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Carbon–Carbon Bond Cleavage in Fluorescent Pyronin Analogues Induced by Yellow Light

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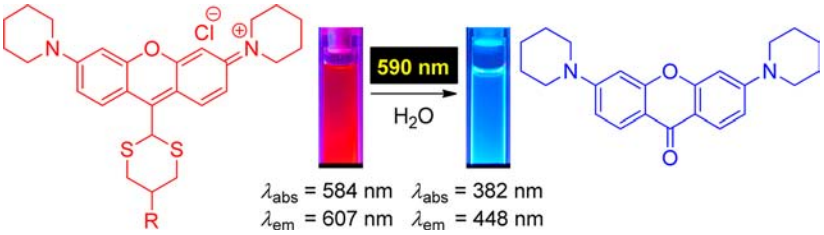
ABSTRACT



A novel class of pyronin analogues, which undergoes a photochemically induced cleavage of the C–C bond in the presence of water in both solution and on a silica gel surface upon direct irradiation with visible light, is reported. The reaction course can be monitored by characteristic fluorescence of both the starting compound and the final product. This system could find useful applications in the field of photoremovable protecting groups or caged fluorophores.

Carbon–carbon bond cleavage is among the most important chemical transformations in both the synthetic laboratory and nature. Such reactions are usually conducted in the presence of a catalyst,[1] but they can also be promoted by UV light.[2] In general, photosensitizers, photocatalysts, or photoinitiators are required for visible light-promoted reactions.[3] Photochemical processes often involve electron or energy transfer in the primary step. For example, Kutateladze and co-workers have developed a strategy of aldehyde and ketone protection using the 1,3-dithiane moiety, which is efficiently removed by oxidative electron transfer in the presence of a sensitizer, such as benzophenone, upon irradiation with UV light.[4] Visible-light initiated C–C bond scission reactions are rare.[5]

Rhodamines, pyronins, and xanthenones, well-known dyes with strong absorption and fluorescence in the visible region, have found wide application in molecular biology and medicine.[6] Wirz and co-workers have recently investigated the photochemistry of fluorescein analogues, 6-hydroxy-3-oxo-3*H*-xanthen-9-yl)methyl derivatives, which release diethyl phosphate or carboxylate via C–O bond scission upon irradiation at over 500 nm.[7] Here, we report on analogous fluorescent pyronin-based derivatives that undergo C–C bond cleavage upon irradiation with visible light in the absence of any photosensitizer.

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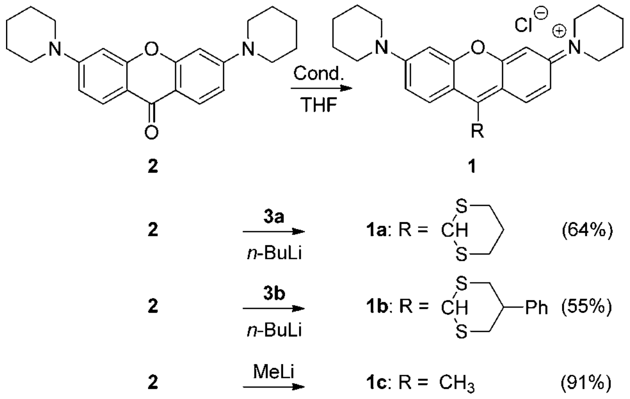
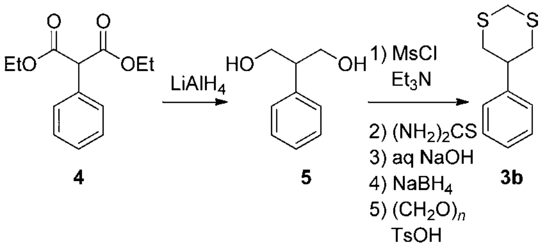
10.1021/ol302244f

The 1,3-dithian-2-yl derivatives of a 6-amino-3*H*-xanthen-3-iminium moiety (pyronin analogues) **1a** and **1b** were synthesized from xanthen-9-one (**2**), prepared by cyclizative condensation of $2,2',4,4'$-tetrahydroxybenzophenone and its subsequent derivatization[8] (Supporting Information), and 1,3-dithiane (**3a**) or 5-phenyl-1,3-dithiane (**3b**), respectively, according to Scheme 1. The synthetic intermediate **3b** was prepared by reduction of diethyl 2-phenyl malonate (**4**) to give the diol **5** and successive steps in 55% overall yield (Scheme 2). The model compound **1c** was synthesized according to a known procedure.[9] The xanthenone **2** was treated with a 5-fold excess of 1,3-dithiane-2-yl anion generated in situ from equimolar amounts of **3** and *n*-BuLi (an excess of *n*-BuLi causes its addition to **1**) at 0 °C. The subsequent elimination of water upon addition of an acid produced the target dark-purple products in good yields (55−64%). In the dark, **1a** and **b** are stable in the solid state for months but show signs of decomposition in a methanolic solution after several weeks.

Scheme 1



Scheme 2



Compounds **1a** and **b** exhibit strong absorption (methanol: λ_{max} = 584 nm; $\varepsilon_{max} \approx 6 \times 10^4$ M^{-1} cm^{-1}; water: λ_{max} (abs) = 588 nm) and emission (methanol: λ_{max} (em) = 608 nm) bands (Figure 1), which are slightly bathochromically shifted compared to those of Rhodamine 101 and Rhodamine B[6a] or their analogues.[10] The fluorescence quantum yields were determined to be Φ_{fl} = (14.7 ± 0.1) and (16.1 ± 0.1)% for **1a** and **1b**, respectively, in methanol. The absorbance of both **1a** and mixed **1c**/1,3-dithiane solutions was found to be proportional to the concentration (Beer's law) over 3 orders of magnitude (Figures S22 and S23, Supporting Information), which ruled out conceivable intermolecular charge transfer interactions of **1** in the ground state.

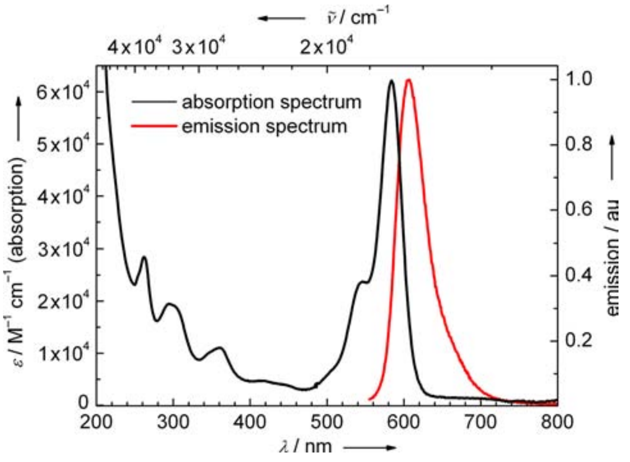


Figure 1. Absorption and uncorrected emission spectra of **1a** in methanol (c = 10^{-5} M).

Irradiation of **1a** and **b** ($c \approx 2 \times 10^{-5}$ M) in methanol at either 546 or 590 nm led to their decomposition. Figure 2 shows a decrease of the absorption band intensity of the parent, red-fluorescing compound **1a** (λ_{max} = 584 nm, red line; right cuvette) and the simultaneous appearance of a new band at λ_{max} = 499 nm (green line) during the course of irradiation. This intermediate was then thermally (in the dark; although a coincident photochemical pathway is not ruled out) converted to a stable product (λ_{max} = 382 nm, blue line). The addition of water slowed down the photoreaction but accelerated the second process. The resulting samples (> 95% conversion) exhibited a strong blue fluorescence (Figure 2, left cuvette), subsequently attributed to the xanthenone **2**. This compound was identified as an exclusive chromophoric product by both NMR (Figure S21) and LC-HRMS (the spectroscopic data were compared to those of the authentic compound).

The HRMS analysis of the irradiated degassed solutions of **1b** identified a side product with the formula $C_{10}H_{11}S_2^+$, whereas secondary, evidently oxidation products with formulas $C_{10}H_{10}OS_2^+$ and $C_9H_{10}OS_2^+$ were found in the nondegassed samples. None of the spectra corresponded to that of **3b**. The observed species can be daughter ions of (photo)products formed during the course of irradiation, such as adducts with methanol or water. Figures S17−19 offer the proposed structures, which are analogous to those

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obtained by anodic oxidation of 1,3-dithiane derivatives.[11] In addition, multiple ^{1}H NMR signals attributed to the phenyl ring in an exhaustively irradiated **1b** solution (Figure S21) demonstrate that the mixture of side products derived from the phenyl-1,3-dithiane moiety is complex and cannot be easily analyzed.

In order to identify a transient species observed by absorption spectroscopy (Figure 2, green line), the methanolic solution of **1b** was irradiated using a 500 W halogen lamp until more than 90% of the starting material was consumed. The solvent was immediately removed under reduced pressure at 20 °C to prevent photoproduct decomposition. ^{1}H, ^{13}C, and 2D NMR analyses in conjunction with the HRMS data (Figure S18) suggested that the thermally unstable intermediate is the methoxy derivative **6** (Scheme 3). In addition, irradiation of **1b** in CD_3OD provided the corresponding CD_3-labeled compound **6** ($\Phi = 0.03\%$), and a subsequent addition of an excess of $D_2{}^{18}O$ or benzylthiol resulted in thermal (dark) substitution of the methoxy group and formation of the adducts **2** or **7**, respectively. Irradiation of **1b** in $CH_3OH/D_2{}^{18}O$ (4:1, v/v) gave **2** with the isotopically labeled carbonyl group (Figure S20). The carbonyl oxygen thus unambiguously originates from water present in a solvent. The fact that dry acetonitrile had a detrimental effect on the photolysis rate confirms that water is indispensable for the formation of **2**. Although irradiation of **1** in pure water also provided **2** as an exclusive final product, the reaction was slower than that carried out in solvent containing only small amounts of water (Table S1).

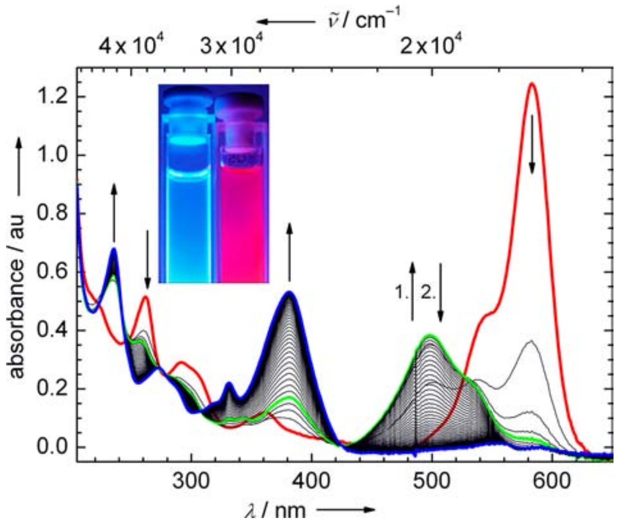
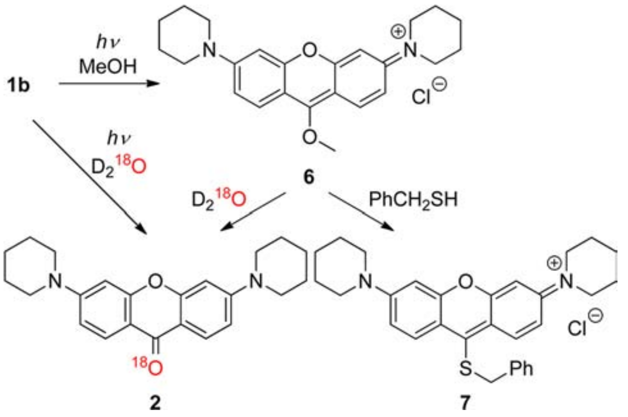


Figure 2. Absorption spectra measured following irradiation at 590 nm of a **1a** solution in methanol ($c_{\text{init}} \approx 2 \times 10^{-5}$ M; one spectrum taken every 30 min). Inset: Blue fluorescence of the irradiated mixture (left cuvette) compared to red fluorescence of **1a** (right cuvette; excited by 366 nm light).

A concentration-dependent quantum yield[12] could be an indication of a photoinitiated bimolecular process. In this work, the quantum yield of disappearance of **1b** in methanol, determined by ferrioxalate actinometry, was found to be approximately the same ((0.029 ± 0.019)%) in the concentration range of 5×10^{-7} to 2×10^{-4} M. Nevertheless, we still cannot fully disregard the contribution of a self-photoinitiated bimolecular mechanism based on this experiment. Addition of an excess of 1,3-dithiane did not affect the quantum yield.

Scheme 3. Formation of the Products from **1b**



Electron transfer could be an initial step in the phototransformation of the excited pyronin chromophore. The dithiane oxidation is known to take place in the presence of a triplet sensitizer, such as benzophenone, resulting in the mesolytic C−C bond cleavage.[4c] The triplet state of rhodamine dyes can be photochemically reduced by electron donors, such as thiols[13] or amines.[14] Its formation by intersystem crossing is known to be inefficient (< 1%),[15] which could be related to our observation of a low disappearance quantum yield of **1b** and also to the fact that the photolysis efficiency was unaffected by deoxygenation in the concentration range 10^{-6} to 2×10^{-4} M (e.g., Table S1).

We also estimated that the driving force for the one-electron reduction potential of pyronin Y (−0.81 V vs SCE[16]), its singlet energy $E_S = 51.4$ kcal mol^{-1},[17] and the oxidation potential of 1,3-dithiane (+1.05 V vs SCE;[18] Eq S1, Supporting Information) is exergonic. However, due to the relatively short singlet-state lifetime of rhodamine

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dyes (units of ns[19]), only intramolecular electron transfer from the sulfur atom to the excited pyronin moiety would be feasible at such low concentrations (fluorescence of Rhodamine 6G in methanol is quenched only at very high concentrations (> 10^{-2} M[20])).

Photoinitiated formation of the products **2** and **6** from **1b** indicates that nucleophilic attack at the pyronin 9-position is one of the key processes which either precedes or follows the mesolytic C−C bond cleavage (subsequent nucleophilic substitution at this position, such as in **6**→**2** or **6**→**7**, is possibly a dark process). It has been shown that a considerable amount of Rhodamine B exists in its lactone form even in polar alcohols.[21] Therefore, more than one pyronin form can exist in nucleophilic solvents, and their participation on the reaction should not be neglected. The reaction mechanism of this reaction is currently under investigation in our laboratory.

Due to its polar nature, **1a** was adsorbed on silica gel from its methanolic solutions. We tested whether it can be photochemically converted into **2** after the solvent is removed by evaporation. Thus **1a** was deposited on a nonfluorescent chromatographic silica gel plate, and green light from a portable laser was used to create a macroscopic pattern (Figure 3). The blue fluorescence of **2** was conveniently detected instead of the original red fluorescence of **1a** at the site of irradiation. **2** was then selectively eluted from the surface by acetonitrile resulting in a blue fluorescent solution. Formation of **2** suggests that the residual amount of water on a silica gel surface was sufficient to enable the reaction.

Figure 3. **1a** adsorbed on a chromatographic silica gel plate visualized by 366 nm light. The *hv* symbol was created by a green laser light.

In conclusion, we report on a novel class of pyronin analogues **1a, b**, which undergo photochemically induced cleavage of the C−C bond, probably via intramolecular electron transfer, in the presence of water in both solution and on a silica gel surface upon direct irradiation with visible light. The final chromophoric photoproduct is a stable compound absorbing below 430 nm. The course of the reaction can be monitored by characteristic fluorescence of both the starting compound and the final product. As one of the rare examples of a visible-light triggered system, it could find useful applications in the field of photoremovable protecting groups[22] or caged fluorophores.

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Supporting Information Available. Experimental details, calculations of the Gibbs energy of electron transfer, epifluorescent microscopy images and NMR, UV−vis, and HRMS characterization data for compounds are provided. This material is available free of charge via the Internet at http://pubs.acs.org.

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The authors declare no competing financial interest.