Electronic Supplementary Information (ESI)

A green-light-emitting, spontaneously blinking fluorophore based on intramolecular spirocyclization for dual-colour super-resolution imaging

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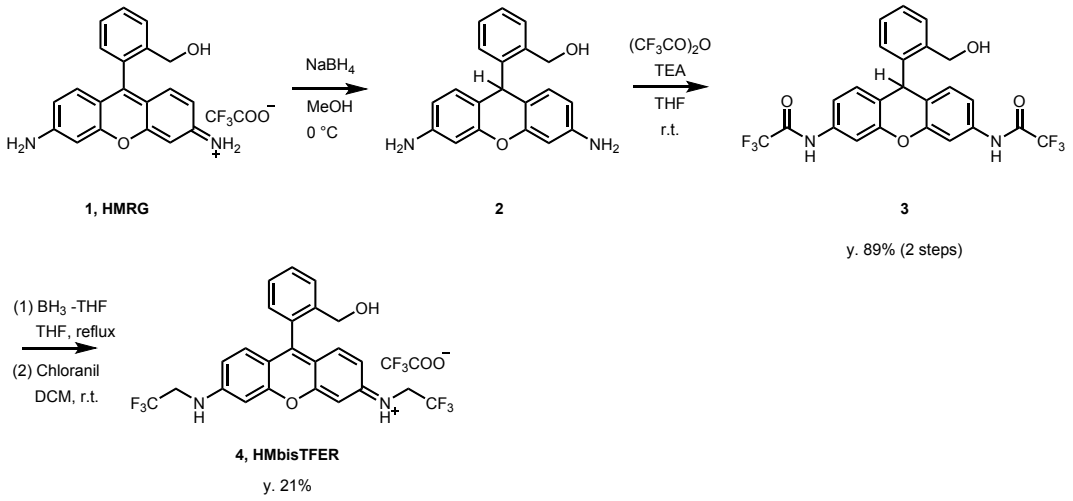
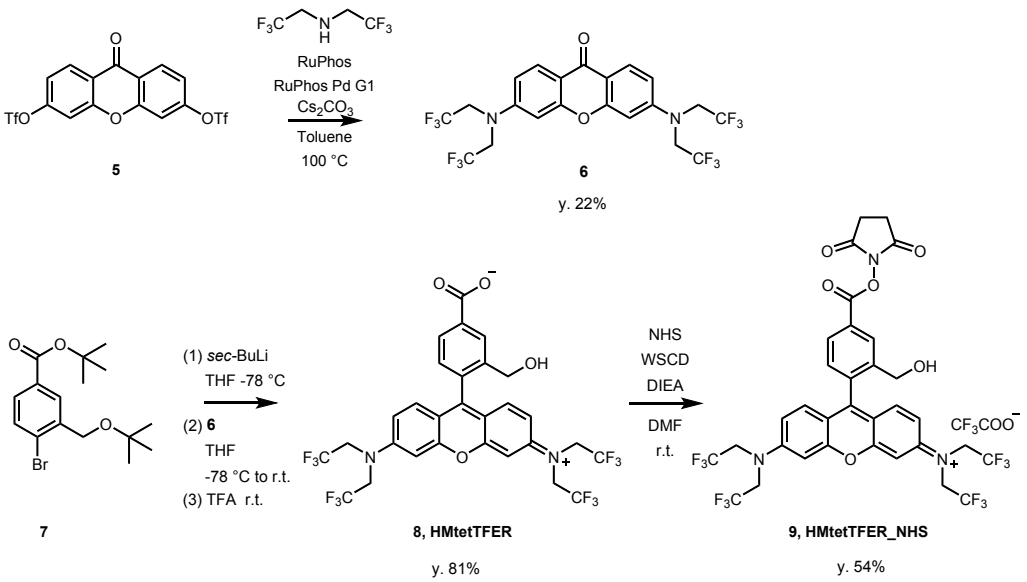
Materials and general information

General chemicals were purchased from commercial suppliers (Wako Pure Chemical, Tokyo Chemical Industries, Aldrich Chemical Company and Dojindo) and were used without further purification. The composition of mixed solvents is given as volume ratio (v/v). Flash chromatography separation was undertaken using an EPCLC-AI-580S chromatograph (Yamazen, Osaka, Japan). ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on a Bruker AVANCEIII 400 (400 MHz for ^{1}H and 101 MHz for ^{13}C) with chemical shifts (δ) reported in ppm relative to the residual solvent signals of $CDCl_3$ (7.26 ppm for ^{1}H, 77.16 ppm for ^{13}C), CD_3OD (3.31 ppm for ^{1}H, 49.00 ppm for ^{13}C), or acetone-*d*6 (2.05 ppm for ^{1}H, 29.84 ppm for ^{13}C). Coupling constants are reported in Hz. High-resolution mass spectra (HRMS) were measured on a Bruker micrOTOFII with electron spray ionization (ESI). HPLC purification and analyses were performed on a reverse-phase column (GL Sciences (Tokyo, Japan), Inertsil ODS-3 10 mm × 250 mm and Inertsil ODS-3 20 mm × 250 mm for purification and Inertsil ODS-3 4.6 mm × 250 mm for analyses) using an HPLC system composed of a pump (Jasco PU-2080 or PU-2087) and a detector (Jasco MD-2010 or MD-2018).

Synthetic procedures



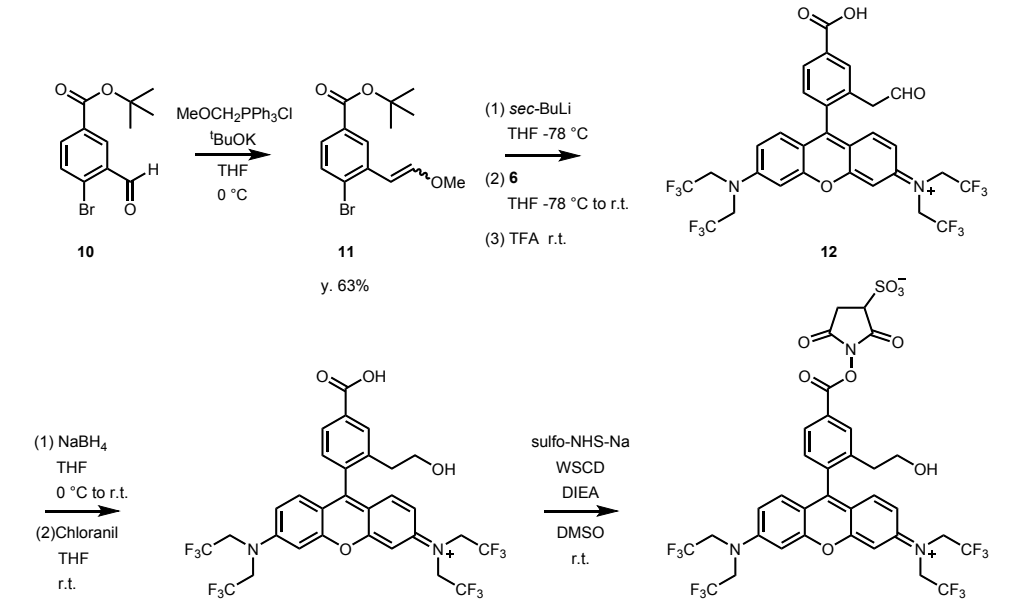
Scheme S1 Synthetic scheme of HMbisTFER



5 **6** **7** **8, HMtetTFER** **9, HMtetTFER_NHS**

y. 81% y. 54%

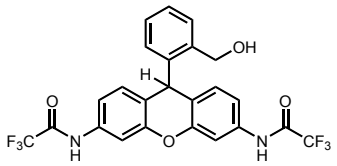
Scheme S2 Synthetic scheme of HMtetTFER



10 **11** **12** **13, HEtetTFER** **14, HEtetTFER-sulfoNHS**

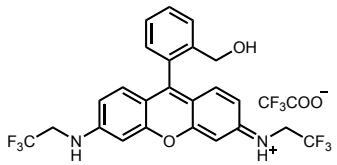
y. 63% y. 23% (5 steps) y. 19%

Scheme S3 Synthetic scheme of HEtetTFER



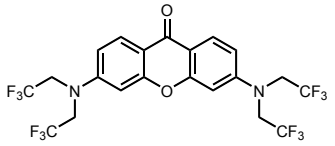
Compound 3: To a solution of HMRG (1)[1] (30.1 mg, 0.095 mmol, 1 eq) in MeOH (5 mL) was added $NaBH_4$ (60 mg) at 0 °C. The solution was stirred at 0 °C for 10 min, then quenched with water (2 mL). The resulting solution was diluted with sat. NH_4Cl and extracted with AcOEt. The organic extract was washed with brine, dried over Na_2SO_4, filtered, and evaporated. The residue was dissolved in THF (3 mL), and triethylamine (56.9 μL, 0.4 mmol, 5 eq) was added. The solution was cooled to 0 °C, and trifluoroacetic anhydride (57.6 μL, 0.4 mmol, 5 eq) was added. The reaction mixture was stirred at room temperature for 13 h, then the solvent was evaporated. The residue was purified by flash column chromatography (silica gel, *n*-hexane/EtOAc = 67/33 to 46/54) to give **compound 3** (31.6 mg, 89%).

^{1}H NMR (400 MHz, CD_3OD): δ 7.53 (d, *J* = 2.1 Hz, 2 H), 7.44-7.42 (m, 1 H), 7.21-16 (m, 4 H), 7.03-7.01 (m, 1 H), 6.98 (d, *J* = 8.5 Hz, 2 H), 5.65 (s, 1 H), 4.73 (s, 2 H); ^{13}C NMR (101 MHz, CD_3OD): δ 156.7 (q, $^2J_{C\text{-}F}$ = 37.6 Hz, CO), 151.9 (C), 145.8 (C), 139.5 (C), 137.4 (C), 132.0 (CH), 131.4 (CH), 129.7 (CH), 129.5 (CH), 127.9 (CH), 123.1 (C), 117.4 (q, $^1J_{C\text{-}F}$ = 289.9 Hz, CF_3), 117.1 (CH), 109.8 (CH), 63.4 (CH_2), 39.9 (CH); HRMS (m/z): $[M+Na]^+$ calcd. for $C_{24}H_{16}F_6N_2NaO_4$, 533.09065; found, 533.09010.



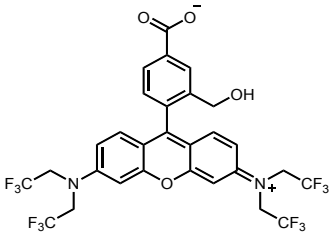
Compound 4, HMbisTFER: To a solution of **compound 3** (18.0 mg, 0.035 mmol, 1 eq) in THF (5 mL) was added 1 M borane tetrahydrofuran complex solution (1 mL) at 0 °C, and the solution was refluxed with stirring under an argon atmosphere for 21 h. The solution was cooled to room temperature, then MeOH (3mL) was added and the solvent was evaporated. The residue was diluted in EtOAc, and the organic solution was washed with water and brine, dried over Na_2SO_4, filtered, and evaporated. The residue was dissolved in DCM (3 mL), and chloranil (10 mg) was added. The solution was stirred at room temperature for 1 h, and the solvent was evaporated. The residue was purified by semi-preparative HPLC using eluent A (H_2O with 0.1% TFA and 1% CH_3CN) and eluent B (CH_3CN with 1% H_2O) (A/B = 90/10 to 0/100 for 40 min) to give **compound 4, HMbisTFER** (4.5 mg, 21%).

^{1}H NMR (400 MHz, CD_3OD): δ 7.74 (d, *J* = 7.1 Hz, 1 H), 7.70 (dt, *J* = 7.6, 1.3 Hz, 1 H), 7.59 (dt, *J* = 7.5, 1.3 Hz, 1 H), 7.33-7.28 (m, 3 H), 7.19 (s, 2 H), 7.06 (d, *J* = 8.6 Hz, 2 H), 4.35 (s, 2 H), 4.28 (q, $^2J_{H\text{-}F}$ = 9.0 Hz, 4 H); HRMS (m/z): $[M]^+$ calcd. for $C_{24}H_{19}F_6N_2O_2$, 481.13507; found, 481.13504.



Compound 6: A double-necked flask was charged with **compound 5**[2] (149.7 mg, 0.30 mmol, 1 eq), Ruphos (14.0 mg, 0.03 mmol, 0.1 eq), RuPhos Pd G1 (21.9 mg, 0.03 mmol, 0.1 eq), Cs_2CO_3 (249.9 mg, 0.76 mmol, 2.5 eq). The flask was evacuated and backfilled with argon three times. A solution of bis(2,2,2-trifluoroethyl)amine (500 μL, 3.0 mmol, 10 eq) in anhydrous toluene (10 mL) was added. The reaction mixture was then stirred at 100 °C for 19 h. The mixture was cooled to room temperature, diluted with AcOEt, washed with water and brine, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by flash column chromatography (silica gel, n-hexane/EtOAc = 88/12 to 67/33) to give **compound 6** (37.3 mg, 22%).

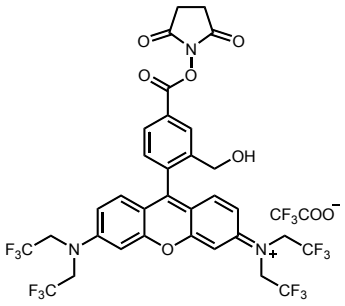
^{1}H NMR (400 MHz, $CDCl_3$): δ 8.21 (d, *J* = 9.0 Hz, 2 H), 6.90 (dd, *J* = 9.0, 2.6 Hz, 2 H), 6.83 (d, *J* = 2.6 Hz, 2 H), 4.18 (q, $^2J_{H\text{-}F}$ = 8.3 Hz, 8 H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 174.9 (C), 157.9 (C), 151.4 (C), 128.5 (CH), 125.0 (q, $^1J_{C\text{-}F}$ = 284.3 Hz, CF_3), 115.2 (C), 110.3 (CH), 100.5 (CH), 51.6 (q, $^2J_{C\text{-}F}$ = 33.9 Hz, CH_2); ^{19}F NMR (376 MHz, $CDCl_3$ + C_6F_6 as an internal standard): δ -70.2 (t, $^2J_{H\text{-}F}$ = 8.1 Hz); HRMS (m/z): $[M+Na]^+$ calcd. for $C_{21}H_{14}F_{12}N_2NaO_2$, 577.07614; found, 577.07488.



Compound 8, HMtetTFER: In an argon-flushed flask fitted with a septum cap, **compound 7**[3] (88.9 mg, 0.26 mmol, 5 eq) was dissolved in dry THF (8 ml) and the solution was cooled to –78 °C. A 1 M *sec*-BuLi solution in cyclohexane and *n*-hexane (260 μL, 0.26 mmol, 5 eq) was slowly added dropwise with stirring, and stirring was continued at the same temperature for 15 min. **Compound 6** (28.7 mg, 0.052 mmol, 1 eq) in dry THF (6 mL) was added as a bolus via a syringe at -78 °C, and the solution was further stirred at -78 °C for 15 min and room temperature for 5 h. Then, 1 N HCl aq. was added to the reaction mixture, and the solution was basified with saturated $NaHCO_3$ aqueous solution and extracted twice with EtOAc. The combined organic phase was washed with brine, dried over Na_2SO_4, filtered, and evaporated. The residue was dissolved in TFA (2 mL) and stirred at room temperature for 16 h. The solvent was evaporated and the residue was purified by semi-preparative HPLC using eluent A (H_2O with 0.1% TFA and 1% CH_3CN) and eluent B (CH_3CN with 1% H_2O) (A/B = 90/10 to 0/100 for 40 min) to give **compound 8, HMtetTFER** (28.7 mg, 81%).

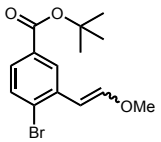
^{1}H NMR (400 MHz, acetone-d_6): δ 8.13 (s, 1 H), 7.97 (dd, *J* = 8.0, 1.1 Hz, 1 H), 6.98 (d, *J* = 8.0 Hz, 1 H),

6.93 (d, *J* = 8.8 Hz, 2 H), 6.90 (d, *J* = 2.4 Hz, 2 H), 6.85 (dd, *J* = 8.8, 2.4 Hz, 2 H), 5.35 (s, 2 H), 4.41 (q, $^{2}J_{H\text{-}F}$ = 8.8 Hz, 8 H); ^{13}C NMR (101 MHz, acetone-d_6): δ 167.3 (C), 152.0 (C), 151.1 (C), 148.7 (C), 140.9 (C), 131.6 (C), 130.8 (CH), 130.6 (CH), 126.6 (q, $^{1}J_{C\text{-}F}$ = 283.8 Hz, CF_3), 124.5 (CH), 123.7 (CH), 117.1 (C), 111.2 (CH), 102.0 (CH), 83.7 (C), 72.4 (CH_2), 52.6 (q, $^{2}J_{C\text{-}F}$ = 33.3 Hz, CH_2); ^{19}F NMR (376 MHz, acetone-d_6 + C_6F_6 as an internal standard): δ -70.0 (t, $^{2}J_{H\text{-}F}$ = 8.7 Hz); HRMS (m/z): $[M+H]^+$ calcd. for $C_{29}H_{21}F_{12}N_2O_4$, 689.13042; found, 689.13244.



Compound 9, HMtetTFER-NHS: **Compound 8, HMtetTFER** (7.3 mg, 0.011 mmol, 1 eq), *N*-hydroxysuccinimide (NHS) (6.1 mg, 0.053 mmol, 5 eq), and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (WSCD) (10.2 mg, 0.053 mmol, 5 eq) were dissolved in dry DMF (2 mL) and the solution was stirred at room temperature for 26 h under an argon atmosphere. The solvent was evaporated and the residue was purified by semi-preparative HPLC using eluent A (H_2O with 0.1% TFA and 1% CH_3CN) and eluent B (CH_3CN with 1% H_2O) (A/B = 90/10 to 0/100 for 60 min) to give **compound 9**, **HMtetTFER-NHS**. (5.3 mg, 54%).

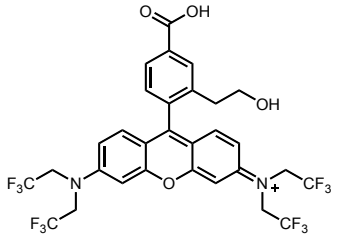
^{1}H NMR (400 MHz, acetone-d_6): δ 8.24 (d, *J* = 0.7 Hz, 1 H), 8.05 (dd, *J* = 8.0, 1.5 Hz, 1 H), 7.11 (d, *J* = 8.0 Hz, 1 H), 6.96 (d, *J* = 8.8 Hz, 2 H), 6.91 (d, *J* = 2.7 Hz, 2 H), 6.86 (dd, *J* = 8.8, 2.7 Hz, 2 H), 5.40 (s, 2 H), 4.42 (q, $^{2}J_{H\text{-}F}$ = 8.8 Hz, 8 H), 2.98 (s, 4 H); ^{13}C NMR (101 MHz, acetone-d_6): δ 170.6 (C), 162.7 (C), 153.3 (C), 151.9 (C), 148.8 (C), 141.8 (C), 131.3 (CH), 130.7 (CH), 126.6 (q, $^{1}J_{C\text{-}F}$ = 283.9 Hz, CF_3), 126.2 (C), 125.4 (CH), 124.5 (CH), 116.7 (C), 111.3 (CH), 102.0 (CH), 83.8 (C), 72.4 (CH_2), 52.6 (q, $^{2}J_{C\text{-}F}$ = 33.2 Hz, CH_2), 26.4 (CH_2); ^{19}F NMR (376 MHz, acetone-d_6 + C_6F_6 as an internal standard): δ -70.5 (t, $^{2}J_{H\text{-}F}$ = 8.7 Hz); HRMS (m/z): $[M+H]^+$ calcd. for $C_{33}H_{24}F_{12}N_3O_6$, 786.14680; found, 786.14734.



Compound 11: To a solution of (methoxymethyl)triphenylphosphonium chloride (1.8 g, 5.26 mmol, 1.5 eq) in THF (20 mL) was added potassium *tert*-butoxide (1.18 g, 10.5 mmol, 3 eq), and the solution was stirred at 0 °C for 1 h. **Compound 10**[4] in dry THF (10 mL) was added dropwise for 10 min, then the reaction mixture was stirred at room temperature for 1 h. Saturated $NaHCO_3$

aqueous solution was added, and the whole was extracted twice with DCM. The combined organic phase was washed with water and brine, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by flash column chromatography (silica gel, n-hexane/DCM = 95/5 to 35/65) to give **compound 11** (697.4 mg, *E/Z* = 1/1, 63%).

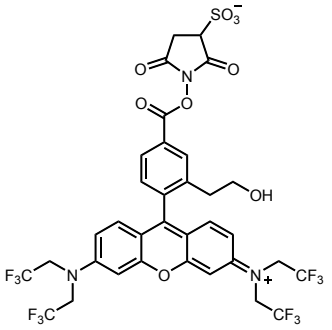
^{1}H NMR (400 MHz, $CDCl_3$): δ 8.65 (d, *J* = 1.9 Hz, 1 H), 7.92 (d, *J* = 2.0 Hz, 1 H), 7.56-7.49 (m, 4 H), 7.03 (d, *J* = 12.8 Hz, 1 H), 6.24 (d, *J* = 7.2 Hz, 1 H), 6.03 (d, *J* = 12.8 Hz, 1 H), 5.54 (d, *J* = 7.2 Hz, 1 H), 3.76 (s, 3 H), 3.69 (s, 3 H), 1.55 (s, 18 H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 165.3 (C), 165.1 (C), 151.3 (CH), 150.0 (CH), 136.4 (C), 135.2 (C), 132.8 (CH), 132.4 (CH), 131.3 (C), 131.2 (CH), 130.9 (C), 127.53 (CH), 127.50 (CH), 127.4 (C), 127.1 (C), 126.4 (CH), 103.8 (CH), 103.1 (CH), 81.3 (C), 81.0 (C), 60.9 (CH_3), 56.7 (CH_3), 28.1 (CH_3), 27.9 (CH_3); HRMS (m/z): $[M+Na]^+$ calcd. for $C_{14}H_{17}BrNaO_3$, 335.02533; found, 335.02542.



Compound 13, HEtetTFER: In an argon-flushed flask fitted with a septum cap, **compound 11** (103.4 mg, 0.33 mmol, 10 eq) was dissolved in dry THF (6 ml) and the solution was cooled to –78 °C. A 1 M *sec*-BuLi solution in cyclohexane and *n*-hexane (330 μL, 0.33 mmol, 10 eq) was slowly added dropwise and stirring was continued at the same temperature for 15 min. **Compound 6** (18.3 mg, 0.033 mmol, 10 eq) in dry THF (6 mL) was added as a bolus via a syringe at -78 °C, and the solution was stirred at -78 °C for 15 min and then at room temperature for 1 h. Next, saturated $NaHCO_3$ aqueous solution was added and the whole was extracted twice with EtOAc. The combined organic phase was washed with brine, dried over Na_2SO_4, filtered, and evaporated. The residue was dissolved in TFA (3 mL) and the solution was stirred at room temperature for 1 h. The solvent was evaporated, and the residue was dissolved in dry THF (5 mL). The resulting solution was cooled to 0 °C, and $NaBH_4$ (100 mg) was added. The reaction mixture was stirred at room temperature for 1 h, then 1 N HCl aq. was added, and the mixture was extracted twice with EtOAc. The combined organic phase was washed with brine, dried over Na_2SO_4, filtered, and evaporated. The residue was dissolved in THF (5 mL), and chloranil (16.2 mg, 0.066 mmol, 2 eq) was added. The mixture was stirred at room temperature for 30 min. The solvent was evaporated and the residue was purified by semi-preparative HPLC using eluent A (H_2O with 0.1% TFA and 1% CH_3CN) and eluent B (CH_3CN with 1% H_2O) (A/B = 90/10 to 0/100 for 60 min) to give **compound 13, HEtetTFER** (5.4 mg, 23%).

^{1}H NMR (400 MHz, acetone-d_6 + 1 drop of D_2O): δ 7.96 (d, *J* = 1.1 Hz, 1 H), 7.76 (dd, *J* = 8.2, 1.5 Hz, 1 H), 6.93 (d, *J* = 2.0 Hz, 2 H), 6.87 (d, *J* = 8.2 Hz, 1 H), 6.83-6.77 (m, 4 H), 4.41 (q, $^2J_{H\text{-}F}$ = 8.8 Hz, 8 H),

3.87 (t, *J* = 5.5 Hz, 2 H) ,3.07 (t, *J* = 5.5 Hz, 2 H); ^{13}C NMR (101 MHz, acetone-d_6): δ 167.4 (C), 153.2 (C), 148.5 (C), 144.9 (C), 137.5 (C), 131.5 (CH), 130.9 (CH), 130.7 (CH), 130.0 (C), 127.8 (CH), 126.6 (q, $^1J_{C\text{-}F}$ = 283.8 Hz, CF_3), 118.5 (C), 110.2 (CH), 102.3 (CH), 73.7 (C), 59.7 (CH_2), 52.7 (q, $^2J_{C\text{-}F}$ = 33.4 Hz, CH_2), 29.7 (CH_2); ^{19}F NMR (376 MHz, acetone-d_6 + C_6F_6 as an internal standard): δ -70.5 (t, $^2J_{H\text{-}F}$ = 8.7 Hz); HRMS (m/z): [M+H]+ calcd. for $C_{30}H_{23}F_{12}N_2O_4$, 703.14662; found, 703.14787.



Compound 14, HEtetTFER-sulfoNHS: A 10 mM solution of **Compound 13, HEtetTFER** in DMSO (300 μL, 3 μmol, 1 eq), 100 mM solution of *N*-hydroxysulfosuccinimide (sulfoNHS) in DMSO (60 μL, 6 μmol, 2 eq), and a 100 mM solution of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (WSCD) (60 μL, 6 μmol, 2 eq), and *N,N*-diisopropylethylamine (5 μL, 15 μmol, 5 eq) were mixed and stirred at room temperature for 20 h. The mixture was purified by semi-preparative HPLC using eluent A (H_2O with 0.1% TFA and 1% CH_3CN) and eluent B (CH_3CN with 1% H_2O) (A/B = 90/10 to 0/100 for 60 min) to give **Compound 14, HEtetTFER-sulfoNHS** (0.5 mg, 19%).
^{19}F NMR (376 MHz, acetone-d_6 + C_6F_6 as an internal standard): δ -70.4 (t, $^2J_{H\text{-}F}$ = 8.7 Hz); HRMS (m/z): $[M\text{-}H]^-$ calcd. for $C_{34}H_{24}F_{12}N_3O_9S$, 878.10416; found, 878.10783.

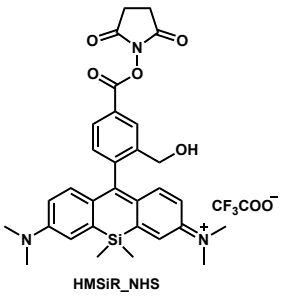
Measurements of photophysical properties

Compounds were dissolved in anhydrous DMSO to obtain 10 mM stock solutions. These stock solutions were diluted with 0.2 M sodium phosphate buffer to a final concentration of 0.5 μM. Absorption spectra were obtained with a UV-2450 UV/Vis spectrometer (Shimadzu) and fluorescence spectra were obtained with an F-7000 fluorescence spectrometer (Hitachi) at room temperature. Absolute fluorescence quantum yields were recorded on a Quantaurus QY C11347-1211 (Hamamatsu Photonics) at room temperature. To determine pK_a values of compounds with *n* acid-base equilibria (*n* = 1 or 2), the pH profiles of absorbance (Abs) or fluorescence intensity (FI) were fitted to the following formula with KaleidaGraph software (version 4.1). The pK_a value was adopted for the pK_{cycl} value in case of n = 1. In contrast, the larger pK_a value was adopted for pK_{cycl} value in case of n = 2. **HMtetTFER** and **HEtetTFER** gave 3.3/5.0 and 3.1/5.5, respectively, and so the pK_{cycl} values were determined to be 5.0 and 5.5 for **HMtetTFER** and **HEtetTFER**, respectively.

$$Abs\ or\ FI = \frac{c_0 + \sum_{k=1}^{n} c_k \cdot 10^{\,k \cdot pH - \sum_{l=1}^{k} pK_{al}}}{1 + \sum_{k=1}^{n} 10^{\,k \cdot pH - \sum_{l=1}^{k} pK_{al}}}$$

(pK_{a1} < pK_{a2} < ••• < pK_{an}; c_n = constant).

Labelling of antibody with NHS or sulfoNHS esters of dyes



HMSiR_NHS

HMtetTFER-NHS, HEtetTFER-sulfoNHS and **HMSiR-NHS**[3] were dissolved in DMSO to obtain 10 mM stock solutions. Secondary antibody was incubated with each dye (20 eq) in 0.2 M sodium phosphate buffer pH 8.5 at 37 °C for 30 min. Goat anti-mouse IgG (whole molecule) (Sigma-Aldrich) was labelled with **HMtetTFER-NHS** or **HEtetTFER-sulfoNHS**, and AffiniPure donkey anti-rabbit IgG (H+L) (Jackson ImmunoResearch Laboratories, Inc.) was labelled with **HMSiR-NHS**. Labelled proteins were purified on a PD MiniTrapTM G-25 (GE Healthcare) with PBS (pH 7.4) as the eluent. The degree of labelling (DOL) ratio (Dye [mol] / Protein [mol]) was determined by measuring the absorbance of the labelled IgG at a pH where the dyes exist in open, coloured form, on the assumption that the protein was obtained without loss at the separation step. DOLs were 5.7, 5.1, and 1.5 for **HMtetTFER-IgG$_{mouse}$**, **HEtetTFER-IgG$_{mouse}$** and **HMSiR-IgG$_{rabbit}$**, respectively.

Immunostaining of β-tubulin and Tom20 in Vero cells

Immunostaining of β-tubulin and Tom20 was performed as described elsewhere[5]. Vero cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Wako) supplemented with 10% FBS (fetal bovine serum, Invitrogen) and 1% penicillin-streptomycin (Invitrogen). Cells were cultured in eight-well LabTek II chamber slides (Nunc, Thermo Fischer Scientific) at 37 °C in a 5%/95% CO_2/air incubator. The cells were washed with pre-warmed PBS, and fixed with 3% PFA and 0.1% glutaraldehyde in PBS at room temperature for 10 min, and the solution was aspirated. A solution of 0.1% $NaBH_4$ in PBS, prepared immediately before use, was added, and the mixture was left at room temperature for 7 min. The cells were washed with PBS three times and then permeabilized and blocked with blocking buffer (3% BSA and 0.5% (v/v) Triton X-100 in PBS) at room temperature for 20 min. The fixed cells were stained with the primary antibody against β-tubulin (mouse monoclonal anti-β-tubulin antibody, 1:100 dilution in blocking buffer, Sigma-Aldrich) and/or Tom20 (rabbit polyclonal anti-Tom20 (FL-145) antibody, 1:50 dilution in blocking buffer, Santa Cruz) at room temperature for 30 min. The sample was rinsed with washing buffer (0.2% BSA and 0.1% (v/v) Triton X-100 in PBS) three times for 10 min each. The corresponding secondary antibodies labelled with the fluorophores as described above were added to the cells (10 μg/μL final concentration, diluted in blocking buffer), and the sample was incubated at room temperature for 30 min, then washed with washing buffer three times for 10 min each, and with PBS once. The cells were post-fixed with 3% PFA and 0.1% glutaraldehyde in PBS at room temperature for 10 min, and then washed with PBS three times.

SMLM of β-tubulin and Tom20 in fixed cells

SMLM was carried out in PBS at room temperature (24 °C) with N-STORM (Nikon), and images were recorded at 60 and 15 ms/frame for **HEtetTFER** and **HMSiR**, respectively. The super-resolution image was reconstructed with NIS-Elements Advanced Research software (Nikon). Cutoff values used to screen switching events were 400 and 1000 photons for **HEtetTFER** and **HMSiR**, respectively. The excitation intensities at 488 and 647 nm were 240 and 1000 W cm^{-2}, respectively. Photon number and localization accuracy of detected signals were analyzed with NIS-Elements Advanced Research software (Nikon). The conventional image was reconstructed by calculating standard deviation from 20,000 frames, because averaging gave only very low contrast images.

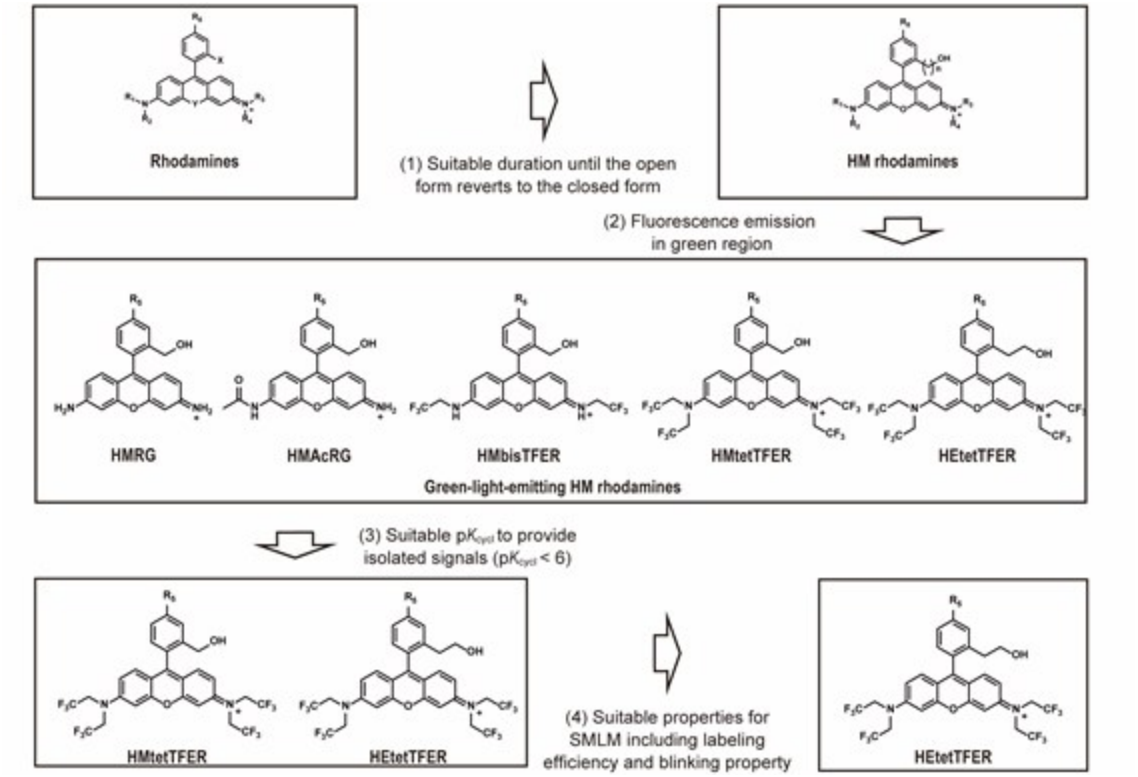
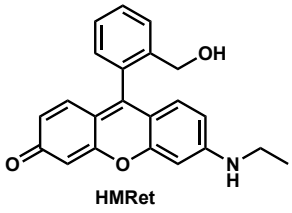
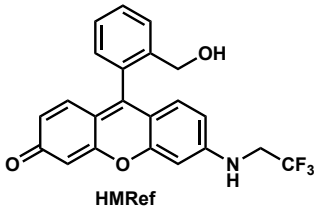


Fig. S1 Selection scheme for green-light-emitting SBFs.



HMRet



HMRef

	λ_{abs} [nm]	λ_{em} [nm]	pK_{cycl}
HMRet	510	534	10.9
HMRef	498	518	10.2

Fig. S2 Chemical structures and photophysical properties of **HMRet** and **HMRef**[6].

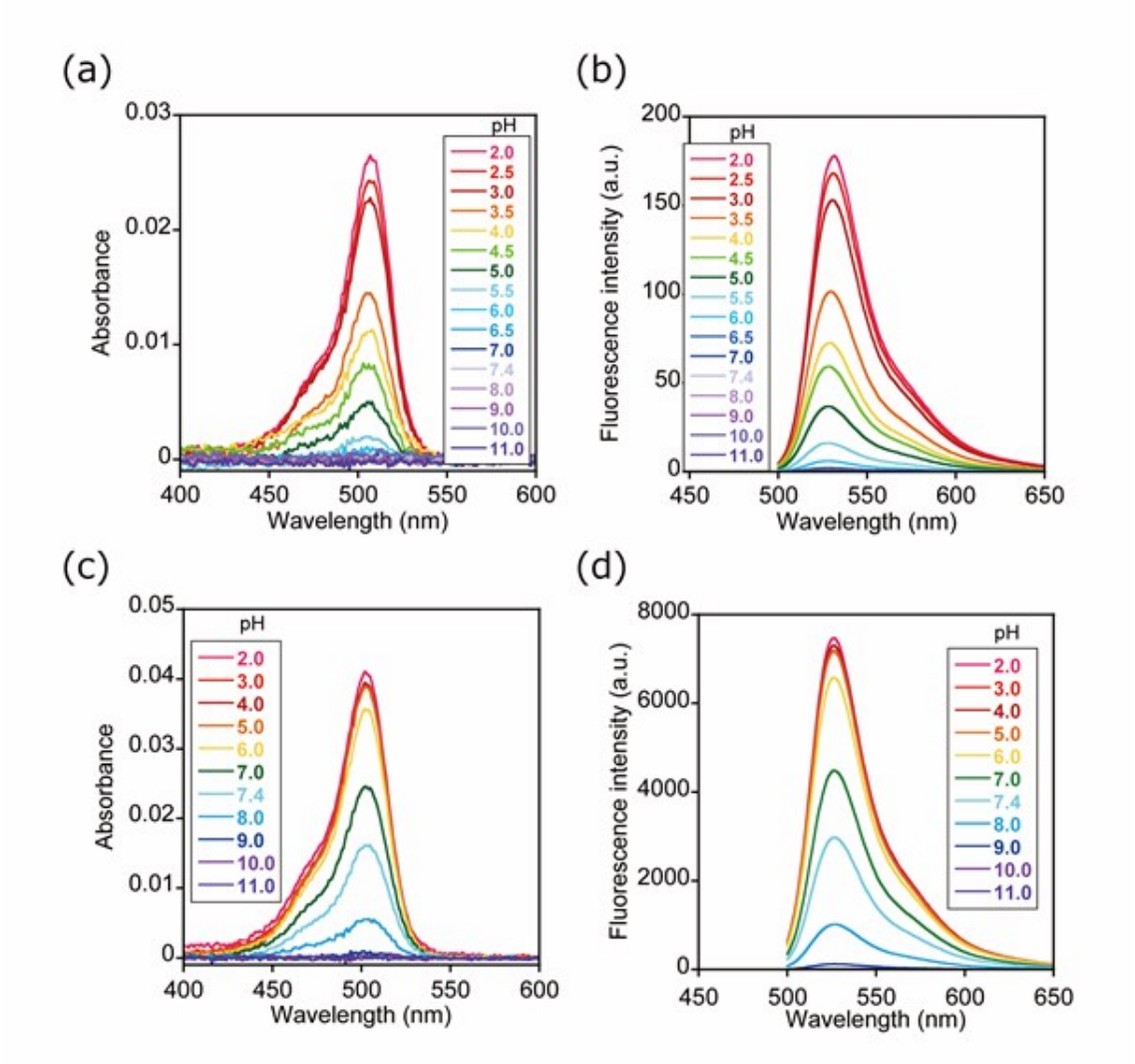


Fig. S3 (a) Absorption and (b) fluorescence spectra of **HMtetTFER**. (c) Absorption and (d) fluorescence spectra of **HMbisTFER**. Measured in 0.2 M sodium phosphate buffer. Excitation wavelength was 480 nm for both compounds.

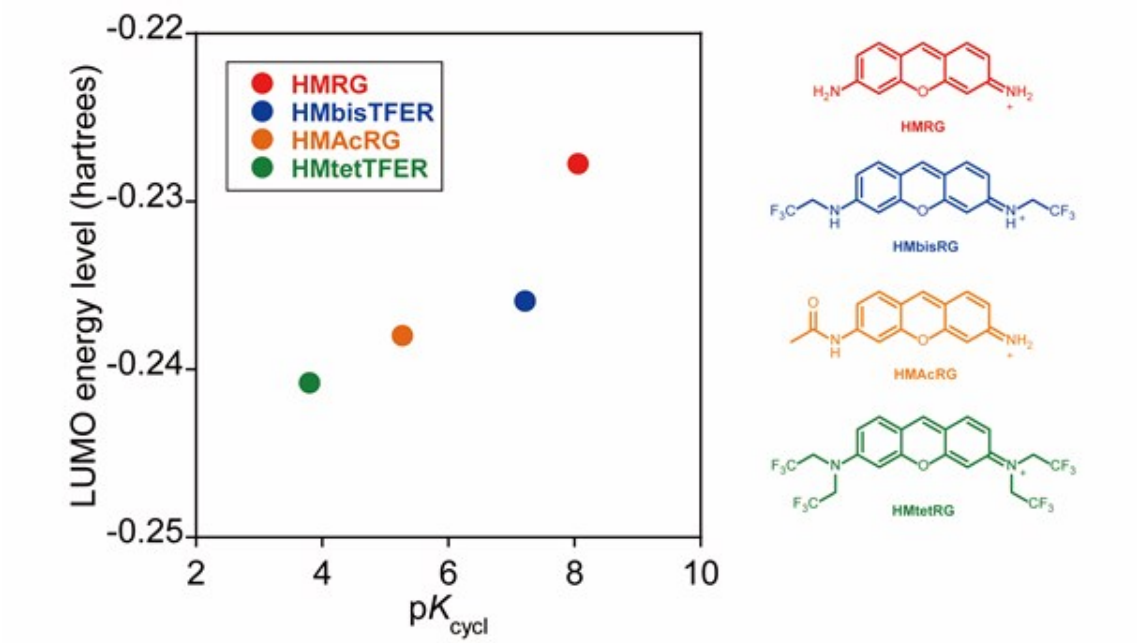


Fig. S4 Relationship between pK_{cycl} and LUMO energy level of green-light-emitting HM rhodamines. LUMO energy levels of corresponding xanthene moieties (shown on the right) were calculated at the B3LYP/6-31+G* level with Gaussian 09.

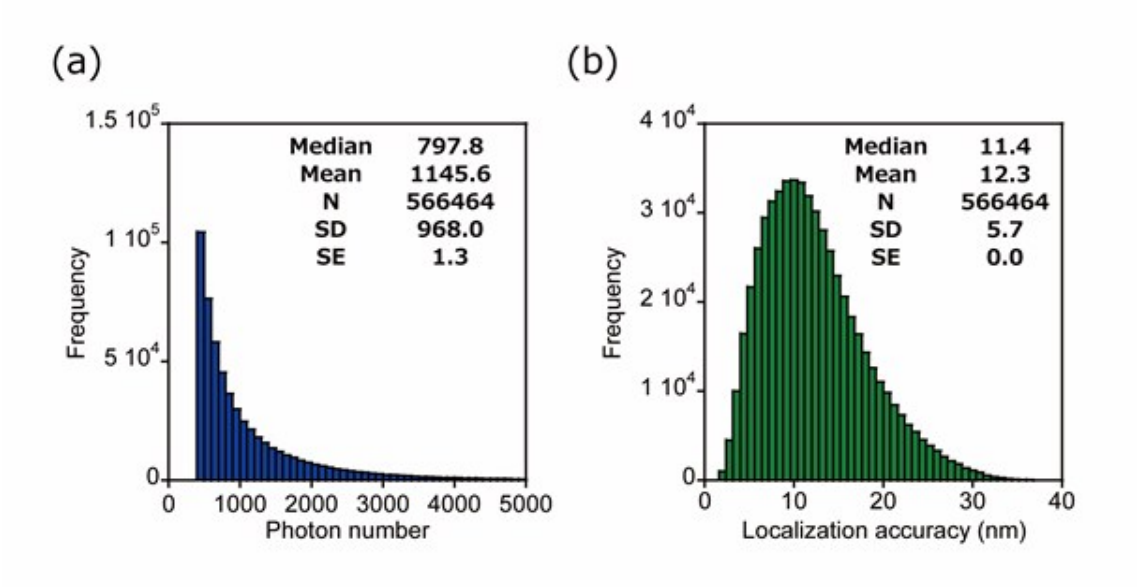


Fig. S5 (a) Photon number and (b) localization accuracy for SMLM with **HEtetTFER**, corresponding to Fig. 3. Measured in PBS. Excitation at 488 nm (240 W cm^{-2}). Cutoff value was 400 photons to filter analyzed signals. Analyzed by NIS Elements installed in N-STORM (Nikon).

Movie S1 Spontaneous blinking of **HEtetTFER** bound to β-tubulin in fixed cells. Measured in PBS. Excitation at 488 nm (240 W cm^{-2}). Recorded at 60 ms/frame just after irradiation. Scale bar, 5 μm.

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