved in PBS (pH 7.4) at 100 mg/mL concentration. To a sample of photolysate solution (1 mL), the BSA solution (100 mg/mL; 10 μ L) was added, and the resulting mixtures (final BSA concentration 15 μ M; dye/protein ratio 1:1.5) were incubated for 18 h at 25 °C in the dark with continuous shaking. Afterwards, the samples were mixed with 4x Laemmli Sample Buffer (Bio-Rad) supplemented with 2-mercaptoethanol to 10% final concentration. Samples were heated for 5 min at 95 °C, and 10 μ L aliquot of each sample was loaded into the gradient 4 – 15% Mini-PROTEAN® TGX™ precast protein gels (Bio-Rad). SDS-PAGE was performed in Mini-PROTEAN Tetra Cell (Bio-Rad) apparatus with voltage set to 150 V until the loading dye had run out of the gel. In gel fluorescence was acquired with Imager 600RGB system (Amersham), see Figure S32. Afterwards, the gels were stained with Coomassie Brilliant Blue G-250 for protein quantification and imaged again on Imager 600RGB system (Figure S32). Image quantification was performed using Fiji software region measurement tool.

Supplementary Tables.



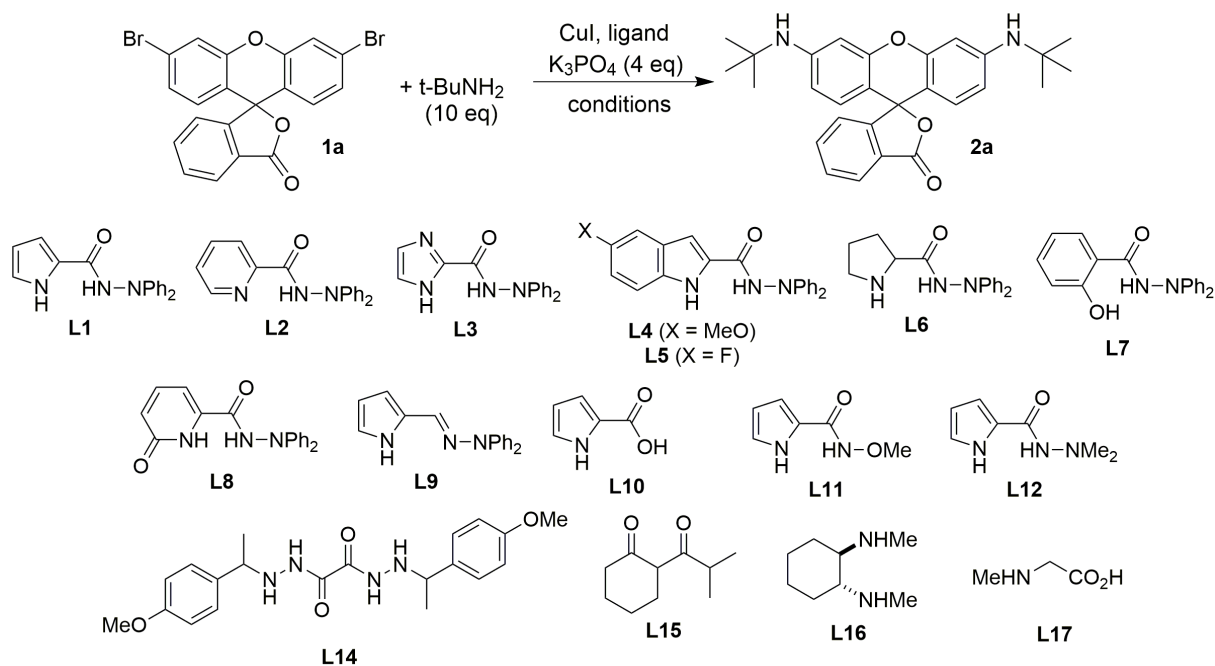
X	M(0) precursor	ligand	base	conditions	yield of 2a
OTf	Pd ₂ (dba) ₃ (10 mol%)	BINAP (30 mol%)	K ₂ CO ₃ (3 eq)	dioxane, 100 °C, 16 h	–
OTf	Pd ₂ (dba) ₃ (10 mol%)	XPhos (30 mol%)	K ₂ CO ₃ (3 eq)	dioxane, 100 °C, 16 h	trace
OTf	Pd ₂ (dba) ₃ (10 mol%)	XPhos (30 mol%)	Cs ₂ CO ₃ (3 eq)	dioxane, 100 °C, 16 h	29% (isolated)
OTf	Pd ₂ (dba) ₃ (10 mol%)	Xantphos (30 mol%)	K ₂ CO ₃ (3 eq)	dioxane, 100 °C, 16 h	–
OTf	Pd ₂ (dba) ₃ (10 mol%)	SIPr·HCl (30 mol%)	Cs ₂ CO ₃ (3 eq)	dioxane, 100 °C, 16 h	–
OTf	tBuBrettPhos Pd G3 (15 mol%) BrettPhos (15 mol%)		Cs ₂ CO ₃ (3 eq)	dioxane, 100 °C, 16 h	–
OTf	RuPhos Pd G3 (15 mol%) RuPhos (15 mol%)		K ₂ CO ₃ (3 eq)	dioxane, 110 °C, 16 h	11% (isolated)
OTf	Pd ₂ (dba) ₃ (10 mol%)	(tBu)PhCPhos (30 mol%)	LiHDMS (4 eq)	dioxane, 100 °C, 20 h	15% (isolated)
OTf	Pd ₂ (dba) ₃ (10 mol%)	(tBu)PhCPhos (30 mol%)	K ₂ CO ₃ (3 eq)	dioxane, 100 °C, 20 h	trace
OTf	(AlPhosPd) ₂ ·COD (3 mol%)		DBU (4 eq)	MTBE, 60 °C, 20 h	–
OTs	NiCl ₂ (DME) (10 mol%), PhBpin (4 eq)	L13 (20 mol%)	t-BuONa (6 eq)	dioxane, 110 °C, 16 h	–
Br	Pd ₂ (dba) ₃ (10 mol%)	(tBu)PhCPhos (30 mol%)	t-BuONa (3 eq)	dioxane, 100 °C, 16 h	trace
Br	(AlPhosPd) ₂ ·COD (3 mol%)		DBU (4 eq)	MTBE, 60 °C, 20 h	–

Table S1. Buchwald-Hartwig amination of fluorescein ditriflate (and related starting materials) with *tert*-butylamine.



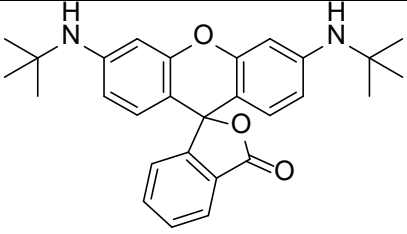
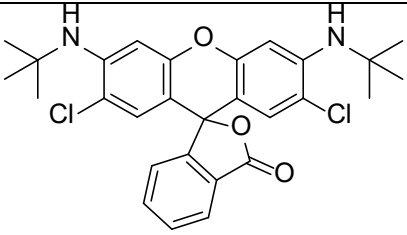
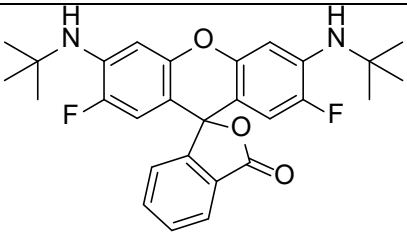
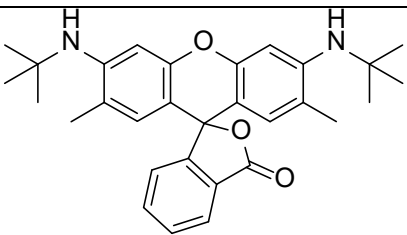
Leaving group (X)	[M] catalyst	Nucleophile (Y)	Isolated yield, %
OMs	[Cp* <i>Ru</i> (MeCN) ₃](OTf) (5 mol%)	Br	–
OTs	[Cp* <i>Ru</i> (MeCN) ₃](OTf) (5 mol%)	Br	–
OTf	[Cp* <i>Ru</i> (MeCN) ₃](OTf) (5 mol%)	Br	80
OTf	[Cp* <i>Rh</i> (MeCN) ₃](SbF ₆) ₂ (5 mol%)	Br	–
OTf	[Cp* <i>Ru</i> (MeCN) ₃](OTf) (5 mol%)	I	96
OTf	[Cp* <i>Ru</i> Cl ₂] (5 mol%)	I	96
OSO ₂ F	[Cp* <i>Ru</i> (MeCN) ₃](OTf) (5 mol%)	I	91
ONf	[Cp* <i>Ru</i> (MeCN) ₃](OTf) (10 mol%) ^a	I	96

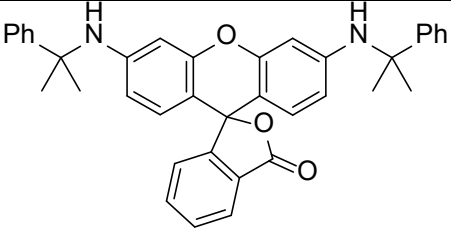
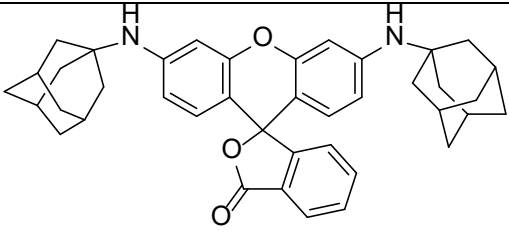
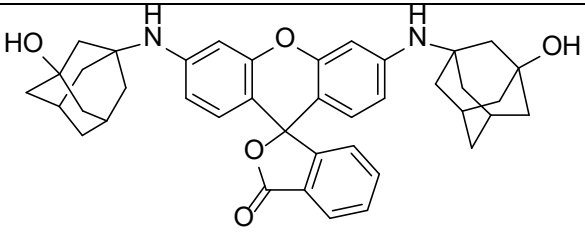
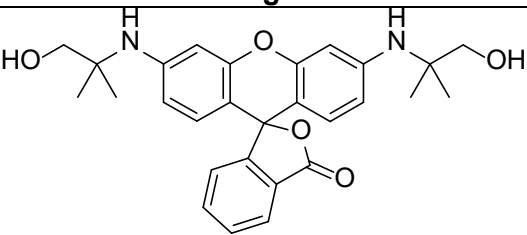
Table S2. Ru-catalyzed synthesis of 3',6'-dihalofluorane: leaving group and catalyst scope.
^a reaction time 23 h.

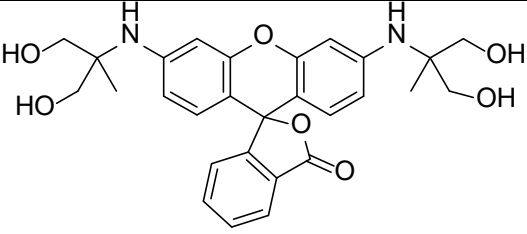
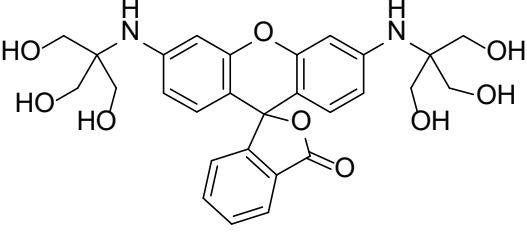
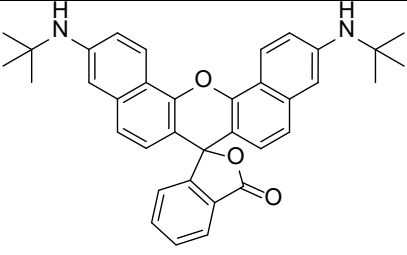
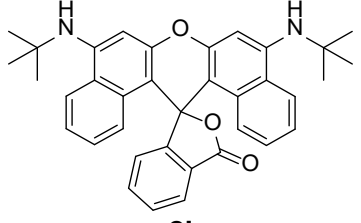


entry	ligand (mol%)	CuI, mol%	solvent	temp, °C	time, h	Isolated yield, %
1	none	20	EG	80	18	nd
2	L1 (10)	10	DEG	60	13	29
3	L1 (20)	20	DEG	80	15	68 (66 ^a)
4	L1 (20)	20	DMF	80	18	traces
5	L1 (20)	20	2-ME	80	16	52
6	L1 (40)	20	2-ME	80	18	6
7	L1 (20)	40	2-ME	80	18	60
8	L1 (20)	40	2-ME	100	18	44
9	L1 (40)	80	2-ME	80	18	76
10	L2 (20)	20	DEG	80	15	nd
11	L3 (20)	20	DEG	80	15	16
12	L4 (20)	20	DEG	80	15	36
13	L5 (20)	20	DEG	80	15	38
14	L6 (20)	20	DEG	80	15	traces ^b
15	L7 (20)	20	DEG	80	15	nd
16	L8 (20)	20	DEG	80	15	traces
17	L9 (20)	20	DEG	80	15	nd
18	L10 (20)	20	DEG	80	15	traces
19	L11 (20)	20	DEG	80	17	48
20	L12 (20)	20	DEG	80	15	<5
21	L14 (20)	20	2-ME	80	20	<10
22	L15 (20)	20	DMF	80	22	7
23	L16 (40)	20	2-ME	80	18	nd ^b
24	L17 (40)	20	DMSO	80	22	nd ^b

Table S3. Ullmann amination of 3',6'-dibromofluorane **1a** with *tert*-butylamine: investigation of the reaction parameters. Solvents: EG: ethylene glycol; DEG: diethylene glycol, DMF: *N,N*-dimethylformamide, 2-ME: 2-methoxyethanol, DMSO: dimethyl sulfoxide. nd: product not detected; ^a aryl iodide (**1b**) used instead of **1a**; ^b *N*-arylation of the ligand was observed.

Structure/ name	in 0.1% (v/v) TFA – EtOH			in PBS (pH 7.4)		
	Absorption $\lambda_{\max}, \text{nm}$ (extinction $\epsilon, \text{M}^{-1}\text{cm}^{-1}$)	Emission $\lambda_{\max}, \text{nm}$ (quantum yield QY)	Fluorescence lifetime τ, ns	Absorption $\lambda_{\max}, \text{nm}$ (extinction $\epsilon, \text{M}^{-1}\text{cm}^{-1}$)	Emission $\lambda_{\max}, \text{nm}$ (quantum yield QY)	Fluorescence lifetime τ, ns
 <p>2a</p>	531 (115000)	554 (0.93)	3.8	526 ^a (85000 ^a)	553 ^a (0.83 ^a)	3.7 ^a
 <p>2b</p>	528 (100000)	555 (0.19)	0.9	–	–	–
 <p>2c</p>	524 (114000)	550 (0.69)	2.9	–	–	–
 <p>2d</p>	525 (109000)	551 (0.61)	2.7	–	–	–

Structure/ name	in 0.1% (v/v) TFA – EtOH			in PBS (pH 7.4)		
	Absorption λ_{\max} , nm (extinction ϵ , $M^{-1}cm^{-1}$)	Emission λ_{\max} , nm (quantum yield QY)	Fluorescence lifetime τ , ns	Absorption λ_{\max} , nm (extinction ϵ , $M^{-1}cm^{-1}$)	Emission λ_{\max} , nm (quantum yield QY)	Fluorescence lifetime τ , ns
 <p>2e</p>	530 (99000)	553 (0.94)	4.0	–	–	–
 <p>2f</p>	537 (108000)	561 (0.88)	3.7	–	–	–
 <p>2g</p>	535 (117000)	559 (0.90)	3.7	–	–	–
 <p>2h</p>	531 (88000)	557 (0.89)	3.8	522 (75000)	550 (0.82)	3.6

Structure/ name	in 0.1% (v/v) TFA – EtOH			in PBS (pH 7.4)		
	Absorption λ_{\max} , nm (extinction ϵ , $M^{-1}cm^{-1}$)	Emission λ_{\max} , nm (quantum yield QY)	Fluorescence lifetime τ , ns	Absorption λ_{\max} , nm (extinction ϵ , $M^{-1}cm^{-1}$)	Emission λ_{\max} , nm (quantum yield QY)	Fluorescence lifetime τ , ns
 <p>2i</p>	532 (111000)	558 (0.80)	3.6	519 (90000)	548 (0.83)	3.6
 <p>2j</p>	533 (111000)	559 (0.84)	3.7	517 (83000)	546 (0.88)	3.7
 <p>2k</p>	646 (18000)	701 (0.35)	2.6	–	–	–
 <p>2l</p>	559 (115000)	589 (0.74)	4.0	–	–	–

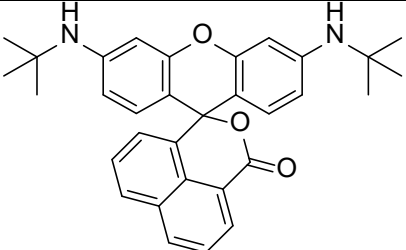
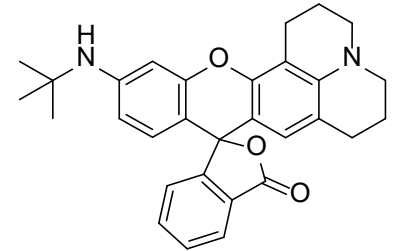
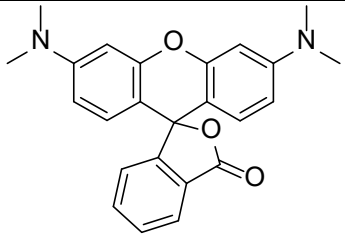
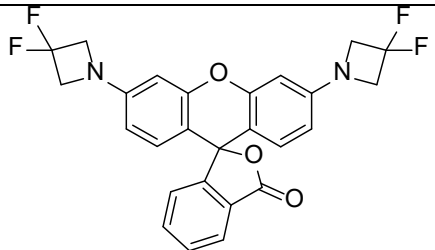
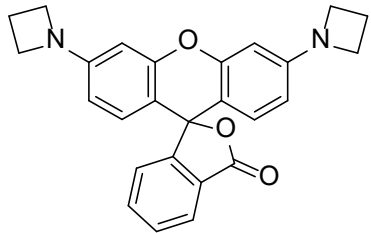
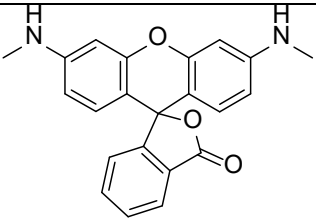
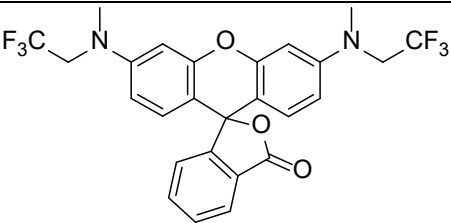
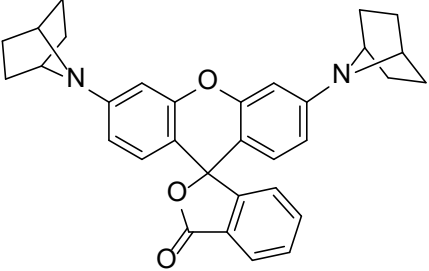
Structure/ name	in 0.1% (v/v) TFA – EtOH			in PBS (pH 7.4)		
	Absorption $\lambda_{\max}, \text{nm}$ (extinction $\epsilon, \text{M}^{-1}\text{cm}^{-1}$)	Emission $\lambda_{\max}, \text{nm}$ (quantum yield QY)	Fluorescence lifetime τ, ns	Absorption $\lambda_{\max}, \text{nm}$ (extinction $\epsilon, \text{M}^{-1}\text{cm}^{-1}$)	Emission $\lambda_{\max}, \text{nm}$ (quantum yield QY)	Fluorescence lifetime τ, ns
 <p>2m</p>	538 (96000)	562 (0.91)	4.3	535 ^a (nd)	563 ^a (0.83) ^a	4.0
 <p>2n</p>	555 (120000)	581 (0.84)	4.6	–	–	–

Table S4. Photophysical properties of the dyes of the present study. ^a values recorded in 10% (v/v) DMSO – PBS 7.4.

Structure/ name	in 0.1% (v/v) TFA – EtOH			in 10% (v/v) DMSO – PBS 7.4		
	Absorption λ_{\max} , nm (extinction ϵ , $M^{-1}cm^{-1}$)	Emission λ_{\max} , nm (quantum yield QY)	Fluorescence lifetime τ , ns	Absorption λ_{\max} , nm (extinction ϵ , $M^{-1}cm^{-1}$)	Emission λ_{\max} , nm (quantum yield QY)	Fluorescence lifetime τ , ns
 <p>TMR</p>	548 (113000)	574 (0.55)	2.7	–	–	–
 <p>JF₅₂₅</p>	529 (106000)	556 (0.97)	3.8	526 (57000)	554 (0.94)	3.7
 <p>JF₅₄₉</p>	550 (113000)	574 (0.90)	3.9	–	–	–

Structure/ name	in 0.1% (v/v) TFA – EtOH			in 10% (v/v) DMSO – PBS 7.4		
	Absorption λ_{\max} , nm (extinction ϵ , $M^{-1}cm^{-1}$)	Emission λ_{\max} , nm (quantum yield QY)	Fluorescence lifetime τ , ns	Absorption λ_{\max} , nm (extinction ϵ , $M^{-1}cm^{-1}$)	Emission λ_{\max} , nm (quantum yield QY)	Fluorescence lifetime τ , ns
 <p>3a</p>	525 (100000)	549 (0.94)	4.0	520 (57000)	547 (0.89)	4.0
 <p>3b</p>	524 (111000)	550 (0.95)	3.9	–	–	–
 <p>3c</p>	548 (122000)	573 (0.96)	3.9	–	–	–

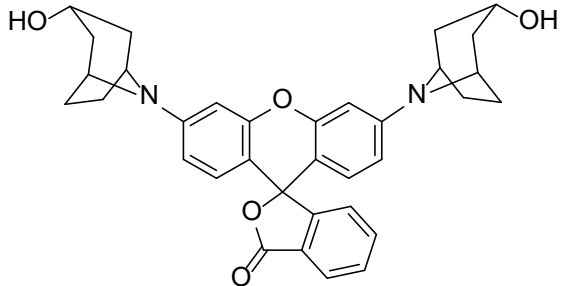
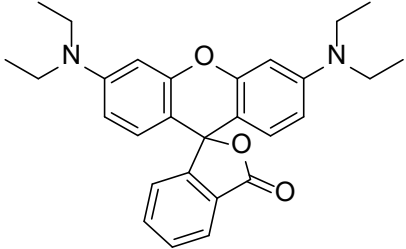
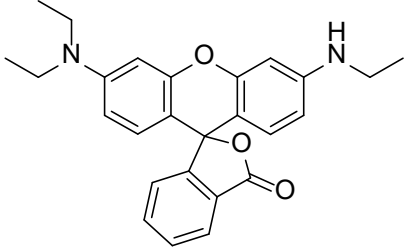
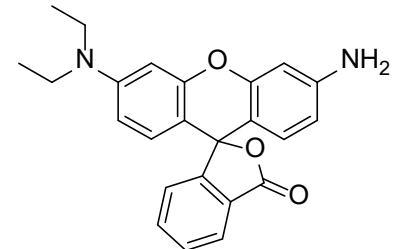
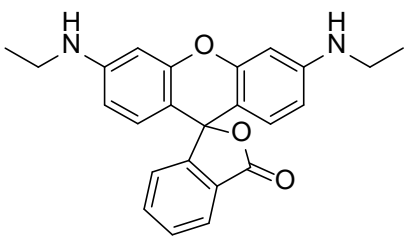
Structure/ name	in 0.1% (v/v) TFA – EtOH			in 10% (v/v) DMSO – PBS 7.4		
	Absorption λ_{\max} , nm (extinction ϵ , M ⁻¹ cm ⁻¹)	Emission λ_{\max} , nm (quantum yield QY)	Fluorescence lifetime τ , ns	Absorption λ_{\max} , nm (extinction ϵ , M ⁻¹ cm ⁻¹)	Emission λ_{\max} , nm (quantum yield QY)	Fluorescence lifetime τ , ns
 <p style="text-align: center;">3d</p>	563 (99000)	587 (0.87)	3.9	560 (82000)	586 (0.79)	3.7

Table S5. Photophysical properties of the known and reference dyes.

Name and structure	NMR	MS (ESI)
 <p style="text-align: center;">RhB-1</p>	identical with Rhodamine B	
 <p style="text-align: center;">RhB-2</p>	¹ H NMR (600 MHz, acetone- <i>d</i> ₆): δ 8.33 (dd, <i>J</i> = 7.9, 1.3 Hz, 1H), 7.90 (td, <i>J</i> = 7.5, 1.4 Hz, 1H), 7.84 (td, <i>J</i> = 7.7, 1.3 Hz, 1H), 7.49 (dd, <i>J</i> = 7.6, 1.3 Hz, 1H), 7.13 (d, <i>J</i> = 9.4 Hz, 1H), 7.09 – 7.04 (m, 2H), 6.96 – 6.92 (m, 2H), 6.84 (d, <i>J</i> = 2.2 Hz, 1H), 3.74 (q, <i>J</i> = 7.2 Hz, 4H), 3.49 (q, <i>J</i> = 7.2 Hz, 2H), 1.34 (dt, <i>J</i> = 7.2 Hz, 3H), 1.32 (t, <i>J</i> = 7.2 Hz, 6H).	HRMS (C ₂₆ H ₂₆ N ₂ O ₃): <i>m/z</i> (positive mode) = 415.2030 (found [M+H] ⁺), 415.2016 (calc. [M+H] ⁺).
 <p style="text-align: center;">RhB-3a</p>	¹ H NMR (600 MHz, acetone- <i>d</i> ₆): δ 8.27 (d, <i>J</i> = 8.3 Hz, 1H), 7.88 (td, <i>J</i> = 7.5, 1.3 Hz, 1H), 7.82 (td, <i>J</i> = 7.7, 1.3 Hz, 1H), 7.45 (d, <i>J</i> = 7.9 Hz, 1H), 7.04 (d, <i>J</i> = 9.4 Hz, 1H), 7.01 – 6.96 (m, 2H), 6.91 – 6.88 (m, 2H), 6.85 (dd, <i>J</i> = 9.1, 1.6 Hz, 2H), 3.70 (q, <i>J</i> = 7.2 Hz, 4H), 1.29 (t, <i>J</i> = 7.1 Hz, 6H).	HRMS (C ₂₄ H ₂₂ N ₂ O ₃): <i>m/z</i> (positive mode) = 387.1707 (found [M+H] ⁺), 387.1703 (calc. [M+H] ⁺).
 <p style="text-align: center;">RhB-3b</p>	¹ H NMR (600 MHz, acetone- <i>d</i> ₆): δ 8.27 (d, <i>J</i> = 8.0 Hz, 1H), 7.87 (td, <i>J</i> = 7.5, 1.3 Hz, 1H), 7.81 (td, <i>J</i> = 7.7, 1.3 Hz, 1H), 7.44 (dd, <i>J</i> = 7.8, 1.1 Hz, 1H), 6.98 (d, <i>J</i> = 9.1 Hz, 2H), 6.86 (dd, <i>J</i> = 9.1, 2.2 Hz, 2H), 6.79 (d, <i>J</i> = 2.2 Hz, 2H), 3.45 (q, <i>J</i> = 7.2 Hz, 4H), 1.33 (t, <i>J</i> = 7.2 Hz, 6H).	HRMS (C ₂₄ H ₂₂ N ₂ O ₃): <i>m/z</i> (positive mode) = 387.1703 (found [M+H] ⁺), 387.1703 (calc. [M+H] ⁺).

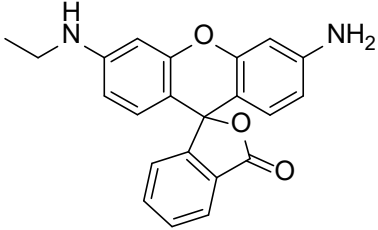
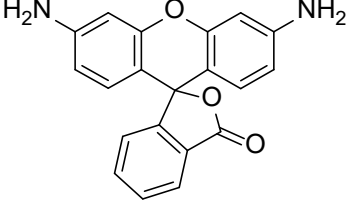
Name and structure	NMR	MS (ESI)
 <p style="text-align: center;">RhB-4</p>	¹ H NMR (600 MHz, acetone- <i>d</i> ₆) δ 8.26 (d, <i>J</i> = 7.5 Hz, 1H), 7.87 (td, <i>J</i> = 7.5, 1.3 Hz, 1H), 7.81 (td, <i>J</i> = 7.6, 1.3 Hz, 1H), 7.44 (dd, <i>J</i> = 7.7, 1.4 Hz, 1H), 7.00 – 6.93 (m, 2H), 6.88 (d, <i>J</i> = 2.1 Hz, 1H), 6.87 – 6.82 (m, 2H), 6.79 (d, <i>J</i> = 2.2 Hz, 1H), 3.45 (q, <i>J</i> = 7.3 Hz, 2H), 1.32 (t, <i>J</i> = 7.2 Hz, 3H).	HRMS (C ₂₂ H ₁₈ N ₂ O ₃): <i>m/z</i> (positive mode) = 359.1393 (found [M+H] ⁺), 359.1390 (calc. [M+H] ⁺).
 <p style="text-align: center;">RhB-5</p>	identical with Rhodamine 110	

Table S6. Identification and characterization of Rhodamine B semipreparative photooxidation products.

dye	$\lambda^{\max}_{\text{abs}}$, nm	$\lambda^{\max}_{\text{em}}$, nm	Φ_{fl}	Stokes shift $\Delta\lambda$, nm ($\Delta\bar{\nu}$, cm ⁻¹)	Fluorescence lifetime τ , ns
Rhodamine B	553	578	0.52	25 (1052)	2.4
RhB-2	539	564	0.49	25 (822)	3.4 (43%), 1.9 (57%)
RhB-3a	532	556	0.27	24 (811)	3.6 (24%), 1.0 (76%)
RhB-3b	529	554	0.83	25 (853)	4.0
RhB-4	519	544	0.79	25 (886)	4.0
Rhodamine 110	509	533	0.93	24 (884)	3.9
RhB-6	500	530	0.69	30 (1132)	4.3

Table S7. Photophysical properties of Rhodamine B photooxidation products in 0.1% TFA – ethanol.

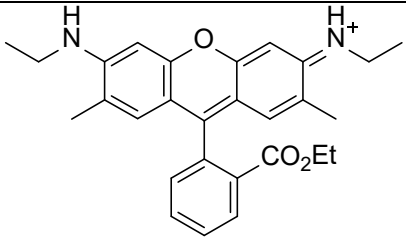
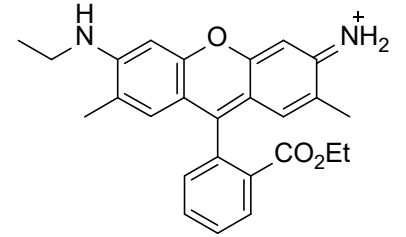
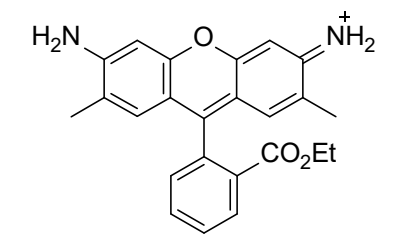
Name and structure	NMR	MS (ESI)
 <p>Rh6G-1</p>	identical with Rhodamine 6G	
 <p>Rh6G-2</p>	¹ H NMR (400 MHz, acetone- <i>d</i> ₆): δ 8.32 (dd, <i>J</i> = 7.8, 1.3 Hz, 1H), 7.92 (td, <i>J</i> = 7.5, 1.5 Hz, 1H), 7.86 (td, <i>J</i> = 7.6, 1.4 Hz, 1H), 7.52 (dd, <i>J</i> = 7.4, 1.5 Hz, 1H), 7.37 (br.t, <i>J</i> = 6.0 Hz, 1H), 7.16 (s, 1H), 7.05 (d, <i>J</i> = 1.0 Hz, 1H), 7.02 (d, <i>J</i> = 1.0 Hz, 1H), 7.00 (s, 1H), 4.00 (q, <i>J</i> = 7.1 Hz, 2H), 3.70 – 3.60 (m, 2H), 2.22 (d, <i>J</i> = 1.0 Hz, 3H), 2.21 (d, <i>J</i> = 1.0 Hz, 3H), 1.38 (t, <i>J</i> = 7.2 Hz, 3H), 0.94 (t, <i>J</i> = 7.1 Hz, 3H).	HRMS (C ₂₆ H ₂₇ N ₂ O ₃): <i>m/z</i> (positive mode) = 415.2026 (found [M] ⁺), 415.2016 (calc. [M] ⁺).
 <p>Rh6G-3</p>	¹ H NMR (400 MHz, acetone- <i>d</i> ₆): δ 8.33 (dd, <i>J</i> = 7.9, 1.4 Hz, 1H), 7.93 (td, <i>J</i> = 7.5, 1.5 Hz, 1H), 7.87 (td, <i>J</i> = 7.6, 1.4 Hz, 1H), 7.53 (dd, <i>J</i> = 7.6, 1.3 Hz, 1H), 7.37 (br.s, 4H), 7.09 (s, 4H), 4.01 (q, <i>J</i> = 7.1 Hz, 2H), 2.23 (d, <i>J</i> = 1.0 Hz, 6H), 0.94 (t, <i>J</i> = 7.1 Hz, 3H).	HRMS (C ₂₄ H ₂₃ N ₂ O ₃): <i>m/z</i> (positive mode) = 387.1711 (found [M] ⁺), 387.1703 (calc. [M] ⁺).

Table S8. Identification and characterization of Rhodamine 6G semipreparative photooxidation products.

dye	$\lambda^{\max}_{\text{abs}}$, nm	$\lambda^{\max}_{\text{em}}$, nm	Φ_{fl}	Stokes shift $\Delta\lambda$, nm ($\Delta\bar{\nu}$, cm ⁻¹)	Fluorescence lifetime τ , ns
Rhodamine 6G	528	553	0.94	25 (856)	3.9
Rh6G-2	523	548	0.91	25 (872)	3.9
Rh6G-3	516	538	0.92	22 (793)	3.8

Table S9. Photophysical properties of Rhodamine 6G photooxidation products in 0.1% TFA – ethanol.

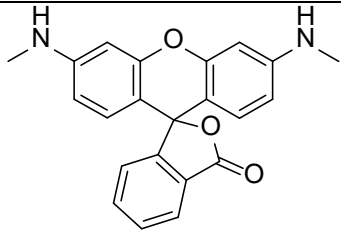
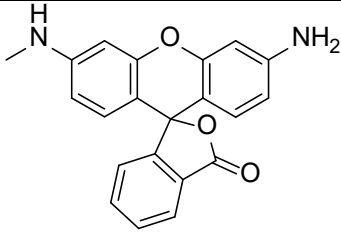
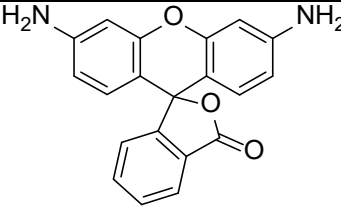
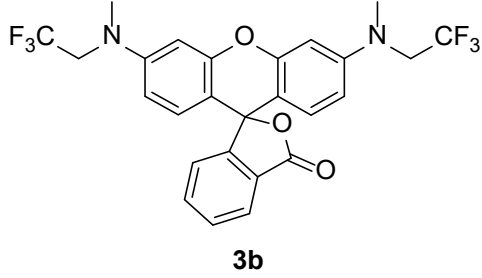
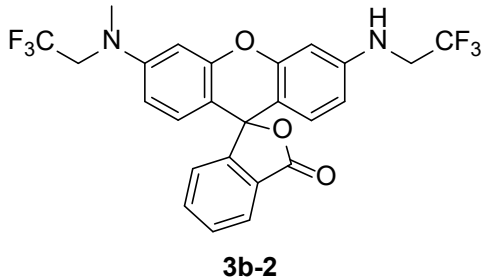
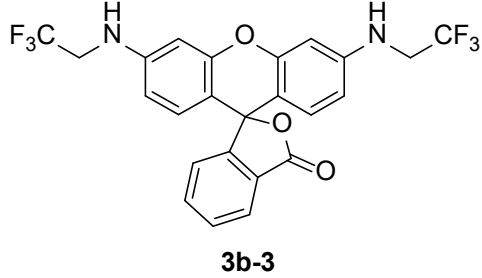
Name and structure	NMR	MS (ESI)
 <p>3a</p>	identical with 3a , see above	
 <p>3a-2</p>	¹ H NMR (400 MHz, CD ₃ OD): δ 8.32 (dd, <i>J</i> = 7.6, 1.5 Hz, 1H), 7.84 (td, <i>J</i> = 7.5, 1.5 Hz, 1H), 7.78 (td, <i>J</i> = 7.6, 1.5 Hz, 1H), 7.39 (dd, <i>J</i> = 7.4, 1.5 Hz, 1H), 7.09 – 7.00 (m, 2H), 6.85 – 6.77 (m, 4H), 3.04 (s, 3H).	HRMS (C ₂₁ H ₁₆ N ₂ O ₃): <i>m/z</i> (positive mode) = 345.1237 (found [M+H] ⁺), 345.1234 (calc. [M+H] ⁺).
 <p>3a-3 (Rh110)</p>	identical with Rhodamine 110	

Table S10. Identification and characterization of *N,N'*-dimethylrhodamine (**3a**) semipreparative photooxidation products.

dye	$\lambda^{\max}_{\text{abs}}$, nm	$\lambda^{\max}_{\text{em}}$, nm	Φ_{fl}	Stokes shift $\Delta\lambda$, nm ($\Delta\bar{\nu}$, cm ⁻¹)	Fluorescence lifetime τ , ns
3a	525	549	0.94	24 (833)	4.0
3a-2	517	541	0.74	24 (858)	4.0
Rhodamine 110	509	533	0.93	24 (884)	3.9

Table S11. Photophysical properties of **3a** photooxidation products in 0.1% TFA – ethanol.

Name and structure	NMR	MS (ESI)
 <p style="text-align: center;">3b</p>	<p style="text-align: center;">identical with 3b, see above</p>	
 <p style="text-align: center;">3b-2</p>		
 <p style="text-align: center;">3b-3</p>		<p style="text-align: center;">identical with compound 1e in ref. [19]</p>

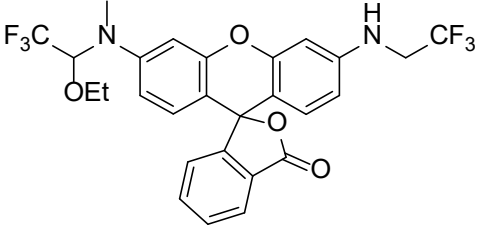
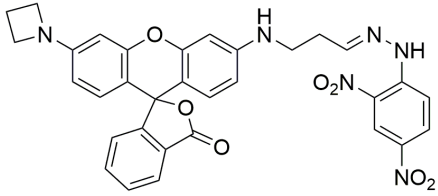
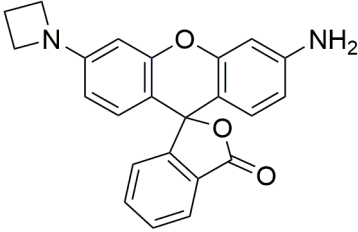
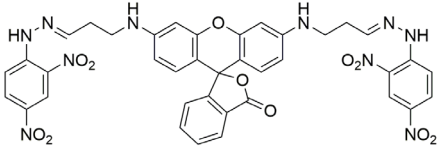
Name and structure	NMR	MS (ESI)
 <p style="text-align: center;">3b-4</p>	¹ H NMR (400 MHz, acetone- <i>d</i> ₆): δ 7.98 (dt, <i>J</i> = 7.6, 1.0 Hz, 1H), 7.81 (tt, <i>J</i> = 7.3, 1.3 Hz, 1H), 7.77 – 7.69 (m, 1H), 7.29 (ddt, <i>J</i> = 7.7, 2.8, 1.0 Hz, 1H), 6.87 (d, <i>J</i> = 2.6 Hz, 1H), 6.83 (dt, <i>J</i> = 8.8, 2.9 Hz, 1H), 6.71 – 6.66 (m, 2H), 6.59 (d, <i>J</i> = 1.4 Hz, 1H), 6.07 (br.t, <i>J</i> = 6.9 Hz, 1H, NH), 5.70 (qd, ³ <i>J</i> _{H-F} = 5.3 Hz, <i>J</i> = 2.0 Hz, 1H), 4.05 (qd, ³ <i>J</i> _{H-F} = 9.4 Hz, <i>J</i> = 6.3 Hz, 2H), 3.74 – 3.57 (m, 2H), 1.28 – 1.19 (m, 3H). ¹⁹ F NMR (376 MHz, acetone- <i>d</i> ₆): δ -72.74 (t, ³ <i>J</i> _{F-H} = 9.5 Hz), -76.45 (s, CF ₃ CO ₂ ⁻), -77.61 (d, ³ <i>J</i> _{F-H} = 5.3 Hz).	HRMS (C ₂₇ H ₂₂ F ₆ N ₂ O ₄): <i>m/z</i> (positive mode) = 553.1558 (found [M+H] ⁺), 553.1557 (calc. [M+H] ⁺).

Table S11. Identification and characterization of **3b** semipreparative photooxidation products.

dye	$\lambda^{\max}_{\text{abs}}$, nm	$\lambda^{\max}_{\text{em}}$, nm	Φ_{fl}	Stokes shift $\Delta\lambda$, nm ($\Delta\bar{\nu}$, cm ⁻¹)	Fluorescence lifetime τ , ns
3b	524	550	0.95	26 (902)	3.9
3b-2	515	541	0.93	26 (933)	3.9
3b-3 (ref. [19])	507	532	0.95	25 (927)	3.9

Table S12. Photophysical properties of **3b** photooxidation products in 0.1% TFA – ethanol.

Name and structure	NMR	MS (ESI)
 <p style="text-align: center;">JF₅₄₉-2-DNPH</p>	¹ H NMR (400 MHz, acetone- <i>d</i> ₆): δ 11.25 (s, 1H), 8.95 (d, <i>J</i> = 2.6 Hz, 1H), 8.34 – 8.27 (m, 2H), 8.08 (br.s, 1H), 8.00 (d, <i>J</i> = 9.6 Hz, 1H), 7.88 (t, <i>J</i> = 7.8 Hz, 1H), 7.82 (t, <i>J</i> = 7.5 Hz, 1H), 7.44 (d, <i>J</i> = 7.8 Hz, 1H), 7.08 (d, <i>J</i> = 9.1 Hz, 1H), 7.02 (d, <i>J</i> = 8.9 Hz, 1H), 6.98 – 6.88 (m, 2H), 6.63 (dd, <i>J</i> = 9.1, 2.1 Hz, 1H), 6.50 (d, <i>J</i> = 2.1 Hz, 1H), 4.34 (t, <i>J</i> = 7.5 Hz, 4H), 3.84 (br.t, <i>J</i> = 6.4 Hz, 2H), 2.90 (br.q, <i>J</i> = 5.4 Hz, 2H), 2.58 (p, <i>J</i> = 7.4 Hz, 2H).	HRMS (C ₃₂ H ₂₆ N ₆ O ₇): <i>m/z</i> (positive mode) = 607.1938 (found [M+H] ⁺), 607.1936 (calc. [M+H] ⁺).
 <p style="text-align: center;">JF₅₄₉-3a</p>	¹ H NMR (400 MHz, acetone- <i>d</i> ₆): δ 8.21 (d, <i>J</i> = 7.7 Hz, 1H), 7.86 (td, <i>J</i> = 7.5, 1.4 Hz, 1H), 7.80 (td, <i>J</i> = 7.5, 1.2 Hz, 1H), 7.40 (d, <i>J</i> = 7.4 Hz, 1H), 6.94 (d, <i>J</i> = 9.0 Hz, 1H), 6.89 (d, <i>J</i> = 8.8 Hz, 1H), 6.81 (d, <i>J</i> = 2.1 Hz, 1H), 6.77 (d, <i>J</i> = 9.0 Hz, 1H), 6.51 (dd, <i>J</i> = 8.9, 2.2 Hz, 1H), 6.46 (d, <i>J</i> = 2.2 Hz, 1H), 4.24 (t, <i>J</i> = 7.5 Hz, 4H), 2.53 (p, <i>J</i> = 7.5 Hz, 2H).	HRMS (C ₂₃ H ₁₈ N ₂ O ₃): <i>m/z</i> (positive mode) = 371.1390 (found [M+H] ⁺), 371.1390 (calc. [M+H] ⁺).
 <p style="text-align: center;">JF₅₄₉-3b-(DNPH)₂</p>	¹ H NMR (400 MHz, acetone- <i>d</i> ₆ + 1% acetic acid- <i>d</i> ₄): δ 11.26 (s, 2H), 8.96 (d, <i>J</i> = 2.6 Hz, 2H), 8.32 (dd, <i>J</i> = 9.6, 2.7 Hz, 2H), 8.27 (d, <i>J</i> = 7.5 Hz, 1H), 8.08 (t, <i>J</i> = 4.8 Hz, 2H), 8.01 (d, <i>J</i> = 9.6 Hz, 2H), 7.91 – 7.85 (m, 1H), 7.85 – 7.77 (m, 1H), 7.42 (d, <i>J</i> = 7.9 Hz, 1H), 7.01 – 6.95 (m, 2H), 6.94 – 6.83 (m, 4H), 3.83 (t, <i>J</i> = 6.6 Hz, 4H), 2.95 – 2.87 (q, <i>J</i> = 6.6 Hz, 4H).	HRMS (C ₃₈ H ₃₀ N ₁₀ O ₁₁): <i>m/z</i> (positive mode) = 803.2167 (found [M+H] ⁺), 803.2168 (calc. [M+H] ⁺).

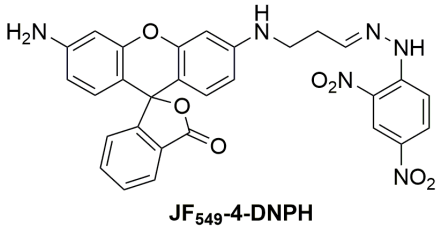
Name and structure	NMR	MS (ESI)
 <p style="text-align: center;">JF₅₄₉-4-DNPH</p>	¹ H NMR (400 MHz, acetone- <i>d</i> ₆): δ 11.26 (s, 1H), 8.97 (d, <i>J</i> = 2.7 Hz, 1H), 8.33 (dd, <i>J</i> = 9.7, 2.7 Hz, 1H), 8.21 (d, <i>J</i> = 7.7 Hz, 1H), 8.08 (t, <i>J</i> = 4.9 Hz, 1H), 8.02 (d, <i>J</i> = 9.6 Hz, 1H), 7.86 (td, <i>J</i> = 7.5, 1.3 Hz, 1H), 7.79 (td, <i>J</i> = 7.5, 1.2 Hz, 1H), 7.40 (d, <i>J</i> = 7.3 Hz, 1H), 6.90 (br.d, <i>J</i> = 8.8 Hz, 4H), 6.86 – 6.81 (m, 2H), 6.78 (dd, <i>J</i> = 8.8, 1.9 Hz, 1H), 3.80 (t, <i>J</i> = 6.7 Hz, 2H), 2.94 – 2.85 (m, 2H).	HRMS (C ₂₉ H ₂₂ N ₆ O ₇): <i>m/z</i> (positive mode) = 567.1619 (found [M+H] ⁺), 567.1623 (calc. [M+H] ⁺).

Table S13. Identification and characterization of JF₅₄₉ semipreparative photooxidation products.

Supplementary Figures

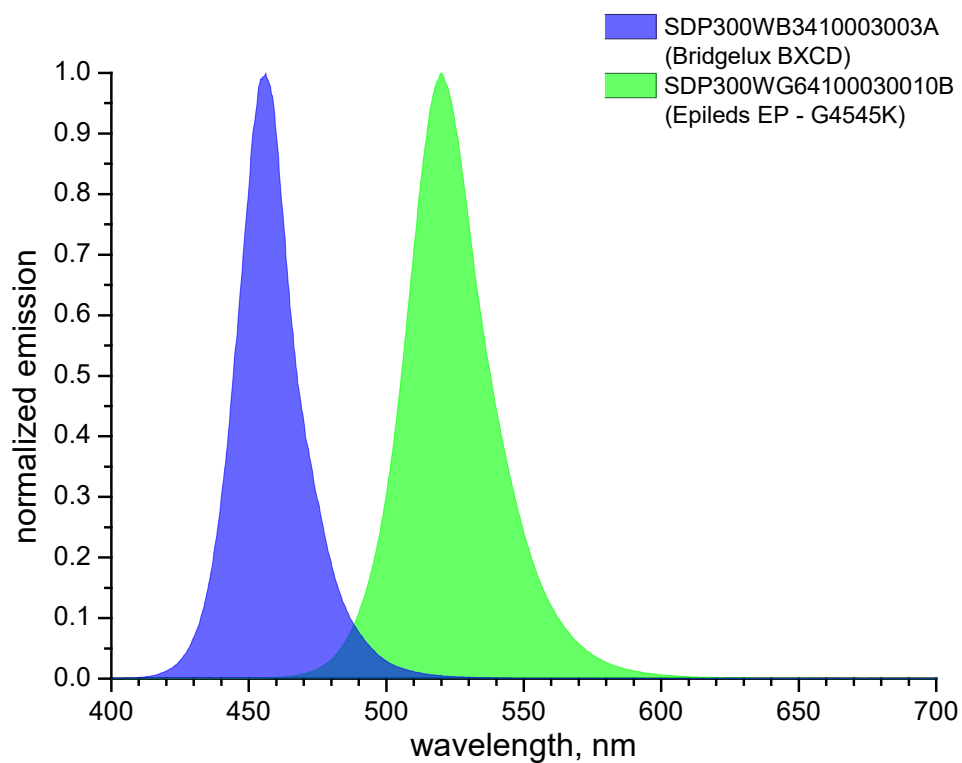


Figure S1. Emission spectra of the LED arrays used as irradiation light sources.

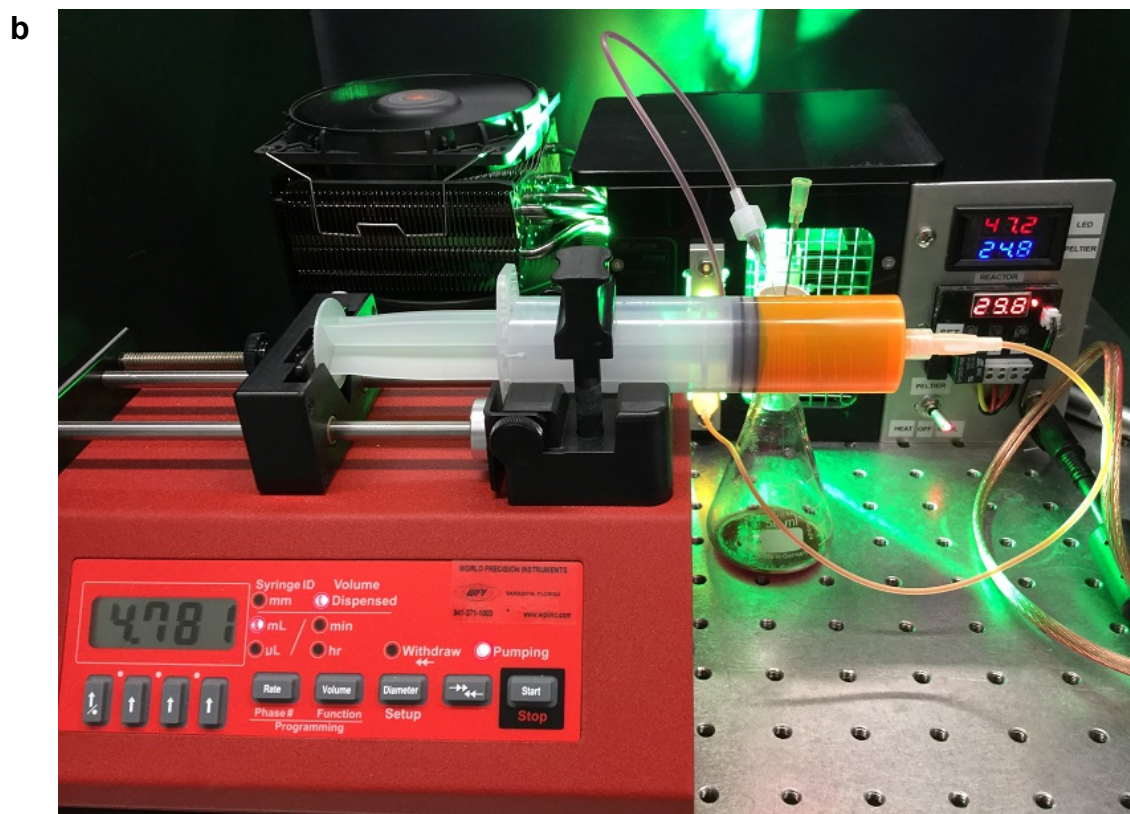
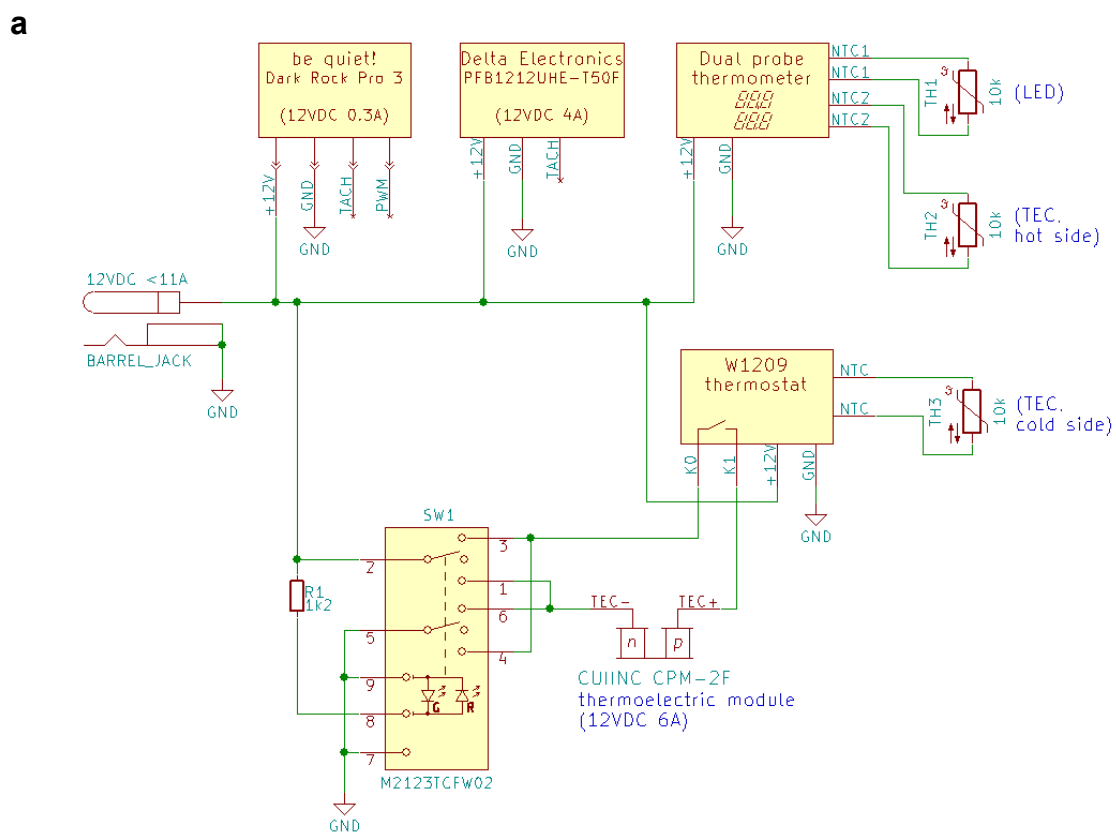
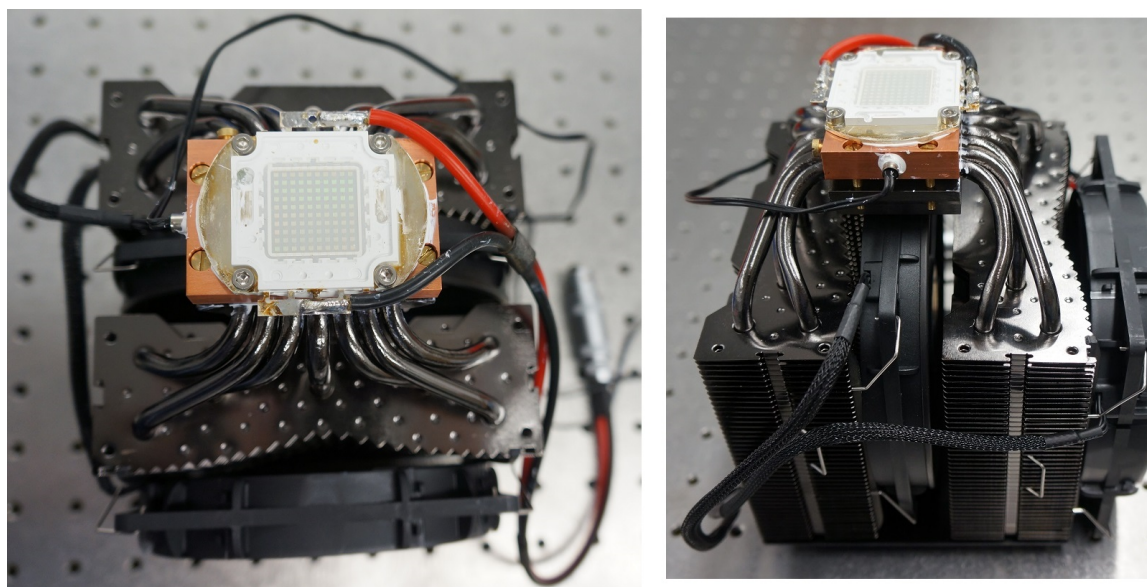
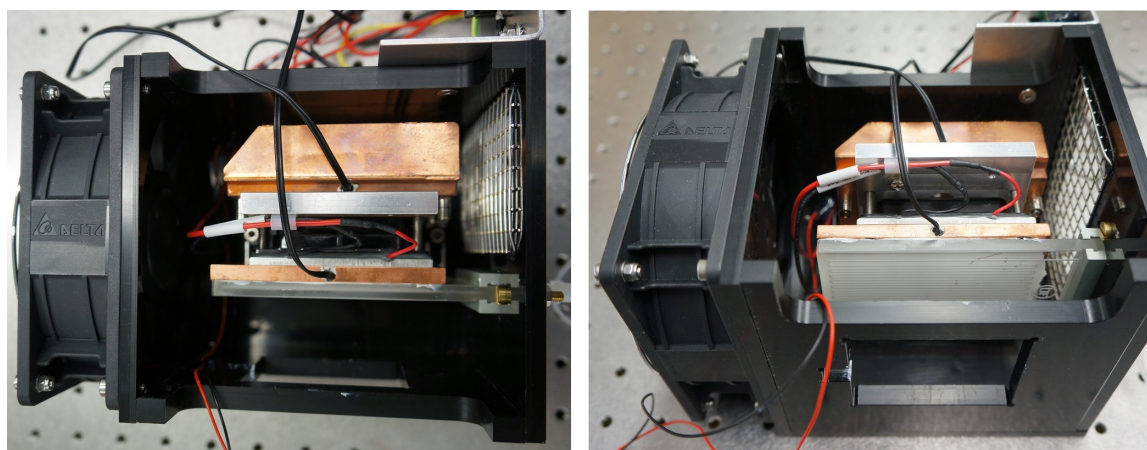


Figure S2. Custom-built flow photoreactor: a) diagram of the temperature control and regulation circuit; b) flow photoreactor in operation.

a



b



c

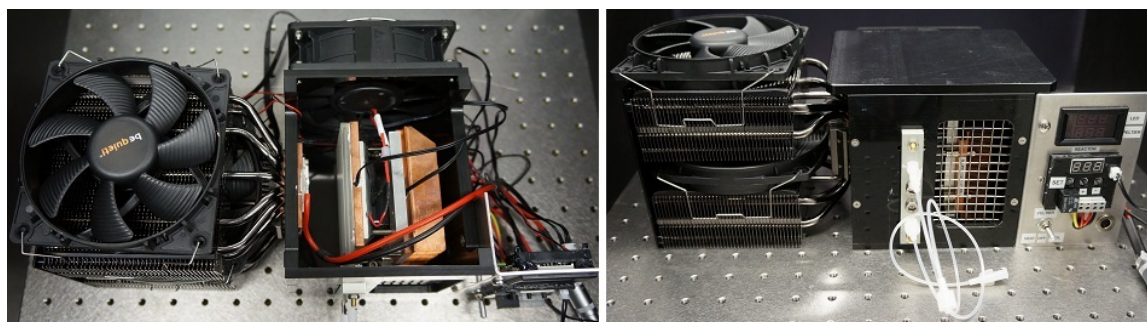


Figure S3. Custom-built flow photoreactor: a) light source (LED array mounted on the cooler); b) flow reactor enclosure with the Peltier cooling element and fan; c) the assembled compact setup allowing easy switching between the light sources.

Photolysis of Rhodamine B

Photooxidation scheme for Rhodamine B (RhB):

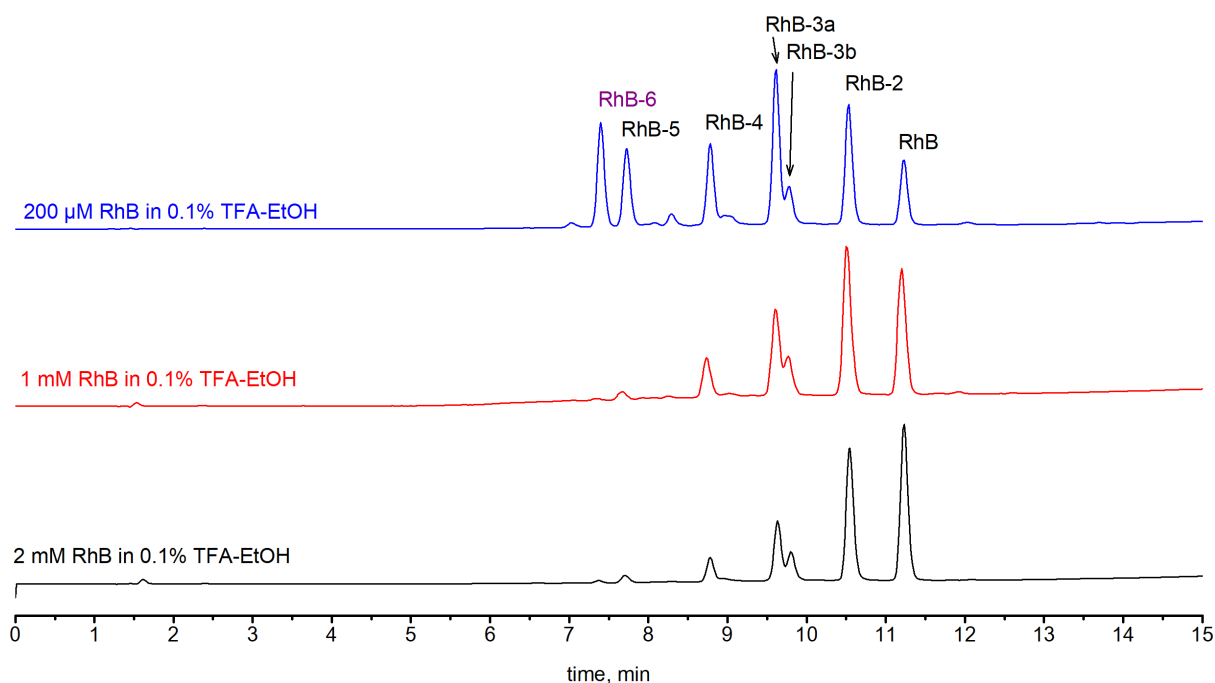
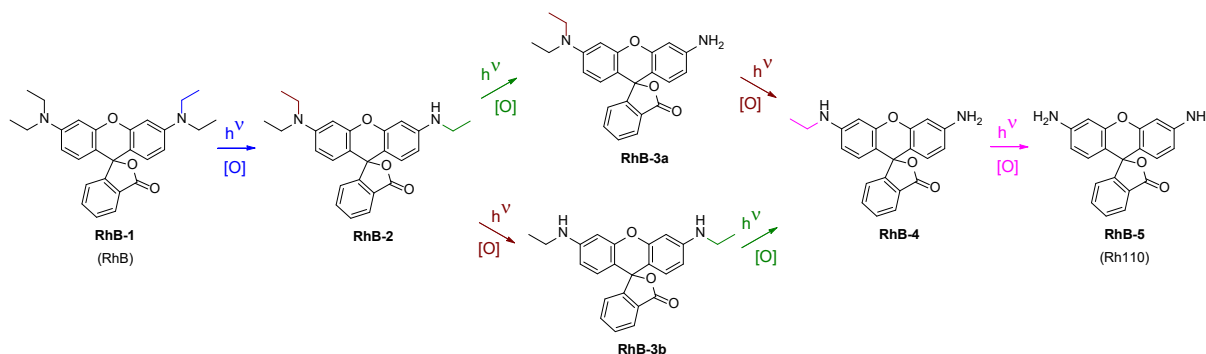


Figure S4. Effect of dye concentration on the degree of photoconversion of Rhodamine B in 0.1% TFA-ethanol (residence time 34 min). HPLC traces (normalized absorption at 254 nm vs. retention time) are shown. **RhB-6** is a side chain oxidation by-product as discussed below.

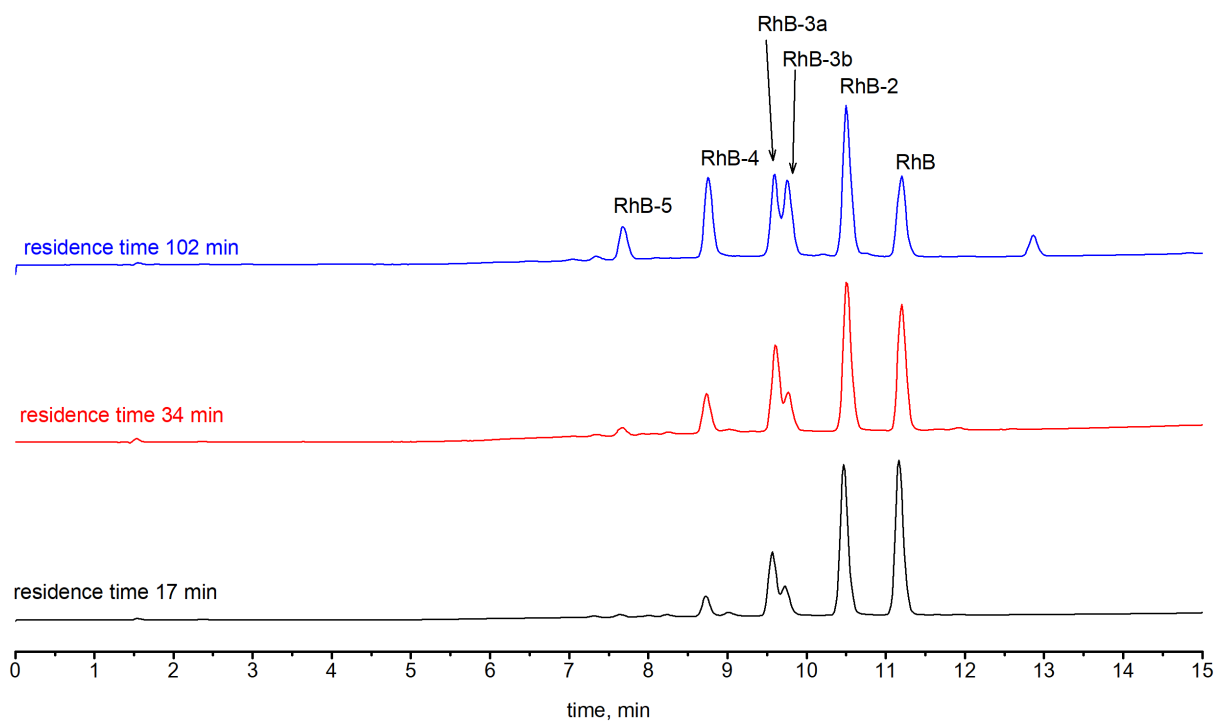


Figure S5. Effect of irradiation time on the degree of photoconversion of Rhodamine B (1 mM) in 0.1% TFA-ethanol. HPLC traces (normalized absorption at 254 nm vs. retention time) are shown.

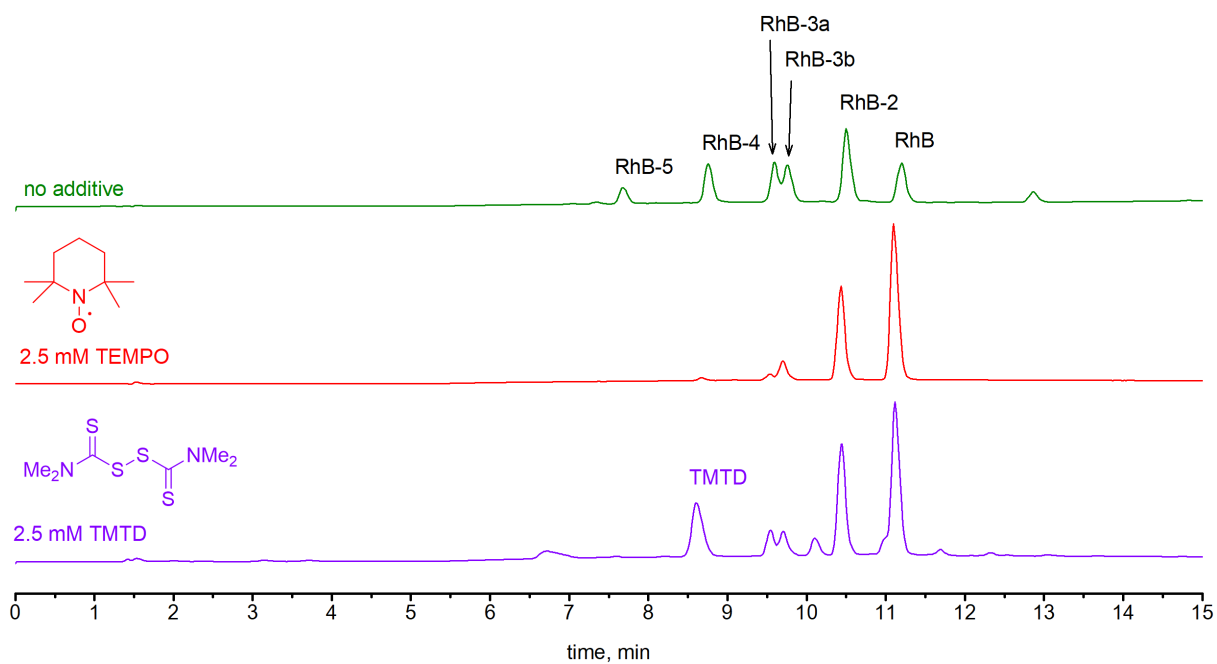


Figure S6. Effect of added free radical scavengers (2.5 mM) on the degree of photoconversion of Rhodamine B (1 mM) in 0.1% TFA-ethanol, residence time 102 min. HPLC traces (normalized absorption at 254 nm vs. retention time) are shown.

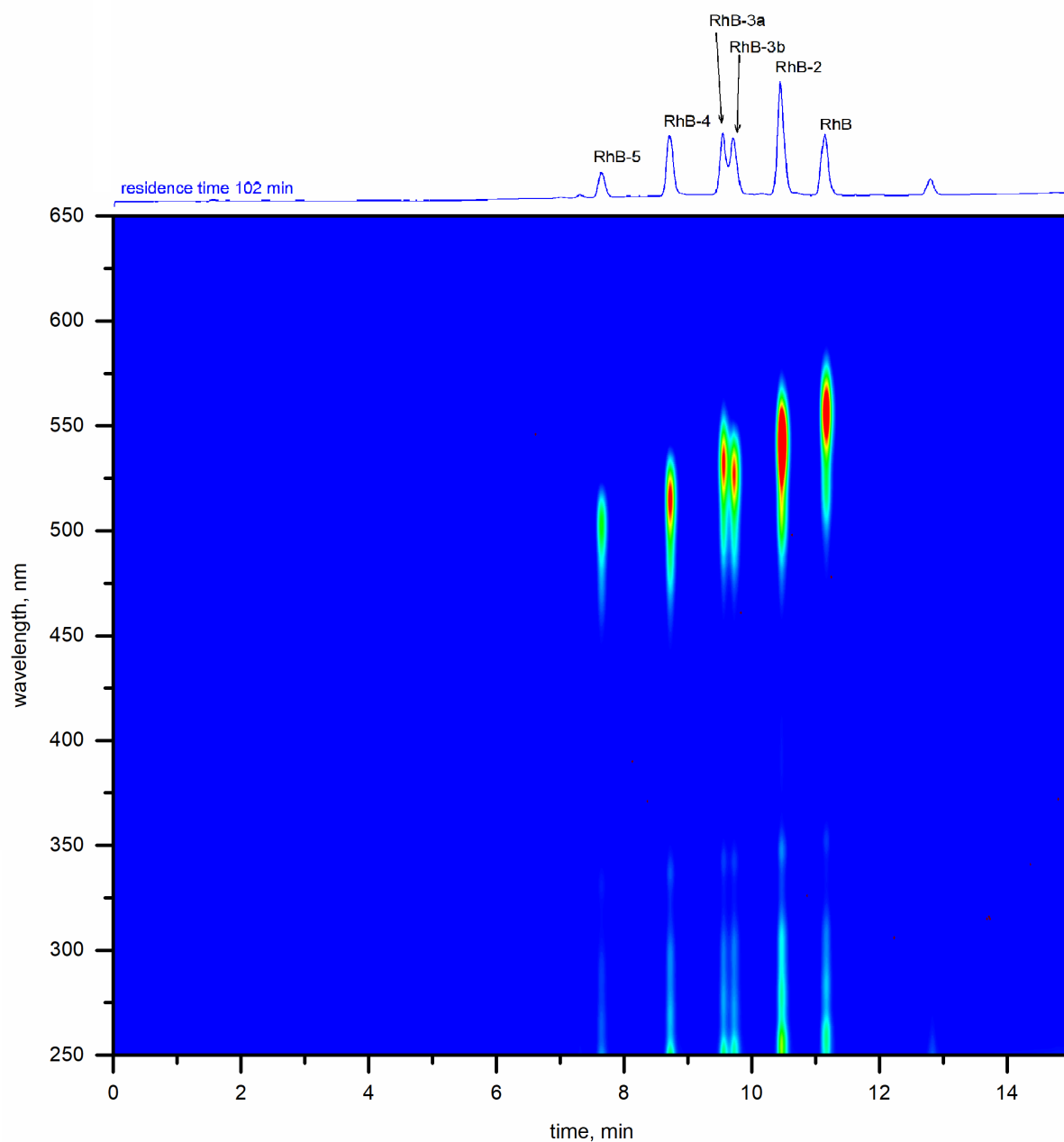


Figure S7. Semipreparative photolysis of Rhodamine B (20 mg in 40 mL of 0.1% TFA-ethanol, ~ 1 mM), irradiation at 525 nm, residence time 102 min. 3D data (absorption spectra vs. HPLC retention time); blue trace indicates normalized absorption at 254 nm.

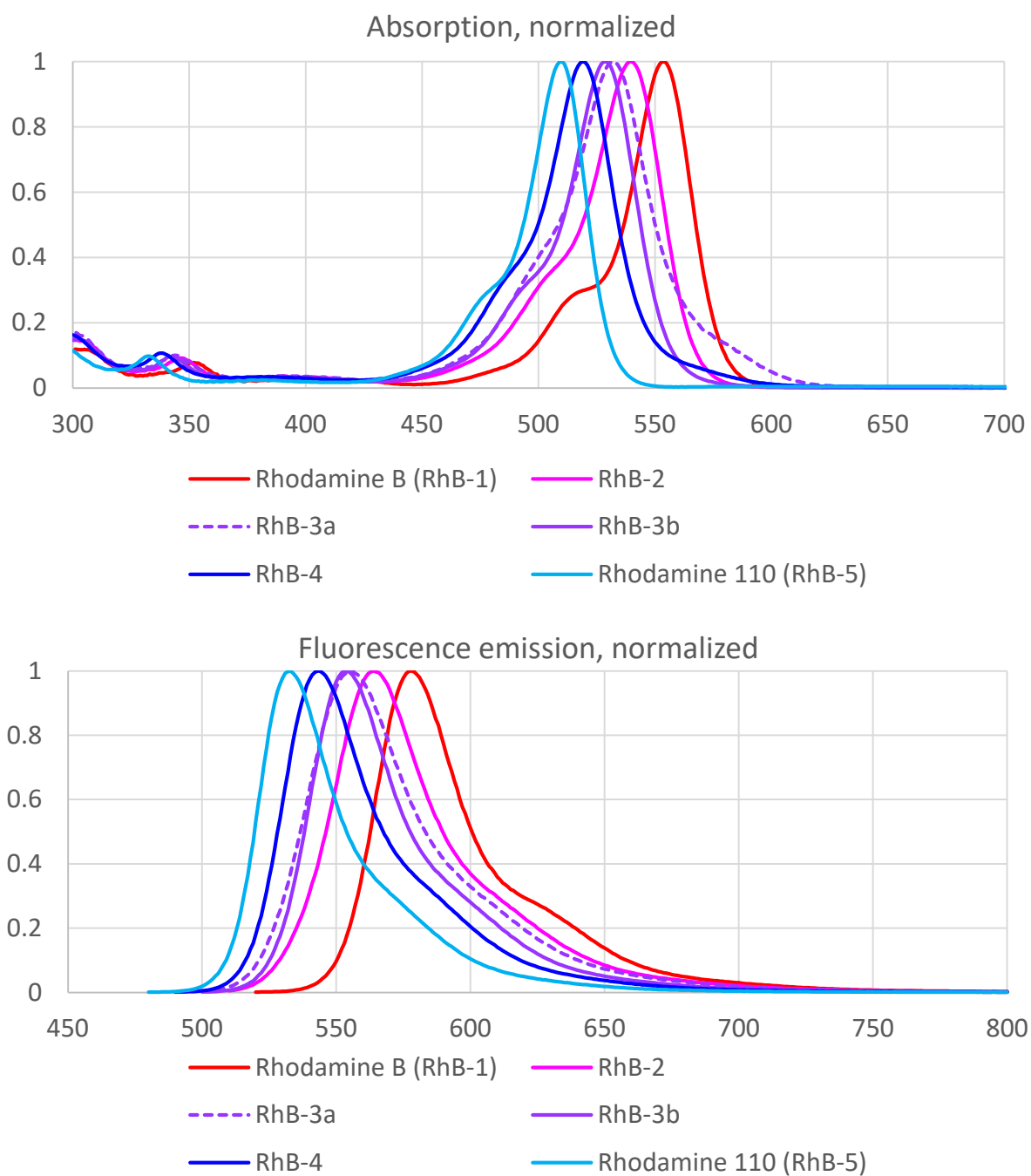


Figure S8. UV-Vis absorption and fluorescence emission spectra of Rhodamine B photooxidation products in 0.1% TFA – ethanol.

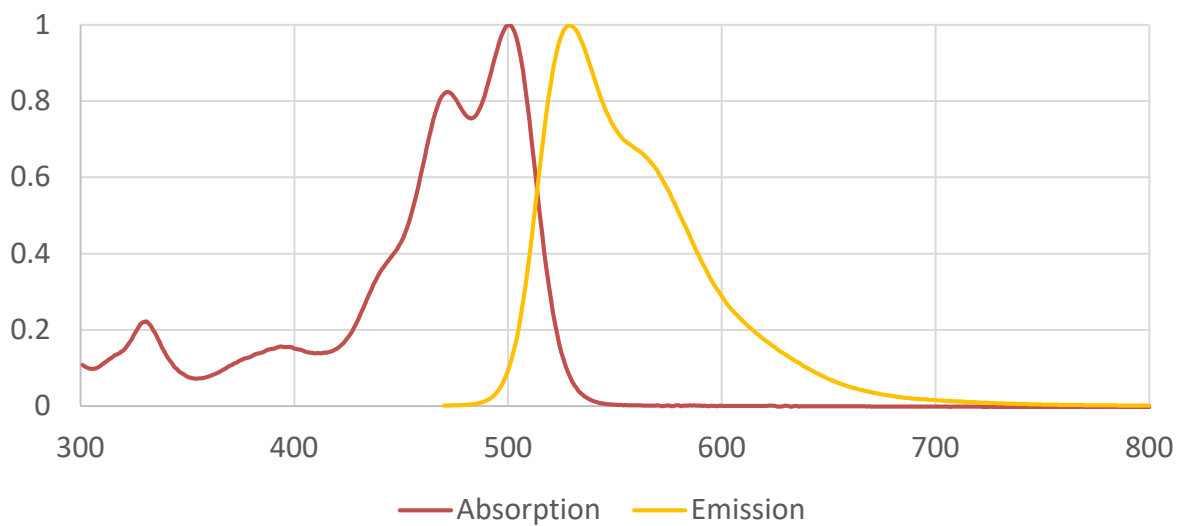
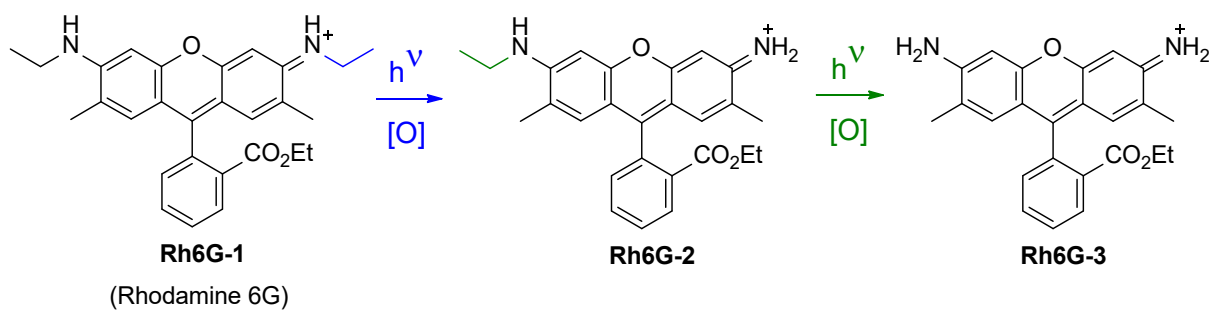


Figure S9. UV-Vis absorption and fluorescence emission spectra of **RhB-6** in 0.1% TFA – ethanol.

Photolysis of Rhodamine 6G

Photooxidation scheme for Rhodamine 6G (irradiation at 465 or 525 nm):



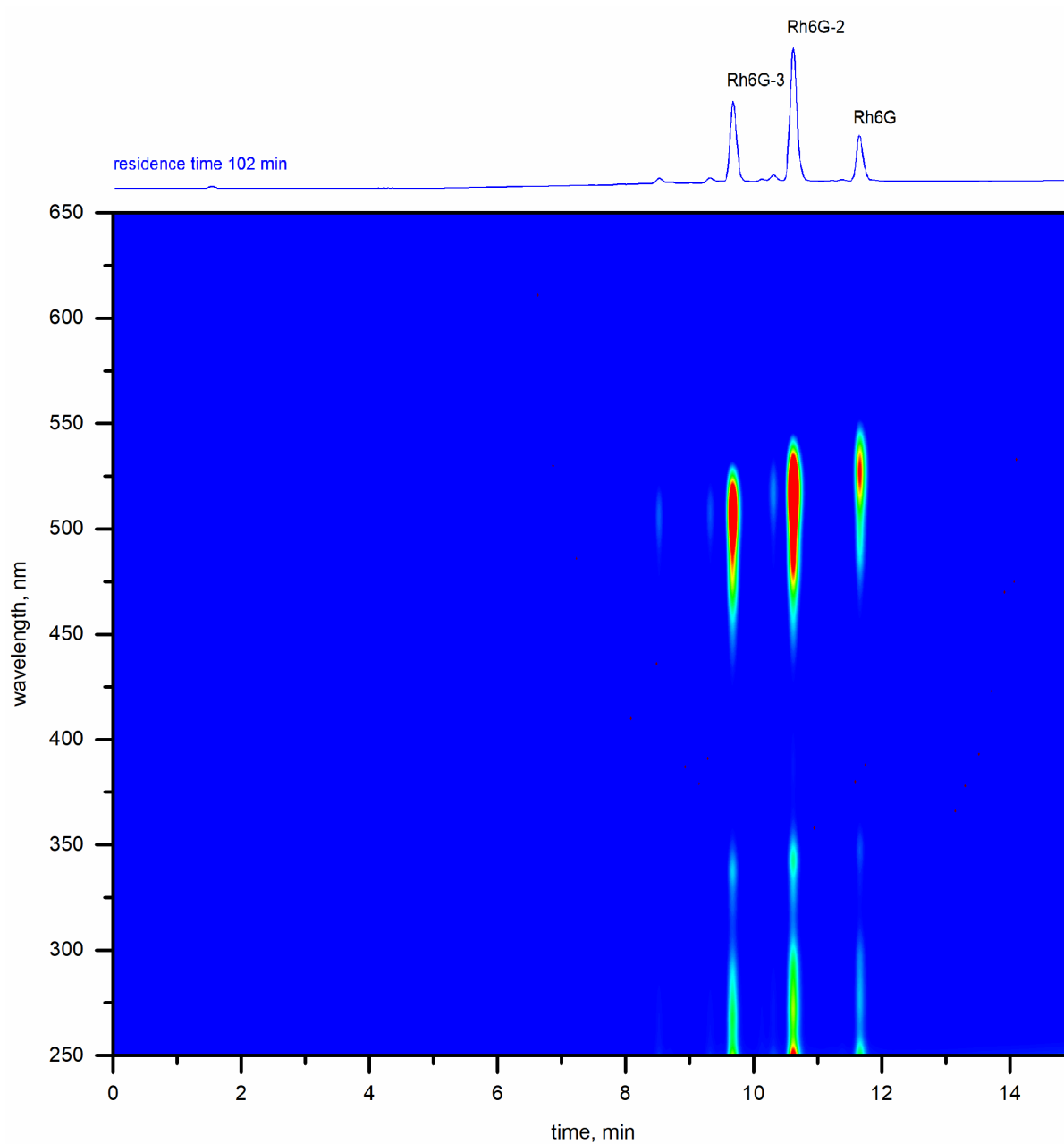


Figure S10. Semipreparative photolysis of Rhodamine 6G (10 mg in 20 mL of 0.1% TFA-ethanol, ~ 1 mM), irradiation at 465 nm, residence time 102 min. 3D data (absorption spectra vs. HPLC retention time); blue trace indicates normalized absorption at 254 nm.

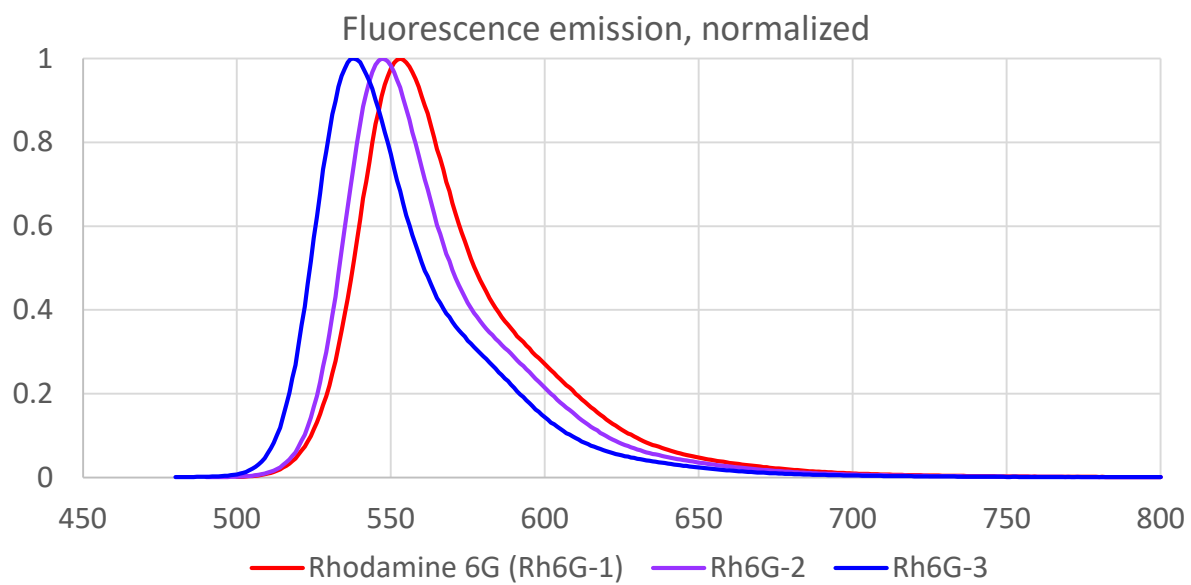
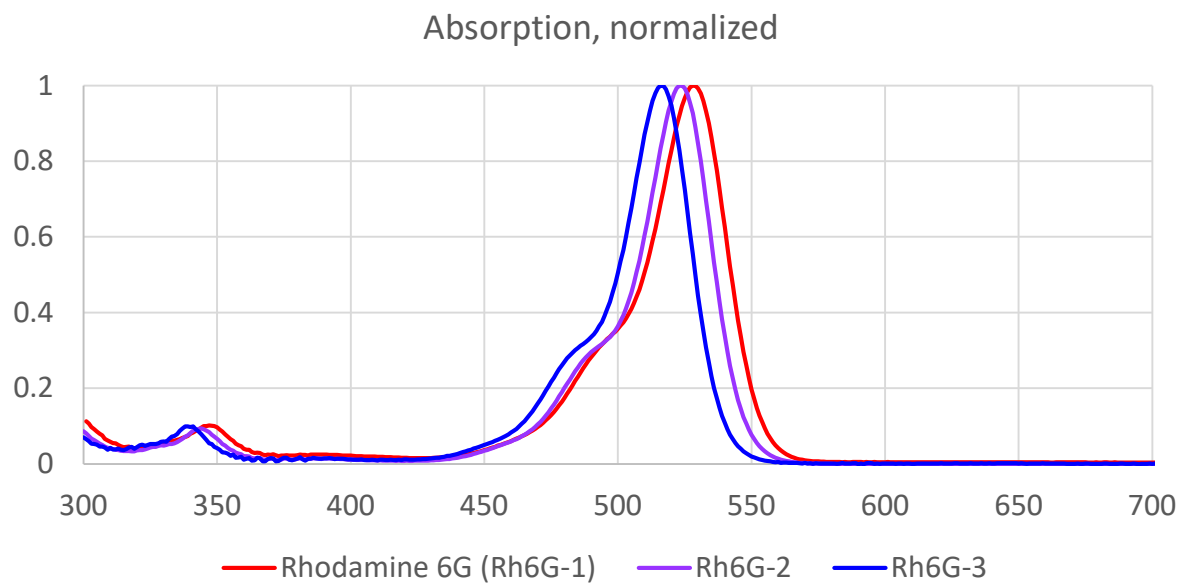
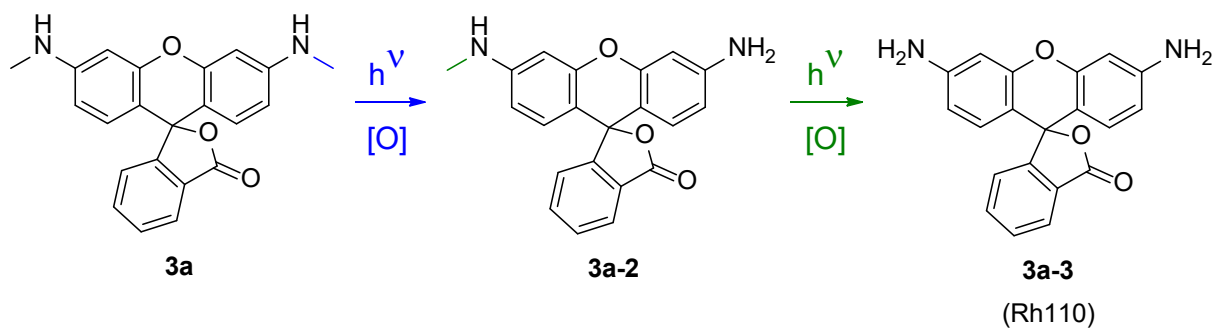


Figure S11. UV-Vis absorption and fluorescence emission spectra of Rhodamine 6G photooxidation products in 0.1% TFA – ethanol.

Photolysis of *N,N'*-dimethylrhodamine (**3a**)

Photooxidation scheme for **3a** (irradiation at 525 nm):



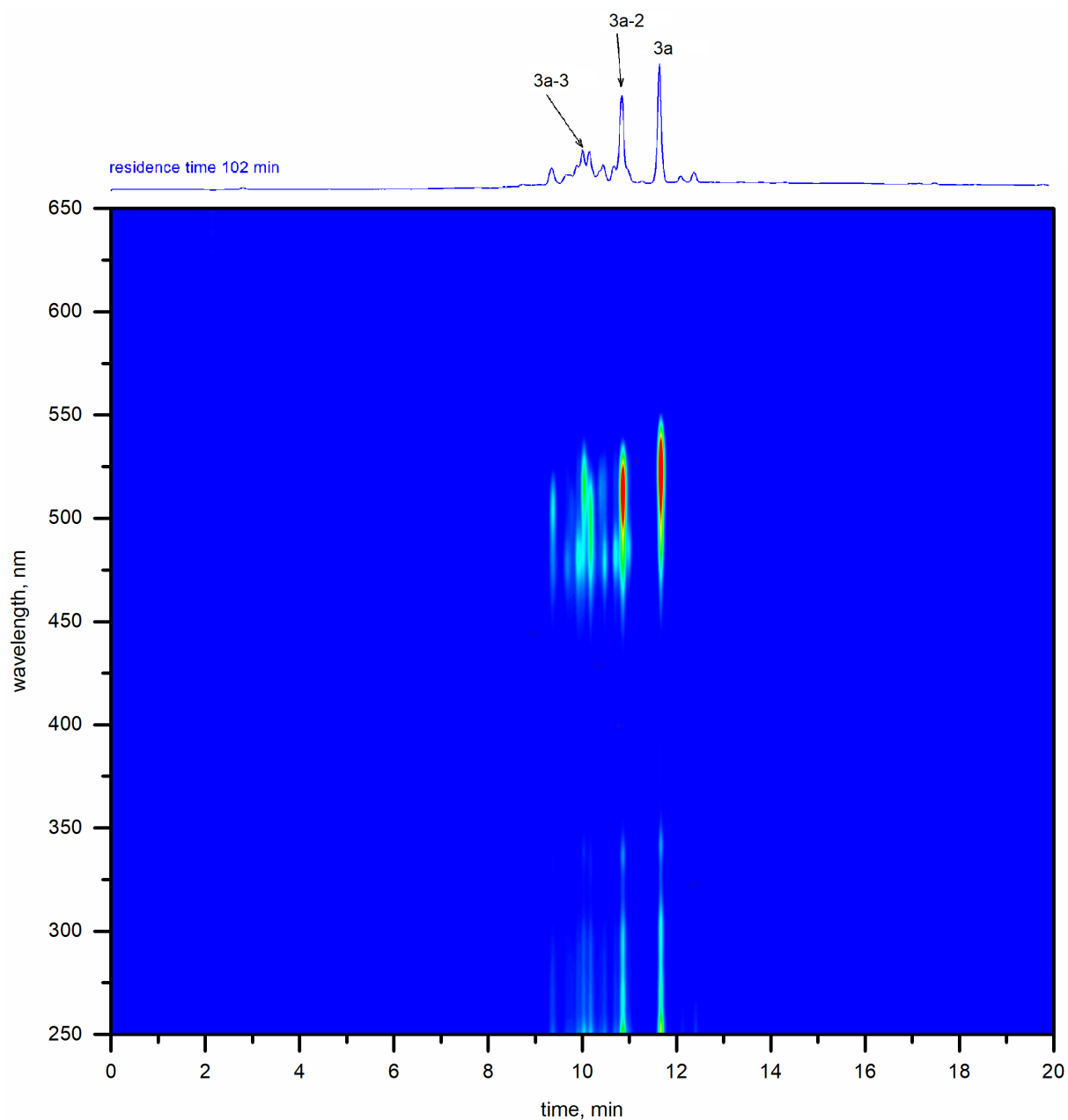


Figure S12. Semipreparative photolysis of **3a** (18 mg in 60 mL of 0.1% TFA-methanol, ~0.8 mM), irradiation at 525 nm, residence time 102 min. 3D data (absorption spectra vs. HPLC retention time); blue trace indicates normalized absorption at 254 nm.

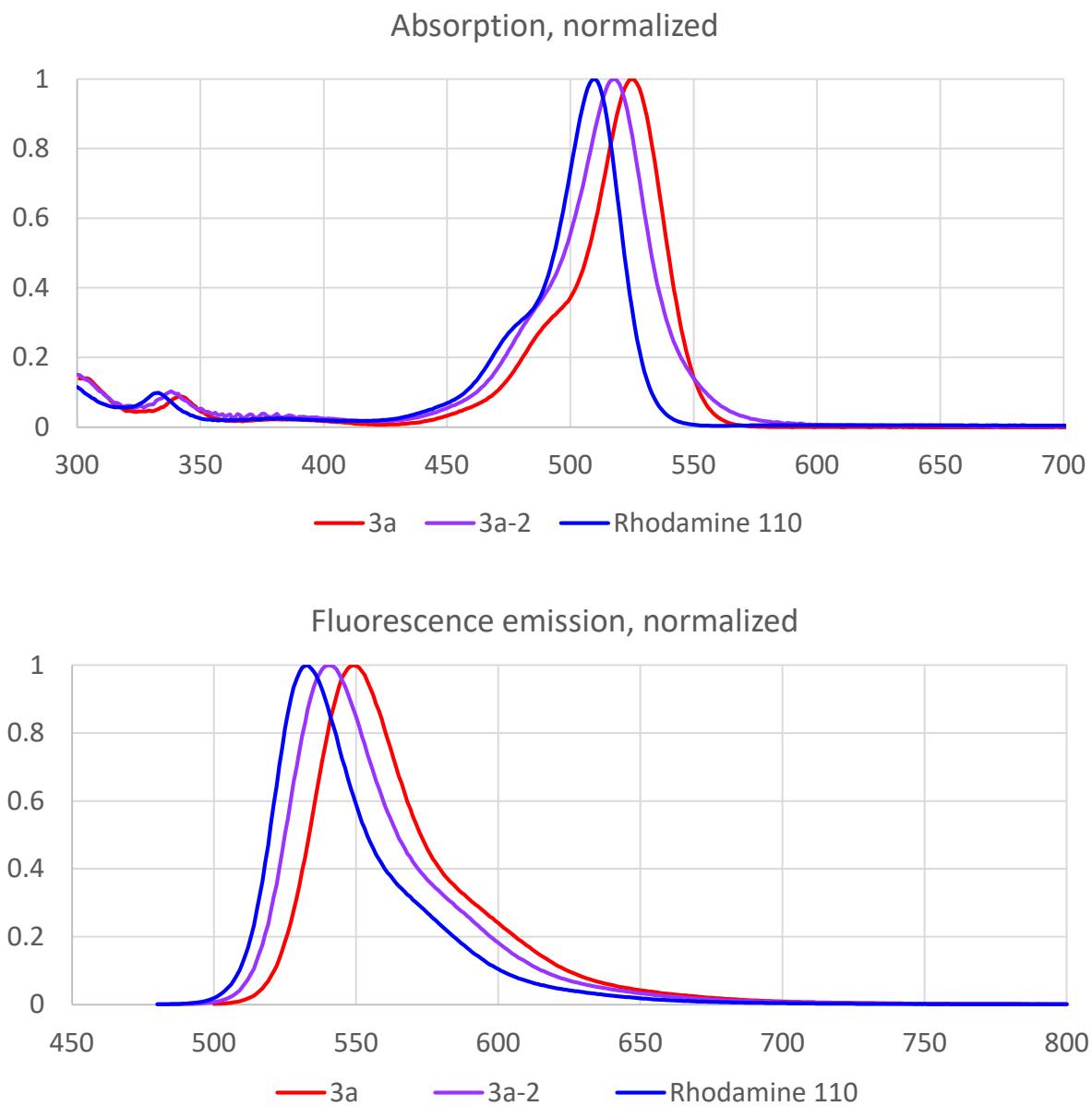
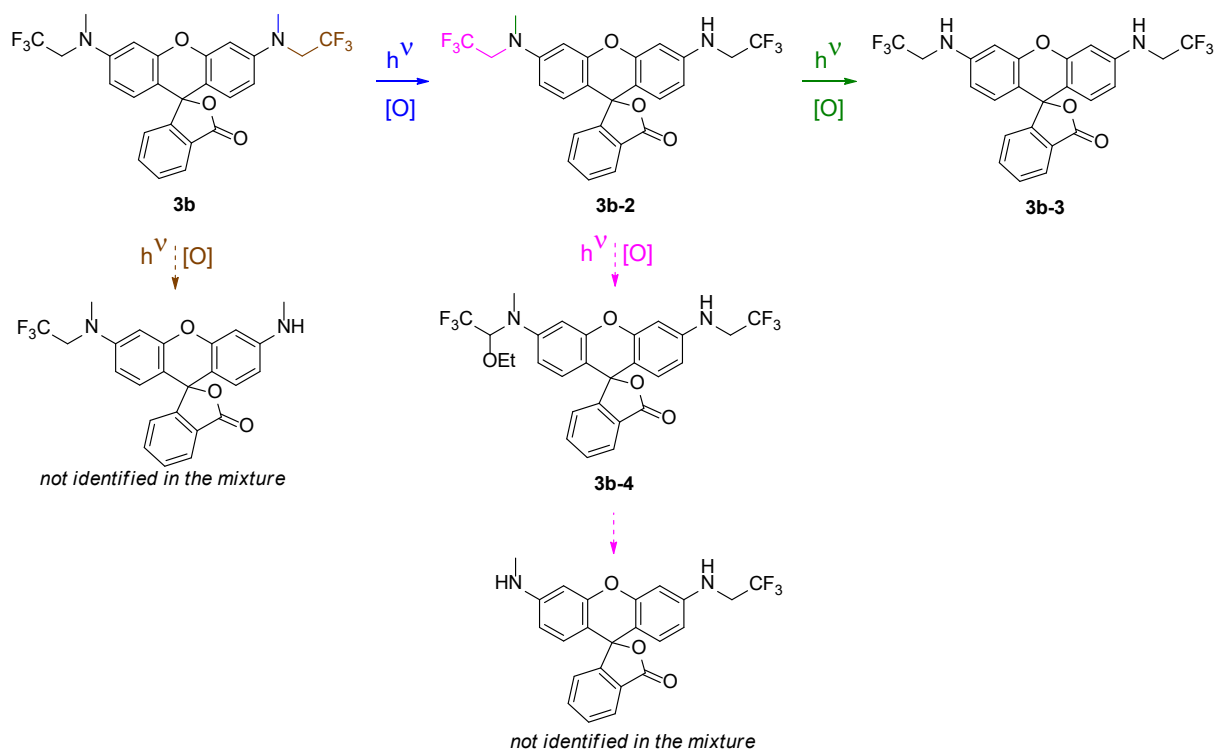


Figure S13. UV-Vis absorption and fluorescence emission spectra of **3a** photooxidation products in 0.1% TFA – ethanol.

Photolysis of 3b

Photooxidation scheme for **3b** (irradiation at 525 nm):



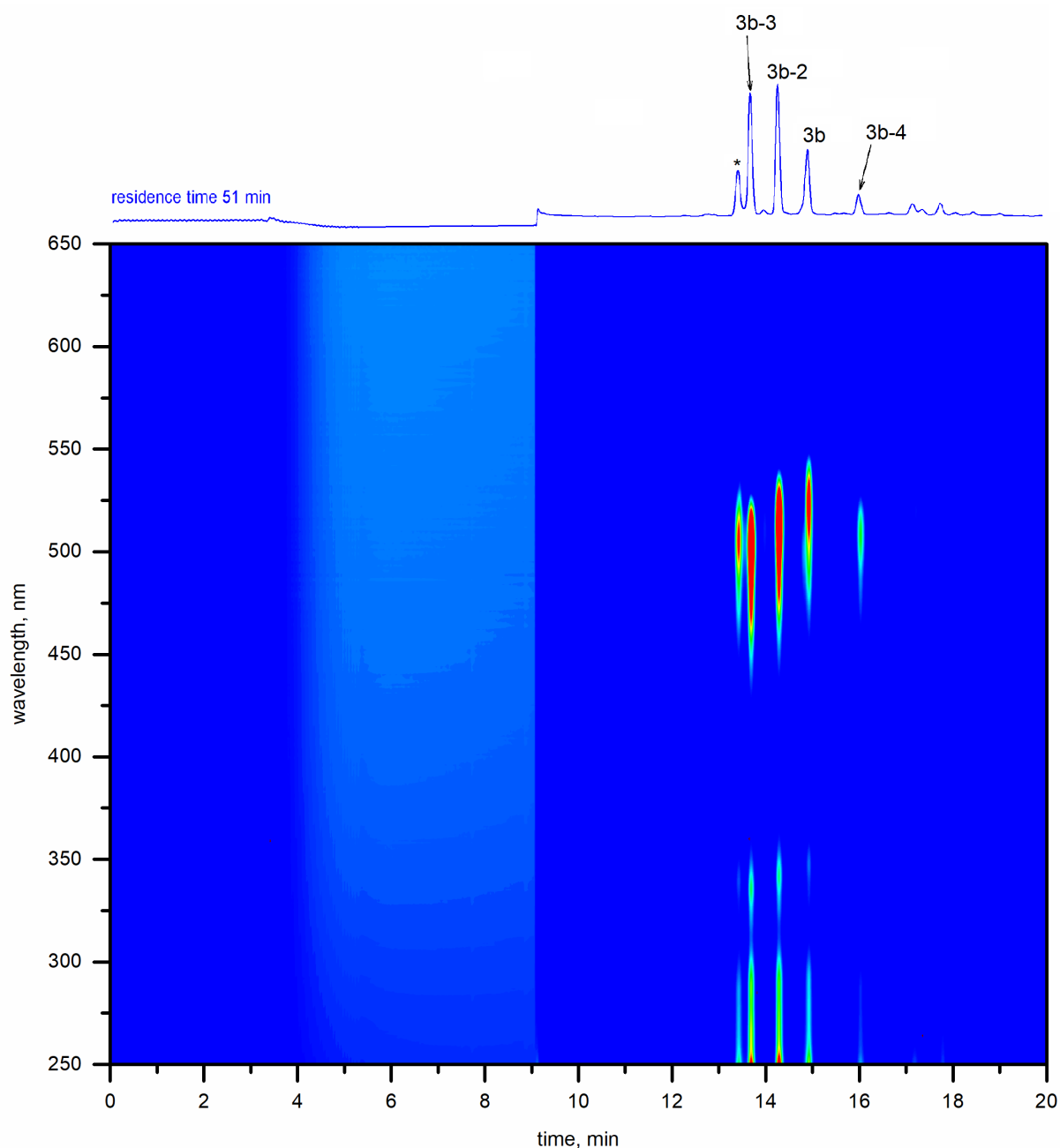


Figure S14. Semipreparative photolysis of **3b** (20 mg in 40 mL of 0.1% TFA-methanol, ~1 mM), irradiation at 525 nm, residence time 51 min. 3D data (absorption spectra vs. HPLC retention time); blue trace indicates normalized absorption at 254 nm. The product marked with an asterisk (*) was not separable under preparative HPLC conditions.

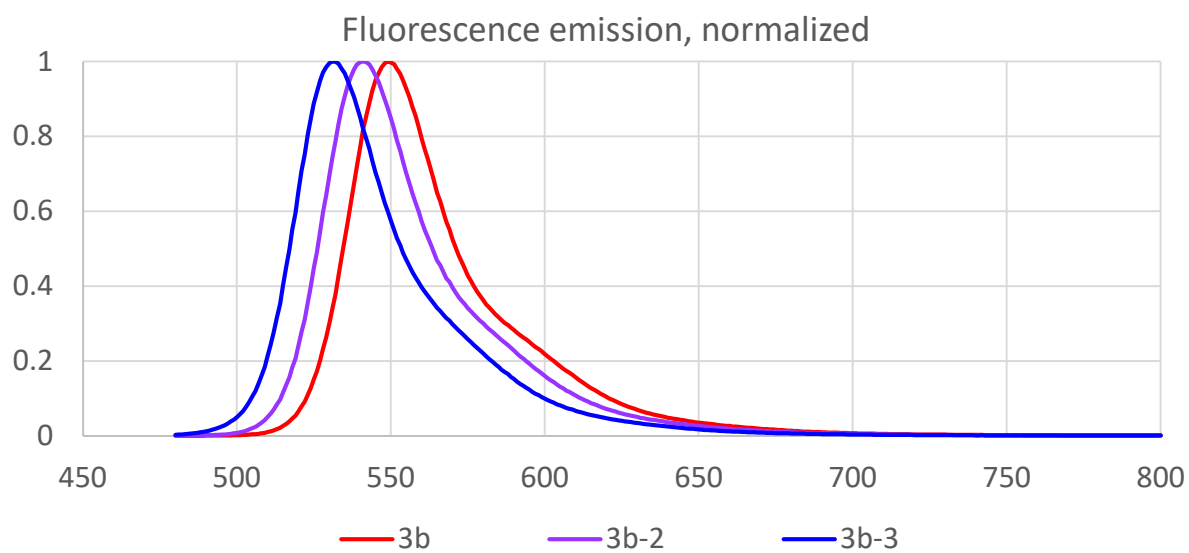
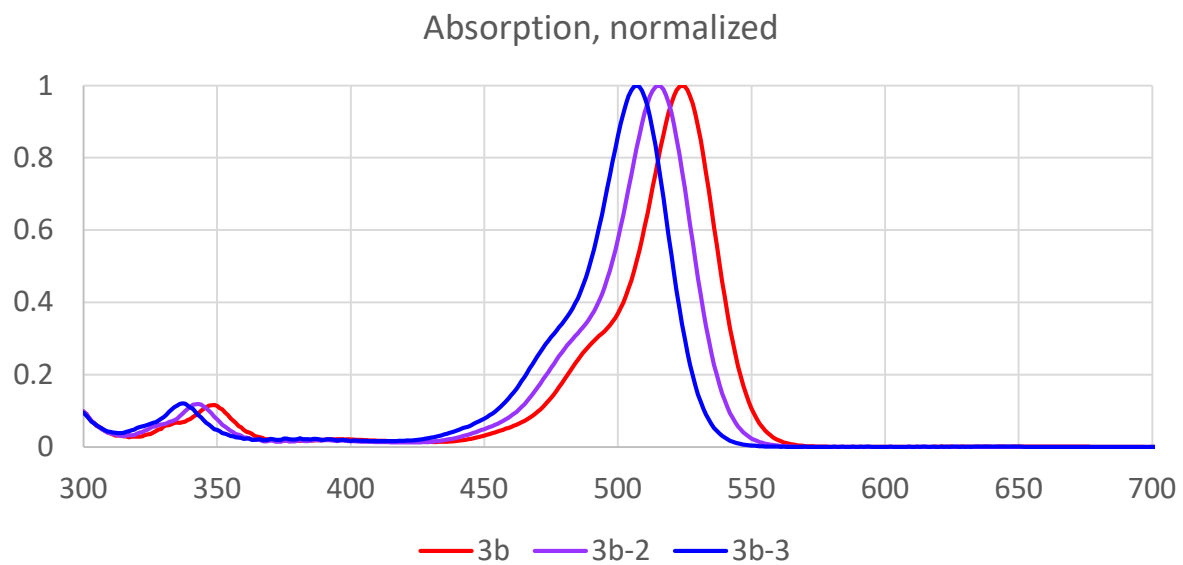
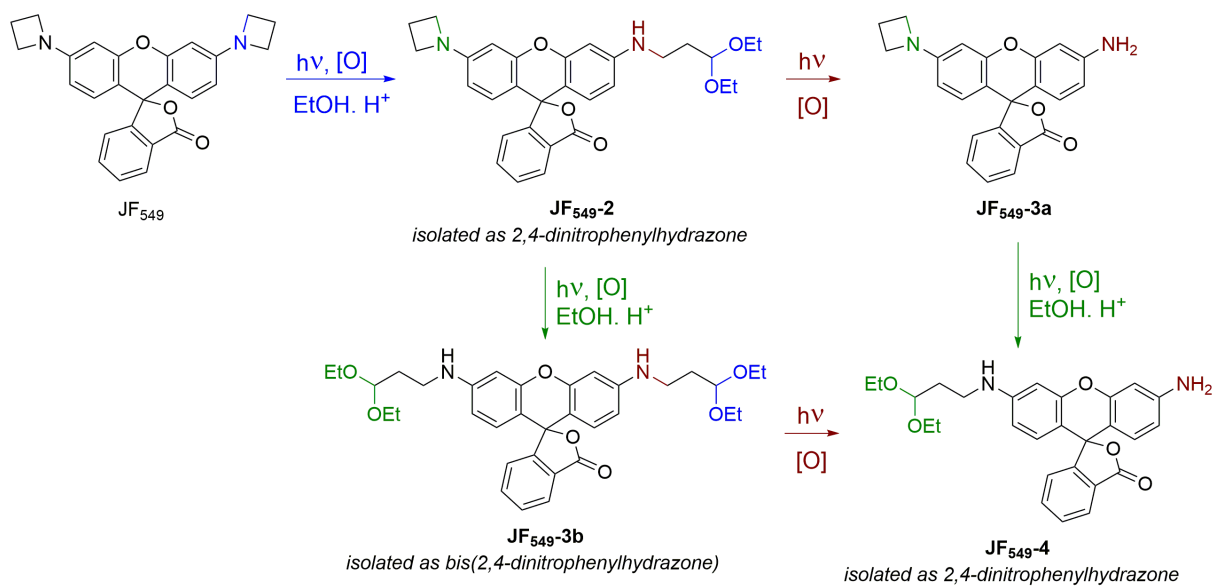


Figure S15. UV-Vis absorption and fluorescence emission spectra of **3b** photooxidation products in 0.1% TFA – ethanol.

Photolysis of JF₅₄₉

Photooxidation scheme for JF₅₄₉ (irradiation at 525 nm):



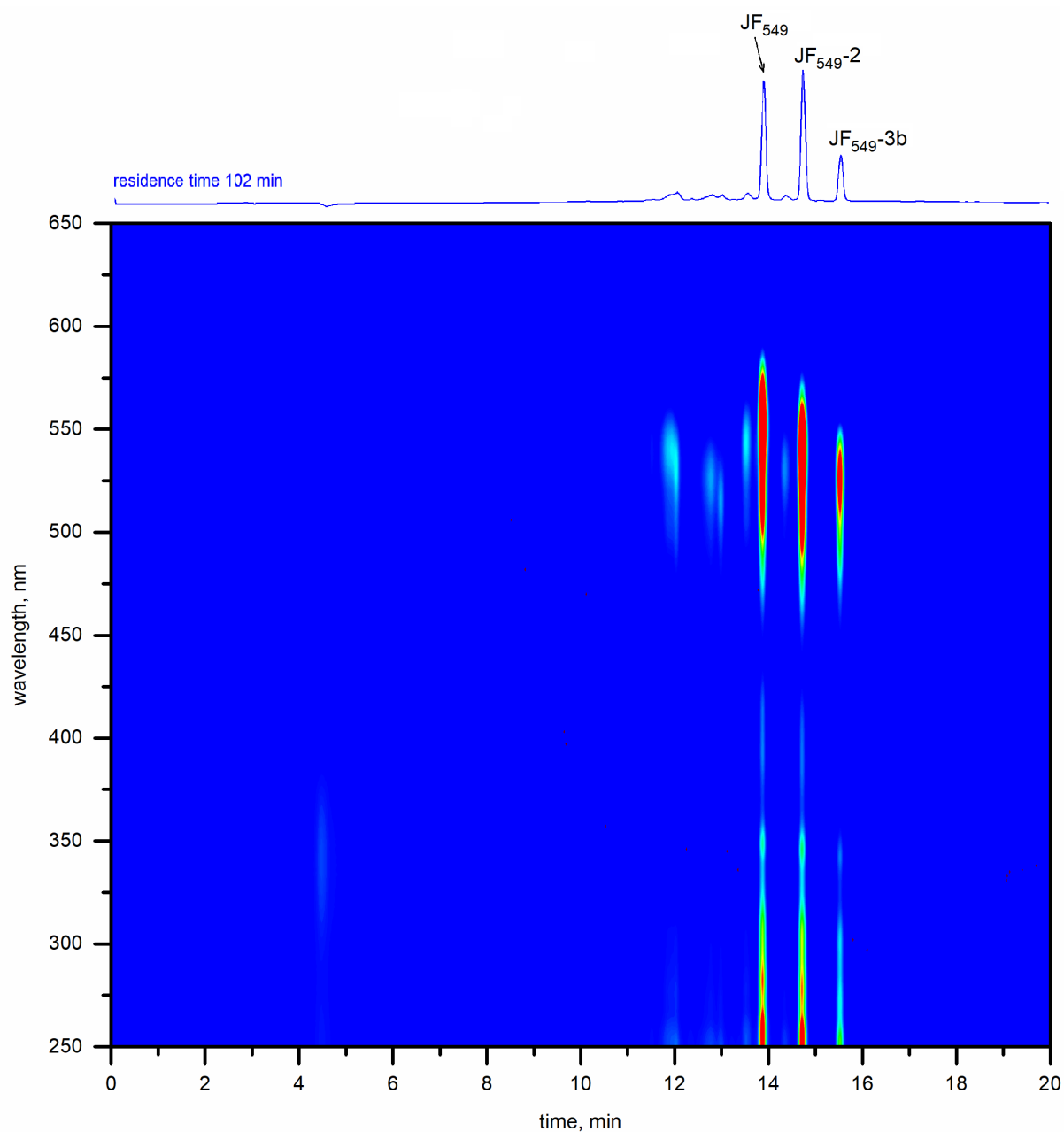


Figure S16. Semipreparative photolysis of JF₅₄₉ (15 mg in 30 mL of 0.1% TFA-ethanol, ~1.2 mM), irradiation at 525 nm, residence time 102 min. 3D data (absorption spectra vs. HPLC retention time); blue trace indicates normalized absorption at 254 nm.

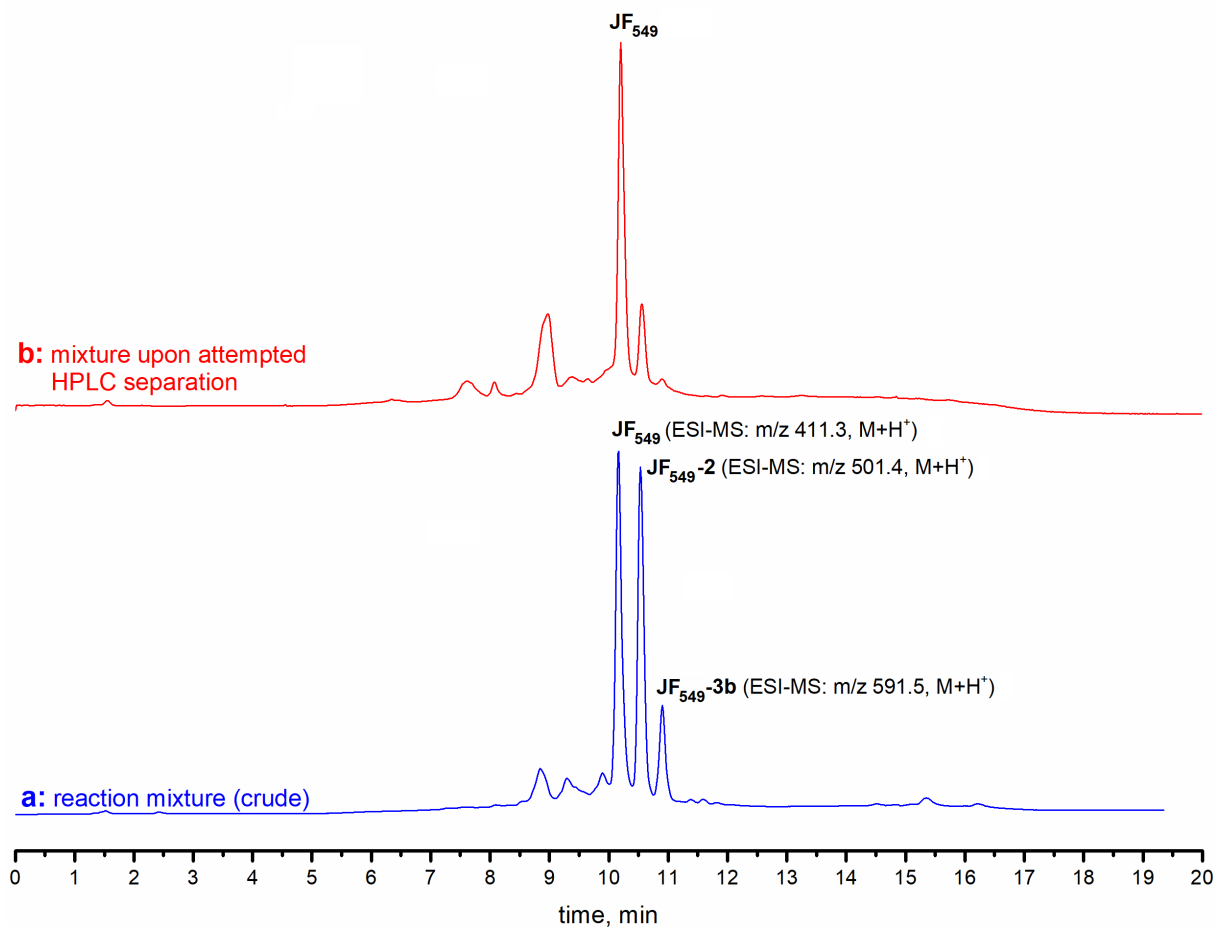


Figure S17. Attempted isolation of diethylacetals **JF₅₄₉-2** and **JF₅₄₉-3b** (trace **a**): the reactive electrophilic species (aldehydes) forming on hydrolysis of acetals in aqueous media undergo extensive self-condensation (trace **b**). HPLC traces (normalized absorption at 254 nm vs. retention time) are shown.

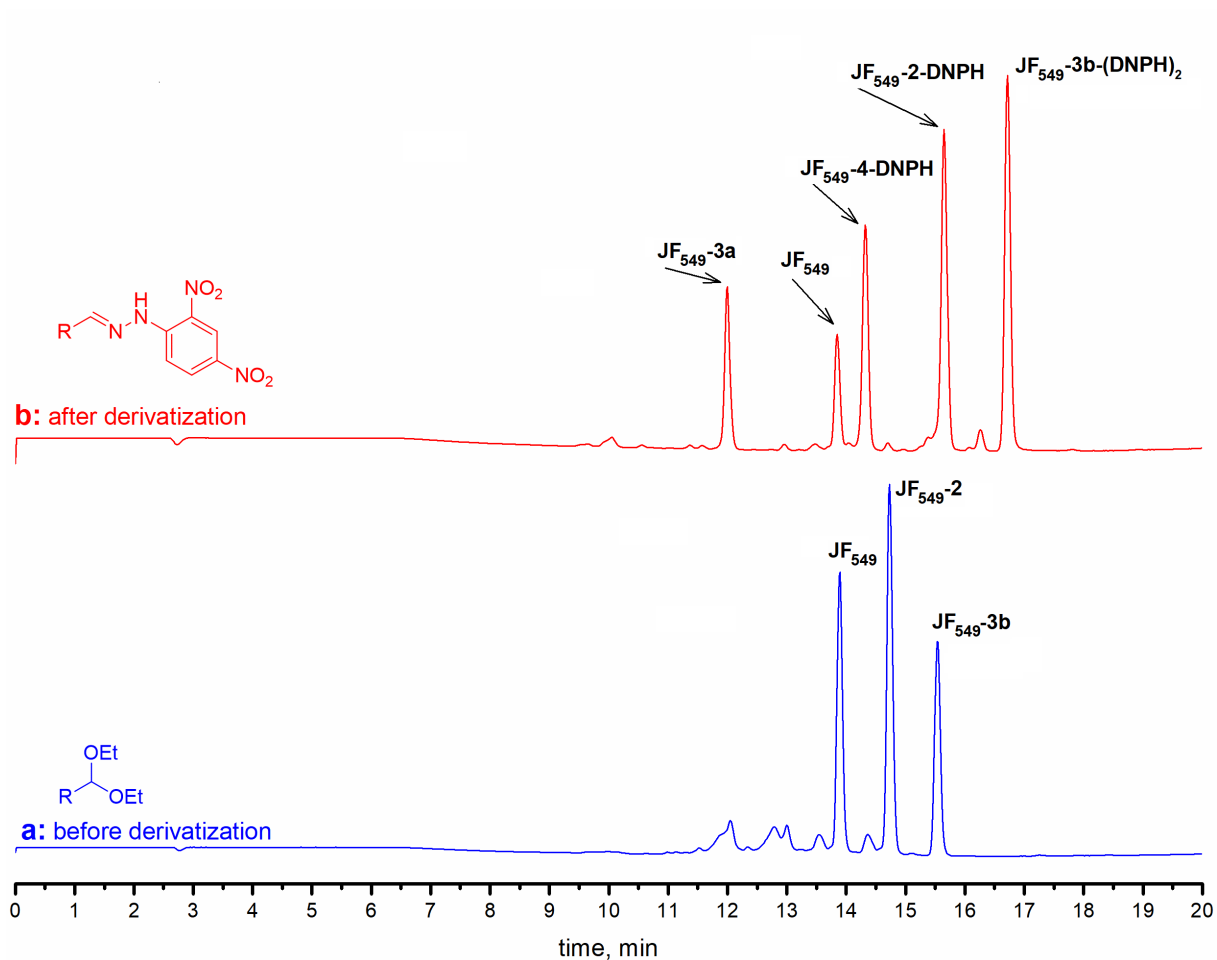


Figure S18. Derivatization of diethylacetals JF_{549-2} , JF_{549-3b} and JF_{549-4} with 2,4-dinitrophenylhydrazine (trace **a** – reaction mixture before derivatization, trace **b** – after derivatization). HPLC traces (normalized absorption at 520 nm vs. retention time) are shown.

Photolysis of JF₅₂₅

Proposed photooxidation scheme for JF₅₂₅:

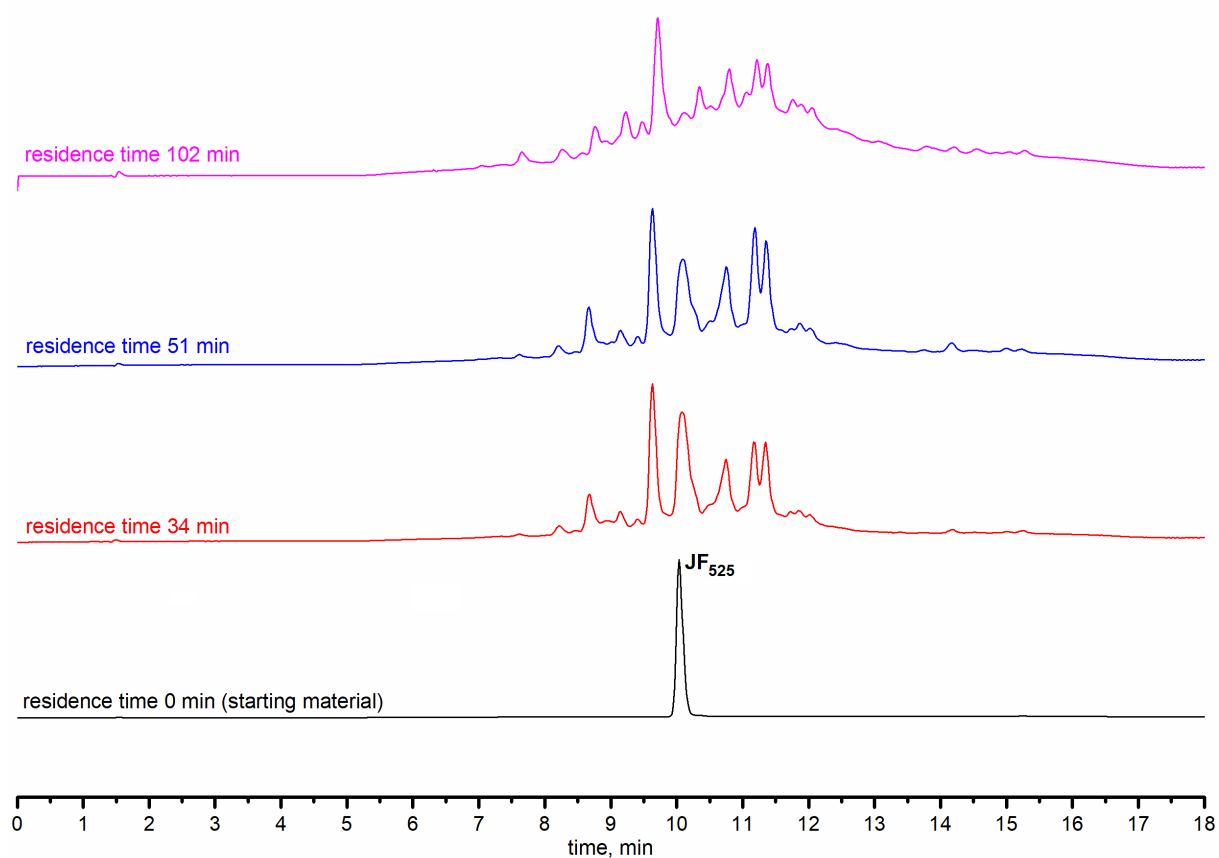
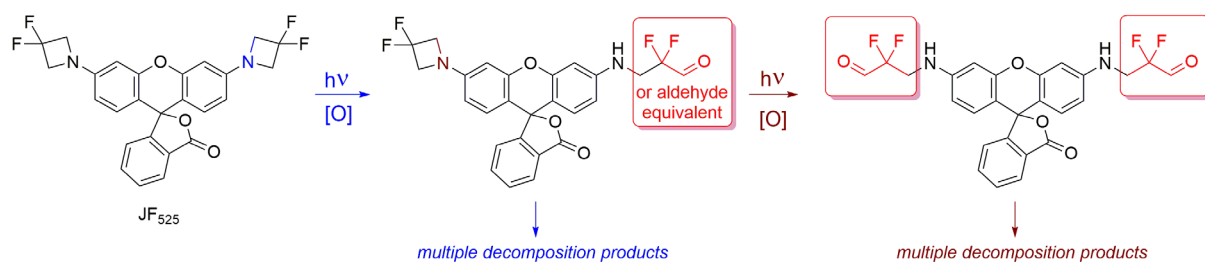


Figure S19. Effect of irradiation time on the degree of photoconversion of JF₅₂₅ (1 mM) in 0.1% TFA-ethanol. HPLC traces (normalized absorption at 254 nm vs. retention time) are shown.

Attempted photolysis of 2a

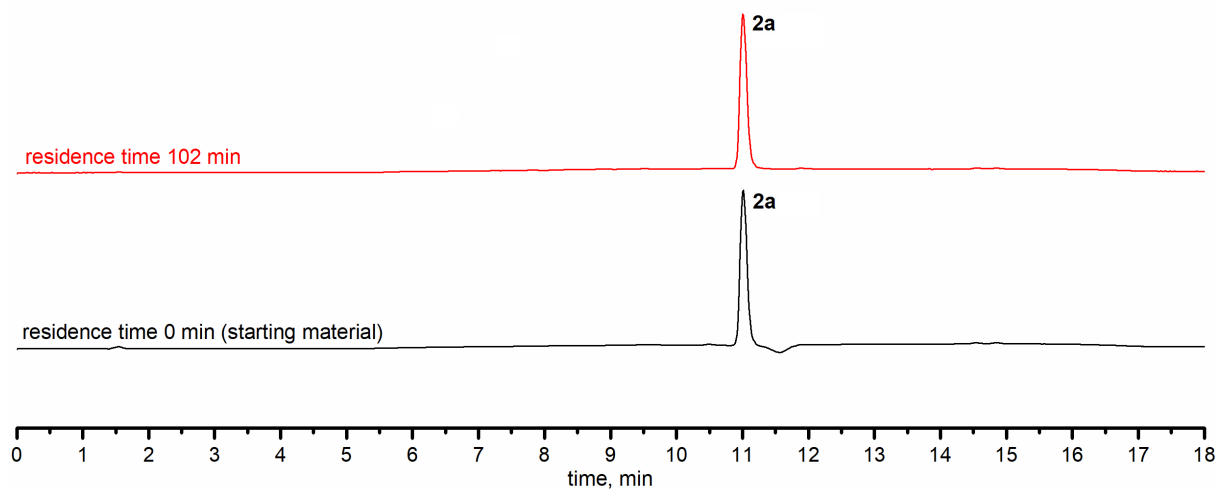


Figure S20. HPLC traces (normalized absorption at 254 nm vs. retention time) of solutions of **2a** (1 mM) in 0.1% TFA-ethanol before and after irradiation (525 nm).

Attempted photolysis of 3c

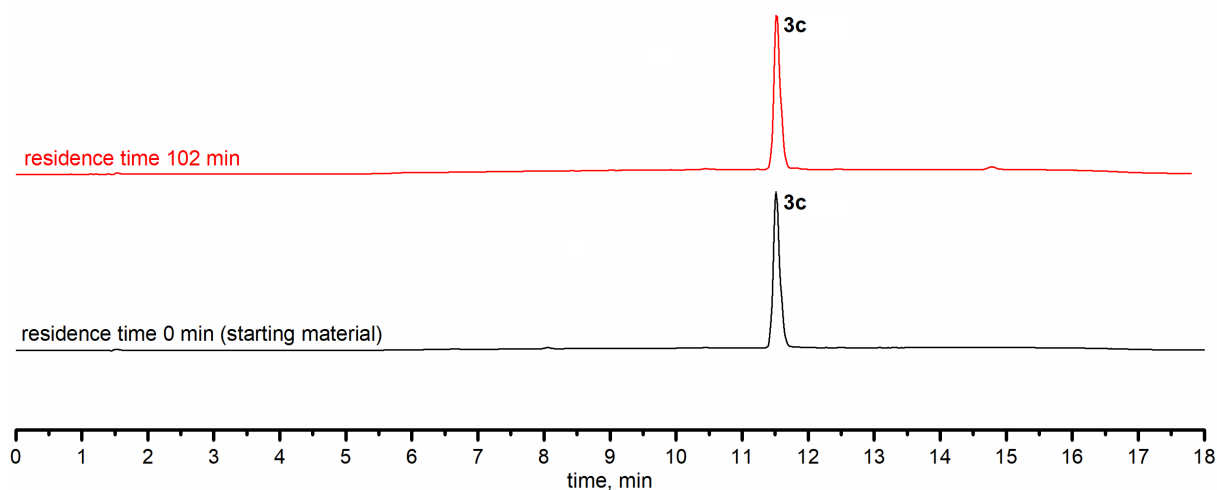


Figure S21. HPLC traces (normalized absorption at 254 nm vs. retention time) of solutions of **3c** (1 mM) in 0.1% TFA-ethanol before and after irradiation (525 nm).

Attempted photolysis of Rhodamine 110

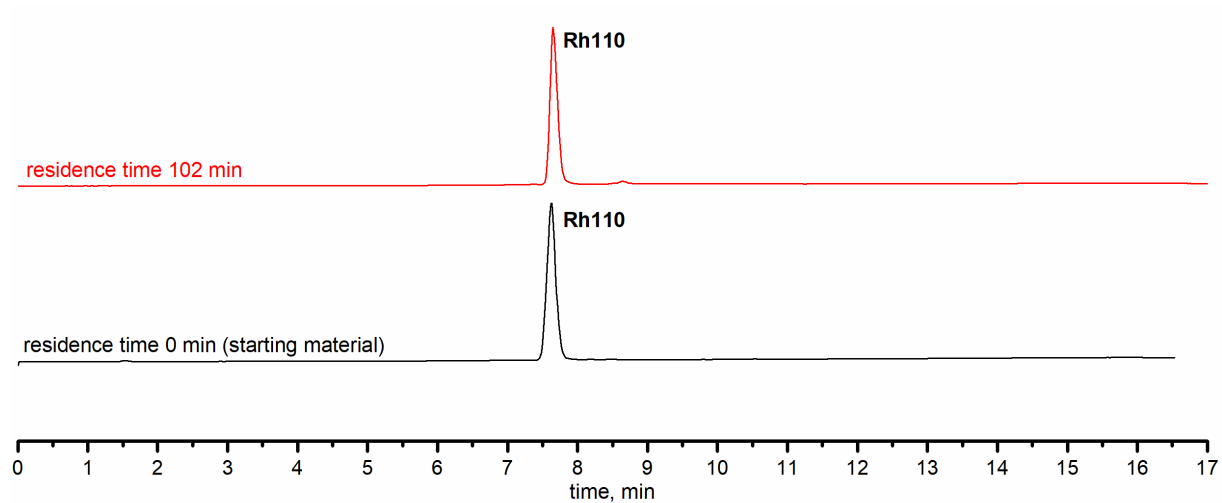


Figure S22. HPLC traces (normalized absorption at 254 nm vs. retention time) of solutions of Rhodamine 110 (1 mM) in 0.1% TFA-ethanol before and after irradiation (465 nm).

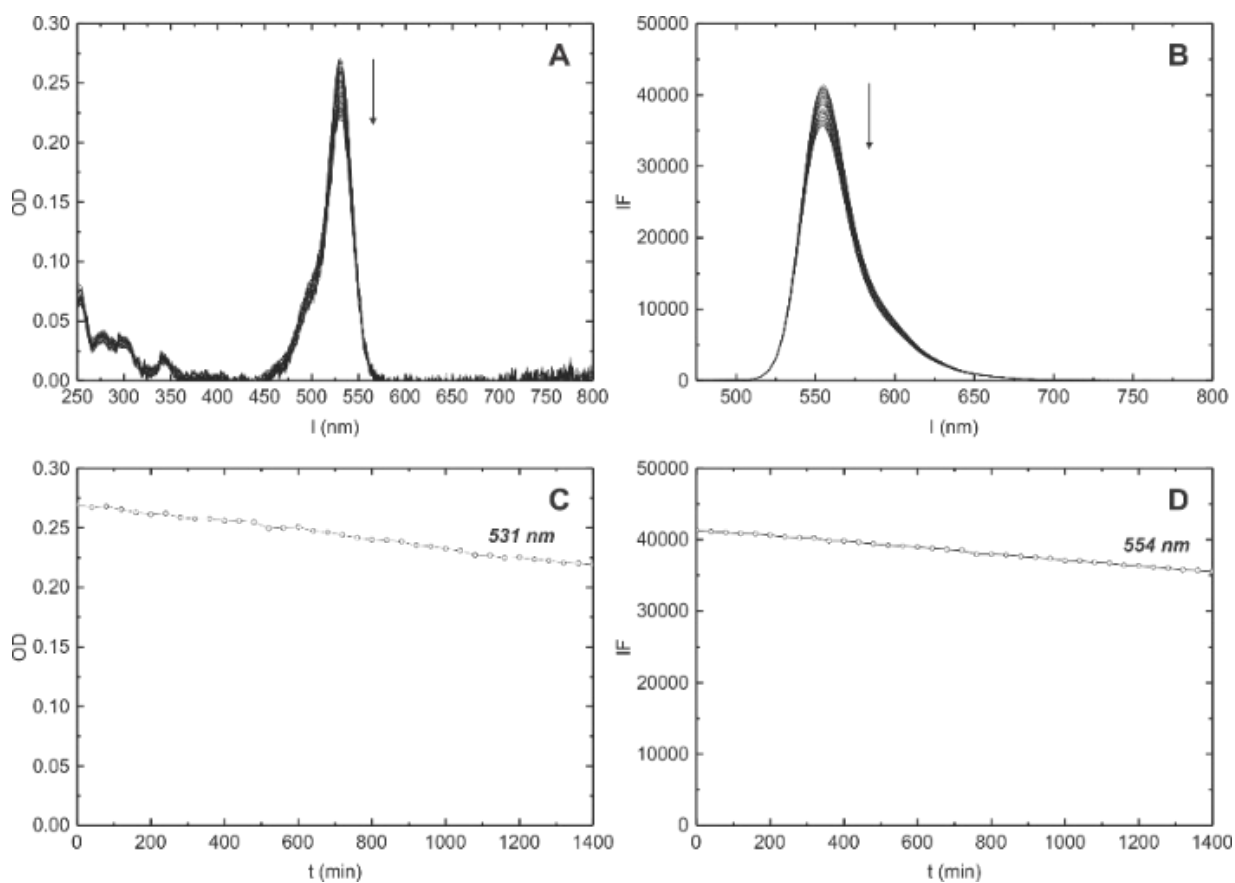


Figure S23. Time-resolved absorption and fluorescence emission spectroscopy during irradiation of **2a** in 0.1%v/v TFA – ethanol (530 nm, 350 mW LED). Absorption spectra (A); emission spectra (B); absorption (C) and emission (D) transients at the indicated wavelengths. The spectra are shown in 40 min intervals.

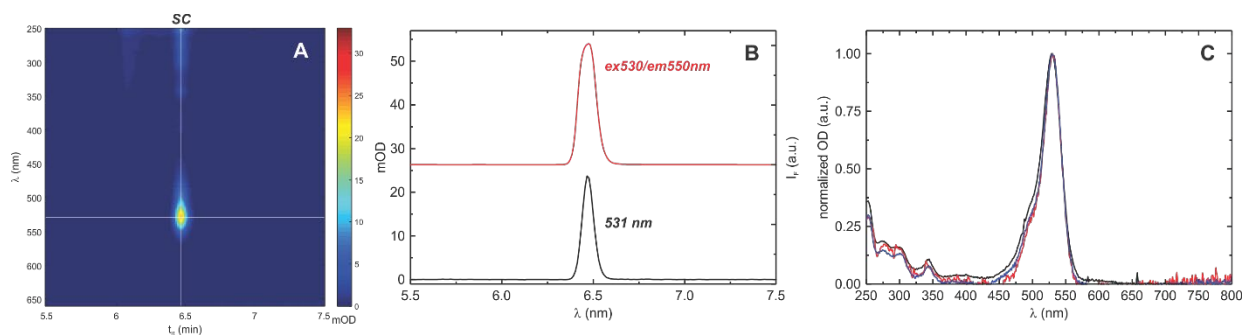


Figure S24. 2D HPLC absorption map at the end of the irradiation of compound **2a** in 0.1%v/v TFA – ethanol ($t = 1400$ min) (A); (B) Absorption (black line) and emission (red line) chromatogram at the indicated wavelengths; (C) normalized absorption spectrum of the retention peak at 6.47 min of the starting **2a** (black line), at the beginning (blue line) and at the end of the irradiation time (red line). Minor differences are due to a change of solvent and errors in baseline correction.

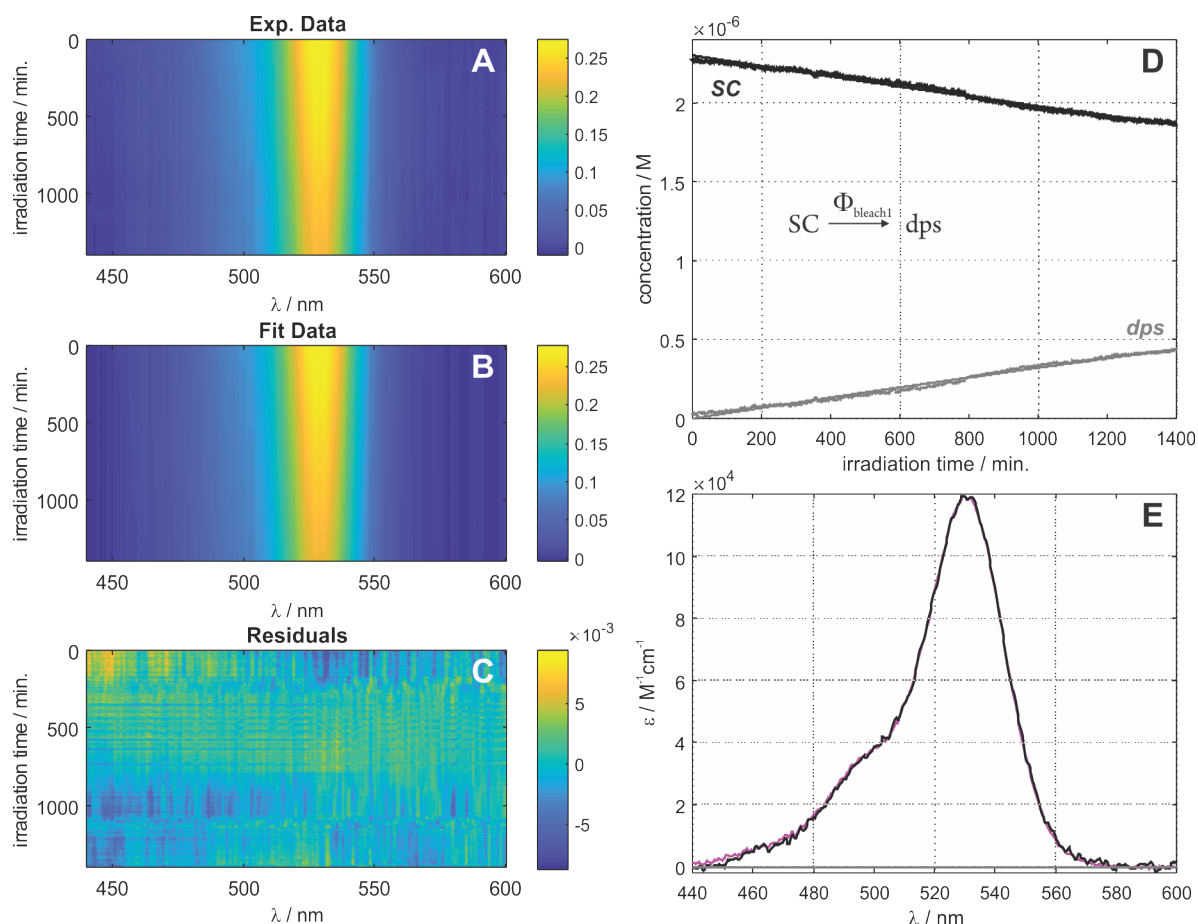


Figure S25. Photobleaching kinetics of the compound **2a** fitted with a simple photobleaching model (one-step bleaching to a dark product, dps). The absorption spectrum of the SC was left as one of the fitting variables (*E*). A) Experimental 2D-data (A^{EXP}); B) Fitted 2D-data (A^{fit}); C) 2D-Residuals map ($A^{EXP} - A^{fit}$); D) Fitted concentrations profiles (*C*), calculated from experimental (symbols, $C = A^{EXP} \times E^T$) and from fitted data (lines, $C = A^{fit} \times E^T$); E) Fitted absorption spectra matrix (*E*, black line), and the initial spectrum at $t = 0$ (magenta line). The resulting photobleaching quantum yield: $\Phi_{bleach1} = 1.4E-7$.

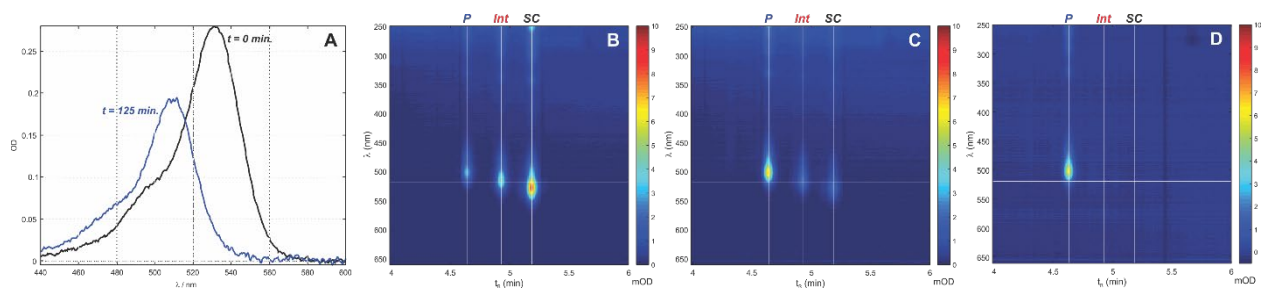


Figure S26. Time-resolved absorption spectroscopy during irradiation of **2h** in 0.1%v/v TFA – ethanol (530 nm, 350 mW LED; selected time frames). (A) Absorption spectra at the beginning of the irradiation experiment ($t = 0$, only SC) and at $t = 125 \text{ min}$ irradiation time. (B-D) 2D HPLC absorption maps at $t = 40 \text{ min}$ (B), $t = 100 \text{ min}$ (C) and $t = 1400 \text{ min}$ (D) irradiation time. Vertical lines show the retention time of the starting compound (SC), the intermediate (*Int*), and the final product (*P*).

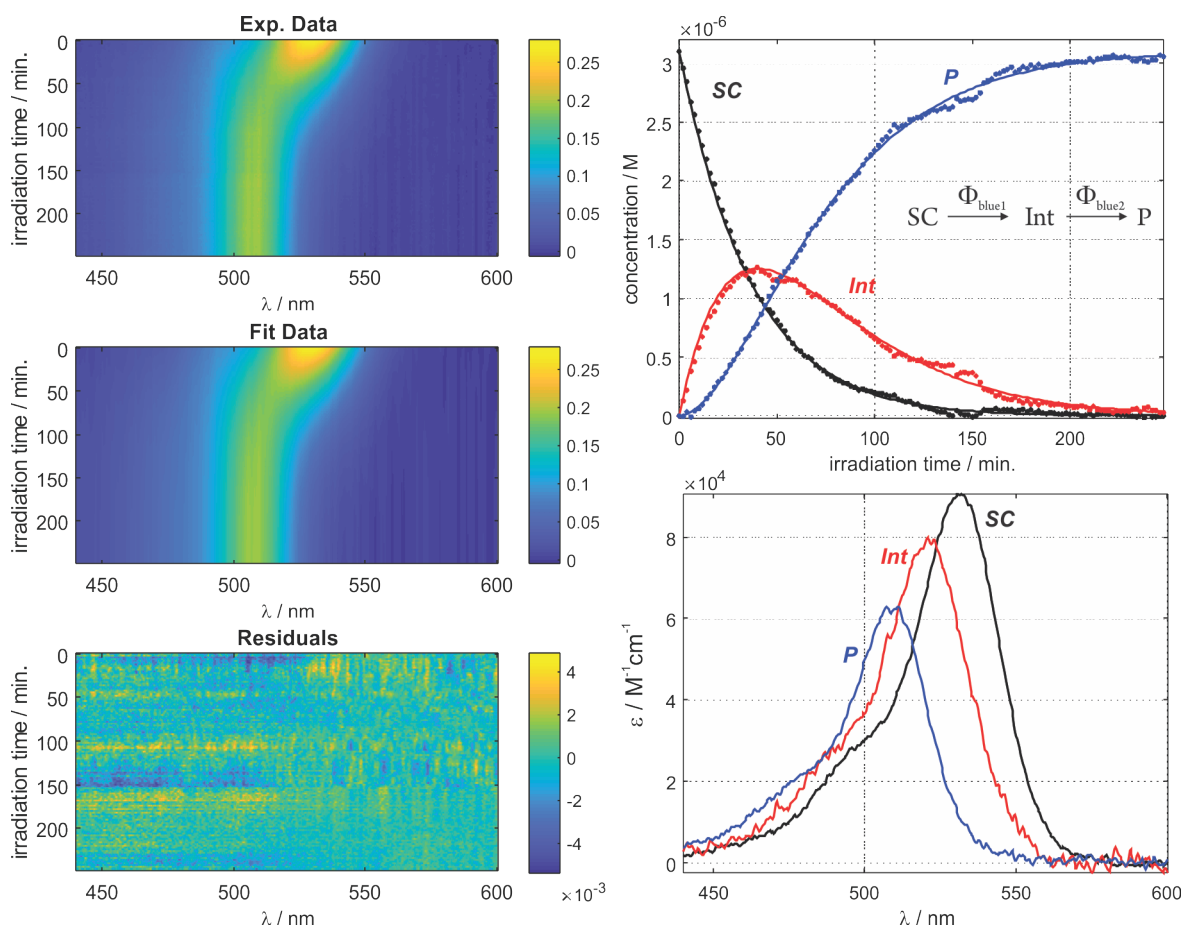


Figure S27. Compound **2h** photobleaching kinetics fitted with test model A (two-step bluing without photobleaching). A) Experimental 2D time-resolved absorption data A^{EXP} ; B) Fitted 2D time-resolved absorption data A^{fit} ; C) 2D-Residuals map, $A^{EXP} - A^{fit}$; D) Fitted concentration profiles (C), calculated from experimental (symbols, $C = A^{EXP} \times E^T$) and from fitted data (lines, $C = A^{fit} \times E^T$); E) Fitted absorption spectra matrix (E), showing the unaccountable progressive loss of $\epsilon(\lambda_{max})$. The resulting photobleaching quantum yield values: $\Phi_{blue1} = 3.4E-5$; $\Phi_{blue2} = 2.9E-5$.

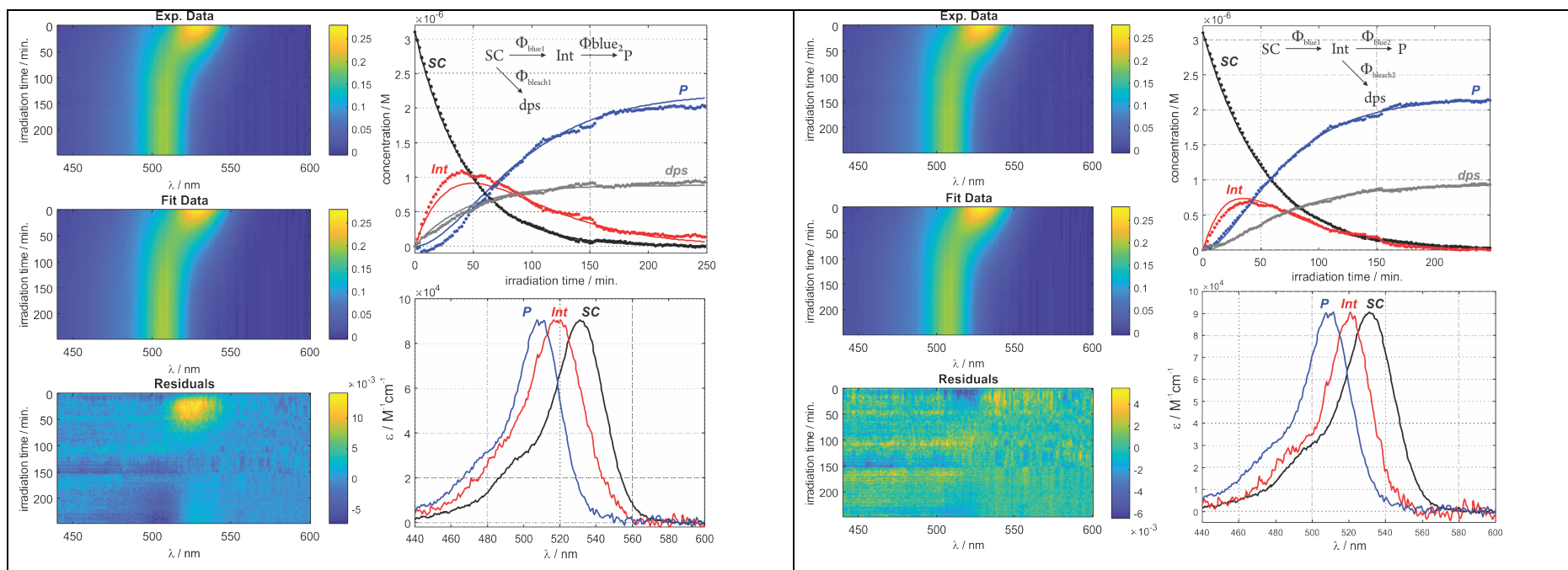


Figure S28. Compound **2h** photobleaching kinetics fitted with test models B and C (two-step blueing with a single photobleaching pathway): **model B** (left panel), **model C** (right panel). All extinction coefficients at the absorption maxima were fixed to the value $\epsilon(\lambda_{\max})$ of the starting compound, but the spectral shapes were left as fitting variables. A) Experimental time-resolved absorption data A^{EXP} ; Fitted 2D time-resolved absorption data A^{fit} ; C) 2D-Residuals map, $A^{EXP} - A^{fit}$; D) Fitted concentration profiles (C), calculated from experimental (symbols, $C = A^{EXP} \times E^T$) and from fitted data (lines, $C = A^{fit} \times E^T$); E) Fitted absorption spectra matrix (E), showing the identical $\epsilon(\lambda_{\max})$ values. The resulting photobleaching and -bleaching quantum yield values: $\Phi_{\text{blue}1} = 2.0\text{E-}5$; $\Phi_{\text{blue}2} = 2.0\text{E-}5$; $\Phi_{\text{bleach}1} = 8.1\text{E-}6$ (model B) and $\Phi_{\text{blue}1} = 2.4\text{E-}5$; $\Phi_{\text{blue}2} = 3.6\text{E-}5$; $\Phi_{\text{bleach}2} = 1.6\text{E-}6$ (model C).

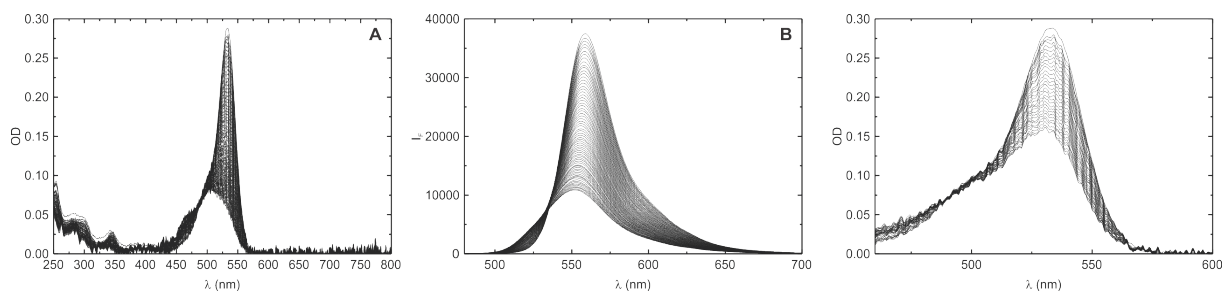


Figure S29. Time-resolved absorption and fluorescence emission spectroscopy during irradiation of **2j** in 0.1%v/v TFA – ethanol (530 nm, 350 mW LED). Absorption (A) and emission (B) spectra; (C) absorption spectra limited to 600 min irradiation time. The spectra are shown in 20 min intervals, but the entire data was used for fitting (up to 600 min).

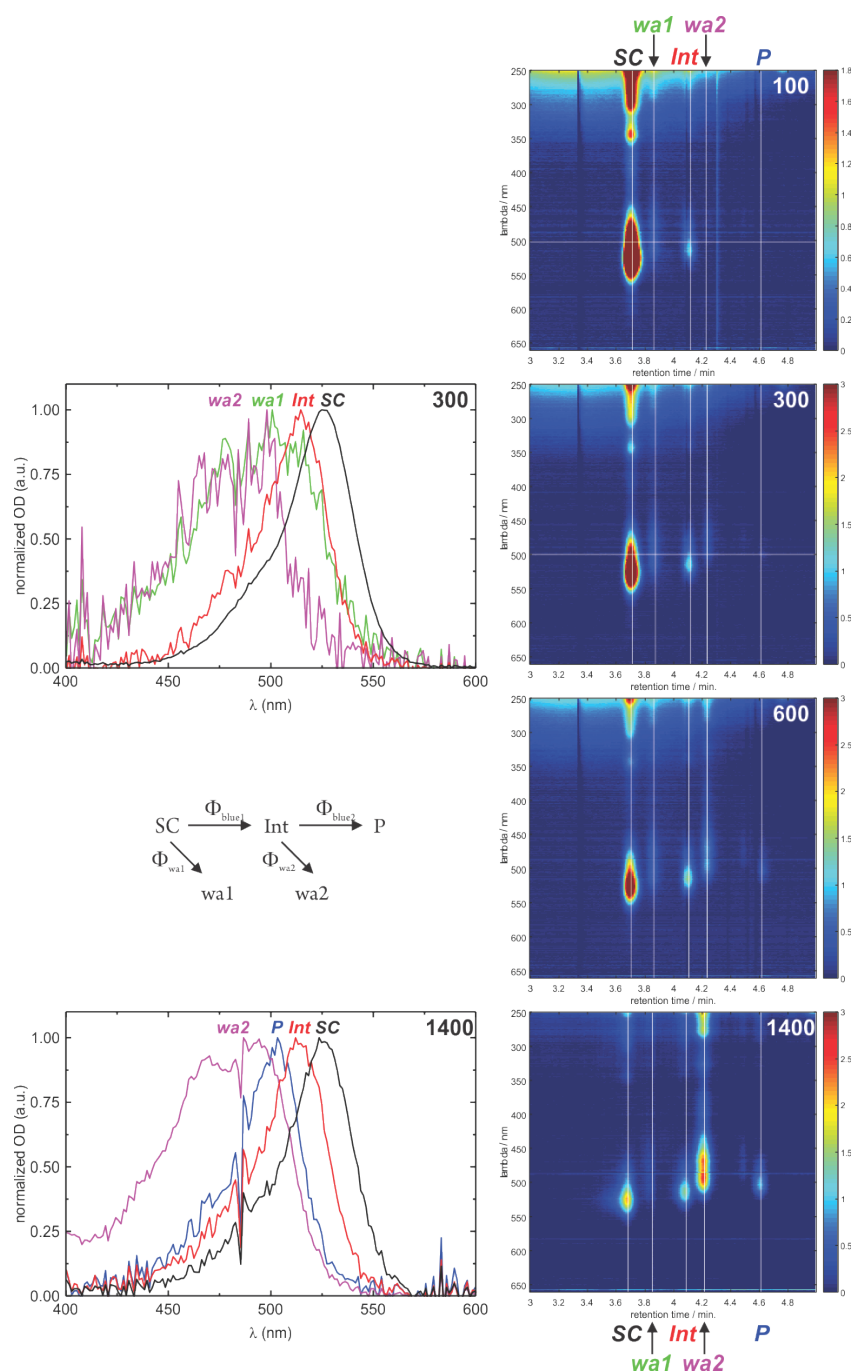


Figure S30. HPLC analysis data for **2j** at selected irradiation times (100, 300 and 600 min) and at a longer irradiation time (1440 min) than the data used for the fitting. At shorter times (100-300 min), the main product is *Int*, with a very low amount of *wa1*. Further irradiation (300-600 min.) increases the amount of *Int* as well of *wa2*. At final stages (1440 min), where almost all *SC* has been consumed, the main product is *wa2* along with a small amount of *Int* and a trace of *P*. Note that all spectra are blue-shifted with respect to those in figure S9, due to a difference in the solvent.

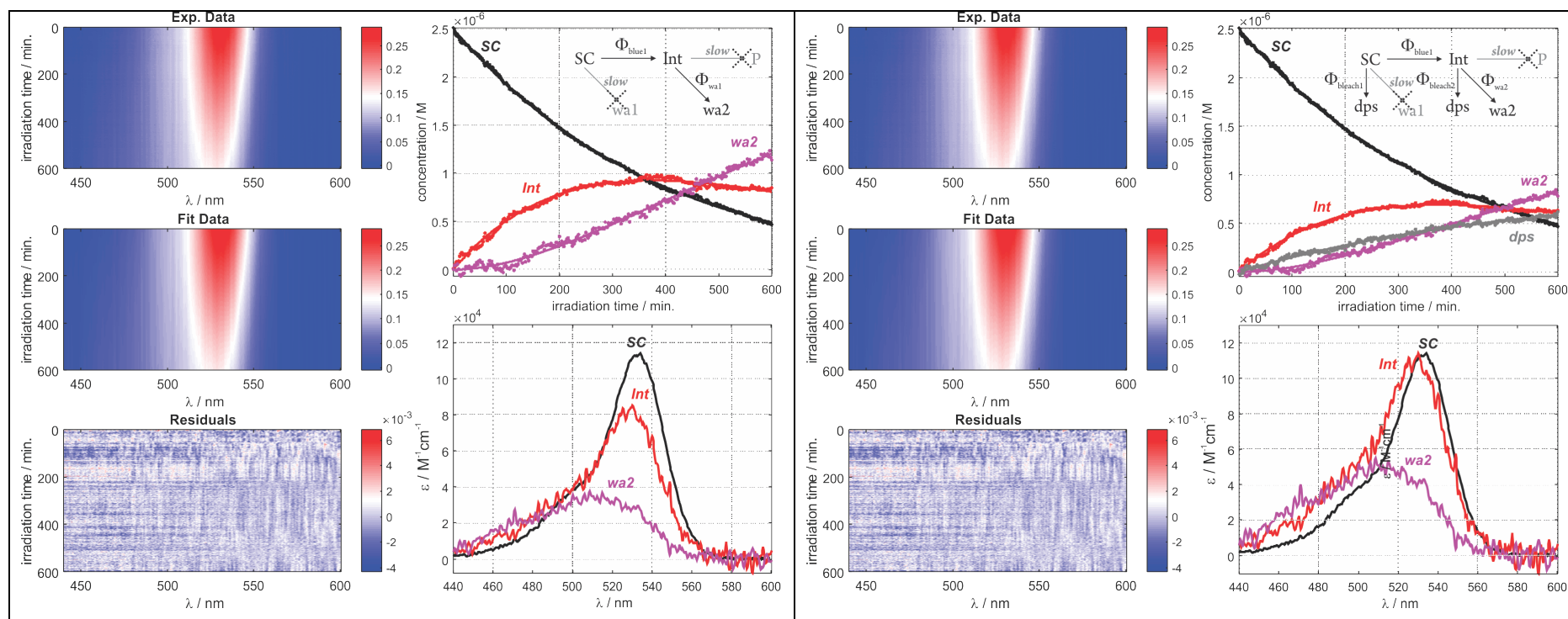
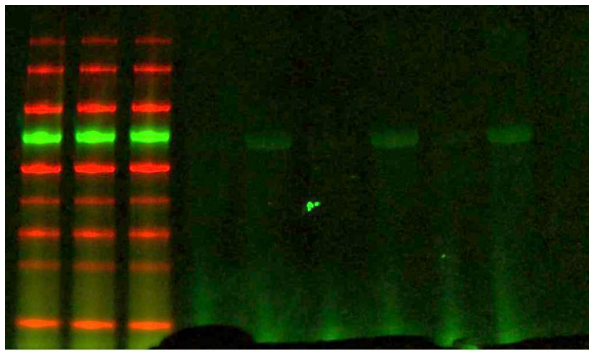
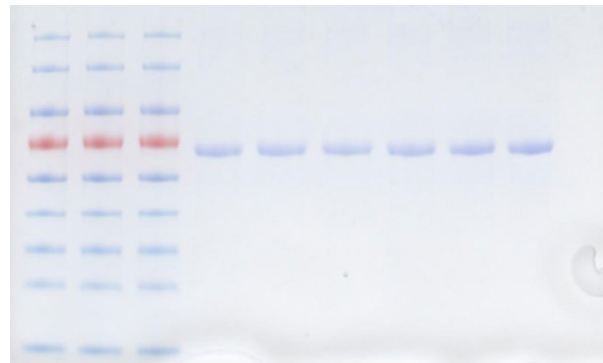


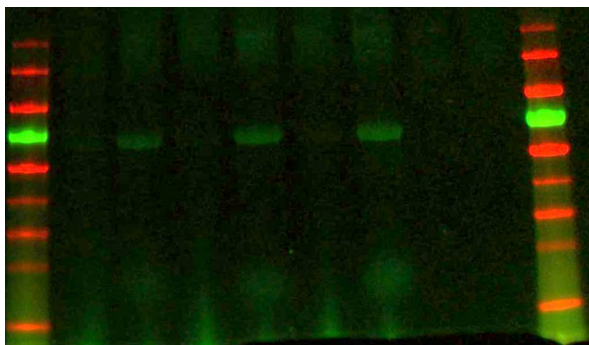
Figure S31. Compound **2j** photobleaching kinetics fitted with test models F and G (two-step bluing with accumulation of byproducts): **model F** (left panel), **model G** (right panel). The extinction coefficients at the absorption maxima were fixed to the values $\varepsilon(\lambda_{\max})$ of the starting compound (for SC and *Int*) and of **RhB-6** (for *wa2*). A) Experimental time-resolved absorption data A^{EXP} ; Fitted 2D time-resolved absorption data A^{fit} ; C) 2D-Residuals map, $A^{EXP} - A^{fit}$; D) Fitted concentration profiles (C), calculated from experimental (symbols, $C = A^{EXP} \times E^T$) and from fitted data (lines, $C = A^{fit} \times E^T$); E) Fitted absorption spectra matrix (E). The resulting photoconversion quantum yield values: $\Phi_{\text{blue1}} = 2.7\text{E-}6$; $\Phi_{\text{wa2}} = 3.1\text{E-}6$ (model F) and $\Phi_{\text{blue1}} = 2.0\text{E-}6$; $\Phi_{\text{wa1}} = 2.1\text{E-}6$; $\Phi_{\text{bleach1}} = 6.7\text{E-}7$; $\Phi_{\text{bleach2}} = 1.9\text{E-}7$ (model G).



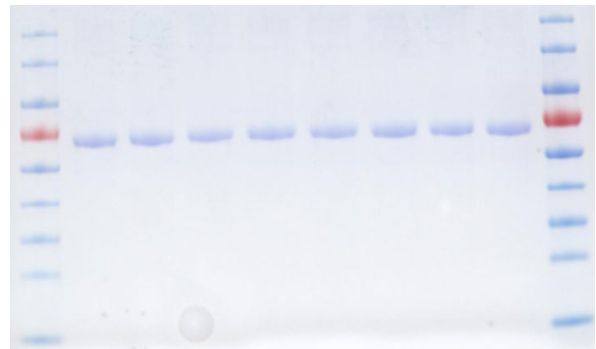
t, min: 0 5.1 0 5.1 0 5.1
2a (fluorescence)



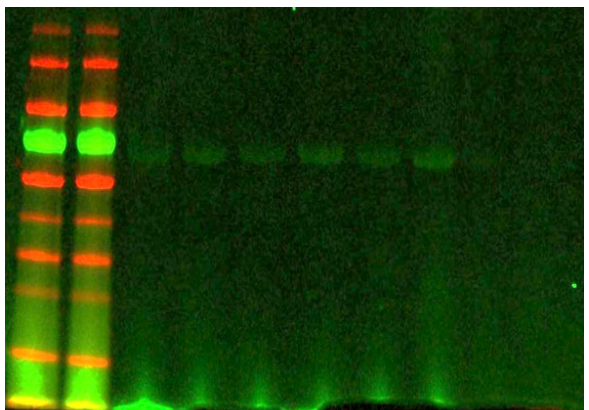
t, min: 0 5.1 0 5.1 0 5.1
2a (Coomassie stain)



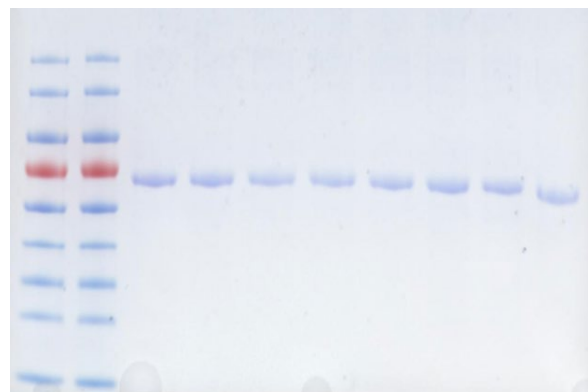
t, min: 0 5.1 0 5.1 0 5.1 BSA control
3c (fluorescence)



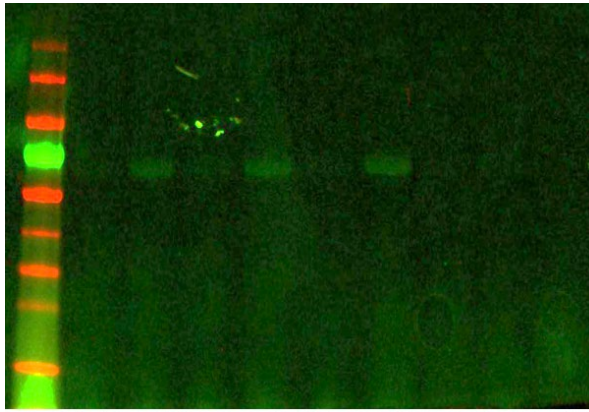
t, min: 0 5.1 0 5.1 0 5.1 BSA control
3c (Coomassie stain)



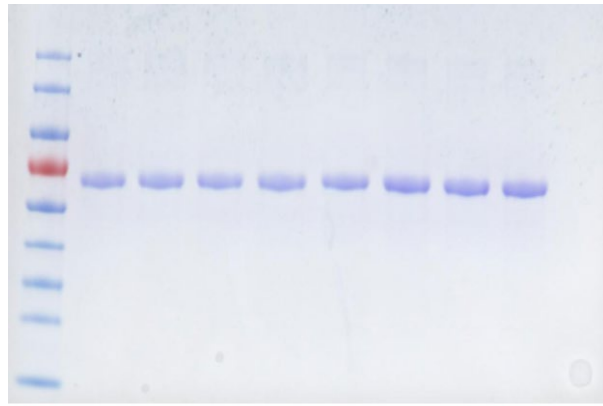
t, min: 0 5.1 0 5.1 0 5.1 BSA control
TMR (fluorescence)



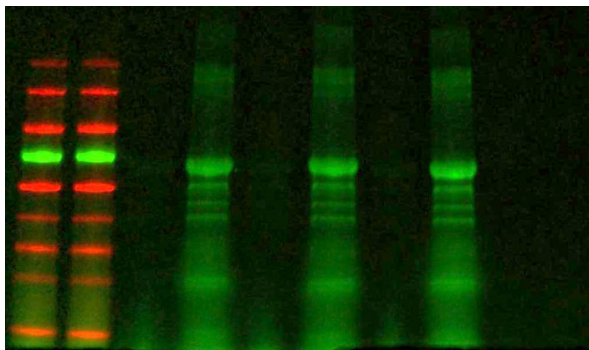
t, min: 0 5.1 0 5.1 0 5.1 BSA control
TMR (Coomassie stain)



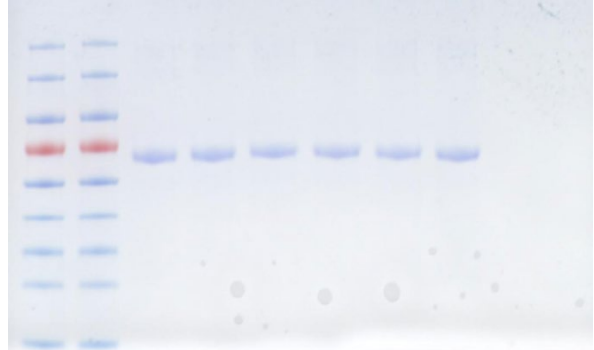
t_i min: 0 5.1 0 5.1 0 5.1 BSA control
Rhodamine 110 (fluorescence)



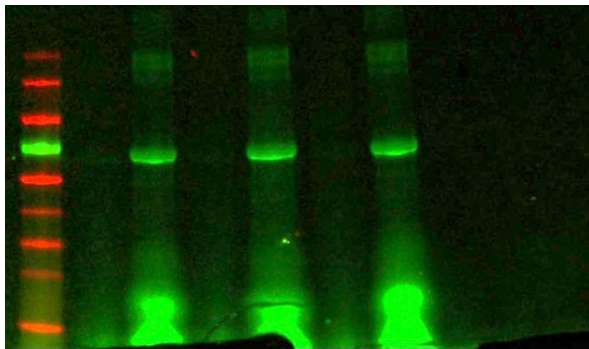
t_i min: 0 5.1 0 5.1 0 5.1 BSA control
Rhodamine 110 (Coomassie stain)



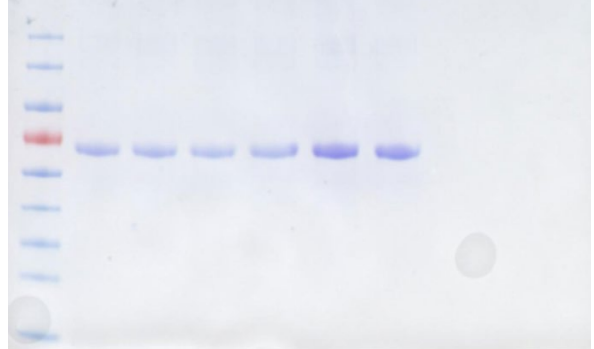
t_i min: 0 5.1 0 5.1 0 5.1
JF₅₄₉ (fluorescence)



t_i min: 0 5.1 0 5.1 0 5.1
JF₅₄₉ (Coomassie stain)



t_i min: 0 5.1 0 5.1 0 5.1
JF₅₂₅ (fluorescence)



t_i min: 0 5.1 0 5.1 0 5.1
JF₅₂₅ (Coomassie stain)

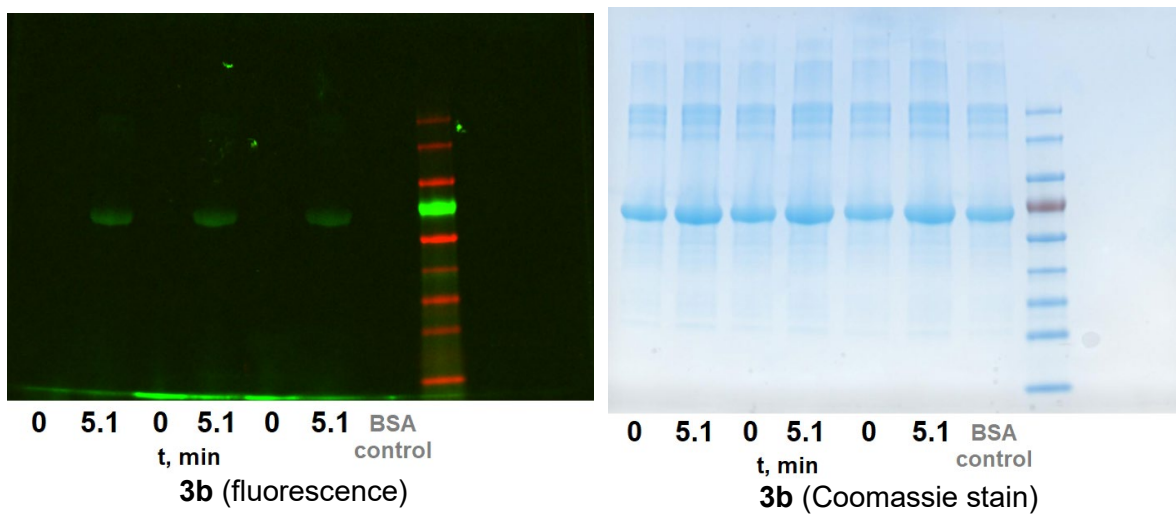


Figure S32. SDS-PAGE gel images (nonspecific BSA labeling experiments). t – sample irradiation time in the flow photoreactor (525 nm).

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