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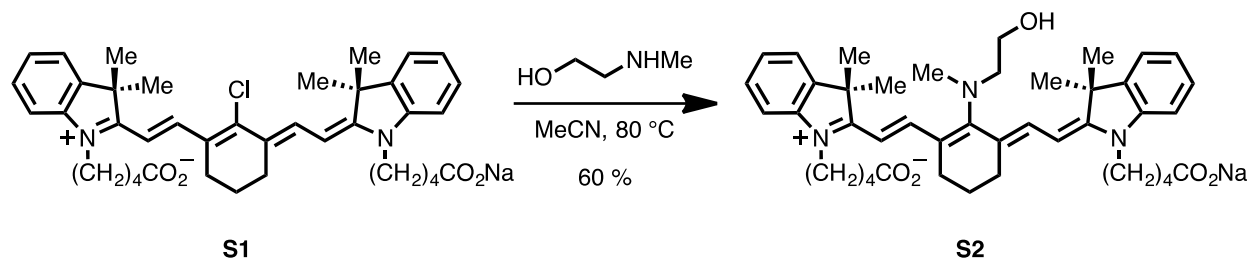
**$^1\text{H}$  and  $^{13}\text{C}$  NMR Spectra**

**General Materials and Methods.** Unless stated otherwise, reactions were conducted in oven-dried glassware under an atmosphere of nitrogen or argon using anhydrous solvents (passed through activated alumina columns). All commercially obtained reagents were used as received. *N*-[(3-(anilinomethylene)-2-chloro-1-cyclohexen-1-yl)methylene]aniline monohydrochloride and Indocyanine Green were purchased from Sigma-Aldrich (St. Louis, MO), IR-Dye800CW was purchased from LI-COR (Lincoln, Nebraska). Flash column chromatography was performed using reversed phase (100 Å, 20-40 micron particle size, RediSep® Rf Gold® Reversed-phase C18 or C18Aq) and silica on a CombiFlash® Rf 200i (Teledyne Isco, Inc.). High-resolution LC/MS analyses were conducted on a Thermo-Fisher LTQ-Orbitrap-XL hybrid mass spectrometer system with an Ion MAX API electrospray ion source in negative ion mode. Analytical LC/MS was performed using a Shimadzu LCMS-2020 Single Quadrupole utilizing a Kinetex 2.6 µm C18 100 Å (2.1 x 50 mm) column obtained from Phenomenex, Inc. Runs employed a gradient of 0→90% MeCN/0.1% aqueous formic acid over 4.5 min at a flow rate of 0.2 mL/min. <sup>1</sup>H NMR and <sup>13</sup>C NMR spectra were recorded on Bruker spectrometers (at 400 or 500 MHz or at 100 or 125 MHz) and are reported relative to deuterated solvent signals. Data for <sup>1</sup>H NMR spectra are reported as follows: chemical shift (δ ppm), multiplicity, coupling constant (Hz), and integration. Data for <sup>13</sup>C NMR spectra are reported in terms of chemical shift. Absorption curves for quantum yield measurements were performed on a Shimadzu UV-2550 spectrophotometer operated by UVProbe 2.32 software. Fluorescence traces were recorded on a PTI QuantaMaster steady-state spectrofluorimeter operated by FelixGX 4.2.2 software, with 5 nm excitation and emission slit widths, 0.1 s integration rate, and enabled emission correction. Data analysis and curve fitting were performed using MS Excel 2011 and GraphPad Prism 8. See *JOC Standard Abbreviations and Acronyms* for abbreviation:

([http://pubs.acs.org/userimages/ContentEditor/1218717864819/jocea\\_h\\_abbreviations.pdf](http://pubs.acs.org/userimages/ContentEditor/1218717864819/jocea_h_abbreviations.pdf)).

## Experimental Procedures

Compound **1**, FNIR-774<sup>1</sup> and UL-766<sup>2</sup> were prepared as described previously.

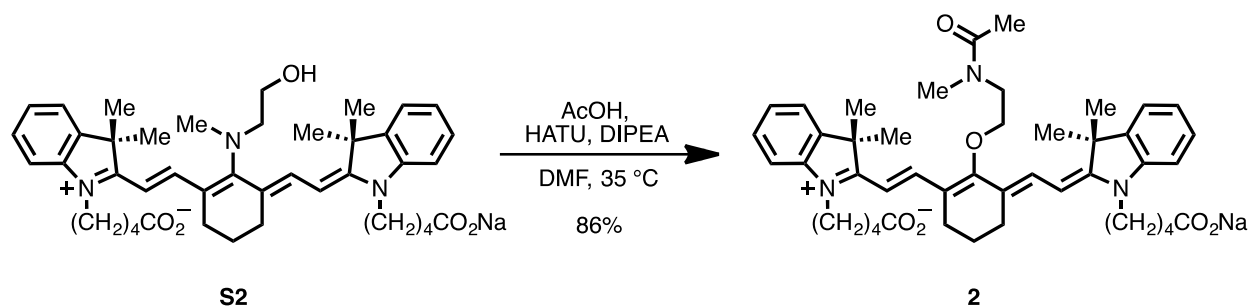


**S2**. **S1**<sup>3</sup> (47 mg, 0.072 mmol) was dissolved in dry MeCN (3.6 mL) in a microwave tube equipped with a magnetic stir bar and sealed. The solution was sparged with argon for 2 minutes. 2-(methylamino)ethanol (23  $\mu$ L, 0.29 mmol, 4 equiv) was added under argon and the reaction mixture was heated to 80  $^{\circ}$ C in a sand bath. After 10 minutes, the reaction changed color from green to dark blue and LC-MS indicated consumption of the starting material. The reaction mixture was concentrated by a gentle  $N_2$  stream and purified by flash chromatography (silica, 0  $\rightarrow$  15% MeOH/ $CH_2Cl_2$ ). The main blue fractions were combined and the solvent removed to afford **S2** (30.0 mg, 60% yield) as a deep blue film. <sup>1</sup>H NMR (400 MHz, Methanol-*d*<sub>4</sub>)  $\delta$  7.77 (d, *J* = 13.4 Hz, 2H), 7.39 (d, *J* = 7.4 Hz, 2H), 7.32 (t, *J* = 7.8 Hz, 2H), 7.17 – 7.10 (m, 4H), 5.94 (d, *J* = 13.4 Hz, 2H), 4.01 (t, *J* = 7.2 Hz, 4H), 3.89 (dd, *J* = 17.4, 5.0 Hz, 4H), 3.47 (s, 3H), 2.54 (t, *J* = 6.5 Hz, 4H), 2.33 (t, *J* = 7.0 Hz, 4H), 1.83 (dd, *J* = 13.9, 6.9 Hz, 6H), 1.77 – 1.72 (m, 4H), 1.65 (s, 12H) ppm; <sup>13</sup>C NMR (125 MHz, Methanol-*d*<sub>4</sub>)  $\delta$  178.97, 177.50, 170.39, 144.30, 144.07, 141.82, 129.49, 125.22, 124.39, 123.11, 110.61, 96.87, 61.15, 60.50, 45.03, 44.03, 36.08, 29.47, 27.51, 25.64, 24.07, 23.25 ppm; HRMS (ESI) calculated for C<sub>43</sub>H<sub>56</sub>N<sub>3</sub>O<sub>5</sub> (M+H)<sup>+</sup> 694.4214, observed 694.4206.

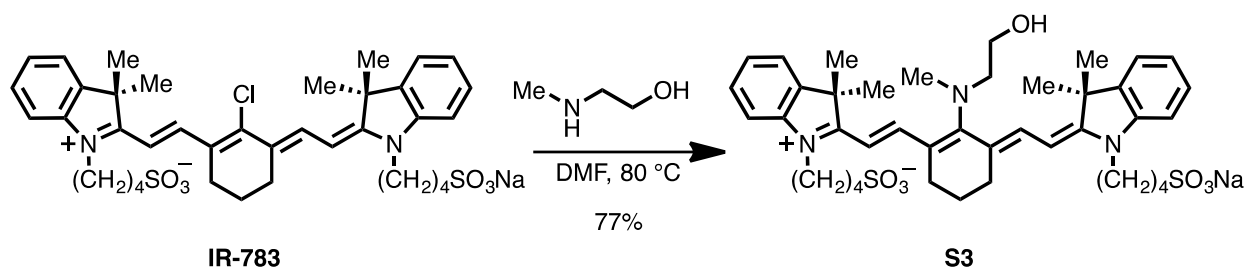
<sup>1</sup> Nani, R. R.; Shaum, J. B.; Gorka, A. P.; Schnermann, M. J. Electrophile integrating smiles rearrangement provides previously inaccessible C4-*O*-alkyl heptamethine cyanine fluorophores. *Org. Lett.* **2015**, 17 (2), 302.

<sup>2</sup> Cha, J.; Nani, R. R.; Luciano, M. P.; Kline, G.; Broch, A.; Kim, K.; Namgoong, J. M.; Kulkarni, R. A.; Meier, J. L.; Kim, P.; Schnermann, M. J. A chemically stable fluorescent marker of the ureter. *Bioorg. Med. Chem. Lett.* **2018**, 28, 2741.

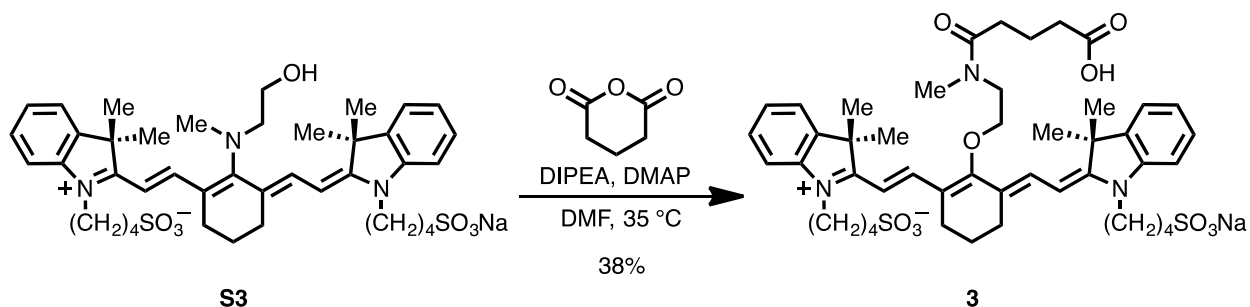
<sup>3</sup> Wang, L.; Jin, J.; Chen, X.; Fan, H.; Li, B. K. F.; Cheah, K.W.; Ding, N.; Ju, S.; Wong, W.T., Li, C. A cyanine based fluorophore emitting both single photon near-infrared fluorescence and two-photon deep red fluorescence in aqueous solution. *Org. Biomol. Chem.* **2012**, 10, 5366.



**2.** AcOH (7  $\mu\text{L}$ , 0.116 mmol) and HATU (40.0 mg, 0.105 mmol) were dissolved in dry DMF (1.0 mL) under argon in a 1-dram vial. DIPEA (20  $\mu\text{L}$ , 0.116 mmol) was added under argon and the light yellow reaction was stirred at ambient temperature for 10 minutes. In a separate 1-dram vial, **S2** (11.0 mg, 0.015 mmol) was dissolved in dry DMF (500  $\mu\text{L}$ ) and sparged with argon. The activated ester solution (150  $\mu\text{L}$ ) was added to the solution of **S2** under argon and heated to 35  $^\circ\text{C}$  in a sand bath for 1.5 h, during which time the color changed from blue to green and LC-MS indicated consumption of the starting material. The solvent was removed by centrifugal evaporation and the crude mixture was dissolved in saturated aqueous sodium bicarbonate (2 mL) and purified by reversed-phased flash chromatography ( $\text{C}_{18}\text{Aq}$ , 10  $\rightarrow$  70% MeCN/ $\text{H}_2\text{O}$ ). The main green fractions were combined and lyophilized to afford **2** (10.0 mg, 86% yield) as an iridescent green solid.  $^1\text{H}$  NMR (500 MHz, Methanol- $d_4$ , compound exists as a mixture of rotamers, major rotamer is designated using an asterisk (“\*”), and minor rotamers are denoted “ $\text{§}$ ”)  $\delta$  8.13 (two overlapping d,  $J = 14.1$  Hz,  $2\text{H}^*$ ,  $2\text{H}^\text{§}$ ), 7.49 (d,  $J = 7.4$  Hz,  $2\text{H}^*$ ,  $2\text{H}^\text{§}$ ), 7.43 – 7.38 (m,  $2\text{H}^*$ ,  $2\text{H}^\text{§}$ ), 7.32 (dd,  $J = 8.0, 2.7$  Hz,  $2\text{H}^*$ ,  $2\text{H}^\text{§}$ ), 7.24 (td,  $J = 7.5, 2.4$  Hz,  $2\text{H}^*$ ,  $2\text{H}^\text{§}$ ), 6.19 (d,  $J = 6.1$  Hz,  $2\text{H}^*$ ), 6.17 (d,  $J = 6.2$  Hz,  $2\text{H}^\text{§}$ ), 4.21-4.17 (m,  $2\text{H}^*$ ,  $2\text{H}^\text{§}$ ), 4.16-4.11 (m,  $4\text{H}^*$ ,  $4\text{H}^\text{§}$ ), 4.02-3.96 (m,  $2\text{H}^*$ ,  $2\text{H}^\text{§}$ ), 3.3 (s,  $3\text{H}^*$ ), 3.21 (s,  $3\text{H}^\text{§}$ ) 3.00 (s,  $3\text{H}^*$ ), 2.86 (s,  $3\text{H}^\text{§}$ ), 2.70 – 2.59 (m,  $4\text{H}^*$ ,  $4\text{H}^\text{§}$ ), 2.26 – 2.21 (m,  $4\text{H}^*$ ,  $4\text{H}^\text{§}$ ), 1.92 (d,  $J = 6.4$  Hz,  $2\text{H}^*$ ,  $2\text{H}^\text{§}$ ), 1.87-1.81 (m,  $4\text{H}^*$ ,  $4\text{H}^\text{§}$ ), 1.77-1.73 (m,  $4\text{H}^*$ ,  $4\text{H}^\text{§}$ ), 1.73 (s,  $12\text{H}^*$ ), 1.70 (s,  $12\text{H}^\text{§}$ ) ppm; HRMS (ESI) calculated for  $\text{C}_{45}\text{H}_{58}\text{N}_3\text{O}_6$  ( $\text{M}+\text{H}$ ) $^+$  736.4320, observed 736.4311.

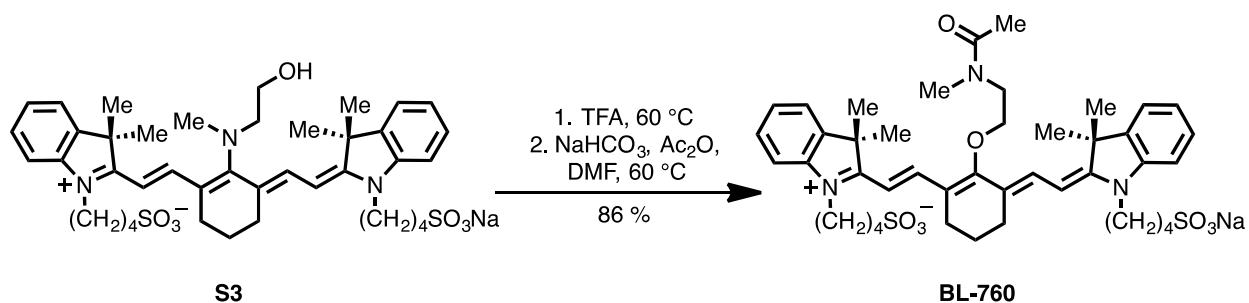


**S3. IR-783** (250 mg, 0.33 mmol) was dissolved in dry DMF (4.2 mL) in a microwave tube equipped with a magnetic stirrer bar and sealed. The solution was sparged with argon for 2 min, followed by the addition of 2-(Methylamino)ethanol (134  $\mu\text{L}$ , 1.7 mmol) under argon. The solution was heated to 80  $^\circ\text{C}$  in a sand bath for 10 min, during which time the color changed from green to blue and LC-MS indicated consumption of the starting material. The reaction mixture was cooled, precipitated into diethyl ether and then centrifuged at 4500 rpm for 5 min. The pellet was dissolved in water (8 mL) and purified by reversed-phase chromatography ( $\text{C}_{18}$ , 0  $\rightarrow$  40% MeCN/ $\text{H}_2\text{O}$ ). The product-containing fractions were combined and lyophilized to afford **S3** (202 mg, 77% yield) as a blue solid.  $^1\text{H}$  NMR (400 MHz, Methanol- $d_4$ )  $\delta$  7.77 (d,  $J = 13.3$  Hz, 2H), 7.37 (d,  $J = 7.4$  Hz, 2H), 7.32 (t,  $J = 7.7$  Hz, 2H), 7.16 (d,  $J = 8.0$  Hz, 2H), 7.11 (t,  $J = 7.5$  Hz, 2H), 5.96 (d,  $J = 13.4$  Hz, 2H), 4.1 – 4.0 (m, 4H), 3.95 – 3.85 (m, 4H), 3.48 (s, 3H), 2.87 (t,  $J = 6.8$  Hz, 4H), 2.55 (t,  $J = 6.6$  Hz, 4H), 2.05 – 1.86 (m, 8H), 1.85 (t,  $J = 6.5$  Hz, 2H), 1.65 (s, 12H) ppm;  $^{13}\text{C}$  NMR (125 MHz, Methanol- $d_4$ )  $\delta$  177.63, 170.31, 144.32, 144.08, 141.79, 129.49, 125.34, 124.33, 123.06, 110.66, 96.89, 61.20, 60.52, 52.01, 45.05, 44.01, 29.50, 26.90, 25.61, 23.68, 23.24 ppm; HRMS (ESI) calculated for  $\text{C}_{41}\text{H}_{56}\text{N}_3\text{O}_7\text{S}_2$  ( $\text{M}+\text{H}$ ) $^+$  766.3554, observed 766.3545.



**3. S3** (94 mg, 0.12 mmol), glutaric anhydride (41 mg, 0.36 mmol) and DMAP (4.4 mg, 0.04 mmol) were dissolved in dry DMF (2.5 mL) in a 20 mL reaction vial under argon. DIPEA (42  $\mu\text{L}$ , 0.24 mmol) was added under argon and the reaction mixture was heated to 35  $^\circ\text{C}$  for 24 h during which

time the color changed from deep blue to green. The reaction mixture was precipitated into diethyl ether and centrifuged for 5 min at 4500 RPM. The pellet was resuspended in H<sub>2</sub>O and directly purified by reversed-phase flash chromatography (C<sub>18</sub>, 0 → 40% MeCN/H<sub>2</sub>O). The main green fractions were pooled and lyophilized to afford **3** in 38% yield (40.0 mg) as an iridescent green solid. <sup>1</sup>H NMR (400 MHz, Methanol-*d*<sub>4</sub>, compound exists as a mixture of rotamers, major rotamer is designated using an asterisk (“\*”), and minor rotamers are denoted “§”) δ 8.13 (two overlapping d, *J* = 14.3 Hz, 2H\*, 2H§), 7.51 (two overlapping d, *J* = 12.9, 7.5, 1.2 Hz, 2H\*, 2H§), 7.40 (t, *J* = 7.5, 2H\*, 2H§), 7.32 (dd, *J* = 7.9, 2.2 Hz, 2H\*, 2H§), 7.24 (td, *J* = 7.4, 2.4, 2H\*, 2H§), 6.22 (d, *J* = 4.4 Hz, 2H\*), 6.18 (d, *J* = 4.4 Hz, 2H§), 4.21-4.12 (m, 6H\*, 6H§), 4.06-3.96 (m, 2H\*, 2H§), 3.3 (s, 3H\*), 3.2 (s, 3H§) 2.88 (t, *J* = 7.1, 4H\*, 4H§), 2.73 – 2.57 (m, 6H\*, 6H§), 2.41 (t, *J* = 7.0 Hz, 2H\*, 2H§), 2.35 (t, *J* = 7.0 Hz, 2H\*, 2H§), 2.03 – 1.90 (m, 12H\*, 12H§), 1.74 (s, 12H\*), 1.71 (s, 12H§) ppm; HRMS (ESI) calculated for C<sub>46</sub>H<sub>62</sub>N<sub>3</sub>O<sub>10</sub>S<sub>2</sub> (M+H)<sup>+</sup> 880.3877, observed 880.3850.



**BL-760.** To a 1-dram vial containing **S3** (115 mg, 0.15 mmol) and a magnetic stirrer bar was added TFA (1.5 mL) under argon. The purple reaction mixture was heated to 60 °C for 5 min and the TFA was removed by nitrogen stream and the residue was dried under vacuum for 30 min (< 0.1 Torr). NaHCO<sub>3</sub> (61 mg, 0.73 mmol) was added to the vial, followed by dry DMF (3.3 mL) and Ac<sub>2</sub>O (41 μL, 0.44 mmol) under argon. The reaction mixture was heated to 60 °C for 2 h, during which time LC-MS indicated formation of the desired product. The reaction mixture was precipitated into diethyl ether and centrifuged at 4500 rpm for 5 min. The pellet was dissolved in H<sub>2</sub>O (12 mL) and purified by reversed-phase chromatography (C<sub>18</sub>, 0→40% MeCN/H<sub>2</sub>O). The product containing fractions were combined and lyophilized to afford **BL-760** (104 mg, 86% yield) as an iridescent green solid. <sup>1</sup>H NMR (400 MHz, Methanol-*d*<sub>4</sub>, compound exists as a mixture of

rotamers, major rotamer is designated using an asterisk (“\*”), and minor rotamers are denoted (“§”)<sup>4</sup>  
δ 8.14 (two overlapping d,  $J = 14.2$  Hz, 2H\*, 2H§), 7.49 (d,  $J = 7.5$ , 2H\*, 2H§), 7.44 – 7.37 (m, 2H\*, 2H§), 7.35 – 7.31 (m, 2H\*, 2H§), 7.24 (t,  $J = 7.4$  Hz, 2H\*, 2H§), 6.22 (d,  $J = 5.9$  Hz, 2H\*), 6.19 (d,  $J = 5.8$  Hz, 2H§), 4.25 – 4.08 (m, 6H\*, 6H§), 4.07 – 3.87 (m, 2H\*, 2H§), 3.29 (s, 3H\*), 3.20 (s, 3H§), 2.88 (td,  $J = 7.2, 1.7$  Hz, 4H\*, 4H§), 2.69-2.63 (m, 4H\*, 4H§), 2.29 (s, 3H\*), 2.22 (s, 3H§), 2.03 – 1.89 (m, 10H\*, 10H§), 1.74 (s, 12H\*), 1.70 (s, 12H§) ppm; HR-MS (ESI) calculated for C<sub>43</sub>H<sub>59</sub>N<sub>3</sub>O<sub>8</sub>S<sub>2</sub> (M+2H)<sup>2+</sup> 404.6866, observed 404.6861.

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<sup>4</sup> The issue of the tertiary amide rotamers was described in ref 1.







