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A Photoactivable Fluorophore Based on Thiadiazolidinedione as Caging Group

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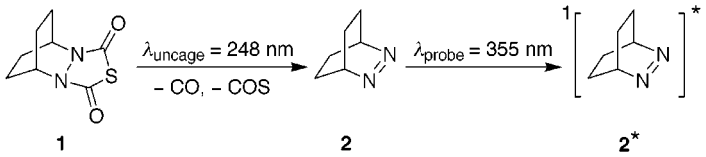
ABSTRACT



Photoactivable ("caged") fluorescent dyes and probes are crucial for temporally and spatially resolved tracer experiments, e.g., in cell biology or fluid physics. The thiadiazolidinedione 1 represents a new class of caged fluorophore. Upon UV-irradiation it releases in a rapid photoreaction with high quantum yield the azoalkane 2. Longer wavelength excitation of 2 to the singlet excited state results in strong and long-lived fluorescence with maximum intensity at 425 nm. It has been demonstrated that one single uncaging laser pulse suffices for time-resolved or steady-state detection of the fluorescence.

Photoactivable or "caged" fluorophores are nonfluorescent molecules that can be converted to a fluorescent form by a photoinduced reaction. They are powerful tools to investigate fluid dynamics in rheology and cell biology. Since their introduction,[1] they have been used in the study of cytoskeleton dynamics, e.g., that of actin microfilament with caged resorufin[2] and that of tubulin with caged fluorescein,[3] or as chemical actinometers for flux determination in biological tissue samples.[4] In rheology they are invaluable for the study of turbulent and laminar hydrodynamic flows and scalar mixing studies.[5]

The design and optimization of appropriate caged fluorescent probes and dyes presents a synthetic and mechanistic challenge. The *caged* fluorophore should meet a number of criteria: thermal stability, water solubility, ease of synthetic accessibility, no fluorescence, UV-only absorption, and an efficient as well as rapid photoactivation. The *uncaged* fluorophore itself should be strongly fluorescent upon long-wavelength excitation. In addition, the application for cellular studies requires the caged fluorophore to be biostable and biocompatible, the photoproducts to be nontoxic, and the uncaged fluorophore to be photostable since the measurements may extend over longer periods of time. In contrast, for rheological applications the main emphasis lies on fast uncaging rates.

To date, most of the established caged fluorophores are fluorescein, rhodamine, and resorufin derivatives which employ variants of the *o*-nitrobenzyl caging group.[6,7] Long-wavelength absorption (>360 nm), poor water solubility, and low photolysis quantum yields may limit the practical use of nitrobenzyl caging groups. The slow uncaging rates in the μs to ms region present another drawback. On the other hand, some fluorescent dyes suffer rapid photobleaching (fluorescein, resorufin) or require more complex syntheses (Q-rhodamines).[6,7] Others like caged resorufins have lifetimes of less than 1 h in cells or already display some fluorescence before their photoactivation.[6−8]

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(5) (a) Lempert, W. R.; Magee, K.; Ronney, P.; Gee, K. R.; Haugland, R. P. *Exp. Fluids* **1995**, *18*, 249−257. (b) Guilkey, J. E.; Gee, K. R.; McMurtry, P. A.; Klewicki, J. C. *Exp. Fluids* **1996**, *21*, 237−242.
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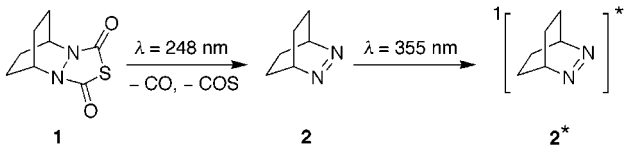
We report in this Letter the suitability of the thiadiazolidinedione derivative **1**[9−11] as a prototype for a new caged fluorophore composed of a new caging group as well as a new fluorescent probe, the 2,3-diazabicyclo[2.2.2]oct-2-ene (**2**).[12,13] 3,4-Dialkyl-1-thia-3,4-diazolidine-2,5-diones are readily accessible by Diels−Alder addition,[14] and their use as precursors for azoalkanes has long been recognized.[9,10,15] Nonetheless, their potential to serve as caged fluorophores has not been addressed. The reaction sequence of the wavelength-selective release of the fluorescent dye and its subsequent excitation to the fluorescent singlet excited state is shown in Scheme 1.

Scheme 1



Of special interest is the released fluorophore **2**, which exhibits an exceedingly long-lived fluorescence (up to 1 μs)[16] and serves as the novel fluorescent probe Fluorazophore-P for monitoring antioxidant activity in biological systems[12] and for investigating supramolecular association kinetics.[13] Accordingly, we refer to **1** as caged Fluorazophore-P.

The photophysical properties of **1** and **2** in different solvents are shown in Table 1. These data are essential to assess the practical suitability of **1** as a caged fluorophore.

Table 1. Photophysical Data for the Caged (**1**) and Uncaged (**2**) Fluorazophore-P

solvent	ϵ(**1**)/M^{-1} cm^{-1} (λ_{max}/nm)	Φ_{uncage}(**1**)[a]	ϵ(**2**)/M^{-1} cm^{-1} (λ_{max}/nm)	Φ_f(**2**)[b]	Φ_d(**2**)[c]
n-hexane	4640 (217)	0.27[d]	193 (377)[e]	0.15	0.03[f]
CH_3CN	5310 (217)	0.15	114 (378)	0.41	0.01
water	5750 (216)	0.05	48 (364)	0.20[g]	0.001

[a] Uncaging quantum yield (λ_{uncage} = 254 nm) determined with preirradiated azobenzene as actinometer, cf. Gauglitz, G.; Hubig, S. *J. Photochem.* **1985**, *30*, 121−125. [b] Fluorescence quantum yield calculated by assuming a natural fluorescence lifetime of 1700 ns according to ref 16. [c] Decomposition quantum yield; Feth, M. P.; Greiner, G.; Rau, H.; Nau, W. M., unpublished results. [d] Independently determined by laser flash actinometry. [e] See ref 18. [f] Value for *n*-heptane. [g] See ref 16.

The thiadiazolidinedione **1**[11] is a colorless compound with thermal stability at ambient temperature and, like **2**, dissolves both in organic solvents and in water. While **1** has a large extinction coefficient in the UV region (ϵ ca. 5000 M^{-1} cm^{-1}, Table 1) it does not absorb above 270 nm. Irradiation of precursor **1** with UV light ($\lambda \leq$ 260 nm) releases quantitatively Fluorazophore-P (**2**)[17] with medium to high quantum yields, depending on solvent (Table 1). The absorption spectrum of the resulting azoalkane **2** shows a UV window (260−320 nm) and a comparatively weak absorption band around 380 nm. The absorption spectra of **1** and its photoproduct **2** are shown in Figure 1.

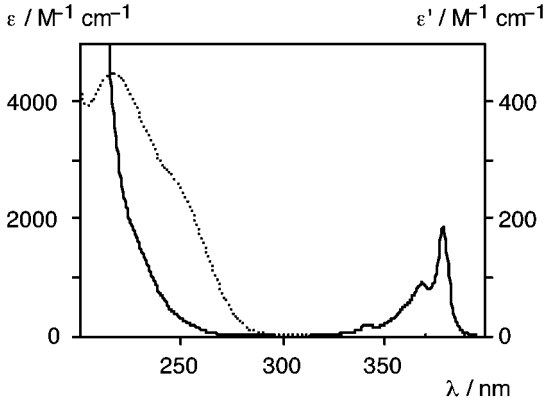


Figure 1. Absorption spectra of the caged Fluorazophore-P **1** (dashed line, ϵ scale) and the uncaged Fluorazophore-P **2** (solid line, ϵ' scale) after 100% conversion of a 0.3 mM solution of **1** in *n*-hexane.

The use of the high-energy UV light for uncaging may present an obstacle for biological but not necessarily for rheological studies. The small extinction coefficient of **2** presents another drawback which, however, is effectively balanced by its high fluorescence quantum yield (ϕ_f up to 0.5, Table 1).[16,18] As illustrated in Figure 2, a single uncaging laser pulse (λ_{uncage} = 248 nm)[19] was sufficient to detect the characteristic broad, unstructured fluorescence of **2** by steady-state fluorimetry (λ_{exc} = 350 nm). In contrast, the nonirradiated solution of **1** showed no fluorescence.

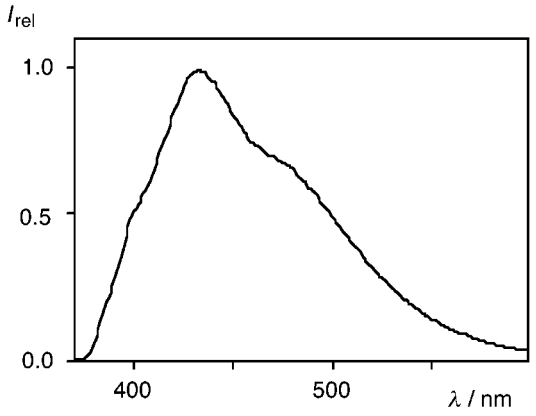


Figure 2. Fluorescence spectrum of Fluorazophore-P **2** upon photolysis of a solution of **1** in *n*-hexane (0.3 mM).

(8) Bendig, J.; Helm, S.; Hagen, V. *J. Fluoresc.* **1997**, *7*, 357−361.
(9) Corey, E. J.; Snider, B. B. *J. Org. Chem.* **1973**, *38*, 3632−3633.
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(11) Beak's method (ref 10) was used for the synthesis of **1**, cf. Supporting Information.
(12) Nau, W. M. *J. Am. Chem. Soc.* **1998**, *120*, 12614−12618.
(13) Nau, W. M. *Chimia* **1999**, *53*, 217.

Time-resolved transient absorption spectroscopy revealed that the uncaging process, monitored through the absorption of **2** (λ_{obs} = 375 nm), occurs faster than 1 μs, e.g., 250 ns in acetonitrile. The photolysis yields CO and COS as the presumed side products, in analogy to the parent compound 1-thia-3,4-diazoline-2,5-dione that decomposes thermally to N_2, CO, and COS.[10]

The suitability of the photoactivable probe **1** for temporally and spatially resolved investigations could be demonstrated by a two-photon two-color flash photolysis experiment. The fluorophore **2** was generated with one single laser pulse[19] at λ_{uncage} = 248 nm from a thiadiazolidinedione **1** solution and subsequently excited with a second laser pulse[20] at λ_{probe} = 355 nm. While the uncaging laser flash results in an inevitable autoluminescence, the probing pulse gives fluorescence with the typical long fluorescence lifetime of **2** (τ = 690 ns)[16] which can be readily differentiated as illustrated in Figure 3.

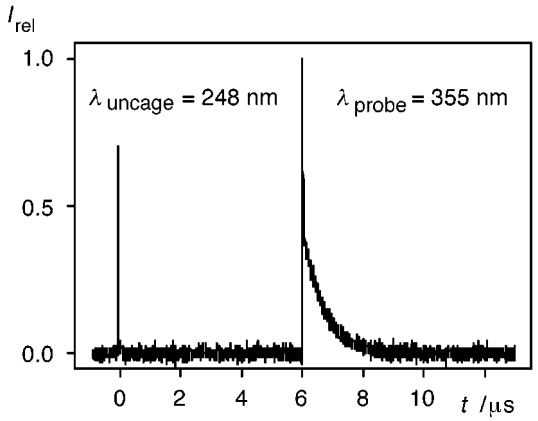

Figure 3. Two-photon two-color flash photolysis experiment. A solution of **1** in acetonitrile (0.2 mM) was irradiated with a single uncaging pulse and subsequently excited with a second laser pulse. The emission was detected at λ_{obs} = 425 nm (arbitrary time delay).

In conclusion our investigations show that the *caged* fluorescent probe **1** fulfills the desirable features for photoactivable fluorophores, namely the UV-induced release from a nonfluorescent precursor, ease of synthesis, low molecular weight, favorable solubility, the long-wavelength absorption of the photoproduct, and its strong fluorescence. Most importantly, the photolytic release is efficient and fast (<1 μs). The *uncaged* fluorophore **2** exhibits additional interesting properties which include the transparency in the UV region, good water solubility, and exceedingly long fluorescence lifetime.[16] The high photostability of **2**, indicated by the low decomposition quantum yields (ϕ_d in Table 1),[16,18] should prevent unwanted photobleaching in long-time measurements, e.g., for biological studies.

For further application several DBO derivatives,[21] e.g., the hydroxymethyl or carboxyl substituted ones **3** and **4**, could be employed in their caged form. These substitution patterns allow covalent attachment to molecules of interest, e.g., peptides.

3 **4**

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Supporting Information Available: Synthesis and characterization for compound **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(14) The Diels−Alder reaction is carried out by addition of the appropriate diene to the in situ generated 1-thia-3,4-diazoline-2,5-dione. Subsequent hydrogenation yields the thiadiazolidinedione product.

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(16) Nau, W. M.; Greiner, G.; Rau, H.; Wall, J.; Olivucci, M.; Scaiano, J. C. *J. Phys. Chem.* A **1999**, *103*, 1579−1584.

(17) This work (by UV) and ref 15 (by NMR).

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(19) COMPex 205 laser, fwhm ca. 20 ns, pulse energy ca. 207 mJ.

(20) Nd:YAG laser (Continuum Surelite II), fwhm ca. 5 ns, pulse energy ca. 10 mJ.

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