JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

pubs.acs.org/JACS

Communication

Water-Soluble BODIPY Photocages with Tunable Cellular Localization

Dnyaneshwar Kand, Pei Liu, Marisol X. Navarro, Logan J. Fischer, Liat Rousso-Noori, Dinorah Friedmann-Morvinski, Arthur H. Winter, Evan W. Miller,* and Roy Weinstain*

Cite This: J. Am. Chem. Soc. 2020, 142, 4970–4974

Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

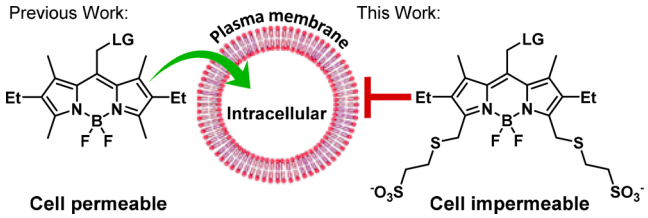
ABSTRACT: Photoactivation of bioactive molecules allows manipulation of cellular processes with high spatiotemporal precision. The recent emergence of visible-light excitable photoprotecting groups has the potential to further expand the established utility of the photoactivation strategy in biological applications by offering higher tissue penetration, diminished phototoxicity, and compatibility with other light-dependent techniques. Nevertheless, a critical barrier to such applications remains the significant hydrophobicity of most visible-light excitable photocaging groups. Here, we find that applying the conventional 2,6-sulfonation to *meso*-methyl BODIPY photocages is incompatible with their photoreaction due to an increase in the excited state barrier for photorelease. We present a simple, remote sulfonation solution to BODIPY photocages that imparts water solubility and provides control over cellular permeability while retaining their favorable spectroscopic and photoreaction properties. Peripherally disulfonated BODIPY photocages are cell impermeable, making them useful for modulation of cell-surface receptors, while monosulfonated BODIPY retains the ability to cross the cellular membrane and can modulate intracellular targets. This new approach is generalizable for controlling BODIPY localization and was validated by sensitization of mammalian cells and neurons by visible-light photoactivation of signaling molecules.

Photoactivation of small bioactive molecules is a powerful approach to manipulate and study cellular events with high spatiotemporal resolution.[1,2] Photoprotecting groups (PPGs) covalently attached to bioactive molecules mask their biological activity and allow subsequent removal upon exposure to light. Established PPGs, including those based on nitrobenzyl,[3] ruthenium[4] (RuBi), coumarin[5] and others,[6] have been used instrumentally in a wide variety of biological[7,8] and materials[9,10] applications. The recent emergence of visible-light excitable photocages, spanning a range of structural classes,[11−15] has the potential to further expand the already significant utility of photocaging in these fields. For example, extension of the excitation wavelength beyond the traditional UV-region[6] expands the operational window in which uncaging light can be delivered, enabling photoactivation of multiple cues through orthogonally caged molecules.[16,17] Furthermore, longer wavelength light can penetrate deeper into tissue and is less harmful to biological matter, opening the door to new applications, such as in drug delivery.[18−20]

We recently introduced *meso*-methyl BODIPYs as photoprotecting groups in the visible range.[21,22] The narrow excitation band, adaptable synthetic chemistry, and overall biocompatibility of BODIPYs[23] make them promising candidates for visible-light photocaging. *Meso*-methyl BODIPY PPGs effectively release cargo in living cells, in part because of their large extinction coefficients.[21,22] Further chemical modification to methyl-BODIPY cages can improve photorelease quantum efficiency,[24] extend excitation wavelength into the far-red,[25] or allow postsynthetic functionalization for targeting to subcellular locations,[26] making them a versatile platform for photorelease.

Nevertheless, all BODIPY PPGs reported to date are inherently highly hydrophobic, which severely limits their potential concentration and thus utility in water-based solutions. Moreover, the hydrophobic nature of BODIPYs makes them highly cell permeable (Scheme 1). While

Scheme 1. Water-Soluble BODIPY Photocages



advantageous when specifically pursuing intracellular interventions, this property makes BODIPY PPGs less effective when targeting extracellular proteins or plasma membrane-residing receptors and complicates analysis when the released molecule can act both intra- and extracellularly.

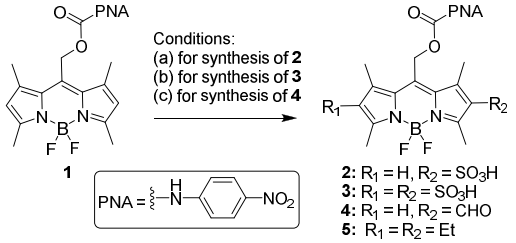
We therefore sought to develop water-soluble BODIPY PPGs with controlled cellular localization, while retaining their

Received: December 8, 2019
Published: February 29, 2020

https://dx.doi.org/10.1021/jacs.9b13219
J. Am. Chem. Soc. 2020, 142, 4970–4974

favorable spectroscopic and photoreaction properties. We initially opted for 2,6-sulfonation[27] as a minimal structural modification[28] that also provides improved photobleaching resistance.[29] 2- and 2,6-sulfo BODIPYs **2** and **3**, bearing *p*-nitroaniline (PNA) as a model leaving group, were synthesized by sulfonation of **1** using a sulfur trioxide−pyridine complex (Scheme 2).[30] Surprisingly, both **2** and **3** did not release PNA

Scheme 2. Synthesis/Structures of BODIPYs 1−5[a]



[a](a) SO_3−Pyridine, DMF, 60 °C, 24 h; (b) SO_3−Pyridine, DMF, 60 °C, 48 h; (c) DMF, $POCl_3$, DCM, 0 °C to rt, 3 h.

when irradiated with green light (545/30 nm, 49 mW/cm^2). In contrast, **1** is an effective photocage, with $\varepsilon \times \Phi_{rel} = 3$ and $t_{1/2} = 3.8$ min (Figure 1a and Table S1). We hypothesized that the

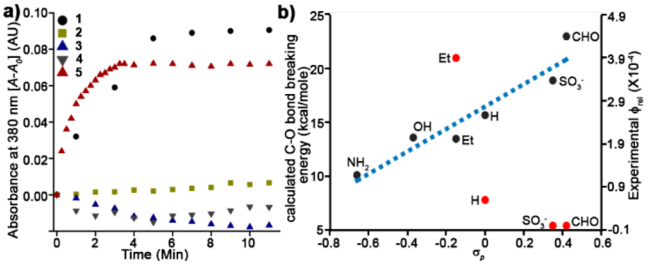


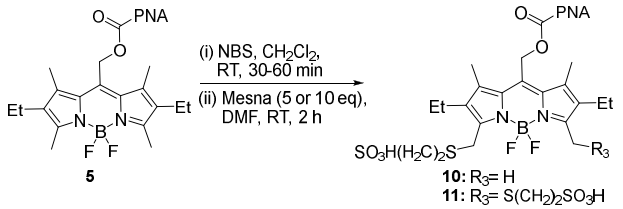
Figure 1. Light-induced release from *meso*-methyl BODIPY PPGs. (a) PNA release from **1**−**5** (10 μM in CH_3CN/water 7/3) irradiated with 545/30 nm (49 mW/cm^2) light for the indicated times. (b) Observed Φ_{rel} and a DFT calculated C−O bond breaking energy for derivatives **1**−**5** as plotted versus σ_p Hammett constants.

electron-withdrawing effect of sulfonate is responsible for the diminished photoreaction. Indeed, a comparable BODIPY with 2-aldehyde (**4**) also failed to release the leaving group, while 2,6-diethyl BODIPY **5** photolyzed with $\varepsilon \times \Phi_{rel} = 19$ and $t_{1/2} = 31$ s, better than **1** (Table S1).

The observed trend in Φ_{rel} (**5** > **1** > **4** ≈ **2** ≈ **3**) suggests a strong effect of the 2,6-positions on the photoreaction with a positive influence of electron donation. We therefore modeled the excited state geometries of **1**−**5** to explore the consequences of 2,6-substitution on photouncaging efficiency (B3LYP/6-31+G(d,p), SMD = H_2O). No fundamental change in the nature of the excited state exists between **1**−**5**. Neither chromophore planarity nor position of the LUMO (*meso* carbon) differs across compounds **1**−**5** (Computation S1−S7). However, a relaxed potential energy scan of the C−O bond breaking coordinates (Computation S8−S13) reveals that electron-withdrawing groups (EWGs) at the 2,6-positions substantially raise the barrier for C−O bond heterolysis on the triplet surface compared to electron-donating groups (Figure 1b), consistent with a previous report of these positions' effect on BODIPY photostability.[31] These computations further support our previous hypothesis regarding photorelease in *meso*-methyl BODIPYs, i.e. formation of a *meso* carbocation diradical intermediate during the photoreaction.[24] Thus, electron-donating groups (EDGs) at the 2,6-positions stabilize the resulting carbocation, lowering the barrier for its formation, and conversely, EWGs increase the barrier to photoreaction.

To circumvent deleterious electronic effects on the BODIPY core when introducing sulfonates, we harnessed our recently reported functionalization method; a one-pot, two-step protocol, to install an unprotected functional group on an *in situ* activated α-methyl.[26] We synthesized (Figure S1a) tertiary amine (**6**), ether (**7**), and thioether (**8**−**9**) containing BODIPY PPGs. Although amine substitution gave the highest synthetic yields, tertiary amines could no longer undergo photorelease (**6**, Figure S1d), likely because of a competing electron transfer (PeT) mechanism.[32−34] Instead, we find thiol nucleophiles best combine high chemical yield with efficient photorelease in BODIPY thioether (Figure S1). Thus, 2-mercaptoethane-sulfonic acid sodium salt (MESNA) was used to introduce sulfonic acid groups, affording BODIPYs **10** and **11** in 42% and 33% yield, respectively (Scheme 3).

Scheme 3. Synthesis of Sulfonated BODIPYs 10 and 11



As expected, both **10** and **11** show improved water solubility compared to **5**. In mixtures of CH_3CN/water, **5** features absorbance λ_{max} at 545 nm, a shoulder at 511 nm, and an ∼2.1 peak/shoulder ratio. But, in water, the peak red shifts and broadens (556 nm), and the ratio collapses to 1.1, a nearly 1.9-fold reduction and characteristic of aggregation[35] (Figure 2a).

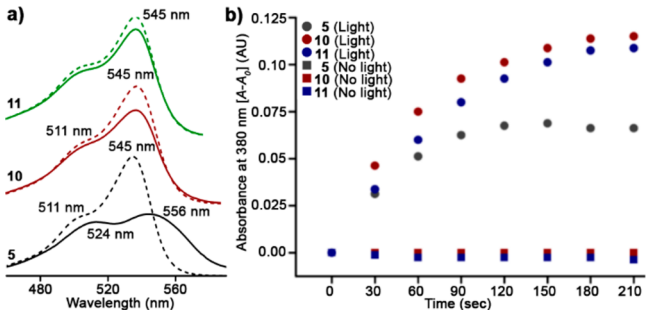


Figure 2. MESNA-BODIPY PPGs. (a) Absorbance spectra of BODIPYs **5**, **10**, and **11** (20 μM) directly dissolved in CH_3CN/water 7/3 (dashed line) or water (solid line). (b) Light-induced release of PNA from **5**, **10**, and **11** (10 μM, CH_3CN/water 7/3) following, or not, irradiation with 545/30 nm light (49 mW/cm^2) for the indicated times.

In contrast, the absorption spectra of **10** and **11** are nearly identical in either pure water or a mixture of CH_3CN/water, establishing their high water solubility (Figures 2a, S2). Critically, both **10** and **11** retain photoreleasing ability, in stark contrast to core-sulfonated **2** or **3**. Both **10** and **11** possess comparable quantum yield to **5** ($\Phi_{rel} = (3.6−5.1) \times 10^{-4}$) (Figures 2b, S3−S5 and Tables S1, S2), but afford

https://dx.doi.org/10.1021/jacs.9b13219
J. Am. Chem. Soc. 2020, 142, 4970−4974

higher photochemical yields (59−60% vs 46%), probably due to their increased solubility. These results establish peripheral MESNA as a small, readily implemented modification to *meso*-methyl BODIPYs that improve solubility while maintaining high photorelease efficiency.

The degree of sulfonation influences the cellular uptake of BODIPY PPGs. HEK 293T cells display intracellular fluorescence, along with bright fluorescent puncta (presumably aggregates), when treated with non-MESNA BODIPY **5** (Figure 3a). In contrast, mono-MESNA **10** shows a higher

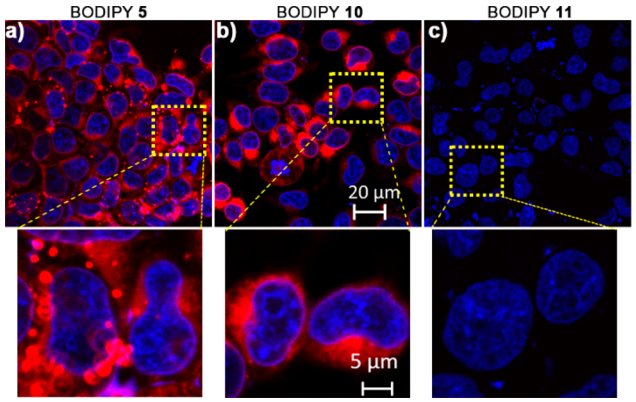
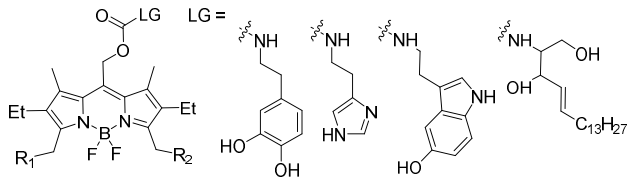


Figure 3. Confocal fluorescence microscopy of HEK 293T cells treated with BODIPY **5**, **10**, or **11** (2 μM) for 30 min and costained with Hoechst dye. Cells were washed thrice and imaged.

degree of cellular fluorescence (2-fold higher than **5**, Figure S6), but without any observable puncta (Figure 3b). The higher intracellular fluorescence may be a result of better water solubility of **10** compared to **5**, leading to higher effective concentration in the buffer. Di-MESNA **11**, with two sulfonates, was completely cell impermeable and showed no intracellular or membrane-associated fluorescence (Figure 3c). These results are in line with previous observations of sulfonated coumarin photocages.[36] No toxicity or phototoxicity was observed for compound **5**, **10**, or **11** (Figure S7).

To evaluate the dependence of sulfonated BODIPY PPG cellular permeability on the nature of the leaving group, we compared sets of non-, mono-, and di-MESNA BODIPYs bearing three caged biogenic amines: serotonin, dopamine, and histamine (compounds **12**−**20**, Scheme 4), forming a series of

Scheme 4. Structures of BODIPYs 12−20



$R_1 = R_2 = H$	**12**	**13**	**14**	**21**
$R_1 = H$, $R_2 = S(CH_2)_2SO_3H$	**15**	**16**	**17**	-
$R_1 = R_2 = S(CH_2)_2SO_3H$	**18**	**19**	**20**	**22**

leaving groups with decreased hydrophobicity. The cellular permeability of non- and mono-MESNA-BODIPYs was highly dependent on the polarity of the leaving group while di-MESNA-BODIPY was completely cell impermeable, irrespective of the leaving group polarity (Figure S6).

Collectively, these data establish that the solubility of BODIPY photocages can be significantly improved by sulfonation without compromising photoreaction properties and that their cellular permeability can be predetermined by tuning the number of sulfonates.

To highlight the ability to tune the cellular accessibility of caged biomolecules with BODIPY compounds, we synthesized two sphingosine-caged BODIPY derivatives, **21** and **22** (Scheme 4). **21** is based on the traditional, nonsulfonated BODIPY photocage, while **22** utilizes the di-MESNA-BODIPY scaffold. Our hypothesis was that **21** could pass through plasma membrane to effect localized uncaging of sphingosine intracellularly, triggering Ca^{2+} release, while **22** would be retained externally and would be incompetent to trigger internal Ca^{2+} release.[37] Consistent with this hypothesis, treatment of HeLa cells with **21**, followed by green uncaging light, results in large Ca^{2+} transients detected by the fluorescent Ca^{2+} indicator, fura-2 (Figure S8a−c). Induction of Ca^{2+} transients requires uncaging light: we observed no Ca^{2+} oscillations in the absence of light (Figure S8d). In contrast, the uncaging of extracellularly targeted **22** results in no Ca^{2+}-associated transient (Figure S8e). Together, these data show that di-MESNA-BODIPY cages can retain even lipophilic bioactive molecules in the extracellular space.

The enhanced solubility of MESNA-BODIPYs makes them promising candidates to modulate cell surface receptors. We utilized them to control the availability of the neuromodulator dopamine. We examined the localization of BODIPY-caged dopamine compounds in cultured neurons. While BODIPY-dopamine **12** shows significant cytosolic accumulation (Figure S9a,d), both mono- (**15**) and di-MESNA-BODIPY-dopamine **18** display little to no cellular uptake (Figure S9b,e and S9c,f), consistent with the localization of di-MESNA-BODIPY cages in HEK cells (Figures 3, S6).

Di-MESNA BODIPY-dopamine **18** delivers dopamine in a light-dependent fashion to neurons. Ca^{2+} imaging in hippocampal neurons treated with dopamine (5 μM) results in fluorescence oscillations (Figure 4a,b).[22,38] Hippocampal neurons treated with di-MESNA-BODIPY-dopamine **18** (5 μM) and irradiated with green light also show Ca^{2+} oscillations (Figure 4c), with 34% of neurons responding compared to 46% with dopamine alone (Figure S10). In the absence of green light, **18** has little effect on the activity of hippocampal neurons (Figure 4d), and green light alone, when **18** is not present, does not evoke a similar Ca^{2+} response (Figure 4e). Finally, preincubation with the dopamine receptor antagonist butaclamol (100 μM) prior to green light uncaging in the presence of **18** results in a substantial reduction in the number of Ca^{2+} transients (Figures 4f, S10).

Complementary experiments using the cell-impermeable mono-MESNA-BODIPY caged histamine **16** reveal that this caged compound can also effectively modulate neuronal physiology and Ca^{2+} signaling in a light-dependent fashion (Figure S11). We further show that the spatial resolution of uncaging can be controlled (Figure S12).

In summary, we introduce biocompatible BODIPY PPGs with substantially improved water-solubility, user-designated control over cellular localization and high photorelease efficiency. Initial efforts to directly sulfonate the BODIPY core improved solubility but abolished photorelease. A combination of computation and *in vitro* characterization suggests that EWGs at the 2,6-positions destabilize the carbocation formed during the photoreaction. We circum-

https://dx.doi.org/10.1021/jacs.9b13219
J. Am. Chem. Soc. 2020, 142, 4970−4974

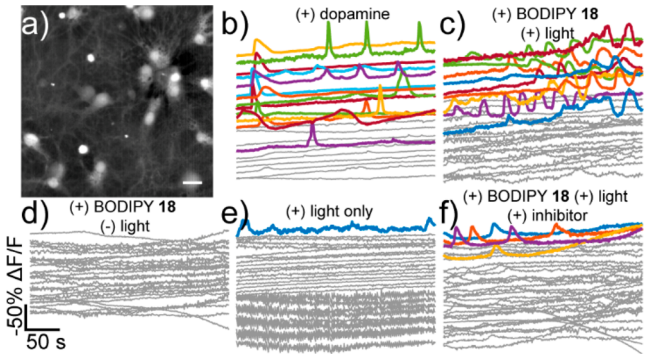


Figure 4. Dopamine uncaging in neurons. (a) Widefield fluorescence image from neurons stained with fura-2. Scale bar is 20 μm. (b–f) Uncaging of dopamine from **18** causes Ca^{2+} oscillations in cultured rat hippocampal neurons. Ca^{2+} imaging in neurons treated with (b) dopamine (5 μM), (c) **18** (5 μM) and uncaging light, (d) **18** (5 μM) without light, or (e) only with uncaging light and (f) **18** (5 μM) and light in the presence of the dopamine receptor antagonist butaclamol (100 μM). Plots represent $\Delta F/F_{max}$ for representative cells vs time and are inverted. A decrease in fluorescence represents a rise in cellular Ca^{2+}. Uncaging light was provided for 10 s at 90 mW/mm^2. Gray traces are neurons which did not show < −20% $\Delta F/F$. Colored traces did show < −20% $\Delta F/F$ response.

vented this barrier by introducing remote sulfonation, resulting in an increase in water solubility and the ability to regulate cellular localization through the degree of sulfonation. The cellular impermeability of peripherally disulfonated BODIPYs makes them promising candidates for use in modulation of extracellular proteins and cell-surface receptors, while monosulfonated BODIPYs retain the ability to cross the cellular membrane and can modulate intracellular targets. Moreover, the peripheral sulfonation strategy presented herein should be applicable to BODIPY fluorophores at large, providing a convenient route to confer water solubility and control cellular permeability.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/jacs.9b13219.

^{1}H and ^{13}C NMR spectra (PDF)

Supplementary data, including supporting figures, procedures, and analysis (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

Roy Weinstain − *School of Plant Sciences and Food Security, Tel-Aviv University, Tel-Aviv 6997801, Israel;* orcid.org/0000-0002-1300-6802; Email: royweinstain@tauex.tau.ac.il

Evan W. Miller − *Departments of Chemistry, Molecular & Cell Biology, and Helen Wills Neuroscience Institute, University of California, Berkeley, California 94720, United States;* orcid.org/0000-0002-6556-7679; Email: evanwmiller@berkeley.edu

Authors

Dnyaneshwar Kand − *School of Plant Sciences and Food Security, Tel-Aviv University, Tel-Aviv 6997801, Israel*

Pei Liu − *Departments of Chemistry, University of California, Berkeley, California 94720, United States*

Marisol X. Navarro − *Departments of Chemistry, University of California, Berkeley, California 94720, United States*

Logan J. Fischer − *Department of Chemistry, Iowa State University, Ames, Iowa 50010, United States;* orcid.org/0000-0003-1166-2379

Liat Rousso-Noori − *School of Neurobiology, Biochemistry and Biophysics, Faculty of Life Sciences, Tel-Aviv University, Tel-Aviv 6997801, Israel*

Dinorah Friedmann-Morvinski − *School of Neurobiology, Biochemistry and Biophysics, Faculty of Life Sciences, Tel-Aviv University, Tel-Aviv 6997801, Israel*

Arthur H. Winter − *Department of Chemistry, Iowa State University, Ames, Iowa 50010, United States;* orcid.org/0000-0003-2421-5578

Complete contact information is available at: https://pubs.acs.org/10.1021/jacs.9b13219

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

R.W. and E.W.M. acknowledge generous support from the Binational Science Foundation (2016060) and from the Rosalinde and Arthur Gilbert Foundation. R.W. acknowledges support from the European Research Council (GAtransport). A.H.W. acknowledges support from the National Science Foundation (CHE-1464956). D.F.M. acknowledges support from the Israel Science Foundation (1310/15). P.L. was supported by an A*STAR graduate fellowship, and M.X.N. was supported in part by the NIH (T32GM066698). D.K. was supported in part by the Planning and Budgeting Committee (PBC) of the Israeli Council for Higher Education.

■ REFERENCES

(1) Mayer, G.; Heckel, A. Biologically active molecules with a "light switch". *Angew. Chem., Int. Ed.* **2006**, *45* (30), 4900–21.

(2) Lee, H. M.; Larson, D. R.; Lawrence, D. S. Illuminating the chemistry of life: design, synthesis, and applications of "caged" and related photoresponsive compounds. *ACS Chem. Biol.* **2009**, *4* (6), 409–27.

(3) Patchornik, A.; Amit, B.; Woodward, R. B. Photosensitive protecting groups. *J. Am. Chem. Soc.* **1970**, *92* (21), 6333–6335.

(4) Zayat, L.; Calero, C.; Alborés, P.; Baraldo, L.; Etchenique, R. A New Strategy for Neurochemical Photodelivery: Metal–Ligand Heterolytic Cleavage. *J. Am. Chem. Soc.* **2003**, *125* (4), 882–883.

(5) Givens, R. S.; Matuszewski, B. Photochemistry of phosphate esters: an efficient method for the generation of electrophiles. *J. Am. Chem. Soc.* **1984**, *106* (22), 6860–6861.

(6) Klan, P.; Solomek, T.; Bochet, C. G.; Blanc, A.; Givens, R.; Rubina, M.; Popik, V.; Kostikov, A.; Wirz, J. Photoremovable protecting groups in chemistry and biology: reaction mechanisms and efficacy. *Chem. Rev.* **2013**, *113* (1), 119–91.

(7) Ankenbruck, N.; Courtney, T.; Naro, Y.; Deiters, A. Optochemical Control of Biological Processes in Cells and Animals. *Angew. Chem., Int. Ed.* **2018**, *57* (11), 2768–2798.

(8) Shao, Q.; Xing, B. Photoactive molecules for applications in molecular imaging and cell biology. *Chem. Soc. Rev.* **2010**, *39* (8), 2835–2846.

(9) Ruskowitz, E. R.; DeForest, C. A. Photoresponsive biomaterials for targeted drug delivery and 4D cell culture. *Nature Reviews Materials* **2018**, *3* (2), 17087.

(10) Zhao, H.; Sterner, E. S.; Coughlin, E. B.; Theato, P. o-Nitrobenzyl Alcohol Derivatives: Opportunities in Polymer and Materials Science. *Macromolecules* **2012**, *45* (4), 1723–1736.

(11) Chaudhuri, A.; Venkatesh, Y.; Behara, K. K.; Singh, N. D. Bimane: A Visible Light Induced Fluorescent Photoremovable Protecting Group for the Single and Dual Release of Carboxylic and Amino Acids. *Org. Lett.* **2017**, *19* (7), 1598−1601.
(12) Chen, Y.; Steinmetz, M. G. Photochemical cyclization with release of carboxylic acids and phenol from pyrrolidino-substituted 1,4-benzoquinones using visible light. *Org. Lett.* **2005**, *7* (17), 3729−32.
(13) Gorka, A. P.; Nani, R. R.; Zhu, J.; Mackem, S.; Schnermann, M. J. A near-IR uncaging strategy based on cyanine photochemistry. *J. Am. Chem. Soc.* **2014**, *136* (40), 14153−9.
(14) Sebej, P.; Wintner, J.; Muller, P.; Slanina, T.; Al Anshori, J.; Antony, L. A.; Klan, P.; Wirz, J. Fluorescein analogues as photoremovable protecting groups absorbing at approximately 520 nm. *J. Org. Chem.* **2013**, *78* (5), 1833−43.
(15) Umeda, N.; Takahashi, H.; Kamiya, M.; Ueno, T.; Komatsu, T.; Terai, T.; Hanaoka, K.; Nagano, T.; Urano, Y. Boron dipyrromethene as a fluorescent caging group for single-photon uncaging with long-wavelength visible light. *ACS Chem. Biol.* **2014**, *9* (10), 2242−6.
(16) Hansen, M. J.; Velema, W. A.; Lerch, M. M.; Szymanski, W.; Feringa, B. L. Wavelength-selective cleavage of photoprotecting groups: strategies and applications in dynamic systems. *Chem. Soc. Rev.* **2015**, *44* (11), 3358−77.
(17) Olson, J. P.; Banghart, M. R.; Sabatini, B. L.; Ellis-Davies, G. C. Spectral evolution of a photochemical protecting group for orthogonal two-color uncaging with visible light. *J. Am. Chem. Soc.* **2013**, *135* (42), 15948−54.
(18) Silva, J. M.; Silva, E.; Reis, R. L. Light-triggered release of photocaged therapeutics - Where are we now? *J. Controlled Release* **2019**, *298*, 154−176.
(19) Vorobev, A. Y.; Moskalensky, A. E. Long-wavelength photoremovable protecting groups: On the way to in vivo application. *Comput. Struct. Biotechnol. J.* **2020**, *18*, 27−34.
(20) Nani, R. R.; Gorka, A. P.; Nagaya, T.; Yamamoto, T.; Ivanic, J.; Kobayashi, H.; Schnermann, M. J. In Vivo Activation of Duocarmycin−Antibody Conjugates by Near-Infrared Light. *ACS Cent. Sci.* **2017**, *3* (4), 329−337.
(21) Goswami, P. P.; Syed, A.; Beck, C. L.; Albright, T. R.; Mahoney, K. M.; Unash, R.; Smith, E. A.; Winter, A. H. BODIPY-derived photoremovable protecting groups unmasked with green light. *J. Am. Chem. Soc.* **2015**, *137* (11), 3783−6.
(22) Rubinstein, N.; Liu, P.; Miller, E. W.; Weinstain, R. meso-Methylhydroxy BODIPY: a scaffold for photo-labile protecting groups. *Chem. Commun. (Cambridge, U. K.)* **2015**, *51* (29), 6369−72.
(23) Loudet, A.; Burgess, K. BODIPY dyes and their derivatives: syntheses and spectroscopic properties. *Chem. Rev.* **2007**, *107* (11), 4891−932.
(24) Slanina, T.; Shrestha, P.; Palao, E.; Kand, D.; Peterson, J. A.; Dutton, A. S.; Rubinstein, N.; Weinstain, R.; Winter, A. H.; Klan, P. Search of the Perfect Photocage: Structure-Reactivity Relationships in meso-Methyl BODIPY Photoremovable Protecting Groups. *J. Am. Chem. Soc.* **2017**, *139* (42), 15168−15175.
(25) Peterson, J. A.; Wijesooriya, C.; Gehrmann, E. J.; Mahoney, K. M.; Goswami, P. P.; Albright, T. R.; Syed, A.; Dutton, A. S.; Smith, E. A.; Winter, A. H. Family of BODIPY Photocages Cleaved by Single Photons of Visible/Near-Infrared Light. *J. Am. Chem. Soc.* **2018**, *140* (23), 7343−7346.
(26) Kand, D.; Pizarro, L.; Angel, I.; Avni, A.; Friedmann-Morvinski, D.; Weinstain, R. Organelle-Targeted BODIPY Photocages: Visible-Light-Mediated Subcellular Photorelease. *Angew. Chem., Int. Ed.* **2019**, *58* (14), 4659−4663.
(27) Wories, H. J.; Koek, J. H.; Lodder, G.; Lugtenburg, J.; Fokkens, R.; Driessen, O.; Mohn, G. R. A Novel Water-Soluble Fluorescent-Probe - Synthesis, Luminescence and Biological Properties of the Sodium-Salt of the 4-Sulfonato-3,3′,5,5′-Tetramethyl-2,2′-Pyrromethen-1,1′-Bf2 Complex. *Recl Trav Chim Pay B* **1985**, *104* (11), 288−291.
(28) Fan, G.; Yang, L.; Chen, Z. J. Water-soluble BODIPY and aza-BODIPY dyes: synthetic progress and applications. *Front. Chem. Sci. Eng.* **2014**, *8* (4), 405−417.
(29) Komatsu, T.; Oushiki, D.; Takeda, A.; Miyamura, M.; Ueno, T.; Terai, T.; Hanaoka, K.; Urano, Y.; Mineno, T.; Nagano, T. Rational design of boron dipyrromethene (BODIPY)-based photobleaching-resistant fluorophores applicable to a protein dynamics study. *Chem. Commun.* **2011**, *47* (36), 10055−10057.
(30) Kim, J.; Kim, Y. A water-soluble sulfonate-BODIPY based fluorescent probe for selective detection of HOCl/OCl− in aqueous media. *Analyst* **2014**, *139* (12), 2986−2989.
(31) Kawatani, M.; Kamiya, M.; Takahashi, H.; Urano, Y. Factors affecting the uncaging efficiency of 500nm light-activatable BODIPY caging group. *Bioorg. Med. Chem. Lett.* **2018**, *28* (1), 1−5.
(32) Zhang, J.; Yang, M.; Li, C.; Dorh, N.; Xie, F.; Luo, F.-T.; Tiwari, A.; Liu, H. Near-infrared fluorescent probes based on piperazine-functionalized BODIPY dyes for sensitive detection of lysosomal pH. *J. Mater. Chem. B* **2015**, *3* (10), 2173−2184.
(33) Zhang, J.; Yang, M.; Mazi, W.; Adhikari, K.; Fang, M.; Xie, F.; Valenzano, L.; Tiwari, A.; Luo, F.-T.; Liu, H. Unusual Fluorescent Responses of Morpholine-Functionalized Fluorescent Probes to pH via Manipulation of BODIPY's HOMO and LUMO Energy Orbitals for Intracellular pH Detection. *ACS Sensors* **2016**, *1* (2), 158−165.
(34) Li, X.; Ji, G.; Jung, J.-M.; Son, Y.-A. A BODIPY based highly selective fluorescence turn-on sensor toward VIB and IIB metal ions. *Mol. Cryst. Liq. Cryst.* **2016**, *636* (1), 159−167.
(35) Niu, S. L.; Ulrich, G.; Ziessel, R.; Kiss, A.; Renard, P. Y.; Romieu, A. Water-soluble BODIPY derivatives. *Org. Lett.* **2009**, *11* (10), 2049−52.
(36) Nadler, A.; Yushchenko, D. A.; Muller, R.; Stein, F.; Feng, S.; Mulle, C.; Carta, M.; Schultz, C. Exclusive photorelease of signalling lipids at the plasma membrane. *Nat. Commun.* **2015**, *6*, 10056.
(37) Wagner, N.; Stephan, M.; Höglinger, D.; Nadler, A. A Click Cage: Organelle-Specific Uncaging of Lipid Messengers. *Angew. Chem., Int. Ed.* **2018**, *57* (40), 13339−13343.
(38) Lezcano, N.; Bergson, C. D1/D5 Dopamine Receptors Stimulate Intracellular Calcium Release in Primary Cultures of Neocortical and Hippocampal Neurons. *J. Neurophysiol.* **2002**, *87* (4), 2167−2175.

https://dx.doi.org/10.1021/jacs.9b13219
J. Am. Chem. Soc. 2020, 142, 4970−4974