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Direct Photorelease of Alcohols from Boron-Alkylated BODIPY Photocages

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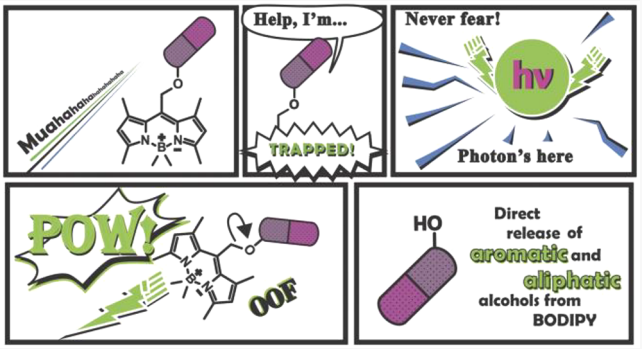
ABSTRACT: BODIPY photocages allow the release of substrates using visible light irradiation. They have the drawback of requiring reasonably good leaving groups for photorelease. Photorelease of alcohols is often accomplished by attachment with carbonate linkages, which upon photorelease liberate CO_2 and generate the alcohol. Here, we show that boron-alkylated BODIPY photocages are capable of directly photoreleasing both aliphatic alcohols and phenols upon irradiation via photocleavage of ether linkages. Direct photorelease of a hydroxycoumarin dye was demonstrated in living HeLa cells.



Photocages are light-sensitive chemical protecting groups that can be covalently linked to a substrate of interest, rendering it inactive. Upon irradiation, the bond is cleaved and the substrate is released, restoring its activity. Photocages are useful in studies that require the spatial and temporal control that can be provided by pulsed light irradiation. These include biological investigations of short-lived species, small molecules, and signaling agents;[1,2] targeted phototherapeutics;[3,4] and microarray synthesis.[5−8] Visible light-absorbing photocages have been an exciting new development as they allow for less toxic visible light irradiation in biological studies.[1,4,9−11] In particular, our group along with others have developed BODIPY photocages that can release leaving groups from the *meso* position after activation with single photons of green to near IR light.[12−18] Many biological targets (e.g., paclitaxel, dopamine) have phenolic or aliphatic alcohol functional groups that remain challenging targets for current photocages.

Previously, *meso*-substituted BODIPY compounds have been used to release alcohols and amines via carbonate or carbamate linkages (Figure 1).[13,15−17] While carbonate linkages permit photorelease of alcohols, they have several potential drawbacks. First, while they are easy to make in theory, carbonates can be less synthetically tractable compared to ether linkages. Moreover, they can, in principle, be cleaved by cellular esterases, which would yield undesirable background thermal deprotection, and in some cases, may be less hydrolytically stable than ethers. In addition, there is a required thermal decarboxylation step after photolysis, which interferes with the temporal control that photoactivation allows. Direct photorelease of alcohols from the corresponding BODIPY ethers would address these disadvantages.

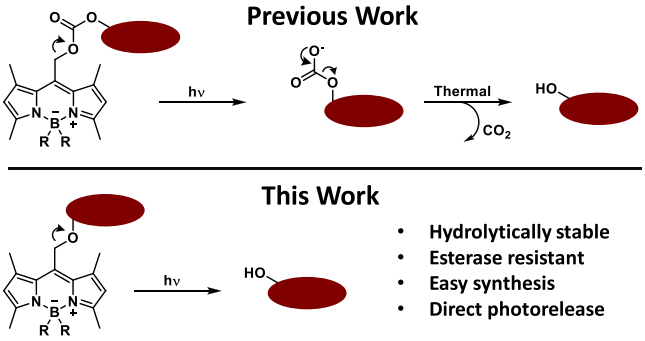


Figure 1. Photochemical reaction followed by thermal decarboxylation to release alcohols via a carbonate linker (top). Direct photochemical release of an alcohol via an ether linkage (bottom).

Recently, Weinstain and co-workers demonstrated the direct release of 2,4-dinitrophenol from a BODIPY photocage, which has a pK_a on par with acetic acid.[13] Our group along with Klan's and Weinstain's groups recently conducted a structure−reactivity investigation of BODIPY photocages and discovered that boron-alkylation leads to a large increase in quantum yields of photorelease for carboxylic acids (see **2-PAA** in

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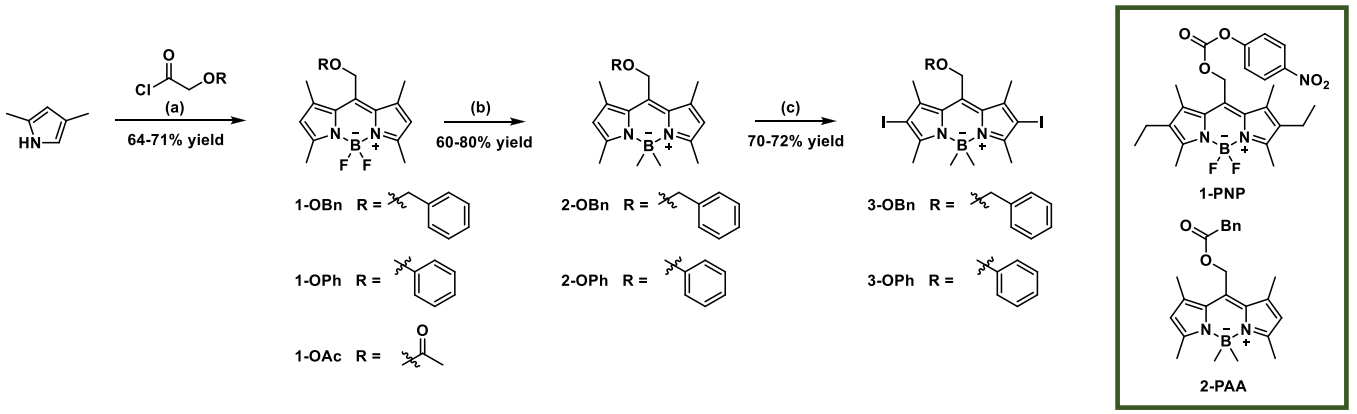
Scheme 1. Synthesis of BODIPY Compounds Ether Linkages from Acetyl Chlorides to Investigate the Release of Alcohols[a]



[a]Conditions: (a) DCM, reflux, triethyamine, boron trifluoride diethyl etherate; (b) DCM, methylmagnesium bromide, rt; (c) THF, *N*-iodosuccinimide, rt. Compound **1-PNP** is an example of a carbonate linkage previously published by Weinstain's group.[16] Compound **2-PAA** is an example of a B-methylated derivative published by our group in a collaborative study with Weinstain and Klan.[18]

Table 1. Photophysical Properties

	λ_{ex} (nm)	λ_{em} (nm)	$\varepsilon \times 10^4$ (M^{-1} cm^{-1})	Φ (%)	photorelease yield (%), (time (h))[e]	remaining ethers (time)	$\varepsilon\Phi$ (M^{-1} cm^{-1})
1-OAc[a]	517	529	7.1	0.099			70
2-PAA[b]	512	550	6.9	5.5 ± 0.02			3800
1-PNP[c]	547		4.8	0.16	>95		77
1-OBn[d]	514	528	6.9	0.10 ± 0.02	12 (20)	83 (20)	69
1-OPh[d]	516	529	6.6	0.38 ± 0.04	30 (20)	56 (20)	251
2-OBn[d]	510	528	5.2	1.0 ± 0.5	71 (24)	0 (24)	340
2-OPh[d]	511	529	5.1	1.0 ± 0.1	65 (24)	0 (24)	510
3-OBn[d]	535	547	5.7	0.15 ± 0.03	32 (4)	0 (4)	86
3-OPh[d]	537	560	6.1	0.8 ± 0.1	28 (6)	0 (6)	104

[a]Values taken from a previous study; quantum yields were calculated in methanol.[12] [b]Values taken from a previous study; quantum yield was calculated in methanol.[18] [c]Values taken from a previous study; quantum yields were calculated in a pH 7.4 aqueous solution with 5% acetonitrile.[16] [d]Compounds synthesized in this study; quantum yields were calculated in 1:1 $CDCl_3/CD_3OD$ using quantitative NMR to follow the release of the substrate using dimethyl sulfone as an internal standard and **1-OAc** as the actinometer. [e]Chemical yields and remaining ether were determined by irradiating NMR tubes with 1 mL of 2 mM solutions of substrates dissolved in 1:1 $CDCl_3/CD_3OD$ with a 500 W halogen lamp. Dimethyl sulfone was used as an internal standard for quantitative NMR.

Scheme 1 and Table 1).[18] We thus considered the possibility that these improved structures might be able to achieve direct photorelease of alcohols. We synthesized BODIPY photocages (Scheme 1) with benzyl and phenyl ethers in the meso position in order to investigate their ability to release aliphatic alcohols and phenols, respectively, upon excitation with visible light.

1-OBn and **1-OPh** were formed in a one-pot synthesis first refluxing 2,4-dimethylpyrrole in dry DCM with benzyloxyacetyl chloride for **1-OBn** or phenoxyacetyl chloride for **1-OPh**. The reaction was then cooled to room temperature, and triethylamine and boron trifluoride diethyl etherate were added sequentially. **1-OBn** and **1-OPh** were transformed into **2-OBn** and **2-OPh** by reaction with excess methylmagnesium bromide. *N*-Iodosuccinimide was used to iodinate the compounds to generate **3-OBn** and **3-OPh**. These photocages have high extinction coefficients (>50 000 M^{-1} cm^{-1}) and λ_{max} values > 500 nm (Table 1). Photocages were irradiated with a 500 W Halogen lamp, and release of phenol or benzyl alcohol occurred, as demonstrated by following the photoreaction by ^1H NMR (Table 1, Figure S1 and S2). Reactivity of the fluorinated derivatives was slow, giving less than 50% conversion when irradiated for 20 h. Interestingly, the methylated derivatives **2-OBn** and **2-OPh** seemed to initially release their respective alcohols quickly; however, over time, the rate of release slowed down (Figures S8 and S9). This may be due to photolability of the methyl groups on the boron, which we have seen in other studies.[19]

Due to low solubility in methanol, a 1:1 mixture with a cosolvent was necessary to dissolve the substrates in this study. Interestingly, in solutions of **2-OBn** dissolved using acetonitrile or DMSO as cosolvents with methanol, benzaldehyde was observed in the NMR after irradiation with a 532 nm Nd:YAG laser. However, benzaldehyde formation was not detected with either a 500 W halogen lamp or a low-intensity green LED (Figure S5). Additionally, benzaldehyde was not detected when **2-OBn** was irradiated with the 532 nm laser using chloroform as the cosolvent (Table S1). The mechanism of this oxidation is currently unknown, and it is unclear why benzaldehyde is only present under certain conditions. To avoid this curiosity, quantum yields were determined using chloroform as the cosolvent for the purpose of this study.

To determine quantum yields of photorelease for the compounds of this study, samples dissolved in 50:50 $CDCl_3/CD_3OD$ were irradiated with a 532 nm Nd:YAG laser. The amount of release over time was calculated using quantitative NMR with dimethyl sulfone as an internal standard and using **1-OAc** as the actinometer (Φ = 0.099%).[12]

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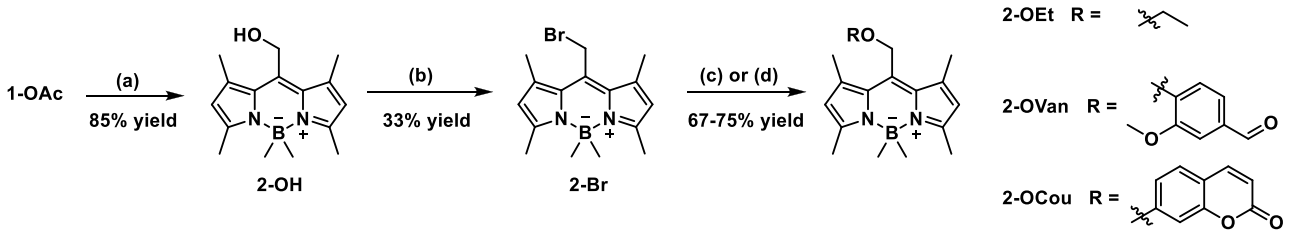
Scheme 2. Synthesis of BODIPY Ethers with Williamson Ether Synthesis From 2-Br[a]



[a]Conditions: (a) DCM, methylmagnesium bromide, rt; (b) DCM, pyridine, phosphorous tribromide, 0 °C; (c) ACN, potassium carbonate, rt; (d) ACN, cesium carbonate, rt.

The boron-fluorinated ethers surprisingly have decent quantum yields of photorelease in a 50:50 methanol/chloroform mixture (Table 1). Upon methylating the boron of the BODIPY ethers, the quantum yield of photorelease increases 2.5−10-fold compared to the corresponding boron-fluorinated ethers. We hypothesized that appending iodines to the BODIPY core would lead to a further increase in the quantum yield by promoting intersystem crossing to a longer-lived triplet excited state. This strategy was previously shown to be effective for increasing the photorelease quantum yields for carboxylic acids.[12,17,18] Curiously, instead of increased quantum yields of release, we observed a lower quantum yield of release and an accelerated rate of photodecomposition compared to the uniodinated derivatives (Figure S6).

Direct photorelease of alcohols with a visible light photoremovable protecting group is an exciting avenue for studies of biologically relevant alcohols or as protecting groups for multistep syntheses. There are three obvious synthetic methods for attaching alcohols to the meso position of BODIPY. First, as used for the synthesis of **1-OBn** and **1-OPh**, the phenoxy or alkyloxy acid chloride could be used for the BODIPY synthesis (Scheme 1). This method is useful for simple alcohols that are readily available. However, it may not be practical to make the acid chloride from sensitive or expensive alcohols. Another potential method of attaching alcohols to the meso BODIPY is via Williamson ether synthesis, using the BODIPY alcohol as the nucleophile and having the desired alcohol replaced with a good leaving group.[13] While this is a reasonable approach, it would be difficult to use this method for precious materials, which are not available with leaving groups in the appropriate positions.

The most generally useful method is to perform a Williamson ether synthesis using the alcohol of interest as the nucleophile. We found that the addition of alkoxides to boron-fluorinated BODIPY can lead to decomposition of the starting material with very low or no yield. However, first substituting the boron with methyl groups allowed this reaction to occur with fair yields (Scheme 2).[20] **1-OAc** was converted to **2-OH** using excess methylmagnesium bromide, which acted to both methylate the boron and hydrolyze the acetyl group. Then, **2-Br** was synthesized by reacting **2-OH** with phosphorus tribromide at 0 °C. The reaction generally occurred with a 10−33% yield accompanied by decomposition. When pyridine is included in the reaction, the yield increased slightly to 38%, and we were able to recover unreacted **2-OH**. Due to low yields of the bromination, we chose to use excess alcohol for the Williamson Ether synthesis in order to conserve **2-Br**. For the phenolic compounds, potassium carbonate was used as the base. However, under these conditions, no reaction was observed between **2-Br** and ethanol after 12 h. When cesium carbonate was used instead, the reaction was complete after stirring overnight. Using the alcohols in excess afforded fair (67−75%) yields of **2-OEt**, **2-OVan**, and **2-OCou** under mild conditions. When *p*-vanillin was reacted with a small excess of **2-Br**, **2-OVan** was achieved in a slightly lowered yield (54%). **2-OEt** and **2-OVan** released ethanol and vanillin, respectively, upon irradiation with white light (Figures S3 and S4). Preliminary data suggests that attachment and photorelease of secondary alcohols may be possible (Scheme S1, Figures S10 and S11).

2-OCou was used as a proof of concept to demonstrate the viability for direct photorelease of alcohols in biological studies. The fluorescence of 7-hydroxycoumarin (**4**) is known to be highly dependent on substitutions on the hydroxy group. The protected coumarin (**2-OCou**) should have a diminished fluorescence, which is recovered after irradiation. HeLa cells were incubated with **2-OCou** and irradiated. Cells irradiated with a green light for intervals showed an increase in fluorescence at 450 nm using short 365 nm excitation (Figure S7). In order to obtain the best signal-to-noise ratio, the cells were irradiated with 350 nm light. This doubled as the activation wavelength for BODIPY, as well as the excitation wavelength for 7-hydroxycoumarin so the fluorescence could be collected continuously at 450 nm (Figure 2). An increase in fluorescence followed by photobleaching was observed for irradiation of cells incubated with

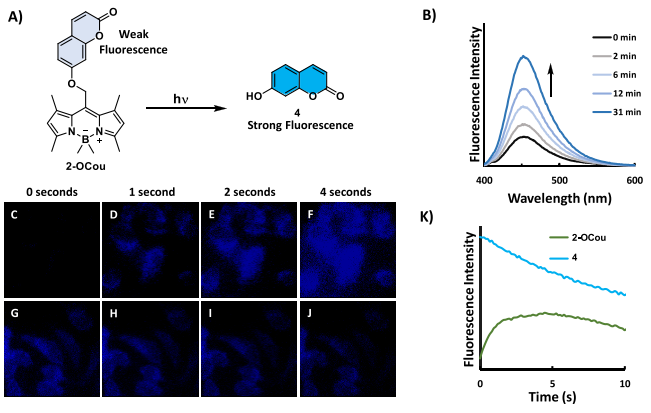


Figure 2. (A) Scheme of **2-OCou**, which has a fluorescence increase upon photorelease of **4**. (B) Fluorescence increase in imaging buffer following the photorelease of 7-hydroxycoumarin upon irradiation with a green LED. (C−F) Fluorescence images of HeLa cells incubated with 25 μM compound **2-OCou** and continuously irradiated with 350 nm light while collecting 450 nm emission. (G−J) Fluorescence images using 25 μM of **4** as a control. (K) Fluorescence intensity with continuous irradiation of cells incubated with **2-OCou** and **4**.

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2-OCou, while only a decrease in fluorescence (photobleaching) was measured in a control experiment irradiating cells incubated only with 7-hydroxycoumarin. These studies indicate that the use of ethers as a linkage to directly photorelease alcohols is a viable strategy in biological systems.

In conclusion, the synthesis of meso-substituted BODIPY ethers was demonstrated, and direct photorelease of the corresponding alcohols has been reported. Swapping the fluorines on the boron for methyls effectively increases the quantum yield of release, while iodination does not. The Williamson ether synthesis between **2-Br** and an alcohol of interest may be a good path for making these ethers, but more work needs to be done to find a better path for the synthesis of **2-Br** itself. Practical use was demonstrated by direct photorelease of the fluorescent dye 7-hydroxycoumarin in living HeLa cells.

EXPERIMENTAL SECTION

General Information. Unless otherwise stated, all purchased chemicals were used without further purification. Solvents were dried for 3 days over activated 4 Å molecular sieves. Compound **1-OAc** was prepared as previously reported.[21] All reactions were done in the dark.

Light Sources. Irradiation with white light was carried out using a Utilitech brand 500 W model no. MPL1025-C500 K9030 halogen work lamp. A 500 mL beaker filled with water was placed in front of the lamp, and a fan was blown on the lamp and the sample to prevent overheating. Samples were irradiated in NMR tubes approximately 25 cm away from the light source.

Irradiation with a green LED was carried out using an Luzchem EXPO-LED photoreactor equipped with 5 LED-GR (4 W) lamps. The photoreactor was placed on its side, and samples were irradiated in NMR tubes approximately 10 cm away from the light source.

Irradiation with a green laser was carried out using a Nd:YAG laser equipped with a 532 nm crystal. Samples were irradiated in quartz cuvettes equipped with stir bars.

Procedure for Determination of Quantum Yields. BODIPY compounds (2−20 mg) were dissolved in 5 mL of deuterated chloroform. The solutions were spiked with a known amount of dimethylsulfone as an internal standard and diluted to 10 mL with deuterated methanol. The solutions were checked to ensure that they had an absorbance of greater than 2 at 532 nm. Three mL of the solutions were transferred to quartz cuvettes and irradiated with a ND:YAG 532 nm laser under air. At varying time intervals of irradiation, 0.6 mL of the samples were transferred to NMR tubes, and ^{1}H NMR spectra were obtained. The solutions were then returned to the cuvettes for further irradiation. Photorelease was monitored at six time points for each compound by ^{1}H NMR, following the growth of the leaving group. The concentration of the released compound was calculated using the internal standard, and the quantum yield was calculated using **1-OAc** (8-acetoxymethyl-1,3,5,7-tetramethyl pyrromethene fluoroborate) as the actinometer. **1-OAc** was irradiated in the same manner as the other photocages.

*General Procedure for the Synthesis of **1-OBn** and **1-OPh**.* To a solution of 2,4-dimethylpyrrole (2 equiv) stirring in 3 mL of dry dichloromethane in a two-neck flask equipped with a condenser under argon was added 1 equiv of acid chloride. The mixture was stirred at reflux in an oil bath for 2 h and turned dark red. The mixture was cooled to room temperature, and triethylamine was added followed by boron trifluoride diethyl etherate. The solvent was reduced under a vacuum to give a dark oily liquid. Methanol was added to precipitate the product, which was then filtered and washed with methanol until the solid was bright orange.

*8-Phenoxymethyl-1,3,5,7-tetramethyl Pyrromethene Fluoroborate (**1-OPh**).* The compound was obtained from 2,4-dimethyl pyrrole (400 μL, 3.9 mmol, 2 equiv) and phenoxyacetyl chloride (270 μL, 1.95 mmol, 1 equiv) as a bright orange solid in 73% yield (511 mg): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34 (t, J = 6 Hz, 2H), 7.04 (t, J = 6 Hz, 1H), 6.98 (d, J = 6 Hz, 2H), 6.07 (s, 2H), 5.15 (s, 2H), 2.54 (s, 6H), 2.30 (s, 6H); ^{13}C{^{1}H} NMR (93 MHz, $CDCl_3$) δ 158.3, 156.5, 141.7, 134.1, 133.0, 129.8, 122.1, 121.7, 114.1, 60.7, 15.4, 14.7; HRMS (ESI/QTOF) m/z [M + H]$^+$ calcd for $C_{20}H_{21}BF_2N_2O$ 355.1793, found 355.1787.

*8-Benzoxymethyl-1,3,5,7-tetramethyl Pyrromethene Fluoroborate (**1-OBn**).* The compound was obtained from 2,4-dimethyl pyrrole (400 μL, 3.9 mmol, 2 equiv) and benzyloxyacetyl chloride (310 μL, 1.95 mmol, 1 equiv) as a bright orange solid in 64% yield (447 mg): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31−7.37 (m, 5 H), 6.04 (s, 2 H), 4.65 (s, 2 H), 4.61 (s, 2 H), 2.52 (s, 6 H), 2.31 (s, 6 H); ^{13}C{^{1}H} NMR (93 MHz, $CDCl_3$) δ 155.9, 141.9, 137.1, 136.2, 133.1, 128.6, 128.4, 128.3, 122.0, 73.1, 63.1, 15.4; HRMS (ESI/QTOF) m/z [M + H]$^+$ calcd for $C_{21}H_{23}BF_2N_2O$ 369.1944, found 369.1952.

General Procedure for Methylation of BODIPY Etherate Photocages. To a solution of **1-OBn** or **1-OPh** in 5 mL of dry dichloromethane was added 11 equiv of methylmagnesium bromide. The solutions were stirred for 1 h, quenched with ammonium chloride, washed 3 times with water and once with brine, and dried over sodium sulfate. The solvent was removed under a vacuum, and the mixtures were purified as listed below.

*1,3,5,7-Tetramethyl-8-phenoxymethyl Pyrromethene Methylborate (**2-OPh**).* The compound was obtained from **1-OPh** (100 mg, 0.28 mmol, 1 equiv) and methylmagnesium bromide (1 mL 3 M solution in THF, 3.0 mmol, 11 equiv). The crude solid was purified with silica gel column chromatography using 90:10 hexanes/methylene chloride as the eluent to give the product as a bright orange solid in 80% yield (75 mg): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36 (t, J = 8 Hz, 2H), 7.05 (t, J = 8 Hz, 2H), 7.00 (d, J = 8 Hz, 2H), 5.20 (s, 2H), 2.56 (s, 6H), 2.35 (s, 6H), 0.22 (s, 6H); ^{13}C{^{1}H} NMR (93 MHz, $CDCl_3$) δ 158.5, 153.1, 137.4, 134.2, 131.6, 129.8, 122.6, 121.5, 114.2, 61.3, 16.7, 15.8, 1.2; HRMS (ESI/QTOF) m/z [M + H]$^+$ calcd for $C_{22}H_{27}BN_2O$ 346.2295, found 347.2288.

*8-Benzoxymethyl-1,3,5,7-tetramethyl Pyrromethene Methylborate (**2-OBn**).* The compound was obtained from **1-OBn** (100 mg, 0.27 mmol, 1 equiv) and methylmagnesium bromide (1 mL 3 M solution in THF, 3.0 mmol, 11 equiv). The crude solid was purified with silica gel column chromatography using 90:10 hexanes/methylene chloride as the eluent to give the product as a bright orange solid in 60% yield (60 mg): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 (m, 5H), 6.09 (s, 2H), 4.77 (s, 2H), 4.65 (s, 2H), 2.49 (s 6H), 2.40 (s, 6H), 0.24 (s, 6H); ^{13}C{^{1}H} NMR (93 MHz, $CDCl_3$) δ 152.5, 137.4, 137.3, 136.1, 131.7, 128.6, 128.5, 128.2, 122.4, 73.4, 63.9, 16.7, 15.7; HRMS (ESI/QTOF) m/z [M + H]$^+$ calcd for $C_{23}H_{29}BN_2O$ 361.2446, found 361.2454.

General Iodination Procedure. To a solution of **2-OBn** or **2-OPh** dissolved in 10 mL of dry THF was added 3 equiv of *N*-iodosuccinimide. The solution was stirred until the color changed to dark pink, after which dichloromethane and water were added. The organic layer was washed with water three times and dried over sodium sulfate. The solvent was removed under a vacuum, and the crude product was purified as listed below.

*8-Benzoxymethyl-2,6-diiodo-1,3,5,7-tetramethyl pyrromethene methylborate (**3-OBn**).* The compound was obtained from **2-OBn** (50 mg, 0.14 mmol, 1 equiv) and *N*-iodosuccinimide (95 mg, 0.42 mmol, 3 equiv). The crude product was purified with silica gel column chromatography using hexanes to a 90:10 hexanes/dichloromethane gradient as the eluent to give the product as a red solid in 70% yield (59 mg): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35 (m, 5 H), 4.73 (s, 2H), 4.63 (s, 2H), 2.52 (s, 6H), 2.42 (s, 6H), 0.17 (s, 6H); ^{13}C{^{1}H} NMR (93 MHz, CDCl3) δ 153.2, 139.9, 136.8, 135.2, 131.6, 128.7, 128.7, 128.5, 87.1, 73.7, 64.5, 18.5, 18.0, 1.2; HRMS (ESI/QTOF) m/z [M − H]$^-$ calcd for $C_{22}H_{25}BI_2N_2O$ 597.0077, found 597.0059.

*2,6-Diiodo-1,3,5,7-tetramethyl-8-Phenoxymethyl pyrromethene methylborate (**3-OPh**).* The compound was obtained from **2-OPh** (50 mg, 0.14 mmol, 1 equiv) and *N*-iodosuccinimide (95 mg, 0.42 mmol, 3 equiv). The crude product was purified with silica gel column chromatography using hexanes to a 90:10 hexanes/dichloromethane gradient as the eluent to give the product as a red solid in 72% yield (62 mg): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36 (dd, J = 8, 7.6 Hz, 2H), 7.06 (t, J = 7.6 Hz, 1H), 7.00 (d, J = 8 Hz, 2H), 5.20 (s, 2H), 2.56 (s,

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6H), 2.35 (s, 6H), 0.23 (s, 6H); $^{13}C\{^1H\}$ NMR (93 MHz, $CDCl_3$) δ 158.0, 153.7, 139.8, 133.3, 131.4, 129.9, 121.8, 114.1, 87.3, 61.8, 18.4, 18.1, 1.1; HRMS (ESI/QTOF) m/z $[M + H]^+$ calcd for $C_{23}H_{27}BI_2N_2O$ 613.0379, found 613.0380.

*8-Hydroxymethyl-1,3,5,7-tetramethyl pyrromethene methylborate (**2-OH**).* To a solution of **1-OAc** (100 mg, 0.31 mmol, 1 equiv) dissolved in dichloromethane was added 15 equiv of methyl magnesium bromide (4.7 mL 1 M solution in diethyl ether, 4.7 mmol, 15 equiv). The solution was stirred for 1 h, after which the reaction was complete by TLC. The reaction was quenched with ammonium chloride, and ethyl acetate was added. The organic layer was washed 3 times with ammonium chloride, with brine, and dried over sodium sulfate. The solvent was reduced under a vacuum, and the product was purified via silica gel column chromatography using methylene chloride as the eluent to give **2-OH** in an 85% yield (71 mg). Characterization matched those previously reported.[21]

*8-Bromomethyl-1,3,5,7-tetramethyl pyrromethene methylborate (**2-Br**).* To a solution of **2-OH** (100 mg, 0.37 mmol, 1 equiv) stirring in 5 mL of dry dichloromethane at 0 °C was added pyridine (60 μL, 0.74 mmol, 2 equiv), followed by phosphorus tribromide (50 μL, 0.55 mmol, 1.5 equiv). The solution was stirred for 30 min, after which it had turned dark red. Ice water was added, and the organic layer was extracted with dichloromethane. The organic layer was washed with saturated sodium carbonate, saturated ammonium chloride, and brine. The organic layer was dried over sodium sulfate, and the solvent was removed under a vacuum. The mixture was purified with column chromatography on silica gel using 90:10 hexanes/dichloromethane as the eluent to give the product as a red-orange solid in a 38% yield (46 mg): ^1H NMR (400 MHz, $CDCl_3$) δ 6.09 (s, 2H), 4.77 (s, 2H), 2.58 (s, 6H), 2.46 (s, 6H), 0.21 (s, 3H), 0.15 (s, 3H); $^{13}C\{^1H\}$ NMR (93 MHz, $CDCl_3$) δ 153.2, 137.6, 136.7, 129.6, 122.9, 26.1, 16.7, 16.5; HRMS (ESI/QTOF) m/z $[M + H]^+$ calcd for $C_{16}H_{22}BN_2Br$ 333.1132, found 333.1133.

*8-Ethoxymethyl-1,3,5,7-tetramethyl Pyrromethene Methylborate (**2-OEt**).* To a solution of 1 mL of ethanol in 5 mL of acetonitrile with stirring with cesium carbonate (5 mg, 0.015 mmol, 1 equiv) was added **2-Br** (5 mg, 0.015 mmol, 1 equiv). The solution was stirred at room temperature overnight. The solvent was evaporated, and the residue was dissolved in dichloromethane and washed with water. The organic layer was dried over sodium sulfate, and the solvent was removed under a vacuum. The residue was purified by silica gel chromatography using 50:50 hexanes/dichloromethane as the eluent to give the product as an orange solid in 67% yield (3 mg): ^1H NMR (400 MHz, $CDCl_3$) δ 6.06 (s, 2H), 4.65 (s, 2H), 3.63 (t, J = 7 Hz, 2H), 2.44 (s, 12H), 1.30 (t, J = 7 Hz, 3H), 0.17 (s, 6H); $^{13}C\{^1H\}$ NMR (93 MHz, $CDCl_3$) δ 152.4, 137.3, 136.0, 131.6, 122.1, 66.5, 64.2, 29.7, 15.6, 15.3, 1.0; HRMS (ESI/QTOF) m/z $[M + H]^+$ calcd for $C_{18}H_{27}BN_2O$ 299.2289, found 299.2299.

***2-OVan*.** (a) Using vanillin in excess, to a solution of **2-Br** (15 mg, 0.04 mmol, 1 equiv) stirring in 5 mL of dry acetonitrile was added *p*-vanillin (7 mg, 0.046 mmol, 1.1 equiv), followed by potassium carbonate (10 mg, 0.07 mmol, 1.8 equiv). The solution was stirred overnight, after which **2-Br** had been consumed by TLC. The mixture was diluted with water and extracted three times with dichloromethane. The combined organic layers were dried over sodium sulfate, and the solvent was removed under a vacuum. The crude mixture was purified via silica gel column chromatography using 20:80 dichloromethane/hexanes as the eluent to give the product as a pink-orange solid in a 75% yield (12 mg). (b) Using **2-Br** in excess, the procedure above was used with 40 mg of **2-Br** (0.12 mmol, 1.2 equiv), 15 mg of *p*-vanillin (0.1 mmol, 1 equiv), and potassium carbonate (20 mg, 0.14 mmol, 1.4 equiv). The above work up gave the product in a 54% yield (26 mg): ^1H NMR (400 MHz, $CDCl_3$) δ 9.89 (s, 2H), 7.50 (dd, J = 8, 2 Hz, 1H), 7.45 (d, J = 2 Hz, 1H), 7.11 (d, J = 8 Hz, 1H), 6.07 (s, 2H), 5.31 (s, 2H), 3.89 (s, 3H), 2.47 (s, 6H), 2.28 (s, 6H), 0.21 (s, 6H); $^{13}C\{^1H\}$ NMR (93 MHz, $CDCl_3$) δ 191.0, 153.5, 153.4, 150.2, 137.4, 132.9, 131.9, 131.0, 126.6, 122.8, 111.6, 109.8, 62.0, 56.2, 16.8, 15.7, 1.2; HRMS (ESI/QTOF) m/z $[M + H]^+$ calcd for $C_{24}H_{29}BN_2O_3$ 404.238, found 404.2375.

***2-OCou*.** To a solution of 7-hydroxycoumarin (20 mg, 0.12 mmol, 1.7 equiv) stirring in 5 mL of acetonitrile was added potassium carbonate (10 mg, 0.07 mmol, 1 equiv), followed by **2-Br** (24 mg, 0.07 mmol, 1 equiv). The solution was stirred until the starting material was consumed by TLC, after which it was diluted with water and ethyl acetate. The organic layer was washed with water 10 times to remove unreacted 7-hydroxycoumarin. The organic layer was dried over sodium sulfate, and the solvent was removed under a vacuum. The crude product was purified via silica gel column chromatography using dichloromethane as the eluent to give the product as a pink solid in 69% yield (20 mg): ^1H NMR (400 MHz, $CDCl_3$): δ 7.67 (d, J = 8 Hz, 1H), 7.43 (d, J = 8, 1H), 6.96 (m, 2H), 6.30 (d, J = 8 Hz, 1H), 6.09 (s, 2H), 5.27 (s, 2H), 2.48 (s, 6H), 2.29 (s, 6H), 0.22 (s, 6H); $^{13}C\{^1H\}$ NMR (140 MHz, $CDCl_3$) δ 161.6, 161.1, 156.1, 153.6, 143.4, 137.2, 132.8, 131.6, 129.3, 122.9, 113.8, 112.9, 101.3, 62.3, 16.8, 15.9, 1.2; HRMS (ESI/QTOF) m/z $[M + H]^+$ calcd for $C_{25}H_{27}BN_2O_3$ 415.2187, found 415.2197.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.joc.0c00044.

^1H NMR of irradiated samples, additional information on cell studies, formation of benzaldehyde, percent release studies, and characterization data of all new compounds including ^1H NMR, $^{13}C\{^1H\}$ NMR, and HRMS (PDF)

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Bardhan, A.; Deiters, A. Development of photolabile protecting groups and their application to the optochemical control of cell signaling. *Curr. Opin. Struct. Biol.* **2019**, *57*, 164−175.

(2) Stanton-Humphreys, M. N.; Taylor, R. D. T.; McDougall, C.; Hart, M. L.; Brown, C. T. A.; Emptage, N. J.; Conway, S. J. Wavelength-orthogonal photolysis of neurotransmittersin vitro. *Chem. Commun.* **2012**, *48* (5), 657−659.

(3) Toupin, N. P.; Arora, K.; Shrestha, P.; Peterson, J. A.; Fischer, L. J.; Rajagurubandara, E.; Podgorski, I.; Winter, A. H.; Kodanko, J. J. BODIPY-Caged Photoactivated Inhibitors of Cathepsin B Flip the Light Switch on Cancer Cell Apoptosis. *ACS Chem. Biol.* **2019**, *14* (12), 2833−2840.

(4) Sarode, B. R.; Kover, K.; Friedman, S. H. Visible-Light-Activated High-Density Materials for Controlled in Vivo Insulin Release. *Mol. Pharmaceutics* **2019**, *16* (11), 4677−4687.

(5) Alexandre Specht, F. B.; Omran, Ziad; Jean-François, Nicoud; Goeldner, M. Photochemical tools to study dynamic biological processes. *HFSP J.* **2009**, *3* (4), 255−264.

(6) Haitao Yu, J. L.; Wu, D.; Qiu, Z.; Zhang, Y. Chemistry and biological applications of photo-labile organic molecules. *Chem. Soc. Rev.* **2010**, *39*, 464−473.

(7) Li, W.-h.; Zheng, G. Photoactivatable fluorophores and techniques for biological imaging applications. *Photochem. Photobiol. Sci.* **2012**, *11*, 460.

(8) 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*, 119−191.

(9) Shembekar, V. R.; Chen, Y.; Carpenter, B. K.; Hess, G. P. A Protecting Group for Carboxylic Acids That Can Be Photolyzed by Visible Light. *Biochemistry* **2005**, *44* (19), 7107−7114.

(10) Olson, J. P.; Banghart, M. R.; Sabatini, B. L.; Ellis-Davies, G. C. R. Spectral Evolution of a Photochemical Protecting Group for Orthogonal Two-Color Uncaging with Visible Light. *J. Am. Chem. Soc.* **2013**, *135* (42), 15948−15954.

(11) 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), 14153−14159.

(12) Goswami, 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*, 3783−3786.

(13) 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*, 4659−4663.

(14) Palao, E.; Slanina, T.; Muchova, L.; Solomek, T.; Vitek, L.; Klan, P. Transition-Metal-Free CO-Releasing BODIPY Derivatives Activatable by Visible to NIR Light as Promising Bioactive Molecules. *J. Am. Chem. Soc.* **2016**, *138* (1), 126−133.

(15) 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*, 7343−7346.

(16) Rubinstein, N.; Liu, P.; Miller, E. W.; Weinstain, R. meso-Methylhydroxy BODIPY: a scaffold for photo-labile protecting groups. *Chem. Commun.* **2015**, *51*, 6369−6372.

(17) Sitkowska, K.; Feringa, B. L.; Szymański, W. Green-Light-Sensitive BODIPY Photoprotecting Groups for Amines. *J. Org. Chem.* **2018**, *83* (4), 1819−1827.

(18) Slanina, T.; Shrestha, P.; Palao, E.; Kand, D.; Peterson, J. A.; Dutton, A. S.; Rubinstein, N.; Weinstain, R.; Winter, A. H.; Klan, P. In Search of the Perfect Photocage: Structure-Reactivity Relationships in *meso*-Methyl BODIPY Photoremovable Protecting Groups. *J. Am. Chem. Soc.* **2017**, *139*, 15168−15175.

(19) Wijesooriya, C. S.; Peterson, J. A.; Shrestha, P.; Gehrmann, E. J.; Winter, A. H.; Smith, E. A. A Photoactivatable BODIPY Probe for Localization-Based Super-Resolution Cellular Imaging. *Angew. Chem., Int. Ed.* **2018**, *57* (39), 12685−12689.

(20) More, A. B.; Mula, S.; Thakare, S.; Sekar, N.; Ray, A. K.; Chattopadhyay, S. Masking and Demasking Strategies for the BF2−BODIPYs as a Tool for BODIPY Fluorophores. *J. Org. Chem.* **2014**, *79* (22), 10981−10987.

(21) Krumova, K.; Cosa, G. Bodipy Dyes with Tunable Redox Potentials and Functional Groups for Further Tethering: Preparation, Electrochemical, and Spectroscopic Characterization. *J. Am. Chem. Soc.* **2010**, *132* (49), 17560−17569.

https://dx.doi.org/10.1021/acs.joc.0c00044
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