Supporting Information for

A fluorescent photochromic compound for labeling biomolecules

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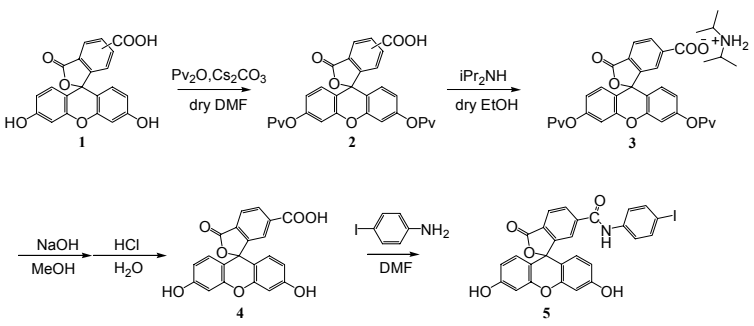
General.

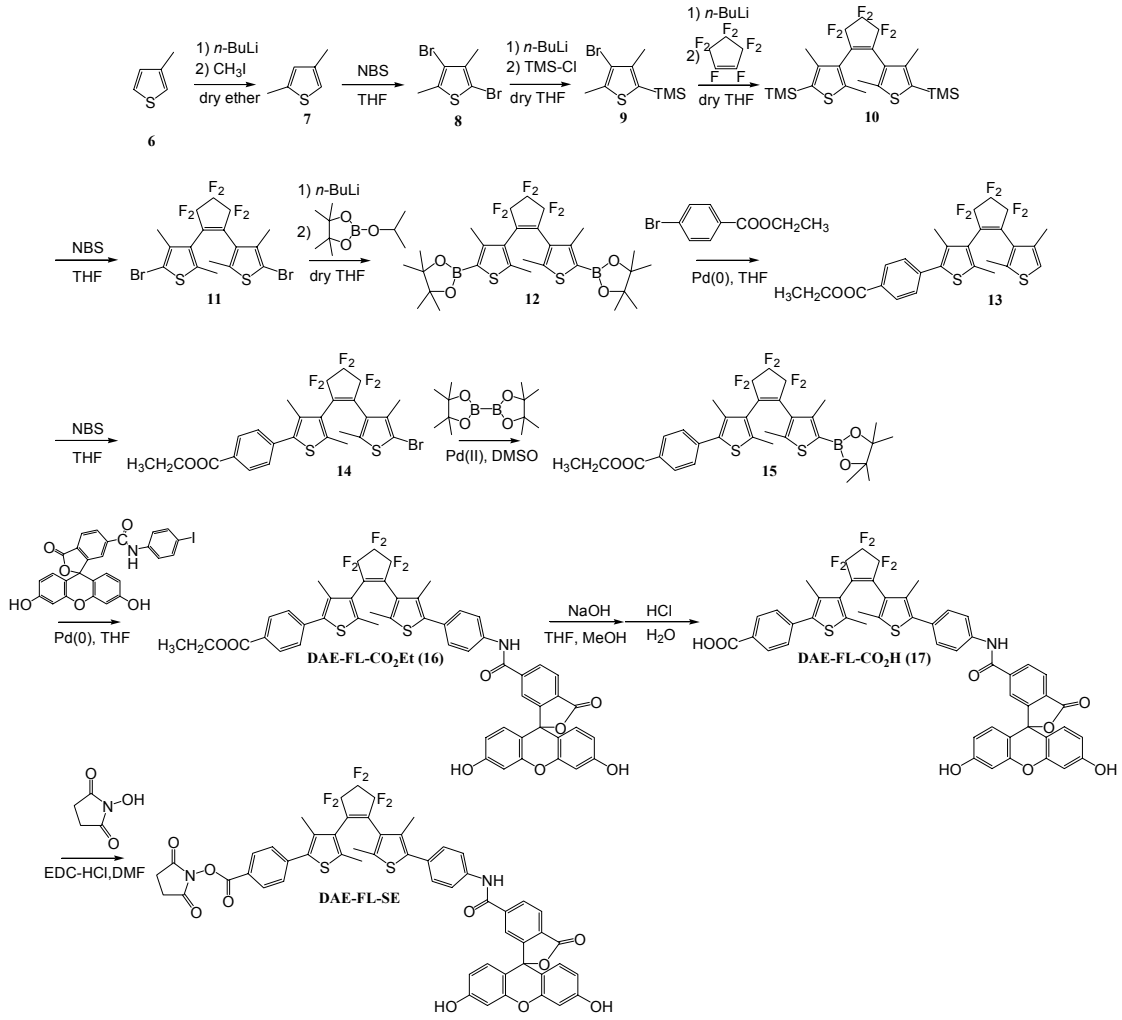
^{1}H NMR spectra were recorded on a NMR spectrometer (Bruker AVANCE-400, 400 MHz) and mass spectra were measured with a mass spectrometer (Shimadzu GCMS-OP5050A, JEOL JMS mate II, and Bruker autoflexII). Absorption and fluorescence spectra were measured with absorption spectrophotometer (JASCO V-560) and a fluorescence spectrophotometer (Shimadzu RF-5300PC), respectively. Photoirradiation was carried out using a UV light source (Moritex MUV-202) and a high-pressure mercury lamp (USHIO, 1000 W) by passing the light through appropriate band-pass filters.

Synthesis.

DAE-FL-SE was synthesized as illustrated in Scheme S1. Compounds **2**-**4**[1] and **6**-**10**[2, 3] were synthesized referring to previous reports.[1-3]

Scheme S1





4-(4-Iodophenylcarbamoyl)-2-(3-hydroxy-6-oxo-6H-xanthen-9-yl)benzoic acid (5).

To a solution of **4** (200 mg, 0.532 mmol) in DMF (6.6 mL), *p*-iodoaniline (1.75 g, 7.99 mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide, hydrochloride (340 mg, 1.77 mmol), and 1-hydroxybenzotriazole (232 mg, 1.72 mmol) were added and stirred overnight at 0 ^{o}C. 2N HCl was added to the reaction mixture and the mixture was extracted with ethyl acetate, dried over anhydrous magnesium sulfate, filtered, and concentrated. The residue was purified by silica gel column chromatography (hexane) to give an orange solid (210 mg, 68%). ^{1}H NMR (400 MHz, MeOD): δ 6.55-6.58 (d, 2H, Ar), 6.65-6.67 (t, 4H, Ar), 7.45-7.47 (d, 2H, Ar), 7.62-7.65 (d, 2H, Ar), 7.74 (s, 1H, Ar) , 8.11-8.13 (d, 1H, Ar), 8.22-8.24 (d, 1H, Ar); MS *m/z* = 578.579 $[M+H]^{+}$.

2-Bromo-4-(2-(5-bromo-2,4-dimethylthiophen-3-yl)-3,3,4,4,5,5-hexafluorocyclopent-1-enyl)-3,5-dimethylthiophene (11).

To a solution of **10** (1.90 g, 3.51 mmol) in THF (40 mL), *N*-bromosuccinimide was added at 0 ^{o}C. The mixture was refluxed overnight. The reaction was stopped with water and the reaction mixture was extracted with ether. The organic layer was dried over anhydrous magnesium sulfate, and concentrated. The residue was purified by silica gel column chromatography (hexane) to give a pink crystal (1.82 g, 94%). ^{1}H NMR (400 MHz, MeOD): δ 1.96-1.98 (m, 6H, CH_3), 2.24-2.25 (m, 6H, CH_3); MS *m/z* = 554 $[M]^+$.

2-(4-(3,3,4,4,5,5-Hexafluoro-2-(2,4-dimethyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)thiophen-3-yl)cyclopent-1-enyl)-3,5-dimethylthiophen-2-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (12).

To a solution of compound **11** (778 mg, 1.40 mmol) in dry THF (11 mL), *n*-BuLi (1.6 M in hexane, 1.90 mL, 3.04 mmol) was added dropwise at -78 ^{o}C and stirred for 1.5 h at that temperature. Then 2-isopropoxy-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (0.70 mL, 3.53 mmol) in dry THF (4 mL) was added slowly and stirred for 2 h. The reaction mixture was allowed to warm up to room temperature. Water was added to the reaction mixture and extracted with ether, washed with water, dried over anhydrous magnesium sulfate, filtered, and concentrated. The residue was recrystallized from hexane to give a white crystal (546 mg, 60%). ^{1}H NMR (400 MHz, MeOD): δ 1.30 (s, 24H, CH_3), 2.17-2.23 (m, 6H, CH_3) 2.32-2.38 (m, 6H, CH_3); MS *m/z* = 648 $[M]^+$.

Ethyl 4-(3,5-dimethyl-4-(2-(2,4-dimethylthiophen-3-yl)cyclopent-1-enyl)thiophen-2-yl)benzoate (13).

To a solution of **12** (1.00 g, 1.54 mmol) in THF (100 mL) and 2N Na_2CO_3 (100 mL), 4-bromobenzoic acid ethyl ester (168 μL, 1.00 mmol) and $Pd(PPh_3)_4$ (340 mg) were added and refluxed for 23 h. The reaction mixture was extracted with ether. The organic layer was dried over anhydrous magnesium sulfate, filtered, and concentrated. The residue was purified by silica gel column chromatography (chloroform/hexane, 1/1) to give a purple oil (179 mg, 21%). ^{1}H NMR (400 MHz, MeOD): δ 1.38-1.42 (t, 3H, CH_3), 2.07-2.09 (m, 6H, CH_3), 2.33-2.35 (m, 6H, CH_3), 4.36-4.42 (m, 2H, CH_2), 6.74 (d, 1H), 7.40-7.42 (d, 2H, Ar), 8.04-8.06 (d, 2H, Ar); MS *m/z* = 544 $[M]^+$.

Ethyl 4-(4-(2-(5-bromo-2,4-dimethylthiophen-3-yl)-3,3,4,4,5,5-hexafluorocyclopent-1-enyl)-3,5-dimethylthiophen-2-yl)benzoate (14).

To a solution of **13** (306 mg, 0.563 mmol) in THF (1.2 mL), *N*-bromosuccinimide (150 mg, 0.843 mmol) was added and the mixture was stirred at room temperature for 4 h. The reaction was stopped with water and the reaction mixture was extracted with ether. The organic layer was dried over anhydrous magnesium sulfate, filtered, and concentrated. The residue was purified by silica

gel column chromatography (chloroform/hexane, 1/1) to give a purple oil (281 mg, 80%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.39-1.42 (t, 3H, CH_3), 1.99-2.10 (m, 6H, CH_3), 2.28-2.35 (m, 6H, CH_3), 4.37-4.42 (m, 2H, CH_2), 7.42-7.44 (d, 2H, Ar), 8.05-8.07 (d, 2H, Ar).

4-(4-(4-(2-(5-(4-(Ethoxycarbonyl)phenyl)-2,4-dimethylthiophen-3-yl)-3,3,4,4,5,5-hexafluorocyclopent-1-enyl)-3,5-dimethylthiophen-2-yl)phenylcarbamoyl)-2-(3-hydroxy-6-oxo-6H-xanthen-9-yl)benzoic acid (DAE-FL-CO_2Et) (16).

To a solution of **14** (281 mg, 0.450 mmol) in DMSO (3 mL), bis(pinacolato)diboron (171 mg, 0.673 mmol), potassium acetate (133 mg, 1.36 mmol), and dichloro[1,1'-ferrocenylbis(diphenyl-phosphine)]palladium(II) dichloromethane (11.0 mg, 13.5 μmol) were added and stirred for 4 h at 80 ^{o}C. The reaction mixture was extracted with ether. The organic layer was dried over anhydrous magnesium sulfate, filtered, and concentrated to give ethyl 4-(4-(3,3,4,4,5,5-hexafluoro-2-(2,4-dimethyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl) thiophen-3-yl)cyclopent-1-enyl)-3,5-dimethylthiophen-2-yl)benzoate (**15**) as a black oil.

To a solution of **15** in THF (30 mL), **5** (210 mg, 0.364 mmol), $Pd(PPh_3)_4$ (105 mg), and 2N Na_2CO_3 (30 mL) were added and refluxed overnight. The organic layer was dried over anhydrous magnesium sulfate, filtered, and concentrated. The residue was purified by silica gel column chromatography (dichloromethane/methanol, 9/1) to give an ocher powder (105 mg, 29%). ^{1}H NMR (400 MHz, MeOD): δ 1.36-1.40 (t, 3H, CH_3), 2.07-2.11 (m, 6H, CH_3), 2.35-2.42 (m, 6H, CH_3), 4.34-4.39 (m, 2H, CH_2), 6.56-6.59 (m, 2H, Ar), 6.69-6.70 (m, 4H, Ar), 7.32-7.34 (m, 2H, Ar), 7.48-7.51 (m, 2H, Ar), 7.69-7.71 (d, 2H, Ar), 7.76 (s, 1H, Ar), 8.02-8.05 (m, 2H, Ar), 8.12-8.14 (d, 1H, Ar), 8.12-8.14 (d, 1H, Ar); HRMS (FAB^+) *m/z* = 993.1892 $[M]^+$ (calcd. for $C_{53}H_{37}F_6NO_8S_2$: 993.1865).

DAE-FL-CO_2H (17).

To a solution of **16** in THF (90 μL) and methanol (90 μL), 2N NaOH (60 μL) was added and stirred for 24 h at room temperature. 2N HCl (90 μL) was added to the reaction mixture and the mixture was extracted with ethyl acetate, dried over anhydrous magnesium sulfate, and concentrated to give an orange solid quantitatively. ^{1}H NMR (400 MHz, MeOD): δ 2.05-2.14 (m, 6H, CH_3), 2.34-2.38 (m, 6H, CH_3), 6.53-6.56 (m, 2H, Ar), 6.61-6.64 (m, 2H, Ar), 6.69 (d, 2H, Ar), 7.26-7.29 (m, 2H, Ar), 7.42-7.46 (t, 2H, Ar), 7.64-7.66 (m, 2H, Ar), 7.74 (s, 1H, Ar), 7.99-8.03 (m, 2H, Ar), 8.09-8.11 (d, 1H, Ar), 8.21-8.23 (d, 1H, Ar); HRMS *m/z* = 966.1622 $[M+H]^+$ (calcd. for $C_{51}H_{33}F_6NO_8S_2$: 965.1552).

DAE-FL-SE.

To a solution of **17** (18 mg, 19 μmol) in DMF (120 μL), *N*-hydroxysuccinimide (8 mg, 70 μmol),

1-ethyl-3-(3-dimethylaminopropyl)carbodiimide, and hydrochloride (8 mg, 42 μmol) were added and stirred for 13 h at room temperature. 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide, hydrochloride (8 mg, 42 μmol) was added to the reaction mixture and the mixture was stirred for 3 days at room temperature to give a yellow solid. MS *m/z* = 1062.182 $[M]^+$.

Labeling of ERK protein with DAE-FL-SE and SDS-PAGE.

10 mM DAE-FL-SE (20 μL) and 14.3 μM ERK (Upstate (Millipore)) (14 μL) were mixed in 200 mM Na_2CO_3-$NaHCO_3$ buffer (26 μL) and shaken for 12 h at room temperature. To the reaction mixture, water (440 μL) was added, and the mixture was purified by Microcon (Millipore). The resultant residue was mixed with sample buffer (NuPAGE LDS sample buffer, Invitrogen), heated at 90°C for 3 min, and the samples contain different amounts of proteins (5 μg, 1 μg, 0.5 μg, 0.1 μg, respectively) were loaded on the gel (SuperSep™10%, Wako) with ERK (1 μg) and protein markers (Molecular Weight Marker, High Range, Wako). After electrophoresis, fluorescent bands in the gel were taken by a molecular imager system (PharosFX, BIO-RAD) and proteins in the gel were then stained by quick-CBB plus (Wako).

Absorption and fluorescence spectral data for DAE-FL-CO_2Et (16).

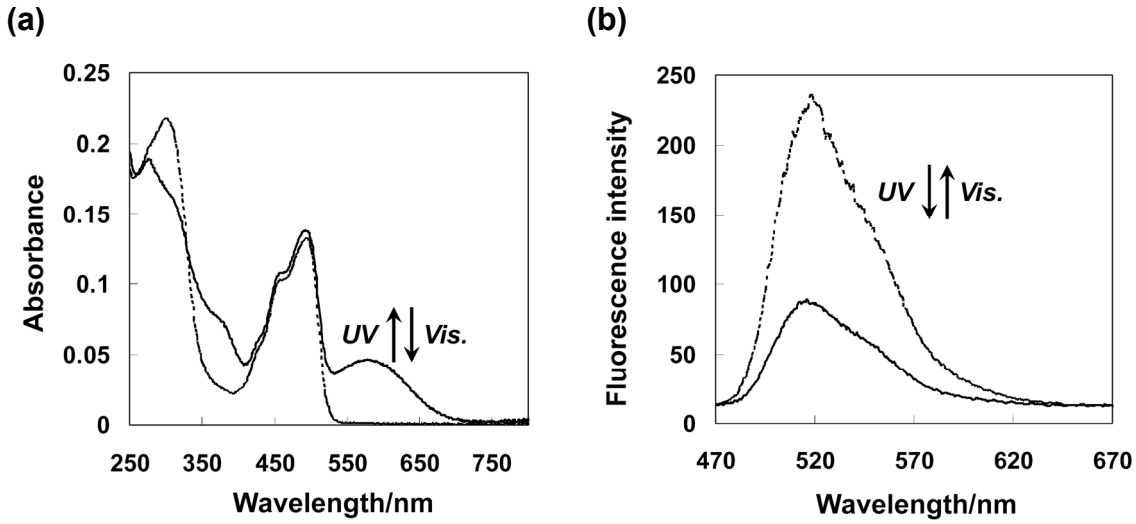


Fig. S1 (a) Absorption and (b) fluorescence spectral changes of DAE-FL-CO_2Et (**16**) in methanol upon irradiation with visible and UV light: open-ring isomer (dashed line), photostationary state (solid line).

^{1}H NMR spectral data (1.95 -2.55 ppm) for DAE-FL-CO_2Et (16).

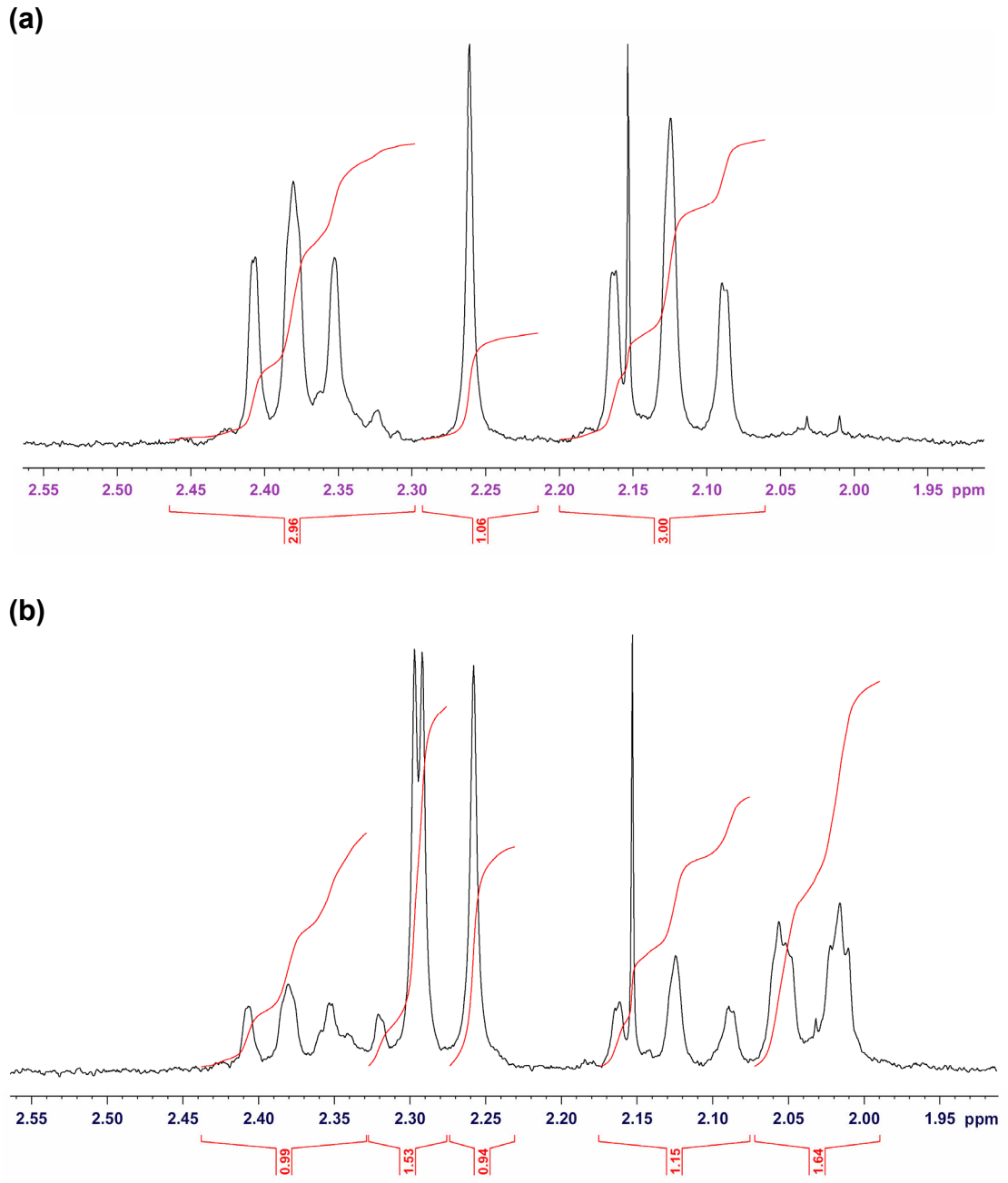


Fig. S2 ^{1}H NMR spectral changes (1.95-2.55 ppm) of DAE-FL-CO_2Et (**16**) in methanol-*d*4: (a) open-ring isomer, (b) photostationary state.

References.

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