Communication

A General Strategy for Visible-Light Decaging Based on the Quinone Trimethyl Lock

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A General Strategy for Visible-Light Decaging Based on the Quinone Trimethyl Lock

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Supporting Information Placeholder

ABSTRACT: Visible-light triggered quinone trimethyl locks are reported as a general design for long-wavelength photoremovable protecting groups for alcohols and amines. Intramolecular photoreduction unmasks a reactive phenol that undergoes fast lactonization to release alcohols and amines. Model substrates are released in quantitative yield along with well-defined, colorless hydroquinone byproducts. Substituent modifications of the quinone core allow absorption from 400-600 nm.

The trimethyl lock (TML) is a highly modular motif that has found many applications[1] as a protecting group for alcohols and amines (Figure 1). Once a phenolic oxygen is revealed, rapid cyclization of the propionic ester or amide occurs, releasing **HX** in seconds (HX = HOR) to minutes (HX = HNR_2) in a process often referred to as decaging.[2] The reaction is quite general, and scores of examples are found in the literature. The phenol is frequently part of a hydroquinone, and chemical, enzymatic, and electrochemical reductions of the corresponding quinone have been used to initiate the trimethyl lock (Figure 1). Photochemical initiation of the trimethyl lock, however, has not been reported, except for the trivial case of nitrobenzyl decaging of a phenol using UV light.[1-18]

We describe here a photochemically-initiated trimethyl lock system that operates well into the visible range and has the potential for extension into the near-infrared. Decaging reactions at longer wavelengths are rare and would be useful in many contexts.[19-29] Importantly, *the photochemical step and the trimethyl lock decaging are completely distinct processes.* An intramolecular photochemical quinone reduction is followed by a *thermal* trimethyl lock reaction. As such, this system innately spans the broad substrate applicability of the trimethyl lock reaction. In addition, the photochemical step can be optimized separately regarding quantum yields, wavelength, etc. without impacting the decaging step.

There are many examples of intermolecular photochemical reductions of quinones[30-34], and several variants where an appended amine[28, 35-41] or sulfide[42-44] mediates the photochemical reduction. As such, we considered compounds such as **1**, in which a photochemical reduction would launch the trimethyl lock process. We describe here the synthesis and photochemistry of variants of **1**.

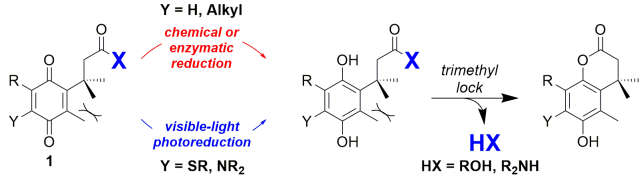
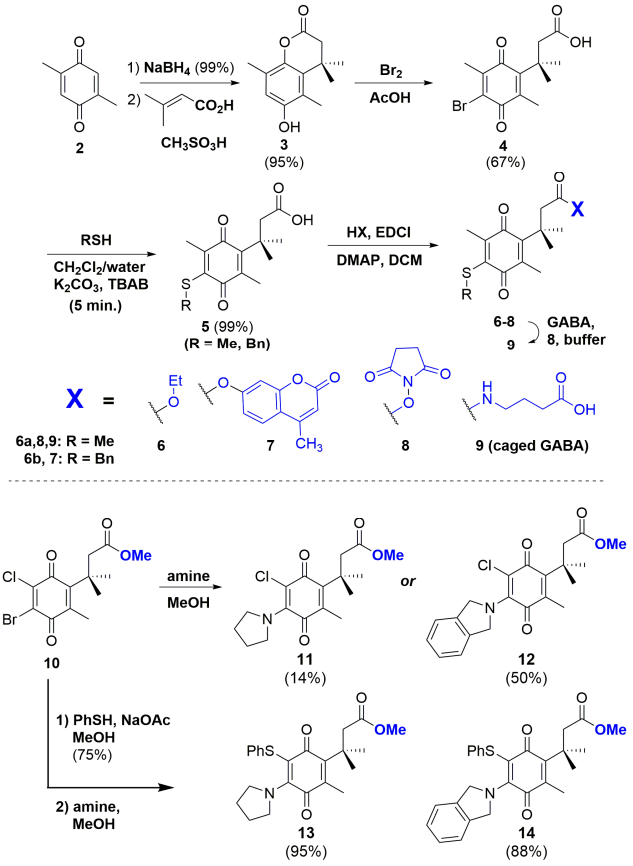


Figure 1. Decaging strategies for the quinone trimethyl lock.

Our initial approach to compounds such as **1** is outlined in Scheme 1. The bromoquinone acid intermediate **4** is quickly obtainable in gram quantities as a crystalline solid without the need for chromatography. Sulfide substituents were then introduced by conjugate addition to **4** (or esters of **4**) in a reaction that is rapid at room temperature under mild conditions and is highly selective for displacement of the bromine. Immediate Steglich esterification affords the methyl, ethyl, and N-hydroxysuccinimide (NHS) esters. Chromatography is only required after esterification, and simply involves collecting the yellow band that elutes. γ-Aminobutyric acid was caged by reaction of the NHS ester in acetonitrile / bicarbonate buffer with the free amino acid.

Scheme 1: Synthesis of Quinone TML Derivatives 7-12.



Surprisingly, conjugate addition of amines was unsuccessful for substrate **4** or **5**. Amine derivatives **11-14** were instead synthetized from **10**, prepared from 2-chloro-5-methylphenol (see SI). Yields of the desired substitution pattern, with the amine para to

the trimethyl lock chain, were increased by first treating **10** with thiophenol before addition of amine to give **13** and **14**.

The UV-vis spectra of compounds **6-9** and **11-14** are similar, with charge transfer absorptions in the visible (Figure 2). In both systems, the absorption bands are broad enough that efficient and clean photolysis is possible at wavelengths both below and well beyond λ_{max}. Irradiation with visible light (300 W Hg arc lamp with >400 nm glass filter) leads to clean and efficient photolysis for both compounds. In practice, a simple 455 nm 1 watt portable LED is suitable for photolysis of both amine and sulfide derivatives. For the amines, a longer-wavelength, 565 nm, 1 watt LED was also practical, allowing for orthogonal decaging (see below).

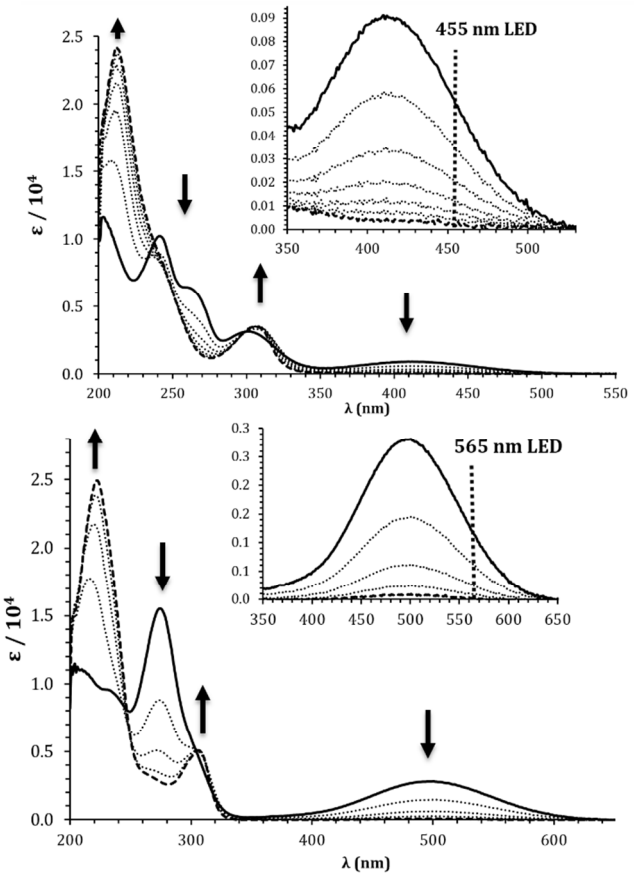
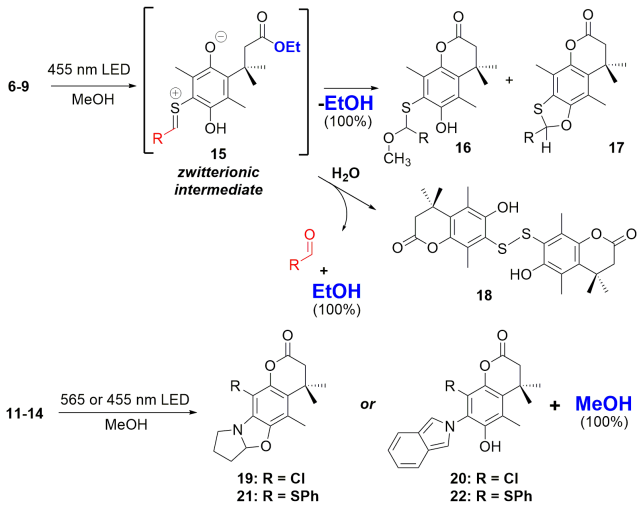


Figure 2. Representative time course UV-vis spectra during photolysis for compounds **6a** (top) and **11** (bottom) irradiated with 455 nm and 565 nm LEDs, respectively. Expansions of the visible bands are shown as insets. Arrows indicate changes in the spectra over time. Traces are 1 minute apart. For both compounds, 3 mL of 0.1-0.2 mM solutions fully converted in under 5 minutes with a standard 1W mounted LED.

Reduction of the quinone core and subsequent trimethyl lock occurs in all cases, releasing caged alcohols in quantitative yield. The reaction is remarkably clean; after removal of the solvent, the crude reaction is pure by NMR and LCMS with few exceptions. Monitoring the reaction by NMR in CD_3OD shows clean conversion and quantitative release of the methanol or ethanol. The hydroquinone byproducts vary depending on the solvent and substituent (Scheme 2). Irradiation of **6a** in methanol with a 455 nm LED releases ethanol and generates methanol-trapped **16a** (R = CH_3) as the exclusive product (Φ_{420} = 1.2%). Photolysis of **6b** is more efficient (Φ_{420} = 6.3%) and produces **16b** (R = Ph), along with a cyclized product **17** (R = Ph).[45] In aqueous solution, the disulfide **18** is cleanly produced, presumably via aqueous trapping of zwitterionic intermediate **15**, subsequent hydrolysis to release a carbonyl and oxidation. Interestingly, a ketone masked as the thiol substituent can be released simultaneously with an alcohol or amine in water. In aprotic solvents, the sulfides undergo slow photolysis to a complex mixture of products.

In contrast to the sulfides **6-9**, photolysis of amines **11-14** to the hydroquinone not only proceeds smoothly in a range of solvents, but is more efficient in non-polar solvents. Pyrrolidine derivatives **11** and **13** cyclize to form oxazolidines **19** and **21**, whereas isoindoline derivatives **12** and **14** form isoindoles **20** and **22**. For the closely related series **11-14**, the relative rate of reaction is **10** > **9** > **12** > **11**. As with the sulfides, an activated benzylic hydrogen on the substituent facilitates a more rapid reaction. The aryl sulfide substituent of **13** and **14** appears to impede the reaction relative to Cl in **11** and **12**. We estimate the quantum yields in methanol for **11** and **12** to be similar to that of **6a** and **6b**, and that of **13** and **14** to be lower, however, detailed photophysical studies are still in progress.

Scheme 2. Photolysis of 6-14.



As described in detail elsewhere[45], we have performed extensive mechanistic studies on the photoreaction of sulfides such as **6**.[41] Based on substituent effects, kinetic isotope effects, stereochemical and radical clock probes, and nanosecond transient-absorption spectroscopy, we consider the reaction to proceed as follows. Photochemically induced electron transfer is followed by a critical and irreversible hydrogen transfer, which leads to a net two-electron intramolecular reduction of the quinone and formation of a zwitterionic intermediate such as **15**. Capture by solvent or the phenolic oxygen, followed by trimethyl lock ring closure completes the decaging. In some cases, the intermediate hydroquinone can be directly observed, and the thermal trimethyl lock ring closure can be monitored (Supporting Information). Given the importance of the hydrogen transfer step, it is not surprising that the benzylic derivative **6b** is more efficient than the methyl derivative, **6a**.

To illustrate the potential usefulness of these compounds, we sought to demonstrate release of a fluorophore, release of a biologically relevant molecule, and the orthogonality of the two developed systems. Figure 3 shows that irradiation of **7** at 455 nm in aqueous acetonitrile rapidly releases a fluorescence sensor (hymecromone). The uncaged coumarin can be selectively excited at 355 nm in aqueous solvents, allowing release to be monitored by fluorescence (Figure 3).

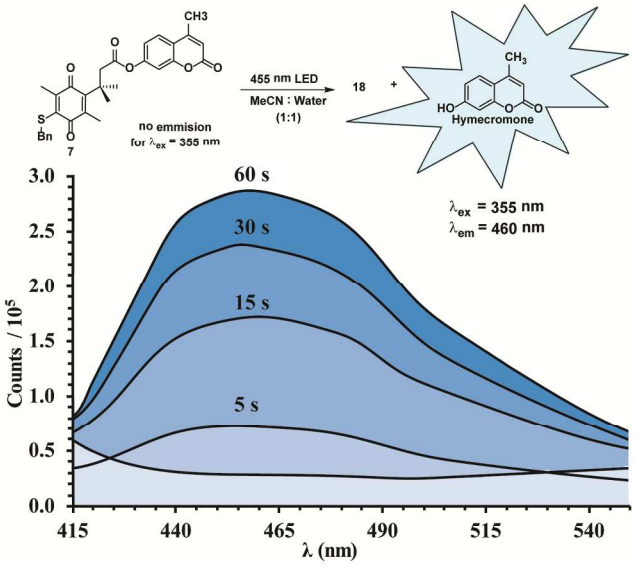


Figure 3. Release of 4-Methylumbelliferone (hymecromone) monitored by fluorescence with 355 nm excitation after samples were irradiated with a 1 watt 455 nm LED for 0 s, 5 s, 15 s, 30 s, and 60 s.

Decaging is also possible in a biological setting, permitting optical control of protein targets. Figure 4 shows decaging of the neurotransmitter γ-aminobutyric acid (GABA) using precursor **9**. When expressed in *Xenopus* oocytes, $GABA_A$ receptors are activated after incubation with **9** and exposure to a 455 nm LED (see SI for details). For caged amines, such as **9**, photolysis is much faster than trimethyl lock closure, as expected from previously determined reaction rates of redox triggered quinone trimethyl lock amine conjugates.[2]

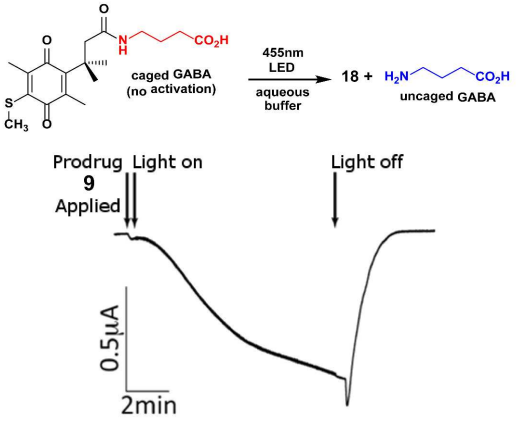


Figure 4. Release of the neurotransmitter γ-amminobutyric acid (GABA) to activate $GABA_A$ receptors in *Xenopus* oocytes upon photolysis with a 455 nm LED. Shown is current produced by activation of the receptor, as probed by whole-cell, two-electrode, voltage clamp electrophysiology.

Having demonstrated the release of biologically meaningful concentrations of model substrates in seconds to minutes, we sought to demonstrate selectivity possible with the amine and sulfide systems.[25] Solutions of **6** and **11** in methanol with matched optical densities were mixed 1:1 to give a solution with a broad absorbance from 360 to 620 nm (Figure 5). Irradiation with first a 565 nm LED through a 530 nm long-pass filter cleanly released only caged methanol from **11** (red). Subsequent irradiation with a 455 nm LED released ethanol from **6**. LCMS (see SI) and UV-vis indicated that no decaging of **6** occurred with the long-wavelength irradiation, allowing complete conversion of **11** orthogonally to **6**. Irradiating the solution with a 455 nm LED led to both compounds bleaching at similar rates. The orthogonality of the two similar systems allows for rapid release of two different compounds in quick succession.

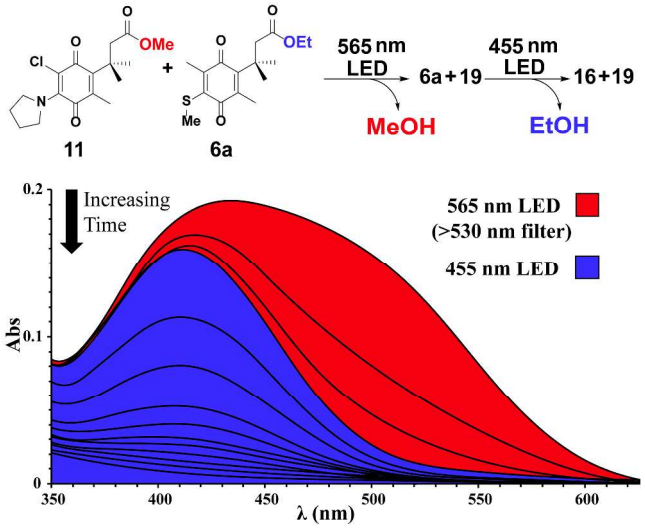


Figure 5. Orthogonal photolysis of **11** and **6** in methanol. Photolysis of a solution of **11** and **6** with >530 nm light followed by >420 nm light allows selective decaging.

We believe compounds of the sort described here will be useful in many contexts. Photoremovable protecting groups, or photocages, offer the ability to spatio-temporally control the release (decaging) of molecules with light. The most widely used examples are 2-nitrobenzyl derivatives, which require UV irradiation and generate toxic byproducts. In fact, there are few transition-metal free photoremovable protecting groups that absorb significantly above 450 nm.[19, 21-28] Because of the well-established increase in tissue penetrance as wavelength increases, longer wavelength decaging strategies such as that described here have great potential as chemical biology tools or in a therapeutic setting. The present systems are already well into the visible range, and a great deal is known about photo-induced electron transfer reactions of the sort that initiate this process, providing valuable guidance on how to move to even longer wavelengths.

In summary, we have created a modular system in which a photoinduced electron transfer of a quinone leads to a thermal trimethyl lock release of an alcohol or amine. The reaction works well with visible light in aqueous media to produce non-absorbing products. The entire range of substrates established to be compatible with the trimethyl lock is now available through visible light activation. Current work is focused on extending the absorption wavelength and expanding the scope of photochemical reductions and thermal cyclizations that are compatible.

ASSOCIATED CONTENT

Supporting Information

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

Synthetic procedures, compound characterization, UV-vis during photolysis, *Xenopus* oocyte experiments and orthogonal photolysis LCMS traces (PDF).

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Notes

The authors declare no competing financial interests.

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ABSTRACT FIGURE

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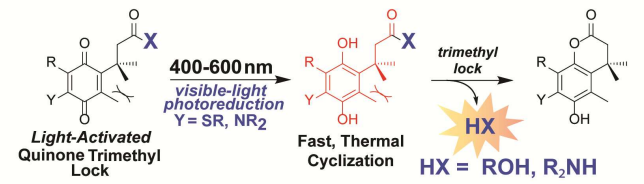
400-600 nm
visible-light photoreduction
Y = SR, NR2
trimethyl lock
HX
Light-Activated Quinone Trimethyl Lock
Fast, Thermal Cyclization
HX = ROH, R2NH