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Family of BODIPY Photocages Cleaved by Single Photons of Visible/Near-Infrared Light

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Abstract

Photocages are light-sensitive chemical protecting groups that provide external control over when, where, and how much of a biological substrate is activated in cells using targeted light irradiation. Regrettably, most popular photocages (e.g., *o*-nitrobenzyl groups) absorb cell-damaging ultraviolet wavelengths. A challenge with achieving longer wavelength bond-breaking photochemistry is that long-wavelength-absorbing chromophores have shorter excited-state lifetimes and diminished excited-state energies. However, here we report the synthesis of a family of BODIPY-derived photocages with tunable absorptions across the visible/near-infrared that release chemical cargo under irradiation. Derivatives with appended styryl groups feature absorptions above 700 nm, yielding photocages cleaved with the highest known wavelengths of light via a direct single-photon-release mechanism. Photorelease with red light is demonstrated in living HeLa cells, Drosophila S2 cells, and bovine GM07373 cells upon ∼5 min irradiation. No cytotoxicity is observed at 20 μM photocage concentration using the trypan blue exclusion assay. Improved B-alkylated derivatives feature improved quantum efficiencies of photorelease ∼20-fold larger, on par with the popular *o*-nitrobenzyl photocages (εΦ = 50–100 M–1 cm–1), but absorbing red/near-IR light in the biological window instead of UV light.

Disciplines

Biochemical and Biomolecular Engineering | Medicinal-Pharmaceutical Chemistry

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Supporting Information Placeholder

ABSTRACT: Photocages are light-sensitive chemical protecting groups that provide external control over when, where, and how much of a biological substrate is activated in cells using targeted light irradiation. Regrettably, most popular photocages (e.g. *o*-nitrobenzyl groups) absorb cell-damaging ultraviolet wavelengths. A challenge with achieving longer wavelength bond-breaking photochemistry is that long-wavelength-absorbing chromophores have shorter excited state lifetimes and diminished excited state energies. However, here we report the synthesis of a family of BODIPY-derived photocages with tunable absorptions across the visible/near-IR that release chemical cargo under irradiation. Derivatives with appended styryl groups feature absorptions above 700 nm, yielding photocages cleaved with the highest known wavelengths of light via a direct single-photon release mechanism. Photorelease with red light is demonstrated in living HeLa cells, Drosophila S2 cells, and bovine GM07373 cells in ~ 5 min irradiation. No cytotoxicity is observed at 20 μM photocage concentration using the trypan blue exclusion assay. Improved B-alkylated derivatives feature improved quantum efficiencies of photorelease ~20-fold larger, on par with the popular o-nitrobenzyl photocages ($\varepsilon\Phi$ = 50 – 100 $M^{-1}cm^{-1}$), but absorbing red/near-IR light in the biological window instead of UV light.

Photocages are light-sensitive moieties that are prized for giving investigators spatial and temporal control over the release of covalently-linked substrates.[1-4] When a photocage blocks a critical functional group on the substrate (e.g. a carboxylic acid), the substrate is rendered biologically inert. Light-induced cleavage of the photocage-substrate bond restores the reactivity or function of the substrate. Photocages have been used to activate proteins,[5, 6] nucleotides,[7, 8] ions,[9-11] neurotransmitters,[12, 13] pharmaceuticals,[14, 15] and fluorescent dyes using light.[16-18] The vast majority of photocaging studies used in biological studies exploit the *o*-nitrobenzyl photocages[19, 20] and their derivatives, but others include those based on the phenacyl,[21, 22] acridinyl,[23] benzoinyl,[24, 25] coumarinyl,[26] and o-hydroxynaphthyl[27] structures.

A major limitation of these popular photocages is that their chromophores absorb mostly in the ultraviolet region of the optical spectrum. Ultraviolet light has limited tissue penetration, restricting photocaging studies to cells and tissue slices. In addition, exposure of cells or tissues to intense UV light results in phototoxic cell damage or death. The ideal wavelength range to be used in animal tissues, known as the biological window, is ~600-1000 nm, where tissues have maximal light transmittance. At these far red/near-IR wavelengths, light achieves maximal tissue penetration while minimizing phototoxicity.[28] Photorelease using these wavelengths holds promise for studies in living animals or as targeted photoactivated therapeutics.

The drawbacks of using UV light as a biological initiator has led to a number of different approaches to permit photorelease of biologically-active substrates using visible light. The *de facto* method for releasing target molecules with visible light is to exploit multiphoton absorption methods, wherein a UV-absorbing photocage is excited with two or more visible light photons.[29-31] Other methods to achieve photorelease using visible light include photorelease initiated via photoinduced electron transfer,[32-34] via metal-ligand photocleavage,[35] or by using photosensitizers that generate reactive singlet oxygen that can initiate reaction cascades leading to substrate release.[36-38]

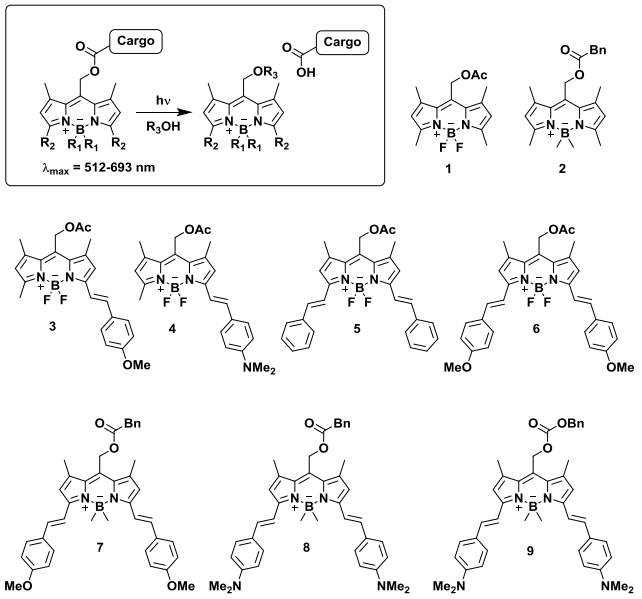

Figure 1. Substrates described in this study. Substrates 1 and 2 from our prior work[39, 40] while 3-9 are from this work.

However, it is advantageous to achieve photorelease with simple biologically benign organic structures that directly release substrates with single photons of visible light. Recently, a new class of photoremovable protecting groups derived from *meso*-

substituted BODIPY dyes was reported by us and Weinstain.[39, 41] For these photocages (**1** and **2** Figure 1), cleavage occurs using green wavelengths ~520 nm. In related recent work, Klan and coworkers have also demonstrated the photorelease of carbon monoxide from irradiation of BODIPY-[42] and xanthene-[43] substituted carboxylic acids. Also of relevance, aminoquinones are reported to undergo neighboring H atom abstraction with long-wavelength light that can lead to hydroquinones that release appended leaving groups via a self-immolative release pathway.)[44] A recent structure-activity relationship varying substituents and leaving groups om BODIPY photocages was conducted in collaborative work between us, Weinstain, and Klan. Sitkowska, Feringa, and Szymanski have recently demonstrated amine-releasing BODIPY derivatives by irradiation of BODIPY carbamates.[45]

Table 1. Optical and Photochemical Properties of 1-9

	λ_{max} (nm)	λ_{em} (nm)	ε ($x10^4$ M^{-1} cm^{-1})	Φ (%)	$\varepsilon\Phi$ ($M^{-1}cm^{-1}$)
1[b]	515	526	7.1	0.099	70
2[b]	512	550	6.9	5.5	3800
3	586	607	6.1	0.0098	6.0
4	633	650	6.0	0.0069	4.1
5	640	656	6.5	0.0045	2.9
6	661	684	6.5	0.0041	2.7
7	647	660	4.9	0.084	41
8	689	728	7.8	0.11	86
9	693	745	5.2	0.14	73

[a] Quantum yields (Φ) determined by ferrioxalate actinometry in MeOH with a 532 nm ND:YAG laser source and release followed using quantitative LC-UV (average of 3 runs). [b] [b]Values from reference[39, 40].

We synthesized compounds **3-9** that have extended conjugation and a red-shifted λ_{max} values between 580-690 nm (λ_{em} between 600-750 nm). (See Figure 2). Compounds **3-9** are thermally stable, showing no change by NMR when refluxed in the dark for 1 hour (SI16), yet release acetic acid when irradiated with visible light (SI6). The quantum yields of the release of acetic acid (ϕ) of **3-9** were determined by ferrioxalate actinometry (SI18) and are reported as the average value of three trials. The extinction coefficient (ε) at the λ_{max} was determined by UV-vis spectroscopy (Table 1). These photocages feature high extinction coefficients typical of BODIPY dyes (~50,000-120,000 $M^{-1}cm^{-1}$ at λ_{max}). In general, the quantum yields (ϕ) for photorelease of acetic acid for the longest wavelength absorbing photocages **3-6** are an order of magnitude lower than those of the unconjugated substrates (**1** or **2**).

However, boron-alkylation leads to a large increase in the quantum yield of release, while not significantly perturbing the λ_{max} of the chromophore. For example, the boron-alkylated **7** has a quantum yield ~20-fold larger than **6**, which is boron-fluorinated. Because many biologically interesting compounds have alcohols, we also synthesized compound **9** to demonstrate release of benzyl alcohol. This compound releases benzyl alcohol with a similar quantum yield as the carboxylates via a decarboxylation mechanism that liberates CO_2 as a byproduct. For comparison, the most popular photocages, the o-nitrobenzyl photocages, have $\varepsilon\Phi$ of ~ 100 $M^{-1}cm^{-1}$ at λ_{irr} = 350 nm, and much less at λ_{irr} > 400 nm. [1a] Thus, the quantum efficiencies of **8** and **9** are on the same order of magnitude as nitrobenzyl photocages, but absorb light in the biological window ~700 nm instead of UV light.

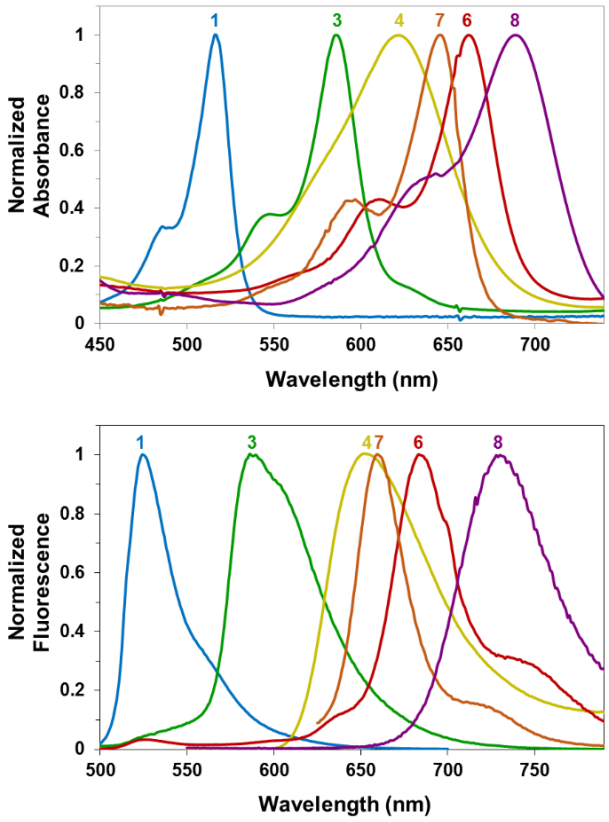

Figure 2. Absorbance and emission spectra of select BODIPY photocages. Data for all photocages can be found in the SI.

Compound **10** was synthesized to test the usefulness of these photocages in live cell imaging. 4-nitrobenzoic acid was used as the leaving group, since nitrobenzoic acids are known fluorescence quenchers of BODIPY dyes. A short ethylene glycol chain was appended to the styryl groups to aid with water solubility. Compound **10** does not exhibit detectable fluorescence when kept in the dark at room temperature. We anticipated that upon irradiation and release of 4-nitrobenzoic acid, a fluorescent photoproduct would be formed with a λ_{em} at ~685 nm. However, we found that when **10** is irradiated in MeOH or cell culture buffer under air we instead observe growth in fluorescence at 610 nm. Similarly, when **6** is irradiated in methanol we see a peak grow in at 610 nm, as the peak at 685 nm decreases (See Fig. 3 and SI12). This new blue-shifted fluorescent peak is similar to the emission wavelength of **3**. ^{1}H NMR studies of the photolysis of **10** confirm that the quencher is released upon irradiation (SI15). Photoproduct studies of **6** and **10** under air show that in addition to acetic acid, *p*-anisaldehyde is a minor photoproduct for **6** and the tri(ethylene) glycol-substituted anisaldehyde for **10** (SI8 and 15). Formation of the benzaldehyde and a λ_{em} at a similar wavelength to **3** indicates that photocage byproducts have either cleaved or broken the conjugation of a styryl group (further information on product studies of **6** can be found in SI13-15), explaining the wavelength shift.

We considered two possible mechanisms for this cleavage and aldehyde formation. First, the photocage could sensitize singlet

oxygen, which could then oxidatively cleave the olefin. This process would be independent of photorelease. Alternatively, oxidative cleavage could arise from oxygen trapping a cation intermediate (possibly in its triplet state), a mechanism that is correlated with the photorelease process.

The simplest release mechanism to consider is a photoheterolysis mechanism, either from the singlet or triplet excited state. The putative carbocation formed by heterolysis of **6** is computed to be a triplet ground state carbocation by ca. 3 kcal/mol using density functional theory (UB3LYP/6-31G(d), including correction for singlet spin contamination; see SI24). Photolysis occurring through the triplet excited state would be expected to yield the triplet carbocation to conserve spin. Indeed, we selected *meso*-substituted BODIPY dyes as potential photocages based on these ions having low-lying diradical states, which we had previously suggested might be critical for providing a surface crossing (conical intersection) between the excited state surface and the ground state ion pair surface.[46] Inspection of the computed SOMOs of the triplet carbocation indicates that the radicals are located on the styryl groups. Little is known about triplet carbocation reactivity,[47] but triplet diradicals are known to react with oxygen, which could lead to a pathway involving oxidative cleavage at one of the styryl groups. A similar styryl BODIPY compound has been demonstrated to be reactive to radicals in the ground state, breaking the conjugation of the olefin and causing a similar wavelength shift to what we observe.[48]

To corroborate that this fluorescence blue shift was correlated with release of the carboxylic acid, compound **8** was synthesized as a control without a leaving group and showed no fluorescence blueshift upon photolysis (fluorescence studies on SI12 and ^{1}HNMR studies on SI10). Irradiation of this compound simply led to a slow fluorescence bleaching at long irradiation times. If oxidative cleavage were independent of the photorelease process, this compound, which is identical except for the absence of the leaving group, would be expected to have a similar fluorescence blueshift. That it does not supports the mechanism involving trapping of a reactive intermediate following the photorelease.

This blue-shifted fluorescence provides a convenient assay by which to follow the photorelease process using fluorescence microscopy, since the photocaged acid **6** does not absorb at 605 nm, the wavelength the blueshifted byproduct absorbs. NMR studies showed that there is mass balance between compound **6** and acetic acid over 3 hr of irradiation (SI11).

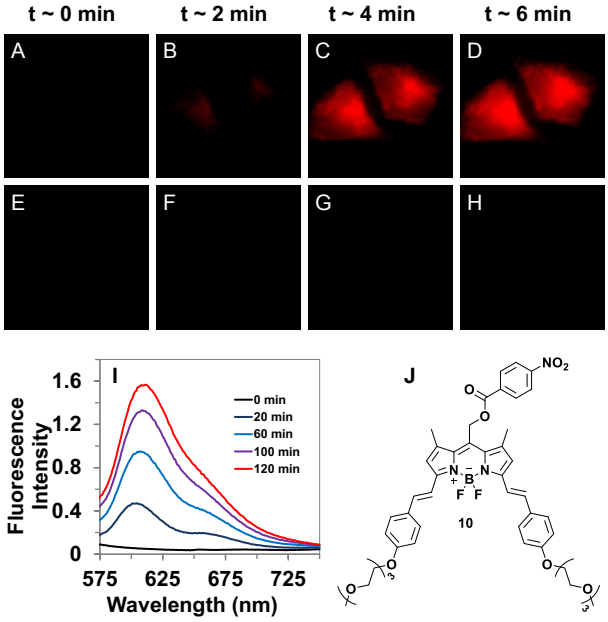

Figure 3. Fluorescence images of HeLa cells incubated with 20 μM compound **10** irradiated with 635 nm light (A-D) and cells incubated with compound **10** with no irradiation (E-H) as a function of time. Average fluorescence intensity v. time of **10** incubated in HeLa cells (n = 10) with (i) and without (ii) 635 nm irradiation (I). Increase in fluorescence of compound 7 in H_2O with 5% BSA when irradiated.

As a proof of principle demonstration that photorelease could be performed in cells, 20 μM compound **10** was incubated with HeLa cells at 36±2 ºC. The cells were irradiated with 635 ±15 nm light. Fluorescence emission was measured at 605 nm. Control experiments were performed without irradiation. An increase in fluorescence was observed at 605 nm when irradiated (Figure 3 A-D). In control studies, no increase in fluorescence was observed in the dark (Fig. 3 E-H). Similar imaging studies were performed in bovine GM07373 cells and Drosophila S2 cells (SI22), yielding similar results. Cytotoxicity studies showed no decrease in cell viability (trypan blue exclusion assay) with treatment of **10** (90±3% for HeLa cells with no treatment, 92±5% for HeLa cells with **10**, 95±2% for S2 cells without treatment, 93±2% for S2 cells with 10, 90±1% for GM07373 cells without treatment, and 92±5% for GM07373 cells with **10)**.

In conclusion, a family of BODIPY-derived photocages have been synthesized that release chemical cargos at a range of wavelengths in the visible absorptions range from ~450-700 nm. The longest-wavelength absorbing derivatives (**8**, **9**) represent photocages that can release substrates with the highest known wavelengths of light via a single-photon direct photorelease pathway. The wide range of wavelengths of absorbance allows photorelease to be achieved using essentially any color of visible light. This work opens up the possibility of the targeted and controlled release of pharmaceuticals or other biomolecules using irradiation with red light in the biological window. It is remarkable that a net photoheterolysis reaction can be achieved using red light given the much diminished photon energies associated with these wavelengths (~40 kcal/mol for 700 nm vs ~100 kcal/mol for a UV photon), and it will be interesting to test the maximum wavelengths of photorelease possible, as increasing wavelengths correspond to increasingly diminished photon energies.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.

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Notes

The authors declare no competing financial interests.

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SYNOPSIS TOC