Unexpected photo-instability of 2,6-sulfonamide-substituted BODIPYs and its application to caged GABA

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Abstract: Investigation of the unexpected photo-instability of 2,6-sulfonamide-substituted derivatives of the boron dipyrromethene (BODIPY) fluorophore led to discovery of a photoreaction accompanied with multiple bond scissions. We characterized the photoproducts, and utilized the photoreaction to design a caged gamma-aminobutyric acid (GABA) derivative that can release GABA upon irradiation in the visible range (> 450 nm). This allowed us to stimulate neural cells in mouse brain slices.

Introduction

In molecular, synthetic, and systems biology, there is increasing interest in the use of precisely controlled stimulation of cellular mechanisms in order to understand cellular behaviors. An efficient approach to achieve this is the use of caged compounds that release the stimulant specifically in response to photoirradiation within a selected area and time window[1]. For example, spatiotemporally photo-controlled release of neurotransmitters, such as glutamic acid and gamma-aminobutyric acid (GABA), from caged derivatives is useful to study neural cell responses[1b]. Further, caged RNAs can be used to achieve spatiotemporally controlled protein expression[2], and caged drugs are potential tools for targeting drugs to specific cells or tissues[3]. Recent progress in microscopy has provided effective instrumentation for studies of cellular behaviors at the single-cell level[1c]. However, although many small-molecular probes, such as fluorescence probes, are available to monitor cellular behaviors, there has been less progress in the development of small molecules to control or modulate cellular responses. One reason for this is the lack of suitable reactions for controlled release of modulators, especially efficient photoreactions working in the visible range. So far, many caged compounds have been developed based on derivatization of 2-nitrobenzyl group (e.g., 4,5-dimethoxy-2-nitrobenzyl; DMNB)[4] or benzyl group modifications (e.g., 6-bromo-7-hydroxycoumarin-4-ylmethyl; Bhc)[5], but novel photoreactions for uncaging would be beneficial to extend the range of available caged compounds[6].

In the present work, we discovered that a novel derivative of the boron dipyrromethene (BODIPY) fluorophore is highly unstable to light irradiation. Based on the discovered reaction, we designed and synthesized a caged GABA derivative and confirmed that it worked as expected.

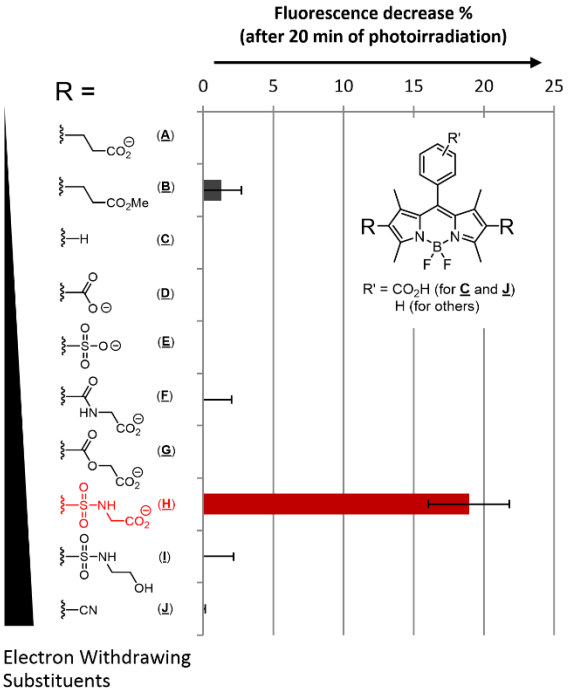

Figure 1. BODIPYs with various 2,6-substituents (1 μM) were dissolved in phosphate buffer (pH 7.4) containing 10% methanol, and fluorescence was monitored for 20 min (480 ± 20 nm) with a microplate reader, EnVision. Photobleaching is shown as fluorescence decrease (%) during the measurement. n = 12. Error bar represents S. D..

Results

BODIPY is a fluorophore that is widely used for protein labelling and as a major scaffold for fluorescent probes[7]. Also, the uses of BODIPY scaffold as the antenna to control photoreactions were also reported[8].

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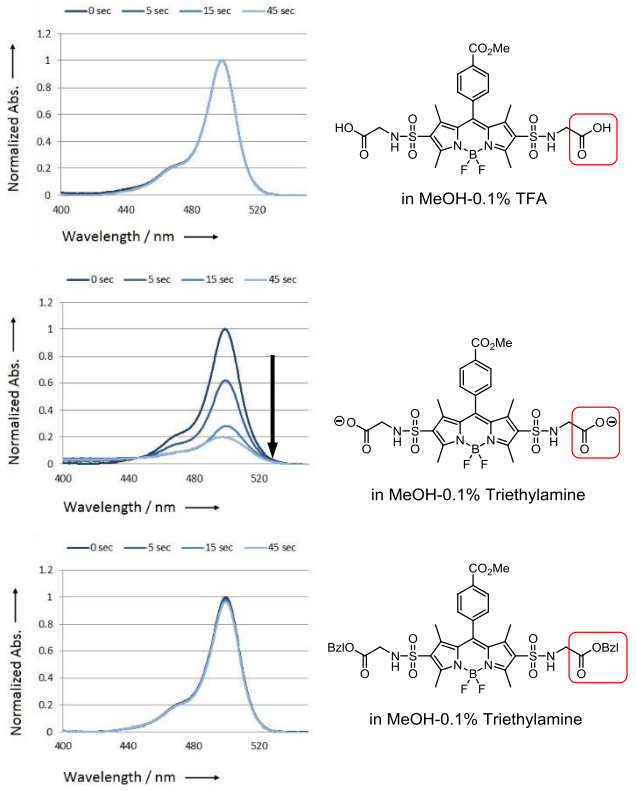


Figure 2. Normalized absorbance spectrum of 2,6-$SO_2NHCH_2CO_2R$-substituted BODIPYs (R = H for left and center, and R= Bzl for right) after photoirradiation (a xenon lamp, 490 ± 5 nm, 5.45 mW/cm^2) in methanol-0.1% trifluoroacetic acid (TFA, left) or in methanol-0.1% triethylamine (TEA, center and right).

The advantages of BODIPY fluorophore include solvent polarity-insensitive and pH-insensitive absorbance and emission in the visible light region[9]. We recently showed that the photostability of BODIPY is dependent on the electron density of the fluorophore[10], and can be controlled by means of modifications at the 2,6-positions; introduction of electron withdrawing substituents (with higher σ_p value) makes the fluorophore less subject to phorobleaching. Based on the finding, we set out to develop a novel BODIPY scaffold with sulfonamide groups as strongly electron-withdrawing substituents (σ_p ~ 0.60) at 2,6-positions. The scaffold was synthesized in a straightforward manner, as shown in **Scheme S1**. However, when we studied its photostability, we found that the 2,6-$SO_2NHCH_2CO_2H$-modified BODIPY showed significant photoinstability even on mild exposure to visible light, though photobleaching of other BODIPYs is hardly detectable under such conditions (**Figure 1**). Even the quite similar 2,6-$SO_2NHCH_2CH_2OH$-modified BODIPY did not show any photobleaching under the same conditions (**Figure S1**), so it seemed that the photoinstability was highly specific to the structure of the compound. One of the major causes of photobleaching in general fluorophores is destruction of the fluorophore by singlet oxygen generated by photoactivation[10-11], but the reaction of our compound was not affected by the presence/absence of oxygen (**Figure S2**). Therefore, the reaction seemed to be a photoreaction rather than photobleaching, and we thus investigated the mechanism involved using a series of 2,6-sulfonamide BODIPY derivatives of glycine, alanine, sarcosine (*N*-methylglycine), and β-alanine. In preparing those derivatives, the initially established reaction scheme, in which the pyrrole substituents were introduced at an early stage of synthesis, was inefficient, so we developed a novel synthetic scheme based on direct chlorosulfation of BODIPY (**Scheme S2**). For this purpose, we modified the reaction conditions for sulfonation of the BODIPY fluorophore developed by Burgess et al.[12]; chlorosulfation was achieved by addition of excess chlorosulfonic acid to 2,6-unsubstituted BODIPY with an electron-withdrawing modification (methyl carboxylate) at the 8-phenyl group, and the resultant chlorosulfated BODIPY was reacted with various amines to afford the 2,6-sulfonamide-modified BODIPYs with comparable overall yield to that of the initial scheme. Chlorosulfated BODIPY could be purified simply by column chromatography, which would be useful in the case of reactions using amines of low availability. We then examined the photochemical characteristics of the synthesized BODIPY derivatives.

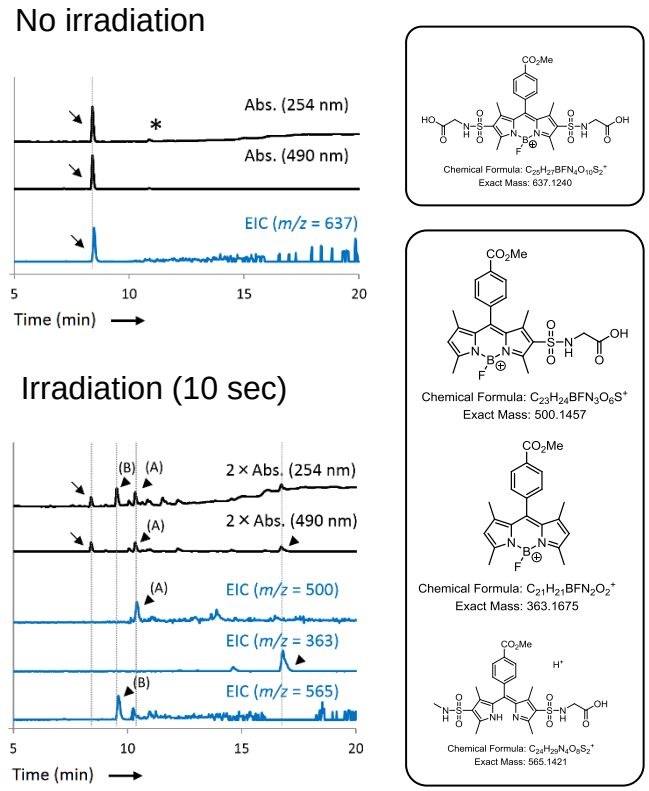
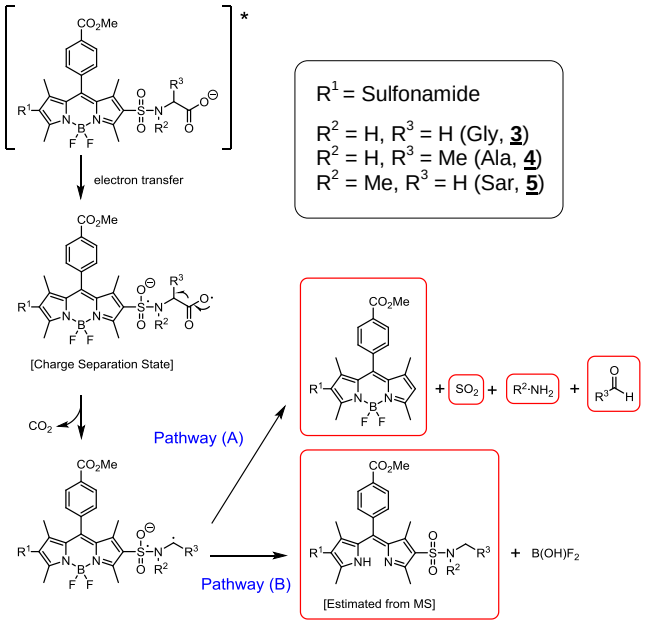


Figure 3. Observed photoproducts of BODIPY-SA-Glycine (**3**, 10 μM) in MeOH after irradiation (Xe lamp, 470 ± 20 nm, >30 mW/cm^2, 10 sec). 2-Me-4-OMe-TG (490 nm absorbance, *m/z* = 333, R_T = 10.8 min (*)) was used as the internal standard. Arrow indicates the intact fluorophore, and arrowheads indicate BODIPY after release of sulfonamide (A) and the decarboxylated and deboronated product (B). Expected *m/z* values of estimated products (ESI$^+$) are shown.



Scheme 1. Observed photoproducts (red squared) formed from 2,6-sulfonamide-substituted BODIPYs, and estimated reaction scheme. See **Table 1** , **Figure S6**-**S11** for details.

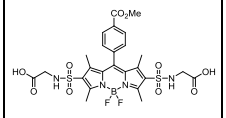
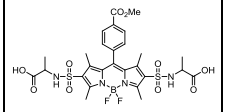
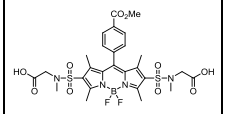
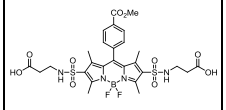
Name	Structure	$\Phi_{Photoreaction}$	
BODIPY-SA-Glycine (**3**)		0.048	・BODIPY (LC-MS, **Figure 3**) ・Formaldehyde (Hydrazide formation, **Figure S8**, **S10**) ・Sulfur dioxide (GC-MS, **Figure S11**)
BODIPY-SA-Alanine (**4**)		0.11	・Acetaldehyde (Hydrazine formation, **Figure S9**)
BODIPY-SA-Sarcosine (**5**)		0.022	・Methylamine (OPA-RSH system, **Figure S7**)
BODIPY-SA-β-Alanine (**6**)		0.001	-

Table 1. Photoreaction quantum yields ($\Phi_{Photoreaction}$) of BODIPY derivatives in MeOH-0.1% TEA and summary of detected photoproducts after photoirradiation in MeOH. $\Phi_{Photoreaction}$ was calculated as reported [ref. 8e].

Our findings can be summarized as follows. (1) The derivatives of glycine, alanine, and sarcosine (all α-amino acids) exhibited rapid photoreaction ($\Phi_{Photoreaction}$ = 0.022-0.11, **Table 1 and S1**), while the derivative of β-alanine (a β-amino acid) did not (**Figure S3**). (2) For all derivatives that exhibited detectable photoreaction, the reaction rate was much slower under acidic conditions (**Figure 2, S4**). (3) All derivatives in which the carboxylic acid moiety was protected showed no photoreaction (**Figure S5**). These results indicate that the presence of amine-linked methylenecarboxylic acid ($-CH_2CO_2H$) structure in carboxylate form is indispensable for the photoreaction to occur.

Based on these findings, we considered that the photoreaction proceeded via formation of a charge-separation state between carboxylate and the fluorophore, as had been proposed for the photolysis of other sulfonamides[13]. Consequently, proximity of the carboxylate to the sulfonamide was important[14], and this is the reason for the different reactivities of α- and β-amino acid derivatives. The photoreaction afforded two major products with aromatic-type absorbance, i.e., non-substituted BODIPY (Pathway (A)), and dipyrromethene with sulfonamide in decarboxylated form (Pathway (B), **Figure 3** and **Scheme 1**)). Thus, at least two competing pathways seemed to be operating. We were especially interested in Pathway (A), since the product linked to the sulfonamide moiety should be released in the course of the reaction, which could provide a basis to develop novel caged compounds. Thus, we set out to characterize the products. We firstly examined whether any amine is generated via the photoreaction, but direct release of amino acids (glycine, alanine, and sarcosine) attached to sulfonamide was not detected (**Figure S6**). This result suggested that radical homolysis of S-N bond[13] did not occur after charge-separation state, and a reaction accompanied with decarboxylation is likely to occur, in which methylamine should be formed from the sarcosine derivative. Indeed, we were able to detect methylamine using the OPA-RSH system[15] after photoreaction of BODIPY-SA-Sarcosine (**Figure S7**). In this reaction scheme, the single carbon unit corresponding to the α-carbon of the amino acid should be released as aldehyde, and this was also confirmed by detection of formaldehyde from glycine derivatives (**Figure S8**) and acetoaldehyde from alanine derivatives, by means of hydrazide formation (**Figure S9**). Since the chemical yield of aldehyde formation was proportional to the formation of de-sulfonated BODIPY (**Figure S10**), we think that these photoproducts were generated via Pathway (A). As for the course of amine and aldehyde formation, it is possible that an imine intermediate is firstly generated and then undergoes hydrolysis, but so far we have insufficient evidence to identify the short lifetime intermediates. We also detected the formation of sulfur dioxide under these conditions (**Figure S11**). Overall, multiple products summarized in **Scheme 1** and **Table 1** seemed to be generated during the course of the reaction. Among observed photoproducts in Pathway (A), formation of amines after decarboxylation seemed to be useful for the photorelease of primary and secondary alkyl amines. Based on this photoreaction, we developed caged GABA compound (**Figure 4a**). There are several useful caged GABA compounds reported to date[16], but we thought that the caged GABA excitable at visible light was still valuable. The design was as

follows. A methylenecarboxylic acid (-CH_2CO_2H) moiety was linked to sulfonamide, so that photocleavage via pathway (A) should result in the release of GABA together with carbon dioxide, formaldehyde, and sulfur dioxide. We prepared both di-substituted (**7**) and mono-substituted (**9**) compounds.

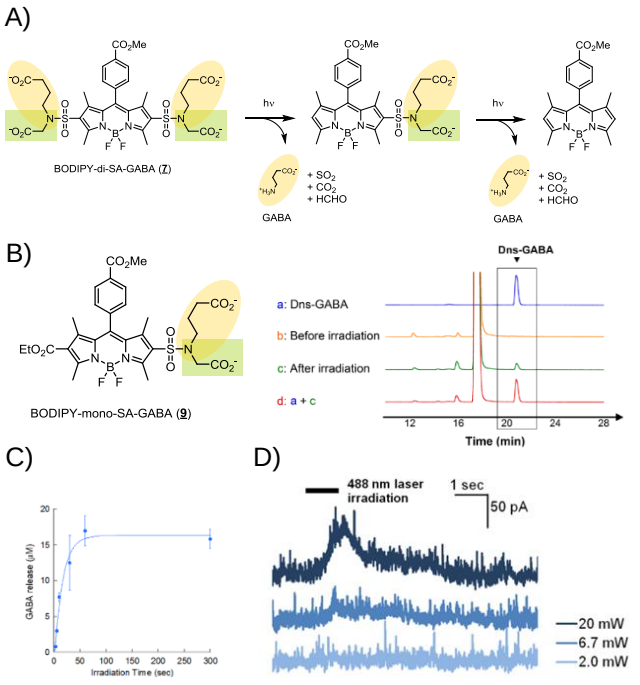


Figure 4. Characterization of the photoreactions of caged GABA. (A) Structure of BODIPY-di-SA-GABA (**7**) and its expected photoreaction to release GABA. The green square indicates the methylcarboxylic acid moiety, which is released as carbon dioxide and formaldehyde. (B) Detection of GABA generated by photoirradiation (Xe lamp, 470 ± 20 nm, >30 mW/cm^2, 5 min) of BODIPY mono-SA-GABA (**7**, 50 μM) in phosphate buffer (pH 7.4) containing 2.5% MeOH as a co-solvent. (C) Formation of GABA from BODIPY-mono-SA-GABA (**9**, 35 μM) in phosphate buffer (pH = 7.4) containing 2.5% MeOH after photoirradiation (Xe lamp, 470 ± 20 nm, 23.7 mW/cm^2). (D) Response of mouse basolateral amygdala neurons to uncaging of BODIPY-SA-GABA, monitored using the whole-cell patch-clamp technique. ACSF containing BODIPY-di-SA-GABA (**7**, 100 μM) was perfused over the mouse brain slice, and irradiation was carried out with a 488 nm laser as indicated. Membrane potentials were held at 0 mV.

The mono-substituted derivative was prepared by using asymmetric BODIPY as a starting material (**Scheme S3**) and was used to study the kinetics of GABA formation. The synthesized compounds showed a rapid photoreaction in aqueous media as well as in methanol. GABA formation via pathway (A) was observed (**Figure 4b**), with the yield reaching almost to 50% under the optimized conditions (**Figure 4c**). Therefore, developed caged GABA compound worked as designed, and we examined whether GABA generated via this photoreaction could be used to modulate neural cell behavior.

After confirming that photolysis products from the reaction did not show notable toxicity to cultured cells (**Figure S12**, **S13**, **S14**), we employed the caged GABA compound (**7**, **Figure S15**) to stimulate neuronal cells in mouse brain slices from the basolateral amygdala area. Using the patch-clamp method, we confirmed a light intensity-dependent cellular response (**Figure 4d**). The activation was specific to caged GABA; the photoreaction to generate *N*-methylamine did not induce any cellular response, and GABA receptor antagonists picrotoxin and baclofen blocked the response (**Figure S16**). Although we have yet to characterize GABA release on a millisecond time scale, which is sometimes required in GABA-uncaging experiments[16], we have confirmed that we can generate sufficient amounts of GABA to trigger neural behaviors. Photo-activated events were able to be triggered even in the visible region (488 nm laser), which we think is a valuable feature of the novel photoreaction.

Discussion

During our studies of a novel BODIPY scaffold bearing sulfonamide modifications, we unexpectedly discovered that one of the derivatives exhibited very rapid photobleaching (i.e., high photoinstability). As novel photoreactions suitable for the development of caged compounds are highly desirable to provide tools for biological research, we investigated the underlying photoreaction. Indeed, this compound proved to be the first example of a sulfonamide that undergoes rapid photocleavage on irradiation in the visible light region. Therefore, we prepared several derivatives and studied their photoreactions under various conditions. The results indicated that (1) formation of a charge separation state after excitation is the key to the reaction, (2) one of the two major photoreaction pathways is accompanied with loss of sulfonamide, and (3) the loss of sulfonamide occurs with release of the free amine. In accordance with the current understanding of the photoreactions of sulfonamides[17], we speculate that the reaction proceeds as illustrated in **Scheme 1**. Firstly, photoexcitation generates the charge separation state between carboxylate and the fluorophore, then decarboxylation occurs, and after that radical reaction takes place, resulting in (A) cleavage of sulfonamides with release of non-substituted BODIPY, or (B) deboronation. In both cases, back electron transfer terminates the reaction. It remains to be fully established whether carboxylate, which is not an ideal electron donor, has the capacity to transfer a single electron to excited BODIPY to trigger the formation of the charge separation state, but the results of Stern-Volmer quenching (**Figure S17**) and calculation of free energy for the photoreaction (**Figure S18**) both support the idea that electron transfer can occur from carboxylate to the excited state of 2,6-sulfonamide-modified BODIPY. In addition, we confirmed that the fluorescence of the 2,6-sulfonamide-modified BODIPYs that exhibit rapid photoreaction (**3**-**5**, **Table 1**) was quenched at neutral pH, where carboxylic acid remains in the carboxylate form (**Figure S19**). This result supports the existence of a photoinduced electron transfer process that competes with fluorescence emission in the excited state. These results are in sharp contrast with the case of another sulfonamide, dansyl-glycine; this compound did not show the efficient photoreaction, and also did not exhibit fluorescence quenching in the

carboxylate form (compared to its ester form) (**Figure S20**). The occurrence of the two competing reaction pathways is a limitation of the current system, and we are planning further studies in order to find ways of directing the overall reaction through the desired pathway.

Conclusions

In conclusion, investigation of the unexpected photo-instability of 2,6-sulfonamide-substituted derivatives of BODIPY led us to discover an efficient photoreaction, accompanied by multiple bond scissions, that was highly specific to the 2,6-sulfonamide structure. Product analysis revealed the involvement of at least two competitive photoreactions, and we considered that one of them would be applicable to the design of novel caged compounds. To validate this idea, we utilized this reaction to design a caged derivative that efficiently releases GABA upon irradiation in the visible range (> 450 nm), and we confirmed that it could be used to stimulate neuronal cells in mouse brain slices in a spatiotemporally controlled manner. We believe that the reaction can be the basis to design more caged compounds in the future.

Experimental Section

Preparative HPLC. HPLC pump: PU-2080 plus; gradient unit: MX-2080-32; degasser: DG-2080-53; multi-wavelength detector: MD-2010-Plus (JASCO); column: Inertsil ODS-3, 5 μm, 10 × 250 mm (GL Science Inc.). All experiments were done with a flow rate of 5 mL/min.

Analytical HPLC. HPLC pump: PU-980; gradient unit: LG-980-02; degasser: DG-980-50; multi-wavelength detector: MD-2015-Plus; fluorescence detector: FP-2025-Plus; autosampler: AS-2055 plus (JASCO); column: Inertsil ODS-3, 5 μm, 4.6 × 250 mm (GL Science Inc.). All experiments were done with a flow rate of 1 mL/min.

LC-MS Analysis. Quaternary pump: G1311A; degasser: G1322A; multi-wavelength detector: G1315D; autosampler: G1367E; Quadrupole LC/MS: 6130 (Agilent); column: Inertsil ODS-3, 5 μm, 2.1 × 250 mm (GL Science Inc.). All experiments were done with a flow rate of 0.5 mL/min.

Photoirradiation. Lamp: Xe Lamp; control unit: MAX-301(Asahi Bunko); 410 ± 5 nm filter: MX0410 (Asahi Bunko); 470 ± 20 nm filter: HQ 470/40 (Chroma Technology Corp.); 490 ± 5 nm filter: MX0490 (Asahi Bunko). Irradiation power was measured with a Nova Display (OPHIR) equipped with photodiode sensor (PD300-UV).

Detection of glycine. A solution of *ortho*-phthalaldehyde (0.2 mmol) and 20 μL 2-mercaptoethanol in sodium borate buffer (0.4 M, pH 9.5) was prepared as a derivatization solution. 20 μL sample and 100 μL derivatization solution were mixed, and an aliquot was subjected to analytical HPLC after 2 min (A: 0.1 M TEAA-H_2O, B: 0.1 M TEAA-80% acetonitrile-20% H_2O, A : B = 50 : 50 → 20 min, 0 : 100). Fluorescence detection: $\lambda_{ex.}$ = 335 nm, $\lambda_{em.}$ = 455 nm.

Detection of *N*-methylamine. 40 μL of *ortho*-phthalaldehyde (7.5 mM) solution in sodium borate buffer (0.4 M, pH 9.5) and 40 μL *N*-acetyl-*L*-cysteine (2.5 mM) solution in sodium borate buffer (0.4 M, pH 9.5) were mixed. After 20 min, 20 μL sample was added, and an aliquot was subjected to analytical HPLC after 2 min (A: 0.1 M TEAA-H_2O, B: 0.1 M TEAA-80% acetonitrile-20% H_2O, A : B = 50 : 50 → 20 min, 0 : 100). Fluorescence detection: $\lambda_{ex.}$ = 335 nm, $\lambda_{em.}$ = 455 nm.

Detection of GABA. 12 μL of sample was mixed with 15 μL dansyl chloride (1.5 mM) in acetonitrile and 18 μL sodium borate buffer (0.4 M, pH 9.5), and after 30 min, 10 μL 2% methylamine in H_2O was added to quench the reaction. An aliquot was subjected to analytical HPLC (A: 0.1 M TEAA-H_2O, B: 0.1 M TEAA-80% acetonitrile-20% H_2O, A : B = 50 : 50 → 20 min, 0 : 100).

Detection of aldehydes. 2,4-Dinitrophenylhydrazine (25 mM) in conc. HCl was diluted10-fold with H_2O, and washed 3 times with chloroform (DNPH derivatization solution). 25 μL sample was mixed with 10 μL derivatization solution, and after 50 min, an aliquot was subjected to analytical HPLC (A: 0.1 M TEAA-H_2O, B: 0.1 M TEAA-80% acetonitrile-20% H_2O, A : B = 50 : 50 → 20 min, 0 : 100). Detection: absorbance at 360 nm.

Absorbance and fluorescence. Absorption spectra were measured with a UV-2550 (Shimadzu). Absorbance change on a fast timescale (seconds) during photoirradiation was recorded with an 845X UV-Visible System (Agilent). Fluorescence spectra were measured with a F-7000 (Hitachi). Quantum yields were calculated using the following equation. Standard: fluorescein in 0.1 M NaOH (Φ_{FL} = 0.85).

$$\emptyset_{fl}^{\text{sample}} = \emptyset_{fl}^{\text{std}} \frac{Abs^{\text{std}}}{Abs^{\text{sample}}} \frac{\int F^{\text{sample}}}{\int F^{\text{std}}} \frac{n^{\text{sample}^2}}{n^{\text{std}^2}} \frac{I^{\text{std}}}{I^{\text{sample}}}$$

Quantification of quantum yields of photobleaching. The calculation was done based on the rate of absorbance decrease after photoirradiation (Xenon lamp, 490 ± 5 nm, 5.45 mW/cm^2). $\Phi_{\text{Photobleaching}}$ was calculated using the following equation[8e].

$$\phi_{\text{Photobleaching}} = \frac{1}{(\ln 10) I \sigma \tau}$$

I: light intensity [einstein/sec/cm^2], σ: average of ε [cm^2/mol], τ: half-life [sec]

Cell culture. HeLa cells were cultured in DMEM (Sigma) containing 10% FBS (JRH Bioscience) and 1% penicillin-streptomycin (GIBCO) at 37˚C in an atmosphere of 5% CO_2 in air.

Confocal microscopy. TCS SP-5 (Leica). $\lambda_{ex.}$ = 488 nm, $\lambda_{em.}$ = 520-600 nm. Cells were plated on poly-*D*-lysine-coated glass-bottomed dishes (MatTek). The medium was changed to DMEM (phenol red free) without FBS before experiments.

Live/dead assay. Photoirradiation was performed 35 times ($\lambda_{ex.}$ = 488 nm) in the presence of the compound. After 1 hr, calcein AM (1 μM) and ethidium homodimer (1 μM) were added. Fluorescence was monitored after 30 min. Dead cell positive control: EtOH-treated cells.

CCK assay. HeLa cells were plated in 96-well plates (Nunc) at ~ 50% confluency. The medium was changed to BODIPY-SA-GABA in DMEM (phenol red free), photoirradiation (470 ± 20 nm, 19 mW/cm^2, 1 min) was done, and incubation was continued at 37˚C for 1 hr. Cells were washed with DMEM, cultured for 24 hr, and stained with Cell Counting Kit-8 (Dojindo; standard protocol). Absorbance at

450 nm and 650 nm was measured with a plate reader (SH-9000, Corona Electric).

Patch-clamp experiments. Experiments were approved by the animal experiment ethics committee of the University of Tokyo, and were in accordance with the University's guidelines for care and use of laboratory animals (approval number: P24-8). Mice (4 weeks old) were deeply anesthetized with diethyl ether and decapitated. Brains were removed quickly, and coronal slices (300 μm thick) containing the basolateral amygdala were prepared with a vibratome (VT 1200S, Leica) in ice-cold, oxygenated (95% O_2/5% CO_2) modified artificial cerebrospinal fluid (mACSF) containing 222.1 mM sucrose, 27 mM $NaHCO_3$, 1.4 mM NaH_2PO_4, 2.5 mM KCl, 0.5 mM ascorbic acid, 1 mM $CaCl_2$, and 7 mM $MgSO_4$. Whole-cell patch-clamp recordings were performed with glass microelectrodes (3-8 MΩ) filled with internal solution (120 mM $CsMeSO_4$, 5 mM CsCl, 10 mM HEPES, 1 mM $MgCl_2$, 10 mM phosphocreatine disodium salt, 2 mM ATP-Mg, 0.1 mM GTP-2Na, 0.2 mM EGTA, pH 7.2-7.3, 280-295 mOsm). Recordings were performed in oxygenated ACSF (NaCl 127 mM, KCl 1.6 mM, KH_2PO_4 1.24 mM, $MgSO_4$ 1.3 mM, $CaCl_2$ 2.4 mM, $NaHCO_3$ 26 mM, glucose 10 mM) containing TTX (1 μM), NBQX (10 μM), AP5 (20 μM). Currents were recorded at a holding potential of 0 mV from basolateral amygdala neurons. Photoirradiation (488 nm laser) was done at 5 min after loading BODIPY-SA-GABA (100 μM) in ACSF.

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Keywords: boron dipyrromethene (BODIPY) · chemical biology · caged compounds · photoreaction · sulfonamide

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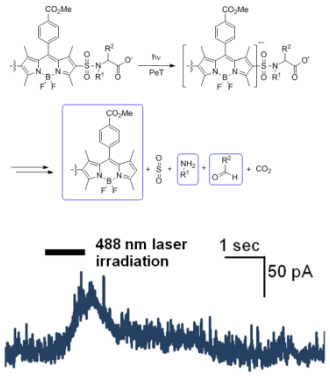
Entry for the Table of Contents

FULL PAPER

Investigation of the unexpected photo-instability of 2,6-sulfonamide-substituted derivatives of the boron dipyrromethene (BODIPY) fluorophore led to discovery of a photoreaction accompanied with multiple bond scissions. We characterized the photoproducts, and utilized the photoreaction to design a caged gamma-aminobutyric acid (GABA) derivative that can release GABA upon irradiation in the visible range (> 450 nm).



Aoi Takeda, Toru Komatsu, Hiroshi Nomura, Masamitsu Naka, Norio Matsuki, Yuji Ikegaya, Takuya Terai, Tasuku Ueno, Kenjiro Hanaoka, Tetsuo Nagano, and Yasuteru Urano**

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Unexpected photo-instability of 2,6-sulfonamide-substituted BODIPYs and its application to development of caged GABA