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

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**Materials and Methods.** Unless otherwise stated, reactions were carried out under ambient conditions in air. Commercially available reagents were obtained from Sigma Aldrich, AK Scientific, Alfa Aesar, or Acros Organics and used without further purification. Solvents used for photolysis and UV-vis were EMD Millipore (OmniSolv®) grade. Other solvents were used as received or dried by elution through activated alumina where noted. If a photoproduct was found to be unstable to hydrolysis, methanol was distilled from magnesium. Thin-layer chromatography with Sigma Aldrich silica gel coated plates with fluorescent indicator (0.25 mm) was used to monitor reactions. Silica gel chromatography was conducted as described by Still et al. (W. C. Still, M. Kahn, A. Mitra, *J. Org. Chem.* **1978**, *43*, 2923), with silica gel purchased from Alfa Aesar (60 Å, 230-400 mesh). NMR spectra were recorded on Varian (300, 400, 500, or 600 MHz), or Bruker (400 MHz) spectrometers. HRMS (ESI) were obtained with an Agilent 6200 Series TOF. UV-vis spectra were recorded on a Cary 60 spectrometer. Samples were irradiated with a 300 W Hg arc lamp with glass filter (>400 nm), M455L3 (455 nm, 900 mW), or a M565L3 (565 nm, 880 mW) mounted LED purchased from Thor Labs. Photolysis was conducted inside the UV-vis spectrometer cavity in a 3 mL cuvette and side on through a glass flask or NMR tube in air-equilibrated methanol, methanol-d₄, or benzene-d₆. Isolated yields are reported except for photolysis yields, which were determined by NMR. Preparatory photolysis was conducted under ambient conditions (air) in glass round bottom flasks with stir bar and side-on illumination. Steady-state luminescence spectra were collected with a Jobin Yvon Spex Fluorolog-3-11 spectrometer. Samples were excited with 355 nm light from a 450 W xenon arc lamp selected with a monochromator. A scanning monochromator was used to collect emission, detecting with a Hamamatsu R928P photomultiplier tube in photon counting mode.

![6-hydroxy-4,4,5,8-tetramethylchroman-2-one (3)](image)

**6-hydroxy-4,4,5,8-tetramethylchroman-2-one (3).** Compound **3** is conveniently prepared from commercially available 2,5-dimethyl-1,4-benzoquinone via an adapted synthetic route.⁴ To a vigorously stirred mixture of 2,5-dimethyl-1,4-benzoquinone (1.0181 g, 7.5 mmol) in ether (20 mL), MeOH (10 mL), and water (40 mL), was added NaBH₄ (1.422 g, 37.6 mmol) portion wise,
and the yellow mixture turned brown then colorless after 15 min. The mixture was then quickly extracted with ether (3 x 50 mL). The combined ether layers were washed with brine, dried over MgSO₄, and evaporated to yield 2,5-dimethyhydroquinone as a white solid 1.033 g (99%). ¹H NMR (500 MHz, DMSO-d₆) δ 8.32 (s, 2H), 6.45 (s, 2H), 1.99 (s, 6H). ¹³C NMR (126 MHz, dmso) δ 147.46, 116.84, 39.52, 15.82. The crude product, 2,5-dimethyhydroquinone (1.033 g, 7.5 mmol), and 3,3-dimethylacrylic acid (0.8453 g, 8.4 mmol) were dissolved in methanesulfonic acid (30 mL) the resulting mixture was heated to 70 °C under argon overnight. The resulting red solution was poured into ice-water and extracted with ethyl acetate (4 x 90mL). The combined organic layers were washed with brine, dried (MgSO₄), and the solvent was removed to yield 3 as a tan solid 1.59 g (95%). ¹H NMR (500 MHz, DMSO-d₆) δ 9.16 (s, 1H), 6.60 (s, 1H), 2.59 (s, 2H), 2.21 (s, 3H), 2.10 (s, 3H), 1.34 (s, 6H). ¹³C NMR (126 MHz, dmso) δ 168.15, 151.60, 142.19, 130.98, 122.90, 119.50, 115.10, 44.96, 39.52, 35.17, 27.12, 15.93, 13.86. HRMS (ESI) calculated 219.1027 for C₁₃H₁₅O₃ [M-H]⁻, found 219.1016.

3-(4-bromo-2,5-dimethyl-3,6-dioxocyclohexa-1,4-dien-1-yl)-3-methylbutanoic acid (4). To a solution of lactone 3 (1.59 g, 7.218 mmol) in acetic acid (70 mL) was added bromine (0.817 mL) as a solution in 9.5 mL acetic acid. The resulting red solution was stirred overnight, open to air but protected from light, then poured into waster and extracted with dichloromethane until the aqueous solution was colorless. The combined organic layers were then extracted with 10% sodium bicarbonate solution until the bicarbonate layer was colorless. The combined bicarbonate extracts were washed with CH₂Cl₂, then carefully acidified with conc. HCl and extracted with ethyl acetate until colorless. The yellow ethyl acetate extracts were then dried (MgSO₄) and concentrated to give a yellow solid. Isolated yield: 1.5301 g (67%). ¹H NMR (500 MHz, Chloroform-d) δ 10.22 (s, 1H, COOH), 3.02 (s, 2H, CH₂), 2.21 (s, 3H), 2.14 (s, 3H), 1.45 (s, 6H). ¹³C NMR (126 MHz, cdcl₃) δ 187.59, 179.88, 177.66, 152.78, 148.52, 138.72, 131.84, 46.94, 38.07, 28.54, 17.14, 15.15. HRMS (ESI) calculated 313.0081, 315.0060 for C₁₃H₁₄BrO₄ [M-H]⁻, found 313.0060, 315.0051.
3-(2,5-dimethyl-4-(methylthio)-3,6-dioxocyclohexa-1,4-dien-1-yl)-3-methylbutanoic acid (5a). To a 20 mL scintillation vial charged with 4 (119.9 mg, 0.38 mmol) in a mixture of dichloromethane / water (1:1, 10.8 mL total) was added tetrabutylammonium bromide (5.5 mg; PTC, 4.5%), followed by sodium methanethiolate (53 mg, 0.76 mmol). The vial was capped and vigorously shaken for 2 minutes, then allowed to stand until the layers separated. 1 M HCl was added with stirring until the aqueous layer was colorless, the yellow organic layer was then separated, dried over MgSO₄, and evaporated to give 5a as a yellow solid (106.8 mg, 99%). ¹H NMR (300 MHz, Chloroform-d) δ 3.59 (s, 2H), 2.97 (s, 2H), 2.47 (s, 3H), 2.17 (s, 2H), 2.13 (s, 2H), 1.42 (s, 5H). ESI-MS(-) calculated for C₁₄H₁₇O₄S [M-H]⁻ 281.1, found 281.1.

ethyl 3-(4-bromo-2,5-dimethyl-3,6-dioxocyclohexa-1,4-dien-1-yl)-3-methylbutanoate. To 4 (2.6526 g, 8.4 mmol) dissolved in dichloromethane (10 mL) under argon was added ethanol (4.9 mL, 84 mmol), and the solution was cooled to 0 °C under argon. N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (1.7908 g, 9.3 mmol) and 4-(dimethylamino)pyridine (105.3 mg, 0.86 mmol) were added in one portion, and the resulting solution was stirred for 5 min, then allowed to warm to room temperature. The crude product was diluted in hexanes and purified by flash column chromatography (SiO₂, 10% EtOAc in hexanes), collecting the yellow band to yield 1.5952 g (55%) of the bromoquinone ester 23 as an oil. ¹H NMR (400 MHz, Chloroform-d) δ 4.04 (q, J = 7.1 Hz, 2H), 2.95 (s, 2H), 2.21 (s, 3H), 2.17 (s, 3H), 1.44 (s, 6H), 1.20 (t, J = 7.1 Hz, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 187.60, 179.88, 172.70, 153.61, 148.77, 138.04, 131.63,
ethyl 3-(2,5-dimethyl-4-(methylthio)-3,6-dioxocyclohexa-1,4-dien-1-yl)-3-methylbutanoate (6a). To a solution of 4 (40 mg, 0.11 mmol) in dichloromethane (1 mL) was added water (1 mL), tetrabutylammonium bromide (1.8 mg, 5%), and sodium methanethiolate (16 mg, 0.23 mmol). The resulting mixture was shaken vigorously for 2 min, then the organic layer was separated, dried, and concentrated. The crude was purified by flash column chromatography (SiO₂, 10% EtOAc in hexanes) to yield 6a as a yellow oil (30 mg, 83%). ¹H NMR (300 MHz, Chloroform-d) δ 4.03 (q, J = 7.1 Hz, 2H), 2.95 (s, 2H), 2.47 (s, 2H), 2.16 (s, 3H), 2.13 (s, 2H), 1.42 (s, 6H), 1.19 (t, J = 7.1 Hz, 2H). ¹³C NMR (101 MHz, CDCl₃) δ 188.26, 183.32, 172.70, 152.99, 146.36, 141.61, 139.54, 60.26, 47.62, 38.12, 28.64, 17.09, 14.64, 14.51, 14.18. HRMS (ESI) calculated for C₁₆H₂₃O₄S [M+H]+ 311.1312, found 311.1316.

ethyl 3-[4-(benzylsulfanyl)-2,5-dimethyl-3,6-dioxocyclohexa-1,4-dien-1-yl]-3-methylbutanoate (6b). To a solution of 4 (1 eq) in methanol was added benzyl mercaptan (1.1 eq), followed by K₂CO₃ (1.1 eq), at which point the pale solution turned dark mustard yellow. The resulting solution was stirred, protected from light, until LCMS indicated the reaction was complete, then 1 equivalent of acetic acid is added, and the solvent was removed. The crude product was dissolved in a small amount of CH₂Cl₂, then diluted in hexanes and filtered through a cotton plug to remove inorganic salts before purification by flash column chromatography (SiO₂, 10% EtOAc in hexanes) to yield 6b as a yellow oil (>95%). ¹H NMR (300 MHz, Chloroform-d) δ 7.36 – 7.14 (m, 5H), 4.18 (s, 2H), 3.98 (q, J = 7.1 Hz, 2H), 2.90 (s, 2H), 2.16 (s, 3H), 2.00 (s,
4-methyl-2-oxo-2H-chromen-7-yl 3-(4-(benzylthio)-2,5-dimethyl-3,6-dioxocyclohexa-1,4-dien-1-yl)-3-methylbutanoate (7). To a solution of 4 (250.0 mg, 0.793 mmol) in methanol (50 mL) was added benzyl mercaptan (0.15 mL, 1.3 mmol), followed by K₂CO₃ (219.1 mg, 1.58 mmol). The resulting solution was stirred, protected from light, until HPLC indicated the reaction was complete. The solvent was removed, and the mixture was partially purified on silica, removing residual benzyl mercaptan by first eluting with dichloromethane, then eluting 5b (yellow band) with 1% CH₃CO₂H / 5% CH₃OH / CH₂Cl₂. Due to a known intramolecular Michael addition on similar compounds, the pooled yellow fractions were quickly concentrated and used immediately in the next step. To a solution of 5b (250 mg, 0.7 mmol) in dichloromethane was added 7-hydroxy-4-methyl-2H-chromen-2-one (300 mg, 1.7 mmol). Ethyl acetate was added to solubilize the coumarin, and once the solution was homogenous excess EDCI·HCl and DMAP (14.5 mg, 0.12 mmol) were added. Once TLC indicated the reaction was complete, the crude product was purified by flash chromatography to yield 122 mg of 7 as a yellow solid (34%). ¹H NMR (400 MHz, Chloroform-d) δ 7.58 (d, J = 8.7 Hz, 1H), 7.34 – 7.09 (m, 5H), 7.02 (d, J = 2.3 Hz, 1H), 6.95 (dd, J = 8.6, 2.3 Hz, 1H), 6.26 (q, J = 1.3 Hz, 1H), 4.15 (s, 2H), 3.24 (s, 2H), 2.42 (d, J = 1.3 Hz, 3H), 2.20 (s, 3H), 1.97 (s, 3H), 1.51 (s, 6H). ¹³C NMR (101 MHz, CDCl₃) δ 188.41, 183.53, 170.52, 160.38, 154.09, 152.74, 151.90, 151.84, 148.45, 140.35, 140.25, 137.49, 128.97, 128.49, 127.23, 125.39, 117.92, 117.86, 114.55, 110.39, 47.42, 38.30, 38.20, 28.75, 18.73, 14.83, 14.77.
2,5-dioxopyrrolidin-1-yl 3-(2,5-dimethyl-4-(methylthio)-3,6-dioxocyclohexa-1,4-dien-1-yl)-3-methylbutanoate (8). To a solution of 5a in CH₂Cl₂ (5 mL) was added DMAP (8.4 mg, 0.07 mmol) and NHS (80.8 mg, 0.7 mmol). After cooling to 0 °C, EDCI was added (134.3 mg, 0.7 mmol), and the resulting solution was stirred for 1 h, then allowed to warm to r.t. over 24 h. Solvent was removed, then the crude material was purified by flash chromatography, eluting with 30% Ethyl acetate/Hexanes, collecting the major yellow band. Yield: 72 mg (29%). ¹H NMR (400 MHz, Chloroform-d) δ 3.26 (s, 2H), 2.79 (s, 4H), 2.47 (d, J = 0.9 Hz, 3H), 2.19 (s, 3H), 2.11 (s, 3H), 1.53 (s, 6H). ¹³C NMR (101 MHz, cdcl₃) δ 187.53, 183.31, 168.83, 167.59, 150.17, 145.60, 142.47, 141.49, 44.11, 38.95, 29.70, 29.09, 25.54, 17.10, 14.55.

4-(3-(2,5-dimethyl-4-(methylthio)-3,6-dioxocyclohexa-1,4-dien-1-yl)-3-methylbutanamido)-butanoic acid (9). To a solution of 8 (62.6 mg, 0.16 mmol) in 5 mL MeCN / 5 mL aqueous buffer (pH =7), followed by gamma-aminobutryic acid (29 mg, 0.28 mmol). K₂CO₃ was added to adjust the pH (20 mg, 0.14 mmol), and the stirred reaction was monitored by TLC. After 3 hours, removed solvent and flashed (7.5% MeOH, 1% AcOH, DCM), collecting the first eluting yellow band. ¹H NMR (400 MHz, Chloroform-d) δ 3.26 (s, 2H), 2.79 (s, 4H), 2.47 (s, 3H), 2.19 (s, 3H), 2.11 (s, 3H), 1.53 (s, 6H). ¹³C NMR (101 MHz, cdcl₃) δ 188.75, 183.54, 177.58, 172.51, 153.56, 147.06, 141.39, 138.88, 49.12, 38.74, 38.43, 31.41, 28.92, 24.72, 17.18, 14.83, 14.49. HRMS (ESI) calculated 366.1380 for C₁₈H₂₄NO₅S [M-H]⁺, found 366.1389.
ethyl 3-(2,5-dimethyl-3,6-dioxo-4-(((2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)thio)cyclohexa-1,4-dien-1-yl)-3-methylbutanoate (24). To a solution of 4 in methylene chloride / water / acetonitrile with tetrabutylammonium bromide (5%) was added excess sodium 1-thio-β-D-glucose, and the reaction was vigoursly stirred until the complete consumption of 4 was observed by HPLC. The mixture was diluted with methylene chloride and brine to form two layers, then the yellow organic layer was separated, washed with brine, dried, then filtered through a plug of silica gel with 100% ethyl acetate to yield 24 as a pale yellow oil. $^1$H NMR (500 MHz, Chloroform-$d$) $\delta$ 4.78 (dd, $J = 9.7$, 2.9 Hz, 1H), 4.03 (q, $J = 7.1$ Hz, 2H), 3.76 (qd, $J = 12.4$, 3.6 Hz, 2H), 3.60 (dt, $J = 33.9$, 9.0 Hz, 2H), 3.44 – 3.25 (m, 2H), 3.09 (d, $J = 16.2$ Hz, 1H), 2.72 (d, $J = 16.2$ Hz, 1H), 2.18 (d, $J = 26.3$ Hz, 6H), 1.41 (d, $J = 6.1$ Hz, 6H), 1.19 (t, $J = 7.1$ Hz, 3H). $^{13}$C NMR (126 MHz, cdcl$_3$) $\delta$ 188.76, 184.31, 173.05, 154.45, 153.94, 138.90, 135.65, 85.51, 79.98, 77.64, 73.58, 69.76, 62.10, 60.59, 47.48, 38.09, 28.52, 28.15, 15.84, 14.68, 14.16.
Synthesis of amine derivatives. An alternative approach was developed to allow incorporation of more diverse substituents, such as amines (Scheme S1). The procedure is general and can be carried out in large scale reactions from commercially available 2-R-5-methylphenols and inexpensive reagents. For 2,5-dimethylphenol, the procedure is equally applicable and intercepts the synthesis of 6-9 at compound 3.

Scheme S1: Synthesis of Amine Quinone Trimethyl Lock Derivatives 11-14.
8-chloro-4,4,5-trimethylchroman-2-one (25). Prepared according to literature procedure.\textsuperscript{4} 2-Chloro-5-methylphenol (14.4066 g, 0.10 mol) and 3,3,3-dimethylacrylic acid (11.1237 g, 0.11 mol) were dissolved in methanesulfonic acid (100 mL), and the resulting mixture was heated at 70 °C for 48 h. The resulting solution was poured into water and extracted with ethylacetate (3 x 100 mL). The organic layer was washed with 5% KOH (5x50 mL), then 5% phosphoric acid (50 mL), and finally brine before drying (MgSO\textsubscript{4}). The solvent was evaporated to yield an oil, which was recrystallized with ether/hexanes to give 25 as a white solid (3.31g, 14.6%).\textsuperscript{1}H NMR (500 MHz, Chloroform-\textit{d}) δ 7.19 (d, \(J = 8.3\) Hz, 1H), 6.85 (dd, \(J = 8.2, 0.8\) Hz, 1H), 2.63 (s, 2H), 2.48 (d, \(J = 0.7\) Hz, 3H), 1.46 (s, 6H).\textsuperscript{13}C NMR (126 MHz, cdc\textsubscript{3}) δ 166.77, 147.16, 134.83, 131.53, 128.60, 128.26, 120.35, 45.27, 35.90, 27.40, 27.39, 23.04.

8-chloro-6-hydroxy-4,4,5-trimethylchroman-2-one (26). The Elbs persulfate oxidation of 25 to 26 was carried out following general methods.\textsuperscript{2,3} A mixture of 25 (2.2473 g, 10 mmol) and NaOH (2.0549 g, 51 mmol) in 20 mL water and 20 mL methanol was refluxed for 12 h under argon to hydrolyze the lactone, then cooled to 15 °C. Ammonium persulfate (2.2827 g, 10 mmol) was added as a solution in 60 mL of water slowly over 5 h while keeping the temperature below 20 °C. The resulting dark brown solution was carefully acidified to pH = 5 and filtered to recover unreacted starting material. The aqueous solution was then extracted twice with ether to further recover starting material, then strongly acidified to pH = 0 with HCl (aq) and refluxed until LCMS indicated complete conversion of the intermediate sulfate to the product (1 h). The resulting orange solution was cooled to precipitate 26 as a yellow solid (0.9296 g, 38.6%) collected by filtration.\textsuperscript{1}H NMR (500 MHz, Chloroform-\textit{d}) δ 6.83 (d, \(J = 0.5\) Hz, 1H), 5.08 (s, 1H), 2.60 (s, 2H), 2.34 (d, \(J = 0.5\) Hz, 3H), 1.48 (s, 6H).\textsuperscript{1}H NMR (500 MHz, DMSO-\textit{d}6) δ 9.75
(s, 1H), 6.85 (s, 1H), 2.69 (s, 2H), 2.23 (s, 3H), 1.37 (s, 6H). $^{13}$C NMR (126 MHz, DMSO-$d_6$) δ 167.20, 152.23, 139.52, 133.15, 121.92, 117.63, 113.74, 44.47, 35.81, 26.87, 13.88. HRMS (ESI) calculated 239.0480 for C$_{12}$H$_{12}$ClO$_3$ [M-H$^-$], found 239.0485.

3-[(4-bromo-5-chloro-2-methyl-3,6-dioxocyclohexa-1,4-dien-1-yl)-3-methylbutanoic acid (27). To a solution of 26 (1.0531 g, 4.38 mmol) dissolved in glacial acetic acid (50 mL) was added Br$_2$ (0.5 mL, 9.6 mmol). The resulting solution was stirred in the dark overnight, then diluted with water and extracted with ethyl acetate. The combined organic layers were washed with brine, dried (MgSO$_4$) and concentrated to give a 1.47 g (100%) of 27 as a yellow solid. The crude product was used without further purification. $^1$H NMR (500 MHz, Chloroform-$d$) δ 3.04 (s, 2H), 2.25 (s, 3H), 1.47 (s, 6H). $^{13}$C NMR (126 MHz, cdcl$_3$) δ 180.26, 178.66, 178.30, 152.91, 145.20, 139.60, 132.38, 47.15, 38.66, 28.58, 15.41. HRMS (ESI) calculated 332.9535 for C$_{12}$H$_{11}$BrClO$_4^-$, found 334.9517.

methyl 3-[(4-bromo-5-chloro-2-methyl-3,6-dioxocyclohexa-1,4-dien-1-yl)-3-methylbutanoate (10). To a solution of 27 (1.5241 g, 4.5 mmol) in 5 mL dichloromethane at 0 °C was added anhydrous methanol (0.73 mL, 18 mmol) followed by N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (877.4 mg, 4.5 mmol) and 4-(dimethylamino)pyridine (56.8 mg, 10%) in one portion. The resulting solution was protected from light and stirred for 5 min, then allowed to warm to room temperature overnight. The solvent was removed, and the crude product was taken up in a minimal amount of dichloromethane, diluted with hexanes and purified by flash chromatography (10% ethylacetate / hexanes), with the product eluting as
yellow band. The combined fractions were concentrated to give a yellow oil (0.9686 g, 61%). $^1$H NMR (500 MHz, Chloroform-$d$) $\delta$ 3.60 (s, 3H), 2.98 (s, 2H), 2.24 (s, 3H), 1.45 (s, 6H). $^{13}$C NMR (126 MHz, cdcl$_3$) $\delta$ 180.43, 178.82, 173.21, 153.69, 145.48, 139.27, 132.50, 51.81, 47.47, 39.02, 28.70, 15.50.

methyl 3-(5-chloro-2-methyl-3,6-dioxo-4-(pyrrolidin-1-yl)cyclohexa-1,4-dien-1-yl)-3-methylbutanoate (11). To a solution of 10 (471 mg, 1.35 mmol) in methanol (50 mL) was added 0.25 mL pyrrolidinone, and the resulting solution was stirred, protected from light, until TLC indicated the reaction was complete. The desired product (11; cherry-red band) was carefully separated from the other substitution product (orange-red band) by flash chromatography (30% ether / hexanes) as a red solid (14% yield by NMR). $^1$H NMR (500 MHz, Chloroform-$d$) $\delta$ 3.85 – 3.78 (m, 4H), 3.60 (s, 3H), 3.09 (s, 2H), 2.13 (s, 3H), 1.93 – 1.84 (m, 4H), 1.45 (s, 6H). $^{13}$C NMR (126 MHz, Chloroform-$d$) $\delta$ 186.05, 181.00, 173.12, 152.46, 147.88, 135.08, 106.40, 52.94, 51.29, 47.88, 38.92, 29.32, 25.50, 13.95. HRMS (ESI) calculated 340.1310 for C$_{17}$H$_{23}$ClNO$_4$[M+H]$^+$, found 340.1311. UV/vis: $\lambda_{\text{max}}$ = 497 nm ($\varepsilon$ = 2,800 M$^{-1}$cm$^{-1}$).

methyl 3-(5-chloro-4-(isoindolin-2-yl)-2-methyl-3,6-dioxocyclohexa-1,4-dien-1-yl)-3-methylbutanoate (12). To a solution of 10 (359 mg) in methanol (20 mL) was added 0.26 mL isoindoline, and the resulting solution was stirred, protected from light, until TLC indicated the reaction was complete. The desired product, 12, (cherry-red band) was carefully separated from the other substitution product (orange-red band) by flash chromatography (30% ether / hexanes)
as a red solid (50% yield by NMR). $^1$H NMR (400 MHz, Chloroform-$d$) $\delta$ 7.38 – 7.27 (m, 4H), 5.22 (s, 4H), 3.59 (s, 3H), 3.07 (s, 2H), 2.17 (s, 3H), 1.45 (s, 6H). HRMS calculated 388.1310, found 388.1311. UV/vis: $\lambda_{\text{max}} = 483$ nm.

methyl 3-methyl-3-(2-methyl-3,6-dioxo-5-(phenylthio)-4-(pyrrolidin-1-yl)cyclohexa-1,4-dien-1-yl)butanoate (13). To a 25 mL round bottom flask equipped with stirbar under argon was added 10 (351 mg, 1 mmol), thiophenol (122 mg, 1.1 mmol), and methanol (15 mL), and the resulting solution was cooled to -78 $^\circ$C. Sodium acetate (180 mg, 2.2 mmol) was added dropwise as a 0.845 M solution in methanol, and the resulting mixture was allowed to warm to room temperature. When TLC indicated the reaction was complete, the solvent was removed and the crude product was purified on silica gel (7.5 % ethyl acetate / hexanes). The first band (yellow) eluting was starting material, the second band (orange) was product, and the third band (red-orange) is the disubstituted byproduct. The pure fractions from the orange band were pooled and concentrated to yield 28 as an orange oil (319.8 mg, 75%). $^1$H NMR (500 MHz, Chloroform-$d$) $\delta$ 7.58 – 7.51 (m, 2H), 7.36 – 7.25 (m, 3H), 3.61 (s, 3H), 2.60 (s, 2H), 2.20 (s, 3H), 1.29 (s, 6H). $^{13}$C NMR (126 MHz, cdcl$_3$) $\delta$ 183.21, 177.86, 172.60, 154.91, 152.44, 138.34, 133.97, 130.86, 129.04, 128.93, 128.90, 77.41, 77.16, 76.91, 51.62, 46.73, 38.80, 28.61, 15.04. To a solution of 28 (57 mg, 0.135 mmol) in methanol (5mL) was added pyrroldine (20.8 mg, 0.292 mmol) as a 10% (v/v) solution in methanol. The resulting solution was stirred, protected from light, as it turned from orange to red. Once TLC indicated the reaction was complete, the solvent was evaporated, and the crude residue was purified on silica (10% EtOAc / Hexanes) to yield 53 mg of 13 as a red solid (95%). $^1$H NMR (500 MHz, Chloroform-$d$) $\delta$ 7.17 (tt, $J = 7.4, 1.8$ Hz, 2H), 7.14 – 7.07 (m, 2H), 7.06 – 6.98 (m, 1H), 3.86 – 3.71 (m, 4H), 3.59 (s, 3H), 3.04 (s, 2H), 2.17 (s, 3H), 1.85 – 1.75 (m, 4H), 1.44 (s, 6H). $^{13}$C NMR (126 MHz, cdcl$_3$) $\delta$ 187.53, 183.74, 173.27, 156.18, 155.25, 139.77, 134.71, 128.60, 125.32, 124.38, 97.41, 52.71, 51.20, 47.69, 38.81, 29.05,
25.22, 13.68. HRMS: Calculated 414.1734 for \( \text{C}_{23}\text{H}_{28}\text{NO}_{4}\text{S} [\text{M+H}]^+ \), found 414.1753. UV/vis: \( \lambda_{\text{max}} = 476 \text{ nm} \ (\varepsilon = 2,700 \ \text{M}^{-1}\text{cm}^{-1}) \).

![Reaction Scheme](image)

**methyl 3-(4-(isoindolin-2-yl)-2-methyl-3,6-dioxo-5-(phenylthio)cyclohexa-1,4-dien-1-yl)-3-methylbutanoate (14).** To a solution of 28 (114 mg, 0.27 mmol) in methanol (20 mL), was added 70 μL isoinodoline. The solution was stirred in the dark until TLC indicated the reaction was complete. The solvent was removed and the crude material was purified by silica gel (10% ethylacetate / hexanes) to yield 14 as a red solid (110 mg, 88%). \(^1\)H NMR (400 MHz, Chloroform-\(d\)) \( \delta \) 7.29 – 7.16 (m, 9H), 7.12 – 7.04 (m, 1H), 5.21 (s, 4H), 3.62 (s, 3H), 3.04 (s, 2H), 2.24 (s, 3H), 1.46 (s, 6H). \(^{13}\)C NMR (101 MHz, CDCl\(_3\)) \( \delta \) 187.34, 183.85, 173.17, 156.50, 155.54, 139.37, 135.43, 134.63, 128.79, 127.57, 125.51, 124.71, 122.00, 98.00, 58.33, 51.27, 47.60, 38.83, 28.95, 13.59. HRMS (ESI) calculated 462.1734 for \( \text{C}_{27}\text{H}_{28}\text{NO}_{4}\text{S} [\text{M+H}]^+ \), found 462.1763. UV/vis: \( \lambda_{\text{max}} = 467 \text{ nm} \ (\varepsilon = 3,000 \ \text{M}^{-1}\text{cm}^{-1}) \).

**4,4,5,8-tetramethylchroman-2-one (29).** Prepared according to a literature procedure.\(^1\) 2,5-Dimethylphenol (14.4066 g, 0.10 mol), 3,3,3-dimethylacrylic acid (11.1237 g, 0.11 mol), and methanesulfonic acid (100 mL) were added to a round bottom flask equipped with a magnetic stir bar and the resulting mixture was heated at 70 °C for 48 h. The deep red solution was poured into water and the precipitated oil was extracted with ethylacetate (3 x 100 mL). The organic layer was washed with 5% KOH (5x50 mL), then 5% phosphoric acid (50 mL), and finally brine before drying (MgSO\(_4\)). The solvent was evaporated to yield an oil, which was recrystallized with ether/hexanes to give 29 as a white solid (3.31 g, 14.6%). \(^1\)H NMR (300 MHz, Chloroform-
δ 6.98 (d, J = 7.7 Hz, 1H), 6.81 (d, J = 7.7 Hz, 1H), 2.59 (s, 2H), 2.46 (s, 3H), 2.26 (s, 3H), 1.45 (s, 6H). ¹³C NMR (101 MHz, CDCl₃) δ 168.32, 149.78, 133.53, 129.51, 129.10, 127.98, 124.55, 45.64, 35.36, 27.65, 23.07, 16.25. HRMS (ESI) calculated for C₁₃H₁₇O₂⁺ requires 205.1223, found 205.1231.

6-hydroxy-4,4,5,8-tetramethylchroman-2-one (3). The Elbs persulfate oxidation of 29 to 3 was carried out following general methods.²³ A mixture of 29 (10.070 g, 49 mmol) and NaOH (9.78 g, 244 mmol) in 100 mL water and 75 mL methanol was refluxed for 12 h under argon to hydrolyze the lactone, then cooled to 15 °C. Ammonium persulfate (11.249 g, 49 mmol) was added as a solution in 150 mL of water slowly while keeping the temperature below 20 °C. The resulting dark brown solution was carefully acidified to pH = 5 and filtered to recover unreacted starting material. The aqueous solution was then extracted twice with ether to further recover starting material, then strongly acidified to pH = 0 with HCl (aq) and refluxed until LCMS indicated complete conversion of the intermediate sulfate to the product. The resulting orange solution was cooled to precipitate the 3 as a yellow solid (3.8 g, 35 %) collected by filtration.

**General Photolysis Procedure and Equipment:** Approximately 5-20 mg of compound was dissolved in methanol (25-50 mL). The resulting solution was exposed to the focused output of a 455 nm, 900 mW mounted LED (M455L3) or a 565 nm, 880 mW mounted LED (M565L3) under ambient conditions with stirring. When the solution became colorless, the solvent was removed, and the crude NMR and LCMS taken. In almost all cases, the crude NMR and LCMS were pure product. The yield of released methanol and ethanol were inferred from that of the lactone products or observed directly in deuterated solvents.

The 455 nm and 565 nm LEDs were used for convenience of use, but a 300 W Hg arc lamp with long-pass filters >400 nm gave clean photolysis as a generic visible light source. The mounted LEDs and the LEDD1B T-Cube driver used to power them were purchased from Thor
Labs. For the selective photolysis experiment, a >530 nm glass long-pass filter was required to remove the weak emission at 400-500 nm for the 565nm LED.

**Photolysis of 6a.** A dilute solution of 6a in methanol was irradiated with a 455 nm LED until colorless. The solvent was removed to give the product, 6-hydroxy-7-((methoxymethyl)thio)-4,4,5,8-tetramethylchroman-2-one (16a), in quantitative yield by NMR. $^1$H NMR (400 MHz, Chloroform-$d$) $\delta$ 7.17 (s, 1H), 4.69 (s, 2H), 3.48 (s, 3H), 2.55 (s, 2H), 2.50 – 2.42 (m, 3H), 2.41 – 2.35 (m, 3H), 1.46 (s, 6H). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 168.23, 152.64, 143.19, 132.95, 128.26, 119.73, 117.69, 79.56, 57.43, 45.75, 35.80, 27.43, 15.06, 15.02. HRMS (ESI) calculated for C$_{15}$H$_{19}$O$_4$S [M-H]$^-$ 295.1010, Found 295.1009.

**Photolysis of 6b.** A dilute solution of 6b in methanol was irradiated with a 455 nm LED until colorless. The solvent was removed to give the product, 6-hydroxy-7-((methoxy(phenyl)methyl)thio)-4,4,5,8-tetramethylchroman-2-one (16b), in 85% yield by NMR. $^1$H NMR (400 MHz, Chloroform-$d$) $\delta$ 7.44 (s, 1H), 7.35 – 7.15 (m, 5H), 5.47 (s, 1H), 3.44 (s, 3H), 2.54 (s, 2H), 2.34 (s, 3H), 2.27 (s, 3H), 1.46 (s, 6H). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 168.33, 153.63, 142.99, 137.78, 133.24, 129.10, 128.63, 128.34, 125.88, 119.64, 116.33, 92.23, 57.64, 45.81, 35.81, 27.56, 27.37, 15.01. HRMS (ESI) calculated for C$_{21}$H$_{23}$O$_4$S [M-H]$^-$ 371.1323, Found 371.1327.
Photolysis of 11. A dilute solution of 11 in methanol was irradiated with a 565 nm LED until colorless. The solvent was removed to give the product, 11-chloro-4,4,5-trimethyl-3,4,6,7,8,9-hexahydro-2H-chromeno[7,6-d][pyrrolo][2,1-b]oxazol-2-one (19), in quantitative yield by NMR. $^1$H NMR (500 MHz, Chloroform-d) δ 5.91 (dd, $J = 5.0$, 2.6 Hz, 1H), 3.45 (dt, $J = 10.7$, 7.1 Hz, 1H), 3.35 – 3.17 (m, 1H), 2.57 (d, $J = 2.5$ Hz, 2H), 2.27 (s, 4H), 1.94 – 1.83 (m, 2H), 1.43 (d, $J = 10.4$ Hz, 6H). $^{13}$C NMR (126 MHz, cdcl$_3$) δ 167.38, 149.38, 141.61, 137.26, 125.51, 114.04, 107.42, 103.01, 54.62, 45.56, 35.93, 32.67, 27.75, 27.66, 23.76, 14.58. HRMS (ESI) calculated 308.1048 for C$_{16}$H$_{19}$ClNO$_3$ [M+H]$^+$, found 308.1049.

Photolysis of 12. A dilute solution of 12 in methanol was irradiated with a 565 nm LED until colorless. The solvent was removed to give the product, 8-chloro-6-hydroxy-7-(2H-isooindol-2-yl)-4,4,5-trimethylchroman-2-one (20), in quantitative yield by NMR. $^1$H NMR (400 MHz, Chloroform-d) δ 7.72 – 7.44 (m, 2H), 7.11 (d, $J = 0.5$ Hz, 2H), 7.06 – 6.86 (m, 2H), 5.71 (s, 1H), 2.61 (s, 2H), 2.52 (s, 3H), 1.53 (s, 7H). $^{13}$C NMR (101 MHz, CDCl$_3$) δ 166.11, 146.70, 141.26, 130.99, 124.44, 124.36, 121.35, 119.99, 117.31, 117.26, 53.43, 45.47, 36.21, 29.71, 27.51, 15.14. HRMS (ESI) calculated for C$_{20}$H$_{19}$ClNO$_3^+$ 356.1048, found 356.1030.
Photolysis of 13. A dilute solution of 13 in methanol was irradiated with a 455 nm LED until colorless. The solvent was removed to give the product, 4,4,5-trimethyl-11-(phenylthio)-3,4,6a,7,8,9-hexahydro-2H-chromeno[7,6-d]pyrrolo[2,1-b]oxazol-2-one (21), in quantitative yield by NMR. HRMS (ESI) calculated 382.1471 for C_{22}H_{24}NO_3S [M+H]^+, found 382.1475.

![Chemical structure of 13 and 21](image)

Photolysis of 14. A dilute solution of 14 in CD_{3}OD sparged with argon was irradiated with a 455 nm LED until colorless. When followed by NMR and LCMS in degassed solvents, the reaction was relatively clean and gave the expected product cleanly, but attempts at preparatory photolysis led to complex mixtures, presumably due to the photoproduct’s instability. The proton spectrum was immediately taken to show two products, assigned to the hydroquinone intermediate and lactone final product. After 10 min in the dark the major species in solution was assigned as 22 and free methanol. $^1$H NMR (600 MHz, methanol-d_4) δ 7.53 – 7.43 (m, 2H), 7.17 – 7.11 (m, 2H), 7.11 – 7.03 (m, 1H), 7.02 – 6.97 (m, 2H), 6.90 – 6.80 (m, 2H), 2.56 (s, 2H), 2.50 (s, 2H), 1.52 (s, 7H).
**UV-vis Spectra:** Photolysis was conducted with 3 mL of dilute methanol solution of compound under ambient conditions with an overhead LED inside the UV-vis cavity, scanning every 0.1 min for all compounds except 13. Changes in absorbance over time are indicated by arrows.

**Figure 1. Photolysis of 6a in methanol**

**Figure 2. Photolysis of 6b in methanol**
Figure 3. Photolysis of 11 in methanol

Figure 4. Photolysis of 12 in methanol
Figure 5. Photolysis of 13 in methanol. Due to a slow rate of photolysis, traces were >> 0.1 min apart and the sample was sparged and sealed to avoid evaporation.

Figure 6. Photolysis of 14 in methanol
Figure 7. Stepped Release of Hymecromone Monitored by UV-vis. Normalized absorption vs. time for controlled release of Hymecromone from 7. Release of the coumarin is complete during the photolysis cycles, with no dark release.
**Photolysis of 12 in CD$_3$OD.** Photolysis rapidly converted the starting material to a mixture of the hydroquinone intermediate and the final lactone product in only a few minutes (figure 8). The intermediate hydroquinone is assigned based on an overall similarity to the starting material, disappearance of the isoindolino N-CH$_2$ that undergoes an H-shift (peak at 5.23 ppm), and only a slight shift in the methyl ester peak from 3.28 to 3.26 ppm. Although from preparatory photolysis studies we expected to see an isoindole peak for the hydroquinone and lactone, deuterium exchange occurred (figure 8), presumably due to equilibration with the oxazolidine isomer. The hydroquinone converted to lactone 20 in the dark, with concomitant release of methanol. The final spectra shows quantitative chemical yield of both methanol and hydroquinone.

![Diagram](image)

**Figure 8.** Photolysis of 12 in CD$_3$OD; the hydroquinone intermediate and subsequent methanol release are observable.
Oocyte Preparation, Injection and Electrophysiology

![Figure 9](image)

**Figure 9.** A. Response from 50 mM GABA. B. Response from irradiation of caged GABA prodrug PRTL-GABA with 455 nm LED. C. Dose-response from “ex-vivo” irradiation of PRTL-GABA solutions before application to oocyte.

*Xenopus laevis* oocytes were harvested at stage V and VI according to standard protocols approved by the Caltech Institutional Animal Care and Use Committee (IACUC). Oocytes were injected with 5 ng total mRNA as a 50 nL solution of \((\alpha_1)_{2}(\beta_{2s})_{2}(\gamma_{2s})_{1} = 2:2:1\) mRNA ratio by weight before incubation at 18 °C for 24–48 hours.

Electrophysiology experiments were conducted with the two-electrode voltage clamp mode of an OpusXpress 6000A (Axon Instruments). A holding potential of 60 mV was used. HEPES-free ND96 medium was used for all experimental running/wash buffers (96 mM NaCl, 1.8 mM
CaCl$_2$, 2 mM KCl, 1 mM MgCl$_2$, 5 mM NaHCO$_3$ [pH 7.5], 190-240 mOsm). Three doses of EC$_{50}$ concentration (GABA) of which the first dose was ignored and were used to equilibrate the cell for testing. Responses from the second and third doses were averaged together for comparison of the next step (Figure 9a). For photolysis experiments, an approximately 300 μM solution of 9 was applied to the cell and aspiration was paused for the duration of irradiation (Figure 9b). Electrical noise from the LED was filtered out with the ClampFit software. After washing the photolysis products off the cell, a dose of EC$_{50}$ concentration (GABA) was applied to compare the level of signal recovery to pre-photolysis responses. For dose-response curves (Figure 9c), approximately 300 μM solutions of 9 in HEPES-free ND96 buffer were irradiated ex-vivo for various durations before the crude photolysis solutions were applied to cells. In control experiments, no response was observed for irradiation of the parent acid, 5a, which releases water in place of GABA when irradiated.

**Selective Photolysis Experimental Details**

*Figure 10. Selective photolysis of a mixture of 11 and 6a in methanol.*
A solution of 11 (1.5 mL; A = 0.3) was mixed with a solution of 6a (1.5 mL; A = 0.3) to give a mixture with the broad visible absorbance shown above. The sample was transferred to a screw-cap cuvette (to avoid evaporation) and irradiated with a 565 nm 1 watt LED with > 530 nm long-pass filter. UV-vis spectra were taken periodically until no change was detected. An aliquot was analyzed by LCMS to ensure no decaging of 6a had occurred during the complete conversion of 11 (see below). The now yellow sample was then irradiated until colorless (blue traces). The sample was then analyzed by LCMS to ensure complete decaging of 6a. Finally, a dark control sample showed no decaging without light when mixed. LCMS spectra are shown below (PES10A, 40-95% MeCN(aq)).
LCMS Data for Selective Photolysis.

![Figure 11](image)

**Figure 11.** LCMS of mixture before any irradiation.
Figure 12. LCMS of mixture after irradiation with 565 nm LED (with >530 nm long-pass).
Figure 13. LCMS of mixture after irradiation with 565 nm LED, followed by 455 nm LED.
References: