Electronic Supplementary Material (ESI) for RSC Advances

A novel strategy to design latent ratiometric fluorescent pH probes based on self-assembled SNARF derivatives

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Supporting Information

Contents

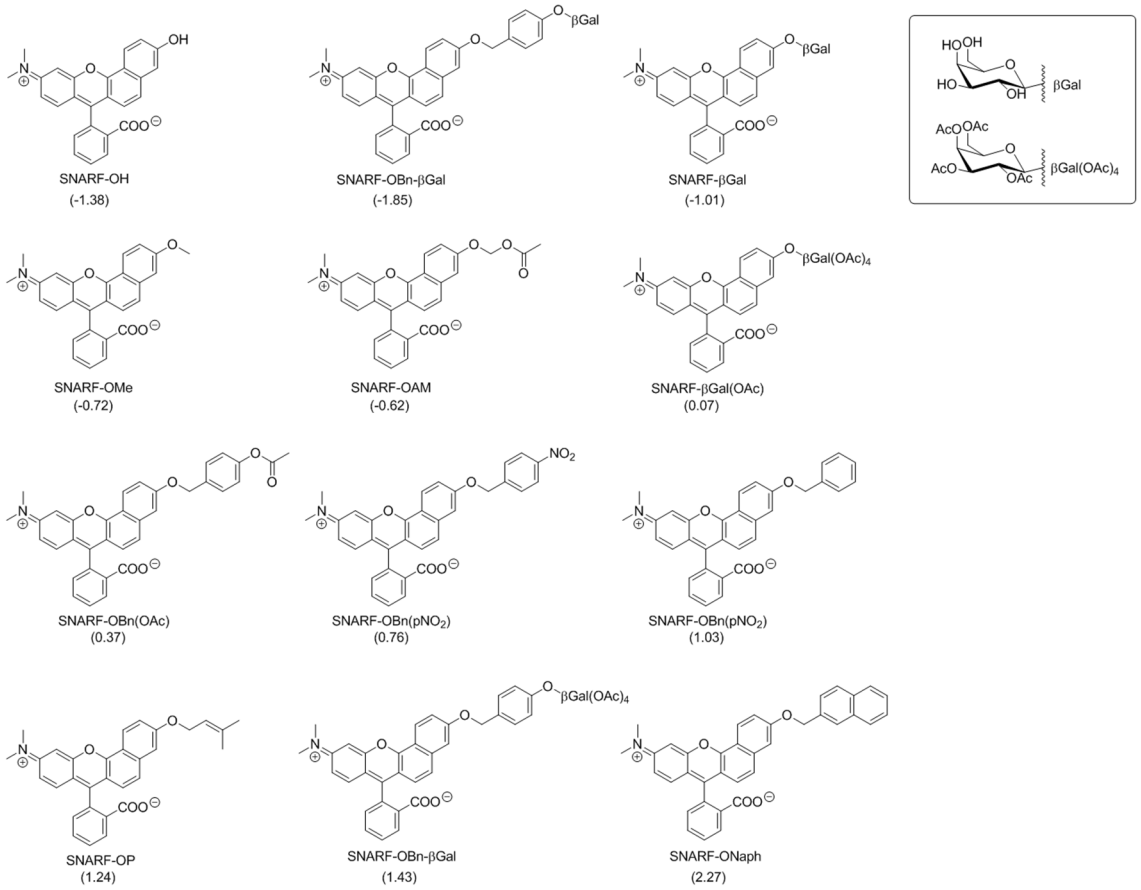


Fig. S1 SNARF derivatives used in this study. The π values of R were shown in parentheses.

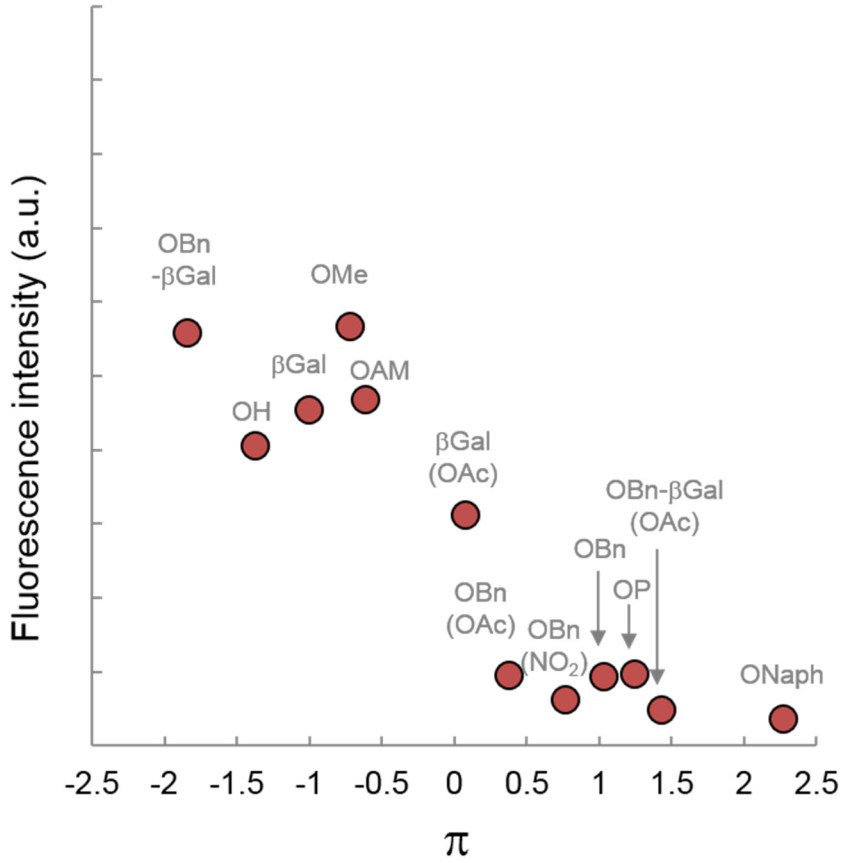


Fig. S2 Relationships between the π value of the inserted substituent and the fluorescence intensity measured the following condition. [SNARF derivatives] = 10 μM in pH 5.0 10 mM Tris, HEPES, and Acetate buffer (excited at 534 nm). The detail of each π value were shown in table 2. The chemical structure of these SNARF derivatives were shown in Fig. S1. The name of SNARF derivatives (SNARF-R) were shown inside the graph.

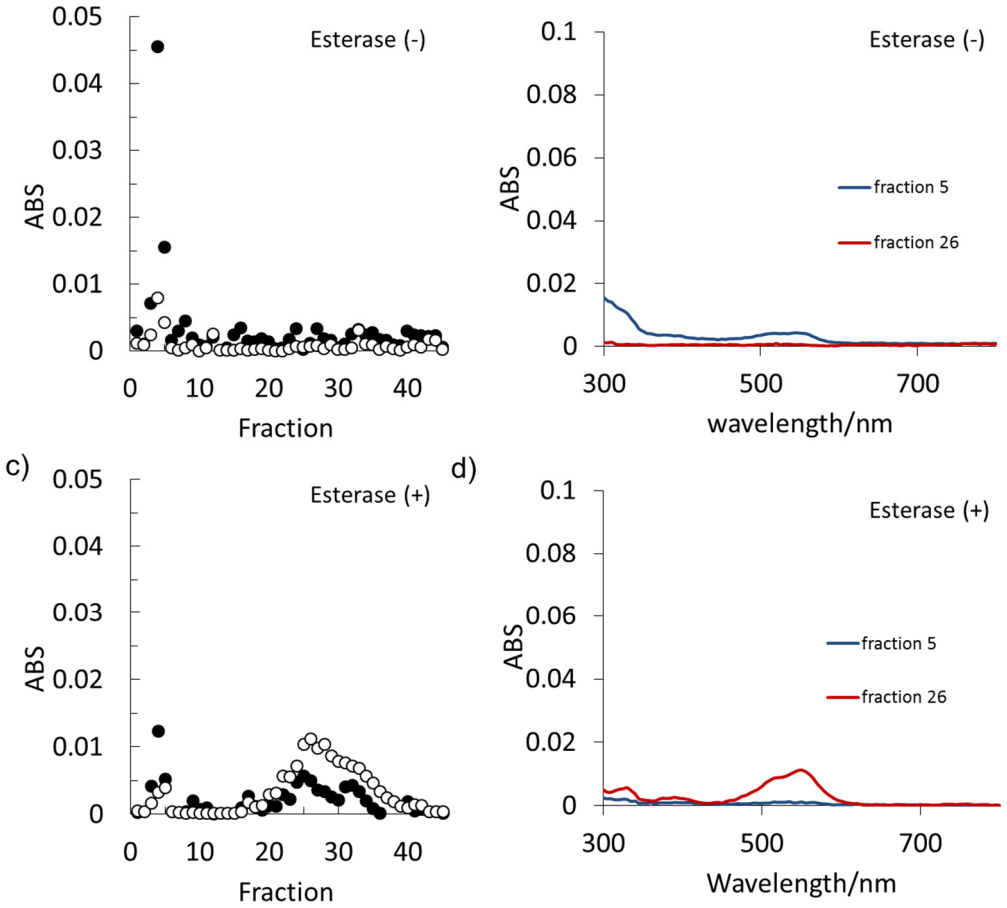


Fig. S3 The size exclusion gel chromatographic analysis of SNARF-OBn(OAc) before and after esterase treatment. (A and C) Fraction curves of ABS of SNARF-OBn(OAc) (A) before or (C) after esterase treatment, respectively. Filled circle: $ABS_{300\ nm}$; opened circle: $ABS_{550\ nm}$ (B and D) UV-visible spectra of SNARF-OBn(OAc) at the fractions 5 and 26 (B) before and (D) after esterase treatment, respectively.

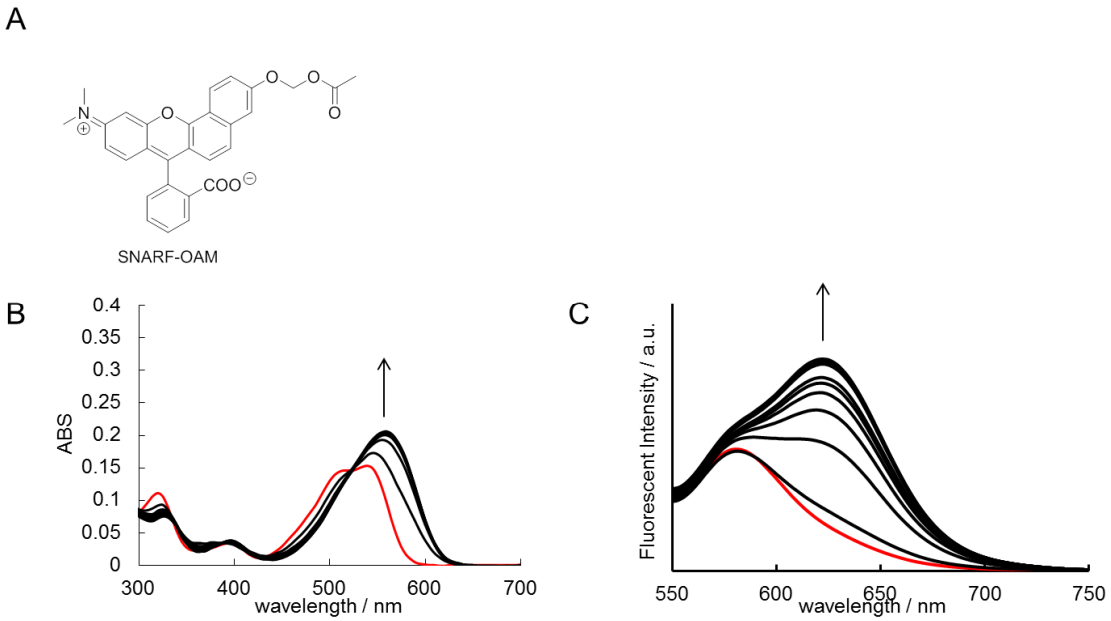


Fig. S4 (A) The chemical structure of SNARF-OAM. (B) and (C) Real time absorbance (B) and fluorescence (C, excited at 534 nm) change via esterase catalyzed ester hydrolysis of SNARF-OAM.

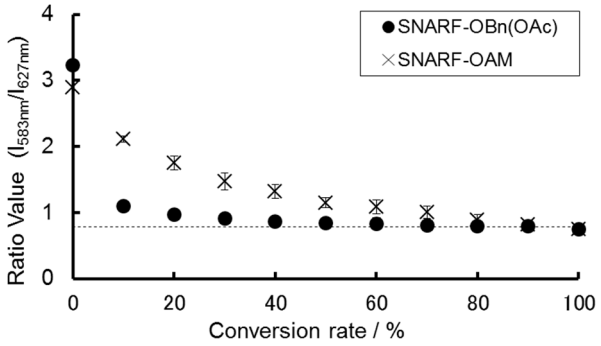


Fig. S5 The theoretical curve of ratiometric change ($I_{583\ nm}$ / $I_{627\ nm}$) of SNARF-OBn(OAc) and SNARF-OAM during ester hydrolysis. The dot line was indicated the ideal ratio value under the condition (10 mM Tris, HEPES, Acetate buffer (pH7.5), excited at 534 nm)).

[SNARF-OBn(OAc) + SNARF-OH] = [SNARF-OAM + SNARF-OH] = 10 μM

Calculation of conversion rate was determined the following formula.

$$\text{conversion rate (\%)} = [\text{SNARF-OH}] / \{[\text{SNARF-OH}] + [\text{X}]\}$$

X = SNARF-OBn(OAc) or SNARF-OAM

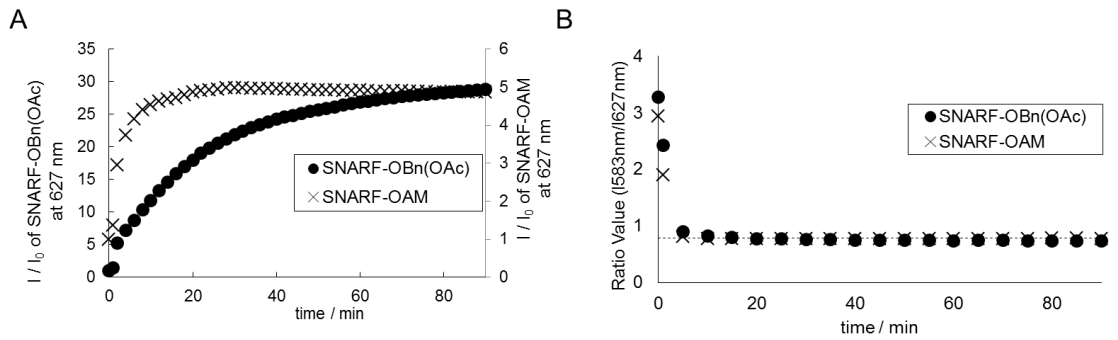


Fig. S6 (A) Real time fluorescent intensity change (I / I_0) at 627 nm and (B) the fluorescent ratio change ($I_{583\ nm}$ / $I_{627\ nm}$) of SNARF-OBn(OAc) and SNARF-OAM *via* esterase-catalyzed ester hydrolysis. The dot line was indicated the ideal ratio value under the condition (10 mM Tris, HEPES, Acetate buffer (pH7.5), excited at 534 nm).

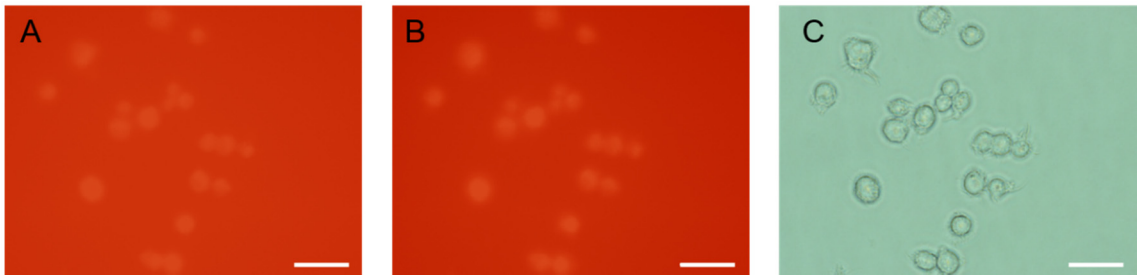


Fig. S7 (A-B) Fluorescent images and (C) bright-field transmission of NR8383 cells after addition SNARF-OAM (A : 0 min, B : 15 min) to extracellular solution (FBS containing F-12K medium). The scale bar is 50 μm.

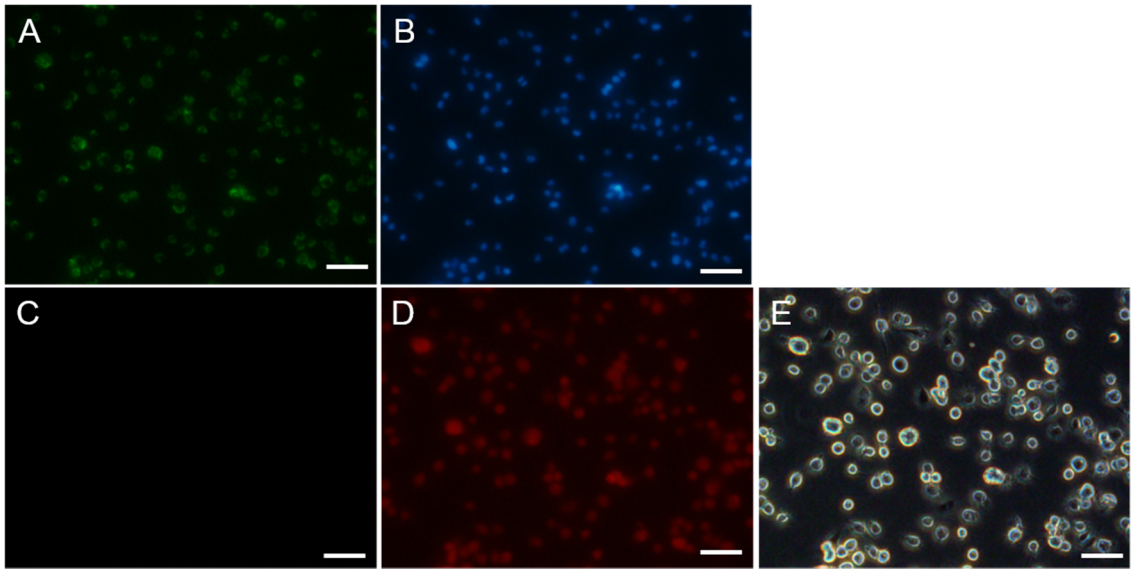


Fig. S8 (A-D) Fluorescent images and (F) bright-field transmission of NR8383 cells pre-treated with Mito tracker Green® FM and Hoechst 33258 after the addition SNARF-OBn(OAc) to extracellular solution (FBS-containing F-12K medium). NR8383 cells were imaged simultaneously for (A) Mito Tracker Green® FM(WIB filter), (B) Hoechst 33258(WU filter) and (C and D) SNARF-OBn(OAc)(WIY filter)(C: 0 min, D: 15 min). (E) is a transmission image. The scale bar is 50 μm.

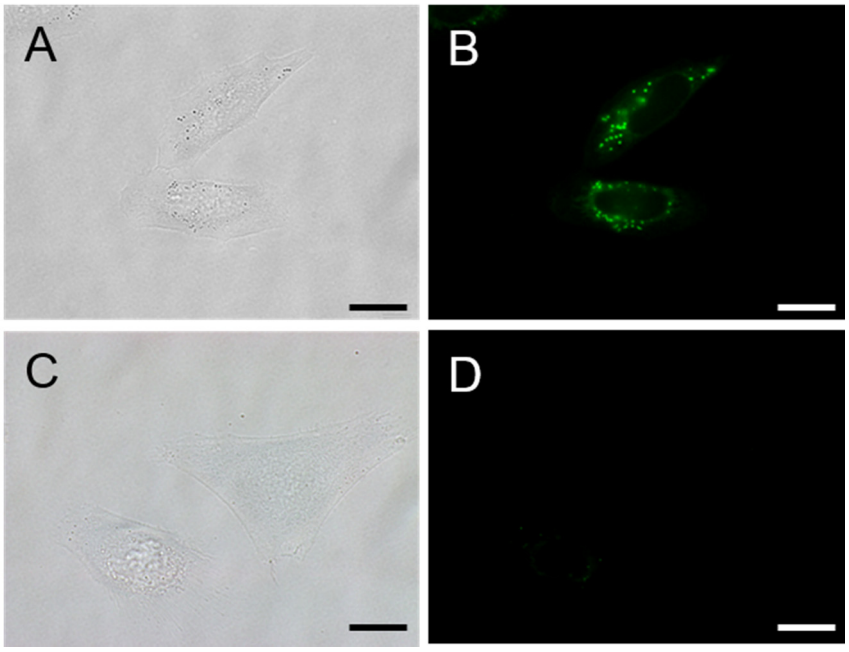


Fig. S9 (A, C) Bright-field transmission and (B, D) fluorescent image (WU filter for Dansyl fluorophore) of HeLa cells after incubated with SNARF-Dan (10 μM) under different temperature condition (A-B: 37 °C, C-D：4 °C). After 1 h incubation, the cells were washed with EMEM(+) and observed with fluorescent microscopy under the same machine condition. The scale bar is 20 μm.

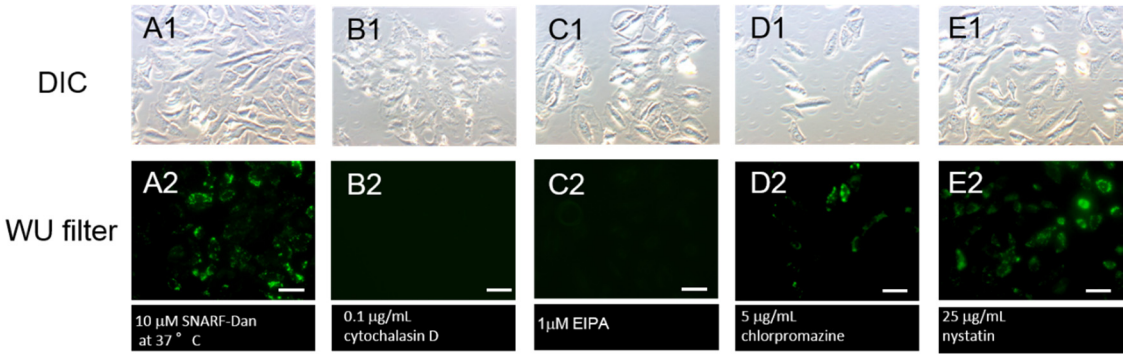


Fig. S10 (A1-E1) Bright-field transmission and (A2-E2) fluorescent image of HeLa cells after incubated with SNARF-Dan (10 μM). (A-E) Internalized SNARF-Dan in HeLa cells after incubation for 1 h at 37°C were observed (WU filter).(A) Without inhibitors (B) cytochalasin D (0.1 μg/ml), (C) EIPA (1 μM), (D) chlorpromazine (5 μg/ml) (E) nystatin (25 μg/ml). The scale bar is 20 μm.

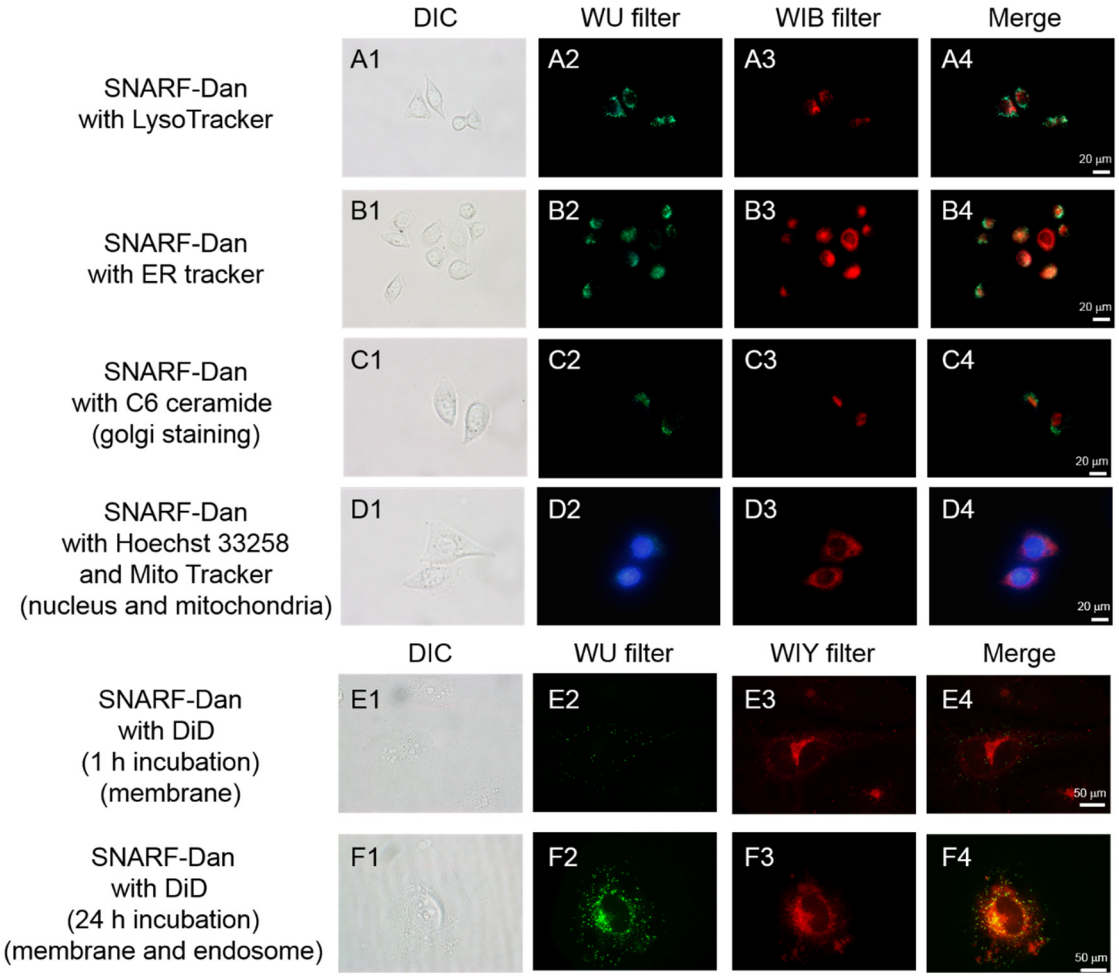


Fig. S11 (A1-F1) Bright-field transmission and (A2-F2, A3-F3 and A4-F4) fluorescent image of HeLa cells after incubated with SNARF-Dan (10 μM) and the organelle specific probes. After appropriate incubation time (A-E : 1h, F : 12 h), the cells were washed with EMEM(+) and observed with fluorescent microscopy by using appropriate filters. The images co-stained with SNARF-Dan and (A) LysoTracker Yellow HCK-123 (L-12491 : invitrogen), (B) ER tracker Green (E34251 : invitrogen), (C) NBD C6 ceramide complexed to BSA (N22651 : invitrogen), (D) Hoechst 33258 (H1398 : invitrogen) and Mito Tracker Green FM (M7514 : invitrogen), or (E and F) DiD (D307 : invitrogen). (A2-C2, and E2-F2) WU filter for Dansyl fluorophore. (D2) WU filter for Dansyl and Hoechst 33258. (A3-D3) WIB filter for the organelle specific probes (LysoTracker Yellow HCK-123, ER tracker Green, NBD C6 ceramide, or Mito Tracker Green FM) (E3-F3) WIY filter for DiD. (A4-F4) The overlay image of A2-F2 and A3-F3, respectively. The scale bar are (A-D) 20 μm or (E-F) 50 μm, respectively.

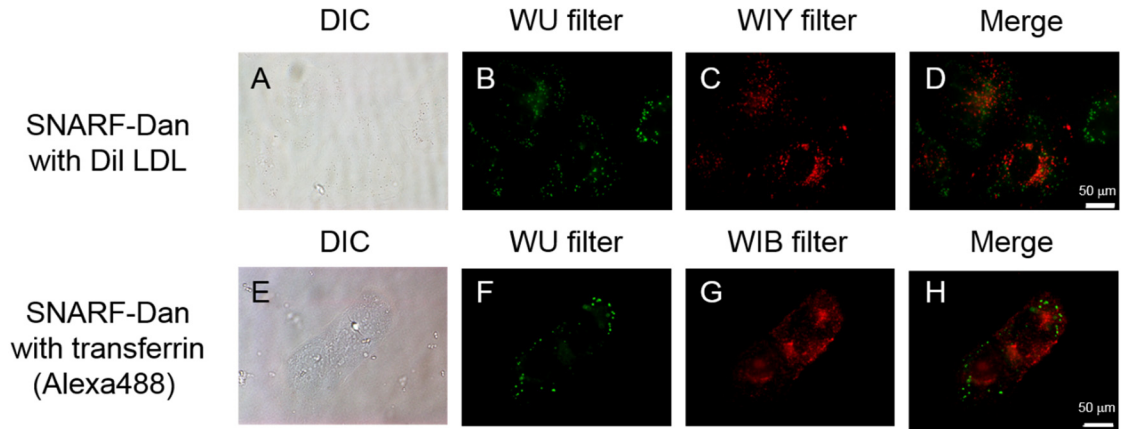
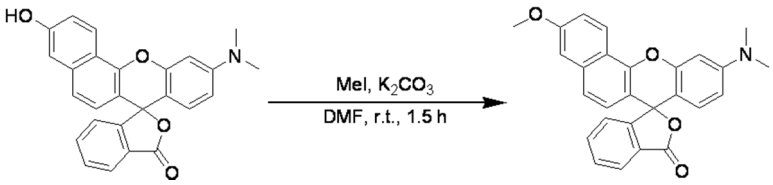


Fig. S12 (A and E) Bright-field transmission and (B-D and F-H) fluorescent image of HeLa cells after incubated with SNARF-Dan (10 μM) and indicator of a kind of endocytosis. After 1 h incubation, the cells were washed with EMEM(+) and observed with fluorescent microscopy by using appropriate filters. (A-D) The images co-stained with SNARF-Dan and Dil-LDL (L-3482: invitrogen) as the indicator of receptor-mediated endocytosis. (E-F) The images co-stained with SNARF-Dan and transferrin-Alexa 488 conjugate (T-13342: invitrogen) as the indicator of clathrin-mediated endocytosis. (B and F) WU filter for Dansyl fluorophore. (C) WIY filter for Dil. (G) WIB filter for Alexa488 (D and H) The overlay image of B-C and F-G, respectively. The scale bar are 50 μm.

Materials and Methods

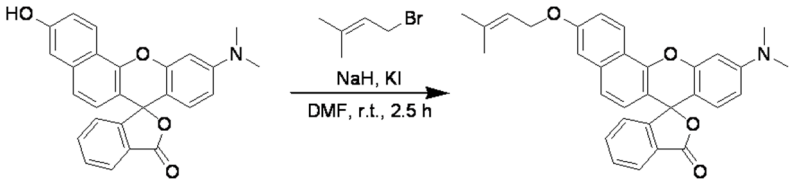
1. Synthesis:

1-1. Synthesis of 10-(dimethylamino)-3-[methoxy]-spiro-[7*H*-benzo[*c*]xanthene-7,1'(3'*H*)-isobenzofuran]-3'-one (SNARF-OMe)



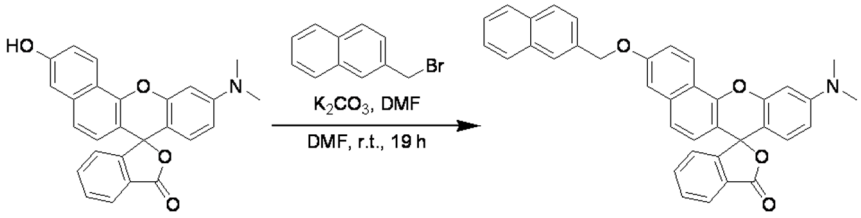
A mixture of SNARF (20.0 mg, 48.9 μmol), K_2CO_3 (121 mg, 930 μmol), and MeI (50 μL, 803.2 μmol) in 2.5 mL dry DMF was stirred for 1.5 h at room temperature. The reaction mixture was diluted with CH_2Cl_2 and washed sat. $NaHCO_3$ aq. (×2) followed by drying over anhydrous Na_2SO_4. The organic layers were evaporated *in vacuo*. The residue was purified by column chromatography on silica gel (CH_2Cl_2/EtOAc: a linear gradient from 1:0 to 6:1 (v/v)) to give SNARF-OMe as a pale pink powder in 65% yield (13.4 mg, 31.7 μmol).: ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.50 (d, J = 9.0 Hz, 1H), 8.04 (d, J = 6.8 Hz, 1H), 7.65-7.60 (m, 2H), 7.32 (d, J = 8.8 Hz, 1H), 7.27 (d, J = 8.5 Hz, 1H), 7.15 (d, J = 7.0 Hz, 1H), 7.12 (d, J = 2.0 Hz, 1H), 6.74-6.67 (m, 3H), 6.47 (dd, J = 8.8, 6.5 Hz, 1H), 3.93 (s, 3H), 3.03 (s, 6H). HRMS (FAB$^+$): calcd for $C_{27}H_{22}NO_4^+$ ([M+H]$^+$), 424.1543; found 424.1556.

1-2.Synthesis of 10-(dimethylamino)-3-[3-methyl-2-butenyloxy]-spiro-[7*H*-benzo[*c*] xanthene-7,1'(3'*H*)-isobenzofuran]-3'-one (SNARF-OP)



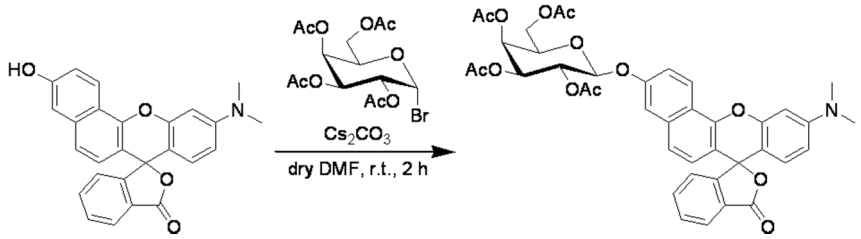
A mixture of SNARF (30.5 mg, 74.5 μmol), K_2CO_3 (42.5 mg, 327 μmol, 4.4 equiv), and 1-bromo-3-methyl-2-butene (10 μL, 86.7 μmol, 1.2 equiv) in 2.5 mL dry DMF was stirred for 2.5 h at room temperature. The reaction mixture was diluted with sat. $NaHCO_3$ aq., extracted to EtOAc (×2) and washed with brine (×2) followed by drying over anhydrous Na_2SO_4. The organic layers were evaporated *in vacuo*. The residue was purified by column chromatography on silica gel (CH_2Cl_2/EtOAc: a linear gradient from 100:1 to 20:1(v/v)) to give **6** (SNARF-OP) as a pale pink powder in 54% yield (19.3 mg, 40.4 μmol).: ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.49 (d, J = 9.0 Hz, 1H), 8.44 (d, J = 6.8 Hz, 1H), 7.62 (t, J = 8.58, 2H), 7.32-7.26 (m, 2H), 7.16-7.12 (m, 2H), 6.73-6.67 (m, 3H), 6.47 (d, J = 8.05 Hz, 1H), 5.56 (brs, 1H), 4.64 (d, J = 6.10 Hz, 2H), 3.02 (s, 6H), 1.81 (d, J = 14.1 Hz, 6H). HRMS (CI$^+$): calcd for $C_{31}H_{28}NO_4^+$ ([M+H]$^+$), 478.2013; found 478.2022.

1-3. Synthesis of 10-(dimethylamino)-3-[naphthalene-2-ylmethoxy]-spiro-[7*H*-benzo [c]xanthenes-7,1'(3'*H*)-isobenzofuran]-3'-one (SNARF-ONaph)



A mixture of SNARF (30.0 mg, 72.5 μmol), K_2CO_3 (60 mg, 434 μmol) and 2-(chloromethyl) naphthalene (29.3 mg, 166 μmol) in dry DMF (2 mL) was stirred for 19 h at room temperature. The reaction mixture was diluted with CH_2Cl_2 and washed with sat. $NaHCO_3$ aq. followed by drying over anhydrous Na_2SO_4. The solvent was removed *in vacuo*. The residue was purified by column chromatography on silica gel (CH_2Cl_2/EtOAc = 1:0 to 50:1 (v/v)) to give a pale pink powder in 58% yield (23.6 mg).: ^{1}H NMR ($CDCl_3$, 400MHz): δ 8.54 (d, *J* = 12Hz, 1H), 8.04 (dd, *J* = 4Hz, 1H), 7.95 (s, 1H), 7.85–7.90 (m, 3H), 7.58–7.63 (m,3H), 7.49–7.51 (m, 2H), 7.40 (dd, *J* = 8Hz, 1H), 7.31 (d, *J* = 12Hz, 1H), 7.24 (m, 1H), 7.15 (dd, *J* = 4Hz, 1H), 6.67–6.74 (m, 4H), 6.47 (dd, J = 8Hz, 1H), 5.30(s, 2H), 3.03(s, 6H).; HRMS (FAB$^+$): calcd for $C_{33}H_{26}NO_4^+$ ([M+H]$^+$) 550.2013, found 550.2023.

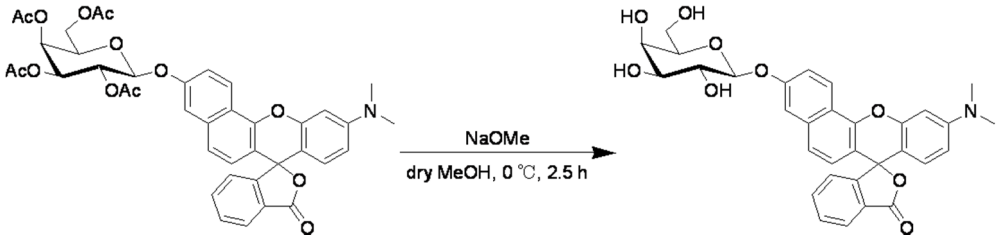
1-4. Synthesis of 10-(dimethylamino)-3-(2',3',4',6'-tetra-*O*-acetyl-β-D-galactopyranosyloxy)-spiro[7*H*-benzo[*c*]xanthene-7,1'(3'*H*)-isobenzofuran]-30-one (SNARF-βGal(OAc))



A mixture of 2,3,4,6-tetra-*O*-acyl-α-D-galactopyranosyl bromide (500 mg, 500 μmol), SNARF (50.0 mg, 122.1 μmol) and Cs_2CO_3 (325 mg, 1.0 mmol) in dry DMF (1 mL) was stirred at room temperature, and then NaH (100 mg, 4.17 mmol) was added and stirred at room temperature for 2 h. The reaction mixture was diluted with EtOAc and filtrated. The filtrate was evaluated *in vacuo*. The residue was dissolved in CH_2Cl_2 and washed with brine. The organic layer was dried over anhydrous Na_2SO_4 and evaluated *in vacuo*. The residue was purified by column chromatography on silica gel (CH_2Cl_2/MeOH: 15:1 (v/v)) to give SNARF-βGal(OAc) as a red product in 78% yield (69.9 mg, 94.5 μmol).: ^{1}H NMR (CD_3OD, 400 MHz): δ 8.53 (dd, *J* = 9.3, 3.4 Hz, 1H), 8.05 (dd, *J* = 6.1, 1.5 Hz, 1H), 7.65–7.61 (m, 2H), 7.34–7.30 (m, 3H), 7.15-7.14 (m, 1H), 6.76 (dd, *J* = 9.5, 0.7 Hz, 1H),

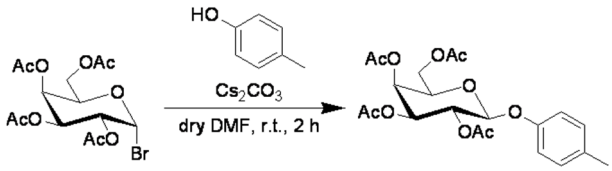
6.70–6.67 (m, 2H), 6.47 (dd, *J* = 8.8, 2.2 Hz, 1H), 5.59–5.55 (m, 1H), 5.49 (t, *J* = 3.1 Hz, 1H), 5.24 (dd, *J* = 7.8, 2.9 Hz, 1H), 5.17 (dd, *J* = 10.5, 3.7 Hz, 1H), 4.25–4.16 (m, 3H), 3.03 (s, 6H), 2.20 (d, *J* = 1.7 Hz, 3H), 2.09 (d, *J* = 3.9 Hz, 3H), 2.05 (d, *J* = 4.6 Hz, 3H), 2.03 (s, 3H). HRMS (FAB $^+$): m/z calcd for $C_{48}H_{38}NO_{13}^+$ ([M + H]$^+$), 740.2338; found, 740.2352.

1-5. Synthesis of 10-(dimethylamino)-3-(β-D-galactopyranosyloxy)-spiro[7H-benzo [c]xanthene-7,1'(3'H)-isobenzofuran]-3'-one (SNARF-βGal)



The mixture of NaOMe (6.95 mg, 128.0 μmol, 9 equiv) and SNARF-βGal(OAc) (10.0 mg, 13.5 μmol) was stirred in 2.5 mL dry MeOH at 0 ˚C for 2.5 h. The reaction mixture was added the Dowex* 50W-X4 ion exchange resin (The Dow Chemical Company) for neutralization. The reaction mixture was filtrated to remove the Dowex* 50W-X4. The solvent was evaporated *in vacuo*. The residue was dissolved in DMSO and purified by HPLC on ODS-3 column (0.1% TFA/0.1% TFA in CH_3CN: 95:5 to 0:100 (v/v) for 40 min) to give purple solid, SNARF-βGal, in 71% yield (5.50 mg, 9.62 μmol).: ^{1}H NMR (CD_3OD, 400 MHz): δ 8.53 (d, *J* = 9.2 Hz, 1H), 8.05 (d, *J* = 7.0 Hz, 1H), 7.76 (td, *J* = 1.5, 7.6 Hz, 1H), 7.71 (td, *J* = 1.5, 7.6 Hz, 1H), 7.51 (d, *J* = 2.2 Hz, 1H), 7.48 (dd, *J* = 1.5, 9.0 Hz, 1H), 7.43 (d, *J* = 8.9 Hz, 1H), 7.20 (dd, *J* = 1.0, 7.6 Hz, 1H), 6.79 (d, *J* = 2.4 Hz, 1H), 6.71–6.65 (m, 2H), 6.60 (dd, *J* = 2.4, 8.9 Hz, 1H), 5.06 (dd, *J* = 2.9, 7.8 Hz, 1H), 4.57 (s, 1H), 3.94 (d, *J* = 3.4 Hz, 1H), 3.90–3.89 (m, 1H), 3.83–3.76 (m, 1H), 3.65–3.61 (m, 1H), 3.05 (s, 6H). HRMS (FAB $^+$): m/z calcd for $C_{32}H_{30}NO_9^+$ ([M + H]$^+$), 572.1915; found, 572.1928.

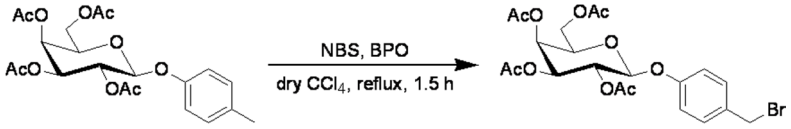
1-6. Synthesis of 4-methylphenyl-2',3',4',6'-tetra-*O*-acetyl-β-D-galactopyranoside



The mixture of *p*-cresol (500 mg, 4.62 mmol), Cs_2CO_3 (3.0 g, 9.24 mmol) and 2,3,4,6-tetra-*O*-acyl-α-D-galactopyranosyl bromide (1.0 g, 2.43 mmol) in dry DMF (5 mL) was stirred at room temperature for 2.5 h. The reaction mixture was filtered. The solution was dissolved in EtOAc and washed with brine. The organic layer was dried over anhydrous Na_2SO_4 and evaporated *in vacuo*. The residue was purified by column chromatography on silica gel (CH_2Cl_2/MeOH: a linear gradient from 1:0 to 50:1 (v/v)) to give target compound (449.6 mg, 930

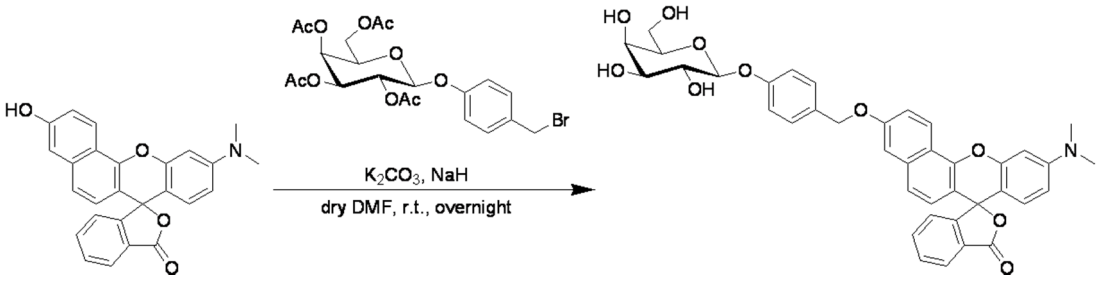
μmol, 40%).: ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.09 (d, *J* = 8.0 Hz, 2H), 6.90 (dd, *J* = 8.5, 2.9 Hz, 2H), 5.49–5.44 (m, 2H), 5.10 (dd, *J* = 10.4, 3.5 Hz, 1H), 4.89 (d, *J* = 8.0 Hz, 1H), 4.25–4.18 (m, 2H), 4.04 (t, *J* = 6.36 Hz, 1H), 2.30 (s, 3H), 2.18 (s, 3H), 2.06 (s, 3H), 2.04 (s, 3H), 2.01 (s, 3H). MS (FAB $^+$): m/z calcd for $C_{21}H_{27}NO_{10}^+$ ([M + H]$^+$), 439.2; found, 439.3.

1-7. Synthesis of 4-(bromo)methylphenyl-2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside



Powdered *N*-bromosuccinimide (NBS) 195.8 mg (1.10 mmol) and benzoylperoxide (BPO) 24.0 mg (0.1 mmol) were added to the solution of 4-methylphenyl-2',3',4',6'-tetra-*O*-acetyl-β-D-galactopyranoside 440 mg (1.00 mmol) in distilled CCl_4 (5 mL). The reaction mixture was refluxed for 1.5 h, and then cooled to ambient temperature. After the precipitate was filtered off, the filtrate was concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (CH_2Cl_2/MeOH: a linear gradient from 1:0 to 30:1 (v/v)) to give target compound (428.5 mg, 0.82 mmol, 83 %).: ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.33 (d, *J* = 7.6 Hz, 2H), 6.98 (d, *J* = 7.6 Hz, 2H), 5.49–5.44 (m, 2H), 5.17-5.09 (m, 2H), 4.49 (s, 2H), 4.22-4.14 (m,3H), 2.17 (s, 3H), 2.07 (s, 6H), 2.01 (s, 3H).

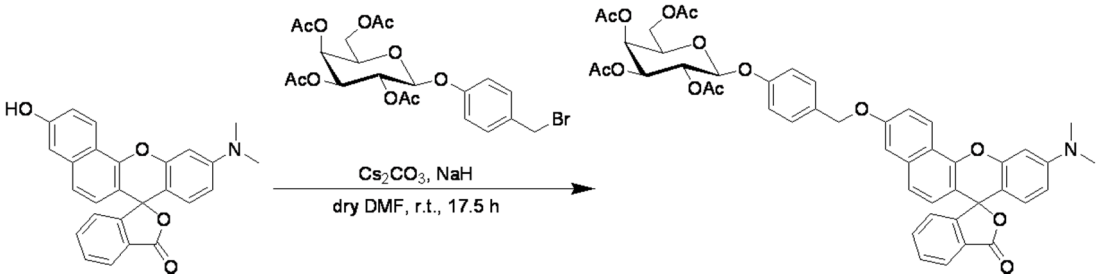
1-8. Synthesis of 10-(dimethylamino)-3-{[4-(β-D-galactopyranosyloxy)phenyloxy] methoxy}-spiro[7*H*-benzo[*c*]xanthene-7,1'(3'*H*)-isobenzofuran]-3'-one (SNARF-OBn-βGal)



A mixture of 4-(bromo)methylphenyl-2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside (70.0 mg), SNARF (50.0 mg, 122.1 μmol) and K_2CO_3 (150.0 mg, 1.09 mmol) in dry DMF (2 mL) was stirred at room temperature, and then NaH (100 mg, 4.17 mmol) was added and stirred at room temperature for overnight. The reaction mixture was filtrated and the filtrate was neutralized with 0.1% TFA and purified by HPLC on ODS-3 column (0.1% TFA/0.1% TFA in CH_3CN: a gradient from 95:5 to 95:5 for 5 min, from 95:5 to 55:45 for 10 min, from 55:45 to 5:95 (v/v) for 25 min. The retention time; 29.608 min) to give SNARF-OBn-βGal in 6% yield (5.30 mg, 7.82 μmol).: ^{1}H NMR (CD_3OD, 400 MHz): δ 8.68 (d, *J* = 8.8 Hz, 1H), 8.41 (dd, *J* = 7.8, 1.5 Hz, 1H), 7.91 (td, *J* = 7.6, 1.5 Hz, 1H), 7.87 (td, *J* = 7.8, 1.5 Hz, 1H), 7.68 (d, *J* = 9.0 Hz, 1H), 7.48–7.42 (m, 5H), 7.32 (d, *J* = 9.3 Hz, 1H), 7.25–

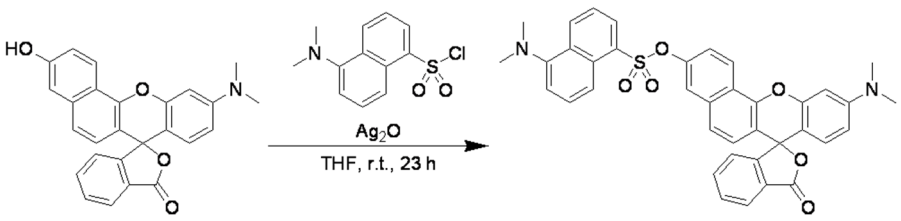
7.21 (m, 2H), 7.16–7.10 (m, 3H), 5.22 (s, 2H), 3.90 (d, J = 3.2 Hz, 1H), 3.82–3.67 (m, 5H), 3.58 (dd, J = 9.7, 3.4 Hz, 1H). HRMS (FAB $^+$): m/z calcd for $C_{39}H_{36}NO_{10}^+$ ([M + H]$^+$), 678.2334; found, 678.2361.

1-9. Synthesis of 10-(Dimethylamino)-3-{[4-(2',3',4',6'-tetra-*O*-acetyl-β-D-galactopyranosyloxy)phenyl]methoxy}-spiro[7*H*-benzo[*c*]xanthene-7,1'(3'*H*)-isobenzofuran]-3'-one (SNARF-OBn-βGal(OAc))



A mixture of 4-(bromo)methylphenyl-2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside (70.0 mg), SNARF (50.0 mg, 122.1 μmol) and Cs_2CO_3 (300.0 mg, 920 μmol) in dry DMF (3 mL) was stirred at room temperature for 17.5 h. The reaction mixture was neutralized with sat. $NaHCO_3$ aq., and then extracted with CH_2Cl_2. The organic layer was dried over anhydrous Na_2SO_4 and evaluated *in vacuo*. The residue was diluted with DMSO and purified by HPLC on ODS-3 column (0.1% TFA/0.1% TFA in CH_3CN: a gradient from 95:5 to 95:5 for 5 min, from 95:5 to 55:45 for 10 min, from 55:45 to 5:95 (v/v) for 25 min. The retention time; 25.667 min) to give purple solid **18** (SNARF-OBn-βGal(OAc)) in 3% yield (3.60 mg, 4.26 μmol).: ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.53 (d, J = 9.3 Hz, 1H), 8.09 (dd, J = 7.8, 1.7 Hz, 1H), 7.65–7.65 (m, 2H), 7.43 (d, J = 8.8 Hz, 2H), 7.34 (dd, J = 9.0, 2.9 Hz, 2H), 7.19 (d, J = 2.4 Hz, 1H), 7.16 (dd, J = 6.6, 2.2 Hz, 1H), 7.04 (d, J = 6.8 Hz, 1H), 6.76 (d, J = 13.2, 2.4 Hz, 1H), 6.55 (dd, J = 9.0, 2.4 Hz, 1H), 5.52-5.46 (m, 2H), 5.11 (dd, J = 10.5, 3.4 Hz, 1H), 5.06 (d, J = 8.0 Hz, 1H), 4.24–4.14 (m, 2H), 4.08-1.07 (m, 1H), 3.49 (s, 2H), 3.07 (s, 6H), 2.19 (s, 3H), 2.07 (s, 3H), 2.06 (s, 3H), 2.02 (s, 3H). HRMS (FAB $^+$): m/z calcd for $C_{47}H_{44}NO_{14}^+$ ([M + H]$^+$), 846.2756; found, 846.2775.

1-10. Synthesis of 10-(Dimethylamino)-3-(5-dimethylaminonaphthalene-1-sulfonyl)-spiro-[7*H*-benzo[*c*]xanthene-7,1'(3'*H*)-isobenzofuran]-3'-one (SNARF-Dan)



A mixture of SNARF (32.2 mg, 78.7 μmol), Dansyl chloride (43.4 mg, 161 μmol) and Ag_2O (60.0 mg, 259 μmol) in 2mL THF was stirred for 23 h at room temperature. A reaction mixture was filtrated to remove Ag_2O and evaporated *in vacuo*. The residue was purified by silica gel column chromatography with CH_2Cl_2/n-hexane (1:5, 1:1, 1:0, stepwise) to give straw solid (5.1 mg , 7.94 mmol , 10.0%).: ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.59-8.54 (m, 2H), 8.41 (d, 1H, *J* = 9.12 Hz), 8.07-8.02 (m, 2H), 7.72 (t, 1H, *J* = 8.68 Hz). 7.64-7.58 (m, 2H), 7.40 (t, 1H, *J* = 7.76 Hz), 7.33 (s, 1H), 7.27-7.26 (m ,1H), 7.20 (d , 1H, *J* = 9.16 Hz), 7.14 (d, 1H , *J* = 10.5Hz), 7.09 (d, 1H, *J* = 7.32 Hz), 6.72 (d, 1H, *J* = 10.0), 6.65 (d, 1H , *J* = 7.8Hz), 6.60 (s, 1H), 6.46 (d, 1H, *J* = 8.68Hz), 3.00 (s, 6H), 3.00 (s, 6H). HRMS (FAB$^+$): calcd for $C_{38}H_{31}O_6N_2S^+$ ($[M+H]^+$) 643.1903, found 643.1910.