Electronic Supplementary Information (ESI)

A photochromic prototype based on
difurylperhydrocyclopentene with remarkable photoswitching
behaviors and in vivo application

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1. Materials and instrumentations

1,2-Dibromocyclopentene\(^{S1}\) (compound 1) and 5-methyl-4-bromo-2-furaldehyde\(^{S2}\) (compound 3) was prepared and purified according to literature procedures. Other starting materials were commercially available and purified before use. All other reagents were of analytical purity and used without further treatment. Thin-layer chromatography (TLC) analyses were performed on silica-gel plates, and flash chromatography was conducted by using silica-gel column packages purchased from Qing-dao Haiyang Chemical Company, China.

\(^1\)H NMR and \(^{13}\)C NMR spectra in CDCl\(_3\) were recorded on Brucker AM-400 spectrometers with tetramethylsilane (TMS) as the internal standard. High-resolution mass spectrometry (HR-MS) were recorded on a Waters GCT Premier XE spectrometer with methanol as solvent. Infrared spectra were obtained using a PerkinElmer Spectrum400 FTIR spectrometer using a PIKE ATR attachment.

UV–vis absorption spectra were recorded on a Shimadzu 1800 spectrophotometer, while the fluorescent emission spectra were taken with a Jobin Yvon Fluorolog-3 spectrofluorometer (Model FL-TAU3); both spectrophotometers were standardized. The fluorescence quantum yields of solutions were measured on QM40 with an integrating sphere (φ 150 mm) from Photo Technology International, Inc. (PTI, USA). Under magnetic stirring, the photochromic reaction was induced in situ by continuous irradiation using an Hg/Xe lamp (Zolix Instruments Co., Ltd., GLORIA-X500A, 500W, slit width of 3 mm) with a tunable outputting monochromatic light for \(\lambda_{\text{irr}} = 365\) nm, \(\lambda_{\text{irr}} = 313\) nm, and \(\lambda_{\text{irr}} = 517\) nm, respectively; the 365 nm light power was \(~2.6\) mW cm\(^{-2}\) upon the sample, the 517 nm light power was \(~1\) mW upon the sample, and the distance between the light source and the sample was kept within 5 cm; all spectrophotometers were standardized. For DFC (10 \(\mu\)M) and DTC (10 \(\mu\)M) in various solvents, the irradiation time is 780 s and 660 s, respectively, until each photostationary state (PSS) reached. HPLC spectra were recorded on a high-performance liquid chromatograph (Agilent Technologies) with
an Eclipse XDB-C18 column (5 μm particle size, 4.6×250 mm, Agilent, USA). The photochromic reaction quantum yields of compounds DFC and DTC were evaluated by the standard procedures using 1,2-bis[2-methylbenzo[b]thiophen-3-yl] perfluorocyclopentene (BTF6) as the references for photocyclization and cycloreversion. The rates of isomerization in the initial stage of the reaction (0-3%) were compared with references whose \( \Phi_{\text{o\to c}} \) (35%) and \( \Phi_{\text{c\to o}} \) (35%) in hexane\(^{S3}\) are known, which brought forth 3% uncertainties on the calculations of quantum yields.

2. Synthesis

Synthesis of 5-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)furan-2-carbaldehyde (4)

A mixture of 5-methyl-4-bromo-2-furaldehyde 3 (9.45 g, 50.0 mmol), bis(pinacolato)diboron (19.05 g, 75.0 mmol), \([1,1']\text{-bis(diphenylphosphino)ferrocene}][\text{dichloropalladium(II)}\) (PdCl\(_2\)(dpff), 1.83 g, 2.5 mmol) and potassium acetate (14.70 g, 150.0 mmol) in absolutely degassed DMF (50.0 mL), then the solution was heated to 100 °C under argon for 12 h. After cooling to room temperature, the mixture was poured into water (200.0 mL), and extracted with ethyl acetate. The organic layer was dried over anhydrous MgSO\(_4\), filtrated, and concentrated. The residue was purified by column chromatography (silica gel, petroleum ether/ ethyl acetate 10:1) to give the compound 4 (9.80 g, 83%) as a light-yellow solid. \(^1\text{H NMR}\) (400 MHz, CDCl\(_3\)) \(\delta\) (ppm): 1.306 (s, 12H, -C(CH\(_3\))-), 2.551 (s, 3H, -CH\(_3\)), 7.353 (s, 1H, furyl-H), 9.485 (s, 1H, -CHO). \(^13\text{C NMR}\) (100 MHz, CDCl\(_3\)) \(\delta\) (ppm): 14.517, 24.818, 24.989, 83.454, 83.768, 128.831, 151.791, 168.727, 176.723. HRMS (EI+, \(m/z\)): [M]** calcd for C\(_{12}\)H\(_{17}\)O\(_4\)B, 235.1256; found, 235.1251. IR (KBr) \(\nu_{\text{max}}\) (cm\(^{-1}\)): 451.4, 505.0, 518.7, 547.4, 576.7, 638.5, 666.9, 699.7, 714.8, 758.4, 809.4, 852.4, 951.7, 960.3, 999.7, 1052.6, 1108.7, 1119.6, 1146.7, 1170.6, 1210.9, 1251.0, 1286.2, 1316.7, 1336.4, 1380.2, 1428.2, 1534.3, 1588.3, 1678.7, 2359.7, 2733.1, 2817.3, 2930.9, 2979.7, 3338.1, 3644.9, 3666.5, 3730.1, 3849.0.
Synthesis of bis-furaldehyde (DFC)

A mixture of 1,2-dibromocyclopentene 1 (1.92 g, 8.50 mmol), compound 4 (4.01 g, 17.0 mmol), anhydrous potassium phosphate (7.21 g, 34.0 mmol), tetrabutylammonium chloride (0.118 g, 0.425 mmol), and tetrakis(triphenylphosphine)palladium (Pd(PPh₃)₄, 0.393 g, 0.340 mmol) in absolutely degassed DMF (100.0 mL), then the solution was heated to 100 °C under argon for 16 h away from light. After cooling to room temperature, the mixture was poured into water (500.0 mL), and extracted with ethyl acetate. The organic layer was dried over anhydrous MgSO₄, filtrated, and concentrated. The residue was purified by column chromatography (silica gel, petroleum ether/ethyl acetate 5:1) to give the compound DFC (1.54 g, 64%) as a light-yellow solid. ¹H NMR (400 MHz, CDCl₃) δ (ppm): 2.055 (m, 2H, -CH₂-), 2.099 (s, 6H, -CH₃), 2.752 (t, J 7.6 Hz, 4H, -CH₂-), 7.006 (s, 2H, furyl-H), 9.456 (s, 2H, -CHO). ¹³C NMR (100 MHz, CDCl₃) δ (ppm): 13.732, 22.424, 37.821, 120.880, 122.865, 130.553, 151.044, 155.668, 176.891. HRMS (EI+, m/z): [M]+ calcd for C₁₇H₁₆O₄, 284.1049; found, 284.1052. IR (KBr) νmax (cm⁻¹): 423.4, 458.9, 495.9, 522.5, 545.7, 575.6, 631.2, 673.0, 682.7, 770.4, 781.5, 815.1, 858.2, 867.8, 884.1, 918.2, 950.4, 975.1, 1004.0, 1028.4, 1041.1, 1068.2, 1119.7, 1171.8, 1213.3, 1305.0, 1320.5, 1336.0, 1371.9, 1410.9, 1428.3, 1442.0, 1520.6, 1679.0, 2819.9, 2847.9, 2915.8, 2961.5, 3085.9, 3186.4, 3337.5, 3436.4.

3. Thin-film preparation

Solutions of compound DFC (6 mM in dichloromethane) were spin-coated (2500 rpm, 45 s) on quartz glass slides. The thicknesses of the films were 60–70 nm. The quartz glass slides were inserted into the cuvette holder for the optical tests.
4. Cell culture

HeLa cells (human neuroblastoma cells) were grown in RPMI-1640 supplemented with 10% fetal bovine serum (FBS) at 37 °C in a humidified 5% CO₂ atmosphere. Cells (1.0×10⁵ cells mL⁻¹) were plated on 14 mm glass cover slips and allowed to adhere for 12 h.

5. Living cell imaging

Before the experiments, HeLa cells (10 μM) were washed with phosphate buffered saline (PBS, pH = 7.4), and then incubated with compounds DFC, DFC-c, DTC and DTC-c (20 μM) in RPMI-1640/DMSO (99:1, v/v) solution for 30 min at 37 °C, respectively. Cell imaging was then carried out after washing cells with PBS. Confocal fluorescence imaging was performed with an OLYMPUS IX81 laser scanning microscope with a 60P oil-immersion objective lens. Fluorescence images of compounds DFC, DFC-c, DTC, and DTC-c loaded cells were monitored at 450–525 nm with excitation wavelength of 404 nm using a HeNe laser.

6. In Vitro cytotoxicity assay

The cytotoxicity of compounds DFC and DTC was quantitatively determined by the Cell Counting Kit-8 (CCK-8) assays. MC3T3-E1 cells obtained from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China) were seeded into a 96-well plate at a density of 1.0×10⁴ cells/well in MEM-α medium. After the cells grew for 12 h, the medium was changed into a new medium (200 μL/well) containing compounds DFC and DTC at various concentrations. The cells then were incubated for another 48 h before conducting CCK-8 assay by replacing the medium with 200 μL of new medium containing 20 μL of CCK-8 solution. After incubation for 3 h, the absorbance at 450 nm in each well was determined using a microplate reader (Multiskan Mk 3). The relative cell viability was calculated to
quantify the cytotoxicity; the control group without treatment of any material in culture medium was defined as 100% viability; the SDS and compounds DFC and DTC at different concentrations were set as positive and negative controls, respectively.

7. Computational details

The geometries of ring-open DFC and DTC molecules were optimized by density functional calculations (DFT) using the B3LYP functional and the 6-31G(d) basis set, as implemented in the Gaussian 09 program. At optimized geometries, time-dependent density functional theory (TDDFT) calculations were carried out using the range-separated CAM-B3LYP functional and the 6-311+G(d,p) basis set. Solvent effects of acetonitrile were taken into account by the polarizable continuum model in both DFT and TDDFT calculations. The absorption spectra were generated by Gaussian broadening of the stick spectra with a full-width at half-maximum of 0.2 eV.
8. 1,2-bis(furan-3-yl)hexafluorocyclopentene derivatives reported

Scheme S1. 1,2-bis(furan-3-yl)hexafluorocyclopentene derivatives reported in literatures.

9. Synthetic route to the compound DFC

Scheme S2. Synthetic route to the compound DFC.
10. Absorption spectra of DFC

Fig. S1 UV–vis absorption changes of compound DFC (10 μM) upon irradiation with 365 nm light at 298 K in various solvents: (A) dimethyl formamide; (B) dichloromethane; (C) toluene; (D) hexane.
11. Absorption spectra of DTC

Fig. S2 UV–vis absorption changes of compound DTC (10 μM) upon irradiation with 365 nm light at 298 K in various solvents: (A) dimethyl formamide; (B) dichloromethane; (C) toluene; (D) hexane.
12. Absorption data

Table S1. Absorption data of compound DFC (1.0×10⁻⁵ M) in different solvents at 298 K.

<table>
<thead>
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<td>$\lambda_{max}$ (nm)</td>
<td>$\epsilon_{max}$ (M⁻¹cm⁻¹)</td>
<td>$\lambda_{max}$ (nm)</td>
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<tr>
<td>toluene</td>
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<td>1.79×10⁴</td>
<td>523</td>
<td>0.46×10⁴</td>
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<td>dichloromethane</td>
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<td></td>
<td>309</td>
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<tr>
<td>acetonitrile</td>
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<td>2.68×10⁴</td>
<td>522</td>
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<td>dimethyl formamide</td>
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<td>306</td>
<td>1.63×10⁴</td>
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Table S2. Absorption data of compound DTC (1.0×10⁻⁵ M) in different solvents at 298 K.

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<td>$\epsilon_{max}$ (M⁻¹cm⁻¹)</td>
<td>$\lambda_{max}$ (nm)</td>
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<td>hexane</td>
<td>312</td>
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<td>toluene</td>
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<td>0.85×10⁴</td>
<td>587</td>
<td>0.45×10⁴</td>
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<tr>
<td>dichloromethane</td>
<td>271</td>
<td>3.09×10⁴</td>
<td>590</td>
<td>0.75×10⁴</td>
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<tr>
<td></td>
<td>323</td>
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<tr>
<td>acetonitrile</td>
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<td>3.05×10⁴</td>
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<td>317</td>
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<td>dimethyl formamide</td>
<td>272</td>
<td>3.16×10⁴</td>
<td>583</td>
<td>0.74×10⁴</td>
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<tr>
<td></td>
<td>319</td>
<td>0.86×10⁴</td>
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13. \textit{\textsuperscript{1}H NMR analysis for the photocyclization conversion of DFC}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figS3}
\caption{The \textsuperscript{1}H NMR (400 MHz) spectra of compound DFC (8.8×10^{-2} \text{ M}) in CDCl\textsubscript{3} without (above) and with (below) irradiation of 313 nm light at 298 K.}
\end{figure}

Note: the photocyclization conversion of compound DFC can be calculated to be 39.8\%, based on the ratio of integral areas of corresponding peaks in Fig. S3 (below), respectively.
**14. HPLC analysis for the photocyclization conversion of DFC**

![HPLC Spectrum](image)

**Fig. S4** The HPLC spectra of compound **DFC** \(1 \times 10^{-4} \text{ M}\) in acetonitrile without (above) and with (below) irradiation of 313 nm light at 303 K under following conditions: Agilent Eclipse XDB-C18 column, methanol-water (90:10, v/v) as mobile phase, the detection wavelength of 308 nm.

Note: a peak with the residue time (R.T.) of 3.559 min appeared in the spectrum without UV light of 313 nm irradiation (Fig. S4 above). Upon irradiation with 313 nm light within the frame of 780 s, compound **DFC** reached the photostationary state in acetonitrile, resulting in the appearance of one new peaks (R.T. = 3.070 min) in the HPLC spectrum (Fig. S4 below). This new peak is attributed to the formation of the closed-ring isomer **DFC-c**, and the area is determined to be as high as 40.032%.
15. \textit{\textsuperscript{1}H NMR analysis for the photocyclization conversion of DTC}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig_s5}
\caption{The \textsuperscript{1}H NMR (400 MHz) spectra of compound \textbf{DTC} (8.8\times10^{-2} M) in CDCl$_3$ without (above) and with (below) irradiation of 313 nm light at 298 K.}
\end{figure}

Note: the photocyclization conversion of compound \textbf{DFC} can be calculated to be 27.5\%, based on the ratio of integral areas of corresponding peaks in Fig. S5 (below), respectively.
16. **HPLC analysis for the photocyclization conversion of DTC**

![HPLC spectra](image)

**Fig. S6** The HPLC spectra of compound **DTC** (1×10⁻⁴ M) in acetonitrile without (above) and with (below) irradiation of 313 nm light at 303 K under following conditions: Agilent Eclipse XDB-C18 column, methanol-water (90:10, v/v) as mobile phase, the detection wavelength of 337 nm.

Note: a peak with the residue time (R.T.) of 4.081 min appeared in the spectrum without UV light of 313 nm irradiation (Fig. S6 above). Upon irradiation with 313 nm light within the frame of 660 s, compound **DTC** reached the photostationary state in acetonitrile, resulting in the appearance of one new peaks (R.T. = 3.458 min) in the HPLC spectrum (Fig. S6 below). This new peak is attributed to the formation of the closed-ring isomer **DTC-c**, and the area is determined to be 27.916%.
17. Emission spectra of DFC

Fig. S7 Fluorescence changes of compound DFC (10 μM) upon irradiation with 365 nm light at 298 K in various solvents: (A) dimethyl formamide; (B) dichloromethane; (C) toluene. \( \lambda_{\text{ex}} = 326 \) nm. Slits: 5 nm/ 5 nm.
18. Computational results

Fig. S8 Simulated absorption spectra of DFT and DTC.

Table S3. Molecular orbital compositions of lowest excited states of DFC.

<table>
<thead>
<tr>
<th>State</th>
<th>Energy (eV)</th>
<th>Wavelength (nm)</th>
<th>Oscillator Strength</th>
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<tbody>
<tr>
<td>S₁</td>
<td>4.06</td>
<td>305</td>
<td>0.4389</td>
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</table>

H → L (49%)
H-3 → L+1 (16%)
H-4 → L (14%)
$S_2$: 4.08 eV, 304 nm, $f = 0.0025$
H-4 $\rightarrow$ L+1 (38%)
H-3 $\rightarrow$ L (33%)
H-4 $\rightarrow$ L (12%)

$S_3$: 4.09 eV, 302 nm, $f = 0.3331$
H $\rightarrow$ L (28%)
H-3 $\rightarrow$ L+1 (24%)
H-4 $\rightarrow$ L (20%)
<table>
<thead>
<tr>
<th>28%</th>
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<tbody>
<tr>
<td>24%</td>
<td>→</td>
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<tr>
<td>20%</td>
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Table S4. Molecular orbital compositions of lowest excited states of DTC.

<table>
<thead>
<tr>
<th>State</th>
<th>Energy (eV)</th>
<th>Wavelength (nm)</th>
<th>Oscillator Strength (f)</th>
<th>H → L(%)</th>
<th>H-1 → L+1(%)</th>
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<tr>
<td>S₁</td>
<td>3.96</td>
<td>313</td>
<td>0.3821</td>
<td>75</td>
<td>10</td>
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<tr>
<td>S₂</td>
<td>4.03</td>
<td>307</td>
<td>0.0114</td>
<td>55</td>
<td>12</td>
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<tr>
<td>S₃</td>
<td>4.06</td>
<td>305</td>
<td>0.0134</td>
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</tbody>
</table>

- **S₁**: 3.96 eV, 313 nm, f = 0.3821
  - H → L (75%)
  - H-1 → L+1 (10%)

- **S₂**: 4.03 eV, 307 nm, f = 0.0114
  - H → L+1 (55%)
  - H-6 → L (12%)

- **S₃**: 4.06 eV, 305 nm, f = 0.0134
  - H-5 → L (36%)
  - H-6 → L+1 (31%)
The computational study provided an impressive sight from the aspect of transition oscillator strength. We can find that the oscillator strength of $S_0$-$S_1$ transition ($f = 0.4389$) in DFC is greater than that ($f = 0.3821$) in DTC. More significantly, the oscillator strength of $S_0$-$S_3$ transition ($f = 0.3331$) in DFC completely overwhelmed the corresponding one ($f = 0.0134$) in DTC. These results suggest that the π-electron in DFC has larger chance to be excited into higher energy levels, and probably easier to undergo a radiative decay in case the other competitive factors are fair.
19. Fatigue resistance

Fig. S9  Fatigue resistance of compounds DFC (A) and DTC (B) upon alternating irradiation with UV (365 nm) and visible light ($\lambda \geq 520$ nm) at 298 K.

Fig. S10  The photoswitching cycles of compound DFC monitored by the fluorescence intensity at 465 nm upon alternating irradiation with UV (365 nm) and visible light ($\lambda \geq 520$ nm) at 298 K, $\lambda_{ex} = 326$ nm. Slits: 5 nm/ 5 nm.
20. Possible route to photogenerated byproduct of DFC

Scheme S3. Possible route of the byproduct formation from compound DFC.
21. Thermal fading kinetics

Kinetic studies are important as they concern the behavior of photochromic molecules of applicative interest. The thermal fading kinetics that is determined from the absorption ratio \( \frac{A_t}{A_0} \) - time data sets was analyzed using the single exponential function.

This is the most common model used for the analysis of the photochromic system based on diarylethenes and makes is beneficial to compare with other literature reported values. It was found that the thermal fading kinetics of DFC-c and DTC-c is well described by the single exponential function with correlation coefficients \( R^2 > 0.99 \) in all analyses, respectively. The fading rate constants \( k \) can be estimated from the slope of the single exponential (first-order) kinetic plots. Additionally, the convenient measurement of thermal fading rate can be described by the \( \tau_{1/2} \) and \( \tau_{3/4} \) values, which are the times that take for the absorbance to reduce by 1/2 and 3/4 of the initial absorbance, respectively.

![Graph showing single exponential decay model](image)

**Fig. S11** The single exponential (first-order) decay model with \( R^2 = 0.994 \) and experimental thermal fading kinetics of DFC-c in toluene solution (1.0×10⁻⁴ M) at 383 K under argon atmosphere (black square: data measured; red line: the first-order decay model fitted). The monitored wavelength was at 522 nm.
Fig. S12 The single exponential (first-order) decay model with $R^2 = 0.995$ and experimental thermal fading kinetics of DTC-c in toluene solution ($1.0 \times 10^{-4}$ M) at 383 K under argon atmosphere (black square: data measured; red line: the first-order decay model fitted). The monitored wavelength was at 578 nm.

From Fig. S11, $\tau_{1/2}$ and $\tau_{3/4}$ obtained for DFC-c in toluene solution at 383 K were 178 min and 216 min with the fading rate constants ($k = 0.373 \text{ s}^{-1}$), respectively. And from Fig. S12, $\tau_{1/2}$ and $\tau_{3/4}$ obtained for DTC-c in toluene solution at 383 K were 316 min and 417 min with the fading rate constants ($k = 0.147 \text{ s}^{-1}$), respectively.
22. Absorption and fluorescent change of DFC in solid-state thin films

Fig. S13 Compound DFC in solid-state thin-films. (A) Absorption changes upon irradiation with 365 nm light at 298 K. (B) Fluorescence changes upon irradiation with 365 nm light at 298 K, $\lambda_{ex} = 326$ nm. Slits: 5 nm/ 5 nm. Inset: the corresponding photographic images upon irradiation with UV and visible light.
23. **Cell imaging of DTC and DTC-c**

![Confocal fluorescence and bright field images of HeLa cells](image)

**Fig. S14** Confocal fluorescence and bright field images of HeLa cells. (A) Fluorescence image, (B) bright field image, and (C) overlay image of HeLa cells incubated with compound **DTC** (20 μM) in the growth media for 30 min at 37 °C. (D) Fluorescence image, (E) bright field image, and (F) overlay image of HeLa cells supplemented with 20 μM of **DTC-c** in the growth media for 30 min at 37 °C. $\lambda_{ex} = 404$ nm. Collecting region: 450–525 nm. Scale Bar, 25 μm.
24. **Fluorescence decay profiles**

Time-resolved fluorescence spectra were measured with an Edinburgh FLS920 spectrofluorometer (excitation wavelength 326 nm). All decay profiles were fitted reasonably well using a single exponential function.

**Fig. S15** Fluorescence decay profile of compound **DFC** at ambient temperature in toluene for the wavelength of 465 nm ($\tau_1 = 4.74$ ns, $\chi^2 = 0.991$).

**Fig. S16** Fluorescence decay profile of compound **DTC** at ambient temperature in toluene for the wavelength of 465 nm ($\tau_1 = 7.09$ ns, $\chi^2 = 0.981$).
25. Calculation of the photocyclization conversion yield and quantum yields

The absorptions of the closed forms of compounds DFC and DTC were determined by the Fischer’s method. The ratio of the equilibrium concentrations of the open form ($C_o$) and closed forms ($C_c$) at a given photostationary state (PSS) is expressed as follows:

$$\frac{C_o}{C_c} = \frac{\Phi_{c\to o} \times \varepsilon_c}{\Phi_{o\to c} \times \varepsilon_o} = \frac{\Phi_{c\to o} \times A_c}{\Phi_{o\to c} \times A_o}$$  \hspace{1cm} \text{(E. 1)}$$

where $\varepsilon_o$ and $\varepsilon_c$ are the molar absorption coefficients of the open and closed forms, $A_o$ and $A_c$ are the absorption of a sample of same chromophore concentration containing only the open or closed form, $\Phi_{c\to o}$ and $\Phi_{o\to c}$ are quantum yields of cycloreversion and cyclization, respectively. By comparing the PSS’s obtained under irradiation at two different wavelengths $\lambda'$ and $\lambda''$, a couple of equations of type (E. 1) are obtained. Assuming that the ratio $\Phi_{c\to o} / \Phi_{o\to c}$ does not depend on the irradiation wavelength, we get:

$$\frac{C_o'}{C_c'} = \frac{A_c'}{A_o'} = \frac{A_c''}{A_o''}$$  \hspace{1cm} \text{(E. 2)}$$

We introduce the open form to closed form conversion yield $\alpha$, equation (E. 2) evolves to:

$$\frac{1 - \alpha'}{1 - \alpha''} = \frac{A_c'}{A_o'} = \frac{A_c''}{A_o''}$$  \hspace{1cm} \text{(E. 3)}$$

In order to introduce experimental data into equation (E. 3), we can write that the absorbance $A$ measured at any particular wavelength $\lambda$ of a mixture of open and closed forms, where the overall concentration $C_o + C_c$ is constant, is given by:

$$A = (1 - \alpha)A_o + \alpha A_c$$

or
\[ A_c = A_o + \frac{A - A_o}{\alpha} \]  
(E. 4)

This can be combined to equation (E. 3) and yields:

\[
\frac{1 - \alpha'}{\alpha} \left/ \frac{1 - \alpha''}{\alpha''} \right. = 1 + \frac{\Delta'}{\alpha'} \left/ 1 + \frac{\Delta''}{\alpha''} \right. 
\]  
(E. 5)

where \( \Delta = (A - A_o)/A_o \) denotes the relative change of absorbance observed when a solution of open form is irradiated to the PSS. Furthermore, the ratio \( \rho = \alpha'/\alpha'' \) of the conversion yields at two different PSS’s, resulting from irradiation at two different wavelengths, is equal to the ratio of the \( \Delta \)'s measured at any given wavelength (the wavelength that maximizes the \( \Delta \)'s is usually chosen). Equating and developing (E. 5) yields the final formula:

\[ \alpha'' = \frac{\Delta' - \Delta''}{1 + \Delta' - \rho(1 + \Delta'')} \]  
(E. 6)

where all the parameters \( \Delta \) and \( \rho \) are experimentally accessible. The numerical value determined by this equation may then be used to calculate the absorption spectrum of the pure closed form by means of equation (E. 4).

The photocyclization quantum yield can be calculated according to a simple photochromic model involving the open form (OF) and closed form (CF) isomers by means of a numerical integration procedure\textsuperscript{12} using the differential equation (E. 7) and phenomenological equation (E. 8).

\[
\frac{dC_c}{dt} = I_o \times \frac{1 - 10^{-Abs(\lambda_{irr})}}{V \times Abs(\lambda_{irr})} \times (\Phi_{o \rightarrow c} \times \varepsilon_{o \lambda_{irr}} \times l \times C_o - \Phi_{c \rightarrow o} \times \varepsilon_{c \lambda_{irr}} \times l \times C_c) 
\]  
(E. 7)

\[
Abs(\lambda_{obs}) = \varepsilon_{c \lambda_{obs}} \times l \times C_c + \varepsilon_{o \lambda_{obs}} \times l \times C_o 
\]  
(E. 8)

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where $C_o$ and $C_c$ are the concentrations of open form and closed form, $I_o$ is the incident irradiation intensity, $V$ is the volume of the solution, $\lambda_{irr}$ and $\lambda_{obs}$ are the irradiation and observation wavelengths, $\epsilon_o$ and $\epsilon_c$ are molar absorption coefficients of open and closed forms at irradiating wavelength (313 nm), respectively.\textsuperscript{13}

Equation (E. 7) is too complicated to for calculating $\Phi_{o\rightarrow c}$, and thus practically, we employ an approximation method.\textsuperscript{14} When the photocyclization produces less than 5% closed form, $C_c$ would be defined as 0, which brings about an error about ±0.05 in quantum yields. And due to $\varepsilon_o \lambda_{irr} = 0$ at 517 nm, equations (E. 7) and (E. 8) can be simplified as:

\[
\frac{dC_c}{dt} = \frac{I_o \Phi_{o\rightarrow c} \left( 1 - 10^{-\varepsilon_o \lambda_{irr} C_o} \right)}{V} \quad \text{(E. 9)}
\]

\[
Abs(\lambda_{obs}) = \varepsilon_c \lambda_{obs} \times l \times C_c \quad \text{(E. 10)}
\]

Combining equations (E. 9) and (E. 10), we get equation (E. 11):

\[
\Phi_{o\rightarrow c} = \frac{V}{I_o \left( 1 - 10^{-\varepsilon_o \lambda_{irr} C_o} \right)} \frac{dAbs(\lambda_{obs})}{dt} \quad \text{(E. 11)}
\]

\[
\frac{dAbs(\lambda_{obs})}{dt} \quad \text{can be easily obtained from the slope of time-dependent absorption changes, and } I_o \text{ can be calculated from reference compound BTF6 with known } \Phi_{o\rightarrow c}. \text{ In this way, we acquired } \Phi_{o\rightarrow c}, \text{ with an error about ±0.05.}
\]

As can be determined from $^1$H NMR analysis, the photocyclization conversion yields of compounds DFC and DTC at 313 nm were 39.8% and 27.5%, respectively. In addition, we obtained that the cyclization quantum yield and cycloreversion quantum yield (in a similar way using BTF6 as reference) were 22.3% at 313 nm and 15.1% at 517 nm for compound DFC, whereas compound DTC showed
somewhat lower value on the order of 16.6% at 313 nm and 20.3% at 517 nm in acetonitrile solution, respectively.

26. \textit{\textsuperscript{1}H NMR spectrum of 4}
Fig. S17 $^1$H NMR (CDCl$_3$, 400 MHz) spectrum of compound 4.

$^{27}$ $^{13}$C NMR spectrum of 4
Fig. S18 $^{13}$C NMR (CDCl$_3$, 100 MHz) spectrum of compound 4.

28. HRMS spectrum of 4
**Fig. S19 HRMS (EI+) spectrum of compound 4.**

**29. $^1H$ NMR spectrum of DFC**
Fig. S20 $^1$H NMR (CDCl$_3$, 400 MHz) spectrum of compound DFC.

30. $^{13}$C NMR spectrum of DFC
Fig. S21 $^{13}$C NMR (CDCl$_3$, 100 MHz) spectrum of compound DFC.

31. HRMS spectrum of DFC
Fig. S22 HRMS (EI+) spectrum of compound DFC.

**References**


