BODIPY-Derived Photoremovable Protecting Groups Unmasked with Green Light

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Supporting Information Placeholder

ABSTRACT: Photoremovable protecting groups derived from *meso*-substituted BODIPY dyes release acetic acid with green wavelengths >500 nm, and photorelease is demonstrated in cultured *S2* cells. The photocaging structures were identified by our previously proposed strategy of computationally searching for carbocations with low-energy diradical states. The superior optical properties of these photocages make them promising alternatives to the popular *o*-nitrobenzyl photocage systems.

Photoremovable protecting groups, sometimes called photocages or phototriggers, are popular light-sensitive chemical moieties that mask substrates through covalent linkages that render the substrates inert. Upon irradiation, the masked substrates are released, restoring their reactivity or function. While photocages have important applications in areas such as organic synthesis,[1-3] photolithography,[4,5] and light-responsive organic materials,[6-8] these structures are particularly prized for their ability to trigger biological activity with high spatial and temporal resolution[9-13]. Examples of such chemical tools include photocaged proteins,[14-16] nucleotides,[17,18] ions,[19-23] neurotransmitters,[24,25] pharmaceuticals,[26,27] fluorescent dyes,[28-30] and small molecules[31,32] (e.g. caged ATP). These biologically relevant caged molecules and ions can be released from the caging structure within particular biological microenvironments using pulses of focused light. The most popular photocages used in biological studies are the *o*-nitrobenzyl systems[31-33] and their derivatives, but other photocages that see significant use include those based on the phenacyl,[34] acridinyl,[35] benzoinyl,[36,37] coumarinyl,[38] and *o*-hydroxynaphthyl structures.[39] Unfortunately, with few exceptions described below,[40,41] a serious limitation of most popular photocages is that they absorb mostly in the ultraviolet where the limited penetration of UV light into tissues largely restricts these studies to fixed cells and tissue slices. Furthermore, prolonged exposure of cells or tissues to intense UV light can lead to cellular damage or death.

Consequently, new photocaging structures that absorb visible light are urgently needed. Advantages of visible light irradiation include diminished phototoxicity compared to UV light and deeper optical penetration into tissue. Additionally, visible light photolysis can be performed with cheap lamps and Pyrex glassware, while UV photolysis requires expensive UV sources. Unfortunately, the major problem that has hindered the development of new photocages that absorb visible light is the lack of a structure-reactivity relationship for excited state heterolysis. That is, it is difficult to predict *a priori* which structures, when irradiated with light, will undergo an efficient photoheterolysis reaction. Thus, attempts to prepare visible light absorbing photocages have mostly bypassed this problem by using metal-ligand photoreleasing systems[41-43] or by using creative indirect schemes. Examples of such creative schemes include upconverting nanoparticles with surface-attached UV-absorbing photocages[44-46] or release mediated by photoinduced electron transfer with a sacrificial electron donor.[47]

However, visible light absorbing organic structures that offer simple photorelease schemes and structures would potentially make a more compelling case for widespread use in biologically oriented labs.[48] A recent computational study performed in our lab suggested the hypothesis that photoheterolysis reactions may be under conical intersection control.[49] That is, photoheterolysis of C-LG (carbon—leaving group) bonds to generate ion pairs[50] may be favored if the ion pair has access to a nearby productive conical intersection that provides an efficient channel for the excited state of the photoprecursor to decay to the ground-state ion pair. Because conical intersections are challenging to compute, we further suggested the idea of using the vertical energy gap of the carbocation to its first excited state as a simple predictor of a nearby conical intersection (CI). A low S_0-S_1 energy gap of the cation would suggest the possibility of a nearby CI between the two surfaces, and the potential for a productive channel from the excited state of the photocaged precursor to the ion pair.

Thus, to find visible light absorbing photocages we searched for potential photocaging structures that would generate carbocations with low-lying diradical states. A time-dependent density functional theory (TD-DFT) computational investigation of carbocations attached to the BODIPY scaffold at the <u>meso</u> position indicated that these ions have low-lying excited states. For example, the TD-DFT computed S_0-S_1 vertical energy gap of the carbocation derived from C-O scission of **2** is 8 kcal/mol (TD-B3LYP/6-311+G (2d,p)), suggesting a near-degenerate diradical configuration. Indeed, all of the cations derived from C-O scission of **1-6** have vertical gaps < 13 kcal/mol (see SI for computational details), and have singlet states with considerable diradical character. Large singlet stabilizations upon switching from restricted → spin-purified unrestricted singlet computations indicate that the singlet states can be described as diradicals or possessing considerable diradical character (see SI for details). Thus, the exact vertical energies from the TD-DFT computations are to be viewed with suspicion, but it is clear that there are low-energy diradical forms for these ions. Further, the singlet-triplet gaps of all the carbocations derived from **1-6** are ~5 kcal/mol in favor of the triplet state, suggesting that the "carbocations" produced by heterolysis of **1-6** may in fact be better described as ion diradicals than by traditional closed-shell carbocation structures.[51]

Encouraged by these computational studies, we synthesized structures **1-6** as photocages for acetic acid. Advantages of the BODIPY scaffold include simple syntheses, a compact structure, known biological compatibility,[52] and high extinction coefficients in the visible.[53] Photorelease studies, described below, indicate that these structures release acetic acid upon photolysis with wavelengths >500 nm.
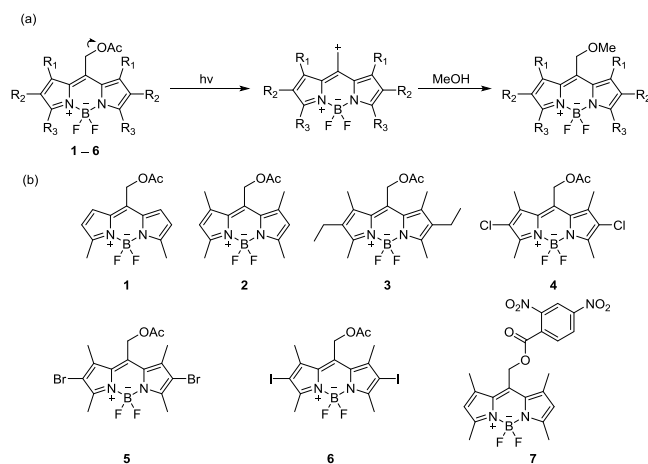

Figure 1. (a) Possible pathway for the photolysis of photocaged acetic acid; (b) BODIPY-derived photocages described in this study.

Photorelease studies and quantum efficiencies. The observed substrate release rate as a function of photolysis time is quantified by the quantum efficiency parameter ($\varepsilon\,\Phi$), which is the product of the extinction coefficient at the irradiation wavelength (ε) and the quantum yield (Φ). Extinction coefficients for **1-6** were determined by UV-Vis spectroscopy (see Table 1). To compute the quantum yields of photorelease (Φ), the flux of a 532 nm laser excitation beam (ND:YAG, 1st harmonic) was determined using potassium ferrioxalate actinometry. Release of acetic acid as a function of laser irradiation time in MeOH was followed by quantitative LC/UV (see SI for details). Each quantum yield reported is the average of 3 separate runs. Identical actinometry measurements performed after photolysis demonstrated high flux stability of the laser. Additionally, repeating the quantum yield measurement for **2** on a different day with a different laser power setting (in triplicate) gave essentially the same value for the quantum yield, indicating reproducibility. A preparative photolysis of **2** in MeOH gave the methyl ether adduct as the isolated end product of the BODIPY photocage, possibly from solvent trapping of an intermediate carbocation. Curiously, unlike **1-4** and **6**, the brominated compound **5** was found to be unstable. It decomposes after 1 day stored on the shelf in the dark, and photolysis of freshly prepared and purified **5** led to secondary products in addition to acetic acid release, and photolysis was accompanied by rapid solution bleaching. Consequently, we were not confident in our quantum yield measurements for **5** and excluded it from Table 1. Probably, it also has access to alternative photochemical pathways (e.g. C-Br homolysis) and thermal degradation channels.

In general, the quantum efficiencies for **1-4** and **6** are slightly lower or comparable with the popular caged o-nitrobenzyl systems.[9] Quantum yields for **1-4** are lower than those for typical o-nitrobenzyl photocaged structures, but this lower quantum yield is compensated by the much higher extinction coefficients of the BODIPY chromophores compared to the o-nitrobenzyl chromophore, leading to reasonable quantum efficiencies. The iodinated derivative **6** has the largest quantum efficiency, comparable to

that of some caged o-nitrobenzyl systems, but with a λ_{max} at ~550 nm rather than in the UV (the parent o-nitrobenzyl system has a λ_{max} of ~280 nm while a popular dimethoxy analog has a λ_{max} of ~350 nm). A plausible explanation for the higher quantum yield of **6** is that the iodine atoms promote intersystem crossing to a triplet state, which are usually longer lived than singlet excited states. Given that the triplet state of the carbocation is the computed ground state by ~ 5 kcal/mol, it is certainly energetically reasonable that heterolysis could occur in the triplet excited state leading to the triplet "carbocation", similar to the phenacyl photocage derivatives described by Givens.[34] This hypothesis is supported by the very weak fluorescence of solutions of **5** and **6**, compared to solutions of **1-4**.

Table 1. Optical properties and quantum efficiencies of **1-6**.

	λ_{max} (nm)	λ_{em} (nm)	ε (x 10^4 M^{-1} cm^{-1})	Φ (x 10^{-4})	$\varepsilon\,\Phi$ ($M^{-1}cm^{-1}$)
1	519	527	5.7	6.4	37
2	515	526	7.1	9.9	70
3	544	560	6.2	9.5	59
4	544	570	4.8	4.0	19
5	545	575	--	--	--
6	553	576	4.9	23.8	117

Thermal stability studies. Photocaged compounds **1-4** and **6** are thermally stable in the dark. Boiling these compounds in MeOH for 1 h in a foil-wrapped vessel led to no change in the ¹H NMR spectrum (see SI for spectra). Compound **5** is unstable in all forms. The other compounds **1-4, 6** are shelf stable for at least a week.

Optical properties of 1-6. The UV-Vis spectra and fluorescence spectra of **1-6** are shown in Figure 2. These structures absorb between 515 nm and 553 nm (and emit between 520 nm and 580 nm), typical of simple BODIPY dyes, and feature large extinction coefficients (~50,000-70,000 $M^{-1}cm^{-1}$).
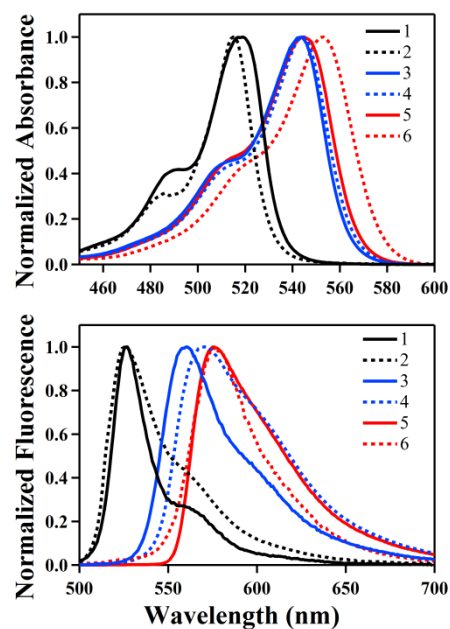

Figure 2. Normalized absorbance and fluorescence spectra of **1-6**.

Cell Studies: To test the viability and usefulness of the BODIPY derived photocages in biological systems, compound **7** was syn-

thesized. 2,4-dinitrobenzoic acid is a known [54] fluorescence quencher for BODIPY compounds. This quencher was coupled with our BODIPY moiety using a simple DCC/DMAP ester coupling reaction. We anticipated that 7 would be weakly fluorescent, but upon photorelease of the quencher the fluorescence would increase. Indeed, when **7** was irradiated with a mercury lamp (excitation = 500 nm, see SI) and its fluorescence was plotted over time (Figure 3 bottom right). There was a steady growth in fluorescence attributed to release of the quencher. Photorelease of the quencher was also confirmed by ^{1}H NMR. As a control, similar steady state fluorescence measurements were performed over time for compound **7** in the dark without light exposure, leading to essentially no change in fluorescence.
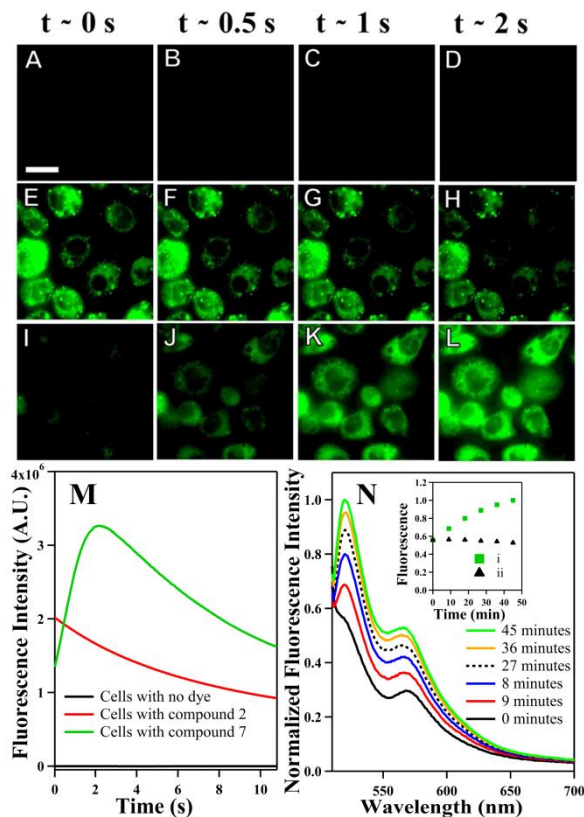

Figure 3. Fluorescence image of *S2* cells with no BODIPY compound (A-D), cells incubated with compound 2 (E-H) and cells incubated with compound 7 (I-L) as a function of irradiation time (top). Scale bar is 20 μm (shown in panel A) and is the same for all the images. Images were adjusted to same contrast in each row. Average fluorescence intensity profile versus irradiation time using 100% lamp power for excitation in cells (bottom left). Increase in free BODIPY fluorescence signal over time with quencher release from compound 7 in BES buffer (bottom right). Plot insert (bottom right) depicts the difference in growth of fluorescence vs time for compound 7 with (i) and without (ii) light irradiation.

Compound **2** and **7** were incubated with Drosophila *S2* cells and monitored using fluorescence microscopy (Figure 3 top). The Drosophilia S2 cells loaded with 2 and 7 were irradiated continuously with 500 nm light. Fluorescence images were collected every 36 ms for a total of 10.8 seconds. The fluorescence intensity for compound **7** inside cell as shown in Figure 3I-L increases rapidly. This increase in fluorescence can be attributed to the release of the quencher. The same fluorescence study with **2** as a control in Figure 3E-H shows no such increase in fluorescence. For 2 the leaving group is acetate, which is not a quencher. Thus,

little change in the fluorescence would be anticipated upon photo-release of acetic acid from this moiety. The background decay in fluorescence for both **2** and **7** can be attributed to photobleaching under intense focused light. Parts A-D of Figure 3 show that there is a minimal change in fluorescence of cells when they are irradiated without being loaded with compound **2** or **7**. Figure 3 bottom left shows the fluorescence intensity change over time for cells incubated with compound **2, 7**, and the control experiments without any compound.

Cytotoxicity of compounds were measured with trpan blue exclusion assay. All values are normalized with the control cells which were not incubated with any compound. At a compound concentration of 25 μM, 97% for compound 2 and 92% for compound 7 remained viable after 1h.

Conclusion. In conclusion, BODIPY-derived photocages unmask acetic acid with green light excitation >500 nm and photocleavage can be carried out in living cells. These photocages are promising alternatives for the popular *o*-nitrobenzyl photocaging systems, being easy to synthesize, utilizing a biocompatible chromophore, and having superior optical properties to the most popular photocages in current use. More generally, our strategy of identifying new photocages by searching for carbocations with low-energy diradical states seems to be a promising one. BODIPY derivatives that release functional groups other than carboxylic acids and that have red-shifted absorptions into the biological window (600-1000 nm) are currently under investigation.

ASSOCIATED CONTENT

Supporting Information

Synthetic procedures and compound characterization data, quantum efficiency determination details, laser photolysis data, computational data, and cellular fluorescence movies. These materials are available free of charge via the Internet at http://pubs.acs.org.

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Notes
The authors declare no competing financial interests.

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