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Substituent Effects on the Sensitivity of a Quinoline Photoremovable Protecting Group to One- and Two-Photon Excitation

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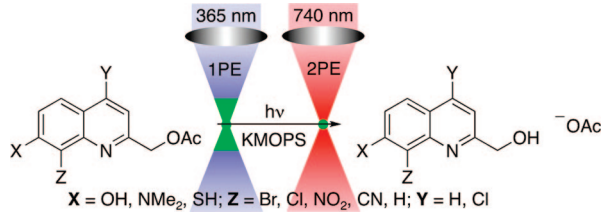

Photoremovable protecting groups that can reveal biologically important functional groups through one- and two-photon excitation (1PE and 2PE, respectively) have promise in regulating physiological function in a temporally and spatially restricted manner. Only a few chromophores have sufficient sensitivity to 2PE suitable for use as "caging groups" in physiology experiments. It would be useful to develop structure−property relationships of chromophores, so that chromophores with high two-photon uncaging action cross-sections (δ_u) can be designed. The 8-bromo-7-hydroxyquinolinyl chromophore (BHQ) releases a variety of functional groups through 1PE and 2PE. Swapping the bromine substituent for a nitro (NHQ), cyano (CyHQ), or chloro (CHQ) or exchanging the hydroxy for dimethylamino (DMAQ and DMAQ-Cl) or sulfhydryl (TQ) significantly alters the photochemical and photophysical properties of the quinoline chromophore. CyHQ-OAc demonstrated a 3-fold increase in sensitivity for acetate release, whereas NHQ-OAc was photochemically insensitive. The quantum efficiencies (Q_u) of the amino and sulfhydryl derivatives were about an order of magnitude lower than that of BHQ-OAc. All of the chromophores showed diminished sensitivity to 2PE compared to BHQ-OAc, but the CyHQ, DMAQ, and DMAQ-Cl chromophores are sufficiently sensitive for physiological use. The high sensitivity of CyHQ to 1PE will be useful in biological applications requiring short exposure with low light intensity.

Introduction

One of the barriers to using two-photon excitation (2PE) to release biological effectors in laser-scanning multiphoton microscopy applications is the small number of chromophores with sufficient sensitivity to 2PE that can be used as photoremovable protecting groups or "caging" groups.[1−4] So-called "caged compounds" have the promise to regulate cellular function in a time-dependent and highly localized manner, because the excitation volume achieved with 2PE can be restricted to that of an *E. coli* bacterium (1 fL), a volume smaller than a mammalian cell. The small dimension of excitation results from a nonlinear optical process in which the caging group absorbs two IR photons nearly simultaneously, which requires the high photon flux found at the focus of a laser beam. Other advantages of 2PE include deeper penetration into tissues and reduced photodamage because lower energy IR light is used in the excitation rather than the UV wavelengths classically employed.

To be useful for the study of physiological processes, a caging group must render the biological effector inactive or reduce its activity by several orders of magnitude. Upon exposure to light, the effector should be released rapidly in high yield. The uncaging must be on a time scale shorter than that of the process under investigation and diffusion in aqueous media (τ = 113−900 μs)[4] to ensure that release occurs within the focal volume of the laser beam. Cell permeability and high solubility in aqueous environments are also desirable properties.

(1) Dore, T. M. In *Dynamic Studies in Biology: Phototriggers, Photoswitches, and Caged Biomolecules*; Givens, R. S., Goeldner, M. , Eds.; Wiley-VCH: Weinheim, Germany, 2005; pp 435−459.
(2) Ellis-Davies, G. C. R. *Nat. Methods* **2007**, *4*, 619–628.
(3) Kiskin, N. I.; Chillingworth, R.; McCray, J. A.; Piston, D.; Ogden, D. *Eur. BioPhys. J.* **2002**, *30*, 588–604.
(4) Kiskin, N. I.; Ogden, D. *Eur. BioPhys. J.* **2002**, *30*, 571–587.

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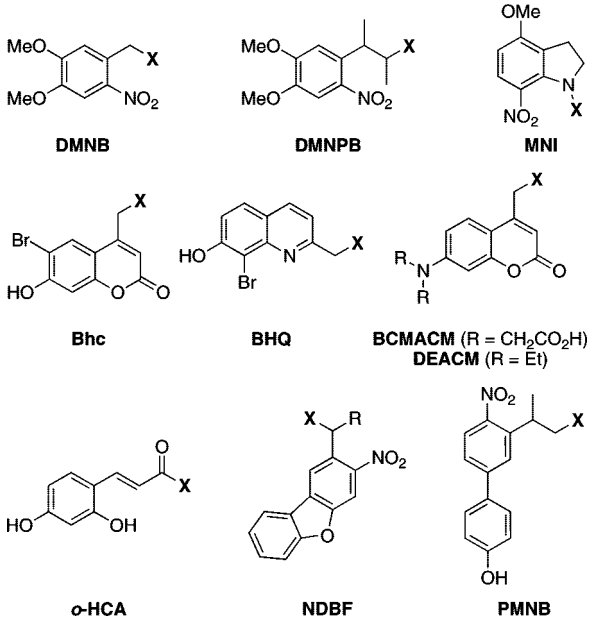


FIGURE 1. Photoremovable protecting groups used with 2PE.

A measure of the sensitivity of a chromophore to 2PE and release of a biological effector is the two-photon uncaging action cross-section (δ_u), which is the product of the absorption cross-section (δ_a) and the quantum efficiency of the uncaging reaction (Q_u) and has the units of Goeppert-Mayer (GM, 10^{-50} (cm^4•s)/photon). Several chromophores have been tested for their sensitivity to 2PE (Figure 1). The well-known and commercially available 4,5-dimethoxy-2-nitrobenzyl group (DMNB) has poor sensitivity to two-photon excitation (δ_u = 0.03 GM at 740 nm),[5,6] although a variant of this protecting group, 3-(4,5-dimethoxy2-nitrophenyl)-2-butyl (DMNPB), has a slightly higher cross-section (δ_u = 0.17 GM at 720 nm).[7] Ellis-Davies developed MNI to release glutamate, but its uncaging action cross-section is also quite low (δ_u = 0.06 GM at 720 nm).[8] Coumarin- and quinoline-based caging groups have much higher sensitivity to two-photon excitation; values of δ_u for 6-bromo-7-hydroxycoumarin (Bhc)[5] and 8-bromo-7-hydroxyquinoline (BHQ)[9,10] are 0.72 and 0.59 GM, respectively.[9] The {7-[bis(carboxymethyl)amino]coumarin-4-yl}methyl (BCMACM) chromophore is sensitive to 2PE with δ_u estimated to be in the range of 0.4−2.6 GM, depending on the protected group.[11] The related *o*-hydroxycinnamic acid derivatives (*o*-HCA) for releasing alcohols with fluorescent reporting have cross-sections that exceed 1 GM at wavelengths around 750 nm.[12,13] Nitrodibenzofuran (NDBF) has been used to release Ca^{2+} with an estimated

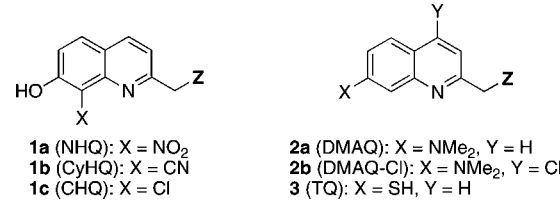


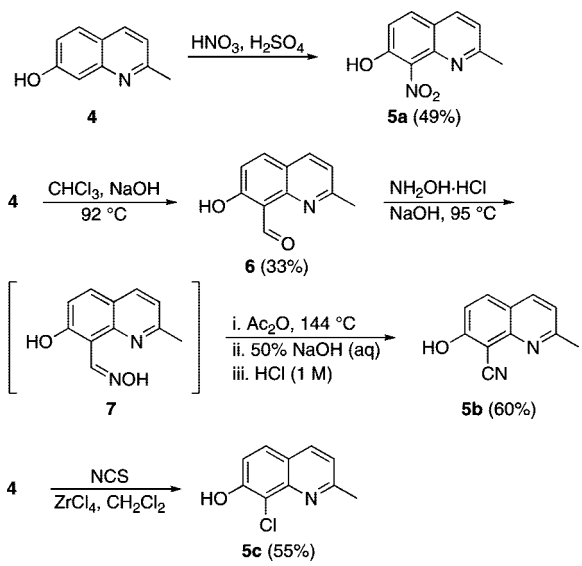
FIGURE 2. Next generation quinoline-based photoremovable protecting groups (Z = biological effector).

δ_u of 0.6 GM,[14] and recently, Bolze and Goeldner have reported a 3-(2-propyl)-4-methoxy-4-nitrobiphenyl (PMNB) that releases glutamate through efficient 2PE at longer wavelengths (δ_u = 0.45 GM at 800 nm).[15] This value compares to δ_u = 0.42 GM measured for Bhc-protected glutamate by Tsien and co-workers.[5]

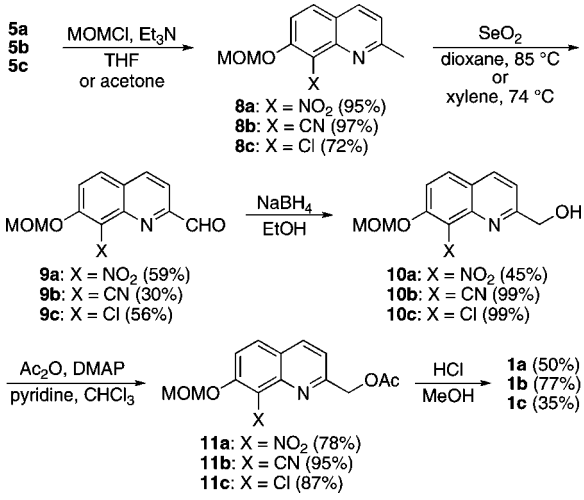
Despite the progress made in discovering chromophores with greater sensitivity to 2PE in the near IR, designing caging groups with large δ_u remains a challenge, because general rules for predicting δ_u from structure do not yet exist. It would be useful to generate structure−property relationships for chromophores, so that better predictive tools can be created. To this end, we synthesized a series of BHQ derivatives (**1**−**3**, Z = OAc, Figure 2) to study the role that substituent effects play on the sensitivity of quinoline-based photoremovable protecting groups to 1PE and 2PE.[16] Mechanistic studies on BHQ suggested that the photolysis reaction proceeds on the sub-microsecond time scale by a solvent-assisted photoheterolysis (S_N1) reaction mechanism from the singlet excited state.[10] Through the heavy atom effect, the bromine substituent of BHQ promotes ISC to the triplet state, which is nonproductive in the reaction. ISC competes with the photochemistry; therefore, we modified the quinoline core in an effort to improve Q_u and δ_u. One role for the bromine in BHQ is to lower the pK_a of the phenol so that the phenolate is the predominant form of the chromophore present at physiological pH. The phenolate has λ_{max} and molar absorptivity larger than those of the phenol, enabling the use of longer wavelength and less intense light to mediate the photochemical uncaging reaction. Other electron-withdrawing groups might serve the same purpose without promoting ISC, so we created 8-nitro-7-hydroxyquinoline (NHQ), 8-cyano-7-hydroxyquinoline (CyHQ), and 8-chloro-7-hydroxyquinoline (CHQ). 7-Dimethylaminoquinoline (DMAQ) and 7-dimethylamino-4-chloro quinoline (DMAQ-Cl) were created on the basis of studies on the coumarin chromophore that revealed that a 7-dialkylamino group increases λ_{max} compared to 7-hydroxycoumarins.[17] Sulfur possesses entirely different electronic properties at neutral pH than hydroxy or amino groups, so 7-mercaptoquinoline (TQ) was created. These six new chromophores were evaluated for their ability to release a carboxylate through 1PE and 2PE in order to investigate how substituents on the quinoline core influence the photophysical and photochemical properties of the chromophore.

(5) Furuta, T.; Wang, S. S. H.; Dantzker, J. L.; Dore, T. M.; Bybee, W. J.; Callaway, E. M.; Denk, W.; Tsien, R. Y. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 1193–1200.
(6) Aujard, I.; Benbrahim, C.; Gouget, M.; Ruel, O.; Baudin, J.-B.; Neveu, P.; Jullien, L. *Chem.−Eur. J.* **2006**, *12*, 6865–6879.
(7) Specht, A.; Thomann, J.-S.; Alarcon, K.; Wittayanan, W.; Ogden, D.; Furuta, T.; Kurakawa, Y.; Goeldner, M. *ChemBioChem* **2006**, *7*, 1690–1695.
(8) Matsuzaki, M.; Ellis-Davies, G. C. R.; Nemoto, T.; Miyashita, Y.; Iino, M.; Kasai, H. *Nat. Neurosci.* **2001**, *4*, 1086–1092.
(9) Fedoryak, O. D.; Dore, T. M. *Org. Lett.* **2002**, *4*, 3419–3422.
(10) Zhu, Y.; Pavlos, C. M.; Toscano, J. P.; Dore, T. M. *J. Am. Chem. Soc.* **2006**, *128*, 4267–4276.
(11) Hagen, V.; Dekowski, B.; Kotzur, N.; Lechler, R.; Wiesner, B.; Briand, B.; Beyermann, M. *Chem.−Eur. J.* **2008**, *14*, 1621–1627.
(12) Gagey, N.; Neveu, P.; Benbrahim, C.; Goetz, B.; Aujard, I.; Baudin, J.-B.; Jullien, L. *J. Am. Chem. Soc.* **2007**, *129*, 9986–9998.
(13) Gagey, N.; Neveu, P.; Jullien, L. *Angew. Chem., Int. Ed.* **2007**, *46*, 2467–2469.
(14) Momotake, A.; Lindegger, N.; Niggli, E.; Barsotti, R. J.; Ellis-Davies, G. C. R. *Nat. Methods* **2006**, *3*, 35–40.
(15) Gug, S.; Charon, S.; Specht, A.; Alarcon, K.; Ogden, D.; Zietz, B.; Leonard, J.; Haacke, S.; Bolze, F.; Nicoud, J.-F.; Goeldner, M. *ChemBioChem* **2008**, *9*, 1303–1307.
(16) Quinoline-based protecting groups for carbonyls, amines, and alcohols have been reported for use in synthesis. The photodeprotection reactions have not been conducted in biologically relevant media, and the reaction mechanisms are likely radical in nature. See: (a) Rukachaisirikul, V.; Koert, U.; Hoffmann, R. W. *Tetrahedron* **1992**, *48*, 4533–4544. (b) Rukachaisirikul, V.; Hoffmann, R. W. *Tetrahedron* **1992**, *48*, 10563–10568. (c) Epling, G. A.; Walker, M. E. *Tetrahedron Lett.* **1982**, *23*, 3843–3846. (d) Epling, G. A.; Provatas, A. A. *Chem. Commun.* **2002**, 1036–1037.
(17) Eckardt, T.; Hagen, V.; Schade, B.; Schmidt, R.; Schweitzer, C.; Bendig, J. *J. Org. Chem.* **2002**, *67*, 703–710.

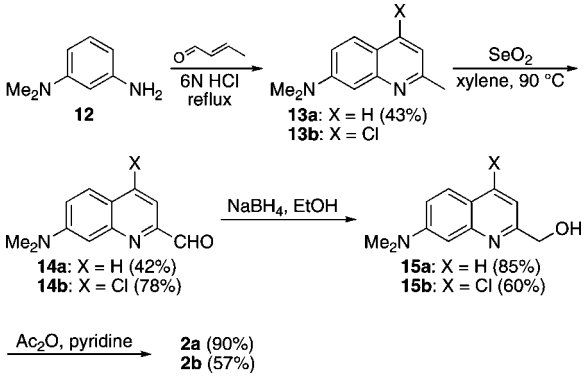
SCHEME 1. Synthesis of Substituted Quinolines 5a, 5b, and 5c



SCHEME 2. Synthesis of NHQ-OAc, CyHQ-OAc, and CHQ-OAc



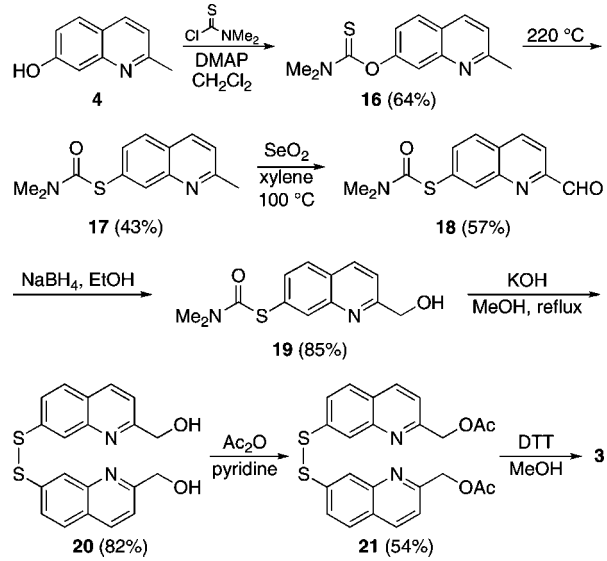
SCHEME 3. Synthesis of DMAQ-OAc and DMAQ-Cl-OAc



Results

Chromophores **1**−**3** with acetate as the simulated biological effector (Z = OAc, Figure 2) were synthesized as shown in Schemes 1−4. 8-Substituted quinolines NHQ-OAc (**1a**), CyHQ-OAc (**1b**), and CHQ-OAc (**1c**) were prepared from appropriately substituted quinolines **5a**, **5b**, and **5c** (Scheme 1). Treatment of

SCHEME 4. Synthesis of TQ-OAc



7-hydroxyquinoline **4** with fuming nitric acid selectively nitrated the 8-position of the quinoline to give **5a**. Exploiting the selectivity for electrophilic aromatic substitution at the 8-position of quinoline, a Reimer−Tiemann reaction generated aldehyde **6**, which could be converted to the nitrile **5b** through intermediate oxime **7**. Chlorination of **4** with *N*-chlorosuccinimide generated 8-chloro-7-hydroxyquinoline **5c**.

Each of the 8-substituted quinolines **5a**, **5b**, and **5c** was converted to NHQ-OAc (**1a**), CyHQ-OAc (**1b**), and CHQ-OAc (**1c**), respectively, by similar synthetic procedures (Scheme 2). Protection as the methoxymethyl ether followed by oxidation with selenium dioxide yielded aldehydes **9a**, **9b**, and **9c**. Reduction with sodium borohydride and treatment of the resulting alcohol with acetic anhydride yielded acetates **11a**, **11b**, and **11c**, which could be selectively deprotected under acidic conditions to provide **1a**, **1b**, and **1c** (Z = OAc).

DMAQ-OAc (**2a**) and DMAQ-Cl-OAc (**2b**) were synthesized from quinolines **13a** and **13b**, respectively (Scheme 3). Condensation of *N*,*N*-dimethyl-1,3-benzenediamine (**12**) with crotonaldehyde in refluxing 6 N hydrochloric acid provided **13a**,[18] and **13b** was prepared from a known literature procedure.[18] Oxidation with selenium dioxide of the benzylic methyl group to the aldehyde followed by reduction with sodium borohydride provided **15a** and **15b**, which were acetylated to generate **2a** and **2b**.

TQ-OAc (**3**) was synthesized in seven steps from hydroxyquinoline **4**[9,19] (Scheme 4). Reaction of dimethylthiocarbamoyl chloride with **4** produced thiocarbamate **16**, which rearranged to carbamate **17** when heated to 220 °C.[20] Oxidation followed by reduction provided alcohol **19**. Removal of the carbamate from the sulfur by refluxing **19** in base resulted in dimerized product **20**, which could be acetylated with acetic anhydride to yield **21**. Because of its stability, characterization of TQ was performed on dimer **21**. The monomeric form **3** was obtained by stirring **21** with DTT in methanol for 3 h, monitoring by

(18) Nasr, M.; Drach, J. C.; Smith, S. H.; Shipman, C., Jr.; Burckhalter, J. H. *J. Med. Chem.* **1988**, *31*, 1347–1351.
(19) Song, Z.; Mertzman, M.; Hughes, D. L. *J. Heterocycl. Chem.* **1993**, *30*, 17–21.
(20) Chen, Y.; Zhang, Q.; Zhang, B.; Xia, P.; Xia, Y.; Yang, Z.-Y.; Kilgore, N.; Wild, C.; Morris-Natschke, S. L.; Lee, K.-H. *Bioorg. Med. Chem.* **2004**, *12*, 6383–6387.

TABLE 1. Photophysical and Photochemical Properties of Quinoline Chromophores[a]

Chromophore	λ_{max} (nm)	ε ($M^{-1}\cdot cm^{-1}$)	λ_{em}[b] (nm)	Q_u[c]	sensitivity $Q_u \times \varepsilon$	δ_u (GM)[d]	δ_a[e] (GM)[c]	τ_{dark}[f] (h)
BHQ-OAc	369[g]	2600[g]	478	0.29[g]	754	0.59[g]	2.0	71[g]
NHQ-OAc (**1a**)	350	6500		0.00	0	0.00		278
CyHQ-OAc (**1b**)	364	7700	436	0.31	2387	0.32	1.0	500
CHQ-OAc (**1c**)	370	2800	492	0.10	280	0.12	1.2	49
DMAQ-OAc (**2a**)	368	4600	496	0.046	211	0.13	2.8	31
DMAQ-Cl-OAc (**2b**)	386	3300	493	0.090	234	0.47	5.2	34
TQ-OAc (**3**)	369	5200		0.063	328	0.42	6.7	29

[a] Measured in KMOPS, pH 7.2. [b] $\lambda_{ex} = 365$ nm. [c] Measured at 365 nm. [d] Measured at 740 nm, GM $= 10^{-50}$ ($cm^4\cdot s$)/photon. [e] Calculated two-photon absorbance cross-section, $\delta_u = \delta_a \times Q_u$. [f] Time constant for hydrolysis in the dark. [g] Value taken from Fedoryak and Dore.[9]

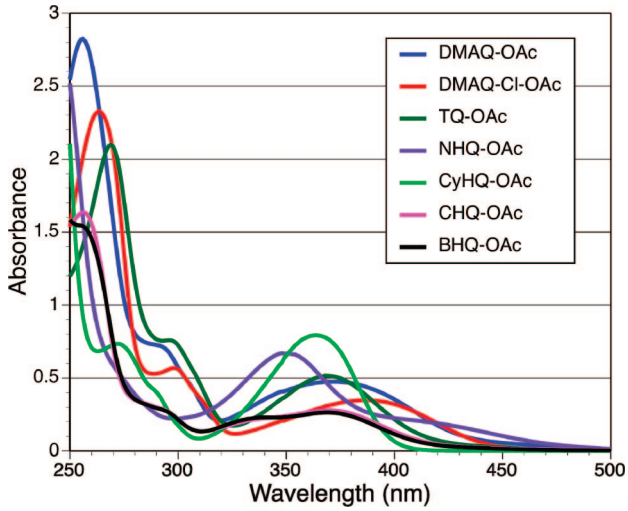


FIGURE 3. UV−vis spectra of BHQ, NHQ-OAc, CyHQ-OAc, CHQ-OAc, DMAQ-OAc, DMAQ-Cl-OAc, and TQ-OAc (100 μM in KMOPS).

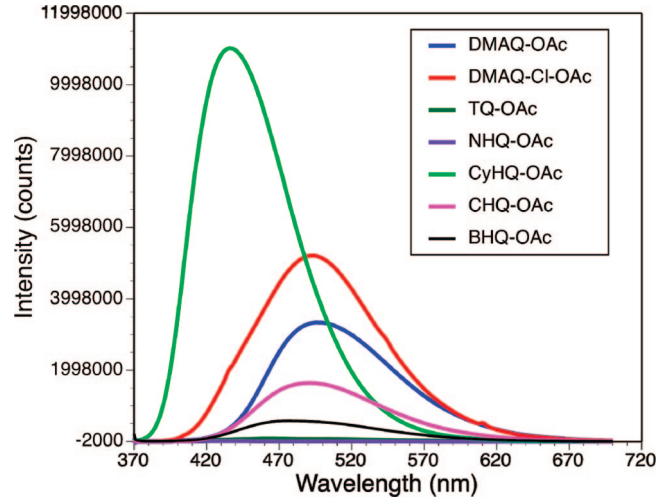


FIGURE 4. Fluorescence spectra of BHQ, NHQ-OAc, CyHQ-OAc, CHQ-OAc, DMAQ-OAc, DMAQ-Cl-OAc, and TQ-OAc (15 μM in KMOPS, $\lambda_{ex} = 365$ nm)

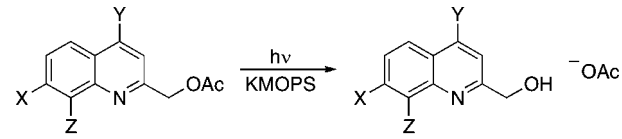
HPLC to ensure reaction completion. The methanolic solution of **3** and DTT was used for all photochemical experiments. DTT is water-soluble, biocompatible, and nonabsorbent in the near UV wavelengths, so it does not present a problem for photophysical and photochemical experiments.

UV−vis spectroscopy reveals that CyHQ-OAc, CHQ-OAc, DMAQ-OAc, and TQ-OAc, have λ_{max} values similar to that of the parent compound BHQ-OAc, whereas λ_{max} for DMAQ-Cl-OAc is slightly elevated, probably due to the presence of the chlorine, and λ_{max} for NHQ-OAc is slightly lower (Table 1). All of the new chromophores exhibited molar absorptivities greater than that of BHQ-OAc. The UV−vis spectra (Figure 3) of BHQ-OAc and CHQ-OAc in aqueous KMOPS buffer (100 mM KCl, 10 mM MOPS, pH 7.2) each show bands at 320 and 370 nm, which correspond to the phenolic and phenolate forms, respectively. In contrast, NHQ-OAc and CyHQ-OAc display only one band at 350 and 364, respectively, suggesting that these two compounds exist as the phenolate in neutral aqueous buffer.

There are distinct differences in the fluorescence spectra of the chromophores (Figure 4). Compared to BHQ-OAc, which exhibits little fluorescence emission, CyHQ-OAc, CHQ-OAc, DMAQ-OAc, and DMAQ-Cl-OAc have stronger emission bands with the most fluorescent chromophore, CyHQ-OAc, exhibiting a strong emission band at 436 nm. NHQ-OAc and TQ-OAc exhibit almost no fluorescence emission.

Compounds **1**−**3** (Z = OAc) in KMOPS were each irradiated with 365-nm light, and the time course of each photochemical reaction to the corresponding alcohols **22**−**24** and acetate (Scheme 5) was monitored by HPLC (Figure 5). From the single exponential decay fit of the data, the uncaging quantum

SCHEME 5. Photolysis Reaction of BHQ-OAc, NHQ-OAc, CyHQ-OAc, CHQ-OAc, DMAQ-OAc, DMAQ-Cl-OAc, and TQ-OAc



1a: X = OH, Z = NO_2, Y = H
1b: X = OH, Z = CN, Y = H
1c: X = OH, Z = Cl, Y = H
2a: X = NMe_2, Y = Z = H
2b: X = NMe_2, Y = Cl, Z = H
3: X = SH, Y = Z = H

No Reaction

22a
22b
22c
23a
23b
24

efficiency (Q_u) was calculated using eq 1 as previously described:[5,9,10,21]

$$Q_u = (I\sigma t_{90\%})^{-1} \quad (1)$$

where I is the radiant power in einstein$\cdot cm^{-2}\cdot s^{-1}$ (measured by potassium ferrioxalate actinometry[22]), σ is the decadic extinction coefficient ($10^3 \times \varepsilon$, molar extinction coefficient) in $cm^2\cdot mol^{-1}$, and $t_{90\%}$ is the irradiation time in seconds for 90% conversion to product.

The quantum efficiencies for photolysis of CHQ-OAc, DMAQ-OAc, DMAQ-Cl-OAc, and TQ-OAc under simulated physiological conditions were significantly lower than the value for BHQ-OAc (Table 1), while the value for CyHQ-OAc was

(21) Lu, M.; Fedoryak, O. D.; Moister, B. R.; Dore, T. M. *Org. Lett.* **2003**, *5*, 2119–2122.
(22) Hatchard, C. G.; Parker, C. A. *Proc. R. Soc. London, Ser. A* **1956**, *235*, 518–536.

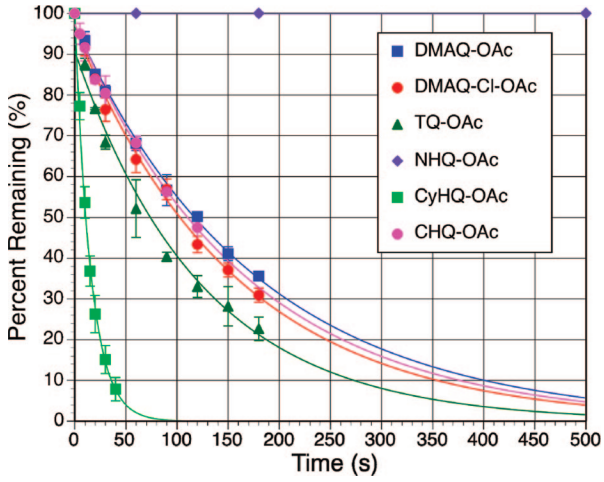


FIGURE 5. Time course of one-photon photolyses of NHQ-OAc, CyHQ-OAc, CHQ-OAc, DMAQ-OAc, DMAQ-Cl-OAc, and TQ-OAc at 365 nm. The percent remaining was determined by HPLC and is the average of 3 runs. Lines are least-squares fits of a single exponential decay. Error bars represent the standard deviation of the measurement.

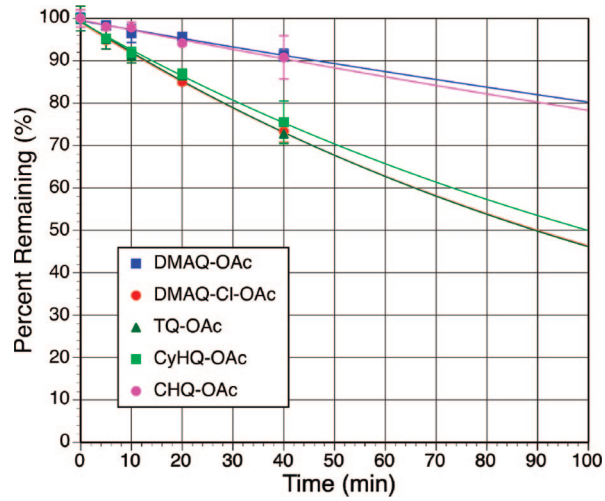


FIGURE 6. Time course of two-photon photolysis of CyHQ-OAc, CHQ-OAc, DMAQ-OAc, DMAQ-Cl-OAc, and TQ-OAc at 740 nm. The percent remaining was determined by HPLC (corrected for background hydrolysis in the dark) and is the average of 3 runs. Lines are least-squares fits of a single exponential decay. Error bars represent the standard deviation of the measurement.

similar. NHQ-OAc did not show evidence of photolysis after 10 min of exposure to 365-nm light. Correspondingly, the sensitivities ($Q_u \times \varepsilon$) of CHQ-OAc, DMAQ-OAc, DMAQ-Cl-OAc, and TQ-OAc were low, whereas the sensitivity of CyHQ-OAc was high.

To measure the time constant for dark hydrolysis (τ_{dark}), each caged compound **1**−**3** was dissolved in KMOPS buffer at pH 7.2 and stored in the dark at room temperature. HPLC analysis was performed periodically to determine the extent of hydrolysis of the acetate. NHQ-OAc and CyHQ-OAc were quite robust under these conditions. The other compounds showed adequate resistance to dark hydrolysis but were inferior to BHQ-OAc in this regard.

The two-photon uncaging action cross-section (δ_u) was measured by exposing a small volume of each of the caged compounds **1**−**3** in KMOPS to 740 nm from a Ti:sapphire laser and relating the rate of photolysis to the fluorescence output of a fluorophore with known 2PE properties using eq 2 as previously described.[5,9,10,21]

$$\delta_u = \frac{N_p \varphi Q_f \delta_a C_F}{<F(t)> C_S} \qquad (2)$$

N_p is the number of molecules photolyzed per unit time (molecules/s,); ϕ is the collection efficiency of the detector used to measure the fluorescence of fluorescein emitted at a right angle to the beam and passed though a 535/45 nm bandpass filter; Q_f is the fluorescence quantum yield of fluorescein (0.9[23,24]); δ_a is the absorbance cross-section of fluorescein (30 GM at 740 nm[23,24]); C_F is the concentration of the fluorescein standard (mol/L); $<F(t)>$ is the time-averaged fluorescent photon flux (photons/s) of the fluorescein standard collected by the detector; and C_S is the initial concentration of the caged compound (mol/L). The average power of the laser was 300−350 mW, which is significantly larger than that used on biological preparations, which is typically less than 10 mW. The higher power is required to enable sufficient reaction progress to detect the disappearance of starting material by HPLC. There was a small but not insignificant amount of degradation due to spontaneous hydrolysis in the dark of CHQ-OAc, DMAQ-OAc, DMAQ-Cl-OAc, and TQ-OAc while irradiated samples waited for HPLC analysis. We compensated for this by subtracting the amount of background hydrolysis (calculated from the time each sample spent in the buffer prior to evaluation by HPLC and the time constant for dark hydrolysis in KMOPS) from the total amount of decay measured by HPLC to give the decay resulting from photolysis. CyHQ-OAc was unaffected by dark hydrolysis on the time scale of the experiment, so no correction was necessary in this case. The corrected (if necessary) time courses for two-photon photolysis of CyHQ-OAc, CHQ-OAc, DMAQ-OAc, DMAQ-Cl-OAc, and TQ-OAc (Figure 6) were used to determine the initial rate of two-photon photolysis, from which N_p was calculated.

Discussion

Our working hypothesis that ISC competes with the photouncaging reaction and diminishes the effectiveness the chromophores might have for controlling cellular processes with light led us to design systems that lacked a bromine, which promotes the singlet to triplet excited-state transition through the heavy atom effect. The quantum efficiencies of the uncaging reaction of the quinoline derivatives **1**−**3** span a broad range of 0−0.31 (Table 1). The 7-hydroxy group is clearly important for a robust photochemical reaction compared to the 7-dimethylamino and 7-sulfhydryl quinolines **2** and **3**. Compared to BHQ-OAc, the 7-dimethylamino analogs DMAQ-OAc and DMAQ-Cl-OAc do not show the ~25-nm red shift in absorption wavelength of the coumarin analogs (compare Bhc-cAMP λ_{max} = 375 nm[25] vs DEACM-cAMP λ_{max} = 402 nm[26]). Placing chlorine at the 4-position red shifts λ_{max} by 18 nm relative to the unsubstituted dimethylamino-quinoline, though at the cost of reducing the

(23) Xu, C.; Webb, W. W. *J. Opt. Soc. Am. B* **1996**, *13*, 481–491.
(24) Xu, C.; Guild, J.; Webb, W. W.; Denk, W. *Opt. Lett.* **1995**, *20*, 2372–2374.
(25) Furuta, T.; Takeuchi, H.; Isozaki, M.; Takahashi, Y.; Kanehara, M.; Sugimoto, M.; Watanabe, T.; Noguchi, K.; Dore, T. M.; Kurahashi, T.; Iwamura, M.; Tsien, R. Y. *ChemBioChem* **2004**, *5*, 1119–1128.
(26) Hagen, V.; Bendig, J.; Frings, S.; Eckardt, T.; Helm, S.; Reuter, D.; Kaupp, U. B. *Angew. Chem., Int. Ed. Engl.* **2001**, *40*, 1046–1048.

molar absorptivity. Substitutions at the 3- and 4-positions of the quinoline system might provide an avenue for red shifting the absorption maximum.

Surprisingly, NHQ-OAc did not photolyze after prolonged exposure to light and is quite stable in neutral aqueous buffer. UV−vis spectroscopy revealed that the chromophore exists primarily as the absorbent phenolate at pH 7.2. The photochemistry does not compete with fluorescence as it exhibits almost no emission upon excitation with 365-nm light. We hypothesize that upon excitation the nitro group enables access to a low-lying triplet state, which decays non-radiatively and without photochemistry to the ground state. Nitro groups are known to have low-lying triplet states,[27] and they promote radiationless decay through interaction with adjacent oxygen atoms.[28]

We expected that swapping bromine for chlorine would enhance the photochemistry quantum efficiency, without significantly changing the ratio of phenol to phenolate present at physiological pH. UV−vis spectroscopy confirms that both phenol and phenolate are present at pH 7.2 in similar ratios as BHQ-OAc. Counter to our expectations, the quantum efficiency of CHQ-OAc ($Q_u = 0.10$) was much lower than the value for BHQ-OAc ($Q_u = 0.29$), and the fluorescence emission was higher. Decay of the excited state through fluorescence and other photophysical processes compete with uncaging; this might explain the lower efficiency of the photochemistry of CHQ-OAc.

CyHQ-OAc has increased sensitivity to 1PE compared to BHQ-OAc and the other chromophores. The increased sensitivity is not attributable to an increase in the quantum efficiency of the photochemistry ($Q_u = 0.31$ and 0.29 for CyHQ-OAc and BHQ-OAc, respectively) but rather a nearly 3-fold rise of the molar absorptivity. UV−vis spectroscopy confirms that the strongly electron-withdrawing nature of the cyano substituent lowers the pK_a of the phenol so that the more absorbent phenolate form is present nearly exclusively at pH 7.2. CyHQ-OAc fluoresces brightly relative to the other chromophores, indicating that decay of the singlet excited state through fluorescence competes with uncaging, thereby limiting the quantum efficiency. The electron-withdrawing strength of the cyano also bequeaths remarkable hydrolytic stability in the dark ($\tau = 500$ vs 71 h for CyHQ-OAc and BHQ-OAc, respectively), because the lone pairs on the quinoline nitrogen are not as available to act as an intramolecular general base catalyst in the hydrolysis of the acetate.

A great deal of effort has been placed into optimizing chromophores for two-photon absorption (2PA) in the past 10 years, particularly for the development of new materials for nonlinear optical applications, such as 3-dimensional data storage.[29] While chromophores have been created with high 2PA cross-sections, mainly composed of a combination of electron donors and acceptors separated by a conjugated system, these systems have not been engineered to mediate photochemical release of biological effectors. Biocompatibility, cell permeability, and toxicity place structural limitations on chromophores that would be used to release biological effectors in a physiological system.

The two-photon uncaging action cross-sections (δ_u) for **1**−**3** spanned a broad range ($\delta_u = 0.0-0.47$ GM) but were lower than BHQ-OAc ($\delta_u = 0.59$ GM). If 1PE does not initiate photochemical uncaging, then 2PE is not expected to do so either, which is what we observed with NHQ-OAc. It has no sensitivity to 2PE. Outside of this example, the sensitivity to 1PE-mediated uncaging does not correlate with sensitivity to 2PE. DMAQ-Cl-OAc had the highest value of δ_u but one of the lowest sensitivities to 1PE, and CyHQ-OAc with its large sensitivity to 1PE did not have an uncaging action cross-section larger than that of BHQ-OAc. CHQ-OAc was far less sensitive to 2PE than BHQ-OAc and CyHQ-OAc. We speculate that bromine might be important for conferring sensitivity to 2PE for quinoline-based photoremovable protecting groups.

Conclusions

We found that CyHQ-OAc had ∼3 times greater sensitivity to 1PE-mediated release of acetate than the parent BHQ-OAc, owing to the increase in molar absorptivity. This will enable its application in physiology experiments that necessitate lower light intensity for the release of biological effectors. None of the quinoline analogs tested had greater sensitivity to 2PE relative to that of BHQ-OAc, but CyHQ-OAc and DMAQ-Cl-OAc have sufficient sensitivity to be potentially useful. These quinoline-based caging groups provide an avenue for developing models for the role substituents play on the 1PE- and 2PE-mediated photochemistry of uncaging. Spectroscopic and computational studies are in progress to help understand the differences in their 1PE- and 2PE-mediated photochemistry.

Experimental Methods

7-Hydroxy-8-nitroquinaldine (5a). Concentrated HNO_3 (4 drops) was added to a solution of 7-hydroxyquinaldine (**4**, 200 mg, 1.258 mmol) in concentrated H_2SO_4 (1.0 mL) at 0 °C, and the mixture was stirred for 10 min. The reaction was neutralized with ammonium hydroxide and concentrated under vacuum. The remaining residue was dissolved in chloroform, and the resulting clear solution was separated from the solid ammonium salts and concentrated to afford **5a** (126 mg, 0.618 mmol, 49%) as a bright yellow solid: ^{1}H NMR (CD_3OD) δ 8.16 (1H, d, $J = 8.4$ Hz), 7.84 (1H, d, $J = 8.8$ Hz), 7.31 (1H, d, $J = 8.4$ Hz), 7.20 (1H, d, $J = 8.8$ Hz), 2.67 (3H, s); ^{13}C NMR (DMSO-d_6) δ 161.8, 150.1, 140.7, 136.9, 134.7, 131.2, 121.6, 120.6, 118.5, 25.7; FTIR (neat) 2916, 2848, 1633, 1585, 1525, 1508, 1375, 1344, 841 cm^{-1}; MS (ESI) m/z calcd for $(C_{10}H_8N_2O_3 + H)^+$ 205, found 205; HRMS (ESI) m/z calcd for $(C_{10}H_8N_2O_3 + H)^+$ 205.0613, found 205.0600.

7-Hydroxy-2-methylquinoline-8-carbaldehyde (6). Chloroform (15 mL) was added to a mixture of **4** (1.000 g, 6.04 mmol) in a solution of NaOH (7 g in 8 mL water). The reaction was stirred at 92 °C for 20 h. After cooling, the reaction was filtered, washing with water, and the filtrate was extracted into chloroform, which was separated and evaporated to give a residue that was purified by column chromatography (1:1 EtOAc/hexane) to give **6** (0.380 g, 2.00 mmol, 33%) as a yellow solid: ^{1}H NMR (acetone-d_6) δ 13.05 (1H, s), 11.20 (1H, s), 8.19 (1H, d, $J = 8.4$ Hz), 8.14 (1H, d, $J = 9.2$ Hz), 7.39 (1H, d, $J = 8.4$ Hz), 8.10 (1H, d, $J = 9.2$ Hz), 2.70 (3H, s); ^{13}C NMR (acetone-d_6) δ 197.4, 165.9, 160.7, 148.3, 137.2, 136.3, 120.6, 120.0, 118.8, 112.6, 25.5; FTIR (neat) 2893, 1629, 1612, 1504, 1292, 1269, 1178, 1140, 781, 692, 662 cm^{-1}; MS (ESI) m/z calcd for $(C_{11}H_9NO_2 + H)^+$ 188, found 188; HRMS (ESI) m/z calcd for $(C_{11}H_9NO_2 + H)^+$ 188.0712, found 188.0746.

7-Hydroxy-2-methylquinoline-8-carbaldehyde Oxime (7). Hydroxylamine hydrochloride (0.106 g, 1.52 mmol) was added to a mixture of **6** (0.200 g, 1.06 mmol) in a solution of NaOH (0.3 in

(27) McGlynn, S. P.; Azumi, T.; Kinoshita, M. *Molecular Spectroscopy of the Triplet State*; Prentice-Hall: Englewood Cliffs, NJ, 1969.

(28) Varma, C. A. G. O.; Plantenga, F. L.; Huizer, A. H.; Zwart, J. P.; Bergwerf, P.; Van der Ploeg, J. P. M. *J. Photochem.* **1984**, *24*, 133–199.

(29) Rumi, M.; Barlow, S.; Wang, J.; Perry, J. W.; Marder, S. R. In *Photoresponsive Polymers I*; Springer-Verlag: Berlin, 2008; Vol. 213, pp 1−95.

6 mL water). The reaction was stirred at 95 °C for 15 min, followed by the addition of glacial acetic acid until the reaction reached pH 6. The resulting mixture was cooled in an ice bath and vacuum filtered to afford **7** (0.176 g, 0.869 mmol, 82% yield) as a yellow solid, which was carried to the next step without further purification.

7-Hydroxy-2-methylquinoline-8-carbonitrile (5b). Compound **7** (0.160 g, 0.792 mmol) was added to acetic anhydride (3.5 mL), and the resulting mixture was stirred at 144 °C under nitrogen atmosphere for 7.5 h. Concentrated NaOH solution (50%) was added to the mixture slowly with stirring until the reaction reached pH 12. The solvent was evaporated, and the resulting dark brown residue was purified by column chromatography (1:1 EtOAc/hexane) to yield **5b** (0.0874 g, 0.475 mmol, 60%) as a yellow solid: ^{1}H NMR (acetone-d_6) δ 8.20 (1H, J = 8.0 Hz), 8.04 (1H, d, J = 8.8 Hz), 7.37 (1H, d, J = 8.4 Hz), 7.36 (1H, d, J = 9.2 Hz), 2.69 (3H, s); ^{13}C NMR (acetone-d_6) δ 163.1, 161.7, 149.1, 136.6, 133.9, 121.0, 117.3, 114.8, 95.7, 24.7; FTIR (neat) 3111, 2922, 2229, 1620, 1577, 1508, 1446, 1342, 1271, 1143, 844 cm^{-1}; MS (ESI) *m*/*z* calcd for $(C_{11}H_8N_2O + H)^+$ 185, found 185; HRMS (ESI) *m*/*z* calcd for $(C_{11}H_8N_2O + H)^+$ 185.0716, found 185.0766.

8-Chloro-7-hydroxyquinaldine (5c). Under a nitrogen atmosphere, 7-hydroxyquinaldine (**4**, 0.3900 g, 2.452 mmol) was added to a solution of NCS (0.3283 g, 2.459 mmol) and zirconium chloride (0.0285 g, 0.122 mmol) in dichloromethane (35 mL). After stirring for 23 h, the solution was diluted with chloroform and washed successively with saturated sodium carbonate solution, water, and brine. The solvent was evaporated to give a brown residue, which was purified by column chromatography (1:3 EtOAc/hexane) to yield **5c** (0.2612 g, 1.349 mmol, 55%) as an oily yellow residue: ^{1}H NMR ($CDCl_3$) δ 8.00 (1H, d, J = 8.0 Hz), 7.64 (1H, d, J = 9.2 Hz), 7.28 (1H, d, J = 8.0 Hz), 7.23, (1H, d, J = 8.4 Hz), 2.80 (3H, s); ^{13}C NMR ($CDCl_3$) δ 160.6, 152.8, 144.9, 136.6, 127.5, 122.7, 120.7, 117.0, 114.6, 25.9; FTIR (neat) 2926, 1619, 1509, 1437, 1343, 1267, 1215, 1141, 1002, 835, 750 cm^{-1}; MS (ESI) *m*/*z* calcd for $(C_{10}H_8ClNO + H)^+$ 194 (35Cl) and 196 (37Cl), found 194 (35Cl) and 196 (37Cl); HRMS (ESI) *m*/*z* calcd for $(C_{10}H_8ClNO + H)^+$ 194.0373 (35Cl) and 196.0343 (37Cl), found 194.0362 (35Cl) and 196.0333 (37Cl).

7-(Methoxymethoxy)-2-methylquinoline-8-carbonitrile (8b). Under a nitrogen atmosphere, triethylamine (0.034 mL, 0.235 mmol) and chloromethyl methyl ether (0.018 mL, 0.235 mmol) were added to a solution of **5b** (0.0200 g, 0.109 mmol) in acetone (8 mL). The reaction was stirred for 1 h. The solvent was evaporated, and the remaining residue was dissolved in chloroform, which was washed with brine, dried over Na_2SO_4, and evaporated to afford **8b** (0.0236 g, 0.106 mmol, 97%) as a white solid: ^{1}H NMR ($CDCl_3$) δ 8.02 (1H, d, J = 8.0 Hz), 7.93 (1H, d, J = 9.2 Hz), 7.48 (1H, d, J = 9.2 Hz), 7.29 (1H, d, J = 8.4 Hz), 5.43 (2H, s), 3.58 (3H, s), 2.79 (3H, s); ^{13}C NMR ($CDCl_3$) δ 162.4, 161.8, 148.7, 135.9, 133.4, 121.8, 121.7, 114.6, 114.9, 95.1, 56.9, 25.7; FTIR (neat) 2924, 2216, 1612, 1504, 1248, 1161, 1143, 1061, 984, 924, 839 cm^{-1}; MS (ESI) *m*/*z* calcd for $(C_{13}H_{12}N_2O_2 + H)^+$ 229, found 229; HRMS (ESI) *m*/*z* calcd for $(C_{13}H_{12}N_2O_2 + H)^+$ 229.0978, found 229.0996.

2-Formyl-7-(methoxymethoxy)quinoline-8-carbonitrile (9b). Under a nitrogen atmosphere selenium dioxide (0.0145 g, 0.131 mmol) was added to a solution of **8b** (0.0300 g, 0.136 mmol) in *m*-xylene (5 mL). The mixture was stirred at 74 °C for 20 h then filtered. The filtrate was evaporated, and the residue was purified by column chromatography (3:7 EtOAc/hexane) to yield **9b** (0.0100 g, 0.041 mmol, 30%) as a white solid: ^{1}H NMR ($CDCl_3$) δ 10.29 (1H, s), 8.34 (1H, d, J = 8.0 Hz), 8.09 (1H, d, J = 9.6 Hz), 8.04 (1H, d, J = 8.0 Hz), 7.73 (1H, d, J = 9.2 Hz), 5.50 (2H, s), 3.61 (3H, s); ^{13}C NMR ($CDCl_3$) δ 193.7, 163.0, 154.2, 148.6, 137.9, 133.9, 125.4, 118.5, 117.3, 114.3, 95.4, 57.3; FTIR (neat) 2922, 2846, 2224, 1710, 1260, 1165, 1043, 922, 856, 775 cm^{-1}; MS (ESI) *m*/*z* calcd for $(C_{13}H_{10}N_2O_3 + H)^+$ 243, found 243; HRMS (ESI) *m*/*z* calcd for $(C_{13}H_{10}N_2O_3 + H)^+$ 243.0769, found 243.0764.

2-(Hydroxymethyl)-7-(methoxymethoxy)quinoline-8-carbonitrile (10b). $NaBH_4$(0.0151 g, 0.400 mmol) was added to a solution of **9b** (0.0240 g, 0.099 mmol) in ethanol (5 mL). The mixture was stirred for 20 min. The solvent was evaporated, and the resulting residue was dissolved in chloroform, washed with water followed by brine, dried over Na_2SO_4, concentrated, and purified by column chromatography (45:55 EtOAc/hexane) to yield **10b** (0.0240 g, 0.98 mmol, 99%) as a white solid: ^{1}H NMR ($CDCl_3$) δ 8.14 (1H, d, J = 8.0 Hz), 8.00 (1H, d, J = 8.8 Hz), 7.56 (1H, d, J = 9.6 Hz), 7.31 (1H, d, J = 8.4 Hz), 5.46 (2H, s), 4.96 (2H, s), 3.59 (3H, s); ^{13}C NMR ($CDCl_3$) δ 202.5, 162.4, 147.6, 137.1, 133.9, 122.9, 118.3, 115.6, 114.6, 95.3, 64.4, 57.2; FTIR (neat) 2926, 1738, 1258, 1078, 795 cm^{-1}; MS (ESI) *m*/*z* calcd for $(C_{13}H_{10}N_2O_3 + H)^+$ 245, found 245; HRMS (ESI) *m*/*z* calcd for $(C_{13}H_{10}N_2O_3 + H)^+$ 245.0922, found 245.0914.

(8-Cyano-7-(methoxymethoxy)quinolin-2-yl)methyl Acetate (11b). Under a nitrogen atmosphere, DMAP (0.0102 g) and acetic anhydride (0.015 mL, 0.158 mmol) were added to a solution of **10b** (0.0090 g, 0.0369 mmol) in chloroform (5 mL) and pyridine (1.5 mL). The reaction was stirred for 24 h. The solvents were evaporated, and the remaining residue was purified by column chromatography (3:7 EtOAc/hexane) to provide **11b** (0.0100 g, 0.0351 mmol, 95% yield) as a white solid: ^{1}H NMR ($CDCl_3$) δ 8.16 (1H, d, J = 8.0 Hz), 7.98 (1H, d, J = 9.2 Hz), 7.55 (1H, d, J = 9.2 Hz), 7.44 (1H, d, J = 8.4 Hz), 5.46 (2H, s), 5.45 (2H, s), 3.58 (3H, s), 2.26 (3H, s); ^{13}C NMR ($CDCl_3$) δ 170.7, 162.2, 159.2, 148.2, 136.9, 133.5, 122.7, 118.5, 115.7, 114.5, 95.1, 66.7, 56.9, 21.0; FTIR (neat) 2924, 2342, 2224, 1738, 1612, 1508, 1246, 1157, 1070, 937, 846 cm^{-1}; MS (ESI) *m*/*z* calcd for $(C_{15}H_{14}N_2O_4 + H)^+$ 287, found 287; HRMS (ESI) *m*/*z* calcd for $(C_{15}H_{14}N_2O_4 + H)^+$ 287.1033, found 287.1059.

(8-Cyano-7-hydroxyquinolin-2-yl)methyl Acetate (CyHQ-OAc, 1b). HCl (12 N, 1 drop) was added to a solution of **11b** (0.0100 g, 0.0350 mmol) in methanol (3 mL), and the mixture was stirred for 35 min. The solvent was evaporated, and the residue was purified by column chromatography (35:65 EtOAc/hexane) to yield **CyHQ-OAc** (0.0065 g, 0.0269 mmol, 77%) as a white solid: ^{1}H NMR ($CDCl_3$) δ 8.14 (1H, d, J = 8.4 Hz), 7.92 (1H, d, J = 8.8 Hz), 7.42 (1H, d, J = 8.8 Hz), 7.262 (1H, d, J = 8.4 Hz), 5.45 (2H, s), 2.26 (3H, s); ^{13}C NMR ($CDCl_3$) δ 171.37, 164.4, 158.9, 148.7, 137.4, 134.0, 121.8, 118.0, 117.5, 114.9, 66.4, 19.6; FTIR (neat) 2924, 2358, 2228, 1744, 1603, 1510, 1238, 1065, 849 cm^{-1}; MS (ESI) *m*/*z* calcd for $(C_{13}H_{10}N_2O_3 + H)^+$ 243, found 243; HRMS (ESI) *m*/*z* calcd for $(C_{13}H_{10}N_2O_3 + H)^+$ 243.0770, found 243.0788.

***N*,*N*,2-Trimethylquinolin-7-amine (13a).**[18] A mixture of *N*,*N*-dimethyl-*m*-phenylene-diamine (800 mg, 3.8 mmol) and 6 N HCl (12 mL) was stirred at reflux. Crotonaldehyde (0.31 mL, 3.8 mmol) dissolved in toluene (1 mL) was added dropwise to the purple solution. The reaction mixture was stirred for 2 h, cooled and neutralized with solid NaOH, and extracted with toluene and then chloroform (×5). The extracts were combined, dried over Na_2SO_4, and evaporated to dryness. The residue was purified by column chromatography (EtOAc) to obtain **13a** (304.3 mg, 1.6 mmol, 43% yield): ^{1}H NMR ($CDCl_3$) δ 7.84 (1H, d, J = 8.4 Hz), 7.58 (1H, d, J = 8.8 Hz), 7.09 (2H, m), 6.98 (1H, d, J = 8. Hz), 3.06 (6H, s), 2.66 (3H, s); ^{13}C NMR ($CDCl_3$) δ 159.3, 151.5, 149.9, 135.8, 128.6, 119.2, 118.2, 115.5, 106.8, 40.8, 25.6; FTIR (neat) 2918, 2804, 1620, 1600, 1514, 1379, 1155, 1062, 827 cm^{-1}.

7-(Dimethylamino)quinoline-2-carbaldehyde (14a). Compound **13a** (304.3 mg, 1.63 mmol) was dissolved in *m*-xylene (2 mL) and stirred under a nitrogen atmosphere. Selenium dioxide (272.29 mg 2.45 mmol) was added, and the yellow-orange mixture was heated to 90 °C for 4 h. The black reaction mixture was filtered through cotton, washing several times with chloroform. The filtrate was directly adsorbed onto silica gel and purified by column chromatography (EtOAc) to afford **14a** (136.4 mg, 0.68 mmol, 42%) as a pale yellow oil: ^{1}H NMR ($CDCl_3$) δ 10.11 (1H, s), 8.11 (1H, d, J = 8.0 Hz), 7.74 (1H, d, J = 8.4 Hz), 7.73 (1H, d, J = 8.0 Hz), 7.31 (1H, dd, J = 9.6, 2.8 Hz), 7.23 (1H, d, J = 1.6 Hz), 3.15 (6H, s); ^{13}C NMR ($CDCl_3$,) δ 194.5, 153.0, 151.9, 150.2, 136.9, 128.6, 123.1, 119.1, 113.8, 107.0, 40.7; FTIR (neat) 2920, 1705, 1620,

1537, 1508, 1382, 1255, 1155, 1066, 835, 761 cm^{-1}; MS(ESI) *m/z* calcd for $(C_{12}H_{12}N_2O + H)^+$ 201, found, 201; HRMS (ESI) *m/z* calcd for $(C_{12}H_{12}N_2O + H)^+$ 201.1028, found, 201.1055.

7-(Dimethylamino)quinolin-2-yl)methanol (15a). Compound **14a** (45 mg, 0.22 mmol) was dissolved in absolute ethanol (0.7 mL), and the solution was cooled with stirring in an ice bath. $NaBH_4$ (8.5 mg, 0.2248 mmol) was added in one portion to the cooled, stirred solution under nitrogen. The reaction was stirred at room temperature and monitored with TLC. The ethanol was evaporated, and the residue was dissolved in chloroform, washed with brine, and dried over Na_2SO_4. The solvent was evaporated, and the remaining residue was purified by column chromatography (EtOAc) to yield **14a** (38.6 mg, 0.19 mmol, 85%) as a yellow oil: ^1H NMR ($CDCl_3$) δ 7.95 (1H, d, $J = 8.4$ Hz), 7.65 (1H, d, $J = 8.4$ Hz), 7.15 (2H, m), 6.98 (1H, d, $J = 8.0$ Hz), 4.84 (2H, s), 3.1 (6H, s); ^{13}C NMR ($CDCl_3$) δ 159.0, 151.5, 148.6, 136.2, 128.3, 120.1, 115.7, 114.1, 106.5, 64.1, 40.5; FTIR (neat) 2918, 1622, 1535, 1508, 1394, 1284, 1207, 1174, 1134, cm^{-1}; MS (ESI) *m/z* calcd for $(C_{12}H_{14}N_2O + H)^+$ 203, found 203; HRMS (ESI) *m/z* calcd for $(C_{12}H_{14}N_2O + H)^+$ 203.1179, found 203.1177.

(7-(Dimethylamino)quinolin-2-yl)methyl Acetate (DMAQ-OAc, 2a). Compound **15a** (38.6 mg, 0.191 mmol) was dissolved in pyridine (0.4 mL). Acetic anhydride (60 μL) was added, and the mixture was stirred under nitrogen overnight. The pyridine was evaporated, and the resulting residue was purified by column chromatography (9:1 EtOAc/hexane) to obtain **DMAQ-OAc** (42 mg, 0.17 mmol, 90% yield) as a bright yellow oil: ^1H NMR ($CDCl_3$) δ 7.99 (1H, d, $J = 8.0$ Hz), 7.65 (1H, d, $J = 9.2$ Hz), 7.18 (1H, d, $J = 2.4$ Hz), 7.16 (1H, d, $J = 3.2$ Hz), 7.13 (1H, d, $J = 2.8$ Hz), 5.31 (2H, s), 3.1 (6H, s), 2.18 (3H, s); ^{13}C NMR ($CDCl_3$) δ 171.0, 156.4, 151.7, 149.8, 136.6, 128.4, 120.4, 116.6, 115.7, 106.8, 68.0, 40.7, 21.3; FTIR (neat) 2929, 1741, 1622, 1514, 1444, 1382, 1222, 1157, 1053, 829, 615 cm^{-1}; MS (ESI) *m/z* calcd for $(C_{14}H_{16}N_2O_2 + H)^+$ 245, found 245; HRMS (ESI) *m/z* calcd for $(C_{14}H_{16}N_2O_2 + H)^+$ 245.1285, found 245.1287.

***O*-2-Methylquinolin-7-yl Dimethylcarbamothioate (16).** Under nitrogen, dimethylthiocarbamoyl chloride (1.35 g, 10.95 mmol) and DMAP (1.33 g, 10.95 mmol) were added to a suspension of **4** (300.0 mg, 0.628 mmol) in CH_2Cl_2 (6 mL). The dark brown solution was stirred for 24 h before it was diluted with chloroform and purified by column chromatography (4:6 to 1:1 EtOAc/hexane) to provide **16** (299 mg, 0.929 mmol, 64%) as an off-white solid: ^1H NMR ($CDCl_3$) δ 8.05, (1H, d, $J = 8.4$ Hz), 7.78 (1H, d, $J = 8.8$ Hz), 7.67 (1H, d, $J = 2.4$ Hz), 7.29 (1H, dd, $J = 8.0$, 2.8 Hz), 7.27, (1H, dd, $J = 8.4$, 3.6 Hz), 3.48 (3H, s), 3.40 (3H, s), 2.73(3H, s); ^{13}C NMR ($CDCl_3$) δ 187.6, 159.9, 154.7, 148.6, 136.2, 128.3, 124.8, 122.7, 122.1, 120.9, 43.5, 39.1, 25.6; FTIR (neat) 3379, 2940, 1624, 1603, 1531, 1504, 1393, 1283, 1206, 1169, 1130, 843, 748 cm^{-1}; MS (ESI) *m/z* calcd for $(C_{13}H_{14}N_2OS + H)^+$ 247, found 247; HRMS (ESI) *m/z* calcd for $(C_{13}H_{14}N_2OS + H)^+$ 247.0905, found 247.0900.

***S*-2-Methylquinolin-7-yl Dimethylcarbamothioate (17).** Compound **16** (598 mg, 2.3 mmol) was heated to 220 °C for 2 h. The reaction was cooled, and the dark solid residue obtained was dissolved in chloroform, adsorbed onto silica gel, and purified by column chromatography (1:1 to 4:6 EtOAc/hexane) to provide **17** (260 mg, 1.05 mmol, 43%) as an off-white solid: ^1H NMR ($CDCl_3$) δ 8.17 (1H, d, $J = 8.8$ Hz), 8.04, (1H, d, $J = 8.8$ Hz), 7.78 (1H, d, $J = 8.4$ Hz), 7.60 (1H, dd, $J = 8.4$, 2.0 Hz), 7.31 (1H, d, $J = 8.8$ Hz), 3.13 (3H, s), 3.04 (3H, s), 2.73 (3H, s); ^{13}C NMR ($CDCl_3$) δ 166.6, 159.7, 147.9, 136.1, 135.6, 132.5, 130.6, 127.9, 126.7, 123.0, 37.2, 25.6; FTIR (neat) 2922, 1663, 1726, 1360, 1094, 843, 752, 689 cm^{-1}; MS (ESI) *m/z* calcd for $(C_{13}H_{14}N_2OS + H)^+$ 247, found 247; HRMS (ESI) *m/z* calcd for $(C_{13}H_{14}N_2OS + H)^+$ 247.0905, found 247.0904.

***S*-2-Formylquinolin-7-yl Dimethylcarbamothioate (18).** Under a nitrogen atmosphere, selenium dioxide (67.3 mg, 0.606 mmol) was added to a solution of **17** (99.1 mg, 0.4027 mmol) in xylenes (3 mL). The mixture was heated to 100 °C for 3 h, then filtered hot through cotton, rinsing with chloroform. The product was adsorbed onto silica gel and purified by column chromatography (4:6 EtOAc/hexane) to afford **18** (60 mg, 0.230 mmol, 57%) as a white solid: ^1H NMR ($CDCl_3$) δ 10.21 (1H, s), 8.41 (1H, d, $J = 8.8$ Hz), 8.33 (1H, d, $J = 8.4$ Hz), 8.06 (1H, d, $J = 8.8$ Hz), 7.91 (1H, d, $J = 8.8$ Hz), 7.80 (1H, dd, $J = 1.6$, 8.4 Hz) 3.17 (3H, s), 3.07 (3H, s); ^{13}C NMR ($CDCl_3$) δ 193.6, 165.8, 152.9, 147.7, 137.2, 136.8, 135.6, 132.21, 129.9, 127.9, 118.1, 37.0; FTIR (neat) 2922, 2876, 1705, 1661, 1366, 853, 758 cm^{-1}; MS (ESI) *m/z* calcd for $(C_{13}H_{12}N_2O_2S + H)^+$ 261, found 261; HRMS (ESI) *m/z* calcd for $(C_{13}H_{12}N_2O_2S + H)^+$ 261.0692, found 261.0661.

***S*-2-(Hydroxymethyl)quinolin-7-yl Dimethylcarbamothioate (19).** $NaBH_4$ (6.2 mg, 0.165 mmol) was added to a solution of **18** (43.1 mg, 0.165 mmol) in ethanol (1 mL), and the resulting mixture was stirred at room temperature. After TLC indicated the reaction was complete, the solvent was evaporated, and the residue obtained was dissolved in chloroform. The solution was then washed with brine (×2), dried over Na_2SO_4, and evaporated to provide **19** (36.5 mg, 0.139 mmol, 85% yield), which was carried to the next step without further purification.

(7,7′-Disulfanediylbis(quinoline-7,2-diyl))dimethanol (20). A solution of KOH (50 mg, 0.19 mmol) in methanol (1 mL) was added to a solution of **19** (50 mg, 0.19 mmol) in methanol (2 mL), and the resulting mixture was refluxed under a nitrogen atmosphere until the reaction was complete as indicated by TLC. The solvent was evaporated, and the yellow residue obtained was neutralized with 1 N HCl and extracted with chloroform. The organic layer was dried over Na_2SO_4. The solvent was evaporated to give a red solid, which was purified by column chromatography (25:1 chloroform/methanol) to obtain **20** (29.5 mg, 77 μmol, 82%) as a yellow solid: ^1H NMR ($CDCl_3$) δ 8.22(1H, s), 8.097 (1H, d, $J = 8.8$ Hz), 7.79 (1H, d, $J = 8.4$ Hz), 7.69 (1H, dd, $J = 8.4$, 1.6 Hz), 7.25 (1H, d, $J = 8.4$ Hz), 4.88 (2H, s); ^{13}C NMR ($CDCl_3$) δ 156.0, 146.9, 138.7, 136.6, 128.6, 126.6, 125.8, 125.1, 118.4, 64.1; FTIR (neat) 2922, 2852, 1664, 1610, 1494, 1361, 1257, 1130, 1097, 906, 844, 688 cm^{-1}; MS (ESI) *m/z* calcd for $(C_{20}H_{16}N_2O_2S_2 + H)^+$ 381, found 381; HRMS (ESI) *m/z* calcd for $(C_{20}H_{16}N_2O_2S_2 + H)^+$ 381.0726, found 381.0734.

(7,7′-Disulfanediylbis(quinoline-7,2-diyl))bis(methylene) Diacetate (21). Acetic anhydride (250 μL) was added to a solution of **20** (15.5 mg, 0.0407 mmol) in pyridine (1 mL). The solution was stirred under nitrogen for 18 h at room temperature. Evaporation of the pyridine under vacuum left a residue that was dissolved in chloroform, adsorbed onto silica gel, and purified by column chromatography (3:7 to 4:6 EtOAc/hexane) to obtain **21** (10.2 mg, 22 μmol, 54%) as a white solid: ^1H NMR ($CDCl_3$) δ 8.22 (1H, s), 8.13 (1H, d, $J = 8.4$ Hz), 7.77 (1H, d, $J = 8.8$ Hz), 7.67 (1H, dd, $J = 8.8$, 1.6 Hz), 7.43 (1H, d, $J = 8.4$ Hz), 5.33 (2H, s), 2.17 (3H, s); ^{13}C NMR ($CDCl_3$) δ 170.9, 157.3, 148.0, 138.9, 137.0, 128.7, 126.7, 126.2, 125.6, 119.8, 67.5, 21.2; FTIR (neat) 2924, 2852, 1743, 1608, 1496, 1375, 1234, 1222, 1053, 844, 758 cm^{-1}; MS (ESI) *m/z* calcd for $(C_{24}H_{20}N_2O_4S_2 + H)^+$ 465, found 465; HRMS (ESI) *m/z* calcd for $(C_{24}H_{20}N_2O_4S_2 + H)^+$ 465.0942, found 465.0914.

(7-Mercaptoquinolin-2-yl)methanol (TQ-OAc, 3). DTT (27.3 mg) was added in three portions to a suspension of **21** (3.5 mg, 7.5 μmol) in methanol (1 mL). After 1 h, the solid dissolved, producing an orange solution. Stirring was continued for 3 h until the reaction, which was monitored by HPLC (65% water (0.1% TFA) and 35% acetonitrile), was complete. MS analysis of the peaks as they eluted from the column revealed that monomer **3** had a retention time of 5.9 min, while **21** eluted at 15 min. The methanol was evaporated, and the resulting solid mixture of DTT and **3** was dissolved in chloroform and washed with brine (×10) to remove DTT. The organic layer was dried over Na_2SO_4, and the chloroform was evaporated to give approximately 1 mg of **TQ-OAc**. This product was unstable in air and easily oxidized to the disulfide. Alternatively, the solid mixture of DTT and **3** was dissolved in methanol and directly used in the photochemical experiments.

Determination of the Uncaging Quantum Efficiency (Q_u). These experiments were carried out using a previously described method.[5,9,10,21] Briefly, KMOPS-buffered solutions (3 mL) of **1a**−**c**, **2**, and **3** (100 μM) were irradiated with 365-nm light from a mercury lamp. Aliquots (20 μL) of the reaction solution were removed for analysis by HPLC at periodic intervals. Q_u of each chromophore was calculated using eq 1.

Determination of the Dark Hydrolysis Rate (τ_{dark}). Substrates were dissolved in KMOPS and stored in the dark at room temperature. HPLC analysis was carried out periodically to determine the extent of decay.

Determination of the 2-Photon Uncaging Action Cross-Section (δ_u). These experiments were carried out using a previously described method.[5,9,10,21] Briefly, successive aliquots (25 μL) of each caged compound in KMOPS were irradiated for 0, 5, 10, 20, and 40 min. Each aliquot was analyzed by HPLC to determine the concentration of the remaining caged compound. δ_u was estimated by referencing to fluorescein, a compound with a known fluorescence quantum yield ($Q_f = 0.9$) and absorbance cross-section ($\delta_a = 30$ GM at 740 nm),[23,24] using eq 2.

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Supporting Information Available: Synthetic procedures for **1a**, **1c**, and **2b**; detailed experimental procedures for photochemical experiments; and characterization data for new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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