Electronic Supplementary Information

Photoisomerization of the Green Fluorescence Protein Chromophore and the meta- and para-Amino Analogues

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Experimental Methods. Electronic spectra were recorded at room temperature $(23 \pm 1 \, {}^{\circ}\text{C})$. UV-visible spectra were measured on a Cary300 double beam spectrophotometer. Fluorescence ¹⁰ spectra were recorded on a PTI QuantaMaster C-60 or the Edinburgh FLS920 spectrometers and corrected for the response of the detector. The optical density (OD) of all solutions was about 0.1 at the wavelength of excitation. A N₂-bubbled (15 min) solution of 9,10-diphenylanthracene ($\Phi_f = 0.93$ in hexane)¹ was

- 15 used as a standard for the fluorescence quantum yield determinations of compounds under N_2 -bubbled solutions with solvent refractive index correction. An error of 10% is estimated for the fluorescence quantum yields. Fluorescence decays were measured at room temperature by the Edinburgh FLS920
- ²⁰ spectrometer with a gated hydrogen arc lamp using a scatter solution to profile the instrument response function. The goodness of the nonlinear least-squares fit was judged by the reduced χ^2 value (<1.2 in all cases), the randomness of the residuals, and the autocorrelation function. Quantum yields of
- ²⁵ photoisomerization were measured on optically dense N₂-bubbled solutions (10⁻³-10⁻⁴ M) at 350 nm using a 75-W Xe arc lamp and monochromator. *N*-phenyl-4-aminostilbenes was used as a reference standard ($\Phi_{tc} = 0.34$ in CH₂Cl₂).² The extent of photoisomerization (<10%) was determined using HPLC analysis
- $_{30}$ (Waters 600 Controller and 996 photodiode array detector, Thermo APS-2 Hypersil, heptane and ethyl acetate mixed solvent) without back-reaction corrections. The reproducibility error was <10% of the average.
- ³⁵ Materials. Solvents for spectra and quantum yield measurements all were HPLC grade and used as received. THF and acetonitrile were dried by sodium metal and casium hydride, respectively, and distilled before use. All the other solvents for spectra and quantum yield measurements were HPLC grade and used as ⁴⁰ received. The synthetic rout for *p*-ABDI and *m*-ABDI is shown in
- Scheme S1. The *p*-HBDI was synthesized according to Niwa's procedure.³
- **Compound** *m*-1. *N*-Acetylglycine (1.5 g, 12.5 mmol), sodium ⁴⁵ acetate (1.0 g, 12.5 mmol), 4-nitrobenzaldehyde (1.51 g, 10 mmol), THF (15 mL) and acetic anhydride (7 mL) were heated at 80 °C with stirring for 12 h. The solvent was removed under reduced pressure, and the residue was dissolved in CH_2Cl_2 and washed with brine. The organic layer was dried over anhydrous
- ⁵⁰ MgSO₄ and the filtrate was concentrated under reduced pressure. The crude product was purified by silica gel column chromatography with ethyl acetate/hexane (1/1) to afford *m*-**1** (2.22 g, 96 %). ¹H-NMR (400 MHz, CDCl₃) : 2.43 (s, 3H), 7.19 (s, 1H), 7.60 (t, J = 8.0 Hz,1H), 8.24 (d, J = 8.0 Hz,1H), 8.31(d, J

- $_{55}$ = 8.0 Hz,1H), 8.99(s. 1H) ppm ; 13 C-NMR(100 MHz, CDCl₃): 15.9, 123.8, 126.1, 127.5, 129.6, 134.4, 124.7, 137.1, 148.3, 166.6, 167.7 ppm. MS (EI, 70 eV): m/z (relative intensity) 232 (M⁺, 100); HRMS calcd. for C₁₁H₈N₂O₄ 232.0484, found 232.0485.
- **Compound** *m*-2. *m*-2 was synthesized using our modification of Niwa's procedure. 40% aqueous methylamine solution (20 mL, 20mmol), Water (100 mL), and 200 mg potassium carbonate were added to *m*-1 (1.16 g, 5 mmol). The reaction mixture was ⁶⁵ stirred for 20 min, and was heated at 80 °C with stirring for 1 h. Than ethyl acetate was added and washed with brine directly. The organic layer was dried over anhydrous MgSO₄ and the filtrate was concentrated under reduced pressure. The crude product was purified by silica gel column chromatography with CH₂Cl₂/ethyl
- ⁷⁰ acetate (3/2) to afford *m*-**2** (1.40 g, 60 %). ¹H-NMR (400 MHz, DMSO-d₆) : 2.39 (s, 3H), 3.33 (s, 3H), 7.11 (s, 1H), 7.15 (t, J = 8.0 Hz,1H), 8.20 (d, J = 8.0 Hz,1H), 8.52 (d, J = 8.0 Hz,1H), 9.16 (s,1H) ppm ; ¹³C-NMR(100 MHz, DMSO-d₆): 15.6, 26.4, 121.4, 123.6, 125.4, 129.8, 135.5, 137.5, 140.5, 147.8, 166.2, 169.3 ppm. ⁷⁵ MS (EI, 70 eV): m/z (relative intensity) 245 (M⁺, 100); HRMS calcd. for C₁₂H₁₁N₃O₃ 245.0800, found 245.0803.

Compound *m*-**ABDI.** Tin (II) chloride dihydrate (3.53 g, 17 mmol) and *m*-**2** (0.49 g, 2 mmol) were suspended in 10 mL of THF and refluxed at 80 °C with stirring for 3 h. THF is removed at the rotary evaporator, and the residue was dissolved in CH₂Cl₂ and washed sequentially with 10% sodium hydrate solution and brine. The organic layer was dried over anhydrous MgSO₄ and the filtrate was concentrated under reduced pressure. The crude ⁸⁵ product was purified by silica gel column chromatography with ethyl acetate/acetone (96/4) to afford *m*-ABDI (0.13 g, 25 %). The production is yellow liquid, and was recrystallized from THF/hexane. ¹H-NMR (400 MHz, DMSO-d₆) : 2.32 (s, 3H), 3.34 (s, 3H), 5.18 (s, 2H), 6.59 (d, *J* = 8.0 Hz,1H), 6.74 (s, 1H), 7.05 (t,

⁹⁰ J = 8.0 Hz, 1H), 7.25 (d, J = 8.0 Hz, 1H), 7.38 (s, 1H) ppm; ¹³C-NMR(100 MHz, DMSO-d₆): 15.9, 26.9, 116.8, 117.1, 121.0, 126.5, 129.7, 135.0, 138.9, 149.4, 164.3, 170.6 ppm. MS (EI, 70 eV): m/z (relative intensity) 215 (M⁺, 100); HRMS calcd. for C₁₂H₁₃N₃O 215.1059, found 215.1057.

Compound *p*-1. The procedure is similar to that for *m*-1, *N*-Acetylglycine (1.755 g, 15 mmol), sodium acetate (1.2 g, 15 mmol), 4-Nitrobenzaldehyde (1.51 g, 10 mmol), and acetic anhydride (20 mL) were heated at 110 °C with stirring for 12 h. ¹⁰⁰ The solvent was removed under reduced pressure, and the residue was dissolved in CH₂Cl₂ and washed with brine. The organic layer was dried over anhydrous MgSO₄ and the filtrate was

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concentrated under reduced pressure. The crude product was purified by silica gel column chromatography with ethyl acetate/hexane (1/1) to afford *p*-**1** (1.88 g, 81 %).¹H-NMR (400 MHz, CDCl₃) : 2.43 (s, 3H), 7.11 (s, 1H), 8.22 (d, J = 9.6 Hz,2H), s 8.25 (d, J = 9.6 Hz,2H) ppm ; ¹³C-NMR(100 MHz, CDCl₃): 15.9. 123.7, 127.3, 132.4, 135.4, 138.7, 148.1, 166.6, 168.2 ppm. MS (EI, 70 eV): m/z (relative intensity) 232 (M⁺, 100); HRMS calcd. for C₁₁H₈N₂O₄ 232.0484, found 232.0476.

- ¹⁰ Compound *p*-2. *p*-2 was synthesized using our modification of Niwa's procedure. THF (50 mL) and a 40% aqueous methylamine solution (0.6 mL, 6 mmol) were added to *m*-1 (1.16 g, 5 mmol). After stirring 20 min, triethylamine (0.6 mL, 9 mmol) was added and was heated at 70 °C with stirring for 12 h. The ¹⁵ solvent was removed under reduced pressure, and CH₂Cl₂ was
- added and washed with brine. The organic layer was dried over anhydrous $MgSO_4$ and the filtrate was concentrated under reduced pressure. The crude product was purified by silica gel column chromatography with CH_2Cl_2 /ethyl acetate (3/2), and the
- ²⁰ solid residue is recrystallized to afford *m*-**2** (0.822 g, 72 %). ¹H-NMR (400 MHz, DMSO-d₆) : 2.40 (s, 3H), 3.33 (s, 3H), 7.08 (s, 1H), 8.27 (d, J = 8.8 Hz, 2H), 8.44 (d, J = 8.8 Hz, 2H) ppm ; ¹³C-NMR(100 MHz, DMSO-d₆): 15.6, 26.4, 121.0, 123.4, 132.2, 140.3, 141.3, 146.8, 167.0, 169.3 ppm. HRMS (ESI⁺) for ²⁵ C₁₂H₁₂N₃O₃⁺; calculated: 246.0800, found: 246.0872.

Compound *p***-ABDI.** Tin (II) chloride dihydrate (4.7 g, mmol), and *p***-2** (0.735 g, 3 mmol) were suspended in 10 mL of THF, and stirred 20 min. Then solvent was refluxed 80 °C with stirring for ³⁰ 1 h. THF is removed at the rotary evaporator, and the residue was dissolved in ethyl acetate and washed with 10% sodium hydrate

- solution and brine. The organic layer was dried over anhydrous MgSO₄ and the filtrate was concentrated under reduced pressure. The crude product was purified by silica gel column ³⁵ chromatography with ethyl acetate to afford *p*-ABDI (0.37 g, 57
- *). ¹H-NMR (400 MHz, DMSO-d₆) : 2.29 (s, 3H), 3.35 (s, 3H), 5.93 (s, 2H), 6.56 (d, J = 8.4 Hz,2H), 6.78 (s, 1H), 7.90 (d, J = 8.4 Hz,2H) ppm ; ¹³C-NMR(100 MHz, DMSO-d₆): 15.2, 26.1, 113.3, 121.3, 126.6, 133.8, 133.9, 151.1, 159.7, 169.4 ppm. MS (EI, 70 eV): m/z (relative intensity) 215 (M⁺, 100); HRMS calcd. for
- $C_{12}H_{13}N_3O$ 215.1059, found 215.1057.



45 Table S1. Crystallographic Data for *m*-ABDI.

Empirical formula	C ₁₂ H ₁₃ N ₃ O			
Formula weight	215.25			
Temperature	295 (2) K			
Wavelength	0.71073 Å			
Crystal system	Orthorhombic			
Space group	Pbca			
Unit cell dimensions	$a = 10.5533 (2) \text{ Å} \alpha = 90^{\circ}$			
	$b = 14.0126 (3) \text{ Å } \beta = 90^{\circ}$			
	$c = 15.1145 (3) \text{ Å } \gamma = 90^{\circ}$			
Volume, Z	2235.12 (8) Å ³ , 8			
Density (calculated)	1.279 Mg/m ³			
Absorption coefficient	0.085 mm ⁻¹			
F (0 0 0)	912			
Crystal size	0.25 x 0.15 x 0.10 mm			
Θ range for data collection	2.77 to 27.48°			
Limiting indices	$-13 \leq h \leq 13, -18 \leq k \leq 17,$			
	$-19 \leq l \leq 19$			
Reflections collected	14829			
Independent reflections	2546 (R _{int} =0.0387)			
Completeness to $\Theta = 27.48^{\circ}$	99.5 %			
Absorption correction	Semi-empirical from			
	equivalents			
Max. and min. transmission	0.9916 and 0.9791			
Refinement method	Full-matrix least-squares on F ²			
Data / restraints / parameters	2546 / 0 / 148			
Goodness-of-fit on F ²	1.084			
Final R indices [$I > 2\sigma$ (I)]	R1 = 0.0564, wR2 = 0.1667			
R indices (all data)	R1 = 0.0743, $wR2 = 0.1799$			
Extinction coefficient	0.033 (9)			
Largest diff. peak and hole	0.249 and -0.181 eÅ ⁻³			



50 Figure S1. The X-ray crystal structure of *m*-ABDI.

Electronic Spectra. The absorption spectra for *p*-ABDI and *m*-ABDI in acetonitrile is shown in Figure S2. The Fluorescence ⁵⁵ spectra for *m*-ABDI in hexane, THF, and acetonitrile are shown in Figure S3. The fluorescence of *p*-ABDI is too week to be measured at room temperature. The corresponding spectroscopic data are shown in Table S2.

- 45 2 J.-S. Yang, K.-L. Liau, C.-M. Wang, C.-Y. Hwang, J. Am. Chem. Soc. 2004, 126, 12325.
 - S. Kojima, H. Ohkawa, T. Hirano, S. Maki, H. Niwa, M. Ohashi, S. Inouye, F. I. Tsuji, *Tetrahedron Lett.* 1998, **39**, 5239-5242.



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Figure S2. The absorption spectra of *p*-ABDI and *m*-ABDI in acetonitrile.



Figure S3. The fluorescence spectra of *m*-ABDI in (a) hexane, (b) THF, 30 and (c) acetonitrile.

Table S2. Maxima of UV Absorption (λ_{abs}) and Fluorescence (λ_t), Stokes Shifts ($\Delta \nu_{st}$), 0,0 Transition ($\lambda_{0,0}$), and Fluorescence Lifetime ³⁵ (τ_t) for *p*-ABDI, and *m*-ABDI in protic and aprotic solvents at room temperature.^{a,b}

Compd	Solvent	λ_{abs} (nm)	λ _f (nm)	Δv_{st}^{c} (cm ⁻¹)	$\lambda_{0,0}{}^d$ (nm)	$\tau_{\rm f}$ (ns)
p-ABDI	Hex	380	n.d.	n.d.	n.d.	n.d.
	THF	397	n.d.	n.d.	n.d.	n.d.
	MeCN	393	n.d.	n.d.	n.d.	n.d.
	MeOH	409	n.d.	n.d.	n.d.	n.d.
m-ABDI	Hex	356	495	7887	429	13.9
	THF	355	545	9820	453	13.9
	MeCN	357	578	10710	456	10.1
	MeOH	351	n.d	n.d	n.d	n.d
a Elucroso	amaa data i	and from	aarraatad	amaatra b	d = not	datarmaina

^a Fluorescence data are from corrected spectra. ^b n.d. = not determined because of extremely low fluorescence intensity. ^c $\Delta v_{st} = v_{abs} - v_{f.}^{d}$ The 40 value of $\lambda_{0,0}$ was obtained from the intersection of normalized absorption and fluorescence spectra.

Notes and references

1 B. J. Birks, *Photophysics of Aromatic Molecules*; Wiley-Interscience: Lonoon, 1970.