SUPPORTING INFORMATION

Carbofluoresceins and Carborhodamines as Scaffolds for High-Contrast Fluorogenic Probes

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S1: Experimental Information

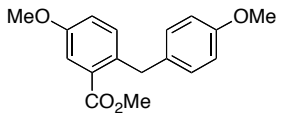
GENERAL EXPERIMENTAL INFORMATION FOR SYNTHESIS

Commercial reagents were obtained from reputable suppliers and used as received (aminophalloidin tosylate was obtained from Enzo Life Sciences, and amino-dPEG$_8$-acid was obtained from Quanta BioDesign). All solvents were purchased in septum-sealed bottles stored under an inert atmosphere; those used in palladium cross-couplings were degassed prior to use by sparging with nitrogen for 20 minutes. All reactions were sealed with septa through which a nitrogen atmosphere was introduced unless otherwise noted. Reactions were conducted in round-bottomed flasks or septum-capped crimp-top vials containing Teflon-coated magnetic stir bars. Heating of reactions was accomplished with a silicon oil bath or an aluminum reaction block on top of a stirring hotplate equipped with an electronic contact thermometer to maintain the indicated temperatures.

Reactions were monitored by thin layer chromatography (TLC) on precoated TLC glass plates (silica gel 60 F_{254}, 250 μm thickness) or by LC/MS (4.6 mm x 150 mm 5 μm C18 column; 5 μL injection; 10–95% or 50–95% CH_3CN/H_2O, linear gradient, with constant 0.1% v/v TFA additive; 20 min run; 1 mL/min flow; ESI; positive ion mode; UV detection at 254 nm). TLC chromatograms were visualized by UV illumination or developed with *p*-anisaldehyde, ceric ammonium molybdate, or $KMnO_4$ stain. Flash chromatography was performed on an automated purification system using pre-packed silica gel columns. High-resolution mass spectrometry was performed by the Mass Spectrometry Center in the Department of Medicinal Chemistry at the University of Washington.

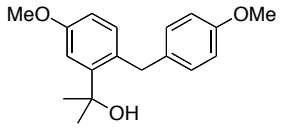
NMR spectra were recorded on a 400 MHz spectrometer. ^{1}H and ^{13}C chemical shifts (δ) were referenced to TMS or residual solvent peaks, and ^{19}F chemical shifts (δ) were referenced to $CFCl_3$. Data for ^{1}H NMR spectra are reported as follows: chemical shift (δ ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublets, m = multiplet), coupling constant (Hz), integration. Data for ^{13}C NMR spectra are reported by chemical shift (δ ppm) with hydrogen multiplicity (C, CH, CH_2, CH_3) information obtained from DEPT spectra.

EXPERIMENTALS AND CHARACTERIZATION DATA FOR ALL COMPOUNDS

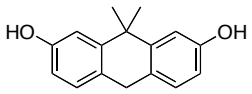


Methyl 5-methoxy-2-(4-methoxybenzyl)benzoate (8): A round bottom flask equipped with a reflux condenser was charged with $Pd(OAc)_2$ (275 mg, 1.22 mmol, 0.05 eq) and SPhos (1.01 g, 2.45 mmol, 0.10 eq). The flask was evacuated and backfilled with nitrogen (3×). Degassed THF (100 mL) was added, followed by methyl 2-bromo-5-methoxybenzoate (**6**, 6.00 g, 24.5 mmol) and 4-methoxybenzylzinc chloride (**7**, 0.5 M in THF, 73.4 mL, 36.7 mmol, 1.5 eq). The reaction was stirred under nitrogen in a 60 °C oil bath for 6 h. It was subsequently cooled to room temperature, quenched with saturated NH_4Cl, diluted with water, and extracted with EtOAc (2×). The combined organic extracts were washed with brine, dried ($MgSO_4$), filtered, and concentrated *in vacuo*. Flash chromatography

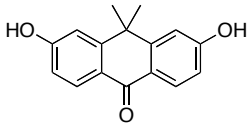
on silica gel (0–20% EtOAc/hexanes, linear gradient) afforded 6.77 g (97%) of **8** as a pale yellow oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.40 (d, *J* = 2.8 Hz, 1H), 7.11 (d, *J* = 8.5 Hz, 1H), 7.07 – 7.01 (m, 2H), 6.97 (dd, *J* = 8.5, 2.8 Hz, 1H), 6.82 – 6.77 (m, 2H), 4.23 (s, 2H), 3.82 (s, 3H), 3.81 (s, 3H), 3.76 (s, 3H); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 168.1 (C), 157.9 (C), 157.8 (C), 134.9 (C), 133.6 (C), 132.7 (CH), 130.8 (C), 129.8 (CH), 118.3 (CH), 115.4 (CH), 113.9 (CH), 55.6 (CH_3), 55.4 (CH_3), 52.1 (CH_3), 38.1 (CH_2); HRMS (ESI) calcd for $C_{17}H_{18}O_4Na$ $[M+Na]^+$ 309.1097, found 309.1099.



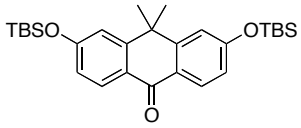
2-(5-Methoxy-2-(4-methoxybenzyl)phenyl)propan-2-ol (9): A solution of ester **8** (6.51 g, 22.7 mmol) in THF (100 mL) was cooled to −78 °C under nitrogen. Methylmagnesium bromide (3 M in Et_2O, 22.7 mL, 68.2 mmol, 3 eq) was added; the reaction was allowed to warm to room temperature and stirred overnight. It was subsequently quenched with saturated NH_4Cl, diluted with water, and extracted with EtOAc (2×). The combined organic extracts were washed with brine, dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The residue was purified by silica gel chromatography (0–25% EtOAc/hexanes, linear gradient) to provide **9** (5.70 g, 88%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.06 – 6.97 (m, 4H), 6.83 – 6.77 (m, 2H), 6.74 (dd, *J* = 8.4, 2.8 Hz, 1H), 4.28 (s, 2H), 3.80 (s, 3H), 3.77 (s, 3H), 1.68 (s, 1H), 1.62 (s, 6H); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 157.8 (C), 157.7 (C), 147.5 (C), 135.0 (C), 134.1 (CH), 130.7 (C), 129.7 (CH), 113.9 (CH), 112.5 (CH), 111.5 (CH), 73.9 (C), 55.34 (CH_3), 55.32 (CH_3), 38.2 (CH_2), 31.8 (CH_3); HRMS (ESI) calcd for $C_{18}H_{22}O_3Na$ $[M+Na]^+$ 309.1461, found 309.1463.



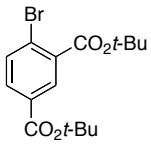
9,9-Dimethyl-9,10-dihydroanthracene-2,7-diol (10): Alcohol **9** (2.13 g, 7.44 mmol) was taken up in CH_2Cl_2 (50 mL) under nitrogen and cooled to 0 °C. Boron tribromide (1 M in CH_2Cl_2, 26.8 mL, 26.8 mmol, 3.6 eq) was added dropwise. The reaction was warmed to room temperature and stirred for 2 h. It was then carefully quenched with water (~50 mL) and vigorously stirred for 30 min. The mixture was neutralized (to pH ~ 7) with saturated $NaHCO_3$ and extracted with CH_2Cl_2 (2×) and EtOAc (2×). The combined organics were dried ($MgSO_4$), filtered, concentrated *in vacuo*, and deposited onto silica gel. Flash chromatography on silica gel (10–50% EtOAc/hexanes, linear gradient; dry load with silica gel) yielded 1.62 g (91%) of **10** as an air-sensitive, off-white solid. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.08 (s, 2H), 7.04 (d, *J* = 8.1 Hz, 2H), 6.91 (d, *J* = 2.4 Hz, 2H), 6.57 (dd, *J* = 8.1, 2.4 Hz, 2H), 3.79 (s, 2H), 1.44 (s, 6H); ^{13}C NMR (DMSO-d_6, 101 MHz) δ 155.7 (C), 145.7 (C), 128.3 (CH), 126.3 (C), 112.5 (CH), 111.1 (CH), 38.8 (C), 33.3 (CH_2), 28.4 (CH_3); HRMS (ESI) calcd for $C_{16}H_{16}O_2Na$ $[M+Na]^+$ 263.1043, found 263.1045.



3,6-Dihydroxy-10,10-dimethylanthracen-9(10*H*)-one (11): Phenol **10** (3.66 g, 15.2 mmol) was taken up in a mixture of CH_2Cl_2 (100 mL) and dioxane (50 mL), and water (12.5 mL) was added. The mixture was cooled to 0 °C, and DDQ (10.37 g, 45.7 mmol, 3 eq) was added. The reaction was warmed to room temperature and stirred overnight. The crude reaction mixture was deposited onto Celite and concentrated to dryness. Flash chromatography (10–100% EtOAc/hexanes, linear gradient; dry load with Celite) afforded **11** (3.34 g, 86%) as a yellow-orange foam. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 10.31 (s, 2H), 8.03 (d, *J* = 8.6 Hz, 2H), 7.09 (d, *J* = 2.3 Hz, 2H), 6.86 (dd, *J* = 8.6, 2.3 Hz, 2H), 1.60 (s, 6H); ^{13}C NMR (DMSO-d_6, 101 MHz) δ 180.2 (C), 162.0 (C), 152.8 (C), 129.1 (CH), 121.8 (C), 114.9 (CH), 112.6 (CH), 37.4 (C), 32.9 (CH_3); HRMS (ESI) calcd for $C_{16}H_{15}O_3$ $[M+H]^+$ 255.1016, found 255.1017.



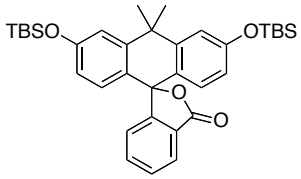
3,6-Bis((*tert*-butyldimethylsilyl)oxy)-10,10-dimethylanthracen-9(10*H*)-one (12): To a solution of **11** (2.99 g, 11.8 mmol) in DMF (60 mL) were added imidazole (2.40 g, 35.3 mmol, 3 eq) and TBSCl (5.32 g, 35.3 mmol, 3 eq). The reaction was stirred at room temperature for 3 h. It was subsequently diluted with water and extracted with EtOAc (2×). The combined organic extracts were washed with water and brine, dried ($MgSO_4$), filtered, and concentrated *in vacuo*. Silica gel chromatography (0–10% EtOAc/hexanes, linear gradient) afforded 5.47 g (96%) of **12** as a colorless solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.26 (d, *J* = 8.6 Hz, 2H), 7.04 (d, *J* = 2.3 Hz, 2H), 6.88 (dd, *J* = 8.6, 2.3 Hz, 2H), 1.67 (s, 6H), 1.02 (s, 18H), 0.27 (s, 12H); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 182.0 (C), 160.3 (C), 152.8 (C), 129.9 (CH), 124.5 (C), 119.3 (CH), 117.5 (CH), 37.9 (C), 33.2 (CH_3), 25.8 (CH_3), 18.5 (C), -4.11 (CH_3); HRMS (ESI) calcd for $C_{28}H_{43}O_3Si_2$ $[M+H]^+$ 483.2745, found 483.2743.



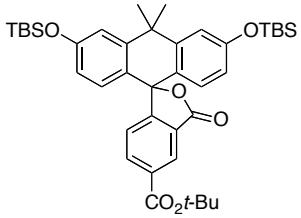
Di-*tert*-butyl 4-bromoisophthalate (14): A suspension of 4-bromoisophthalic acid (2.05 g, 8.37 mmol) in toluene (20 mL) was heated to 80 °C, and *N*,*N*-dimethylformamide di-*tert*-butyl acetal (20.1 mL, 83.7 mmol, 10 eq) was added dropwise over 15 min. The reaction was stirred at 80 °C for 20 min. After cooling the mixture to room temperature, it was diluted with saturated $NaHCO_3$ and extracted with EtOAc (2×). The combined organic extracts were washed with brine, dried ($MgSO_4$), filtered, evaporated. Flash chromatography (0–10% Et_2O/hexanes, linear gradient) provided **14** as a colorless gum (2.26 g, 76%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.25 (d, *J* = 2.2 Hz, 1H), 7.86 (dd, *J* = 8.3, 2.2 Hz, 1H), 7.67 (d, *J* = 8.3 Hz, 1H), 1.62 (s, 9H), 1.59 (s, 9H); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 165.2

(C), 164.5 (C), 134.6 (C), 134.2 (CH), 132.3 (CH), 131.7 (CH), 131.3 (C), 125.8 (C), 83.2 (C), 82.1 (C), 28.3 (CH_3); HRMS (ESI) calcd for $C_{16}H_{21}BrO_4Na$ $[M+Na]^+$ 379.0515, found 379.0514.

General procedure for Br/Mg exchange and addition to ketones: The following procedure for **15** is representative. A vial was charged with *tert*-butyl 2-bromobenzoate (160 mg, 0.621 mmol, 1.5 eq), sealed, and flushed with nitrogen. After dissolving the bromide in THF (1 mL) and cooling the reaction to −15 °C, *i*PrMgCl·LiCl (1.3 M in THF, 478 μL, 0.621 mmol, 1.5 eq) was added. The reaction was warmed to −5 °C and stirred for 6 h. Ketone **12** (200 mg, 0.414 mmol) in THF (1 mL) was then added dropwise. The reaction mixture was warmed to room temperature and stirred for 1 h. It was subsequently quenched with saturated NH_4Cl, diluted with water, and extracted with EtOAc (2×). The combined organics were washed with brine, dried ($MgSO_4$), filtered, and evaporated. Silica gel chromatography (0–20% Et_2O/hexanes, linear gradient) provided 137 mg (56%) of **15** as a colorless, gummy solid.



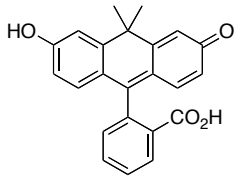
3,6-Bis((*tert*-butyldimethylsilyl)oxy)-10,10-dimethyl-3'*H*,10*H*-spiro[anthracene-9,1'-isobenzofuran]-3'-one (15): (56%, colorless solid) ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.03 – 7.97 (m, 1H), 7.61 (td, *J* = 7.4, 1.4 Hz, 1H), 7.56 (td, *J* = 7.4, 1.3 Hz, 1H), 7.08 – 7.02 (m, 3H), 6.63 – 6.57 (m, 4H), 1.80 (s, 3H), 1.71 (s, 3H), 0.98 (s, 18H), 0.20 (s, 12H); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 170.7 (C), 156.4 (C), 155.4 (C), 147.1 (C), 134.8 (CH), 129.4 (CH), 129.2 (CH), 126.8 (C), 125.2 (CH), 124.7 (C), 124.0 (CH), 119.0 (CH), 117.6 (CH), 86.8 (C), 38.3 (C), 35.3 (CH_3), 32.7 (CH_3), 25.9 (CH_3), 18.4 (C), -4.18 (CH_3), -4.20 (CH_3); HRMS (ESI) calcd for $C_{35}H_{47}O_4Si_2$ $[M+H]^+$ 587.3007, found 587.2997.



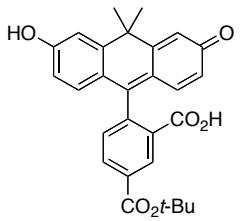
***tert*-Butyl 3,6-bis((*tert*-butyldimethylsilyl)oxy)-10,10-dimethyl-3'-oxo-3'*H*,10*H*-spiro[anthracene-9,1'-isobenzofuran]-5'-carboxylate (16):** (47%, colorless foam) ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.60 (dd, *J* = 1.5, 0.7 Hz, 1H), 8.24 (dd, *J* = 8.0, 1.5 Hz, 1H), 7.11 – 7.04 (m, 3H), 6.62 – 6.55 (m, 4H), 1.80 (s, 3H), 1.71 (s, 3H), 1.63 (s, 9H), 0.98 (s, 18H), 0.21 (s, 12H); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 169.9 (C), 164.4 (C), 158.9 (C), 156.6 (C), 147.1 (C), 135.8 (CH), 133.7 (C), 129.3 (CH), 127.0 (C), 126.7 (CH), 123.99 (C), 123.96 (CH), 119.2 (CH), 117.9 (CH), 86.8 (C),

82.3 (C), 38.3 (C), 35.2 (CH_3), 32.8 (CH_3), 28.3 (CH_3), 25.8 (CH_3), 18.4 (C), -4.20 (CH_3); HRMS (ESI) calcd for $C_{40}H_{55}O_6Si_2$ $[M+H]^+$ 687.3532, found 687.3524.

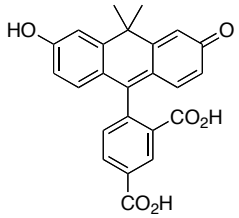
General procedure for deprotection of silyl ethers: The following procedure for **4** is representative. To a solution of silyl ether **15** (314 mg, 0.535 mmol) in THF (8 mL) was added TBAF (1.0 M in THF, 2.14 mL, 2.14 mmol, 4 eq). The reaction was stirred at room temperature for 30 min. It was subsequently acidified with 1 N HCl, diluted with water, and extracted with EtOAc (2×). The organic extracts were dried ($MgSO_4$), filtered, evaporated, and deposited onto silica gel. Flash chromatography (20–100% EtOAc/hexanes, linear gradient, with constant 1% v/v AcOH additive; dry load with silica gel) yielded **4** (190 mg, 99%) as a pale orange solid.



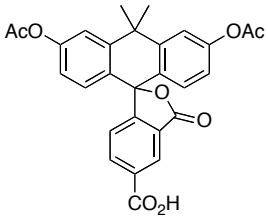
Carbofluorescein (4): (99%, orange solid) ^{1}H NMR (MeOD, 400 MHz) δ 7.98 (dt, *J* = 7.5, 1.0 Hz, 1H), 7.70 (td, *J* = 7.5, 1.2 Hz, 1H), 7.64 (td, *J* = 7.5, 1.0 Hz, 1H), 7.10 (d, *J* = 2.4 Hz, 2H), 7.08 – 7.02 (m, 1H), 6.58 (dd, *J* = 8.6, 2.5 Hz, 2H), 6.52 (d, *J* = 8.6 Hz, 2H), 1.81 (s, 3H), 1.70 (s, 3H); ^{13}C NMR (MeOD, 101 MHz) δ 172.8 (C), 159.5 (C), 156.9 (C), 148.6 (C), 136.3 (CH), 130.5 (CH), 130.2 (CH), 127.7 (C), 125.7 (CH), 125.0 (CH), 123.7 (C), 115.7 (CH), 113.7 (CH), 89.3 (C), 39.2 (C), 35.4 (CH_3), 33.1 (CH_3); analytical HPLC: 98.8% purity (4.6 mm x 150 mm 5 μm C18 column; 5 μL injection; 10–95% CH_3CN/H_2O, linear gradient, with constant 0.1% v/v TFA additive; 20 min run; 1 mL/min flow; ESI; positive ion mode; UV detection at 254 nm); HRMS (ESI) calcd for $C_{23}H_{19}O_4$ $[M+H]^+$ 359.1278, found 359.1277.



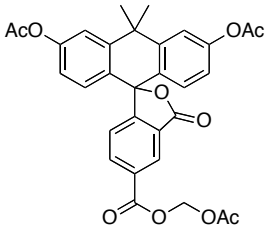
5-(*tert*-Butoxycarbonyl)carbofluorescein (17): (97%, orange solid) ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.72 (s, 2H), 8.40 – 8.30 (m, 1H), 8.20 (dd, *J* = 8.1, 1.4 Hz, 1H), 7.21 (d, *J* = 8.0 Hz, 1H), 7.09 (d, *J* = 2.3 Hz, 2H), 6.60 (dd, *J* = 8.6, 2.4 Hz, 2H), 6.50 (d, *J* = 8.6 Hz, 2H), 1.74 (s, 3H), 1.63 (s, 3H), 1.59 (s, 9H); ^{13}C NMR (DMSO-d_6, 101 MHz) δ 168.8 (C), 163.6 (C), 158.6 (C), 158.1 (C), 146.6 (C), 135.7 (CH), 132.7 (C), 129.0 (CH), 126.2 (C), 125.3 (CH), 124.3 (CH), 121.0 (C), 114.9 (CH), 112.6 (CH), 86.6 (C), 81.8 (C), 37.6 (C), 34.5 (CH_3), 32.8 (CH_3), 27.7 (CH_3); HRMS (ESI) calcd for $C_{28}H_{27}O_6$ $[M+H]^+$ 459.1802, found 459.1808.



5-Carboxycarbofluorescein (18): Ester **17** (150 mg, 0.327 mmol) was taken up in CH_2Cl_2 (5 mL), and trifluoroacetic acid (1 mL) was added. The reaction was stirred at room temperature for 6 h. Toluene (5 mL) was added; the reaction mixture was concentrated to dryness and then azeotroped with MeOH three times to provide **18** as a red-orange solid (130 mg, 99%). Analytical HPLC and NMR indicated that the material was >95% pure and did not require further purification. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 13.48 (s, 1H), 9.72 (s, 2H), 8.43 – 8.35 (m, 1H), 8.23 (dd, *J* = 8.0, 1.5 Hz, 1H), 7.20 (d, *J* = 8.0 Hz, 1H), 7.09 (d, *J* = 2.4 Hz, 2H), 6.62 (dd, *J* = 8.6, 2.4 Hz, 2H), 6.51 (d, *J* = 8.6 Hz, 2H), 1.74 (s, 3H), 1.64 (s, 3H); ^{13}C NMR (DMSO-d_6, 101 MHz) δ 168.9 (C), 166.0 (C), 158.7 (C), 158.1 (C), 146.6 (C), 136.0 (CH), 132.3 (C), 129.0 (CH), 126.2 (C), 125.6 (CH), 124.2 (CH), 121.0 (C), 115.0 (CH), 112.6 (CH), 86.5 (C), 37.6 (C), 34.4 (CH_3), 33.1 (CH_3); analytical HPLC: >99% purity (4.6 mm x 150 mm 5 μm C18 column; 5 μL injection; 10–95% CH_3CN/H_2O, linear gradient, with constant 0.1% v/v TFA additive; 20 min run; 1 mL/min flow; ESI; positive ion mode; UV detection at 254 nm); HRMS (ESI) calcd for $C_{24}H_{19}O_6$ $[M+H]^+$ 403.1176, found 403.1168.

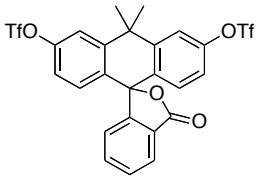


3,6-Diacetoxy-10,10-dimethyl-3'-oxo-3'*H*,10*H*-spiro[anthracene-9,1'-isobenzofuran]-5'-carboxylic acid (19): Carbofluorescein **18** (90 mg, 0.224 mmol), pyridine (90 μL, 1.12 mmol, 5 eq), and acetic anhydride (2.0 mL) were combined in a vial and stirred at 80 °C for 1 h. The reaction was cooled to room temperature, diluted with 1 N HCl, and extracted with EtOAc (2×). The organic layers were washed with brine, dried ($MgSO_4$), filtered, evaporated, and deposited onto silica gel. The residue was purified by flash chromatography (10–100% EtOAc/hexanes, linear gradient, with constant 1% v/v AcOH; dry load with silica gel) to afford 87 mg (80%) of **19** as a colorless solid. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 13.55 (s, 1H), 8.44 (dd, *J* = 1.5, 0.7 Hz, 1H), 8.25 (dd, *J* = 8.0, 1.5 Hz, 1H), 7.61 (d, *J* = 2.3 Hz, 2H), 7.30 (dd, *J* = 8.0, 0.6 Hz, 1H), 7.01 (dd, *J* = 8.7, 2.4 Hz, 2H), 6.82 (d, *J* = 8.7 Hz, 2H), 2.28 (s, 6H), 1.81 (s, 3H), 1.71 (s, 3H); ^{13}C NMR (DMSO-d_6, 101 MHz) δ 169.0 (C), 168.6 (C), 165.9 (C), 158.0 (C), 151.3 (C), 146.1 (C), 136.4 (CH), 132.8 (C), 128.9 (CH), 127.4 (C), 126.0 (CH), 125.6 (C), 124.2 (CH), 121.3 (CH), 120.2 (CH), 84.7 (C), 38.1 (C), 33.9 (CH_3), 33.2 (CH_3), 20.9 (CH_3); HRMS (ESI) calcd for $C_{28}H_{23}O_8$ $[M+H]^+$ 487.1387, found 487.1394.

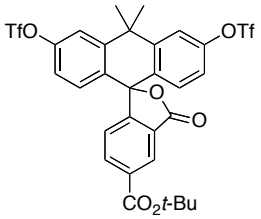


5'-((Acetoxymethoxy)carbonyl)-10,10-dimethyl-3'-oxo-3'*H*,10*H*-spiro[anthracene-9,1'-isobenzofuran]-3,6-diyl diacetate (20): Acid **19** (50 mg, 0.103 mmol) and DIEA (54 μL, 0.309 mmol, 3 eq) were combined in DMF (1 mL), and bromomethyl acetate (25 μL, 0.257 mmol, 2.5 eq) was added. The reaction was stirred at room temperature for 1 h. The mixture was concentrated to dryness and directly purified by silica gel chromatography (10–75% EtOAc/hexanes, linear gradient) to provide **20** as a colorless, gummy solid (48 mg, 84%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.73 (dd, *J* = 1.4, 0.6 Hz, 1H), 8.32 (dd, *J* = 8.1, 1.5 Hz, 1H), 7.38 (d, *J* = 2.3 Hz, 2H), 7.14 (dd, *J* = 8.1, 0.5 Hz, 1H), 6.91 (dd, *J* = 8.7, 2.4 Hz, 2H), 6.74 (d, *J* = 8.7 Hz, 2H), 6.04 (s, 2H), 2.31 (s, 6H), 2.17 (s, 3H), 1.85 (s, 3H), 1.75 (s, 3H); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 169.6 (C), 169.2 (C), 168.9 (C), 163.8 (C), 159.2 (C), 151.7 (C), 146.7 (C), 136.4 (CH), 131.1 (C), 129.2 (CH), 128.0 (C), 127.6 (CH), 126.9 (C), 124.5 (CH), 120.8 (CH), 119.7 (CH), 85.6 (C), 80.2 (CH_2), 38.7 (C), 35.1 (CH_3), 32.9 (CH_3), 21.3 (CH_3), 20.8 (CH_3); HRMS (ESI) calcd for $C_{31}H_{27}O_{10}$ $[M+H]^+$ 559.1599, found 559.1624.

General procedure for formation of ditriflates: The following procedure for **21** is representative. Carbofluorescein **4** (190 mg, 0.530 mmol) was taken up in CH_2Cl_2 (5 mL) and cooled to 0 °C. Pyridine (343 μL, 4.24 mmol, 8.0 eq) and trifluoromethanesulfonic anhydride (357 μL, 2.12 mmol, 4.0 eq) were added, and the ice bath was removed. The reaction was stirred at room temperature for 1 h. It was subsequently diluted with water and extracted with CH_2Cl_2 (2×). The combined organic extracts were washed with brine, dried ($MgSO_4$), filtered, and concentrated *in vacuo*. Flash chromatography on silica gel (0–25% EtOAc/hexanes, linear gradient) afforded 250 mg (76%) of **21** as a colorless foam.

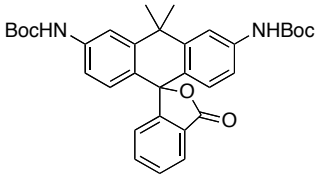


10,10-Dimethyl-3'-oxo-3'*H*,10*H*-spiro[anthracene-9,1'-isobenzofuran]-3,6-diyl bis(trifluoromethanesulfonate) (21): (76%, colorless foam) ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.11 – 8.04 (m, 1H), 7.73 – 7.63 (m, 2H), 7.55 (d, *J* = 2.5 Hz, 2H), 7.09 (dd, *J* = 8.8, 2.5 Hz, 2H), 7.07 – 7.02 (m, 1H), 6.90 (d, *J* = 8.8 Hz, 2H), 1.90 (s, 3H), 1.80 (s, 3H); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 169.6 (C), 153.9 (C), 150.3 (C), 147.2 (C), 135.7 (CH), 131.8 (C), 130.4 (CH), 130.3 (CH), 126.0 (CH), 125.9 (C), 123.8 (CH), 120.4 (CH), 119.7 (CH), 118.9 (CF_3, $^{1}J_{CF}$ = 320.9 Hz), 84.1 (C), 39.0 (C), 35.0 (CH_3), 32.9 (CH_3); ^{19}F NMR ($CDCl_3$, 376 MHz) δ -73.26 (s); HRMS (ESI) calcd for $C_{25}H_{17}F_6O_8S_2$ $[M+H]^+$ 623.0264, found 623.0264.

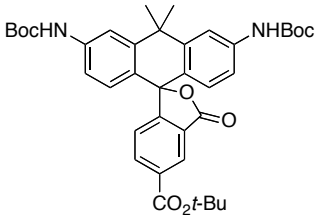


***tert*-Butyl 10,10-dimethyl-3'-oxo-3,6-bis(((trifluoromethyl)sulfonyl)oxy)-3'*H*,10*H*-spiro[anthracene-9,1'-isobenzofuran]-5'-carboxylate (22):** (83%, colorless solid) ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.66 (dd, *J* = 1.4, 0.7 Hz, 1H), 8.31 (dd, *J* = 8.0, 1.5 Hz, 1H), 7.56 (d, *J* = 2.5 Hz, 2H), 7.09 (dd, *J* = 8.8, 2.5 Hz, 3H), 6.87 (d, *J* = 8.8 Hz, 2H), 1.90 (s, 3H), 1.80 (s, 3H), 1.64 (s, 9H); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 168.8 (C), 163.8 (C), 157.1 (C), 150.4 (C), 147.2 (C), 136.6 (CH), 134.8 (C), 131.1 (C), 130.3 (CH), 127.3 (CH), 126.2 (C), 123.8 (CH), 120.5 (CH), 119.8 (CH), 118.9 (CF_3, $^{1}J_{CF}$ = 320.8 Hz), 84.1 (C), 82.8 (C), 39.1 (C), 35.0 (CH_3), 32.9 (CH_3), 28.3 (CH_3); ^{19}F NMR ($CDCl_3$, 376 MHz) δ -73.21 (s); HRMS (ESI) calcd for $C_{30}H_{25}F_6O_{10}S_2$ $[M+H]^+$ 723.0788, found 723.0789.

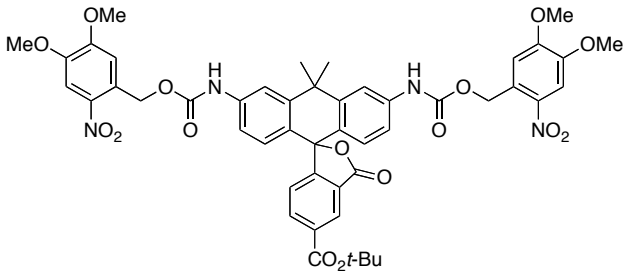
General procedure for C–N cross-coupling of ditriflates: The following procedure for **23** is representative. A vial was charged with **21** (100 mg, 0.161 mmol), *tert*-butyl carbamate (45 mg, 0.386 mmol, 2.4 eq), Pd_2dba_3 (15 mg, 0.0161 mmol, 0.1 eq), Xantphos (28 mg, 0.0482 mmol, 0.3 eq), and Cs_2CO_3 (147 mg, 0.450 mmol, 2.8 eq). The vial was sealed and evacuated/backfilled with nitrogen (3×). Dioxane (1 mL) was added, and the reaction was flushed again with nitrogen (3×). The reaction was stirred at 100 °C for 18 h. It was then cooled to room temperature, filtered through Celite with CH_2Cl_2, and evaporated. The residue was purified by silica gel chromatography (0–50% EtOAc/hexanes, linear gradient) to afford **23** (79 mg, 88%) as a colorless solid.



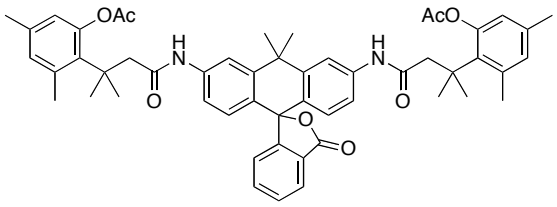
Di-*tert*-butyl (10,10-dimethyl-3'-oxo-3'*H*,10*H*-spiro[anthracene-9,1'-isobenzofuran]-3,6-diyl)dicarbamate (23): (88%, colorless solid) ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.05 – 7.95 (m, 1H), 7.79 (d, *J* = 1.6 Hz, 2H), 7.61 – 7.51 (m, 2H), 7.04 – 6.93 (m, 3H), 6.68 (d, *J* = 8.6 Hz, 2H), 6.53 (s, 2H), 1.87 (s, 3H), 1.76 (s, 3H), 1.52 (s, 18H); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 170.8 (C), 155.5 (C), 152.8 (C), 146.4 (C), 139.3 (C), 134.9 (CH), 129.3 (CH), 128.7 (CH), 126.3 (C), 125.9 (C), 125.3 (CH), 123.8 (CH), 117.5 (CH), 116.1 (CH), 86.3 (C), 80.9 (C), 38.6 (C), 35.0 (CH_3), 33.3 (CH_3), 28.5 (CH_3); HRMS (ESI) calcd for $C_{33}H_{37}N_2O_6$ $[M+H]^+$ 557.2646, found 557.2640.



***tert*-Butyl 3,6-bis((tert-butoxycarbonyl)amino)-10,10-dimethyl-3'-oxo-3'*H*,10*H*-spiro[anthracene-9,1'-isobenzofuran]-5'-carboxylate (24):** (80%, colorless solid) ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.60 (dd, *J* = 1.4, 0.7 Hz, 1H), 8.21 (dd, *J* = 8.0, 1.5 Hz, 1H), 7.87 – 7.77 (m, 2H), 7.02 (dd, *J* = 8.0, 0.6 Hz, 1H), 6.98 (dd, *J* = 8.6, 2.2 Hz, 2H), 6.64 (d, *J* = 8.6 Hz, 2H), 6.53 (s, 2H), 1.87 (s, 3H), 1.77 (s, 3H), 1.63 (s, 9H), 1.52 (s, 18H); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 169.9 (C), 164.3 (C), 158.9 (C), 152.7 (C), 146.4 (C), 139.6 (C), 135.9 (CH), 133.8 (C), 128.6 (CH), 126.8 (CH), 126.6 (C), 125.2 (C), 123.8 (CH), 117.4 (CH), 116.1 (CH), 86.4 (C), 82.4 (C), 81.0 (C), 38.6 (C), 35.1 (CH_3), 33.2 (CH_3), 28.5 (CH_3), 28.3 (CH_3); HRMS (ESI) calcd for $C_{38}H_{45}N_2O_8$ $[M+H]^+$ 657.3170, found 657.3159.

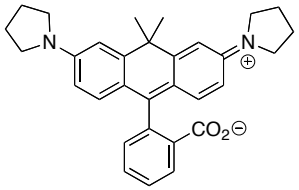


***tert*-Butyl 3,6-bis((((4,5-dimethoxy-2-nitrobenzyl)oxy)carbonyl)amino)-10,10-dimethyl-3'-oxo-3'*H*,10*H*-spiro-[anthracene-9,1'-isobenzofuran]-5'-carboxylate (25):** The preparation of this compound followed the general procedure described above except that the reaction was stirred at 80 °C for 3 h with XPhos (0.3 eq) as ligand (82%, off-white solid). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.61 (dd, *J* = 1.4, 0.7 Hz, 1H), 8.23 (dd, *J* = 8.1, 1.5 Hz, 1H), 7.86 (s, 2H), 7.72 (s, 2H), 7.09 – 7.01 (m, 5H), 6.91 (s, 2H), 6.67 (d, *J* = 8.6 Hz, 2H), 5.61 (s, 4H), 3.97 (s, 6H), 3.96 (s, 6H), 1.85 (s, 3H), 1.74 (s, 3H), 1.63 (s, 9H); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 170.1 (C), 164.2 (C), 158.5 (C), 153.7 (C), 153.0 (C), 148.5 (C), 146.5 (C), 140.1 (C), 138.9 (C), 136.2 (CH), 134.0 (C), 128.7 (CH), 127.2 (C), 126.8 (CH), 126.5 (C), 125.7 (C), 123.9 (CH), 117.7 (CH), 116.5 (CH), 110.8 (CH), 108.4 (CH), 86.4 (C), 82.5 (C), 64.2 (CH_2), 56.7 (CH_3), 56.6 (CH_3), 38.6 (C), 34.9 (CH_3), 33.1 (CH_3), 28.3 (CH_3); HRMS (ESI) calcd for $C_{48}H_{47}N_4O_{16}$ $[M+H]^+$ 935.2982, found 935.2965.

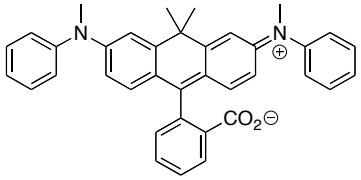


(((10,10-Dimethyl-3'-oxo-3'*H*,10*H*-spiro[anthracene-9,1'-isobenzofuran]-3,6-diyl)bis(azanediyl))bis(2-methyl-4-oxobutane-4,2-diyl))bis(3,5-dimethyl-2,1-phenylene) diacetate (26): The preparation of this compound

followed the general procedure described above except that the reaction was stirred at 80 °C for 6 h (25%, colorless solid). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.00 – 7.93 (m, 1H), 7.60 – 7.49 (m, 2H), 7.27 (s, 2H), 7.42 (d, J = 2.1 Hz, 2H), 6.97 – 6.89 (m, 1H), 6.85 (dd, J = 8.6, 2.1 Hz, 2H), 6.80 (d, J = 1.8 Hz, 2H), 6.66 (d, J = 1.7 Hz, 2H), 6.55 (d, J = 8.6 Hz, 2H), 2.56 (AB quartet, ν_A = 1030.1, ν_B = 1016.3, J_{AB} = 13.1 Hz, 4H), 2.40 (s, 6H), 2.38 (s, 6H), 2.25 (s, 6H), 1.69 (d, J = 1.7 Hz, 9H), 1.68 (s, 6H), 1.61 (s, 3H); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 172.1 (C), 170.6 (C), 169.9 (C), 155.2 (C), 150.3 (C), 146.0 (C), 139.4 (C), 139.0 (C), 137.3 (C), 134.8 (CH), 133.3 (CH), 133.2 (C), 129.3 (CH), 128.4 (CH), 126.6 (C), 126.4 (C), 125.2 (CH), 123.9 (CH), 123.7 (CH), 118.5 (CH), 117.2 (CH), 86.2 (C), 51.3 (CH_2), 40.6 (C), 38.3 (C), 34.9 (CH_3), 32.8 (CH_3), 32.4 (CH_3), 32.3 (CH_3), 25.7 (CH_3), 22.1 (CH_3), 20.3 (CH_3); HRMS (ESI) calcd for $C_{53}H_{57}N_2O_8$ $[M+H]^+$ 849.4109, found 849.4095.

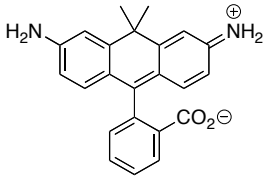


Pyrrolidinocarborhodamine (27): The preparation of this compound followed the general procedure described above except that the reaction was stirred at 100 °C for 4 h with XPhos (0.3 eq) as ligand (94%, light blue solid). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.01 – 7.95 (m, 1H), 7.56 (td, J = 7.4, 1.5 Hz, 1H), 7.52 (td, J = 7.4, 1.3 Hz, 1H), 7.11 – 7.03 (m, 1H), 6.72 (d, J = 2.5 Hz, 2H), 6.58 (d, J = 8.7 Hz, 2H), 6.35 (dd, J = 8.7, 2.5 Hz, 2H), 3.39 – 3.25 (m, 8H), 2.07 – 1.95 (m, 8H), 1.88 (s, 3H), 1.77 (s, 3H); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 171.1 (C), 155.7 (C), 148.2 (C), 147.2 (C), 134.4 (CH), 129.2 (CH), 128.7 (CH), 127.6 (C), 124.9 (CH), 124.1 (CH), 118.9 (C), 111.0 (CH), 108.4 (CH), 89.2 (C), 47.7 (CH_2), 38.6 (C), 36.0 (CH_3), 32.5 (CH_3), 25.6 (CH_2); analytical HPLC: 95.6% purity (4.6 mm x 150 mm 5 μm C18 column; 5 μL injection; 30–95% CH_3CN/H_2O, linear gradient, with constant 0.1% v/v TFA additive; 20 min run; 1 mL/min flow; ESI; positive ion mode; UV detection at 254 nm); HRMS (ESI) calcd for $C_{31}H_{33}N_2O_2$ $[M+H]^+$ 465.2537, found 465.2540.

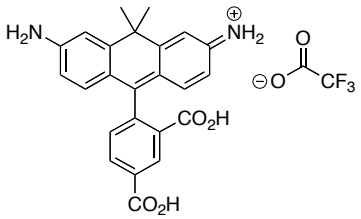


***N*,*N*'-Dimethyl-*N*,*N*'-diphenylcarborhodamine (28):** The preparation of this compound followed the general procedure described above except that the reaction was stirred at 100 °C for 4 h with XPhos (0.3 eq) as ligand (93%, off-white foam). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.01 – 7.97 (m, 1H), 7.61 (td, J = 7.4, 1.3 Hz, 1H), 7.55 (td, J = 7.4, 1.1 Hz, 1H), 7.34 – 7.27 (m, 4H), 7.17 (d, J = 2.4 Hz, 2H), 7.14 – 7.07 (m, 5H), 7.06 – 7.00 (m, 2H), 6.70 (dd, J = 8.7, 2.5 Hz, 2H), 6.60 (d, J = 8.7 Hz, 2H), 3.34 (s, 6H), 1.72 (s, 3H), 1.67 (s, 3H); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 170.7 (C), 155.3 (C), 149.5 (C), 148.5 (C), 146.7 (C), 134.7 (CH), 129.5 (CH), 129.1 (CH), 128.9 (CH), 127.1 (C), 125.1 (CH), 124.0 (CH), 123.3 (C), 122.8 (CH), 122.4 (CH), 117.5 (CH), 115.7 (CH), 87.3 (C), 40.3 (CH_3), 38.6

(C), 35.3 (CH_3), 32.6 (CH_3); analytical HPLC: >99% purity (4.6 mm x 150 mm 5 μm C18 column; 5 μL injection; 50–95% CH_3CN/H_2O, linear gradient, with constant 0.1% v/v TFA additive; 20 min run; 1 mL/min flow; ESI; positive ion mode; UV detection at 254 nm); HRMS (ESI) calcd for $C_{37}H_{33}N_2O_2$ $[M+H]^+$ 537.2537, found 537.2528.

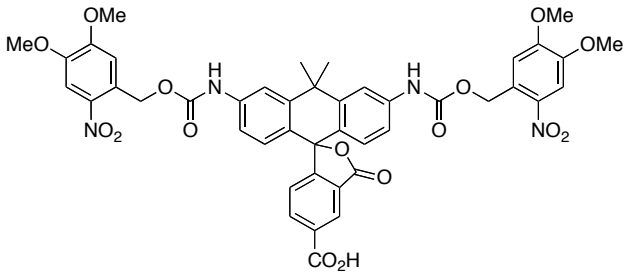


Carborhodamine 110 (5): Dicarbamate **23** (69 mg, 0.124 mmol) was taken up in CH_2Cl_2 (2.5 mL), and trifluoroacetic acid (0.5 mL) was added. The reaction was stirred at room temperature for 6 h. The reaction mixture was concentrated to dryness and then azeotroped with MeOH three times. The residue was taken up in saturated $NaHCO_3$ and extracted with EtOAc (2×). The combined organic extracts were washed with brine, dried ($MgSO_4$), filtered, deposited onto silica gel, and concentrated to dryness. Flash chromatography (0–10% MeOH (2 M NH_3)/CH_2Cl_2, linear gradient; dry load with silica gel) afforded 38 mg (86%) of **5** as a purple solid. ^{1}H NMR (MeOD, 400 MHz, 330 K, TFA salt) δ 8.24 (dd, *J* = 7.8, 1.1 Hz, 1H), 7.76 (td, *J* = 7.5, 1.4 Hz, 1H), 7.70 (td, *J* = 7.6, 1.3 Hz, 1H), 7.29 (dd, *J* = 7.5, 1.0 Hz, 1H), 7.18 (d, *J* = 2.1 Hz, 2H), 6.95 (d, *J* = 9.0 Hz, 2H), 6.61 (dd, *J* = 9.0, 2.1 Hz, 2H), 1.78 (s, 3H), 1.69 (s, 3H); ^{13}C NMR (MeOD, 101 MHz, 330 K, TFA salt) δ 168.4 (C), 159.5 (C), 159.0 (C), 139.7 (C), 138.8 (CH), 133.4 (CH), 132.3 (C), 131.9 (CH), 131.2 (CH), 130.6 (CH), 122.4 (C), 116.0 (CH), 113.9 (CH), 42.4 (C), 34.9 (CH_3), 31.9 (CH_3); ^{19}F NMR (MeOD, 376 MHz, 330 K, TFA salt) δ -76.01 (s); analytical HPLC: 98.3% purity (4.6 mm x 150 mm 5 μm C18 column; 5 μL injection; 10–95% CH_3CN/H_2O, linear gradient, with constant 0.1% v/v TFA additive; 20 min run; 1 mL/min flow; ESI; positive ion mode; UV detection at 254 nm); HRMS (ESI) calcd for $C_{23}H_{21}N_2O_2$ $[M+H]^+$ 357.1598, found 357.1595.

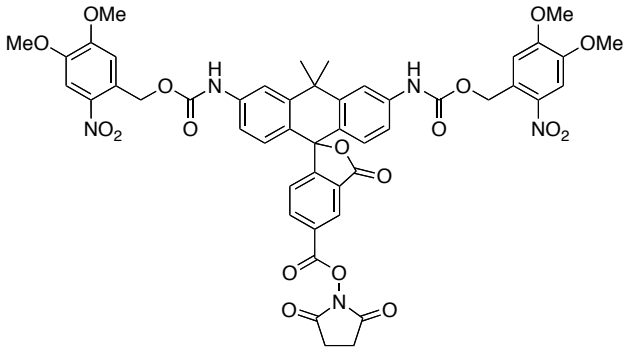


5-Carboxycarborhodamine 110 trifluoroacetate (29): Dicarbamate **24** (65 mg, 0.0990 mmol) was taken up in CH_2Cl_2 (2.5 mL), and trifluoroacetic acid (0.5 mL) was added. The reaction was stirred at room temperature for 5 h. Toluene (3 mL) was added; the reaction mixture was concentrated to dryness and then azeotroped with MeOH three times to provide **29** as a dark purple solid (50 mg, 98%). Analytical HPLC and NMR indicated that the material was >95% pure and did not require further purification. ^{1}H NMR (MeOD, 400 MHz) δ 8.86 (d, *J* = 1.6 Hz, 1H), 8.38 (dd, *J* = 7.9, 1.7 Hz, 1H), 7.44 (d, *J* = 7.9 Hz, 1H), 7.16 (d, *J* = 2.2 Hz, 2H), 6.90 (d, *J* = 9.0 Hz, 2H), 6.59 (dd, *J* = 9.0, 2.2 Hz, 2H), 1.79 (s, 3H), 1.68 (s, 3H); ^{13}C NMR (MeOD, 101 MHz) δ 168.1 (C), 167.5 (C), 159.7 (C), 158.9 (C), 143.8 (C), 138.6 (CH), 134.1 (CH), 133.5 (C), 133.1 (CH), 132.7 (C), 131.9 (CH), 121.9 (C), 116.1 (CH), 114.0

(CH), 42.4 (C), 35.1 (CH_3), 31.7 (CH_3); ^{19}F NMR (MeOD, 376 MHz) δ -75.36 (s); analytical HPLC: 99.0% purity (4.6 mm x 150 mm 5 μm C18 column; 5 μL injection; 10–95% CH_3CN/H_2O, linear gradient, with constant 0.1% v/v TFA additive; 20 min run; 1 mL/min flow; ESI; positive ion mode; UV detection at 254 nm); HRMS (ESI) calcd for $C_{24}H_{21}N_2O_4$ $[M+H]^+$ 401.1496, found 401.1496.

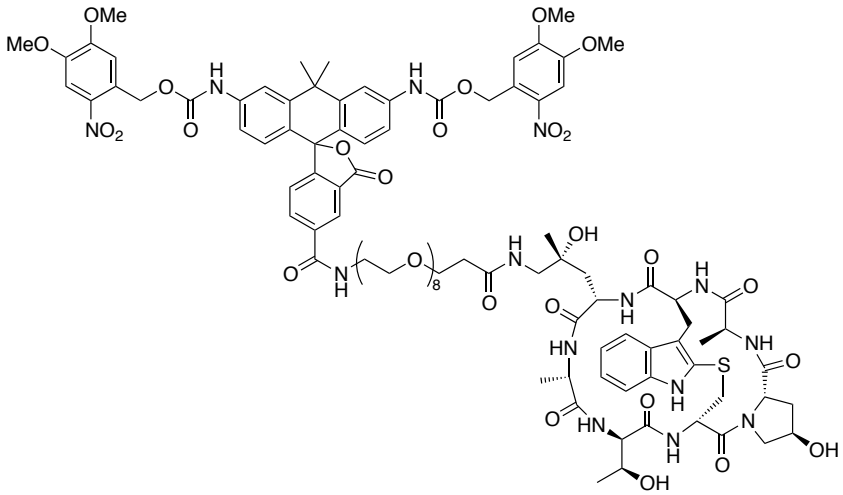


3,6-Bis((((4,5-dimethoxy-2-nitrobenzyl)oxy)carbonyl)amino)-10,10-dimethyl-3'-oxo-3'*H*,10*H*-spiro-[anthracene-9,1'-isobenzofuran]-5'-carboxylic acid (30): Ester **25** (105 mg, 0.112 mmol) was taken up in CH_2Cl_2 (4 mL), and trifluoroacetic acid (0.8 mL) was added. The reaction was stirred at room temperature for 6 h. Toluene (5 mL) was added; the reaction mixture was concentrated to dryness and then azeotroped with MeOH three times to provide **30** as a tan solid (97 mg, 98%). Analytical HPLC and NMR indicated that the material was >95% pure and did not require further purification. ^1H NMR (DMSO-d_6, 400 MHz) δ 13.51 (s, 1H), 10.01 (s, 2H), 8.41 (dd, *J* = 1.4, 0.7 Hz, 1H), 8.22 (dd, *J* = 8.1, 1.5 Hz, 1H), 7.91 (s, 2H), 7.73 (s, 2H), 7.38 – 7.24 (m, 4H), 7.20 (d, *J* = 7.9 Hz, 1H), 6.70 (d, *J* = 8.7 Hz, 2H), 5.47 (s, 4H), 3.91 (s, 6H), 3.88 (s, 6H), 1.77 (s, 3H), 1.66 (s, 3H); ^{13}C NMR (DMSO-d_6, 101 MHz) δ 168.8 (C), 166.0 (C), 158.4 (C), 153.3 (C), 153.0 (C), 148.0 (C), 145.2 (C), 139.9 (C), 139.7 (C), 136.1 (CH), 132.5 (C), 128.3 (CH), 126.6 (C), 125.8 (CH), 124.3 (C), 124.0 (CH), 117.69 (C), 117.67 (CH), 115.9 (CH), 111.6 (CH), 108.3 (CH), 85.4 (C), 63.0 (CH_2), 56.3 (CH_3), 56.1 (CH_3), 37.7 (C), 34.3 (CH_3), 33.4 (CH_3); HRMS (ESI) calcd for $C_{44}H_{39}N_4O_{16}$ $[M+H]^+$ 879.2356, found 879.2380.



2,5-Dioxopyrrolidin-1-yl 3,6-bis((((4,5-dimethoxy-2-nitrobenzyl)oxy)carbonyl)amino)-10,10-dimethyl-3'-oxo-3'*H*,10*H*-spiro[anthracene-9,1'-isobenzofuran]-5'-carboxylate (31): To a solution of acid **30** (52 mg, 0.0592 mmol) and TSTU (27 mg, 0.0888 mmol, 1.5 eq) in DMF (4 mL) was added DIEA (31 μL, 0.178 mmol, 3 eq). The reaction was stirred at room temperature for 18 h while being shielded from light. It was subsequently diluted with 10% w/v citric acid and extracted with EtOAc (2×). The combined organic extracts were washed with brine, dried

($MgSO_4$), filtered, deposited onto silica gel, and concentrated *in vacuo*. Silica gel chromatography (0–50% EtOAc/CH_2Cl_2, linear gradient; dry load with silica gel) yielded 43 mg (74%) of **31** as an off-white solid. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 10.03 (s, 2H), 8.56 (dd, *J* = 1.6, 0.7 Hz, 1H), 8.36 (dd, *J* = 8.1, 1.6 Hz, 1H), 7.93 (s, 2H), 7.73 (s, 2H), 7.36 (dd, *J* = 8.1, 0.6 Hz, 1H), 7.34 – 7.27 (m, 4H), 6.77 (d, *J* = 8.7 Hz, 2H), 5.47 (s, 4H), 3.92 (s, 6H), 3.88 (s, 6H), 2.93 (s, 4H), 1.77 (s, 3H), 1.67 (s, 3H); ^{13}C NMR (DMSO-d_6, 101 MHz) δ 170.1 (C), 168.1 (C), 160.8 (C), 160.2 (C), 153.3 (C), 153.0 (C), 148.0 (C), 145.4 (C), 140.1 (C), 139.7 (C), 136.5 (CH), 128.6 (CH), 127.0 (CH), 126.7 (C), 126.6 (C), 126.0 (C), 125.2 (CH), 123.9 (C), 117.7 (CH), 115.9 (CH), 111.6 (CH), 108.3 (CH), 85.7 (C), 63.1 (CH_2), 56.3 (CH_3), 56.1 (CH_3), 37.8 (C), 34.4 (CH_3), 33.2 (CH_3), 25.6 (CH_2); HRMS (ESI) calcd for $C_{48}H_{42}N_5O_{18}$ $[M+H]^+$ 976.2519, found 976.2512.



NVOC$_2$-CRh$_{110}$-5-dPEG$_8$-phalloidin (32): Ester **31** (13 mg, 13.3 μmol) and amino-dPEG$_8$-acid (11.8 mg, 26.6 μmol, 2 eq) were combined in DMF (2 mL), and DIEA (11.6 μL, 66.6 μmol, 5 eq) was added. The reaction was stirred at room temperature for 18 h while being shielded from light. It was subsequently concentrated *in vacuo* and directly purified by reverse phase HPLC (30–95% MeCN/H_2O, linear gradient, with constant 0.1% v/v TFA additive) to provide 13 mg (75%) of NVOC$_2$-CRh$_{110}$-5-dPEG$_8$-CO_2H.

The resulting acid (13 mg, 9.98 μmol, 2 eq) was combined with TSTU (2.6 mg, 8.73 μmol, 1.75 eq) in DMF (2 mL). After adding DIEA (4.3 μL, 25.0 μmol, 5 eq), the reaction was stirred at room temperature for 18 h while shielded from light. Aminophalloidin tosylate (4.8 mg, 4.99 μmol) in DMF (0.5 mL) was then added. The reaction was stirred an additional 3 h at room temperature. After concentration *in vacuo*, the residue was directly purified by reverse phase HPLC (30–95% MeCN/H_2O, linear gradient, with constant 0.1% v/v TFA additive) to afford **32** (9.5 mg, 92%) as a colorless solid. Analytical HPLC: 96.6% purity (4.6 mm x 150 mm 5 μm C18 column; 5 μL injection; 30–95% CH_3CN/H_2O, linear gradient, with constant 0.1% v/v TFA additive; 20 min run; 1 mL/min flow; ESI; positive ion mode; UV detection at 254 nm); HRMS (ESI) calcd for $C_{98}H_{123}N_{14}O_{34}SNa$ $[M+H+Na]^{2+}$ 1047.3967, found 1047.3986.

OPTICAL SPECTROSCOPY AND MICROSCOPY METHODS

General. Fluorescent and fluorogenic molecules were prepared as stock solutions in DMSO and diluted such that the DMSO concentration did not exceed 1% v/v. Phosphate buffered saline (PBS) was at pH 7.4 unless otherwise noted.

UV–Vis and Fluorescence Spectroscopy. Spectroscopy was performed using 1-cm path length, 3.5-mL quartz cuvettes from Starna Cells or 1-cm path length, 1.0-mL quartz microcuvettes from Hellma. All measurements were taken at ambient temperature (22 ± 2 °C). Absorption spectra were recorded on a Cary Model 100 spectrometer (Varian). Maximum absorption wavelength (λ_{max}) and extinction coefficient (ε) were taken in in 0.1 M NaOH or in 10 mM HEPES buffer, pH 7.3; reported values for ε are averages (n = 3). Fluorescence spectra were recorded on a Cary Eclipse fluorometer (Varian). Normalized spectra for dyes listed in Table 2 are shown in Figure S1.

pK_a Determination (Fig. 2b). The pK_a values for compounds **1** and **4** were determined in buffers containing 150 mM NaCl and 10 mM buffer. The following buffer systems were used: citrate (pH 4.0–6.2); phosphate (pH 5.8–8.0); tris (pH 7.8–9.0); carbonate (pH 9.2–10.0). Absorbance values at λ_{max} were read on 5 μM samples (n = 3) and fitted to a sigmoidal dose response curve (variable slope) using GraphPad Prism software.[1]

Dioxane–H_2O Titration (Fig. 2c). Dioxane–H_2O titrations were performed in spectral grade dioxane (Aldrich) and milliQ H_2O. The solvent mixtures contained 0.01% v/v triethylamine to ensure the dye was in the zwitterionic form. The absorbance values at λ_{max} were read on 5 μM samples (n = 2). Values of dielectric constant (ε_r) were taken from ref [2].

Quantum Yield Determination. Absolute quantum yields (Φ) were measured using a Quantaurus-QY spectrometer (model C11374) from Hamamatsu. This instrument uses an integrating sphere to determine photons absorbed and emitted by a sample. Measurements were carried out using dilute samples ($A < 0.1$) and self-absorption corrections were performed using the instrument software.[3] Since this is a new instrument, we validated the quantum yield values using fluorophores with established quantum yield values. We found good agreement between measured and literature values for all fluorophores tested including fluorescein in 0.1 M NaOH (measured: 0.88; literature: 0.86–0.92; refs [3-5]), Cresyl Violet in ethanol (measured: 0.54; literature: 0.51; ref [4]), and rhodamine 101 in ethanol (measured: 0.86; literature: 0.91; ref [6]).

Cell Culture and Live Cell Confocal Fluorescence Microscopy (Fig. 3). HeLa cells (ATCC) were cultured according to instructions and maintained at 37 °C in a humidified 5% CO_2 v/v environment. For confocal microscopy, 35-mm MatTek petri dishes with glass bottoms were coated with 15 μg/mL human fibronectin (Millipore) prior to culturing of cells in Dulbecco's modified eagle medium (DMEM; Life Technologies) supplemented with 10% v/v fetal bovine serum (FBS; Life Technologies). Compounds **20** or **26** were added and the samples incubated for 1 and 24 h respectively. Nuclear staining was accomplished by addition of 1.0 μg/mL Hoechst 33342 (Life Technologies). Mitochondrial staining was accomplished using MitoTracker Red or MitoTracker Green Life Technologies) at 200–500 nM. The samples were imaged directly without intermediate washing using a Zeiss LSM 510 META confocal microscope with a LD C-APOCHROMAT 40×/1.1 W Korr UV-VIS-IR objective.

Determination of Contrast for Caged Dyes. To minimize background fluorescence from impurities, caged dyes were purified directly before measurement using an Agilent 1200 analytical HPLC system equipped with an autosampler, photodiode array detector, and fraction collector. Stock solutions of 5-carboxy-$NVOC_2$-carborhodamine 110 (**30**) and the isologous 5-carboxy-$NVOC_2$-rhodamine 110 [7] were prepared in 9:1 v/v CH_3CN:DMSO at 720 μM. Using the HPLC autosampler, 10 μL of this solution was injected onto a 4.6 × 150 mm C-18 column (Phenomenex) eluting with a gradient of 50–95% v/v CH_3CN:H_2O containing 0.1% TFA over 20 min. The desired (major) peak eluted at approximately 9 min and was collected in polypropylene 96-well plates by the fraction collector. An aliquot of 20–30 μL of the product-containing peak was dissolved in 3.0 mL of 10 mM HEPES pH 7.3 in a quartz cuvette equipped with a Teflon cap and the background fluorescence measured ($\lambda_{ex/em}$ for carborhodamine 110 = 552/577 nm; $\lambda_{ex/em}$ for carborhodamine 110 = 497/520 nm). This sample was then irradiated in a Luzchem LZC 4V photoreactor equipped with 365 nm lamps and a carousel until full photoconversion was attained (40–60 min), after which the postactivation fluorescence was then measured. Control samples of carborhodamine 110 (**5**) and rhodamine 110 (**2**) at approximately the same concentration (~0.5 μM) were also irradiated and exhibited <2% loss in fluorescence after irradiation. We found on:off contrast ratio of 65000 ± 3000 for carborhodamine **30** and 7000 ± 1000 for caged rhodamine 110 [7] (mean ± SD, n = 2). The contrast value of carborhodamine 110 allows a minimum labeling density of approximately 1 molecule per $(200\ nm)^2/65{,}000 = 0.6\ nm^2$.

Preparation of Mono-Caged Carborhodamine 110. A stock solution of 5-carboxy-$NVOC_2$-carborhodamine 110 (**30**) was prepared in 1:1:2 v/v CH_3CN:H_2O:DMSO at 3.2 mM. This solution was irradiated for 20 minutes in a Luzchem LZC 4V photoreactor equipped with 365 nm lamps and a carousel. The partially photolyzed product was purified using an Agilent 1200 analytical HPLC system equipped with an autosampler, photodiode array detector, and fraction collector. Using the HPLC autosampler, 10 μL of this solution was injected onto a 4.6 × 150 mm C-18 column (Phenomenex) eluting with a gradient of 20–95% v/v CH_3CN:H_2O containing 0.1% TFA over 20 min. The desired (major) peak eluted at approximately 10.3 min and was collected in polypropylene 96-well plates by the fraction collector and the identity was confirmed by LC–MS (expected *m/z*: 640.2; found: 640.1). An aliquot of 30 μL of the product-containing peak was dissolved in 3.0 mL of 10 mM HEPES pH 7.3 in a quartz cuvette equipped with a Teflon cap and the background fluorescence measured ($\lambda_{ex/em}$ = 552/577 nm). This sample was then irradiated as described above for the other dyes. We found on:off contrast ratio of 235 ± 18 for the monocaged carborhodamine compound.

Preparation of Samples for Super-Resolution Microscopy Using Caged Rhodamine–Phalloidin Conjugate. Mouse embryonic fibroblast (MEF) cells (ATCC) were grown on fibronectin-coated 25 mm round coverslips. Cells were then fixed in a buffer containing 10 mM MES pH 6.1, 138 mM KCl, 3 mM $MgCl_2$, 2 mM EGTA, 0.32 M sucrose, and 4% w/v paraformaldehyde for 20 minutes, and subsequently washed with phosphate buffered saline (PBS, 3×). The cells were then permeabilized in PBS containing 0.5% v/v Triton-X (PBS–Triton) for 10 min, and then blocked with a solution of PBS–Triton containing 2% w/v bovine serum albumin (BSA) for 1 h. The cells were then incubated with $NVOC_2$-carborhodamine–PEG_8–phalloidin (**32**; 2 μM) for 3 h. Finally, the cells were washed with PBS–Triton (3×) and rinsed in PBS. 100 μL of PBS was applied to the slide prior to imaging.

Preparation of Samples for Super-Resolution Microscopy Using mEos2. Mouse embryonic fibroblast (MEF) cells (ATCC) were transfected with mEos2–actin as described previously[8] and grown on fibronectin-coated 25 mm round coverslips. Cells were extracted in a buffer containing 100 mM PIPES, 5 mM $MgCl_2$, 5 mM EGTA, 4% w/v PEG, 1% v/v Triton X-100, 10 μM phalloidin, pH 6.9. The cells were then washed (3×) in a buffer containing 100 mM PIPES, 5 mM $MgCl_2$, 5 mM EGTA, 10 μM phalloidin, pH 6.9. Cells were then fixed with 4% w/v paraformaldehyde for 20 minutes, and subsequently washed with PBS (3×). 100 μL of PBS was applied to the slide prior to imaging.

Photoactivated Localization Microscopy (PALM) with Caged Carborhodamine and mEos2 (Figure 4b–c and 4d–e). PALM imaging was performed on instrumentation described previously.[7,9] Laser light was delivered to the rear pupil of a 60×/1.49 NA objective on an Olympus IX81 inverted wide field microscope. Photoactivatable fluorophores were uncaged with a 50 mW 405 nm laser (Coherent 405-50C) at 3–6 μW. Activated fluorophores were excited with a 561 nm laser (Crystal laser GCL-150-561) at 10 mW objective entrance power and emission collection using FF562-Di02-25x36 dichroic and FF02-617/73-25 emission filters (Semrock). Typical power density values ranged from 1–10 W/cm^2 for the 405 nm activation light and 2–4 kW/cm^2 for the 561 nm fluorescence excitation light. Images were detected with an electron multiplying CCD camera (Andor DV897ECS0-BV). Single molecule frame times of 100 ms commonly yielded complete PALM images collected within 80,000 frames (~2 h). Sample drift within this time was corrected by tracking the movement of 100 nm Au rods that were embedded into the coverslip. Molecule localization and image rendering algorithms used were similar to those previously described.[10]

PALM with AlexaFluor 647–phalloidin (Figure 4f–g). MEF cells were grown, fixed, and blocked as described above for the carborhodamine–phalloidin conjugate. The cells were then stained with Alexa Fluor 647–phalloidin (500 nM; Life Technologies) for three hours and washed three times with PBS–Triton, and finally rinsed with PBS. A redox buffer consisting of 100 mM MEA, 50 μg/mL glucose oxidase, 40 μg/mL catalase, 10% w/v glucose, pH 8.5 was used during the experiment.[11,12] The imaging instrumentation was as described above with the following changes: fluorophores were excited with a 700 mW 642 nm laser (MPB Communications VFL-P 700-642) and emission collected using a LP02-647RS-25 long pass (Semrock) and ET700/75 M band pass filter (Chroma). Single molecule frame times of 30 ms yielded images within 120,000 frames (~1 h).

PALM Image Analysis. Molecule localization algorithms were written in MATLAB (Mathworks). Each frame of the image data was bandpass filtered[13] to suppress shot noise and background and enhance molecule peaks. The coordinates of peaks (local maxima) that were more than 6× the image standard deviation above the image mean were identified in each frame. 11×11 pixel sub-images, centered at each of these coordinates were then isolated from the raw (unfiltered) image data and used for molecule localization. From this spatio-temporal dataset (x,y, and t) we next identify if nearby peaks in successive frames correspond to a single emitter or separate molecules. If peaks occur at the same pixel (±1) in consecutive frames we consider them to arise from the same molecule and their summed images are stored across their common fit window. If, however, pixel-identical peaks occur in nearby but not consecutive frames, it is not known whether they correspond to the same molecule (that has temporarily blinked off in the intervening frames) or whether they correspond to distinct molecules. If the former, their signals should be

summed so that higher localization precision is achieved. If the latter, their signals should be localized separately, or else an erroneous localization measurement will arise from their sum. In this analysis, identically localized peaks that were separated by 500 ms or less were considered to arise from the same molecule. This criterion was kept constant across each of the fluorophore labels and imaging conditions.

The image of each molecule, summed across all frames in which it appears, was fit using a Gaussian maximum likelihood estimator for the molecule centroid, total number of photons and image background, following a similar procedure as previously described.[14,15] Prior to fitting, images were converted to photon counts and the camera dark current subtracted. The number of photons was computed according to the EMCCD manufacture's specifications as:

$$P = (S \times T_{A/D})/G$$

where P is the number of photons, S is the signal counts, $T_{A/D}$ is the CCD sensitivity and G is the EMCCD gain. Localization precision was computed as the square root of 2× the variance as described in Mortenson et. al (see: Supplementary Materials, equation 54).[15] PALM datasets were plotted as previously described.[10]

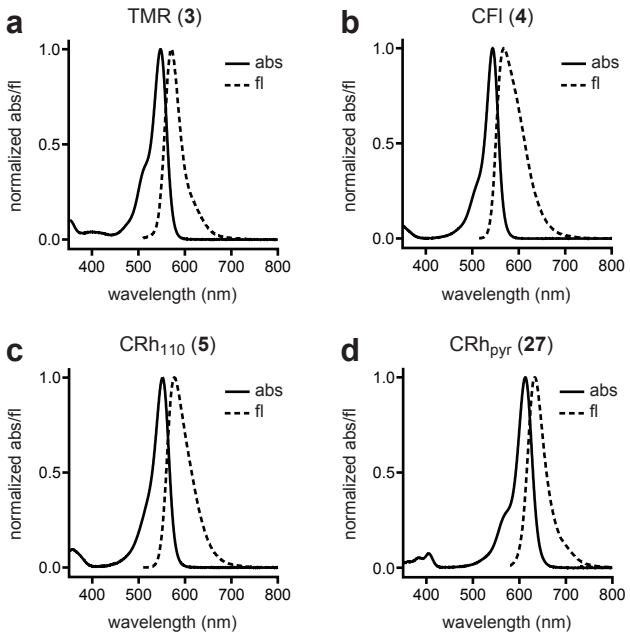


Figure S1. Normalized absorption (abs) and fluorescence emission (fl) spectra for selected fluorophores.

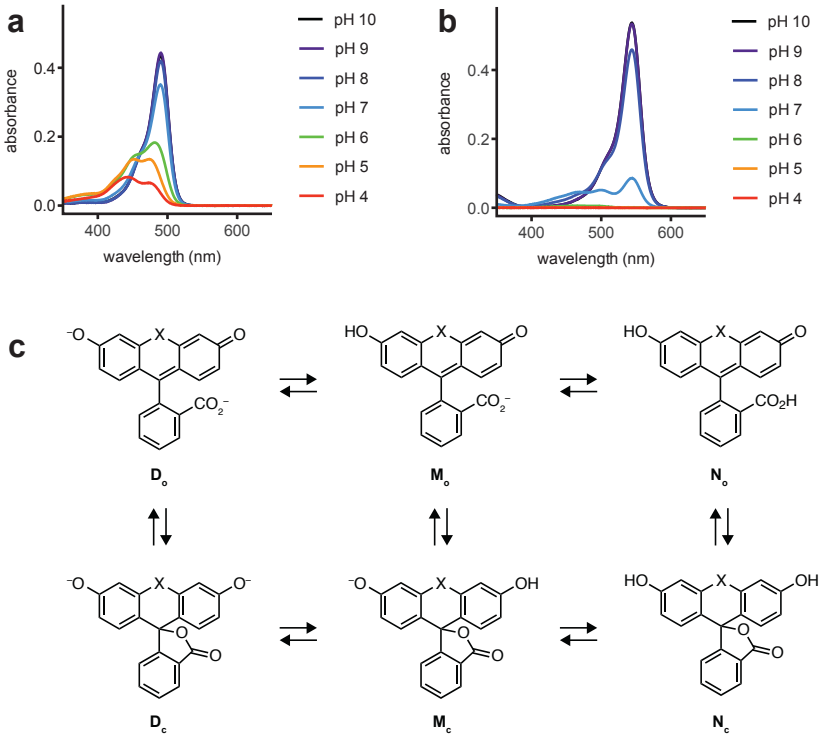


Figure S2. (**a**) Absorption spectra of fluorescein (**1**, 5 μM) at different pH values. (**b**) Absorption spectra of carbofluorescein (**4**, 5 μM) at different pH values. (c) Diagram of the equilibria of dianion (**D**), monoanion (**M**), and neutral (**N**) fluorescein isologues. For carbofluorescein (**4**, X = $C(CH_3)_2$), the monoanion open–closed equilibrium ($\mathbf{M_o}$–$\mathbf{M_c}$) is shifted towards the $\mathbf{M_c}$ form causing cooperative protonation to ultimately form the neutral closed ($\mathbf{N_c}$) dye, similar to phenolphthalein.[16]

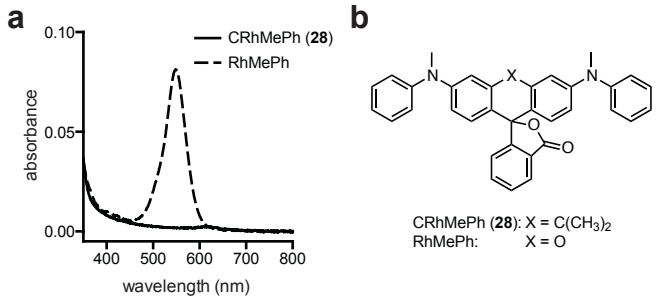


Figure S3. (**a**) Absorption spectra (5 µM in 10 mM HEPES, pH 7.3) of CRhMePh (**28**) and isologous rhodamine dye RhMePh. (**b**) Chemical structures of CRhMePh (**28**) and RhMePh

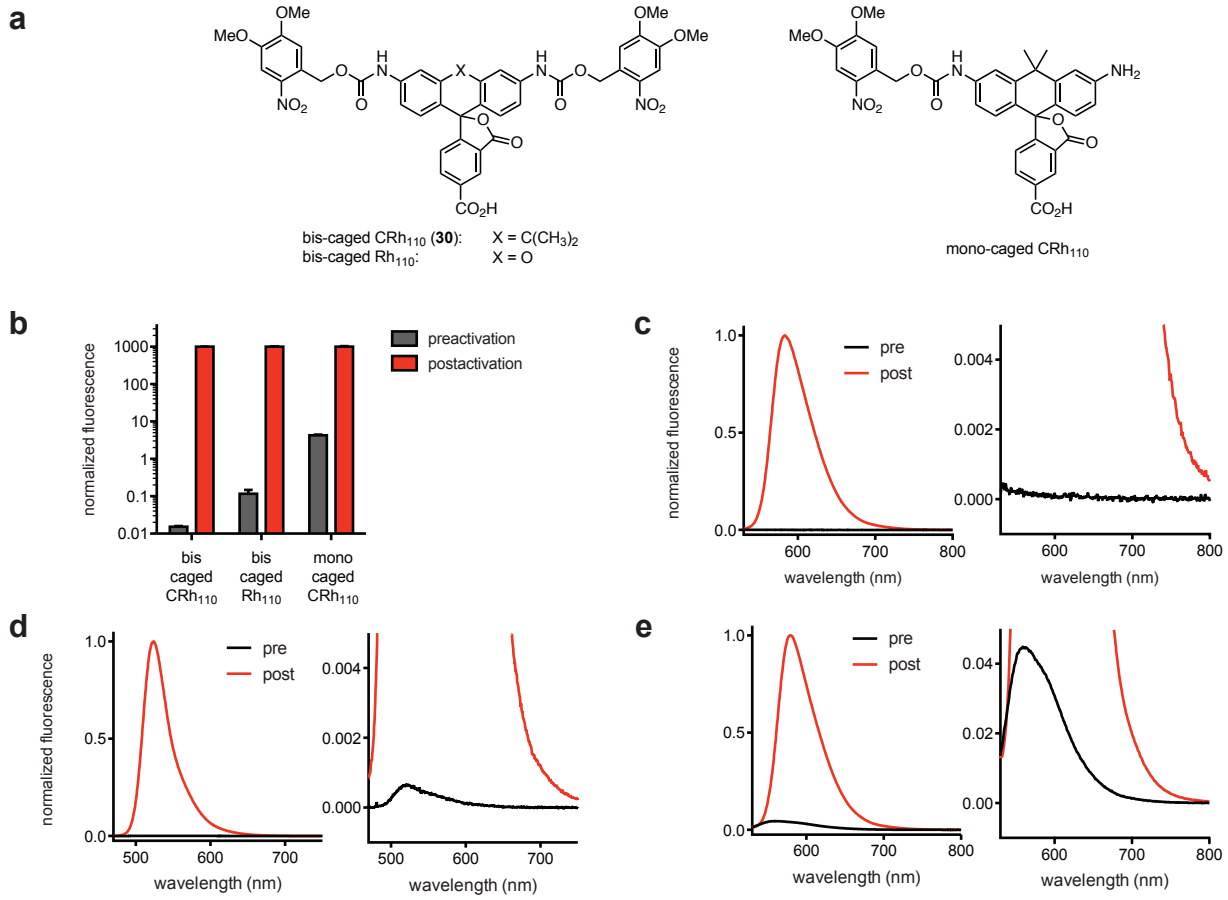


Figure S4. Determination of contrast for caged dyes. (**a**) Chemical structures of 5-carboxy-NVOC$_2$-carborhodamine 110 (i.e., bis-caged CRh$_{110}$, **30**), 5-carboxy-NVOC$_2$-rhodamine 110 (i.e., bis-caged Rh$_{110}$; ref [7]), and 5-carboxy-NVOC-carborhodamine 110 (mono-caged CRh110; generated by partial photolysis of **30**, see S1.17). (**b**) Average fluorescence intensity pre- and post-photoactivation with 365 nm light ($\lambda_{ex/em}$ for carborhodamine 110 dyes = 552/577 nm; $\lambda_{ex/em}$ for rhodamine 110 = 497/520 nm; note logarithmic scale). Found: on:off contrast ratio of 65000 ± 3000 (mean ± SD, n = 2) for carborhodamine **30**; and 7000 ± 1000 for caged rhodamine 110. (**c**–**e**) Full fluorescence emission spectra (left panel) and zoom (right panel) for caged dyes pre- and post-photoactivation (λ_{ex} was chosen at ~40 nm shorter than λ_{max}: for carborhodamine 110 dyes λ_{ex} = 515 nm; for rhodamine 110 λ_{ex} = 455 nm). (**c**) Spectra for 5-carboxy-NVOC$_2$-carborhodamine 110 (i.e., bis-caged CRh$_{110}$). (**d**) Spectra for 5-carboxy-NVOC$_2$-rhodamine 110 (i.e., bis-caged Rh$_{110}$). (**e**) Spectra for 5-carboxy-NVOC-carborhodamine 110 (i.e., mono-caged CRh110).

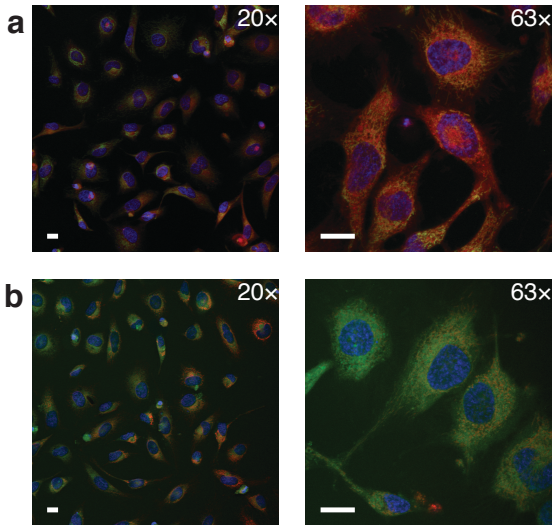


Figure S5. Confocal microscopy of live, unwashed HeLa cells incubated with carborhodamine 110 bis(trimethyl lock acetate) (**26**) and isologous rhodamine 110 bis(trimethyl lock acetate) (refs [17] [18]) for 24 h. (**a**) Cells incubated with compound **26** (red) and counterstained with Hoechst 33342 (blue), and MitoTracker Green. (**b**) Cells incubated with rhodamine 110 bis(trimethyl lock acetate) (green) and counterstained with Hoechst 33342 (blue), and MitoTracker Red. Scale bars for all images: 20 μm.

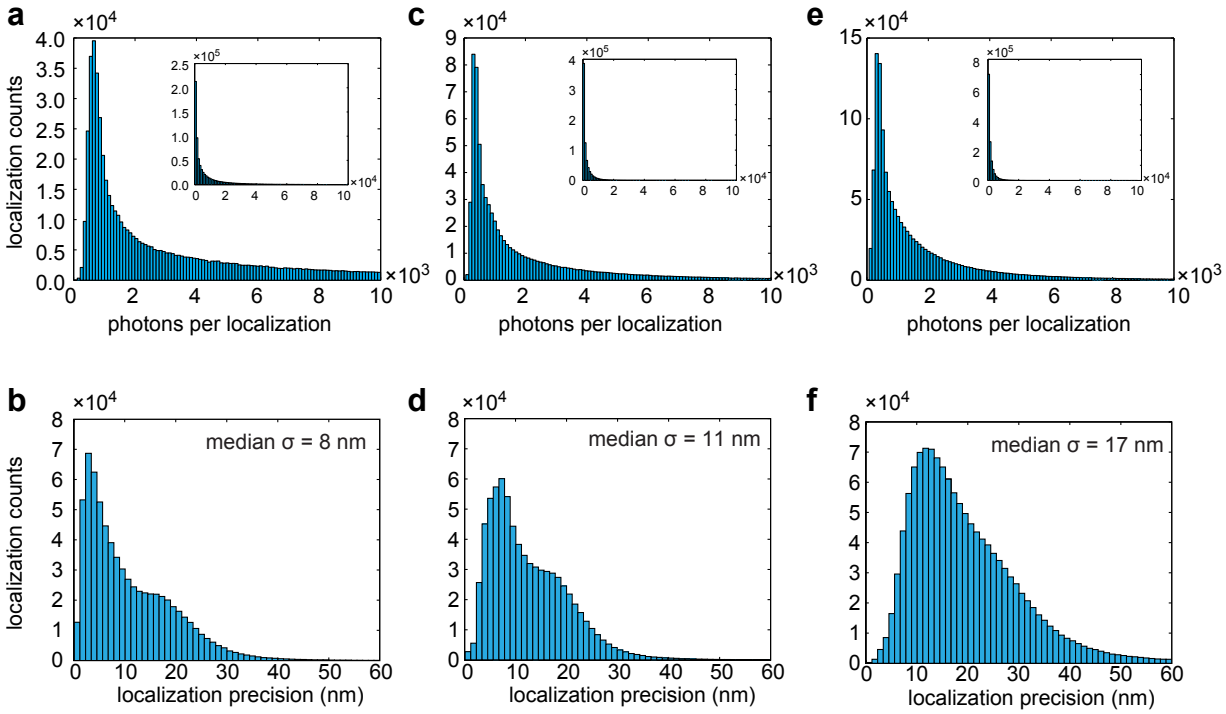


Figure S6. Comparison of photon yields and localization precision of three different labels. Top panels (**a**, **c**, and **e**): Histograms showing localization counts against photon yields per molecule for PALM imaging in mouse embryonic fibroblast (MEF) cells. Large graphs show a cropped histogram of photons per localization event for each condition. The full-range histograms are shown as insets. Bottom panels (**b**, **d**, and **f**): localization precision histogram for different labels for PALM imaging in MEF cells. (**a**–**b**) Carborhodamine 110–PEG_8–phalloidin (**32**)-labeled MEF cells (842,372 localizations/cell). (**c**–**d**) mEos2–actin fusion protein[8] expressed in MEF cells (1,011,424 localizations/cell). (**e**–**f**) AlexaFluor 647–phalloidin-labeled MEF cells (2,074,875 localizations/cell).

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