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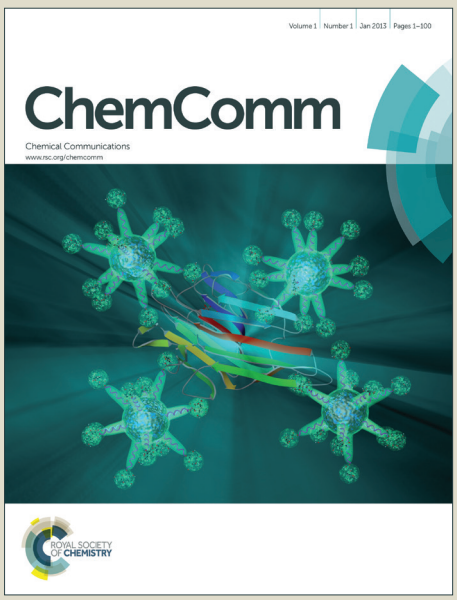

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meso-Methylhydroxy BODIPY: a scaffold for photo-labile protecting groups

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Here, we show that by installing a *meso*-methylhydroxy moiety, the boron dipyrromethene (BODIPY) scaffold can be converted into an efficient caging group, removable by green light. We describe caging and uncaging of important chemical functionalities and demonstrate green light mediated control over biological processes in cultured cell lines and neurons.

The molecular mechanisms that govern biological functions are highly sophisticated and complex. Elucidation and manipulation of such processes on a molecular level requires precise external spatiotemporal control over their function. Light is an attractive way to achieve this goal, as it is non-invasive and readily delivered with high spatial and temporal precision. Thus, light has been harnessed to control biological processes through a method termed "caging"[1], in which the biological activity of a target small- or macro-molecule is abrogated by covalent ligation to a photolabile protecting group (PPG or caging groups). Exposure to light at a specific wavelength releases the molecule in its active form (Figure 1A). This useful method has been applied to investigate and manipulate the activity of a wide range of biological processes by controlling the activity of small-molecules, proteins, RNA and gene expression.[2]

Over the years, dozens of caging groups have been developed, yet most operate in the UV range[3] (250-400 nm), which is problematic due to low tissue penetration and high tissue damage caused by the high energy UV irradiation. In addition, the narrow window limits the ability to control multiple cues through differentially caged compounds. While some of these concerns have been ameliorated through the use of two-photon excitation[4], the technique's applicability to several important fields (synthetic chemistry, material sciences, therapeutics delivery) is inherently limited by its small focal-point and low conversion throughput. Thus, there still exists a strong need for PPGs that operate by one-photon outside of the 250-400 nm window, to enable multiplex imaging and optical control of biological systems. Recently, several efforts have been made to identify novel scaffolds that would address this need.

Specific modifications in the structure of the known caging group 4-methylhydroxycoumarin enabled pushing their absorbance to the blue-cyan range.[5, 6] Very recently, 4-arylalkoxy-boron dipyrromethene were demonstrated to release phenols when photolized with green light.[7] The light-induced cleavage of the boron-arylalkoxy bond was elegantly utilized to uncage a biogenic amine through a self-immolating linker, yet caging with these molecules requires a multi-step synthesis (up to 5 synthetic steps) and the stability of the boron-arylalkoxy bond under physiological conditions is a concern. Cyanine dyes were also converted into near-IR caging groups though uncaging in biological settings required prolonged irradiation times[8] (30 minutes). Metal complexes have likewise been explored as potential scaffolds for photocaging. Photolysis of amines from ruthenium complexes by blue-green light has been demonstrated and was applied in cellular environment.[9-11] Nevertheless, their broad absorption spectrum impedes multiplex fluorescence imaging and their photosensitizer-based design leads to sensitizer-dependent phototoxicity. Another interesting approach is the contact-quenching induced scission of a Co–C bond that enables tuning of the light absorbance wavelength, up to the near-IR range, by selecting an appropriate fluorophore serving as an antenna.[12, 13]

Here, we demonstrate that a specific derivatization of the boron dipyrromethene (BODIPY) core (Figure 1B) enables its translation into an efficient green light excitable caging group.

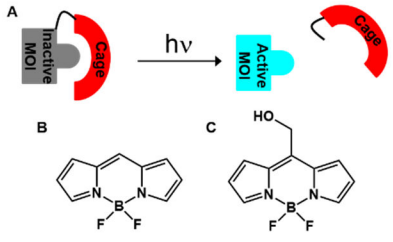
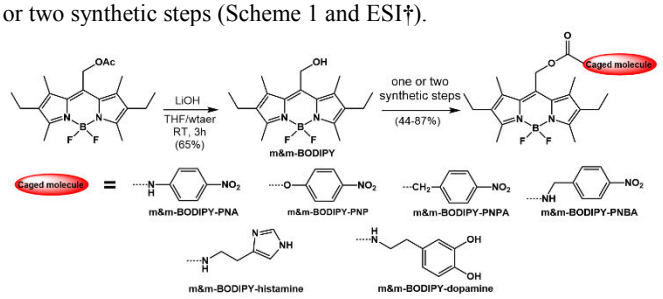


Figure 1. A) Schematic representation of light-mediated control over a small- or macro-molecule of interest (MOI) activity. **B**,**C**) General structure of BODIPY core (**B**) and ***meso*-methylhydroxy** BODIPY (m&m-BODIPY) applied for photocaging **(C)**.

The excellent spectroscopic properties of BODIPYs render them potentially attractive caging groups. This family of chromophores is characterized by sharp and tunable light absorption (490-650 nm), high molar absorption coefficient (30,000-80,000 $L \cdot mol^{-1} \cdot cm^{-1}$) and chemical flexibility and stability, making them ideally suited for biological applications.[14] Taking a cue from the structures of coumarin-based PPGs, the recent efforts to convert xanthenes into PPGs[15, 16], and the ease with which multiple functional groups are incorporated into the *meso* position of BODIPY[14, 17-23], we hypothesized that equipping the BODIPY scaffold with a methylhydroxy moiety on at this position (Figure 1C) will similarly enable caging and uncaging of molecules of interest.

To test our hypothesis, we synthesized 2,6-diethyl-1,3,5,7-tetramethyl-BODIPY bearing a **m**ethylhydroxy group on the ***meso*** position (m&m-BODIPY, Scheme 1). The molecule features strong absorbance centered at 537 nm (SI Figure S1), high molar absorption coefficient (ε_{537} = 53,300 $L \cdot mol^{-1} \cdot cm^{-1}$) and is highly fluorescent (Φ_{fl} = 0.90). The hydroxyl group of m&m-BODIPY can be straightforwardly conjugated to various chemical functionalities. Thus, conjugation of several model leaving groups to m&m-BODIPY through distinct chemical bonds was efficiently accomplished in one or two synthetic steps (Scheme 1 and ESI†).



Scheme 1. Synthesis and chemical structures of m&m-BODIPY and its conjugates. In parentheses: chemical yields.

The photo-induced release of model leaving groups from m&m-BODIPY was first evaluated for m&m-BODIPY-PNA (Figure 2A). The conjugate has comparable spectroscopic properties to m&m-BODIPY (i.e. λ_{max} = 545 nm, ε = 34,500, Φ_{fl} = 0.65), including an additional absorbance maxima at 325 nm, characteristic of caged PNA (SI Figure S1). Irradiation of m&m-BODIPY-PNA (100 μM in PBS pH 7.4, 5% ACN) with green light (540/30 nm, 49 mW/cm^2) led to a rapid release of PNA ($t_{1/2}$ = 120 seconds), as measured by an increase in 377 nm absorbance and by concomitant decrease of conjugated PNA absorbance at 325 nm (Figures 2B,C). Bleaching of both the m&m-BODIPY-PNA and free BODIPY occurs on a slower timescale ($t_{1/2}$ ~ 360 seconds, Figures 2B,C). The photo-chemical yield for the reaction was 33% (agreeing well with 1-Φ_{fl}, or 1-0.65 = 0.35), and negligible hydrolysis was observed in the absence of light (< 1%, SI Figure S2). Independent measurement of the photo-reaction's progress by HPLC-MS corroborates the spectrophotometric findings, showing identical kinetics and photochemical yield (Figure 2D). Based on these measurements, the photo-release quantum efficiency was calculated to be $\Phi_{eff} = 6 \times 10^{-4}$ and $\Phi_{eff} \times \varepsilon = 21$, comparable to the efficiencies recorded for B-O photolysis of BODIPY-phenols.[7]

Next, we characterized the spectroscopic and photochemical properties of additional functional groups caged by m&m-BODIPY (Scheme 1). All photo-reactions were performed under similar conditions (100 μM of appropriate m&m-BODIPY conjugate in PBS pH 7.4 20 mM, 5% ACN, irradiation at 540/30 nm, 49 mW/cm^2) and monitored by HPLC-MS (Table 1 and SI Figure S3). Photo-induced release of a model primary amine (*p*-nitrobenzylamine, PNBA) proceeded ~4 times slower than that of an aromatic amine ($t_{1/2}$ = 600 seconds compared to $t_{1/2}$ = 120 seconds for PNA) but with overall similar chemical yield (37%). In contrast, photolysis of an ester bond to release *p*-nitrophenylacetic acid (PNPA) resulted in an almost complete conversion (chemical yield > 95%), albeit at a slower rate ($t_{1/2}$ = 1,200 seconds). Photolysis of a carbonate bond, to release *p*-nitrophenol (PNP), demonstrated a combination of both fast kinetics ($t_{1/2}$ = 140 seconds) and very efficient conversion (chemical yield > 95%). Collectively, these results demonstrate that *meso*-methylhydroxy BODIPY chromophores can serve as a scaffold for PPGs and suggests it could be applied to cage diverse chemical functionalities. While the values of uncaging quantum efficiency (ϕ_{eff}) for m&m-BODIPY are lower than for many widely used UV-excitable caging groups[3], they are well compensated by high molar absorption coefficients and, as demonstrated below, are sufficient for practical biological applications.

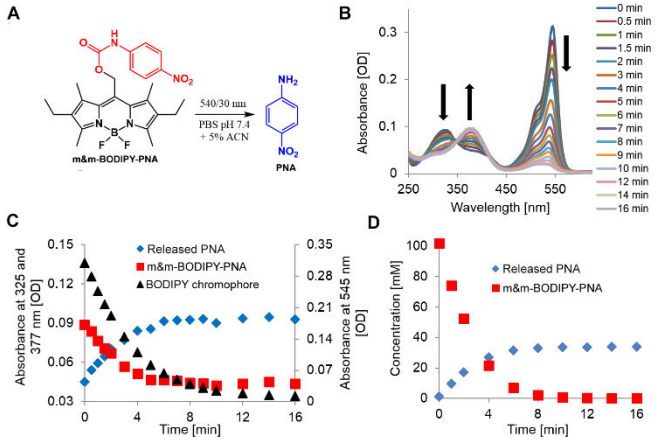


Figure 2. Light-induced release from a BODIPY cage. **A**) Schematic representation of PNA release from m&m-BODIPY-PNA conjugate, following green light irradiation. **B**) Spectral evolution of m&m-BODIPY-PNA (100 μM in PBS pH 7.4 supplemented with 5% ACN) upon 540/30 nm (49 mW/cm^2) light irradiation for the indicated times. Irradiated samples were diluted 20-fold for measurement. **C**) Specific wavelength monitoring of **C** showing accumulation of free PNA (377 nm) coupled to depletion of bound PNA (325 nm). Bleaching rate of the BODIPY chromophore is shown on the right axis (545 nm). **D**) Quantitative monitoring of the reaction in **B** by HPLC-MS shows similar PNA and m&m-BODIPY-PNA accumulation and depletion, respectively, as was observed by spectrometry (B,C).

Table 1. Spectroscopic and photochemical properties of m&m-BODIPY-caged model leaving groups.[a]

Leaving group	λ_{abs} [nm]	ε [$L \cdot mol^{-1} \cdot cm^{-1}$]	Chemical yield [%]	$t_{1/2}$ [sec]	ϕ_{eff}	$\varepsilon \cdot \phi_{eff}$ [b]
PNA	545	34,500	33	120	6×10^{-4}	21
PNBA	544	45,000	37	600	1.5×10^{-4}	6.7
PNP	547	48,400	> 95	140	1.6×10^{-3}	77
PNPA	545	38,600	> 95	1,200	1.8×10^{-4}	7

[a] All measurements were at 100 μM of appropriate M&M-BODIPY conjugate in PBS pH 7.4 20 mM supplemented with 5% ACN and irradiated with 540/30 nm light at 49 mW/cm^2 under constant stirring. [b] units: [$L \cdot mol^{-1} \cdot cm^{-1}$].

To establish the utility of m&m-BODIPY as a platform for photocaging and activation of biomolecules in living cells, we synthesized the histamine-conjugated form of m&m-BODIPY (Scheme 1). Histamine is an important physiological effector molecule, with diverse consequences for human health and disease. Binding of histamine to the histamine H1 receptor initiates a cascade of intracellular events, including Ca^{2+} release.[24] We hypothesized that

capping of the primary amine of histamine with a carbamate-linked m&m-BODIPY would mask the functionality of histamine and enable light-induced photoactivation and delivery of histamine.

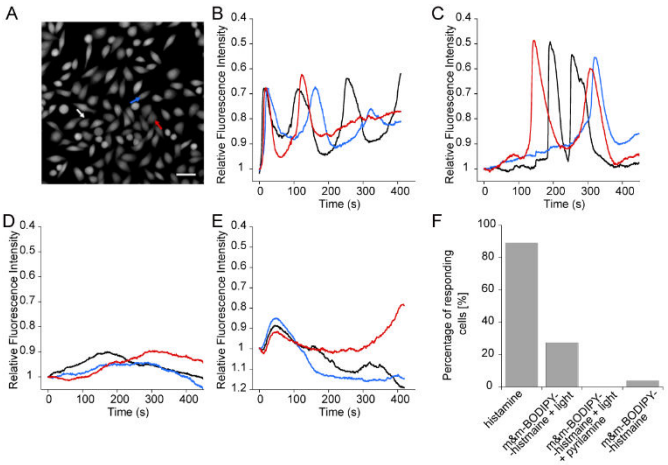


Figure 3. Live cell epifluorscence imaging of Ca^{2+} release triggered by m&m-BODIPY-histamine in HeLa cells. All cells were loaded with fura-2 AM and then treated with either 5 μM histamine (**A**,**B**), 5 μM BODIPY-histamine and green light (**C**), 5 μM BODIPY histamine, light, and the H1 receptor antagonist, pyrilamine (**D**), or 5 μM BODIPY-histamine without light (**E**). Shown are changes in Fura-2 fluorescence as quantified from representative cells. Scale bar is 20 μm. **F**) Percentage of responding cells in each treatment, as quantified for the whole field of view (n > 65 cells in each treatment). Additional control experiments are provided in the Supporting Information.

To test this, we loaded HeLa cells with the Ca^{2+}-selective fluorescent indicator, fura-2. Treatment with histamine (5 μM, Figure 3A,B and F, SI Movie 1) resulted in 89% of the cells (in the field of view) responding by prompt oscillations in intracellular $[Ca^{2+}]_i$, as measured by changes in fura-2 fluorescence. Upon incubation with m&m-BODIPY-caged histamine (5 μM), no oscillations were observed prior to uncaging with 540 nm light. Uncaging light, in the presence of m&m-BODIPY-histamine, recapitulated the oscillations seen with treatment by free histamine in 27% of the cells (Figure 3C,F, SI Movie 2). Pre-incubation of cells with the H1 receptor antagonist pyrilamine completely blocked this response (Figure 3D, SI Movie 3), demonstrating that the Ca^{2+} response is evoked by histamine photochemically liberated from the m&m-BODIPY cage. Importantly, no oscillations were observed in the presence of m&m-BODIPY-histamine but in the absence of light (Figure 3E, Movie 4). Additional control experiments further establish that Ca^{2+} flux depends on caged m&m-BODIPY-histamine and light, as light alone (no m&m-BODIPY-histamine, SI Figure S4A,B, SI Movie 5) and m&m-BODIPY-OH combined with irradiation (as a non-histamine releasing control, SI Figure S4C,D, SI Movie 6) do not promote correspondingly similar $[Ca^{2+}]_i$ changes. Taken together, these data establish m&m-BODIPY cages as an effective platform for photocaging biologically active molecules.

To further explore the versatility of m&m-BODIPY-type PPGs, we sought to apply this strategy to caging the catecholamine neurotransmitter dopamine (Scheme 1). This important neurotransmitter is vital for movement, motivation and cognition, and its misregulation is implicated in a number of human pathologies.[25] Methods to photocage dopamine exist, but rely on photocages with UV uncaging profiles[26] or use of transition metals.[27] A visible-light uncaging strategy with dopamine would be of utility for interrogating the role of dopamine in neuronal signalling. In a manner similar to the caging of histamine, the amino group of dopamine was modified with a carbamate-linked m&m-BODIPY.

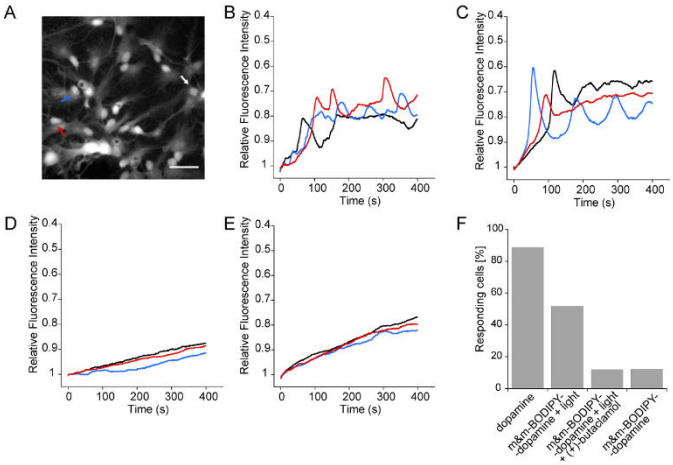


Figure 4. Live cell epifluorscence imaging of Ca^{2+} release triggered by BODIPY-dopamine in KCl-primed rat cortical/hippocampal neurons. All cells were loaded with fura-2 AM and then treated with either dopamine (10 μM) (**A**,**B**), BODIPY-dopamine (10 μM) and green light (**C**), BODIPY dopamine, light, and the dopamine receptor antagonist, (+)-butaclamol (100 μM) (**D**), or BODIPY-dopamine without light (**E**). Shown are changes in Fura-2 fluorescence as quantified from representative cells. Scale bar is 20 μm. **F**) Percentage of responding cells in each treatment, as quantified for the whole field of view (n > 17 cells in each treatment). Additional control experiments are provided in the Supporting Information.

In vitro characterization establish that the photocage shows good stability in the absence of light, but prompt release of dopamine following irradiation. (SI Figure S5). To examine the efficiency of m&m-BODIPY-dopamine, we again used fura-2 to monitor changes in $[Ca^{2+}]_i$ upon photo-induced release of dopamine. Mixed hippocampal/cortical rat neurons loaded with fura-2, sensitized with KCl, and treated with dopamine (10 μM) substantial fluxes in $[Ca^{2+}]_i$ in 88% of the cells, similar to those observed with histamine (Figure 4A,B,F, SI Movie 7). Incubation with m&m-BODIPY-dopamine (10 μM) followed by uncaging with 540 nm light resulted in similar oscillations of Ca^{2+} (51% of cells respond, Figure 4C,F, SI Movie 8), consistent with dopamine receptor agonism.[28] Importantly, control experiments established that hippocampal/cortical neuron activation by m&m-BODIPY-dopamine and light is abolished by the dopamine receptor antagonist (+)-butaclamol (100 μM Figure 4D, SI Movie 9), and that m&m-BODIPY-dopamine with no light (Figure 4E, SI Movie 10), light only (SI Figure S6A,B, Movie 11) or non-photolyzable m&m-BODIPY with light (SI Figure S6C,D, Movie 12), all result in baseline neuronal activity.

Conclusions

The incorporation of a methylhydroxy moiety at the *meso* position of BODIPY core transform this commonly used fluorophore into an effective, green-light excitable photo-labile protecting group. The introduced hydroxyl group can be straightforwardly conjugated to diverse chemical functionalities, enabling simple caging of molecules of interest through a variety of chemical bonds. Caging of 6 separate substrates, including phenol, aniline, a primary amine and acetic acid was readily accomplished within one or two synthetic steps and similar chemistry should be applicable for caging additional functional groups, including alcohols and thiols. These cageable functional groups are frequently represented in many biogenic and bioactive molecules. Uncaging from m&m-BODIPY was effectively

facilitated by green light for all caged functionalities, with small variations in release rate and yield, which seems to depend on the nature of the leaving group, as well as on the chemical bond connecting it to the BODIPY cage.

The bioactivity of two biogenic amines, histamine and dopamine, was successfully controlled in cell cultures and in neurons by biologically benign green light, demonstrating the biological relevance and practical application of this novel caging group. Finally, m&m-BODIPY's structure readily enables tuning of color to encompass the whole cyan-red range, as well as to determine cellular permeability or impermeability. Thus, we expect that the general strategy presented herein will enable expansion of the BODIPY cage to include these properties.

Notes and references

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