A Practical Synthetic Route to Functionalized Rhodamine Dyes

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Supporting Information

General Procedures. Unless otherwise noted, all solvents and reagents were added via syringe to reaction flasks equipped with rubber septa. Reactions were performed under an atmosphere of N₂ with flame-dried glassware. Dichloromethane (CH₂Cl₂) was distilled under N₂ from CaH. Tetrahydrofuran (THF) was distilled under N₂ from Na and benzophenone. HPLC grade methanol (MeOH) and DMF were obtained from EM Science and used as received. Flash column chromatography was carried out with Merck Silica Gel 60. Thin layer chromatography was performed with Silicia Gel 60 F₂₅₄ plates. Trimethyl aluminum was obtained as a 2.0 M solution in toluene from Aldrich. Rhodamine B base was used as obtained from Aldrich. Rhodamine 101 inner salt was purchased from Acros Organics. TLC plates were initially rinsed in the chamber solvent prior to sample application. Wang resin (4-benzyloxybenzyl alcohol bound to 100-200 mesh polystyrene, 1.5-2.0 mmol loading) was purchased from Aldrich and used as obtained. All reactions involving Wang resin were performed using disposable chromatography columns available from Bio-Rad and agitated using a Barnstead/Thermolyne Labquake® rotary shaker. Myoglobin from horse heart was purchased from Sigma and used as received.

Instrumentation. ¹H and ¹³C FT-NMR spectra were measured with a Bruker AMX-300 (300MHz), AM-400 (400MHz) or DRX 500 (500 MHz) spectrometer as noted. Chemical shifts are reported as δ in units of parts per million (ppm) relative to chloroform (δ 7.26, s) or methanol (δ 3.30, septet). Multiplicities are reported as follows: s (singlet), d (doublet), t (triplet), q

(quartet), dd (doublet of doublets), m (multiplet), br (broadened), or app (apparent). Coupling constants are reported as a J value in Hertz (Hz). The number of protons (n) for a given resonance is indicated as nH , and is based on spectral integration values. ^{13}C NMR spectra are reported as δ in units of parts per million (ppm) relative to chloroform- d (δ 77.23, t) or methanol- d_4 (δ 49.15, septet). Fast Atom Bombardment (FAB) and Electron Impact (EI) mass spectra were obtained at the UC Berkeley Mass Spectrometry Facility. Elemental Analyses were obtained at the UC Berkeley Microanalytical Laboratory. Reported atomic percentages are within error limits of $\pm 0.4\%$. Infrared spectra were assigned on thin film samples on a reflecting plate using a Genesis II FTIRTM (ATI Mattson, USA). Uncorrected melting points were determined in glass capillary tubes using a Mel-Temp 3.0[®] apparatus (Laboratory Devices, Inc., USA). Reaction aliquots were often monitored with matrix assisted laser desorption-ionization time-of-flight mass spectrometry (MALDI-TOF MS) on a Voyager-DE instrument from PerSeptive Biosystems. Samples were co-crystallized using with a trihydroxy acetophenone matrix. Electrospray LC/MS analysis was performed using an API 150EX (Applied Biosystems) system equipped with a Turbospray source and an Agilent 1100 series LC pump. Protein mass reconstruction was performed on the charge ladder with Analyst software (version 1.3.1, Applied Biosystems). Extinction coefficients were determined using a WPI Tidas II spectrophotometer. Emission spectra were obtained using a Jobin Yvon-Spex FluoroMax-2 fluorimeter.

Rhodamine B base (4). Rhodamine B (**3**, 3.3 g, 6.9 mmol) was dissolved and partitioned between aqueous 1 M NaOH and EtOAc. After isolation of the organic layer, the aqueous layer was extracted with two additional

portions of EtOAc. The combined organic layers were then washed with NaOH and brine. The resulting organic solution was dried over Na_2SO_4 , filtered, and concentrated under reduced

pressure to yield 2.83 g of product as a pink foam (93%): ^1H NMR (300 MHz, CD_3OD): δ 1.26-1.31 (t, 12, $J=6.9$), 3.61-3.68 (q, 8, $J=6.9$), 6.90-6.91 (d, 2, $J=2.4$), 6.96-7.00 (dd, 2, $J=2.7$, 9.6), 7.23-7.29 (m, 3), 7.57-7.67 (m, 2), 8.07-8.09 (m, 1).

Rhodamine B piperazine amide (5). A 2.0 M solution of trimethyl aluminum in toluene (22.6 mL, 45.2 mmol) was added dropwise to a solution of piperazine (7.8 g, 91 mmol) in 35 mL of CH_2Cl_2 at room

temperature. After one hour of stirring a white precipitate was observed. A solution of rhodamine B base (10.0 g, 22.6 mmol) in 20 mL of CH_2Cl_2 was added dropwise to the heterogeneous solution. Gas evolution was observed during the addition period. After stirring at reflux for 24 h, a 0.1 M aqueous solution of HCl was added dropwise until gas evolution ceased. The heterogeneous solution was filtered and the retained solids were rinsed with CH_2Cl_2 and a 4:1 $\text{CH}_2\text{Cl}_2/\text{MeOH}$ solution. The combined filtrate was concentrated and the residue was dissolved in CH_2Cl_2 , filtered to remove insoluble salts, and concentrated again. The resulting glassy solid was then partitioned between dilute aqueous NaHCO_3 and EtOAc. After isolation, the aqueous layer was washed with 3 additional portions of EtOAc to remove residual starting material. The retained aqueous layer was saturated with NaCl, acidified with 1 M aqueous HCl, and then extracted with multiple portions of 2:1 $^i\text{PrOH}/\text{CH}_2\text{Cl}_2$, until a faint pink color persisted. The combined organic layers were then dried over Na_2SO_4 , filtered, and concentrated under reduced pressure. The glassy purple solid was dissolved in a minimal amount of MeOH and precipitated by dropwise addition to a large volume of Et_2O . The product was collected by filtration as a dark purple solid 8.7 g, (70%): mp 218-220 $^\circ\text{C}$. IR: 1630 cm^{-1} . ^1H NMR (500 MHz, CD_3OD): δ 1.28-1.33 (t, 12, $J=7.5$), 3.12 (br s, 4), 3.64-3.74 (m, 12), 6.97-6.98 (d, 2, $J=2.5$), 7.09-7.11 (dd, 2, $J=2.5$, 10.0), 7.26-7.27 (d, 2, $J=9.5$), 7.51-7.54 (m, 1), 7.76-7.80 (m, 3).

^{13}C NMR (100 MHz, CD_3OD) δ 13.07, 44.34, 45.59, 47.05, 97.51, 114.87, 115.67, 129.04, 131.50, 131.63, 131.97, 132.54, 133.08, 135.75, 156.82, 157.28, 159.32, 169.48. High-resolution FAB-MS, m/z ($\text{M}+\text{H}$) calcd 511.3073 found 511.3078. Anal calcd for : C, 71.69; H, 7.22; N, 8.36. Found: C, 71.41; H, 7.24; N, 8.38.

Rhodamine B 4-(3-Carboxypropionyl)piperazine amide (6).

Triethylamine (0.05 mL, 0.4 mmol) was added to a stirred solution of **5** (0.2 g, 0.3 mmol), succinic anhydride (0.04 g, 0.4 mmol), and DMAP (0.05 g, 0.4 mmol) in CH_2Cl_2 (1.2 mL). After stirring at room temperature for 24 h, the reaction solution was partitioned between EtOAc and 1 M aqueous K_2CO_3 . The aqueous layer was washed with 3 additional portions of EtOAc. Sodium chloride was added to the isolated aqueous layer until saturation was achieved and the solution was then extracted with 2:1 isopropanol/ CH_2Cl_2 . The organic layer was dried over Na_2SO_4 , and concentrated under reduced pressure. The resulting solid was dissolved in CHCl_3 and filtered to remove insoluble salts. Upon concentration, the product was obtained as a dark solid, 144 mg (72 %): mp: 166-168 $^\circ\text{C}$. IR: 1720 cm^{-1} . ^1H NMR (400 MHz, CD_3OD) δ 1.28-1.32 (t, 12H, $J=6.8$), 2.54 (br s, 2H), 2.59 (br s, 2H), 3.41 (br s, 8H), 3.66-3.71 (q, 8H, $J=7.2$), 6.96-6.97 (d, 2H, $J=2.0$), 7.09 (br s, 2H), 7.27-7.29 (d, 2H, $J=9.6$), 7.52-7.53 (m, 1H), 7.69-7.71 (m, 1H), 7.76-7.78 (m, 2H). ^{13}C NMR (100 MHz, CD_3OD) δ 13.03, 28.94, 30.33, 40.40, 43.08, 47.05, 50.00, 97.49, 114.95, 115.57, 129.07, 131.42, 131.90, 132.41, 133.29, 136.66, 140.14, 157.12, 157.29, 169.62, 172.82, 176.67. High-resolution FAB-MS, m/z (M^+) calcd 611.3233, found 611.3233.

Rhodamine B 4-(3-hydroxypropyl)piperazine amide (7). To a solution of **5** (0.20 g, 0.4 mmol) in 0.7 mL DMF were added 3-bromopropanol (0.07 g, 0.5 mmol) and *N,N*-diisopropylethylamine

(0.09 g, 0.7 mmol). After 24 h, additional 3-bromopropanol (0.07 g, 0.5 mmol) and DIPEA (0.09 g, 0.7 mmol) were added, and the resulting solution was stirred for 2 additional hours. The reaction solution was then partitioned between EtOAc and saturated aqueous NaHCO₃ and the aqueous layer was washed with three portions of EtOAc, which were discarded. The resulting aqueous layer was extracted with ¹PrOH/CH₂Cl₂ (1:3), and the organic layer was collected, dried over Na₂SO₄, filtered, and concentrated to afford 0.158 g of the desired alcohol (71%). mp 149-151 °C. IR: 3241 cm⁻¹. ¹H NMR (400 MHz, CD₃OD) δ 1.29-1.33 (t, 12H, *J*=7.2), 1.61-1.65 (m, 2H), 2.21 (br s, 4H), 2.33-2.37 (t, 2H, *J*=7.2), 3.38 (br s, 4H), 3.54-3.57 (t, 2H, *J*=6.4), 3.66-3.72 (q, 8H, *J*=7.2), 6.97-6.98 (d, 2H, *J*=2.8), 7.06-7.09 (dd, 2H, *J*=2.4, 9.6), 7.26-7.29 (d, 2H, *J*=9.6), 7.51-7.53 (m, 1H), 7.64-7.66 (m, 1H), 7.75-7.77 (m, 2H). ¹³C NMR (100 MHz, CD₃OD) δ 13.04, 30.08, 42.63, 47.06, 54.22, 56.59, 61.35, 97.48, 114.99, 115.56, 129.00, 131.28, 131.44, 131.77, 132.20, 133.44, 136.87, 157.12, 157.32, 159.40, 169.39. High-resolution FAB-MS, M⁺ calcd 569.3492, found 569.3487.

Rhodamine B 4-(3-(N-hydroxysuccinimidyl)propyl)piperazine amide (8). To a round bottom flask charged with *N, N'*-disuccinimidyl carbonate (DSC) (0.10 g,

0.5 mmol) and **7** (0.10 g, 0.2 mmol) was added 1.5 mL of distilled pyridine (1.5 mL). After stirring at room temperature for 4 h, additional DSC (0.09 g, 0.3 mmol) was added to achieve full conversion. After a total of 7 h, the excess DSC was quenched by adding H₂O (0.07 g, 4.1 mmol). The resulting solution was immediately coupled to amines, as described below:

a) Reaction with benzylamine

Rhodamine B 4-(3-benzylcarbamoyloxypropyl)piperazine amide (9). To a 1.18 mL portion of the

solution of **8** prepared above was added benzylamine (0.08 g, 0.7 mmol). Shortly after addition, colorless vapor condensation was visible. After stirring at room temperature for 2 hrs, the solution was partitioned between 4:1 CH₂Cl₂/ⁱPrOH and an aqueous HCl solution saturated with NaCl. The organic layer was isolated and the aqueous layer was extracted with 3 additional portions of 4:1 CH₂Cl₂/ⁱPrOH. The combined organic layers were dried over Na₂SO₄, filtered, and concentrated under reduced pressure to yield the product as a dark red glassy solid (0.08 g, 87%): mp 60.8-69.2 °C. ¹H NMR (500 MHz, CD₃OD) δ 1.29-1.32 (t, 12H, *J*=7.0), 2.04 (br, 2H), 3.09 (br s, 4H), 3.63-3.73 (m, 10H), 4.11-4.13 (t, 2H, *J*=6.0), 4.3 (s, 2H), 6.96-6.97 (d, 2H, *J*=2.5), 7.08-7.10 (dd, 2H, *J*=2.5, 9.5), 7.20-7.31 (m, 8H), 7.51-7.54 (m, 1H), 7.75-7.78 (m, 3H). ¹³C NMR (125 MHz, CD₃OD) δ 13.05, 25.36, 45.62, 46.13, 47.06, 52.76, 53.02, 55.58, 62.93, 97.54, 114.96, 115.65, 128.30, 128.50, 129.03, 129.63, 131.55, 131.71, 132.02, 132.56, 133.14, 135.86, 140.67, 156.85, 157.36, 158.79, 159.35, 169.44. High-resolution FAB-MS, M⁺ calcd 702.4019, found 702.4022.

b) Myoglobin labeling. To a solution of horse heart myoglobin (2.2 mg, 0.13 μmol) in 5 mM NaHCO₃ buffer, pH 8 (1 mL) was added a solution of rhodamine derivative **8** (1.3 mg, 1.7 μmol). After 24 h of vortex agitation at room temperature, the solution was subjected to gel filtration through a MicroSpin G-25 column (Amersham Biosciences) according to the manufacturers protocol. Analysis of the resulting protein solution by LC/MS indicated a ratio of 1:2:3:2 of unmodified, singly, doubly, and triply modified protein at 16953, 17550, 18147, and 18739 amu, respectively.

Rhodamine B 4-(2-chloroacetyl)piperazine amide (10). A 5 mL round bottom flask was charged with 0.3 g (0.5 mmol) of **5** and 2 mL of CH₂Cl₂. The reaction was cooled to 0 °C and chloroacetyl chloride

(0.1 g, 1.0 mmol) and pyridine (0.09 g, 1.2 mmol) were added via syringe. The reaction was allowed to warm to ambient temperature over a period of 4 h. The dark red solution was concentrated under reduced pressure and partitioned between Et₂O and aqueous 1.0 M K₂CO₃. After washing with 3 portions of Et₂O, the aqueous layer was isolated, saturated with NaCl, and extracted with 3 portions of 1:4 isopropanol/CH₂Cl₂. The combined organic layers were dried over Na₂SO₄, filtered, and concentrated to afford the desired product (0.3 g, 95%): mp 194-198 °C. IR: 1643.06, 1632.45 cm⁻¹. ¹H NMR (500 MHz, CD₃OD) δ 1.29-1.31 (t, 12H, *J*=7.0), 3.39 (br s, 4H), 3.46 (br s, 4H), 3.66-3.71 (q, 8H, *J*=7.0), 4.21 (s, 2H), 6.96-6.97 (d, 2H, *J*=2.5), 7.06-7.08 (dd, 2H, *J*=2.5, 9.5), 7.27-7.29 (d, 2H, *J*=9.5), 7.51-7.53 (m, 1H), 7.70-7.72 (m, 1H), 7.76-7.78 (m, 2H). ¹³C NMR (100 MHz, CD₃OD) δ 13.02, 31.84, 42.10, 43.06, 47.05, 50.01, 97.51, 114.98, 115.60, 129.09, 131.47, 131.89, 132.39, 133.32, 136.59, 159.08, 157.35, 159.37, 167.84, 169.69. High-resolution FAB-MS, M⁺ calcd 587.2789, found 587.2788.

Rhodamine B 4-(2-iodoacetyl)piperazine amide (11). To a solution of **10** (52.3 mg, 0.09 mmol) in 1:2 MeOH/acetone (3 mL) was added sodium iodide (133 mg, 0.44 mmol). The reaction flask was covered

in foil and heated at reflux for 15 h, at which time additional NaI (66.7 mg, 0.2 mmol) was added. After a total of 48 hrs, the solvent was evaporated under a stream of nitrogen. The residue was taken up in CH₂Cl₂, and the heterogeneous solution was filtered to remove insoluble salts. The solution was then concentrated under reduced pressure to yield a glassy red solid. The product was dissolved in a minimal volume of MeOH and precipitated in a large excess volume of ether. Filtering the solution afforded 54 mg of the desired product as a red solid (88%): mp 174-182 °C. IR: 1640 cm⁻¹. ¹H NMR (500 MHz, CD₃OD) δ 1.28-1.32 (t, 12H, *J*=7.0), 3.36 (br s, 4H), 3.48 (br s, 4H), 3.66-3.71 (q, 8H, *J*=7.0), 3.84 (s, 2H), 6.97-6.97 (d, 2H, *J*=2.5), 7.07-7.09

(m, 2H), 7.28 (m, 2H), 7.53 (m, 1H), 7.70-7.72 (m, 1H), 7.78 (m 2H). ^{13}C NMR (100 MHz, CD_3OD) δ 13.12, 30.87, 42.68, 47.12, 47.63, 79.65, 97.55, 114.96, 115.73, 129.15, 131.48, 131.89, 133.33, 133.38, 136.56, 157.05, 157.32, 159.35, 169.94. High-resolution FAB-MS, M^+ calcd 679.2145, found 679.2133.

Rhodamine B 4-(2-thioacetyl-acetyl)piperazine amide (12).

To a solution of **10** (0.22 g, 0.35 mmol) in DMF (0.7 mL) was added KSAc (0.05 g, 0.42 mmol). After stirring at room temperature for 8 h, the reaction solution was added to a saturated aqueous NaCl solution, and the aqueous solution was extracted with 3 portions of 4:1 $\text{CH}_2\text{Cl}_2/\text{iPrOH}$. The resulting organic layers were combined and dried over Na_2SO_4 , filtered, and concentrated under reduced pressure. The resulting red solid was dissolved in CH_2Cl_2 (8 mL) and precipitated by dropwise addition to a large volume of ether (450 mL). The resulting solid was collected via filtration and washed with ether to afforded 0.15 g of the desired product (64%): mp 221.1-227.8 °C. ^1H NMR (500 MHz, CD_3OD) δ 1.29-1.32 (t, 12H, $J=7.0$), 2.33 (s, 2H), 3.41 (br s, 4H), 3.45 (br s, 4H), 3.67-3.71 (q, 8H, $J=7.0$), 3.82 (s, 2H), 6.97-6.97 (d, 2H, $J=2.0$), 7.06-7.08 (m, 2H), 7.29-7.27 (d, 2H, $J=9.5$), 7.52 (m, 1H), 7.70-7.71 (m, 1H), 7.77-7.78 (m 2H). ^{13}C NMR (125 MHz, CD_3OD) δ 13.10, 30.22, 32.36, 42.64, 47.04, 55.15, 97.47, 114.89, 115.55, 129.05, 131.40, 131.86, 132.37, 133.23, 136.53, 157.03, 157.19, 159.23, 168.65, 169.46, 195.86. High-resolution FAB-MS, M^+ calcd 627.3005, found 627.2990.

Wang resin, 4-nitrophenyl carbonate (17).

A disposable polypropylene chromatography column (BioRad cat # 732-1010) was charged with Wang resin (1.0 g, 2.0 mmol, Aldrich cat # 514748), 10 mL of CH_2Cl_2 , p-nitrochloroformate (1.2 g, 6.0 mmol), and distilled pyridine (0.8 g, 10.0 mmol). After agitating

the column overnight using a rotary shaker, the resin was collected by filtration and washed with CH_2Cl_2 and THF in an alternating fashion, followed by 10 mL of $i\text{PrOH}$, and finally 4 alternating portions of CH_2Cl_2 and THF. The resin was dried under vacuum, and then used immediately. A new IR band was observed at 1773 cm^{-1} (KBr pellet).

Rhodamine 6G piperazine amide (14). A 2.0 M solution of AlMe_3 in toluene (2.1 mL, 4.18 mmol) was added dropwise to a solution of piperazine (0.4 g, 4.2 mmol) in CH_2Cl_2 (10 mL). A white precipitate was

observed. After stirring for 30 min, **13** (1.0 g, 2.1 mmol) was added in a single portion to the heterogeneous solution, and the suspension was heated to reflux for 24 h. The reaction was then quenched with aqueous 0.1 M HCl and filtered. The retained solids were washed with CH_2Cl_2 and then 4:1 $\text{CH}_2\text{Cl}_2/\text{MeOH}$. In order to remove the residual piperazine, the combined filtrates were concentrated, and the resulting solid was taken up in dilute HCl saturated with NaCl. The aqueous layer was extracted with 3 portions of 2:1 $i\text{PrOH}/\text{CH}_2\text{Cl}_2$, and the combined organic layers were dried over NaSO_4 , filtered, and concentrated.

A 0.5 g portion of the crude dye mixture was dissolved in DMF (12.9 mL), after which *N,N*-diisopropylethylamine (0.4 g, 3.0 mmol), DMAP (0.2 g, 2.0 mmol), and resin **17** (1.0 g) were added. The mixture was agitated at room temperature for 12 hrs. Afterwards, the resin beads were isolated via filtration and washed with 4 portions of CH_2Cl_2 (20 mL) and MeOH (20 mL) in an alternating fashion, until no red color persisted in the washing solvent. Subsequent washings were performed with 3 portions of THF (20 mL). The purple resin was then washed with 6 volumes of an aqueous 10% Et_3N solution (20 mL) and 3 washes with aqueous 10% AcOH solution (20 mL). After no color persisted in the washings, the resin was cleaved by exposure to 1:9 TFA/ CH_2Cl_2 for 2 min. The supernatant was collected, and the resin was repeatedly washed

with 4 portions of 1:9 TFA/CH₂Cl₂ (20 mL) until only a faint color persisted in the washing solvent. The combined filtrates were concentrated, and the residue was dissolved in a saturated aqueous solution of NaHCO₃ and brine. The aqueous layers were extracted with 3 portions of a 2:1 ¹PrOH/CH₂Cl₂ solution. The combined organic layers were then dried over Na₂SO₄, filtered, and concentrated to yield 0.2 g of the desired product (48 %): mp 85.8-88.6 °C. ¹H NMR (300 MHz, CD₃OD) δ 1.33-1.38 (t, 6H, *J*=7.2), 2.17 (s, 6H), 2.52 (br s, 4H), 3.34 (s, 4H), 3.49-3.56 (q, 4H, *J*=7.2), 6.92 (s, 2H), 7.02 (d, 2H, *J*=0.9), 7.46-7.49 (m, 1H), 7.63-7.66 (m, 1H), 7.72-7.77 (m, 1H). ¹³C NMR (100 MHz, CD₃OD) δ 14.15, 17.73, 39.60, 43.67, 46.16, 46.68, 49.79, 95.02, 114.96, 117.22, 119.55, 126.83, 128.99, 130.87, 131.87, 131.24, 131.33, 131.70, 132.51, 136.85, 156.28, 157.83, 158.85, 169.43. High-resolution FAB-MS, M⁺ calcd 483.2760, found 483.2764.

Rhodamine 101 piperazine amide (16). A 2.0 M solution of AlMe₃ in toluene (2.0 mL, 4.1 mmol) was added dropwise to a solution of piperazine (0.4 g, 4.1 mmol) in CH₂Cl₂ (7 mL). A white precipitate was observed.

After stirring for 15 min, a solution of **15** (1.0 g, 2.1 mmol) in 3 mL CH₂Cl₂ was added dropwise to the heterogeneous reaction solution, and the suspension was heated to reflux for 24 h. The reaction was then quenched with aqueous 0.2 M HCl and filtered. The retained solids were washed with CH₂Cl₂ and then 4:1 CH₂Cl₂/MeOH. In order to remove the residual piperazine, the combined filtrates were concentrated, and the resulting solid was taken up in dilute HCl saturated with NaCl. The aqueous layer was extracted with 4 portions of 2:1 ¹PrOH/CH₂Cl₂, and the combined organic layers were dried over NaSO₄, filtered, and concentrated.

A 0.8 g portion of the crude dye mixture was dissolved in DMF (20.0 mL), after which *N*, *N*-diisopropylethylamine (0.2 g, 1.4 mmol), DMAP (0.1 g, 0.9 mmol), and resin **17** (0.5 g), were added. The mixture was agitated at room temperature for 12 hrs. Afterwards, the resin beads

were isolated via filtration and washed with 4 portions of CH₂Cl₂ (20 mL) and MeOH (20 mL) in an alternating fashion, until no red color persisted in the washing solvent. Subsequent washings were performed with 3 portions of THF (20 mL) and CH₂Cl₂ (20 mL). The purple resin was then washed with 8 volumes of an aqueous 10% Et₃N solution (20 mL) and 4 washes with aqueous 10% AcOH solution (20 mL). After no color persisted in the washings, the resin was cleaved by exposure to 1:9 TFA/CH₂Cl₂ for 2 min. The supernatant was collected, and the resin was repeatedly washed with 4 portions of 1:9 TFA/CH₂Cl₂ (20 mL) until only a faint color persisted in the washing solvent. The combined filtrates were concentrated to yield 88 mg of the desired product (6 %): mp 99.0-101.3 °C. ¹H NMR (500 MHz, CD₃OD) δ 1.90-1.99 (m, 4H), 2.06-2.10 (m, 4H), 2.66-2.76 (m, 4H), 3.04 (t, 4H, *J*=6.5), 3.07 (br s, 4H), 3.49-3.52 (t, 4H, *J*=5.5), 3.53-3.55 (t, 4H, *J*=5.5), 3.63 (br s, 4H), 6.74 (s, 2H), 7.41-7.44 (m, 1H), 7.68-7.71 (m, 1H), 7.72-7.76 (m, 2H). ¹³C NMR (125 MHz, CD₃OD) δ 20.89, 21.03, 21.84, 28.59, 39.71, 44.24, 44.50, 45.63, 51.55, 52.02, 106.84, 114, 32, 116.43, 118.74, 125.47, 127. 68, 128.82, 131.22, 131.55, 132.04, 133.05, 135.90, 152.75, 153.46, 153.60, 169.80. High-resolution FAB-MS, M⁺ calcd 559.3073, found 559.3086.

Determination of Quantum Yields. Quantum yields were determined according to the method of Fery-Forgues et al.¹ Compounds to be evaluated were weighed on a microbalance and dissolved in 5 mM phosphate buffer, pH 7, such that absorbance values between 0.2-0.5 were obtained at the excitation wavelength. Each sample was then diluted tenfold using additional phosphate buffer, and emission spectra were recorded with a fluorimeter. Rhodamine 6G derivatives were excited at 515 nm, while rhodamine B, and rhodamine 101 derivatives were excited at 545 nm. Emission spectra were integrated using SpectraAcq software. Relative

¹ Fery-Forgues, S.; Lababre, D. *J. Chem. Ed.* **1999**, 76, 1260.

quantum yields were determined using rhodamine 6G in H₂O ($\Phi=0.95$)² and rhodamine 101 in MeOH ($\Phi=1.0$)³ as standards for samples excited at 515 nm and 545 nm, respectively.

Table S1. Spectral properties of rhodamine derivatives reported herein.^a

Compound	λ_{max} (excitation)	λ_{max} (emission)	ϵ (M cm ⁻¹)	Φ	Reference
Rhodamine B	554 nm	583 nm	97,300	0.36	
Compound 5	567 nm	589 nm	96,100	0.24	
Rhodamine 6G	527 nm	557 nm	86,700	0.95	2
Compound 14	537 nm	561 nm	41,600	0.92	
Rhodamine 101	576 nm	602 nm	90,700	0.78	
Compound 16	589 nm	610 nm	22,300	0.48	

^aAll values are reported as solutions in 5 mM aqueous phosphate buffer, pH 7.0.

² Bindhu, C. V.; Harilal, S. S.; Nampoori, V. P.; Vallabhan, C. *Mod. Phys. Lett.* **1999**, *13*, 563.

³ Karstens, T.; Kobs, K. *J. Phys. Chem.* **1980**, *84*, 1871. In order to obtain a quantum yield for this dye in water, refractive index corrections were used as described in reference 1..