Synthesis of Thioxanthone 10,10-Dioxides and Sulfone-Fluoresceins via Pd-Catalyzed Sulfonylative Homocoupling

Gergely Knorr, Mariano L. Bossi, Alexey N. Butkevich,* and Stefan W. Hell*

ABSTRACT: Our report describes the facile and scalable preparation of 9H-thioxanthene-9-one 10,10-dioxides via Pd-catalyzed sulfonylative homocoupling of the appropriately substituted benzophenones. This transformation provides a straightforward route to previously unreported sulfone-fluoresceins and -fluorones. Several examples of these red fluorescent dyes have been prepared, characterized, and evaluated as live-cell permeant labels compatible with super-resolution fluorescence microscopy with 775 nm stimulated emission depletion.

Sulfone-fluorescein (Scheme 1a; X = SO₃H) is the sulfone-bridged analogue of the long-established green-emitting fluorescein dye fluorescein (3′,6′-dihydroxyfluoran). The introduction of a strongly electron-deficient bridging group has been previously demonstrated to shift the absorption and emission maxima, mainly by decreasing the LUMO energy level of the fluorophore, into the red range (>600 nm) preferred for many applications such as phosphorescent fluorescence emitters, in particular in challenging recent reports of Wu and Wang and Jiang relying on sodium dithionite as a masked “SO₂” synthon in their preparation of alkyl aryl sulfones, because the reported systems employing other SO₂ surrogates (K₂S₂O₈, DABSO, or formamidinesulfinic acid) consistently failed when tested with our substrates.

Gratifyingly, after a brief investigation of the reaction conditions (Table S1), we determined that the target thioxanthone 10,10-dioxides formed in good yields (>70%) for many examples (see Scheme 2) with an air-stable and inexpensive Pd(II) catalyst Pd(dppf)Cl₂ when DMSO was used as the reaction solvent with mild heating (80 °C). Distinct from the reported conditions, no addition of an external base or quaternary ammonium salts was needed. At least 1.5 equiv of Na₂S₂O₄ was required to achieve complete conversion of the starting material, but a larger excess of dithionite was well tolerated and found necessary in certain cases. Decreasing the Pd catalyst loading resulted in substantially lower reaction yields.

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The reaction was sensitive to the nature of the solvent, and even though other dipolar aprotic solvents (in particular DMF) were suitable, the reproducibility suffered because of the significant induction time as determined by means of in situ IR spectroscopy (see Figure S1). The substrates bearing strong electron-withdrawing groups yielded xanthones (e.g., 2m and 2o) as the major products, likely via competing hydrolysis of aryl triflates to phenols if the main reaction was slowed, and the presence of aryl halides (as in 1r) other than fluorine was not tolerated. Most peculiarly, and in stark contrast with previous reports, the transformation was successful only in the intramolecular version; otherwise, alternative reactivity with the molecular version; otherwise, alternative reactivity with the sulfoxylate dianion arising by disproportionation from the substrate of choice for the preparation of unsubstituted sulfone-fluoresceins (Scheme 4), the key building block for the synthesis of sulfone-fluoresceins, on a multigram scale by demethylation of 7a-Halo (Scheme 4b and Figure S4).

Test labeling was performed in living U2OS-Vim-Halo cells engineered using the CRISPR-Cas technology, which were preferred for their high cell-to-cell reproducibility as opposed to transient transfection methods. Hydrogenolysis followed by sequential treatment with TFA and DDQ (Scheme 4b and Figure S4).
compatibility of the label with stimulated emission depletion (STED) super-resolution fluorescence imaging. The attainable resolution was limited by the low molecular brightness of fluorophore 5a and the dynamics of intermediate filaments in living cells, despite the distinct fluorogenic response of the dye upon binding to HaloTag7 protein (Figure S6). Therefore, live-cell labeling with postfixation, permitting a longer total imaging time on immobile structures, was attempted and indeed demonstrated improved resolution (Figure S5). The samples stained with 7b-Halo and 7c-Halo were characterized by a significantly inferior quality of labeling, making the imaging impracticable.

In summary, we have developed an original entry in the synthesis of sulfone-fluorescein fluorophores and performed their photophysical characterization and preliminary evaluation as live-cell compatible far-red-emitting fluorescent labels. The proposed synthetic approach to thioxanthone 10,10-dioxides increases the availability of these building blocks for organo-photocatalysis, as well as for photovoltaics, electroluminescence, and other material science applications. Furthermore, we are currently exploring variations of the reported reactivity toward the synthesis of other sulfone-embedded heterocycles.

### Scheme 2. Preparation of Thioxanthone 10,10-Dioxides via Sulfonylative Homocoupling: (a) Scope of Substrates and (b) Limitations and Side Reactions

**a**

- 2a: 77%* (74%)^2
- 2b: 75%
- 2c: 75%^3
- 2d: 61%^2
- 2e: 48%
- 2f: 86%^4

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Yield (%)</th>
</tr>
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<tbody>
<tr>
<td>2g</td>
<td>83%</td>
</tr>
<tr>
<td>2h (5.29 g, 17.4 mmol)</td>
<td>92%^5</td>
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<tr>
<td>2i</td>
<td>49%^6</td>
</tr>
</tbody>
</table>

**b**

- 2m (X = SO_2, 14%)
- 2n (X = O, 70%)
- 2o (X = SO_2, 8%)
- 2o' (X = O, 87%)

<table>
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<tr>
<td>2m</td>
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<td>2o</td>
<td>8%</td>
</tr>
<tr>
<td>2o'</td>
<td>87%</td>
</tr>
</tbody>
</table>

### Scheme 3. Proposed Reaction Mechanism

**Table 1. Properties of Fluorophores 5a–5c and HaloTag Ligands 7a-Halo–7c-Halo**

<table>
<thead>
<tr>
<th>dye</th>
<th>λ_{\text{max}}^\text{abs} (nm)</th>
<th>ε (M\textsuperscript{-1} cm\textsuperscript{-1})</th>
<th>λ_{\text{max}}^\text{em} (nm)</th>
<th>Φ_f</th>
<th>τ (ns)</th>
<th>pK_a</th>
</tr>
</thead>
<tbody>
<tr>
<td>5a</td>
<td>625 (3000)</td>
<td>3000</td>
<td>663 (0.11)</td>
<td>1.35</td>
<td>8.1</td>
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<tr>
<td>5b</td>
<td>631 (42000)</td>
<td>42000</td>
<td>670 (0.08)</td>
<td>1.12</td>
<td>5.7</td>
<td></td>
</tr>
<tr>
<td>5c</td>
<td>629 (25000)</td>
<td>25000</td>
<td>667 (0.11)</td>
<td>1.31</td>
<td>5.3</td>
<td></td>
</tr>
<tr>
<td>7a-Halo</td>
<td>630 (13400)</td>
<td>13400</td>
<td>670 (0.11)</td>
<td>1.35</td>
<td>7.8</td>
<td></td>
</tr>
<tr>
<td>7b-Halo</td>
<td>636 (58000)</td>
<td>58000</td>
<td>677 (0.08)</td>
<td>1.02</td>
<td>5.3</td>
<td></td>
</tr>
<tr>
<td>7c-Halo</td>
<td>633 (27000)</td>
<td>27000</td>
<td>670 (0.10)</td>
<td>1.27</td>
<td>5.3</td>
<td></td>
</tr>
</tbody>
</table>

*aOptical properties measured in 0.1 M phosphate buffer (pH 9.0).
*bFluorescence quantum yield.
*cFluorescence lifetime.

Compatibility of the label with stimulated emission depletion (STED) super-resolution fluorescence imaging. The attainable resolution was limited by the low molecular brightness of fluorophore 5a and the dynamics of intermediate filaments in living cells, despite the distinct fluorogenic response of the dye upon binding to HaloTag7 protein (Figure S6). Therefore, live-cell labeling with postfixation, permitting a longer total imaging time on immobile structures, was attempted and indeed demonstrated improved resolution (Figure S5). The samples stained with 7b-Halo and 7c-Halo were characterized by a significantly inferior quality of labeling, making the imaging impracticable.

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ASSOCIATED CONTENT

Data Availability Statement
The data underlying this study are available in the published article and its Supporting Information.

Supporting Information
The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.orglett.3c04300.

Confocal time-lapse video of living U2OS cells stably expressing the vimentin-HaloTag fusion protein labeled with probe 7a-Halo (Video S1) (AVI)

Additional experimental details, materials, methods, and characterization data for all new compounds, including Figures S1–S6 and Tables S1–S2, and NMR spectra (PDF)

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Notes
The authors declare no competing financial interest.