Electronic Supplementary Material (ESI) for Chemical Communications

Electronic Supplementary Information for

Fluorescence photoswitching based on a photochromic pK_a change in aqueous solutions

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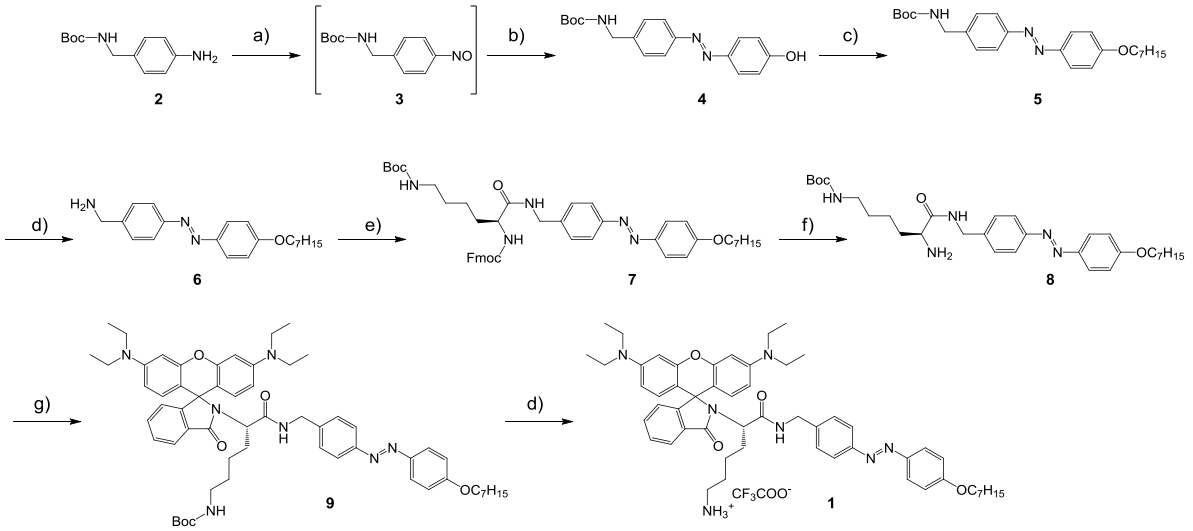
1. General

General chemicals were purchased from Tokyo Chemical Industries, Wako Pure Chemicals, Watanabe Chemical, or Aldrich Chemical Co., and used without further purification. Purifications of almost compounds were performed by silica gel column chromatography. Compound **9** and dyad **1** (Scheme S1) were further purified by GPC and HPLC, respectively. ^{1}H NMR spectra were recorded on a NMR spectrometer (JEOL, ECX-400, 400 MHz). Samples were dissolved in $CDCl_3$ (Chloroform-*d1*), $(CD_3)_2O$ (Acetone-*d6*), $(CD_3)_2SO$ (DMSO-*d6*) or CD_3OD (Methanol-*d4*) with tetramethylsilane as an internal standard. Mass spectra were measured with a mass spectrometer (Applied Biosystems, Voyger). Absorption and fluorescence spectra were measured with a Hitachi U-3100 absorption spectrophotometer and a Hitachi F-2500 fluorescence spectrophotometer, respectively. Fluorescence quantum yields were measured by comparing the yields with that of reference sample, Rhodamine B (Φ_f ~ 0.9 in water).[S1] Photoirradiation was carried out using an USHIO 500 W xenon lamp as the light sources. Monochromic light was obtained by passing the light through a monochromater (Jobin-Yvon) or a band-pass filter ($\Delta\lambda_{1/2}$ = 15 nm). The thermal stability of the open-form of RSA unit was measured by plotting the change of absorbance at 563 nm, which corresponds to only the absorption band of the open-form of RSA unit, versus time. The rate constant of the thermal open-to-closed form of RSA unit (k) was determined by fitting the absorption decay curve with the first-order kinetics.

2. Synthetic Procedure

The synthetic route to compound **1** is illustrated in Scheme S1. Detailed synthetic procedures are described below. *tert*-Butyl 4-nitrosobenzylcarbamate **3** was prepared according with the literature.[S2]

Scheme S1



a) Oxone, CH_2Cl_2/H_2O; b) 4-aminophenol, DMSO/AcOH (1/1); c) 1-iodoheptane, K_2CO_3, KI (cat.), acetone; d) TFA, CH_2Cl_2; e) Fmoc-Lys(Boc)-OH, HOBt·H_2O, DCC, dry DMF; f) piperidine, dry DMF; g) rhodamine B, HOBt·H_2O, DCC, dry DMF

Compound 4

Crude **3** (65 % purity, 2.00 g, 8.4 mmol) was dissolved in 120 ml of DMSO/AcOH 1:1. To the solution was added 4-aminophenol (1.10 g, 10 mmol). The mixture was stirred for 18h at room temperature. The product was precipitated by addition of water, separated and washed with water. The residue was purified by silica gel column chromatography (CH_2Cl_2) to give 1.66 g (5.7 mmol) of **4** in 60 % yield (two steps) as yellow solid.

^{1}H NMR ($CDCl_3$) δ (ppm) = 1.48 (s, 9H), 4.39 (d, *J* = 4.8 Hz, 2H), 5.22 (s, 1H), 6.93 (d, *J* = 8.4 Hz, 2H), 7.40 (d, *J* = 8.4 Hz, 2H), 7.81-7.89 (m, 4H); MS (MALDI): m/z = 328 $[M+H]^+$; Anal. Calcd. for $C_{18}H_{21}N_3O_3$: C, 66.04; H, 6.47; N, 12.84; Found: C, 66.13; H, 6.33; N, 12.69.

Compound 5

1-Iodoheptane (600 mg, 2.66 mmol) was added into 12 ml acetone solution containing **4** (600 mg, 1.83 mmol) and anhydrous potassium (520 mg, 3.72 mmol). The reaction mixture was refluxed overnight. After being cooled, the residue was extracted with $CHCl_3$, dried over anhydrous magnesium sulfate, and concentrated. The residue was purified by silica gel column chromatography ($CHCl_3$/hexane = 6/4) to give 720 mg (1.70 mmol) of **5** in 92 % yield as orange powder.

^{1}H NMR ($CDCl_3$) δ (ppm) = 0.90 (t, *J* = 8.0 Hz, 3H), 1.28-1.54 (m, 17H), 1.82 (q, *J* = 8.0 Hz, 2H), 4.04 (t, *J* = 8.0 Hz, 2H), 4.39 (d, *J* = 4.8 Hz, 2H), 4.91 (brs, 1H), 6.99 (d, *J* = 8.0 Hz, 2H), 7.40 (d, *J* = 8.0 Hz, 2H), 7.84 (d, *J* = 8.0 Hz, 2H), 7.89 (d, *J* = 8.0 Hz, 2H); MS (MALDI): m/z = 426 $[M+H]^+$; Anal. Calcd. for $C_{25}H_{35}N_3O_3$: C, 70.56; H, 8.29; N, 9.87; Found: C, 70.42; H, 8.27; N, 9.76.

Compound 6

Compound **5** (600 mg, 1.4 mmol) was dissolved in 8.0 ml of dry CH_2Cl_2. To the solution was added trifluoroacetic acid (TFA) (0.5 ml, 6.5 mmol). Under argon atmosphere, the mixture was stirred for 3h at room temperature. Removal of the solvent in vacuo was given crude **6** in 98 % yield as an orange powder with a purity of > 96 % as determined by ^{1}H NMR spectroscopy. The crude product was used to the next reaction without further purification.

^{1}H NMR (Methanol-*d4*) δ (ppm) = 0.92 (t, *J* = 8.0 Hz, 3H), 1.27-1.58 (m, 8H), 1.82 (p, *J* = 8.0 Hz, 2H), 4.08 (t, *J* = 8.0 Hz, 2H), 4.19 (s, 2H), 7.07 (d, *J* = 8.0 Hz, 2H), 7.60 (d, *J* = 8.0 Hz, 2H), 7.88-7.97 (m, 4H); MS (MALDI): m/z = 326 $[M+H]^+$; Anal. Calcd. for $C_{20}H_{27}N_3O$: C, 73.81; H, 8.36; N, 12.91; Found: C, 73.83; H, 8.33; N, 13.04.

Compound 7

To an anhydrous DMF (6.0 ml) solution of a mixture of **6** (300 mg, 0.92 mmol) and Fmoc-Lys(Boc)-OH (476 mg, 1.0 mmol) were successively added 1-hydroxybenzotriazol monohydrate ($HOBt\cdot H_2O$) (137 mg, 1.0 mmol), and *N*,*N*'-dicyclohexylcarbodiimide (DCC) (210 mg, 1.0 mmol). After being stirred for 18 h under Ar at room temperature, the reaction mixture was diluted with CH_2Cl_2. The precipitate was removed by filtration and the filtrate was washed with saturated aqueous NH_4Cl. Then, the organic layer was dried over $MgSO_4$ and concentrated. The residue was roughly purified by silica gel column chromatography (CH_2Cl_2/MeOH = 95/5) to give 487 mg (0.63 mmol) of **7** in 68 % yield as yellow powder.

^{1}H NMR (DMSO-*d6*) δ (ppm) = 0.87 (t, *J* = 8.0 Hz, 3H), 1.21-1.81 (m, 25H), 2.85-2.95 (m, 2H), 3.96-4.04 (m, 1H), 4.07 (t, *J* = 8.0 Hz, 3H), 4.18-4.32 (m, 3H), 4.34-4.41 (d, *J* = 4.0 Hz, 2H), 6.79 (t, *J* = 4.0 Hz, 1H), 7.11 (d, *J* = 8.0 Hz, 2H), 7.32 (t, *J* = 8.0 Hz, 2H), 7.36-7.47 (m, 4H), 7.55 (d, *J* = 8.0

Hz, 2H), 7.69-7.81 (m, 4H), 7.82-7.92 (m, 4H); MS (MALDI):m/z = 776 $[M+H]^+$; Anal. Calcd. for $C_{46}H_{57}N_5O_6$: C, 71.20; H, 7.40; N, 9.03; Found: C, 71.13; H, 7.17; N, 9.02.

Compound 8

Compound **7** (400 mg, 0.52 mmol) was dissolved in 4.0 ml of dry CH_2Cl_2. To the solution was added piperidine (0.4 ml, 5.2 mmol). Under argon atmosphere, the mixture was stirred for 3h at room temperature. The reaction mixture was diluted with CH_2Cl_2 and washed with saturated aqueous NH_4Cl. Then, the organic layer was dried over $MgSO_4$ and concentrated. The residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH = 95/5) to give 265 mg (0.48 mmol) of **8** in 93 % yield as orange powder.

^{1}H NMR (Acetone-*d6*) δ (ppm) = 0.88 (t, *J* = 8.0 Hz, 3H), 1.25-1.71 (m, 23H), 1.75 (q, *J* = 8.0 Hz, 3H), 2.82-2.95 (m, 2H), 3.13-3.24 (m, 1H), 4.07 (t, *J* = 8.0 Hz, 3H), 4.30-4.40 (m, 2H), 6.75 (brs, 1H), 7.11 (d, *J* = 8 Hz, 2H), 7.44 (d, *J* = 8.0 Hz, 2H), 7.83 (dd, *J* = 8.0 Hz, 4H), 8.46 (brs, 1H); MS (MALDI):m/z = 554 $[M+H]^+$; Anal. Calcd. for $C_{31}H_{47}N_5O_4$: C, 67.24; H, 8.56; N, 12.65; Found: C, 67.22; H, 8.48; N, 12.62.

Compound 9

To an anhydrous DMF (2.0 ml) solution of a mixture of **8** (100 mg, 0.18 mmol) and Rhodamine B (100 mg, 0.21 mmol) were successively added 1-hydroxybenzotriazol monohydrate ($HOBt\cdot H_2O$, 28 mg, 0.21 mmol), and DCC (43 mg, 0.21 mmol). After being stirred for overnight under Ar at room temperature, the reaction mixture was diluted with CH_2Cl_2 and washed with saturated aqueous NH_4Cl. Then, the organic layer was dried over $MgSO_4$ and concentrated. The residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH = 99/1) and GPC to give 148 mg (0.31 mmol) of **9** in 84 % yield as yellow powder.

^{1}H NMR (Methanol-*d4*) δ (ppm) = 0.88 (t, *J* = 8.0 Hz, 3H), 0.95-1.21 (m, 12H), 1.21-1.55 (m, 21H), 1.60-1.83 (m, 2H), 1.89-2.17 (m, 2H), 2.66-2.88 (m, 2H), 3.19-3.42 (m, 8H), 3.53 (t, *J* = 8.0 Hz, 3H), 3.99 (t, *J* = 8.0 Hz, 3H), 4.05-4.28 (m, 2H), 6.05-6.15 (m, 1H), 6.16-6.25 (m, 1H), 6.28-6.47 (m, 5H), 6.93-7.09 (m, 3H), 7.15 (d, *J* = 8.0 Hz, 2H), 7.45-7.60 (m, 2H), 7.71 (d, 2H), 7.78-7.95 (m, 4H); MS (MALDI):m/z = 979.18 $[M+H]^+$; Anal. Calcd. for $C_{59}H_{75}N_7O_6$: C, 72.44; H, 7.73; N, 10.02; Found: C, 72.51; H, 7.71; N, 10.09.

Compound 1

Compound **9** (50 mg, 0.051 mmol) was dissolved in 1.0 ml of dry CH_2Cl_2. To the solution was added TFA (250 μl, 3.3 mmol). Ander argon atmosphere, the mixture was stirred for 3h at room

temperature. The solvent was removed in vacuo and the crude product was purified by column chromatography (Cosmosil 75C_{18}-OPN, nacalai tesque; MeOH) to give 43 mg (0.049 mmol) of **1** in 96 % yield as red amorphous.

^{1}H NMR (Methanol-*d4*) δ (ppm) = 0.93 (t, J = 8 Hz, 3H), 1.06-1.57 (m, 22H), 1.58-1.70 (m, 2H), 1.77-1.89 (m, 2H), 1.89-2.12 (m, 2H), 2.80-2.95 (m, 2H), 3.49-3.78 (m, 8H), 4.03-4.15 (t, J = 8 Hz, 2H), 4.17-4.35 (m, 3H), 6.46 (brs, 1H), 6.75-6.82 (m, 1H), 6.88-6.94 (m,1H), 6.95-7.02 (m, 1H), 7.04-7.31 (m, 5H), 7.39-7.47 (m, 1H), 7.56-7.65 (m, 1H), 7.67-7.84 (m, 3H), 7.85-7.96 (m, 2H), 7.97-8.10 (m, 2H), 8.21 (t, J = 8 Hz, 1H); HRMS (FAB$^+$): calcd for $[M+H]^+$, m/z = 878.5327; found 878.5324

3. HPLC analyses on the conversion ratio from the *trans*- to the *cis*-isomer of Azo unit

The photoconversion ratio frm the *trans*- to the *cis*-isomer of Azo unit in dyad **1** upon irradiation with 365 nm light was measured with HPLC analyses. HPLC was performed on a Hitachi ELITE LaChrom system equiped with a diode array detector. Silica gel column (Mightysil RP-18 GP, KANTO chemicals) in reversed phase (MeOH/H_2O containing 1% TFA = 96/4) was used to analyze the ratio of each isomer and the isosbestic points in this eluent condition (303 nm and 412 nm) were used as the monitoring wavelength. In this eluent condition, the RSA unit nearly almost leans to the open-state, and therefore we can simply discuss the conversion ratio of Azo unit.

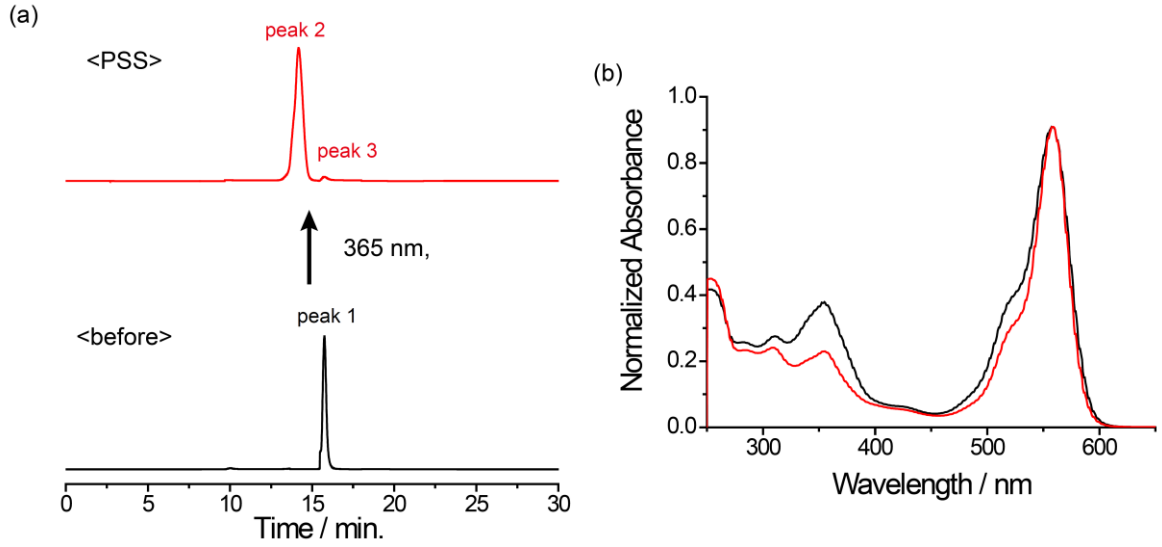


Fig. S1. (a) HPLC charts of before photoirradiation and PSS upon irradiation with 365 nm light monitored at an isosbestic point (412 nm) in this eluent condition. (b) Normalized absorption spectra of peak 2 (red-line) and peak 3 (black-line).

Peak 1 (t = 15.7 min) corresponds to the *trans*-isomer. Peak 2 (t = 14.2 min) newly observed in addition to the small peak 3 (t = 15.7 min), which retention time is same with peak 1, after 365 nm light irradiation. From the detected absorption spectra, peak 2 and 3 were assigned to the *cis*-isomer and the *trans*-isomer, respectively. The conversion ratio was estimated from the ratio of peak area between peak 2 and 3 to be > 95 % (A_2/A_3 = 95.6/4.4).

4. Absorption spectra of dyad 1 in other pH conditions

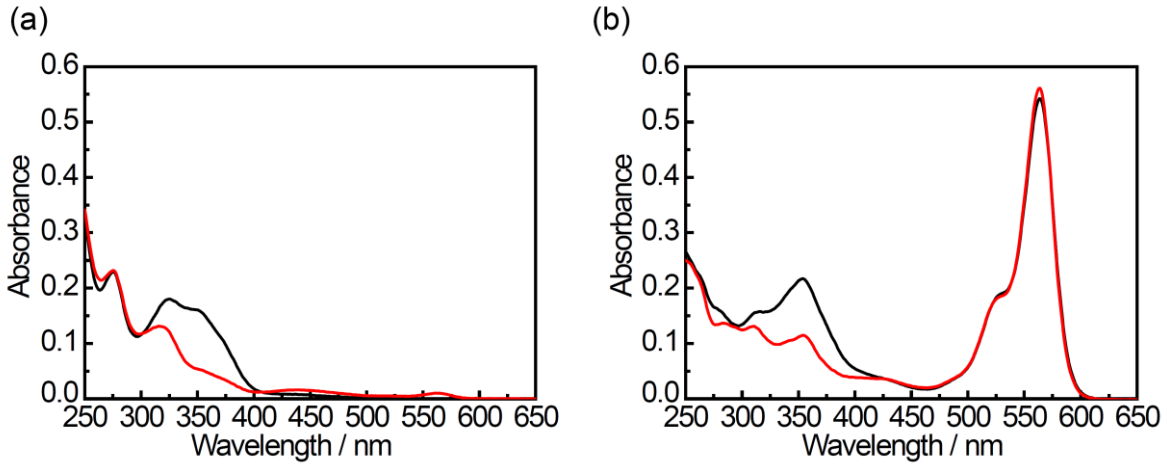


Fig. S2. Absorption spectra in a pH (a) 5.0 and (b) 3.0 solution; (black-line) before photoirradiation, (red-line) PSS upon irradiation with 365 nm light.

5. References

S1) C. V. Bindhu and S. S. Harilal, *Anal. Sci*, **2001**, *17*, 141-144.

S2) B. Priewisch and K. Ruck-Braun, *J. Org. Chem.*, **2005**, *70*, 2350-2352.