Supporting Information : A Photochromic ATP analogue Driving a Motor Protein with Reversible Light-Controlled Motility : Controlling Velocity and Binding Manner of a Kinesin-Microtubule System in an *In Vitro* Motility Assay

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Syntheses of Compounds

General

All solvents and reagents were obtained from commercial sources and used for syntheses without further purification. The reactions were performed under argon or nitrogen when using dry solvents. The silica gel used for flash column chromatography was purchased from Kanto Chemical (silica gel 60N, spherical, neutral, 40-50 μm). The paper filters used for filtration were purchased from Kiriyama (Nos. 4, 5B or 5C). For identification of the synthesized products, ^{1}H NMR and mass spectra were measured with an ECX-400 (400 MHz) (JEOL) and an AccuTOF (JMS-T100LC) (JOEL), respectively.

Compounds Shown in Scheme S1

Compound 3

To a suspension of **2** (4.0 g, 15 mmol) in dry pyridine (60 mL), trimethylsilyl chloride (TMS-Cl) (6.8 mL, 54 mmol) was added dropwise and stirred for 4.5 h at room temperature, then benzoyl chloride (BzCl) (4.2 mL, 36 mmol) was added dropwise in an ice bath. After ~3h stirring at room temperature, water (16 mL) was slowly poured to the mixture in an ice bath, followed by stirring for ~1h at room temperature. The resulting mixture was diluted with 200 mL CH_2Cl_2 and washed with brine (80 mL). After separation, the aqueous layer was further extracted with CH_2Cl_2 (50 mL) three times. The combined extracts were dried with anhydrous Na_2SO_4, evaporated, and dried in vacuo. Silica gel column chromatography (CH_2Cl_2/MeOH=100:1 to 100:6) provided **3** (5.81 g, 81%). ^{1}H-NMR [$CDCl_3$ (TMS)], δ=8.62 [s, 1H], 8.13 [s, 1H], 7.87-7.36 [m, 10H], 5.89 [d, *J*=7.1 Hz, 1H], 5.24-2.85 [m, 8H] ppm. ESI-MS:

m/*z*=498.16 $[M+Na]^+$ (calcd. 498.14).

Compound 4

To a dry pyridine (60 mL) solution of **3** (5.35 g, 11.3 mmol), 1,1,3,3-tetraisopropyl-1,3-dichlorodisilyloxane (TIPDS-Cl2) (5.3 ml, 16.9 mmol) was added and stirred for 8 h at room temperature. The reaction was quenched by the stepwise addition of water (43 mL), then the resulting mixture was diluted with 200 mL CH_2Cl_2 and washed with brine (75 mL) followed by the separation and further CH_2Cl_2 extraction (80 mL, three times). The combined extracts were dried with anhydrous Na_2SO_4, evaporated, and dried in vacuo. Silica gel column chromatographies (CH_2Cl_2/MeOH=100:3 and 100:5) provided **4** (6.17 g, 76%). ^{1}H-NMR [$CDCl_3$ (TMS)], δ=8.60 [s, 1H], 8.16 [s, 1H], 7.87-7.34 [m, 10H], 6.00 [d, *J*=1.3 Hz, 1H], 5.12-3.13 [m, 6H], 1.12-1.03 [m, 28H] ppm. ESI-MS: *m*/*z*=740.30 $[M+Na]^+$ (calcd. 740.29).

Compound 5a, 5b

NaH (60%, dispersion in paraffin liquid) (0.25 g, 6.27 mmol) was suspended in dry DMF {2 mL for **5a**, 3 mL for **5b**, respectively} at -20 ºC, and a dry DMF (32, 18 mL) solution of **4** (3.0 g, 4.18 mmol) was added dropwise followed by dropwise addition of a dry DMF (16, 15 ml solution) of 4-bromomethylazobenzene (1.38 g, 5.01 mmol) or 4'-*tert*-butyl-4-bromomethylazobenzene (1.66 g, 5.01 mmol) (for compound **13a** or **13b**; the synthetic pathway is described in this Supporting Information, Scheme S1). The suspension was stirred for >4.5 h as it was gradually increased in temperature to -1~0 ºC in 2h and then decreased to -20 ºC in 15 min. After termination of the stir, the resulting mixture was placed for 2 h at -20 ºC, and the precipitate was removed. To the supernatant liquid, saturated aqueous solution (10 mL) of $NaHCO_3$ was slowly added at -20 ºC and then added Ac-O-Et (120, 150 mL) at room temperature. After having been washed with saturated aqueous solutions (120, 150 mL) of $NaHCO_3$ (twice) and NaCl (once), the organic layer was dried with anhydrous Na_2SO_4, evaporated, and dried in vacuo. Silica gel column chromatography (Hexane/Ac-O-Et=7:3) provided **5a** (0.97 g, 25%) or **5b** (1.09 g, 27%). ^{1}H-NMR [$CDCl_3$ (TMS)], **5a**: δ=8.64 [s, 1H], 8.28 [s, 1H], 7.93-7.33 [m, 19H], 6.14 [s, 1H], 5.15-4.03 [m, 7H], 1.10-1.02 [m, 28H]. **5b**: δ=8.63 [s, 1H], 8.28 [s, 1H], 7.88-7.33 [m, 18H], 6.13 [s, 1H], 5.15-4.04 [m, 7H], 1.38 [s, 9H], 1.10-1.02 [m, 28H] ppm.

ESI-MS: **5a**, *m*/*z*=934.34 $[M+Na]^+$ (calcd. 934.38), **5b**, *m*/*z*=968.47 $[M+H]^+$ (calcd. 968.46).

Compound 6a, 6b

To a solution of **5** (**a**: 2.80 g, 3.07 mmol or **b**: 2.18g, 2.25 mmol) in 112 or 82 mL of dioxane, concentrated NH_4OH (38 or 27 mL) was added in an ice bath. The mixture was stirred at room temperature overnight and then evaporated and dried in vacuo. Silica gel column chromatography (CH_2Cl_2/MeOH=95:5, 93:7) provided **6a** (1.68 g, 78 %) or **6b** (0.79 g, 46%). ^{1}H-NMR [$CDCl_3$ (TMS)], **6a**: δ=8.36 [s, 1H], 8.12 [s, 1H], 7.93-7.45 [m, 9H], 6.15 [s, 1H], 5.49 [s, br, 2H], 5.17-4.04 [m, 7H], 1.12-0.98 [m, 28H] ppm. **6b**: δ=8.36 [s, 1H], 8.12 [s, 1H], 7.87-7.52 [m, 8H], 6.15 [s, 1H], 5.51 [s, br, 2H], 5.15-4.04 [m, 7H], 1.37 [s, 9H] 1.12-0.97 [m, 28H] ppm. ESI-MS: **6a**, *m*/*z*=704.37 $[M+H]^+$ (calcd. 704.34). **6b**, *m*/*z*=782.41 $[M+Na]^+$ (calcd. 782.39).

Compound 7a, 7b

Tetrabutylammonium fluoride (TBAF) (1M THF solution, 5.1 or 2.4 mL) was added dropwise to a dry THF (64 or 30 mL) solution of **6** (**a**: 1.59 g, 2.26 mmol or **b**: 0.79 g, 1.04 mmol) and stirred for 1.5 h in an ice bath, and then the mixture was evaporated and dried in vacuo. Silica gel column chromatography (CH_2Cl_2/MeOH=92:8, 91:9) provided **7a** (0.88 g, 84%) or **7b** (0.50 g, 93%) . ^{1}H-NMR [$CDCl_3$ (TMS)], **7a**: δ=8.20 [s, 1H], 7.94-7.16 [m, 9H], 7.72 [s, 1H], 6.65 [dd, *J*=12.3, 1.7 Hz, 1H], 5.83-2.71 [m, 9H], 5.39 [s, br, 2H], ppm. **7b**: δ=8.20 [s, 1H], 7.87-7.15 [m, 8H], 7.72 [s, 1H], 6.66 [dd, *J*=10.8, 1.8 Hz, 1H], 5.82-2.73 [m, 9H], 5.39 [s, br, 2H], 1.38 [s, 9H] ppm. ESI-MS: **7a**, *m*/*z*=462.05 $[M+H]^+$ (calcd. 462.19). **7b**, *m*/*z*=540.22 $[M+Na]^+$ (calcd. 540.23).

Compound 8a, 8b

1*H*-Tetrazole (0.36 g, 5.20 mmol or 0.20 g, 2.90 mmol) was added in one portion to a dry DMF (14 or 5 mL) solution of **7** (**a**: 0.80 g, 1.73 mmol or **b**: 0.50 g, 0.97 mmol) and di-*tert*-butyl *N*,*N*-diisopropylphoshoramidite (0.71 ml, 2.25 mmol or 0.40 ml, 1.26 mmol), and stirred for >3h at room temperature. And then, to the mixture was added a solution of 65% m-chloroperoxybenzoic acid (MCPBA) (0.80g, 3.00 mmol or 0.44g, 1.67 mmol) in CH_2Cl_2 (4 or 3.6 mL) and stirred for ~1h in an ice bath. After having

been stirred for 25 min at room temperature, saturated aqueous solution (19 or 10 mL) of $NaHCO_3$ was added and the mixture was further stirred for 40 min. To the resulting mixture was added Ac-O-Et (80 or 40 mL) to be washed with saturated aqueous solutions (80 or 40 mL) of $NaHCO_3$ (twice) and NaCl (once), and then the organic layer was dried with anhydrous Na_2SO_4, evaporated, and dried in vacuo. Silica gel column chromatography (CH_2Cl_2/MeOH=90:10) provided **8a** (0.85 g, 75%) or 8**b** (0.41 g, 60%). ^{1}H-NMR [$CDCl_3$ (TMS)], **8a**: δ=8.36 [s, 1H], 8.06 [s, 1H], 7.93-7.39 [m, 9H], 6.22 [d, *J*=4.0 Hz, 1H], 5.49 [s, br, 2H], 4.84 [s, 2H], 4.59-2.96 [m, 6H], 1.50 and 1.48 [s, 9x2=18H] ppm. **8b**: δ=8.36 [s, 1H], 8.05 [s, 1H], 7.86-7.37 [m, 8H], 6.22 [d, *J*=3.8 Hz, 1H], 5.47 [s, br, 2H], 4.82 [s, 2H], 4.59-2.94 [m, 6H], 1.54-1.46 [m, 18H], 1.38 [s, 9H] ppm. ESI-MS: **8a**, *m*/*z*=676.33 $[M+Na]^+$ (calcd. 676.26). **8b**, *m*/*z*=732.33 $[M+Na]^+$ (calcd. 732.33).

Compound 9a, 9b

To a dry CH_2Cl_2 (4.5 or 6.3 mL) solution of **9** (**a**: 0.27 g, 0.41 mmol or **b**: 0.41g, 0.58 mmol), CF_3COOH (0.5 or 0.7 mL) was added, stirred for >6 h at room temperature and evaporated. And then, after adding 10 mL of methanol, the suspension was exhaustively evaporated and dried in vacuo. This procedure was repeated more than three times for completely removing CF_3COOH residue followed by washing with CH_2Cl_2 and drying in vacuo to obtain **9a** or **9b** (quant). ^{1}H-NMR [DMSO-d_6 (TMS)], **9a**: δ=8.41 [s, 1H], 8.24 [s, 1H], 7.90-7.46 [m, 9H], 6.17 [d, *J*=5.5 Hz, 1H], 4.84-3.98 [m, 7H] ppm. **9b**: δ=8.42 [s, 1H], 8.24 [s, 1H], 7.84-7.44 [m, 8H], 6.17 [d, *J*=5.7 Hz, 1H], 4.84-3.98 [m, 7H], 1.34 [s, 9H] ppm. ESI-MS: **9a**, *m*/*z*=540.27 $[M-H]^-$ (calcd. 540.14). **9b**, ESI-MS: *m*/*z*=598.25 $[M+H]^+$ (calcd. 598.22).

Compound 1a, 1b

Phosphoric acid (0.27g, 2.77 mmol for **1a** or 0.33 g, 3.35 mmol for **1b**) was suspended in 10 ml of dry pyridine and then the suspension was evaporated and dried in vacuo. After three times of this procedure, 10 ml of dry pyridine was added and stirred, and to the mixture was added 1.8 or 2.2 mL of tri-*n*-butylamine at room temperature. To the solution, **9** (**a**: 0.15 g, 0.28 mmol or **b**: 0.20 g, 0.33 mmol) and DCC (063 g, 3.05 mmol or 0.76 g, 3.68 mmol) were added and stirred for >2 d at room temperature. The resultant mixture was filtered, added 80 mL of water, and washed with 90 mL portion

of ether three times. The water layer was evaporated to be reduced to ~90% volume and adsorbed on activated charcoal. Then the adsorbed was washed with 200 ml of water and eluted with 50% aqueous ethanol containing 2% ammonia (150 mL). The eluted solution was evaporated and dried in vacuo. The dried residue was dissolved in 0. 10 or 0.15 M trietylammonium hydrogencarbonate solution and applied to a column (ϕ=2.8 cm, 25 g) of DEAE-cellulose (carbonate form) and eluted with a linear gradient of trietylammonium hydrogencarbonate (0.10 M-0.40 M or 0.15 M-0.45 M, total 1L, 15 mL/tube). Fractions corresponding to **1a** or **1b** (ATP-Azo) confirmed by ESI-mass were collected and evaporated with 15 ml each of methanol for three times to remove triethylammonium hydrogencarbonate. Finally, **1a** or **1b** with Na^+-form was obtained through an weak cationic ion-exchange chromatography (Bio-Rex 70) (conversion ratio > 90 or 80%/molecule, >96 or 93%/phosphate of **1a** or **1b**). Contamination of ADP derivative was negligible according to an HPLC analysis (Fig. S7 for **1b**). The concentration of ATP-Azo was determined by at the absorbance of λ_{max} (327, 338 nm) of the azobenzene moiety using the value, ε=25000, which is derived from an water soluble azobenzen derivative, *p*-phenylazophenyl phosphoric acid as a control compound. Yield; **1a**, 10%, **1b**, 8.8%. ^{1}H-NMR [D_2O (TMS)], **1a**: δ=8.32 [s, 1H], 8.04 [s, 1H], 7.91-7.64 [m, 9H], 5.90 [d, *J*=6.8 Hz, 1H], 4.96-4.15 [m, 7H] ppm. **1b**: δ=8.32 [s, 1H], 8.04 [s, 1H], 7.88-7.25 [m, 8H], 5.91 [d, *J*=6.6 Hz, 1H], 4.84-4.15 [m, 7H], 1.41 [s, 9H] ppm. ESI-MS: **1a**, *m*/*z*=701.22 $[M-H]^-$ (calcd. 701.08). **1b**, *m*/*z*=756.24 $[M-H]^-$ (calcd. 756.13).

Compounds Shown in Scheme S2

Compound 11

Compound **10** (7.44 g, 48.6 mmol) and ammonium chloride (3.28 g, 61.3 mmol) were dissolved in 160 mL of a 2-methoxyethanol-water mixing solvent (10:1) under argon, and then added zinc powder (7.80 g, 119 mmol) at room temperature. After 2 h stirring, the suspension was filtered, followed by pouring into 500 mL aqueous solution of $FeCl_3 \cdot 6H_2O$ (13.24 g, 49.0 mmol) in an ice bath and then stirred overnight. The resultant aqueous solution was extracted by CH_2Cl_2 (100 mL × 1, 50 mL × 3). After evaporation of the extracted solution and drying in vacuo, **11** was obtained as a mixture

of **10** (5.56 g for mixture, 2.73 g for **11** estimated from NMR analysis) and used for synthesizing **12a** and **12b** without further purification. ^{1}H-NMR of the mixture of **10** and **11** [$CDCl_3$ (TMS)], δ=8.23 [d, *J*=8.6 Hz, 2H], 7.91 [d, *J*=8.4, 2H], 7.61 [d, *J*=8.6 Hz, 2H], 7.54 [d, *J*=9.0 Hz, 2H], 4.84 [d, 2H], 1.89 [s, br, 1H] ppm.

Compound 12a

To an ethanol (320 mL) solution of **10** and **11** (3.01 g, 22.0 mmol for **11**), aniline (2.6 mL, 28.6 mmol) and CH_3COOH (36.0 mL) were added and then stirred for 8 h at room temperature. Water (~600 mL) was poured to the solution and the obtained precipitate was dried in vacuo after filtration. Silica gel column chromatography (CH_2Cl_2/MeOH=98:2) provided **12a** (3.35 g, 72%). ^{1}H-NMR [$CDCl_3$ (TMS)], δ=7.94-7.48 [m, 9H], 4.80 [d, *J*=6.0 Hz, 2H], 1.74 [t, *J*=6.0 Hz, 1H] ppm, ESI-MS: *m*/*z*=211.19 $[M-H]^-$ (calcd. 211.08).

Compound 12b

To an ethanol (290 mL) solution of **10** and **11** (2.73 g, 19.9 mmol for **11**), 4-*tert*-butylaniline (4.1 mL, 25.9 mmol) and CH_3COOH (32.2 mL) were added and then stirred for 8 h at room temperature. The solution was reduced to ~30 mL by evaporation and added 200 mL of Ac-O-Et, and then washed with saturated aqueous solutions (230 mL) of $NaHCO_3$ (twice) and NaCl (twice), and then the organic layer was dried with anhydrous Na_2SO_4, evaporated, and dried in vacuo. Silica gel column chromatographys (CH_2Cl_2/MeOH=98:2 and Hexane/Ac-O-Et=55:45) provided **12** (4.68 g, 88%). ^{1}H-NMR [$CDCl_3$ (TMS)], δ=7.92-7.50 [m, 8H], 4.80 [d, *J*=6.0 Hz, 2H], 1.72 [t, *J*=6.0 Hz, 1H], 1.38 [s, 9H] ppm. ESI-MS: *m*/*z*=269.23 $[M+H]^+$ (calcd. 269.17).

Compound 13a

Compound **12a** (3.14 g, 14.8 mmol) and CBr_4 (7.36 g, 22.2 mmol) were dissolved in 160 mL of dry THF and added PPh_3 (5.82 g, 22.2 mmol). After stirring (~4h) at room temperature, the solution was filtered and the filtrate was evaporated and dried in vacuo. Silica gel column chromatography (Hexane/Ac-O-Et=10:1) provided **13a** (3.65 g, 90%). ^{1}H-NMR [$CDCl_3$ (TMS)], δ=7.93-7.48 [m, 9H], 4.56 [s, 2H] ppm. ESI-MS: *m*/*z*=297.05, 299.04 $[M+Na]^+$ (calcd. 297.00, 299.00).

Compound 13b

Compound **12b** (3.0 g, 11.2 mmol) and CBr_4 (5.56 g, 16.8 mmol) were dissolved in 120 mL of dry THF and added PPh_3 (4.40 g, 16.8 mmol). After overnight stirring at room temperature, the solution was reduced to ~10 mL by evaporation and added 120 mL of Ac-O-Et, and then washed with saturated aqueous solutions (130 mL) of $NaHCO_3$ (twice) and NaCl (once), and then the organic layer was dried with anhydrous Na_2SO_4, evaporated, and dried in vacuo. Silica gel column chromatography (Hexane/Ac-O-Et=100:8) provided **13b** (3.44 g, 93%). ^{1}H-NMR [$CDCl_3$ (TMS)], δ=7.89-7.52 [m, 8H], 4.56 [s, 2H], 1.38 [s, 9H] ppm. ESI-MS: *m*/*z*=353.07, 355.05 $[M+Na]^+$ (calcd. 353.06, 355.06).

Observation of Motility of Kinesin-Microtubule System

Protein Purifications and Preparations

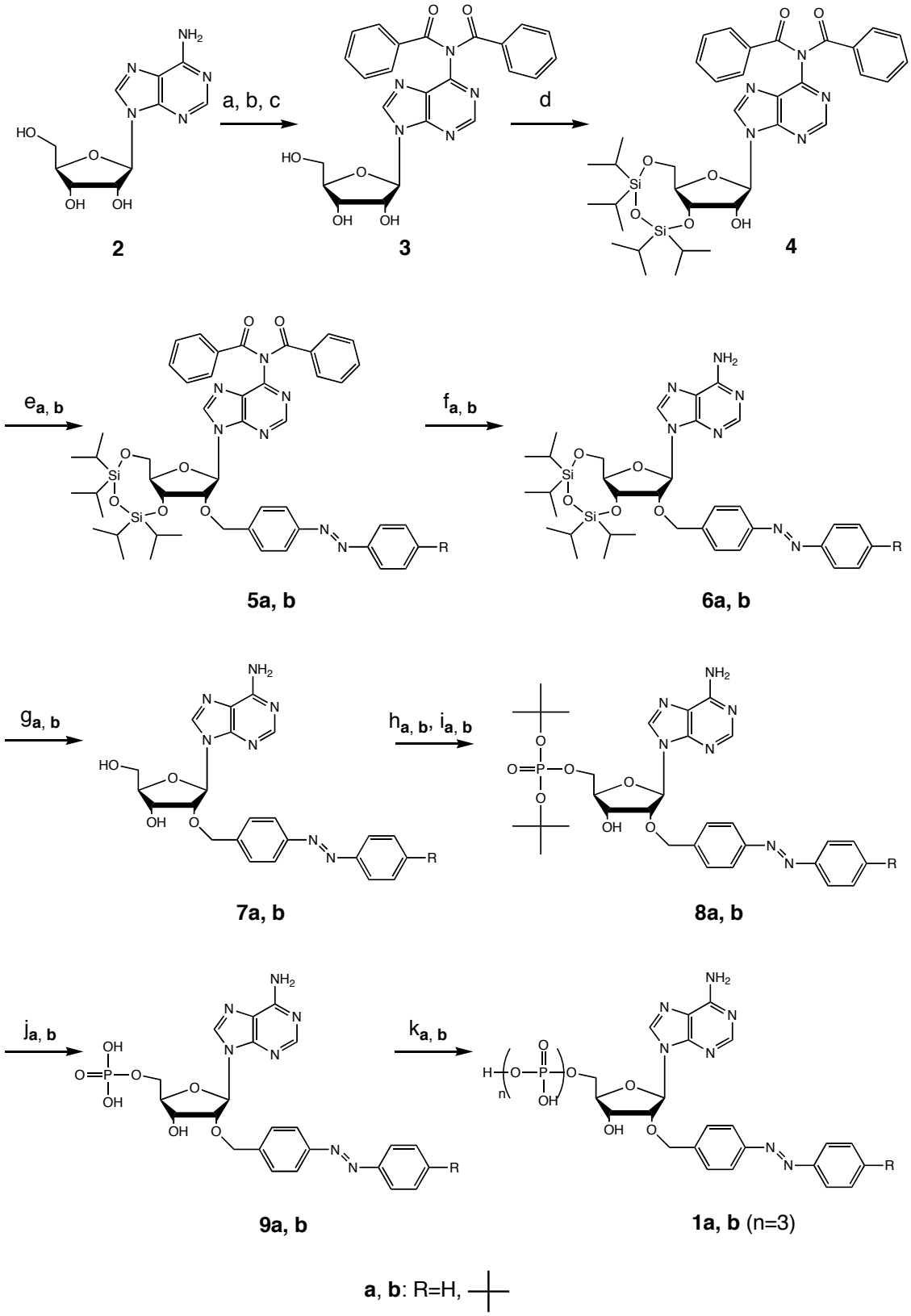
Tubulins were purified from porcin brains through two cycles of polymerization-depolymerization processes in the presence of a high-molarity PIPES buffer. MTs were polymerized using the purified tubulins and labeled with teteramethylrhodamine succinimidyl ester. Kinesin utilized in this research was a recombinant kinesin consist of 573 amino acid residues from N-terminus of a conventional human kinesin. This recombinant kinesin fused with His-tag in the N-terminus (plasmid; pET 30b) was expressed in *E. coli* Rossetta (DE3)pLysS and purified by the general method utilizing Ni-NTA-agarose.

***In Vitro* Motility Assay**

The chamber for microscopy observation was prepared by taping a cover slip (22×22 mm) and a slide glass (76×26 mm) together at the both extremities to make a flow path (~2×22 mm). The kinesin solution containing casein (10 μL; kinesin : ~0.1 mg/mL, casein: ~2 mg/mL) was flowed into the prepared chamber, followed by two times washing with the assay buffer (PIPES: 80 mM, $MgCl_2$: 2mM, EGTA: 1mM) containing Taxol (10 μL; Taxol: 10 μM) after one minute incubation. Then, MTs solution (10 μL; MTs calculated as tubulin dimer: 0.5 μM, Taxol 10 μM) was flowed and incubated for two minutes. After two times flowing the assay buffer (10 μL) containg ATP-Azo

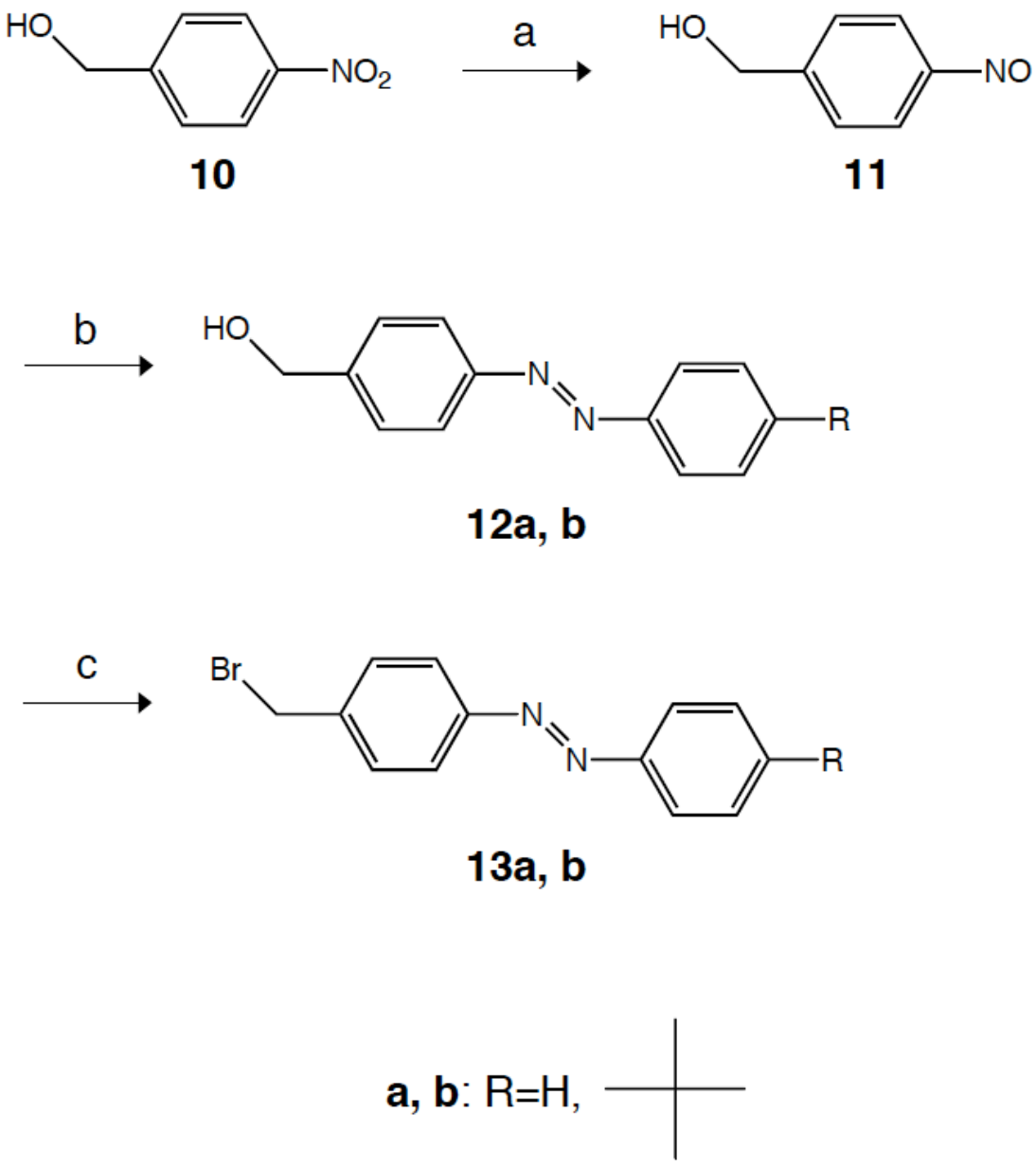
(desired concentrations) and Taxol (10 μM) with an added oxygen scavenger system (casein: 0.5 mg/mL, 2-mercaptethanol: 0.14 M, glucose 20 mM, catalase: 20 μg/mL, glucose oxydase: 100 μg/mL), the prepared chamber was irradiated for 1 min with UV (λ=365 nm) or visible (λ=436 nm) lights from a high pressure mercury lamp (Optical Modulex SX-UID501HAMQ; USHIO) through appropriate filters. Then the chamber was supplied for observation with fluorescent microscopy (BX50 with fluorescent mirror unit, U-MWIG2 and an oil- emersion objective lens, 100× 1.30 NA; OLYMPUS) coupled to a CCD camera (DXC-950; SONY) at room temperature controlled to 23.1~23.6 ºC. During microscopy observation, the chamber was simultaneously irradiated with lights through fiber cable from the mercury lamp in order to keep photostationary state as possible. Same chamber was used for a sequence of observation at each condition of ATP-Azo concentration. Motilities of MTs (Fig. S3) were analyzed by Image J. Sliding velocities of MTs driven by kinesins in the presence of ATP in a control experiment and no effect of UV-light irradiation on kinesin-induced motilities of MTs were shown in Fig. S4. According to the HPLC analysis (Fig. S7), contaminating ADP derivative inhibition of kinesin activity was negligible.

Scheme S1


a, b, c
d
2
3
4
$e_{a, b}$
$f_{a, b}$
5a, b
6a, b
$g_{a, b}$
$h_{a, b}$, $i_{a, b}$
7a, b
8a, b
$j_{a, b}$
$k_{a, b}$
9a, b
1a, b (n=3)
a, b: R=H,

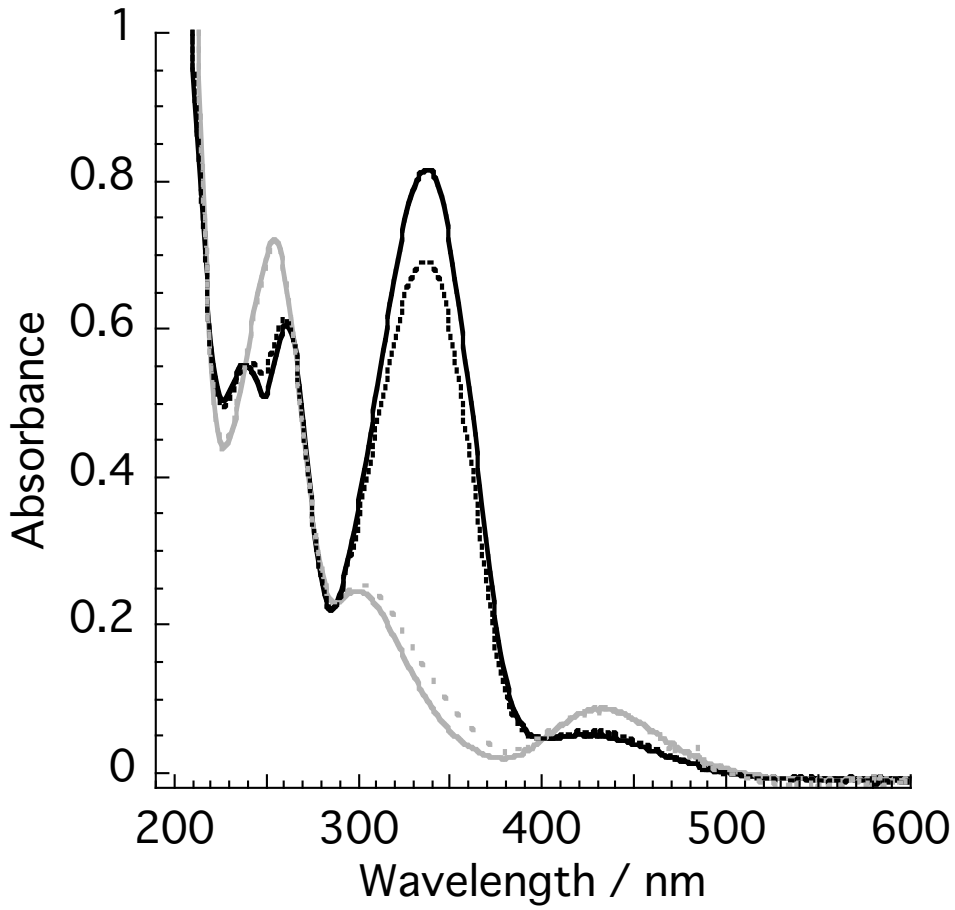
Synthesis of ATP-Azo **1a** and **1b**. a) TMS-Cl, dry pyridine, rt, 4.5 h; b) benzoyl chloride, ca. 0 °C, rt, 3 h; c) H_2O, ca. 0 °C, rt, 1 h, 81%; d) TIPDS-Cl2, dry pyridine, rt, 8 h, 76%; e) 4´-bromomethylazobenzene (**a**) or 4-*tert*-butyl-4´-bromomethylazobenzene (**b**) (see Scheme S2, ESI), NaH, dry DMF, –20 to ca. 0 °C, >4.5 h, 25% (**a**) or 27% (**b**); f) NH_4OH, dioxane, ca. 0 °C, rt, overnight, 78% (**a**) or 46% (**b**); g) TBAF, dry THF, ca. 0 °C, 1.5 h, 88% (**a**) or 93% (**b**); h) di-*tert*-butyl *N,N*-diisopropylphosphoramidite, ^{1}*H*-tetrazole, dry DMF, rt, >3 h; i) MCPBA, ca. 0 °C/55 min then rt/25 min, 75% (**a**) or 60% (**b**); j) trifluoroacetic acid, dry CH_2Cl_2, rt, >6 h, quant.; k) phosphoric acid, DCC, tri-*n*-butylamine, dry pyridine, rt, >2 days, 10% (**a**) or 8.8% (**b**).

Scheme S2



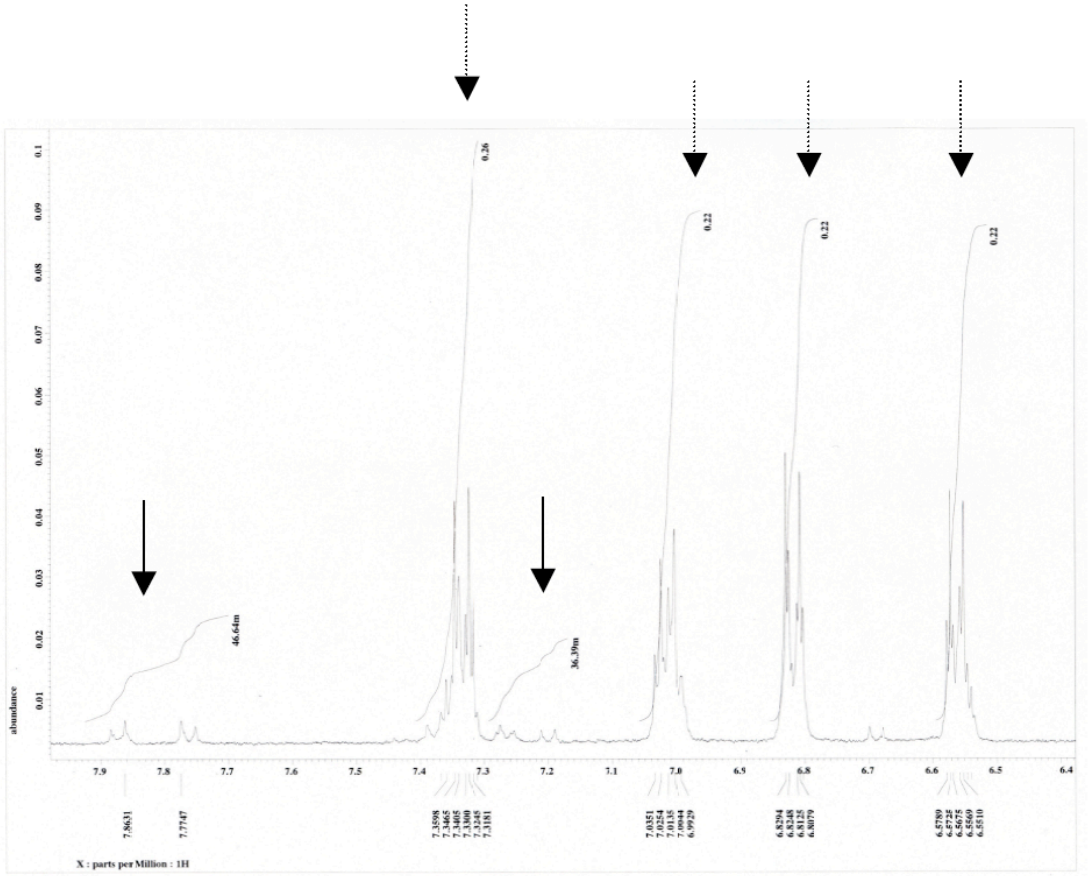
Synthesis of 4-bromomthylbenzene (**13a**) and 4'-*tert*-butyl-4-bromomethylazobenzene (**13b**). a) NH_4Cl, Zn, 2-methoxyethanol/water (10:1), rt, 2h, $FeCl_3 \cdot 6H_2O$, Water, 0 ºC, rt, overnight. b) (**12a**) aniline, CH_3COOH, ethanol, 8h, rt, 72%. (**13b**) 4-*tert*-butylaniline, CH_3COOH, ethanol, 8h, rt, 88%. c) CBr_4, PPh_3, dryTHF, rt, >4h, 90 % for (**13a**), 93% for (**13b**).

Fig. S1



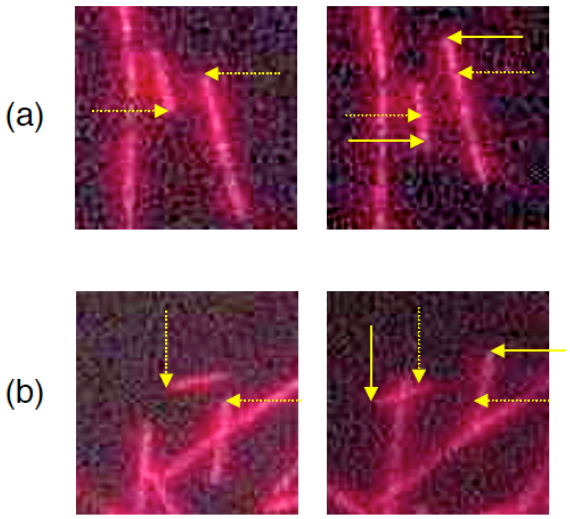
Photoisomerization of **1b** (32.5 μM) at 23.5 ± 0.5 °C in water. Spectral changes of **1b** before (black solid line) and after (gray solid line) irradiation (1 min) with UV light, followed by irradiation with visible light (black dotted line). Thermal recovery after 270 min in the dark is revealed by gray dotted line.

Fig. S2



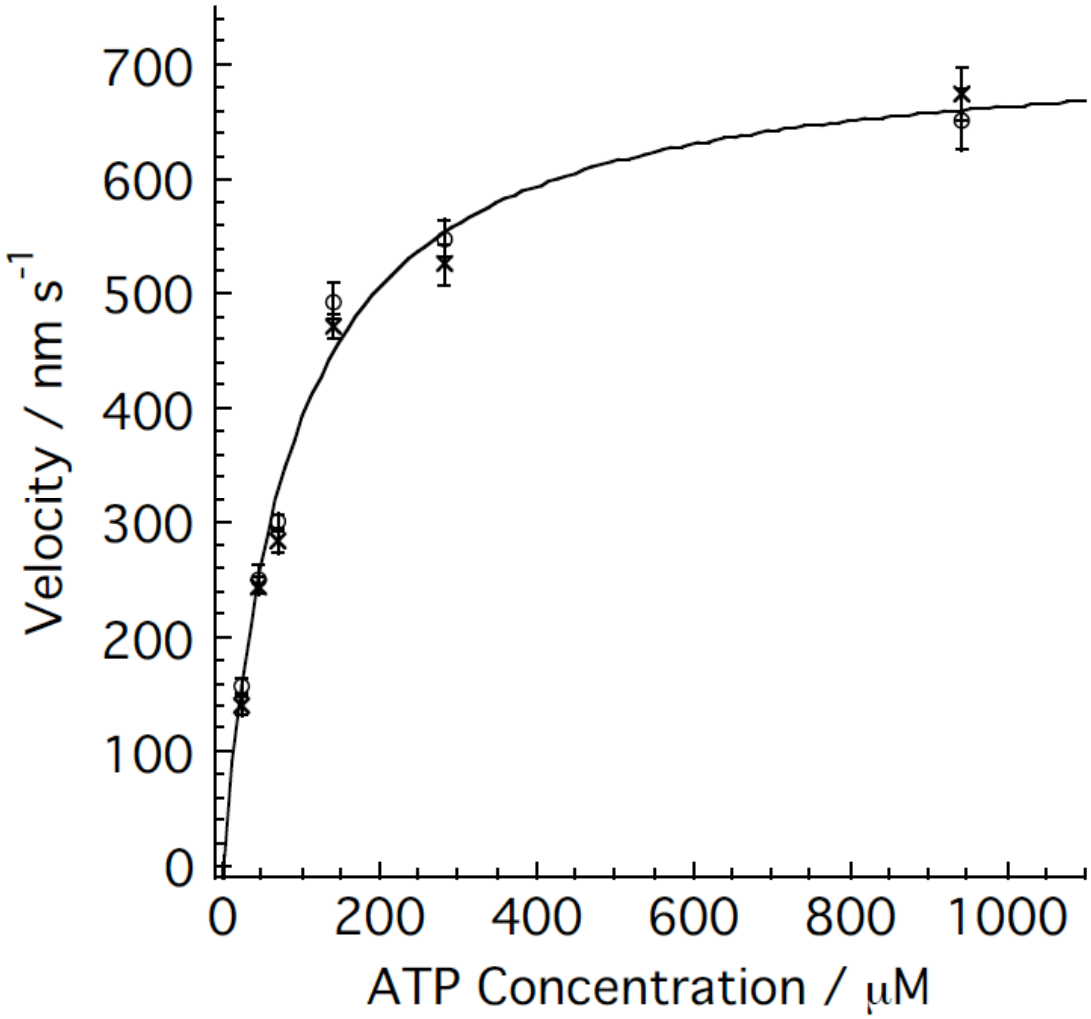
NMR chart of ATP-Azo (**1b**) in D_2O with photostationary state induced by UV light irradiation. Solid arrows and dotted arrows correspond to the peaks of *trans*-form azobenzen moiety and those of *cis*-form, respectively. The fraction ratio of *trans*-form azobenzene moiety tethered in **1b** was ~0.08. Therefore, the ratios of *trans*-**1b** to *cis*-**1b** in the photostationary states upon irradiation with UV and visible light were 8:92 and 86:14, respectively, estimated from analyses of both NMR (Fig. S2, ESI) and UV–Vis absorption (Fig. S1) spectra.

Fig. S3



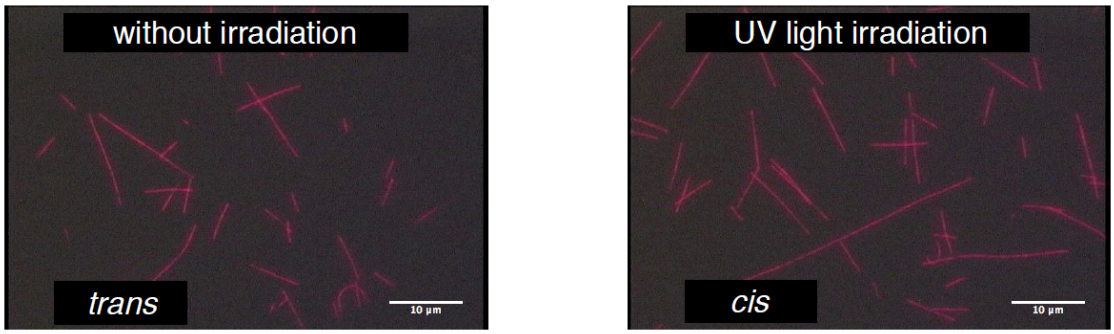
Photographed images of fluorescent-labeled microtubules in the *in vitro* motility assay [300 μM *trans*-**1b** (a) and *cis*-**1b** (b) for instances]. Left images; 0 s. Right images; 8.3 s. Dotted and solid arrows represent the edges of microtubules as starting (0 s) and final (8.3 s) points in the gliding distances, respectively. Field of each image; 6.4x6.4 μm.

Fig. S4



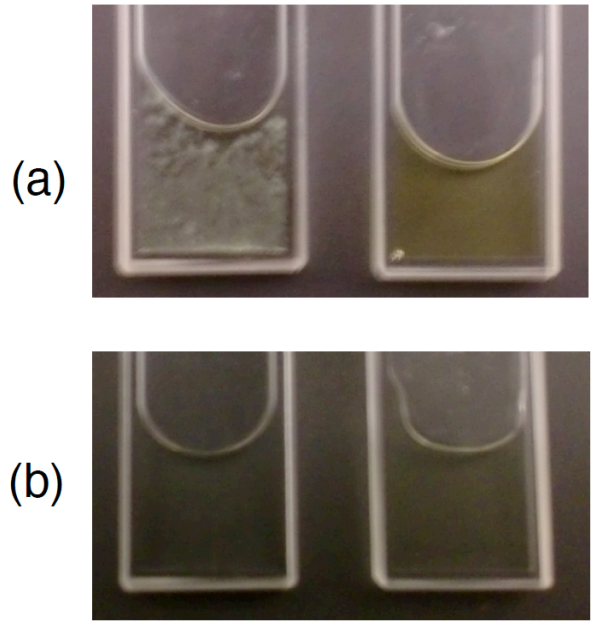
Sliding velocities of MTs driven by kinesin coated on the glass at the various conditions of ATP concentration without (circle) and with UV (cross) light irradiation. The velocities of MTs without irradiation were almost identical to those with irradiation at the same concentrations of ATP. The curve without irradiation was fitted to the data by Michaelis-Menten equation {$V=V_{max}\cdot[ATP]/(K_m^{ATP}+[ATP])$, V_{max}=720 nm/s, K_m^{ATP}=85 μM}.

Fig. S5



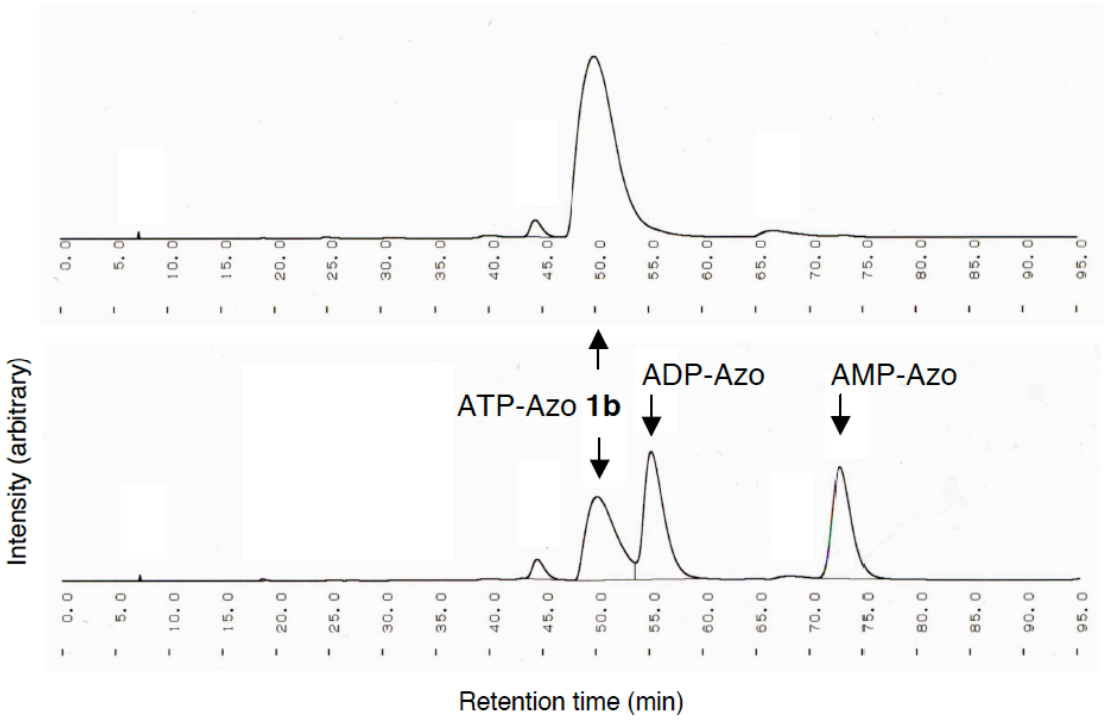
Attachments of MTs to kinesins coated on the glass surface in the presence of ATP-Azo **1b** (300 μM) in *trans*-form (left) and in *cis*-form (right), respectively.

Fig. S6



Photographs of the motility assay buffers in the presence of **1b**, 1 mM (a) and 300 μM (b), respectively. Left; before irradiation (*trans*-**1b**). Right; after irradiation with UV light (*cis*-**1b**). The content of the motility assay buffer is described in the ESI "Observation of Motility of Kinesin-Microtubule System, *In Vitro* Motility Assay".

Fig. S7



Reverse-phased HPLC profiles of ATP-Azo (for **1b**) after the final manipulation of synthesis. Top; Profile of ATP-Azo **1b** (0.27 mM in water) after the final manipulation of synthesis. Bottom; Control profile of the solution of ATP-Azo **1b** (0.13 mM) added with the mixture of ADP (0.13 mM) and AMP (0.13 mM) derivatives (ADP-Azo and AMP-Azo, respectively), which were obtained through the synthesis of **1b**. Retention times of ATP-Azo, ADP-Azo and AMP-Azo were 50. 1, 55.2 and 72.9 min, respectively. Judging from these profiles, the contamination of ADP-Azo was negligible in our *in vitro* motility assay. Conditions of the RP-HPLC analysis; Column: CN-80Ts, 4.6x250 mm (TOSOH). Eluent: Aqueous solution of 0.1 M NaH_2PO_4 (prepared to pH 4.1 before mixing CH_3CN) containing 20% CH_3CN. Monitoring wavelength: λ=338 nm. Flow rate: 0.54 ml/min at room temperature (~22 ºC). Injection volume: 20 μL.