Asymmetric Diarylethenes with Oxidized 2-Alkylbenzothiophen-3-yl Units: Chemistry, Fluorescence, and Photoswitching

Kakishi Uno, Mariano L. Bossi,* Timo Konen, Vladimir N. Belov,* Masahiro Irie,* and Stefan W. Hell

1. Introduction

Recently, a new type of photoswitchable diarylethenes (DAEs), which have no fluorescent units, but emit in the closed-ring forms in a “turn-on” fashion, has been developed.[1–3] These compounds contain a perfluorocyclopentene bridge linking two 2-alkyl-1-benzothiophene-1,1-dioxide residues with accessible variation points: alkyl groups and C-6(6’)- atoms (Figure 1).

The “closed” forms (CFs) of these switches related to asymmetric structures and the presence of “push–pull” substituents are introduced in the present study, and their switching power is studied. The new “simplified” asymmetric DAEs possess higher cycloreversion quantum yields, but lower emission efficiencies than their known analogs with extended \(\pi\)-conjugation paths. Fatigue resistance in organic (acetonitrile and methanol) and aqueous solvents is studied, and the performance in reversible saturable optical fluorescence transition (RESOLFT) microscopy is evaluated. The push–pull structures show an outstanding increase in fatigue resistance, compared with a reference \((H/H)\) diarylethene. Fatigue resistances on the order of thousands of cycles have been observed in acetonitrile solutions without exclusion of air oxygen. The structure–property relationships and facile preparation procedures enable rational design of DAEs applicable as photoswitchable fluorescent probes. A new setup and methodology for measuring switching performance of fluorescent DAEs are introduced.

Reversibly photoswitchable diarylethenes (DAEs) with fluorescent closed forms are applied in biology- and material science-related super-resolution microscopy. Yet, their full photoswitchable capacities remain unresolved and unexplored. Due to limitations in synthesis, only 1,2-[bis-(2-ethyl-1-benzothiophene-1,1-dioxide-3-yl)]perfluorocyclopentenes with two identical or very similar aryl groups attached to C-6 and C-6’ have been prepared so far. DAEs with increased Stokes shifts related to asymmetric structures and the presence of “push–pull” substituents are introduced in the present study, and their switching power is studied. The new “simplified” asymmetric DAEs possess higher cycloreversion quantum yields, but lower emission efficiencies than their known analogs with extended \(\pi\)-conjugation paths.

Fatigue resistance in organic (acetonitrile and methanol) and aqueous solvents is studied, and the performance in reversible saturable optical fluorescence transition (RESOLFT) microscopy is evaluated. The push–pull structures show an outstanding increase in fatigue resistance, compared with a reference \((H/H)\) diarylethene. Fatigue resistances on the order of thousands of cycles have been observed in acetonitrile solutions without exclusion of air oxygen. The structure–property relationships and facile preparation procedures enable rational design of DAEs applicable as photoswitchable fluorescent probes. A new setup and methodology for measuring switching performance of fluorescent DAEs are introduced.

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The “closed” forms (CFs) of these switches related to asymmetric structures and the presence of “push–pull” substituents are introduced in the present study, and their switching power is studied. The new “simplified” asymmetric DAEs possess higher cycloreversion quantum yields, but lower emission efficiencies than their known analogs with extended \(\pi\)-conjugation paths. Fatigue resistance in organic (acetonitrile and methanol) and aqueous solvents is studied, and the performance in reversible saturable optical fluorescence transition (RESOLFT) microscopy is evaluated. The push–pull structures show an outstanding increase in fatigue resistance, compared with a reference \((H/H)\) diarylethene. Fatigue resistances on the order of thousands of cycles have been observed in acetonitrile solutions without exclusion of air oxygen.

The structure–property relationships and facile preparation procedures enable rational design of DAEs applicable as photoswitchable fluorescent probes. A new setup and methodology for measuring switching performance of fluorescent DAEs are introduced.

Therefore, the goal of the present study is to find new synthetic transformations providing DAEs with variable photo-physical properties related to asymmetric structures: only one aryl group at C-6 and an auxochromic residue “directly”
attached to C-6′ “on the other side” of 1,2-[bis-(2-ethyl-1-benzothiophene-1,1-dioxide-3-yl)]perfluorocyclopentene. We expected that the properties of the “simplified” asymmetric DAEs will provide guidelines for the rational design of DAEs applicable as photoswitchable fluorescent probes in light microscopy. In particular, higher cycloreversion quantum yields (due to shorter conjugation path in the closed form) were expected, and this could result in a better fatigue resistance.

2. Synthesis

The synthesis of 6-methoxy substituted DAEs 1-MeO,N and 1-MeO,COMe (Scheme 1) involved diiodide 2 which underwent iodine-lithium exchange followed by the reaction with isopropyl pinacol borate[9] and oxidation of the intermediately formed aryl boronic ester(s).[10] Phenol 3 represents an important building block, as it can be transformed into the corresponding triflate (not shown in Scheme 1), with perspectives to the stepwise and selective Pd-catalyzed substitution reactions involving first the iodine atom (P(t-Bu)3/KF) and then the triflate group (PCy3/KF).[11] Additionally, we expected that it will be possible to selectively oxidize the electron-rich benzothiophene unit (with a methoxy group) to the corresponding sulfone, and that would open a new route to yet unknown DAEs.

The asymmetric DAEs with a cyano group (1-CN,N and 1-CN,COMe in Scheme 2) have been prepared from diiodide 7. A certain degree of selectivity in the reaction with Zn(CN)2 afforded compound 8 in a moderate yield. In fact, this was a surprising result, because we initially expected that the substitution of the first iodine atom will facilitate the displacement of the second one. Less selective was the iodination of compound 9: it afforded monoiodide 10 (precursor of the reference DAE 1-H,H) in low yield.

3. Photoswitching

The stirred solutions of DAEs (OFs) in methanol (20 × 10⁻⁶ m) were irradiated at 20 °C with 365 nm light until the photosationary state (PSS) was reached (αPSS (365 nm)). The degree of conversion to the fluorescent CF in the PSS was calculated from high performance liquid chromatography (HPLC) analysis and spectroscopy procedures (see Figure S1 in the Supporting Information), and the absorption spectrum of the CF was calculated. Then the solutions were irradiated with 470 nm light, until full conversion to the OF was detected (HPLC). From the transients under irradiation with 365 and 470 nm, the isomerization quantum yields were calculated. The emission spectrum and fluorescence quantum yield of the CF, as well as lifetime (τfl), were measured at low conversions assuming the CF being the only species absorbing and emitting at the excitation wavelength (470 nm). The obtained results are summarized in Table 1; the spectra and details of HPLC detection are given in Figures S2 and S3 in the Supporting Information. The irradiation wavelengths (365 and 470 nm) are available in fluorescence microscopes as excitation sources for common markers (4′,6-diamidino-2-phenylindol (DAPI), fluorescein isothiocyanate (FITC), and green fluorescent protein (GFP)). The reference compound 1-H,CH with “neutral” substituents at the extremes (Scheme 1) has a large emission quantum yield and a high value of αPSS (365 nm). It also presents large cyclization (ΦOF→CF) and ring-opening (ΦCF→OF) quantum yields. The introduction of donor groups at the extremes of the core structure (1-MeO,COMe) shifts...
with one of the other DAEs. Both dyes could be switched-off with the same doughnut shaped 488 nm laser beam, and the residual fluorescence of both dyes could be read out with a Gaussian shaped 488 nm laser, followed by on-switching with a 355 nm laser (see Table 3), simplifying the laser configuration needed for a two-color approach. The emission of 1-CN,COMe is redshifted and well separated from any of the other four compounds. This feature enables us to detect the fluorescence of 1-CN,COMe in another spectral window. The relative switching rates were measured in methanol (Table 2), acetonitrile, and water (see the Supporting Information). Variable switching rates may be used for separation of the markers using innovative switching schemes.[12] With respect to the reference compound, all new substituents decrease the cyclization/cycloreversion quantum yields by a factor of 2–60 (except 1-CN,N; Table 2).

4. Fatigue Resistance

The number of cycles a photochromic compound can endure before bleaching (fatigue resistance) is very important for practical applications. The $10 \times 10^{6}$ M solutions of DAEs in acetonitrile, methanol, and water were alternately irradiated with UV (365 nm) and visible light (470 nm) for irradiation times $t_{UV}$ and $t_{VIS}$, respectively (Figure S4, Supporting Information). After each semicycle (OF–CF and CF–OF), the absorption and emission spectra were recorded (Figure 2). As a measure of fatigue resistance ($N_{1/2}$), we used a number of cycles after which a half of the initial amount of CF is left in the PSS-365 nm (measured by absorption). In the literature, there is no uniform point of view of how to assess this parameter, and it was shown that the irradiation conditions affect the fatigue resistance.[13] We established the following procedure (for details, see the Supporting Information). Variable switching rates were measured in methanol (Table 2), acetonitrile, and water (see the Supporting Information). Variable switching rates may be used for separation of the markers using innovative switching schemes.[12] With respect to the reference compound, all new substituents decrease the cyclization/cycloreversion quantum yields by a factor of 2–60 (except 1-CN,N; Table 2).

Table 1. Photophysical properties of the asymmetric DAEs (Figure 1) in methanol.

<table>
<thead>
<tr>
<th>DAE $^{a}$</th>
<th>$\lambda^{\text{max,obs}}$ [nm]</th>
<th>$\xi$ [s$^{-1}$ cm$^{-1}$]</th>
<th>$\lambda^{\text{max,em}}$ [nm]</th>
<th>$\Phi^{b}$ $^{c}$</th>
<th>Stokes shift [nm]</th>
<th>$\tau^{d}$ [ns]</th>
<th>$\Phi_{\text{OF-CF}}$ [165 nm]</th>
<th>$\Phi_{\text{CF-OF}}$ [470 nm]</th>
<th>$\kappa_{\text{REV}}$ [165 nm]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-MeO,N  (D–A)</td>
<td>291/14 000</td>
<td>450/28 000</td>
<td>589, 0.02</td>
<td>139 (5200)</td>
<td>0.14</td>
<td>0.18</td>
<td>8.7 $\times 10^{-2}$</td>
<td>0.95</td>
<td></td>
</tr>
<tr>
<td>1-MeO,COMe  (D–D)</td>
<td>294/14 000</td>
<td>455/42 000</td>
<td>567, 0.07</td>
<td>112 (4400)</td>
<td>0.41</td>
<td>6.2 $\times 10^{-2}$</td>
<td>4.9 $\times 10^{-2}$</td>
<td>0.75</td>
<td></td>
</tr>
<tr>
<td>1-CN,N  (A–A)</td>
<td>295/17 000</td>
<td>432/40 000</td>
<td>542, 0.07</td>
<td>110 (4650)</td>
<td>0.44</td>
<td>0.46</td>
<td>4.6 $\times 10^{-2}$</td>
<td>0.23</td>
<td></td>
</tr>
<tr>
<td>1-CN,COMe  (A–D) $^{a}$</td>
<td>300/15 000</td>
<td>446/34 000</td>
<td>621, 0.11</td>
<td>175 (6300)</td>
<td>0.78</td>
<td>6.7 $\times 10^{-2}$</td>
<td>1.6 $\times 10^{-2}$</td>
<td>0.85</td>
<td></td>
</tr>
<tr>
<td>1-H,CH $^{e}$</td>
<td>296/12 000</td>
<td>429/32 000</td>
<td>526, 0.41</td>
<td>97 (4300)</td>
<td>1.97</td>
<td>0.40</td>
<td>5.5 $\times 10^{-2}$</td>
<td>0.75</td>
<td></td>
</tr>
</tbody>
</table>

$^{a}$Donor (D), acceptor (A) substituted; $^{b}$Fluorescence quantum yield (reference: fluorescein/NaOH 0.1 M); $^{c}$Fluorescence lifetime; $^{d}$Degree of conversion in the photostationary state; $^{e}$Values determined in MeCN due to the high fatigue in MeOH presented by this compound; $^{f}$Reference compound (with “neutral” [H–H] substitution pattern).
maximum of the CF (OD_{\text{CF}}) was measured in the course of irradiation.

First, a cycle with intermediate switching times was performed. The change in absorption at the maximum of the CF (OD_{\text{CF}}) was fitted to a monoexponential function ($A_0 \times \exp(-t/\tau_{\text{UV/VIS}}) + A_\infty$), determining fitting parameters $\tau_{\text{UV}}$ and $\tau_{\text{VIS}}$ (Figure 2). In order to ensure full conversion at the PSS and uniform conditions for different dyes and 

### Table 2. Relative brightness ($\Phi_h \times \epsilon_{\text{CF}}$) and specific switching rates ($\epsilon_{\text{CF}} \times \Phi_{\text{OF-CF}}, \epsilon_{\text{OF}} \times \Phi_{\text{CF-OF}}$) in methanol; fatigue resistances of DAEs.

<table>
<thead>
<tr>
<th>DAE (Figure 1)</th>
<th>$\epsilon_{\text{CF}} \times \Phi_{\text{fl}}$</th>
<th>$\epsilon_{\text{OF}} \times \Phi_{\text{OF-CF}}$</th>
<th>$\epsilon_{\text{CF}} \times \Phi_{\text{CF-OF}}$</th>
<th>Fatigue resistance ($N_{1/2}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH$_3$CN</td>
<td>CH$_3$OH</td>
<td>H$_2$O</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-MeO,N (D–A)</td>
<td>0.09</td>
<td>0.48</td>
<td>0.28</td>
<td>8124</td>
</tr>
<tr>
<td>1-MeO,COMe (D–D)</td>
<td>0.47</td>
<td>0.68</td>
<td>0.25</td>
<td>4940</td>
</tr>
<tr>
<td>1-CN,N (A–A)</td>
<td>0.26</td>
<td>0.32</td>
<td>1.28</td>
<td>202</td>
</tr>
<tr>
<td>1-CN,COMe (A–D)</td>
<td>0.56</td>
<td>0.05</td>
<td>0.06</td>
<td>256</td>
</tr>
<tr>
<td>1-H,CH</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>672</td>
</tr>
</tbody>
</table>

$^a$Units: [L mol$^{-1}$ cm$^{-1}$]; $^b$Number of cycles providing half of the initial amount of the closed form left in the PSS (365 nm); see the text for details; $^c$Reference compound.

Figure 2. Photoswitching of 1-MeO,N in acetonitrile. A,B) First full cycle used to determine the irradiation times $t_{\text{UV}} = 5 \times \tau_{\text{UV}}$ and $t_{\text{VIS}} = 5 \times \tau_{\text{VIS}}$. C,D) 6000 full cycles. E,F) The first and the last 50 cycles, respectively. For clarity, we plot the spectra of every 20th cycle in panel (C). Irradiation intensities: $2.3 \times 10^{-4}$ einstein dm$^{-3}$ s$^{-1}$ (365 nm) and $3.9 \times 10^{-4}$ einstein dm$^{-3}$ s$^{-1}$ (470 nm).
solvents, irradiation times were selected as $t_{UV} = 5 \times t_{UV}$ and $t_{VIS} = 5 \times t_{VIS}$.\[^{[13]}\] The selection of $t_{UV}$ is critical, because bleaching reactions are likely to occur in the CF upon UV irradiation.\[^{[10]}\] To corroborate this dependence, we “cycled” compound 1-MeO,COMe in water using progressive $t_{UV}$ values and found a strong dependence of $N_{1/2}$ upon $t_{UV}$ (Figure S5, Supporting Information). This result suggests bleaching from the CF excited with UV light, as one of the main bleaching paths.\[^{[15]}\] If the photoproducts absorb in the same region as the CF, the time until $OD_{ACF}$ falls to its initial value may be erroneous.\[^{[13]}\] Thus, we selected $N_{1/2}$ as the value when the difference in $OD_{ACF}$ in a semicycle ($OD_{ACF-UV} - OD_{ACF-VIS}$) drops to a half of the value obtained during the first cycle. The results for the donor–acceptor substituted dye 1-MeO,N in acetonitrile are presented in Figure 2. Remarkably, 1-MeO,N endured 6000 full UV–VIS cycles in acetonitrile “without exclusion of air oxygen,” and more than half of the compound was still active. This experiment took a week, at a rate of $\approx 0.6$ cycles min\(^{-1}\), with $t_{UV} = 22$ s and $t_{VIS} = 45$ s (+ time for spectrum recording and pauses between steps). This compound shows very high fatigue resistance ($N_{1/2} = 8100$, Table 2), probably, surpassed by only a limited number of DAEs.\[^{[14b]}\] The fatigue resistance, expressed as $N_{1/2}$, for all DAEs in the three solvents can be found in Table 2 (see the Supporting Information for details). In general, a drastic decrease in photostability was observed by changing the solvent from acetonitrile to methanol and water (Figure S4, Supporting Information). Compounds 1-MeO,N and 1-MeO,COMe are more fatigue resistant than 1-H,CH in all solvents, while 1-CN,N and 1-CN,COMe (with acceptor groups) perform poorly. The donor–acceptor substituted 1-MeO,N presents the best fatigue resistance. The high switching performance of all compounds in acetonitrile makes them interesting candidates for superresolution microscopy in material science.\[^{[5–7]}\] On the other hand, the specific switching rate in aqueous solutions is not much slower than in acetonitrile or methanol (Table S1, Supporting Information). This positive feature indicates that a high photobleaching rate in aqueous solutions is not entirely associated with low cycloreversion quantum yields. Notably, if DAEs are not forced to a complete ring closure, more cycles can be performed. In particular, if the shorter UV irradiation times are applied in aqueous solutions for compound 1-MeO,COMe, up to 550 cycles can be recorded (Figure S5, Supporting Information). However, a shorter UV irradiation yields only 55–60% of the maximal signal (compare the second and the last plots in Figure S5 in the Supporting Information), but this could be compensated in a microscope, if we consider two switching cycles. Under these conditions, compound 1-MeO,COMe can perform about 250 cycles in aqueous media. The drastic decrease in the fatigue resistance in water might be due to changes in the photobleaching paths or aggregation of the closed form. We are now studying the nature of photoproducts trying to reveal the bleaching mechanism.

5. Bioconjugates

Based on the data given in Tables 1 and 2, we have chosen compound 1-MeO,COMe as relatively bright and photoresistant DAE. We prepared its conjugates with bovine serum albumin (BSA) and studied their photoswitching. The published protocol\[^{[48]}\] afforded ~25–40% (analytical yield) of the mono-NHS (N-hydroxysuccinimidy) ester generated in the reaction mixture and detected by means of HPLC. For that, the free dye (2 mg of tetracarboxylic acid in 200 µL N,N-dimethylformamide (DMF)) was treated under stirring with 1.2 equiv. of N-hydroxysuccinimide in DMF (2.5 µL) followed by slow addition of N-ethyl-N’-[3-(N,N-dimethylamino)propyl]carbodiimide (EDCI) (8 equiv. of HCl salt) in DMF (66 µL). All attempts to isolate the individual mono-NHS ester by HPLC failed. Thus, three aliquots of the freshly prepared reaction mixture containing 0.14, 0.27, and 0.55 mg of the reactive dye were slowly added each to a solution containing 1 mg of BSA in phosphate buffered saline (PBS) (550 µL) with pH = 8.1 (added carbonate buffer). The mixture was stirred in the dark for 1 h and then purified in a PD10 exchange column (GE Healthcare). The degrees of labeling (DOL, average number of dye molecules per protein molecule) were estimated by absorption spectroscopy as 5.1, 8.1, and 10.5, respectively (Figure S6, Supporting Information), with ~6% of CF (see the Supporting Information for details). We concluded that the DOL value can be controlled, though it was not linear with the mass of dye introduced, probably due to a limited solubility in the reaction media, where it was present at concentrations of $(0.25–1) \times 10^{-3}$ M.

Switching of the BSA conjugate with DOL 5.1 was studied in PBS, at a concentration of 70 µg mL\(^{-1}\) (corresponds to a dye concentration of $5 \times 10^{-5}$ M). Bound to a protein, dye 1-MeO,COMe retained the photochromic and fluorescent properties. However, the switching behavior was found to be biexponential, with a “fast” fraction having the similar switching kinetics as a free dye in water, and a “slow” fraction with a tenfold longer $t$ (see the section “Fatigue Resistance”). In addition, we found a fraction of the dye in the CF (10%) which was not switching back to the OF, but remained fluorescent. The values of $N_{1/2}$ determined from the absorption and fluorescence modulation were 19 and 30 cycles, respectively, with the second value being more reliable (see the Supporting Information for details).

6. Confocal and RESOLFT Microscopy

Secondary antibodies were labeled with compounds 1-MeO,COMe, 1-CN,COMe, and 1-H,CH using the same protocol, as for BSA.\[^{[48]}\] Bioconjugation with compounds 1-MeO,N and 1-CN,N was not pursued due to a very low fluorescence efficiency in the first case, and poor fatigue resistance in the latter. In general, we obtained similar DOLs for the same protein/dye ratios in reaction mixtures. The antibodies were kept in the fridge without the addition of any stabilizing agent. Solutions showed no sign of aggregation, presented no change, and kept the selectivity for several months. Vero cells were immunostained using standard protocols (see the Supporting Information), mounted in PBS, and sealed. Confocal imaging was performed in a Leica SP5, equipped with a mercury lamp (UV source) and a DAPI filter cube for wide-field illumination. The sample was exposed for 1–5 s to wide-field UV illumination to switch-on (OF→CF) the DAE adducts, and then imaged in confocal mode (Figure 3; Figures S7–S9, Supporting Information). Best images were obtained with fast scanning, because the probes switched off very fast with excitation light
(458, 476, or 488 nm). In fact, even with such fast scanning, a considerable amount of the marker had reverted to the dark isomer (OF) after the first confocal image (Figures S8 and S9, Supporting Information). Specific labeling and low background were observed. In our previous work, we have found that DAEs with eighth carboxylic acid groups (seven carboxylates in bioconjugates) provided the best results.[4,8] Asymmetric DAEs introduced in this work have more compact switching units and “only” four carboxylic acid residues (three after bioconjugation). This number is enough to provide controllable reactivity, sufficient polarity, switching ability, and fluorescent properties of the marker and its bioconjugate (the specificity of the antibody was unaltered).

Finally, reversible saturable optical fluorescence transition (RESOLFT) microscopy images with fixed Vero cells immunostained with secondary antibodies labeled with compounds 1-CN,COMe, with DOL 3.1, and a primary antibody against tubulin.

Figure 3. A,B) Confocal images of Vero cells immunostained with a secondary antibody labeled with compound 1-CN,COMe, with DOL 3.1, and a primary antibody against tubulin.

Although power regimes were well within the range of those used for imaging of reversibly switchable fluorescent proteins (RSFPs),[16] synthetic photoswitchable DAEs are outperformed by RSFPs. The latter can be imaged with “shorter” imaging schemes,[17] are applicable in vivo and significantly better in terms of switching fatigue. All this makes them more suitable for time lapse imaging.[18] Under conditions, as specified in Table S3 (Supporting Information), we were able to acquire only 3–5 RESOLFT images, before brightness of the antibody conjugates was reduced to 50% (Figures S11–S13, Supporting Information). We also used 100 nm amino-modified beads and labeled them with compound 1-MeO,COMe. Under confocal conditions, FWHM was found to be 292 ± 9 nm. In RESOLFT mode, the beads served as a convenient test object for proving the optical resolution (found: 137 ± 9 nm FWHM; Figure S14 and Table S4, Supporting Information). The brightness of the beads was reduced to 50% after about seven frames. From all RESOLFT imaging experiments, we conclude that the most important future task is to improve the fatigue resistance of fluorescent DAEs, in particular in aqueous environments, in order to improve the optical resolution.

Table 3. Imaging parameters and full width at half maximum (FWHM) as a measure of optical resolution achieved for selected DAEs in confocal and RESOLFT imaging.

<table>
<thead>
<tr>
<th>Dye</th>
<th>355 nm activation(^{a}) [µs]</th>
<th>488 nm doughnut(^{a}) [µs]</th>
<th>488 nm readout(^{a}) [µs]</th>
<th>FWHM(^{d})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Confocal [nm]</td>
<td>RESOLFT [nm]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-MeO,COMe(^{b})</td>
<td>200</td>
<td>1800</td>
<td>40</td>
<td>184 ± 13</td>
</tr>
<tr>
<td>1-CN,COMe(^{b})</td>
<td>200</td>
<td>1400</td>
<td>120</td>
<td>187 ± 27</td>
</tr>
<tr>
<td>1-H,CH(^{b})</td>
<td>150</td>
<td>1000</td>
<td>120</td>
<td>183 ± 24</td>
</tr>
</tbody>
</table>

\(^{a}\)361.9 W cm\(^{-2}\); \(^{b}\)15.5 kW cm\(^{-2}\); \(^{c}\)26.1 kW cm\(^{-2}\); \(^{d}\)Average FWHM of 15 line profiles measured over 5 adjacent lines and fitted by Lorentzian nonlinear curve fit; \(^{e}\)100 µs delays between activation (355 nm) and doughnut (488 nm) pulses were introduced.
7. Conclusion and Outlook

Reversibly photoswitchable diarylethenes with fluorescent closed forms were applied in single molecule localization\[^4–7\] and RESOLFT\[^8\] microscopy. However, only fully symmetric 1,2-[bis-(2-ethyl-1-benzothiophene-1,1-dioxide-3-yl)]perfluorocyclopentenes with two carboxylated phenyl groups attached to C-6 and C-6’ have been used in RESOLFT microscopy.\[^8\] Due to structural and synthetic limitations, the full photoswitchable capacity of DAEs remained unrevealed and unexplored. Here we introduced new DAEs with “simplified” structures and increased Stokes shifts related to asymmetric substitution patterns and the presence of “push–pull” substituents. They feature higher cycloreversion quantum yields, but lower emission efficiencies than their analogs with extended π-conjugation paths.\[^4–8\] In view of the relatively large Stokes shifts of 4000–6000 cm\(^{-1}\), moderate fluorescence quantum yields are not surprising. Fatigue resistance in organic (acetonitrile and methanol) and aqueous solvents has been compared (without exclusion of air oxygen). In acetonitrile solutions, DAEs endure several thousands of cycles, in methanol several hundreds, and in water several tens (in cuvette experiments). Photostability in organic solvents is good enough, and compounds of the present study may be interesting as leads for developing photoswitchable probes applicable in material science.\[^5–7\]

Their performance in RESOLFT microscopy and other applications in aqueous media are limited by photobleaching (low fatigue resistance). From the standpoint of the switching performance, the shorter π-conjugation path is a far less limiting factor than photostability (at least in aqueous solutions). The structure–property relationships established in this study, together with simple preparation procedures, will enable rational design of new DAEs useful as photoswitchable fluorescent probes. For example, asymmetric structures are promising for switching with 405 nm laser, when only “one half” of the probe possesses an extended conjugation path. The most important future task is to improve the fatigue resistance of fluorescent DAEs, in particular, in aqueous solutions. Now we are studying the nature of photoproducts forming in aqueous solutions and try to find alternative (to carboxylates) biocompatible and hydrophilic groups protecting DAEs from photobleaching.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest
The authors declare no conflict of interest.

Keywords
photochemistry, photoluminescence, photonics, structure–property relationships

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