Supplementary Material (ESI) for Chemical Communications

Supplementary Information

"Development of 2,6-Carboxy-Substituted Boron Dipyrromethene (BODIPY) as a Novel Scaffold of Ratiometric Fluorescent Probes for Live Cell Imaging"

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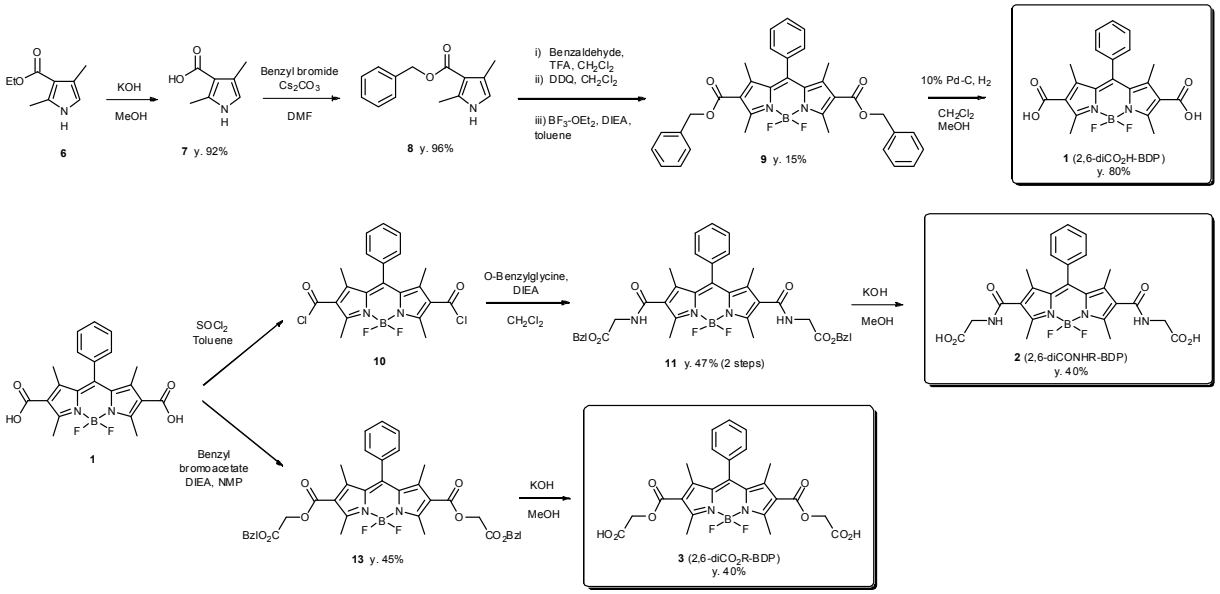
Material and general instruments. General chemicals were of the best grade available, supplied by Tokyo Chemical Industries, Wako Pure Chemical, Aldrich Chemical Co. or Astatech Inc., and were used without further purification. Porcine liver esterase was purchased from Sigma-Aldrich Japan. ^{1}H NMR and ^{13}C NMR spectra were recorded on a JEOL JNM-LA300 instrument (300 MHz for ^{1}H NMR and 75 MHz for ^{13}C NMR); δ values are in ppm relative to tetramethylsilane (TMS). Mass spectra (MS) were measured with a JEOL JMS-T100LC AccuTOF (ESI).

Scheme S1. Synthetic schemes for 2,6-diCO_2H-BDP (**1**) and derivatization to amide (**2**) and ester (**3**).



Preparation of 2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (7).

Ethyl 2,4-dimethyl-1*H*-pyrrole-3-carboxylate (**6**) was prepared according to the literature[1]. Ethyl 2,4-dimethyl-1*H*-pyrrole-3-carboxylate (2.10 g, 12.6 mmol) was dissolved in methanol (100 mL) and H_2O (20 mL). KOH (33.6 g) in methanol (100 mL) was added slowly (final 3 M), and the solution was refluxed for 20 hr. When TLC monitoring (silica; CH_2Cl_2) showed complete consumption of the reactant, the reaction mixture was evaporated, and the remaining solution was neutralized (to pH ~ 7) with 4 N HCl (aq), then acidified (to pH ~ 4) with 10% (v/v) AcOH (aq). Products were extracted with CH_2Cl_2 three times, and the mixed organic extracts were washed with brine, dried over Na_2SO_4, filtered, and evaporated. The crude product was purified by column chromatography over silica gel (silica; CH_2Cl_2-1% (v/v) AcOH) to afford **7** as a pale brown solid (1.62 g, yield 92%). The product was readily oxidized, so it was used immediately after purification. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.08 (s, 3H); 2.33 (s, 3H); 6.34 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 12.58; 13.48; 110.00; 114.46; 119.68; 135.09; 169.94. LRMS (ESI$^-$): *m/z* 138 $(M - H)^-$.

Preparation of benzyl 2,4-dimethyl-1*H*-pyrrole-3-carboxylate (8).

To a solution of **7** (1.59 g, 11.4 mmol) in *N*,*N*-dimethylformamide (DMF; 20 mL), Cs_2CO_3 (7.52 g, 23.1 mmol) was added at 0°C. Benzyl bromide (1.2 mL, 10.0 mmol) was added dropwise under an Ar atmosphere, and the solution was stirred at room temperature for 6 hr. When TLC monitoring (silica; CH_2Cl_2) showed complete consumption of **7**, the reaction mixture was poured into cooled phosphate buffer (pH ~ 7) on an ice bath. The product was extracted with CH_2Cl_2 three times, and the combined organic extracts were washed with brine, dried over Na_2SO_4, filtered, and evaporated. The crude product was purified by column chromatography over silica gel (CH_2Cl_2) to afford **8** as a colorless solid (2.19 g, yield 96%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.24 (s, 3H); 2.48 (s, 3H); 5.29 (s, 2H); 6.34 (s, 1H); 7.3-7.5 (m, 5H); 8.00 (br, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.76; 14.28; 65.06; 110.60; 114.23; 121.84; 127.76; 127.96; 128.44; 136.22; 137.08; 165.90. HRMS (ESI^-): *m/z* calcd for $(M - H)^-$, 228.1025; found, 228.1016.

Preparation of 2,6-diCO_2Bzl-BDP (9). **8** (370 mg, 1.60 mmol) and benzaldehyde (79 μL, 0.78 mmol) were dissolved in CH_2Cl_2 (50 mL). Trifluoroacetic acid (0.1 mL) was added under an Ar atmosphere, and the solution was stirred at room temperature for 12 hr. 2,3-Dichloro-5,6-dicyano-*p*-benzoquinone (DDQ; 178 mg, 0.78 mol) was added, and after 30 min of stirring, the reaction was washed with brine three times, dried over Na_2SO_4, filtered, and evaporated. The remaining red solid was purified on a filtration column (alumina). Acquired crude dipyrromethene was dissolved in toluene and *N*,*N*-diisopropyl-*N*-ethylamine (DIEA; 2 mL). BF_3-OEt_2 (1.5 mL) was added dropwise under an Ar atmosphere, and the solution was stirred at room temperature for 1 hr. Then AcOEt was added, and the mixture was washed with brine, dried over Na_2SO_4, filtered, and evaporated. The crude product was purified by column chromatography over silica gel (CH_2Cl_2), and recrystallized from CH_2Cl_2 to afford **9** as orange crystals (69 mg, yield 15%). Recrystallization was necessary to obtain photochemically pure 2,6-diCO_2H-BDP in the next step. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.64 (s, 6H); 2.82 (s, 6H); 5.26 (s, 4H); 7.25 (m, 2H); 7.3-7.4 (m, 10H); 7.52 (m, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 13.77; 15.11; 66.17; 122.21; 125.42; 127.68; 128.26; 128.60; 129.63; 129.73; 131.47; 134.30; 135.86; 145.98; 147.92; 159.73; 164.00. HRMS (ESI^+): *m/z* calcd for $(M + Na)^+$, 615.2243; found, 615.2276.

Preparation of 2,6-diCO_2H-BDP (1). **9** (100 mg, 0.17 mmol) was dissolved in CH_2Cl_2 / methanol, 2:1 (20 mL). After addition of a small amount of 10% Pd-C, the mixture was stirred under a H_2 atmosphere for 3 hr. When TLC monitoring (silica; CH_2Cl_2-0.1% (v/v) AcOH) showed that the formation of the product was complete, DMF (20 mL) was added to the solution, and the Pd-C was filtered off. Evaporation of the filtrate gave **1** as a red solid. Recrystallization from CH_2Cl_2 afforded the pure product as red crystals (56 mg, yield 80%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.58 (s, 6H); 2.71 (s, 6H); 7.42 (m, 2H); 7.58 (m, 3H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 13.11; 14.58; 127.64; 129.66; 129.88; 130.74; 133.55; 146.18; 146.70; 158.36; 164.93. HRMS (ESI^-): *m/z* calcd for $(M - H)^-$, 411.1328; found, 411.1333.

Preparation of 2,6-diCOCl-BDP (10). To a solution of **1** (35 mg, 0.085 mmol) in toluene (15 mL), $SOCl_2$ (3 mL) was added dropwise at 0°C. The solution was refluxed for 3 hr, then toluene was added, and solvents were removed *in vacuo*. The remaining crude product was used immediately without further purification. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.67 (s, 6H); 2.89 (s, 6H); 7.2-7.3 (m, 2H); 7.60 (m, 3H). LRMS (ESI^+): *m/z* 471 $(M + Na)^+$.

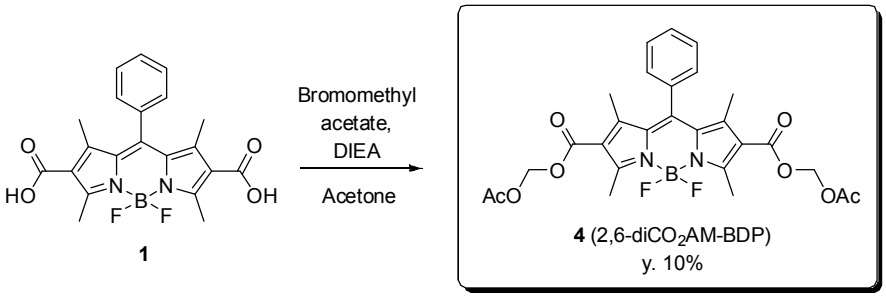
Preparation of 11. To a solution of **10** in CH_2Cl_2 (20 mL), a solution of *O*-benzylglycine *p*-TsOH salt (80 mg, 0.24 mmol) and DIEA (2 mL) in CH_2Cl_2 (10 mL) was added dropwise at 0°C. Pyridine (0.1 mL) was added slowly, and the mixture was stirred for 2 hr at room temperature. When TLC monitoring (silica; CH_2Cl_2-2% (v/v) methanol) showed complete consumption of **10**, CH_2Cl_2 was added, and the resulting solution was washed with 10% (v/v) AcOH (aq) and brine twice, dried over Na_2SO_4, filtered, and evaporated. The crude product was purified by column chromatography over silica gel (CH_2Cl_2-2% (v/v) methanol) to afford **11** as an orange solid (28 mg, yield 47%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.59 (s, 6H); 2.70 (s, 6H); 4.20 (d, 4H, J = 5.4 Hz); 5.18 (s, 4H); 6.02 (t, 2H, J = 5.4 Hz); 7.3-7.4 (m, 12H); 7.52 (m, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 13.02; 13.70; 41.52; 67.45; 127.60; 128.05; 128.50; 128.67; 129.65; 131.34; 134.20; 134.99; 142.22; 144.98; 155.77; 164.67; 169.67. HRMS (ESI^+): *m/z* calcd for $(M + Na)^+$, 729.2672; found, 729.2673.

Preparation of diCONHR-BDP (2). **11** (14 mg, 0.020 mmol) was dissolved in methanol (10 mL). KOH (2.2 g) in methanol (10 mL) was added slowly (final 2 M) at 0°C and the solution was stirred for 1 hr. When TLC monitoring (silica; CH_2Cl_2) showed complete consumption of **11**, the solution was acidified with 2 N HCl (aq), and the product was extracted with CH_2Cl_2. The organic layer was washed with brine twice, dried over Na_2SO_4, filtered, and evaporated. The crude product was purified by column chromatography over silica gel (silica; CH_2Cl_2-0.1% (v/v) AcOH) to afford **2** as a red solid (4.2 mg, yield 40%). ^{1}H NMR (300 MHz, CD_3OD): δ 1.52 (s, 6H); 2.64 (s, 6H); 4.00 (s, 4H); 7.38 (m, 2H); 7.60 (m, 3H). ^{13}C NMR (75 MHz, CD_3OD): δ 13.18; 13.58; 42.12; 129.08; 130.82; 132.41; 135.62; 143.36; 146.56; 156.51; 167.69; 172.92. HRMS (ESI^+): *m/z* calcd for $(M + Na)^+$, 549.1733; found, 549.1741.

Preparation of 13. To a solution of **1** (50 mg, 0.12 mmol) in 1-methylpyrrolidone (2 mL), DIEA (0.5 mL) was added at 0°C. Benzyl bromoacetate (50 μL, 0.31 mmol) was added dropwise, and the solution was stirred at room temperature for 30 min. When TLC monitoring (silica; CH_2Cl_2) showed complete consumption of **1**, CH_2Cl_2 was added, and the solution was washed with phosphate buffer (pH ~ 7) and brine twice, dried over Na_2SO_4, filtered, and evaporated. The crude product was purified by column chromatography over silica gel (CH_2Cl_2-2% (v/v) methanol) to afford **13** as an orange solid (38 mg, yield 45%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.67 (s, 6H); 2.85 (s, 6H); 4.80 (s, 4H); 5.20 (s, 4H); 7.3-7.4 (m, 12H); 7.55 (m, 3H). HRMS (ESI^+): *m/z* calcd for $(M + Na)^+$, 731.2352; found, 731.2318.

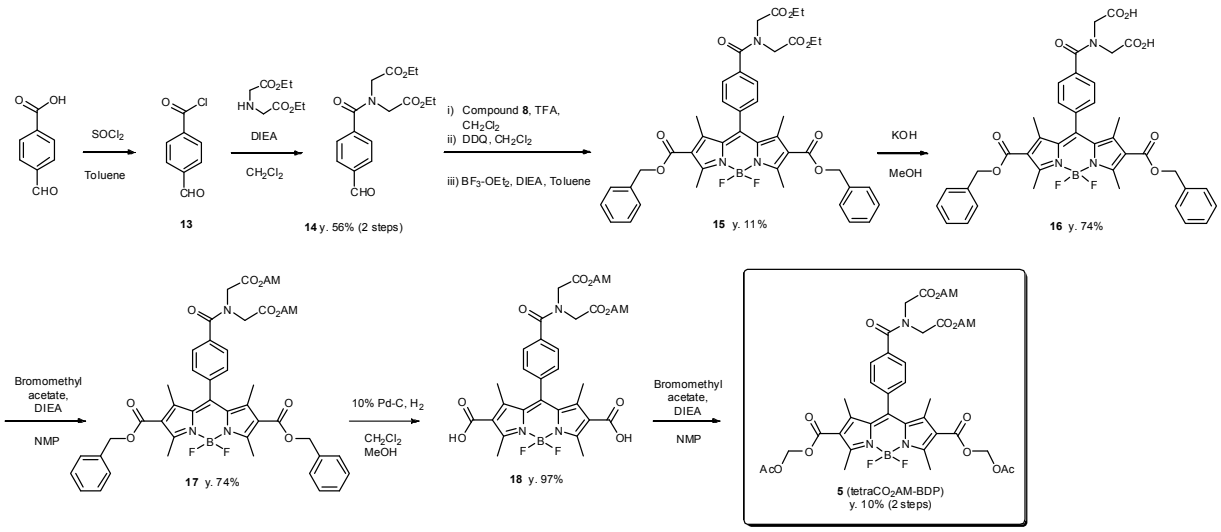
Preparation of 2,6-diCO_2R-BDP (3). **12** (20 mg, 0.028 mmol) was dissolved in methanol (10 mL). KOH (2.2 g) in methanol (10 mL) was added slowly (final 2 M) at 0°C, and the solution was stirred for 1 hr. When TLC monitoring (silica; CH_2Cl_2) showed complete consumption of **12**, the solution was acidified with 2 N HCl (aq), and the product was extracted with CH_2Cl_2. The organic layer was washed with brine twice, dried over Na_2SO_4, filtered, and evaporated. The crude product was purified by column chromatography over silica gel (silica; CH_2Cl_2-0.1% (v/v) AcOH) to afford **3** as a red solid (5.2 mg, yield 35%). ^{1}H NMR (300 MHz, CD_3OD): δ 1.61 (s, 6H); 2.71 (s, 6H); 4.67 (s, 4H); 7.30 (m, 2H); 7.52 (m, 3H). ^{13}C NMR (75 MHz, CD_3OD): δ 13.21; 14.58; 54.94; 122.13; 127.61; 130.06; 130.23; 131.00; 133.27; 147.51; 158.40; 162.77; 169.14. LRMS (ESI^-): *m/z* 527 $(M - H)^-$.

Scheme S2. Synthetic route to 2,6-diCO_2AM-BDP (**4**)



Preparation of 2,6-diCO_2AM-BDP (4). To a solution of **1** (15 mg, 0.035 mmol) in acetone (2 mL), DIEA (0.5 mL) was added at 0°C. Bromomethyl acetate (50 μL) was added dropwise, and the solution was refluxed at 90°C for 30 min. When TLC monitoring (silica; CH_2Cl_2) showed complete consumption of **1**, CH_2Cl_2 was added, and the solution was washed with 10% (v/v) AcOH (aq) and brine twice, dried over Na_2SO_4, filtered, and evaporated. The crude product was purified by column chromatography over silica gel (CH_2Cl_2), and recrystallized from CH_2Cl_2 / hexane to afford **4** as red crystals (2.0 mg, yield 10%). Recrystallization was necessary to obtain a photochemically pure product. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.66 (s, 6H); 2.10 (s, 6H); 2.84 (s, 6H); 5.88 (s, 4H); 7.25 (m, 2H); 7.55 (m, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 13.89; 20.76; 42.29; 54.09; 121.03; 127.56; 129.78; 129.95; 131.65; 134.00; 147.22; 149.17; 160.36; 162.60; 169.73. HRMS (ESI^+): *m/z* calcd for $(M + Na)^+$, 579.1721; found, 579.1729.

Scheme S3. Synthetic route to tetraCO_2AM-BDP (**5**)



Preparation of 4-formylbenzoyl chloride (13). To a solution of 4-formylbenzoic acid (500 mg, 3.3 mmol) in toluene (10 mL), $SOCl_2$ (8 mL) was added dropwise at 0°C. The solution was refluxed for 1 hr, then toluene was added, and the solvents were removed *in vacuo*. The crude product was used immediately without further purification.

Preparation of diethyl 2,2'-(4-formylbenzoylazanediyl)diacetate (14). To a solution of **13** in CH_2Cl_2 (15 mL), a solution of iminodiacetic acid diethyl ester (420 μl, 3.6 mmol) and DIEA (2 mL) in CH_2Cl_2 (15 mL) was added dropwise at 0°C, and the solution was stirred for 2 hr at room temperature. When TLC monitoring (silica; CH_2Cl_2-2% (v/v) methanol) showed complete consumption of **13**, CH_2Cl_2 was added, and the solution was washed with 2 N HCl (aq) and brine twice, dried over Na_2SO_4, filtered, and evaporated. The crude product was purified by column chromatography over silica gel (CH_2Cl_2) to afford **14** as a colorless liquid (606 mg, yield 56%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.26 (t, 3H, *J* = 7.2 Hz), 1.35 (t, 3H, *J* = 7.2 Hz), 4.06 (s, 2H), 4.2-4.3 (m, 4H), 4.33 (s, 2H), 7.61 (d, 2H, *J* = 8.1 Hz), 7.94 (d, 2H, *J* = 8.1 Hz), 10.06 (s, 1H). LRMS (ESI$^+$): *m/z* 344 $(M + Na)^+$.

Preparation of 15. **8** (300 mg, 1.3 mmol) and **14** (200 mg, 0.62 mmol) were dissolved in CH_2Cl_2 (50 mL). Trifluoroacetic acid (0.1 mL) was then added under an Ar atmosphere, and the solution was stirred at room temperature for 12 hr. DDQ (150 mg, 0.66 mmol) was added, and after 30 min of stirring, the reaction mixture was washed with brine three times, dried over Na_2SO_4, filtered, and evaporated. The remaining red solid was purified on a filtration column (alumina). Acquired crude dipyrromethene was dissolved in toluene and DIEA (2 mL). BF_3-OEt_2 (1.5 mL) was added dropwise under an Ar atmosphere, and the solution was stirred at room temperature for 1 hr. Then AcOEt was added, and the mixture was washed with brine, dried over Na_2SO_4, filtered, and evaporated. The crude product was purified by column chromatography over silica gel (CH_2Cl_2) to afford **15** as an orange solid (110 mg, yield 11%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.22 (t, 3H, *J* = 7.1 Hz); 1.33 (t, 3H, *J* = 7.1 Hz); 1.64 (s, 6H); 2.82 (s, 6H); 4.06 (s, 2H); 4.17 (q, 2H, *J* = 7.1 Hz); 4.26 (q, 2H, *J* = 7.1 Hz); 4.36 (s, 2H); 5.26 (s, 4H); 7.3-7.4 (m, 12H); 7.63 (d, 2H, J = 8.4 Hz). HRMS (ESI$^+$): *m/z* calcd for $(M + Na)^+$, 830.3036; found, 830.3029.

Preparation of 16. **15** (55 mg, 0.068 mmol) was dissolved in CH_2Cl_2 (5 mL) and methanol (5 mL). KOH (2.2 g) in methanol (10 mL) was added slowly (final 2 M) at 0°C, and the solution was stirred for 1 hr. When TLC monitoring (silica; CH_2Cl_2) showed complete consumption of **15**, the solution was acidified with 2 N HCl (aq), and the product was extracted with CH_2Cl_2. The organic layer was washed with brine twice, dried over Na_2SO_4, filtered, and evaporated. The crude product was purified by column chromatography over silica gel (silica; CH_2Cl_2-0.1% (v/v) AcOH) to afford **16** as a red solid (38 mg, yield 74%). ^{1}H NMR (300 MHz, CD_3OD): δ 1.69 (s, 6H); 2.74 (s, 6H); 4.07 (s, 2H); 4.31 (s, 2H); 5.26 (s, 4H); 7.3-7.4 (m, 10H); 7.47 (d, 2H, *J* = 8.4 Hz); 7.65 (d, 2H, *J* = 8.4 Hz). HRMS (ESI$^-$): *m/z* calcd for $(M - H)^-$, 750.2434; found, 750.2421.

Preparation of 17. To a solution of **16** (20 mg, 0.026 mmol) in 1-methylpyrrolidone (2 mL), DIEA (0.5 mL) was added at 0°C. Bromomethyl acetate (50 μL) was added dropwise, and the solution was stirred at room temperature for 30 min. When TLC monitoring (silica; CH_2Cl_2) showed complete consumption of **16**, CH_2Cl_2 was added, and the solution was washed with 10% (v/v) AcOH (aq) and brine twice, dried over Na_2SO_4, filtered, and evaporated. The crude product was purified by column chromatography over silica gel (CH_2Cl_2) to afford **17** (22 mg, 97%) as an orange solid. ^{1}H NMR (300 MHz, CDCl3): δ 1.64 (s, 6H); 2.06 (s, 3H); 2.16 (s, 3H); 2.82 (s, 6H); 4.12 (s, 2H); 4.40 (s, 2H); 5.27 (s, 4H); 5.73 (s, 2H); 5.84 (s, 2H); 7.3-7.4 (m, 12H); 7.62 (d, 2H, *J* = 8.1 Hz). HRMS (ESI$^+$): *m/z* calcd for $(M + Na)^+$, 918.2833; found, 918.2851.

Preparation of 18. **17** (15 mg, 0.017 mmol) was dissolved in CH_2Cl_2 / MeOH, 2:1 (20 mL). After the addition of a small amount of 10% Pd-C, the mixture was stirred under a H_2 atmosphere for 3 hr. When TLC monitoring (silica; CH_2Cl_2-0.1% (v/v) AcOH) showed complete formation of the product, the Pd-C was filtered off. Evaporation of the filtrate gave **18** as a red solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.59 (s, 6H); 2.00 (s, 3H); 2.09 (s, 3H); 2.72 (s, 6H); 4.19 (s, 2H); 4.36 (s, 2H); 7.49 (d, 2H, *J* = 8.1 Hz); 7.55 (d, 2H, *J* = 8.1 Hz). HRMS (ESI$^+$): *m/z* calcd for $(M + Na)^+$, 738.1894; found, 738.1829.

Preparation of tetraCO_2AM-BDP (5). To a solution of **18** (12 mg, 0.017 mmol) in 1-methylpyrrolidone (2 mL), DIEA (0.5 mL) was added at 0°C. Bromomethyl acetate (50 μL) was added dropwise, and the solution was stirred at room temperature for 30 min. When TLC monitoring (silica; CH_2Cl_2) showed complete consumption of **18**, CH_2Cl_2 was added, and the solution was washed with 10% (v/v) AcOH (aq) and brine twice, dried over Na_2SO_4, filtered, and evaporated. The crude product was purified by column chromatography over silica gel (CH_2Cl_2), and chromatographed on preparative TLC (silica) with CH_2Cl_2-AcOEt (2:1) as an eluent to give an orange solid (1.2 mg, 8.2%). Recrystallization was necessary to give photochemically pure product. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.67 (s, 6H); 2.10 (s, 3H); 2.11 (s, 6H); 2.17 (s, 3H); 2.84 (s, 6H); 4.13 (s, 2H); 4.41 (s, 2H); 5.78 (s, 2H); 5.84 (s, 2H); 5.89 (s, 4H); 7.37 (d, 2H, J = 8.4 Hz); 7.65 (d, 2H, J = 8.4 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 13.90; 15.23; 20.57; 29.71; 47.63; 51.53; 78.87; 121.41; 128.37; 131.34; 135.90; 136.38; 144.76; 148.62; 160.87; 162.42; 167.70; 169.30; 169.61; 169.68; 171.07. HRMS (ESI$^+$): *m/z* calcd for $(M + Na)^+$, 882.2317; found, 882.2270.

Calculation of apparent pK_a of 2,6-diCO_2H-BDP (1). Phosphate buffer (100 mM) containing 20% (v/v) DMSO was prepared with various pH values; the pH was measured after addition of DMSO. 2,6-diCO_2H-BDP (**1**) was dissolved in these solutions, and the absorbance ratio at 510 nm / 495 nm was calculated, and plotted against pH to determine the apparent pK_a of 2,6-diCO_2H-BDP.

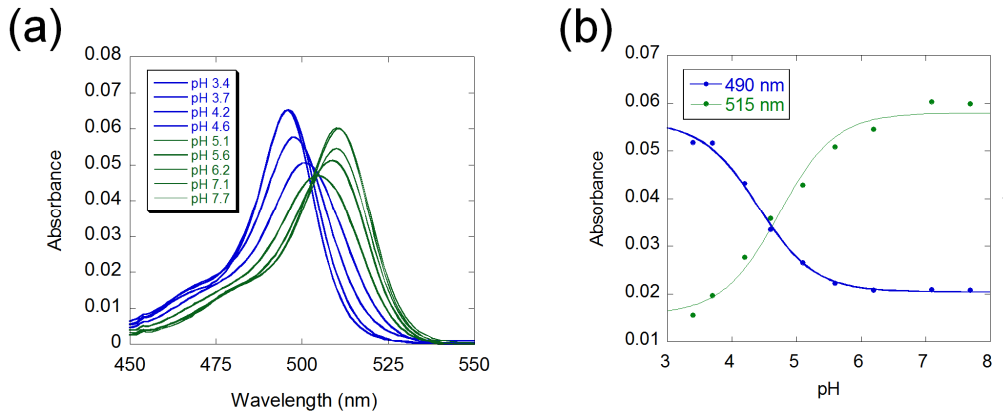


Figure S1. Absorption spectra of 2,6-diCO_2H-BDP (**1**) in phosphate buffer (100 mM) containing 20% (v/v) DMSO (left). The absorption ratio at 510 nm / 495 nm was calculated, and plotted against pH to determine the apparent pK_a of diCO_2H-BDP.

pK_a of two carboxylic acid groups. Since 2,6-diCO_2H-BDP has two carboxylic acid groups, there are theoretically three possible protonated/deprotonated forms, i.e., diCO_2H, CO_2^-/CO_2H, and diCO_2^- form. Close examination showed that the spectra in Figure S1 have two isosbestic points at 500 nm and 505 nm in such a narrow pH range that they apparently give a single isosbestic point. This means that the pK_a values of the two carboxylic acid groups are very similar.

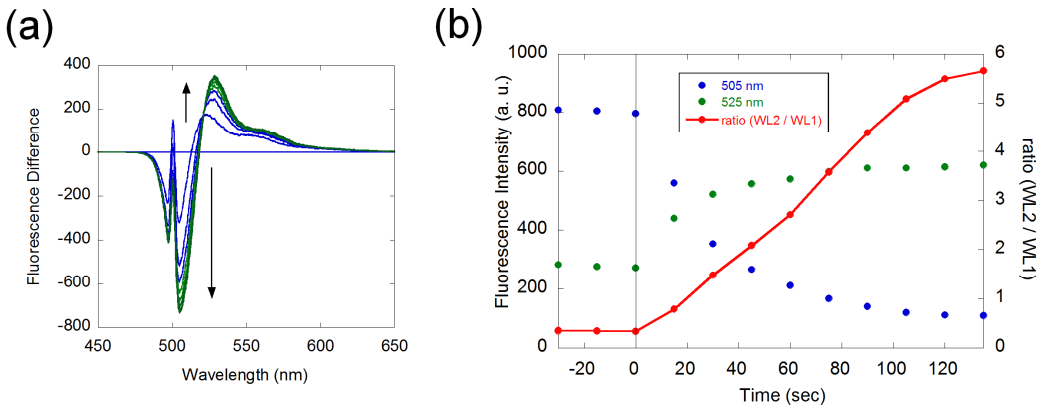
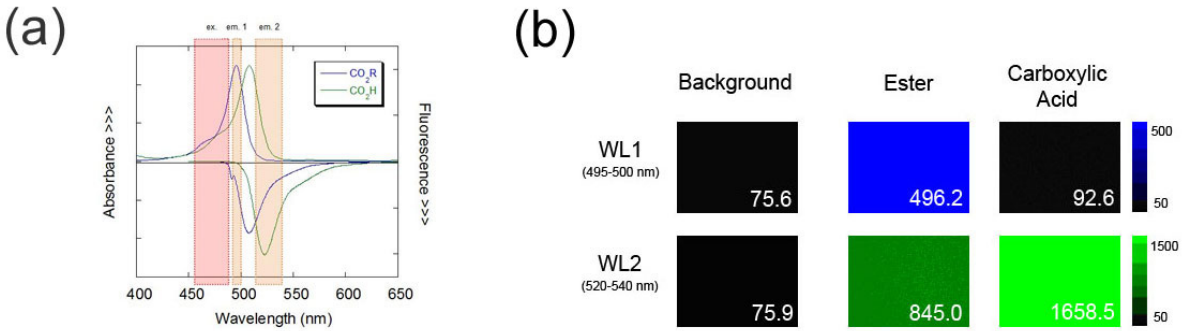


Figure S2. Fluorescence difference spectrum (from 0 min) (a) and time course of fluorescence change (b) in data in Figure 2 (b).



	Excitation (nm)	Emission (nm)
WL1	460–480	495–500
WL2		520–540

Figure S3. (a) Filter conditioning in ratiometric measurement. (b) Fluorescence images of HBSS (background), and 10 mM solution of 2,6-diCO_2R-BDP (ester, **3**) and 2,6-diCO_2H-BDP (carboxylic acid, **1**) in HBSS in each fluorescence emission channel. An ND filter (ND12) was used for image acquisition. The value in each square represents the average fluorescence intensity of the solution.

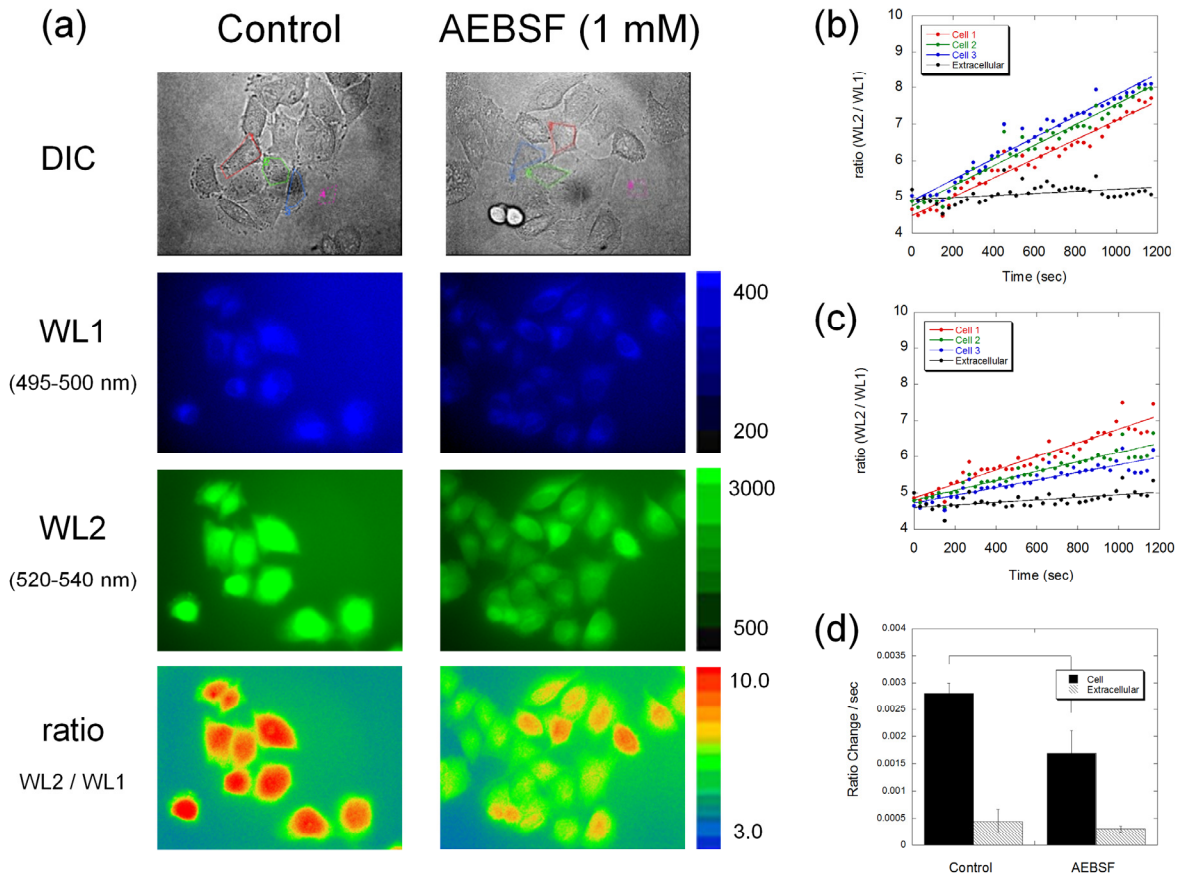


Figure S4. Bright-field transmission and fluorescence images of tetraCO_2AM-BDP (**5**, 10 μM)-loaded HeLa cells in HBSS, pre-incubated with or without AEBSF (1 mM) for 30 min. (a) Bright-field transmission, short wavelength fluorescence (WL1: 495-500 nm), long wavelength fluorescence (WL2: 520-540 nm), and ratio (WL2 / WL1) images at 30 min after probe addition. (b) The ratio increase of three randomly chosen cells in (a) (control). (c) The ratio increase of three randomly chosen cells in (a) (AEBSF treated cells). (d) Slope of ratio increase calculated for 3 independent cells each on 2 dishes (n = 6).

Supporting references. (SR1) *J. Am. Chem. Soc.* 2006, **128**, 10640-41.

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