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8-Bromo-7-hydroxyquinoline as a Photoremovable Protecting Group for Physiological Use: Mechanism and Scope

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Abstract: Two-photon excitation (2PE) of "caged" biomolecules represents a powerful method to investigate the temporal and spatial relevance of physiological function in real time and on living tissue, because the excitation volume can be restricted to 1 fL. Additionally, low-energy IR light is used, which minimizes tissue destruction and enables deeper penetration into tissue preparations. Exploitation of this technology for studying cell physiology requires the further development of photoremovable protecting groups with sufficient sensitivity to 2PE for use in "caged" compounds. 8-Bromo-7-hydroxyquinoline (BHQ) is efficiently photolyzed by classic 1PE (365 nm) and 2PE (740 nm) under simulated physiological conditions (aqueous buffer of high ionic strength, pH 7.2) to release carboxylates, phosphates, and diols—functional groups commonly found on bioactive molecules such as neurotransmitters, nucleic acids, and drugs. It is stable in the dark, soluble in water, and exhibits low levels of fluorescence, which will enable use in conjunction with fluorescent indicators of biological function. BHQ-protected effectors are synthetically accessible. Stern−Volmer quenching, time-resolved infrared (TRIR), and ^{18}O-labeling experiments suggest that the photolysis occurs through a solvent-assisted photoheterolysis (S_N1) reaction mechanism on the sub-microsecond time scale. BHQ has the requisite photochemical and photophysical properties as a photoremovable protecting group to regulate the action of biological effectors in cell and tissue culture with light, especially 2PE.

Introduction

Understanding the temporal and spatial relevance of physiological processes requires a set of probes that selectively act at specific times and locations within a biological preparation. The nonlinear optical process of two-photon excitation (2PE)[1,2] enables the excitation volume of a chromophore to be confined to 1 fL, about the volume of an *Escherichia coli* bacterium. This volume is significantly smaller than the size of a typical mammalian cell or neuron; therefore, excitation in subregions of a cell in a complex tissue sample is conceivably possible. Conventional one-photon excitation (1PE) using UV light enables tight control over the timing of the excitation of a chromophore, but 2PE makes three-dimensional spatial control of the excitation also possible. In addition, 2PE is a particularly noninvasive technique, because it uses IR wavelengths, which minimizes tissue destruction, light absorption, and scattering, facilitating much deeper penetration into complex tissue samples than can be achieved with UV light. When a photoremovable protecting group that is sensitive to 2PE is attached to a biological effector, thereby inactivating it, one achieves control over the timing and the three-dimensional space in which the active form of the effector is released.[3−5] Chromophore−effector conjugates used to study biological systems are often referred to as "caged" compounds,[6−11] because the activity of the effector is "caged" by the connection of the chromophore with the effector through a single covalent bond. Exposure to light causes the covalent bond to break and "uncages" the effector in its active form. Using 2PE to mediate the release of a biological effector represents a powerful technique for probing the temporal and location dependence of biological function in real time and on living tissue.

To be useful in exploring a biological system, photoremovable protecting groups for 2PE should meet the following design criteria. The chromophore must possess a large absorption cross section at wavelengths accessible with lasers employed in two-photon microscopy, typically mode-locked and fs-pulsed Ti:sapphire lasers. The photolysis reaction must proceed in high quantum yield. The photoremovable protecting group must render the biological effector inert to the biological system used with minimal chemical or photochemical toxicity to living cells. Any photoproducts other than the desired messenger should not interact or interfere with the biological system. The chromophore and the effector conjugate must be synthetically accessible.

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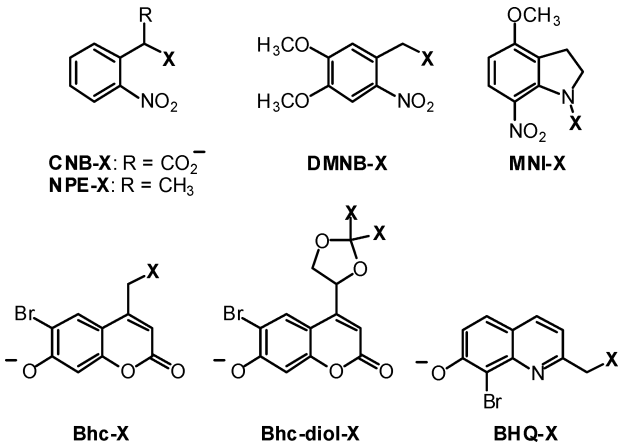
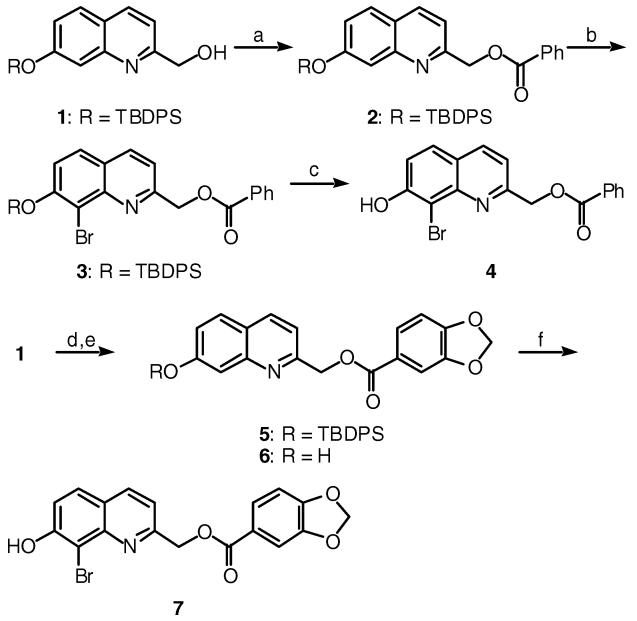
Chart 1. Photoremovable Protecting Groups



Chromophores that can act as a photoremovable protecting group (Chart 1) and have sufficient sensitivity to 2PE for biological use are rare. The nitrobenzyl-based protecting groups such as the dimethoxynitrobenzyl (DMNB or veratryl), carboxynitrobenzyl (CNB), and the nitrophenylethyl (NPE) groups have almost no sensitivity to 2PE at wavelength ranges typically associated with two-photon microscopy, 730−980 nm.[4,12,13] The MNI-based protecting group has been used to release glutamate in hippocampal brain slices,[14,15] but the neuroscience community has not used it widely probably because of its marginal sensitivity to 2PE. Groups based on 8-bromo-7-hydroxycoumarin (Bhc and Bhc-diol) have good sensitivity to 2PE, and they have been used to cage neurotransmitters,[5,12] DNA and RNA,[16] diols,[17] alcohols,[18] cyclic nucleotide monophosphates,[19] ketones and aldehydes,[20] an inhibitor of nitric oxide synthase,[21,22] and an inhibitor of protein synthesis.[23] Nevertheless, the somewhat low solubility of Bhc in aqueous buffers of high ionic strength and the high level of fluorescence observed upon excitation might limit its use, especially in applications where a fluorescent indicator is used to observe a physiological event. We found that the 8-bromo-7-hydroxyquinolinyl group (BHQ) can be efficiently photolyzed by 1PE and 2PE in aqueous buffer at physiological pH and has the additional advantages of a large quantum efficiency for the photolysis, good solubility in aqueous buffers, and low levels of fluorescence.[24] We report that BHQ can release carboxylates, phosphates, and diols efficiently and rapidly using 1PE or 2PE. Through Stern−Volmer quenching, time-resolved infrared (TRIR), and ^{18}O-labeling experiments, we demonstrate that the photochemical reaction proceeds through a singlet excited state and that the release of the effector likely proceeds through a solvent-assisted photoheterolysis (S_N1) reaction mechanism.[25]

Scheme 1. Synthesis of BHQ-Protected Carboxylates[a]



[a] Reagents and conditions: (a) BzCl, Et_3N, DMAP, CH_3Cl, rt, 12 h, 73%; (b) Br_2, AcOH, rt, 24 h, 67%; (c) TBAF, THF, rt, 90%; (d) piperonyloyl chloride, Et_3N, DMAP, CH_3Cl, rt, 12 h, 66%; (e) TBAF, THF, rt, 87%; (f) Br_2, AcOH, rt, 5 h, 64%.

Results

Synthesis. BHQ-protected benzoate and piperonylate were prepared from *tert*-butyldiphenylsilyl-protected quinoline **1**[24] (Scheme 1). Esterification of alcohol **1** with benzoyl chloride, triethylamine, and 4-(dimethylamino)pyridine in chloroform provided benzoate **2** in 73% yield. Bromination of **2** with bromine in acetic acid gave a single regioisomer in 67% yield, and removal of the silyl protecting group with tetrabutylammonium fluoride provided BHQ-protected benzoate **4** in 90% yield. The ^1H NMR spectrum of **3** showed that the H-6 signal condensed to a doublet (δ 7.12 ppm, $J = 8.8$ Hz) from the doublet of doublets (δ 7.10 ppm, $J = 8.8$, 2.8 Hz) found in the spectrum of **2**. The disappearance of the smaller *meta*-coupling indicates the addition of bromine at C-8, rather than at C-6, which would generate a spectrum with two singlets for H-5 and H-8. The regiochemistry of the bromination was further confirmed by the ^1H NMR spectrum of BHQ-OBz (**4**) in which the quinolinyl protons appear as four doublets, each with coupling constants ($J = 8.8$ Hz) indicative of having one neighboring proton in the *ortho* position. No other substitution pattern is consistent with this observation. BHQ-protected piperonylate was synthesized similarly, except the *tert*-butyldiphenylsilyl protecting group was removed prior to bromination. ^1H NMR confirmed the regiochemistry of the bromination. The doublet of doublets

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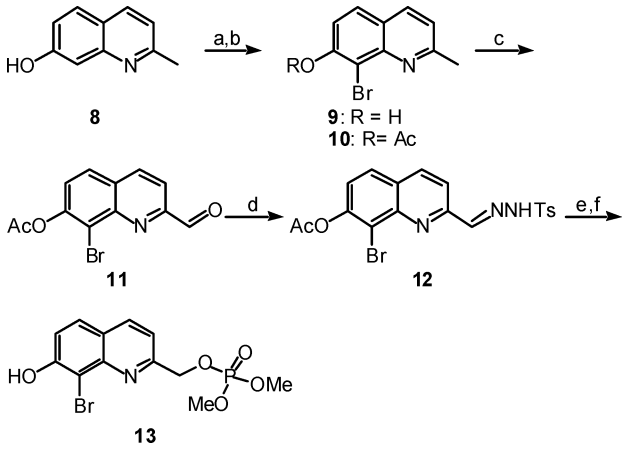
Scheme 2. Synthesis of BHQ-Protected Phosphates[a]



[a] Reagents and conditions: (a) Br_2, AcOH, rt, 8 h, 71%; (b) Ac_2O, pyridine, DMAP, rt, 4 h, 83%; (c) SeO_2, dioxane, 80 °C, 18 h, 60%; (d) H_2NNHTs, EtOH, rt, 48 h, 74%; (e) NaOMe, MeOH, rt, 10 min; (f) dimethyl phosphate, CH_3CN, rt, 48 h, 20%.

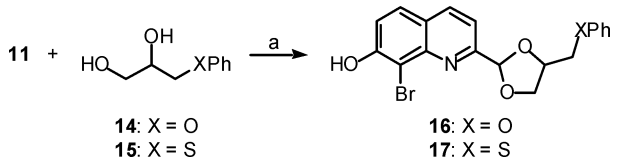
corresponding to H-6 (δ 7.19 ppm, J = 8.8, 2.4 Hz) in the spectrum of **6** gave way to a doublet (δ 7.36 ppm, J = 8.4 Hz) in the spectrum of **7**, and the signal for H-8 (δ 7.29, d, J = 2.0 Hz) in the spectrum of **6** was absent in the spectrum of **7**.

BHQ-protected dimethyl phosphate **13** was synthesized in six steps from quinaldine **8**[24] (Scheme 2). Bromination of **8** with bromine in acetic acid followed by acetylation of the phenol with acetic anhydride in pyridine yielded bromoquinaldine **10**. The regiochemistry of the bromination was determined by ^{1}H NMR. The peak corresponding to H-8 in **8** (δ 7.33 ppm, d, J = 1.9 Hz) was not present in the spectrum of **9**, and the H-6 signal in the spectrum of **8** collapsed from a double of doublets (δ 7.08 ppm, J = 8.8, 1.9 Hz) to a doublet in **9** (δ 7.22 ppm, J = 8.0 Hz). Oxidation of the benzylic methyl group with selenium dioxide generated aldehyde **11**, which upon treatment with tosylhydrazine in ethanol eventually provided the corresponding tosylhydrazone **12** in moderate yields. Generation of BHQ-protected phosphate was accomplished by treating **12** with sodium methoxide to generate the diazo species, which was trapped by dimethyl phosphate to produce the target compound **13**, albeit in poor yield.

The BHQ-protected glycerol derivatives **16** and **17** were synthesized in a single step from aldehyde **11** and 1-phenoxypropane-2,3-diol (**14**)[26] and 1-phenthioxypropane-2,3-diol (**15**)[27] by heating the aldehyde with the diol with pyridinium *para*-toluenesulfonic acid and anhydrous magnesium sulfate in toluene (Scheme 3). The yields for these reactions were modest (55−60%).

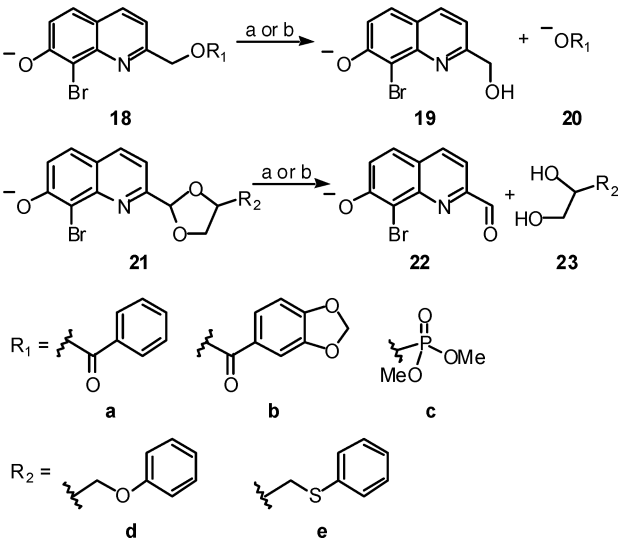
Photochemistry. We evaluated the BHQ-protected compounds for their ability to undergo one- and two-photon photolysis under simulated physiological conditions to generate the caging remnant **19** (BHQ-OH) or **21** (BHQ-CHO) and the respective carboxylate, phosphate, or diol (Scheme 4). Solutions of **18a**−**c** and **21d**,**e** (100 μM) in KMOPS buffer (10 mM 4-morpholinepropanesulfonic acid and 100 mM potassium

Scheme 3. Synthesis of BHQ-Protected Diols[a]



[a] Reagents and conditions: (a) PPTS, $MgSO_4$, toluene, 110 °C, 48 h, 60% for **16**, 55% for **17**

Scheme 4. Single- and Two-Photon Photolyses of BHQ-Protected Compounds[a]



[a] Reagents and conditions: (a) $h\nu$ (365 nm), KMOPS, pH 7.2; (b) $h\nu$ (740 nm), KMOPS, pH 7.2

chloride titrated to pH 7.2 with potassium hydroxide) were excited with light from a 365-nm mercury lamp passed through a pair of filters that narrow the spectral output to 365 ± 15 nm. The pK_a of the phenolic moiety in BHQ-OAc is 6.8, and λ_{max} for the phenolate is ∼370 nm, compared to ∼320 for the phenol;[24] therefore, in KMOPS buffer, the excited state likely arises from the deprotonated form of the hydroxyquinoline. UV spectra indicated that the solutions contained a mixture of the phenol and phenolate forms of the hydroxyquinoline. Nevertheless, excited-state hydroxyquinolines are superacids, and the hydroxy group is expected to deprotonate rapidly upon excitation.[28,29]

The time courses for the one-photon photolysis reactions of **18a**−**c** were monitored by HPLC analysis of 20-μL aliquots taken at periodic intervals, using an external standard to relate the detector response to the concentrations of **18a**−**c** and **20b** (Figure 1). In the case of BHQ-OPip (**18b**), the product of the reaction, piperonylate (**20b**), had sufficient UV signal to monitor its formation in the reaction. The yield of **20b** approaches 70−75%. Similarly, HPLC was used to monitor the photolyses of BHQ-caged diols **21d**,**e** and the generation of **23e** (Figure 2). Comparison with the HPLC retention times under identical conditions of authentic samples of **19**, **22**, **20b**, and **23e** confirmed their identity in the reaction mixture. From these data, the one-photon uncaging quantum efficiency, Q_u, for each

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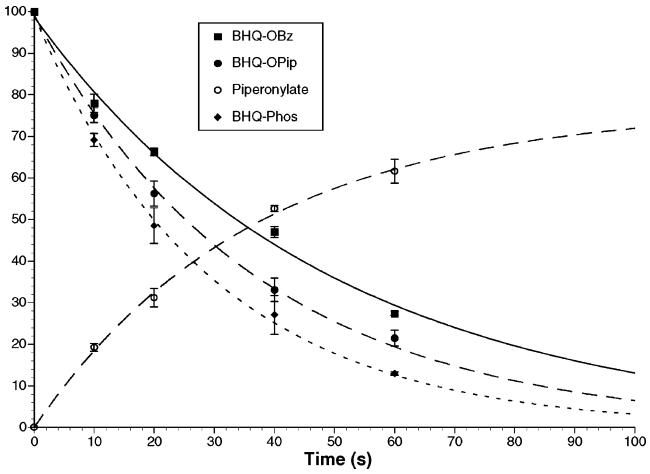

Figure 1. Time course of one-photon photolysis of BHQ-protected carboxylates **18a**,**b** and phosphate **18c** at 365 nm. The percent remaining was determined by HPLC and is the average of 3 runs. Also shown is the relative amount of piperonylate (**20b**) released from **18b**, which was determined by HPLC from 3 runs. Lines are least-squares fits of a simple decaying exponential or exponential rise to max. Error bars represent the standard deviation of the measurement.

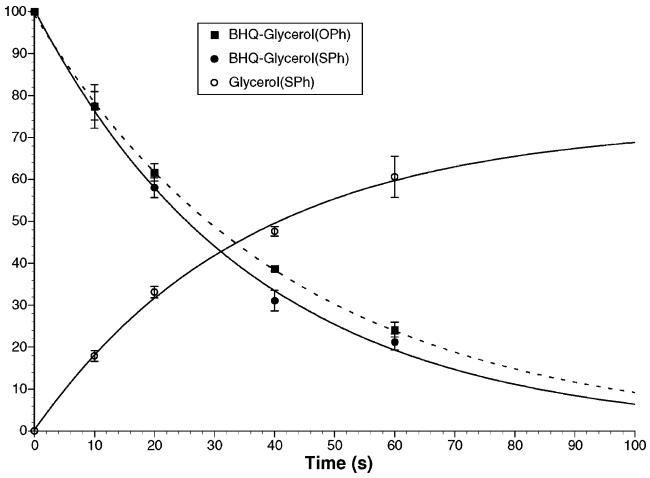

Figure 2. Time course of one-photon photolysis of BHQ-protected diols **21d**,**e**. The percent remaining was determined by HPLC and is the average of at least 3 runs. Also shown is the relative amount of 1-phenthioxypropane-2,3-diol (**23e**) released from **21e**, which was determined by HPLC from at least 3 runs. Lines are least-squares fits of a simple decaying exponential or exponential rise to max. Error bars represent the standard deviation of the measurement.

compound was determined from the following relationship:[30,31]

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

Here I is the irradiation intensity (determined by potassium ferrioxalate actinometry[32]), σ is the decadic extinction coefficient (1000ϵ, the molar extinction coefficient) at 365 nm, and $t_{90\%}$ is the irradiation time for 90% conversion to product. The quantum efficiencies for the BHQ-protected carboxylates and phosphates were found to be 0.30−0.32, while the protected diols had slightly higher values of 0.37 and 0.39 (Table 1).

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Table 1. Photophysical and Photochemical Properties of BHQ-Caged Compounds[a]

compd	λ_{max} (nm)	ϵ_{max} ($M^{-1}\cdot cm^{-1}$)	ϵ_{365} ($M^{-1}\cdot cm^{-1}$)	Q_u	δ_u (GM)[b]	τ_{dark} (h)[c]
BHQ-OAc[d]	369	2600	2580	0.29	0.59	71
18a	368	2400	2400	0.30	0.64	100
18b	296	6400	2900	0.32	0.76	94
18c	370	3900	3800	0.31	0.43	105
21d	375	2300	2200	0.37	0.78	79
21e	374	2500	2400	0.39	0.90	69

[a] Measured in KMOPS, pH 7.2. [b] Measured at 740 nm, GM = 10^{-50} ($cm^4\cdot s$)/photon. [c] Time constant for hydrolysis in the dark. [d] Values taken from ref 24.

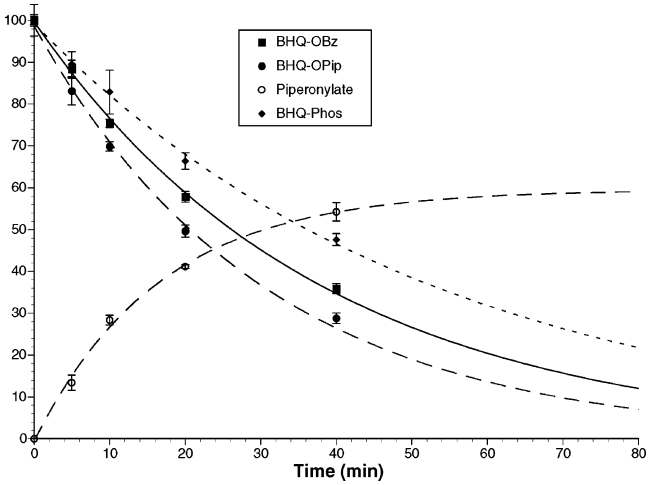


Figure 3. Time course of two-photon photolysis of BHQ-protected carboxylates **18a**,**b** and phosphate **18c** at 365 nm. Also shown is the relative amount of piperonylate (**20b**) released from **18b**. The percent remaining was determined by HPLC and is the average of 3 runs. Lines are least-squares fits of a simple decaying exponential or exponential rise to max. Error bars represent the standard deviation of the measurement.

We followed a previously described method[12,20,24] to determine the two-photon uncaging cross section, δ_u (in Goeppert-Mayer, GM, which is defined as 10^{-50} $cm^4\cdot s$/photon), of compounds **18a**−**c**. This is a measure of the sensitivity of a photoremovable protecting group to undergo a photochemical reaction initiated by 2PE. It is related to the two-photon absorption cross-section, δ_a, by $\delta_u = Q_u\cdot\delta_a$. Briefly, 20-$\mu$L samples were irradiated with a fs-pulsed, mode-locked Ti:sapphire laser tuned to 740 nm (287 fs pulse width and 160−200 mW average power). After a period, HPLC analysis of the entire sample was used to determine the extent of photolysis, as in the one-photon quantum efficiency measurements, and, in the case of **18b**, the yield of piperonylate (**20b**), which approached 60%. The runs were graphed as a function of time and fit to a least-squares decaying exponential (Figure 3). The pulse parameters of the laser were estimated by using fluorescein as an external standard, because it has a known fluorescence quantum yield, Q_{fF}, and two-photon absorbance cross-section, δ_{aF}.[33,34] The time-averaged fluorescence photon flux, $\langle F(t)\rangle$, from the two-photon excitation of fluorescein in the same setup was measured using a radiometer. The initial rate of photolysis was used to determine the number of molecules photolyzed/s, N_p, which is related to δ_u by the following equation:[12]

$$\delta_u = \frac{N_p \phi Q_{fF} \delta_{aF} C_F}{\langle F(t)\rangle C_S} \quad (2)$$

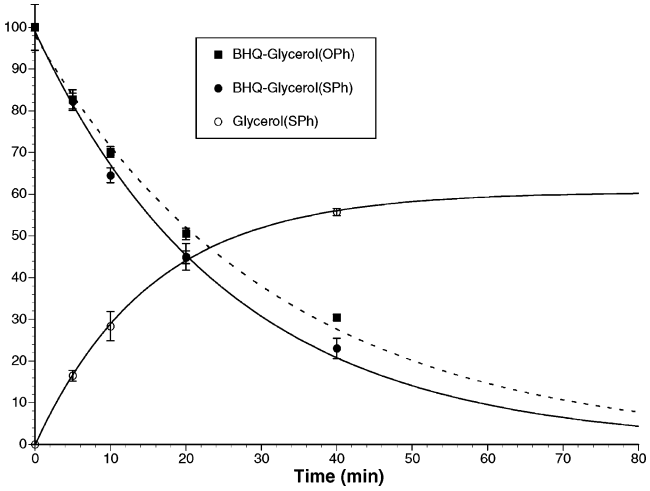


Figure 4. Time course of two-photon photolysis of BHQ-protected diols **21d**,**e**. Also shown is the relative amount of phenthioglycerol **23e** released from **21e**. The percent remaining was determined by HPLC and is the average of 3 runs. Lines are least-squares fits of a simple decaying exponential or exponential rise to max. Error bars represent the standard deviation of the measurement.

Table 2. Stern−Volmer Quenching of BHQ-OAc

[SNS][a] (μM)	[BHQ-OAc] (μM)	Q_u	[PS][b] (μM)	[BHQ-OAc] (μM)	Q_u
0	100	0.29	0	180	0.29
100	100	0.30	180	180	0.30
200	100	0.31	360	180	0.30
300	100	0.29	720	180	0.30

[a] SNS = sodium 2-naphthalene sulfonate. [b] PS = potassium sorbate.

Here ϕ is the collection efficiency of the detector, Q_{fF} is the fluorescence quantum yield of fluorescein, δ_{aF} is the absorbance cross section of fluorescein, C_F is the concentration of fluorescein, and C_S is the concentration of the BHQ-protected substrate. Similarly, the time courses for the BHQ-protected diols **21d**,**e** were measured (Figure 4) and δ_u was calculated. Values of δ_u for **18a**−**c** and **21d**,**e** ranged from 0.43 to 0.90 GM (Table 1).

Dark Hydrolysis Rates. Each of the BHQ-protected compounds was tested for its stability in the dark at room temperature in KMOPS. Compounds **18a**−**c** and **21d**,**e** were placed in KMOPS and stored in the dark. Periodically, 20-μL aliquots were removed to measure the extent of spontaneous hydrolysis by HPLC (data not shown). The time constants measured ranged from 69 to 105 h (Table 1).

Stern−Volmer Quenching Experiments. To determine whether the reaction proceeds through a triplet or singlet excited state, we conducted Stern−Volmer quenching experiments on BHQ-OAc (**23**). The quantum efficiency, Q_u, of the one-photon photolysis of BHQ-OAc was determined in the presence of 1, 2, and 3 times the concentration of a triplet quencher, sodium 2-naphthalene sulfonate (SNS) or potassium sorbate (PS). No statistically relevant change in Q_u was observed with either quencher (Table 2).

Time-Resolved IR Studies. We further analyzed the photolysis of BHQ-OAc by time-resolved infrared (TRIR) spectroscopy. TRIR spectra observed following 266 nm laser photolysis (5 ns, 1 mJ) of BHQ-OAc in argon-saturated acetonitrile-d_3 are shown in Figure 5. Depletion of reactant gives rise to negative signals, and the formation of transient intermediates or products leads to positive bands. The observed

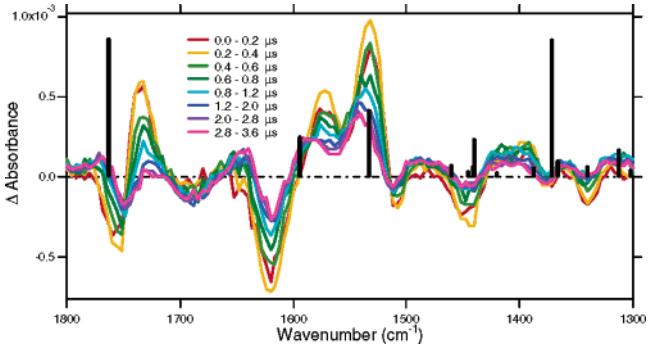


Figure 5. TRIR difference spectra averaged over the time scales indicated following laser photolysis (266 nm, 5 ns, 1 mJ) of BHQ-OAc (5 mM) in argon-saturated acetonitrile-d_3. The vertical bars represent B3LYP/6-31G* calculated frequencies (scaled by 0.96)[33] and relative intensities of the lowest triplet excited state of BHQ-OAc.

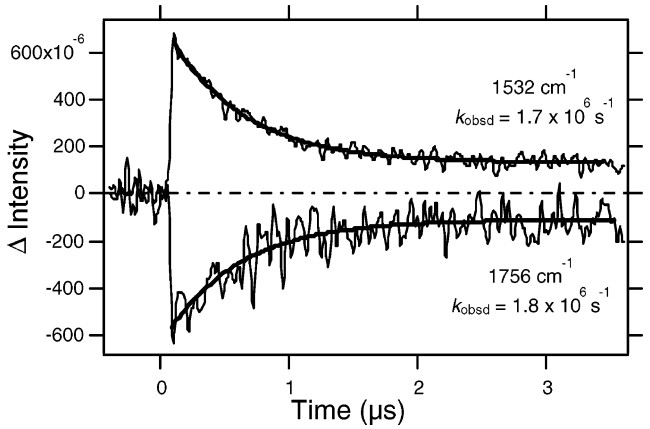


Figure 6. Representative kinetic traces observed following laser photolysis (266 nm, 5 ns, 1 mJ) of BHQ-OAc (5 mM) in argon-saturated acetonitrile-d_3 showing decay of the BHQ-OAc triplet excited state at 1532 cm^{-1} and recovery of the ground-state BHQ-OAc band at 1756 cm^{-1}. The dotted curves are experimental data; the solid curves are the calculated best fit to a single-exponential function.

depletion bands match well with the ground-state IR spectrum of BHQ-OAc. These depletion bands recover (albeit incompletely) with an observed rate constant of 1.8×10^6 s^{-1} (Figure 6). The incomplete recovery of these bands indicates some irreversible conversion (ca. 20%) of BHQ-OAc to products. Two sets of partially overlapping positive bands are observed. One set, with major signals at 1736, 1574, 1532, and 1412 cm^{-1}, is produced within the time resolution (50 ns) of the TRIR experiment and subsequently decays at the same rate within experimental error (±10%) that the BHQ-OAc ground-state bands recover (Figure 6). A second set, with signals at 1764, 1644, 1584, 1540, 1484, 1412, 1360, and 1316 cm^{-1}, is also produced within the time resolution of the TRIR experiment but appear to be stable on the microsecond time scale.

We have assigned the first set of bands to the triplet excited state of BHQ-OAc since they agree well with B3LYP/6-31G* calculated frequencies (Figure 5 and Supporting Information) and are quenched by oxygen (Figure 7). Although oxygen efficiently quenches the BHQ-OAc triplet excited state, TRIR spectral data collected in oxygen-saturated acetonitrile (Figure 7) indicate that the stable product(s) (e.g., observed bands at 1764 and 1644 cm^{-1}) is (are) still formed in the presence of oxygen. This latter observation is consistent with our hypothesis that productive chemistry occurs from a BHQ-OAc singlet

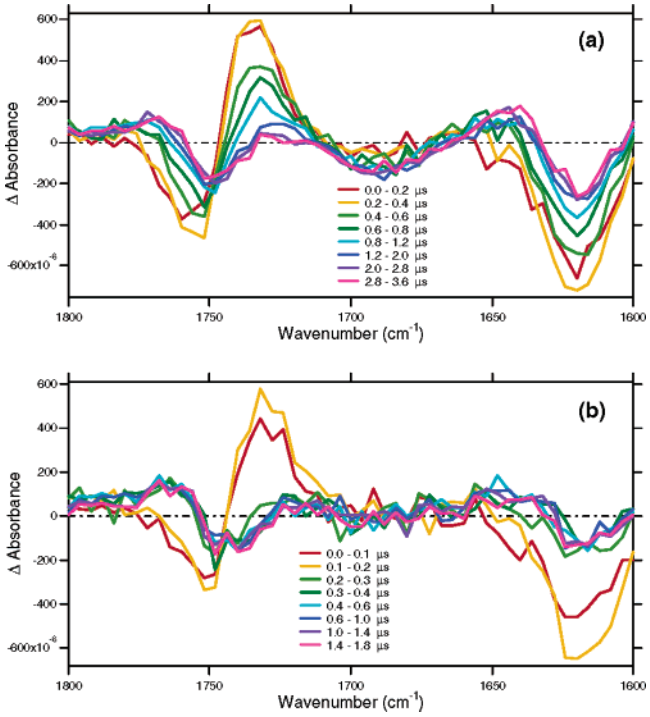


Figure 7. TRIR difference spectra averaged over the time scales indicated following laser photolysis (266 nm, 5 ns, 1 mJ) of BHQ-OAc (5 mM) in (a) argon- and (b) oxygen-saturated acetonitrile.

excited state; the BHQ-OAc triplet excited state merely decays back to ground state. The set of stable product bands are assigned to BHQ-OH (presumably formed via trapping by residual water) and acetic acid on the basis of a comparison with the known IR spectra of these products (see Supporting Information).

The kinetics of BHQ-OAc ground-state recovery at 1756 cm^{-1} were further examined following triplet sensitized photolysis with xanthone. The triplet excited state of xanthone has a broad, weak absorbance between 1800 and 1700 cm^{-1}. Thus, in the absence of BHQ-OAc, a positive transient signal, attributed to the xanthone triplet excited state, is observed at 1756 cm^{-1} following 355 nm laser photolysis (90 ns, 1.5 mJ) of an acetonitrile solution of xanthone (Figure 8a). However, in the presence of 1 mM BHQ-OAc, the xanthone triplet excited state is efficiently quenched and the decay and recovery of the BHQ-OAc 1756 cm^{-1} is now observed (Figure 8b). Note that, in contrast to direct photolysis (Figure 8c), triplet-sensitized photolysis of BHQ-OAc results in complete recovery of the ground-state depletion, further verifying that products are not formed via the BHQ-OAc triplet excited state.

BHQ-phosphate (**18c**) was also examined by TRIR spectroscopy. TRIR spectra observed following 266 nm laser photolysis (5 ns, 1 mJ) of BHQ-phosphate in acetonitrile-d_3 are shown in Figure 9. Analogous to BHQ-OAc, we observe the decay and recovery of ground-state BHQ-phosphate, the corresponding formation and decay of BHQ-phosphate triplet excited state, and the formation of stable product bands. Experimentally observed IR bands of the BHQ-phosphate triplet excited state are well reproduced by B3LYP/6-31G* calculations (Figure 9) and are also quenched by oxygen (Supporting Information). Similar to the case with BHQ-OAc, ground-state bleaching does not fully recover to baseline, indicating some finite depletion of reactant. Although kinetic data indicate that the triplet excited-

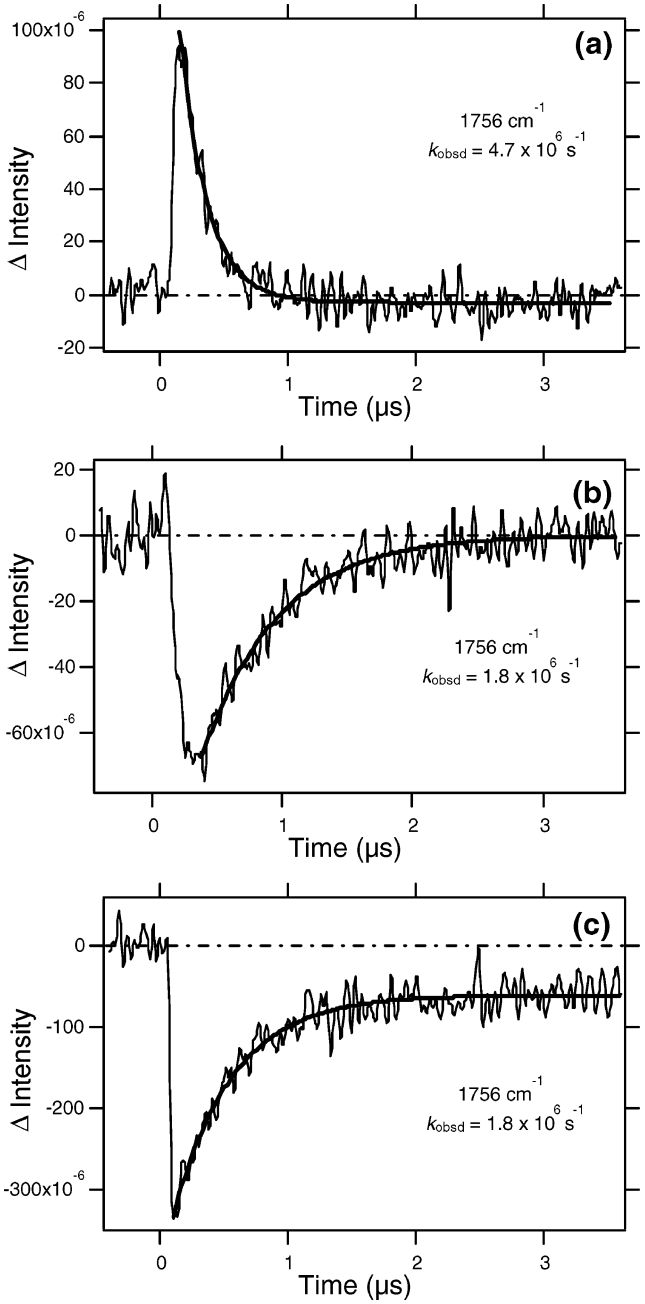


Figure 8. Kinetic traces observed following laser photolysis (355 nm, 90 ns, 1.5 mJ) of an argon-saturated acetonitrile solution of (a) xanthone (A_{355} = 0.25) and of (b) xanthone (A_{355} = 0.25) plus 1 mM BHQ-OAc. (Under these conditions >96% of the incident light is absorbed by xanthone.) Also shown for comparison is (c) the kinetic trace observed following laser photolysis (266 nm, 5 ns, 1 mJ) of an argon-saturated acetonitrile solution of BHQ-OAc (5 mM). The dotted curves are experimental data; the solid curves are the calculated best fit of a single-exponential function.

state lifetimes of BHQ-OAc and BHQ-phosphate are comparable, in the case of BHQ-phosphate no signals were observed (or calculated) in the region between 1800 and 1600 cm^{-1}. The signals observed in this region for BHQ-OAc (Figure 5) have been assigned to the acetate portion of the molecule.

Oxygen-18 Labeling Experiment. In the photolysis of BHQ-caged carboxylates, the oxygen on the primary alcohol of BHQ-OH can come from the aqueous solvent or from the ester (Scheme 5). To determine the origin of this oxygen, the photolysis was conducted in ^{18}O-labeled water. If the oxygen on the remnant of the BHQ-chromophore comes from the

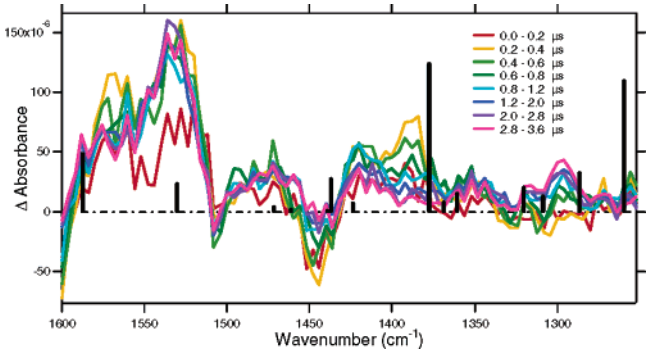
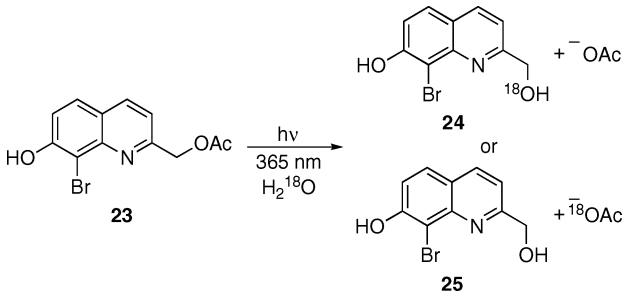


Figure 9. TRIR difference spectra averaged over the time scales indicated following laser photolysis (266 nm, 5 ns, 1 mJ) of BHQ-phosphate (5 mM) in argon-saturated acetonitrile. The vertical bars represent B3LYP/6-31G* calculated frequencies (scaled by 0.96) and relative intensities for the lowest triplet excited state of BHQ-phosphate.

Scheme 5. Oxygen-18 Labeling Experiment

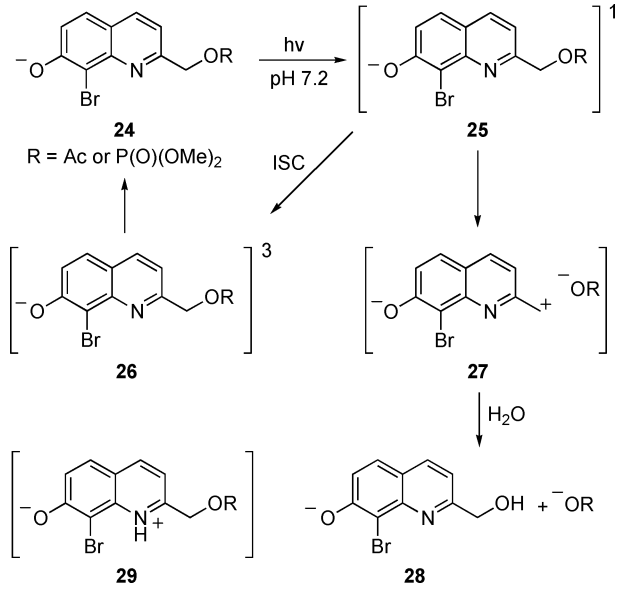


solvent, then the product observed will be the labeled BHQ-^{18}OH (**24**). Alternatively, if unlabeled BHQ-OH (**25**) is observed, then the source of the oxygen would be from the ester. A solution of BHQ-OAc (**18**) in either H_2O or $H_2{}^{18}O$ (97% label) was exposed to 365-nm light for several seconds. The reactions were then analyzed by LC-MS (Supporting Information). For the reaction in H_2O, mass spectral analysis of the peak on the chromatogram corresponding to BHQ-OH gave *m*/*z* 254/256, while the reaction in $H_2{}^{18}O$ gave almost exclusively *m*/*z* 256/258. A comparison of the MS data of the labeled and unlabeled reaction revealed that 96% of the label was incorporated into the chromophore remnant.

Discussion

The BHQ chromophore can be covalently linked to carboxylates, phosphates, and diols, three common functional groups found on bioactive molecules such as neurotransmitters, nucleic acids, and drugs. It is efficiently removed, releasing the active functional group, through 1PE or 2PE at wavelengths that are not deleterious to biological preparations, and all of the compounds tested are sufficiently stable in the dark at pH 7.2. Compared to other chromophores for protecting carboxylates, the sensitivity, as measured by the product of the quantum efficiency and the molar absorptivity ($Q_u\epsilon$),[19] of BHQ (780) to 1PE is higher than DMNB (31),[24] Bhc (555),[12] and MNI (408).[36] CNB[37] and NPE[38] have high sensitivities, 714 and 2184,

Scheme 6. Proposed Mechanism of the BHQ Photolysis Reaction



respectively, but at wavelengths below 270 nm, which are deleterious to biological preparations. The two-photon uncaging cross section is greater than the 0.1 GM threshold for physiological use,[12] and further, it is an order of magnitude larger than the measured values for DMNB, MNI, CNB, and NPE, which have been used in a physiological context. The photolysis kinetics is faster than physiological signal transduction events, which occur on millisecond and slower time scales. Because the kinetics were measured in acetonitrile and not aqueous buffer of high ionic strength at neutral pH, the estimated rate constant for product formation ($\geq 2 \times 10^7$ s^{-1} in acetonitrile) is likely larger in a physiological environment. In conducting flash photolysis experiments on Bhc-OAc, which photochemically decomposes by a mechanism[25] similar to BHQ, Magde found that the photolysis rate constant is proportional to pH.[5] BHQ-caged carboxylates and phosphates will enable tight three-dimensional localization of effector release with 2PE, because the photolysis time scale is much shorter ($\tau = 0.6\ \mu$s) than those typical for diffusion through aqueous media ($\tau = 113-900\ \mu$s).[39] The photochemical reaction is complete and the cargo released before any intermediates can diffuse away from the focus of the laser. Photolysis rates of BHQ-caged diols are likely to be slower than those for the carboxylates and phosphates.

The Stern−Volmer quenching experiments indicated that a triplet excited state is not involved in product formation, because the two triplet quenchers used were unable to diminish the quantum efficiency of the photolysis of BHQ-OAc. TRIR spectroscopy shows that recovery of the ground-state starting material is incomplete, as would be expected for a photochemical reaction where a portion of the excited state provides product rather than decaying back to the ground state. In the presence of oxygen as a triplet quencher, stable products, BHQ-OH and acetate, are still observed, also indicating that the reaction proceeds through a singlet excited state. If the mechanistic path to products involved a triplet state, no product bands would be observed in the TRIR spectra in the presence of a triplet quencher. Triplet-sensitized excitation of BHQ with xanthone results in complete recovery of the original ground state, and

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no product bands are observed, consistent with our hypothesis that the triplet state is not involved in product formation. Intersystem crossing to form the triplet state is probably facilitated by the presence of the bromine in BHQ, but its presence is required to lower the pK_a of the phenolic proton and induce a shift of the absorbance maximum from approximately 320 to 370 nm. Improvements to the quantum efficiency could potentially be made by replacing the bromine with an electron-withdrawing group that does not facilitate intersystem crossing but would still lower the pK_a of the phenol. Conducting the photoreaction in ^{18}O-labeled water reveals that the reaction is not a simple hydrolysis of an ester; the label is incorporated into the BHQ-OH product and not the acetate. This supports a mechanism involving a substitution reaction at the benzylic carbon center.

Taken together, the physical data on the photolysis of BHQ-OAc and BHQ-phosphate suggest a solvent-assisted photoheterolysis (S_N1) reaction mechanism[25] (Scheme 6). Irradiation of BHQ-OR (**24**) in aqueous media at pH 7.2 leads to a singlet excited state **25**, which can undergo intersystem crossing to the triplet state **26** followed by decay back to **24**. Cleavage of the carbon−oxygen bond in **25** proceeds from the singlet state, either by heterolytic cleavage or homolytic cleavage followed by single electron transfer, to generate a zwitterion-like intermediate resembling **27**. Trapping the cation or electropositive carbon by water yields **28** and the carboxylate or phosphate. In the excited state, hydroxyquinolines are protonated on the picosecond time scale even in 9−10 M NaOH;[29] therefore, a transient intermediate such as **29** is probably formed during the photoreaction, but its role on the path to product is unclear.

Conclusions

We have demonstrated that BHQ can mediate the efficient photorelease of carboxylates, phosphates, and diols with 1PE or 2PE under simulated physiological conditions. The carboxylates and diols are produced in good yield (60−70%) via both 1PE and 2PE. BHQ has a high sensitivity to light in 1PE and a large 2PE uncaging cross section at convenient wavelengths, 365 and 740 nm, respectively. The carboxylate and phosphate functional groups are discharged on the sub-microsecond time scale, sufficient for the study of fast kinetics in physiological processes. The physical data support a solvent-assisted photoheterolysis (S_N1) reaction mechanism[25] that proceeds from the singlet excited state. BHQ has the requisite photochemical and photophysical properties as a photoremovable protecting group to regulate the action of biological effectors in cell and tissue culture and should prove useful to the neuroscience community.

Experimental Methods

General Methods. Compounds **1**,[24] **8**,[24] **14**,[26] **15**,[27] and **23**[24] were prepared by their respective literature procedure. All other reagents and solvents were purchased from commercial sources and used without further purification with the following exceptions. Toluene and THF were dried by passing through activated alumina under nitrogen pressure (Solv-Tek, Berryville, VA). Pyridine and acetonitrile were refluxed with calcium hydride under nitrogen and then distilled. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian MercuryPlus 400 MHz spectrometer. FTIR spectra were recorded on an Avatar 360 spectrophotometer (Thermo Nicolet). UV spectra were recorded on a Cary 300 Bio UV−visible spectrophotometer (Varian). HPLC analysis (analytical and preparative) was performed on a Varian ProStar HPLC system with an autosampler and diode array detector using Microsorb C-18 reverse phase columns. Mass spectrometry was performed on a Sciex API-1 Plus quadrupole mass spectrometer with an electrospray ionization (ESI) source or a Bruker Autoflex MALDI-TOF. HRMS was performed on a Micromass QTOF-Ultima with ESI. KMOPS buffer consisted of 100 mM KCl and 10 mM MOPS titrated to pH 7.2 with KOH. Thin layer and column chromatography were performed on precoated silica gel 60 F_{254} plates (Sorbent Technologies) and 230−400 mesh silica gel 60 (Sorbent Technologies), respectively. Melting points were determined on a Mel-Temp (Laboratory Devices, Inc.) and are uncorrected.

TBDPS−HQ-Benzoate (2). Under a nitrogen atmosphere, alcohol **1** (100 mg, 0.242 mmol) was dissolved in $CHCl_3$ (10 mL) and Et_3N (45 μL, 0.36 mmol) and DMAP (10 mg, 0.08 mmol) were added. Benzoyl chloride (30 μL, 0.26 mmol) was added dropwise. The reaction mixture was stirred overnight. The mixture was washed with 15% citric acid, water, and brine. The organic layer was dried over anhydrous Na_2SO_4. The solvent was evaporated and the residue was purified by flash chromatography with EtOAc/hexane (1:9) to afford **2** as a white solid (92 mg, 0.178 mmol, 73% yield), mp = 85−89 °C: ^{1}H NMR ($CDCl_3$) δ 8.11 (2H, dm, J = 8.4 Hz), 8.04 (1H, d, J = 8.4 Hz), 7.76 (4H, m), 7.57 (2H, d, J = 8.8 Hz), 7.41 (10H, m), 7.10 (1H, dd, J = 8.8−2.4 Hz), 5.55 (2H, s), 1.13 (9H, s); ^{13}C NMR ($CDCl_3$) δ 166.27, 156.97, 156.52, 149.02, 136.59, 135.53, 134.83, 133.22, 132.36, 130.07, 129.85, 128.46, 127.91, 127.74, 123.06, 122.59, 117.28, 116.25, 67.91, 22.68, 14.16; FTIR (neat) 3070, 2932, 2857, 2360, 1685, 1583, 1425, 1289, 1113, 933, 809, 702 cm^{-1}; HR-MS (ESI) m/z calcd for $(C_{33}H_{31}NO_3Si + H)^+$ 518.2146, found 518.2130.

TBDPS−BHQ-Benzoate (3). Ester **2** (80.0 mg, 0.155 mmol) was dissolved in acetic acid (5 mL). Bromine (140 mg, 20% in acetic acid solution, 0.175 mmol) was added dropwise. The reaction mixture was stirred for 24 h. The reaction was quenched with saturated $NaHCO_3$, extracted with EtOAc, washed with water and brine, and dried over anhydrous Na_2SO_4. The organic layer was evaporated, and the residue was purified by flash chromatography with EtOAc/hexane (1:9) to afford **3** as a white solid (62 mg, 0.104 mmol, 67% yield), mp = 80−84 °C: ^{1}H NMR ($CDCl_3$) δ 8.64 (1H, d, J = 8.8 Hz), 8.06 (2H, dm, J = 8.4 Hz), 7.56 (6H, m), 7.41 (9H, m), 7.11 (1H, d, J = 8.8 Hz), 6.18 (2H, s), 1.14 (9H, s); ^{13}C NMR ($CDCl_3$) δ 166.15, 158.91, 157.64, 147.13, 136.99, 135.43, 134.88, 132.32, 131.63, 130.17, 129.58, 128.91, 127.85, 127.06, 123.94, 122.08, 118.18, 114.05, 69.17, 22.54, 14.16; FTIR (neat) 3071, 2955, 2858, 1723, 1618, 1506, 1427, 1257, 1109, 862, 700 cm^{-1}; MS (MALDI-TOF) m/z calcd for $(C_{33}H_{30}BrNO_3Si + H)^+$ 596.1 (^{79}Br) and 598.1 (^{81}Br), found 596.2 (^{79}Br) and 598.2 (^{81}Br).

BHQ-Benzoate (4). Ester **3** (50 mg, 0.084 mmol) was dissolved in THF (5 mL). TBAF in THF (1 M, 90 μL, 0.09 mmol) was added dropwise. The reaction was monitored by TLC. When complete, it was diluted with EtOAc and washed with water and brine. The organic layer was dried over anhydrous Na_2SO_4. The solvent was evaporated, and the residue was purified by flash chromatography with EtOAc/hexane (3:7) to afford **4** as a pale yellow solid (27 mg, 0.075 mmol, 90% yield), mp = 180−202 °C (dec): ^{1}H NMR ($CDCl_3$) δ 8.17 (2H, dm, J = 8.8 Hz), 8.13 (1H, d, J = 8.8 Hz), 7.72 (1H, d, J = 9.2 Hz), 7.61 (1H, tm, J = 7.6 Hz), 7.49 (3H, m), 7.33 (1H, d, J = 8.8 Hz), 5.72 (2H, s); ^{13}C NMR ($CDCl_3$) δ 166.25, 158.94, 155.80, 148.13, 137.43, 133.12, 130.19, 129.65, 128.86, 128.30, 122.19, 119.72, 116.68, 108.81, 66.96; FTIR (neat) 2958, 1723, 1612, 1506, 1471, 1269, 1113, 842, 710 cm^{-1}; HR-MS (ESI) m/z calcd for $(C_{17}H_{12}BrNO_3 + H)^+$ 358.0073 (^{79}Br) and 360.0055 (^{81}Br), found 358.0074 (^{79}Br) and 360.0057 (^{81}Br).

TBDPS−HQ-Piperonylate (5). Under a nitrogen atmosphere, alcohol **1** (100 mg, 0.242 mmol) was dissolved in $CHCl_3$ (10 mL). Et_3N (45 μL, 0.36 mmol) and DMAP (10 mg, 0.08 mmol) were added, followed by piperonylic chloride (50 mg, 0.27 mmol). The reaction was stirred overnight. The mixture was washed with 15% citric acid, water, and brine. The organic layer was dried over anhydrous Na_2SO_4. The solvent was evaporated, and the residue was purified by flash

chromatography with EtOAc/hexane (1:9) to afford **5** as a white solid (90 mg, 0.16 mmol, 66% yield), mp = 90−95 °C: ^{1}H NMR (CD_3OD) δ 8.18 (1H, d, J = 8.0 Hz), 7.76 (4H, m), 7.71 (1H, d, J = 8.0 Hz), 7.66 (1H, dd, J = 8.4, 2.0 Hz), 7.40 (8H, m), 7.22 (1H, s), 7.21 (1H, d, J = 7.2 Hz), 6.87 (1H, d, J = 8.4 Hz), 6.03 (2H, s), 5.39 (2H, s), 1.12 (9H, s); ^{13}C NMR (CD_3OD) δ 165.48, 157.18, 156.92, 148.06, 137.39, 135.24, 131.84, 130.02, 128.88, 127.71, 125.33, 123.31, 123.23, 122.49, 117.24, 114.64, 108.85, 107.67, 102.10, 66.57, 25.50, 18.81; FTIR (neat) 3072, 2932, 2858, 1719, 1619, 1506, 1442, 1257, 1113, 1076, 876, 842, 702 cm^{-1}; HR-MS (ESI) m/z calcd for $(C_{34}H_{31}NO_5Si + H)^+$ 562.2044, found 562.2020.

HQ-Piperonylate (6). Ester **5** (80.0 mg, 0.142 mmol) was dissolved in THF (5 mL). TBAF in THF (1 M, 150 μL, 0.15 mmol) was added dropwise. The reaction was monitored by TLC. When complete, it was diluted with EtOAc and washed with water and brine. The organic layer was dried over anhydrous Na_2SO_4. The solvent was evaporated, and the residue was purified by flash chromatography with EtOAc/hexane (4:6) to afford **6** as a white solid (41.0 mg, 0.124 mmol, 87% yield), mp = 130−185 °C (dec): ^{1}H NMR (CD_3OD) δ 8.25 (1H, d, J = 8.4 Hz), 7.79 (1H, d, J = 8.8 Hz), 7.73 (1H, dd, J = 8.0, 1.6 Hz), 7.51 (1H, d, J = 1.6 Hz), 7.43 (1H, d, J = 8.0 Hz), 7.29 (1H, d, J = 2.0 Hz), 7.18 (1H, dd, J = 8.8, 2.4 Hz), 6.92 (1H, d, J = 8.4 Hz), 6.06 (2H, s), 5.51 (2H, s); ^{13}C NMR (CD_3OD) 164.58, 157.67, 155.03, 154.49, 148.29, 143.52, 137.59, 128.75, 125.67, 123.77, 121.83, 120.49, 118.07, 117.68, 110.05, 109.40, 101.87, 66.41; FTIR (neat) 2905, 2361, 1716, 1620, 1442, 1257, 1156, 1104, 1036, 842, 759 cm^{-1}; HR-MS (ESI) m/z calcd for $(C_{18}H_{13}NO_5 + H)^+$ 324.0866, found 324.0868.

BHQ-Piperonylate (7). Ester **6** (30 mg, 0.093 mmol) was dissolved in acetic acid (2 mL). Bromine (100 mg, 20% in acetic acid, 0.125 mmol) was added dropwise. The mixture was stirred for 5 h. The reaction was quenched with saturated $NaHCO_3$, extracted with EtOAc, and washed with water and brine. The organic layer was dried over anhydrous Na_2SO_4, and the solvent was evaporated. The residue was purified by flash chromatography with EtOAc/hexane (4:6) to afford **7** as a white solid (24 mg, 0.06 mmol, 64% yield), mp = 186−194 °C (dec): ^{1}H NMR ($CDCl_3$) δ 8.15 (1H, d, J = 8.4 Hz), 7.77 (1H, dd, J = 8.4, 1.6 Hz), 7.72 (1H, d, J = 8.8 Hz), 7.59 (1H, d, J = 2.0 Hz), 7.46 (1H, d, J = 8.4 Hz), 7.35 (1H, d, J = 8.4 Hz), 6.87 (1H, d, J = 8.4 Hz), 6.07 (2H, s), 5.69 (2H, s); ^{13}C NMR ($CDCl_3$) δ 165.67, 158.58, 158.03, 157.29, 148.49, 141.59, 137.52, 128.25, 125.77, 123.67, 122.43, 120.89, 117.87, 117.40, 109.68, 108.19, 101.90, 67.52; FTIR (neat) 2909, 2359, 1716, 1622, 1504, 1444, 1259, 1229, 1157, 1037, 843, 760 cm^{-1}; HR-MS (ESI) m/z calcd for $(C_{18}H_{12}BrNO_5 + H)^+$ 401.9972 (^{79}Br) and 403.9954 (^{81}Br), found 401.9979 (^{79}Br) and 403.9960 (^{81}Br).

8-Bromo-7-hydroxyquinaldine (9). 7-Hydroxyquinaldine (**8**, 1.0 g, 6.32 mmol) was dissolved in glacial acetic acid (15 mL). Bromine (5.5 g, 20% acetic acid solution, 6.88 mmol) was added dropwise with vigorous stirring. A precipitate was observed. The reaction was stirred for 8 h and then diluted with $CHCl_3$ and washed with saturated $NaHCO_3$, water, and brine. The organic layer was dried over anhydrous Na_2SO_4 and the solvent evaporated, leaving a brown oil, which was purified by silica gel with EtOAc/hexane (3:7). The product **9** (1.07 g, 4.49 mmol, 71% yield) was acquired as a pale yellow solid, mp = 175−180 °C (dec): ^{1}H NMR ($CDCl_3$) δ 7.97 (1H, d, J = 8.4 Hz), 7.66 (1H, d, J = 8.4 Hz), 7.27 (1H, d, J = 9.2 Hz), 7.21 (1H, d, J = 8.0 Hz), 2.79 (3H, s); ^{13}C NMR ($CDCl_3$) δ 160.77, 153.89, 145.52, 136.39, 128.17, 122.57, 120.44, 116.79, 107.65, 25.69; FTIR (neat) 3059, 2360, 1618, 1561, 1503, 1429, 1334, 1262, 984, 836 cm^{-1}; HR-MS (ESI) m/z calcd for $(C_{10}H_8BrNO + H)^+$ 237.9862 (^{79}Br) and 239.9842 (^{81}Br), found 237.9856 (^{79}Br) and 239.9833 (^{81}Br).

7-Acetyl-8-bromoquinaldine (10). Under a nitrogen atmosphere, 8-bromo-7-hydroxyquinaldine (**9**, 1.0 g, 4.2 mmol) was dissolved in pyridine (20 mL). DMAP (50 mg, 0.41 mmol) was added to the reaction, followed by slow addition of acetic anhydride (0.600 mL, 6.23 mmol). The reaction was stirred for 4 h. It was diluted with $CHCl_3$, washed by 15% citric acid, water, and brine, and dried over anhydrous Na_2SO_4. Evaporation of the solvent followed by purification through silica gel with EtOAc/hexane (2:8) provided **10** as a white solid (980 mg, 3.5 mmol, 83% yield), mp = 144−146 °C: ^{1}H NMR ($CDCl_3$) δ 8.05 (1H, d, J = 8.4 Hz), 7.76 (1H, d, J = 8.8 Hz), 7.34 (1H, d, J = 8.0 Hz), 7.29 (1H, d, J = 8.8 Hz), 2.83 (3H, s), 2.45 (3H, s); ^{13}C NMR ($CDCl_3$) δ 168.56, 161.06, 149.49, 145.78, 136.41, 127.73, 125.63, 122.61, 121.85, 117.12, 25.77, 20.97; FTIR (neat) 2932, 1772, 1701, 1612, 1369, 1184, 1009, 841, 722 cm^{-1}; HR-MS (ESI) m/z calcd for $(C_{12}H_{10}BrNO_2 + H)^+$ 279.9968 (^{79}Br) and 281.9948 (^{81}Br), found 279.9975 (^{79}Br) and 281.9953 (^{81}Br).

(7-Acetyl-8-bromoquinolin-2-yl)formaldehyde (11). A mixture of SeO_2 (375 mg, 3.38 mmol) and 1,4-dioxane (10 mL) was heated to over 80 °C. 7-Acetyl-8-bromoquinaldine (**10**, 900 mg, 3.21 mmol) in 1,4-dioxane (5 mL) was added. After stirring at 80 °C for 18 h, the reaction was cooled and vacuum filtered. The filtrate was collected and concentrated, leaving a yellow solid. Purification by silica gel with EtOAc/hexane (3:7) gave **11** as a white solid (561 mg, 1.9 mmol, 60% yield), mp =190−196 °C (dec): ^{1}H NMR ($CDCl_3$) δ 10.31 (1H, s), 8.36 (1H, d, J = 8.0 Hz), 8.09 (1H, d, J = 8.0 Hz), 7.91 (1H, d, J = 8.4 Hz), 7.50 (1H, d, J = 9.2 Hz), 2.48 (3H, s); ^{13}C NMR ($CDCl_3$) δ 193.34, 168.33, 153.39, 150.50, 146.01, 138.03, 129.28, 127.93, 125.51, 118.87, 117.85, 20.98; FTIR (neat) 3054, 2848, 2360, 1751, 1708, 1369, 1194, 1157, 909, 851, 752 cm^{-1}; HR-MS (ESI) m/z calcd for $(C_{12}H_8BrNO_3 + Na)^+$ 315.9580 (^{79}Br) and 317.9561 (^{81}Br), found 315.9587 (^{79}Br) and 317.9559 (^{81}Br).

7-Acetyl-8-bromo-2-(formylquinolinyltosyl)hydrazone (12). *p*-Toluenesulfonhydrazide (507 mg, 2.72 mmol) was added to a stirred solution of aldehyde **11** (400 mg, 1.36 mmol) in ethanol (5 mL). After 2 d, the resulting precipitate was collected by vacuum filtration, washed with minimum amount of ethanol, and dried under vacuum to yield **12** as a white solid (466 mg, 1.01 mmol, 74% yield), mp = 180−190 °C (dec): ^{1}H NMR (DMSO-d_6) δ 8.76 (1H, d, J = 8.8 Hz), 8.19 (1H, d, J = 8.8 Hz), 7.89 (1H, d, J = 8.4 Hz), 7.83 (1H, s), 7.81 (2H, d, J = 8.4 Hz), 7.74 (1H, d, J = 8.4 Hz), 7.42 (2H, d, J = 8.4 Hz), 2.47 (3H, s), 2.36 (3H, s); ^{13}C NMR (DMSO-d_6) δ 168.74, 152.80, 150.92, 144.52, 143.55, 140.08, 137.23, 136.51, 130.53, 129.22, 127.63, 127.09, 125.51, 123.74, 115.88, 21.50, 21.16; FTIR (neat) 3178, 2360, 2339, 1764, 1599, 1502, 1434, 1356, 1162, 1079, 938, 895, 845 cm^{-1}; HR-MS (ESI) m/z calcd for $(C_{19}H_{16}BrN_3O_4S + Na)^+$ 483.9937 (^{79}Br) and 485.9918 (^{81}Br), found 483.9936 (^{79}Br) and 485.9886 (^{81}Br).

8-Bromo-7-hydroxyquinolin-2-ylmethyl Dimethyl Phosphate (13). NaOMe (80 mg, 1.48 mmol) was added to a mixture of compound **12** (300 mg, 0.65 mmol) in MeOH (5 mL). The MeOH was removed by rotary evaporation. The resulting residue was taken up in dry CH_3CN (15 mL), and dimethyl phosphate (492 mg, 3.9 mmol) was added dropwise. The reaction was stirred for 2 d. The solvent was evaporated, and the residue was purified by flash chromatography using EtOAc/hexane (4:6) to afford **13** (47 mg, 0.13 mmol, 20% yield) as a pale yellow oil: ^{1}H NMR ($CDCl_3$) δ 8.02 (1H, d, J = 8.4 Hz), 7.75 (1H, d, J = 8.8 Hz), 7.34 (1H, d, J = 8.4 Hz), 7.24 (1H, d, J = 8.4 Hz), 5.37 (2H, d, J = 8.0 Hz), 3.80 (6H, d, J = 10.8 Hz); ^{13}C NMR ($CDCl_3$) 160.87, 154.59, 144.32, 134.51, 128.47, 121.97, 120.84, 116.74, 104.76, 71.77, 49.56; FTIR (neat) 2929, 2832, 2359, 1614, 1505, 1438, 1332, 1217, 1189, 1108, 1070, 986, 909, 844 cm^{-1}; HR-MS (ESI) m/z calcd for $(C_{12}H_{13}BrNO_5P + H)^+$ 361.9788 (^{79}Br) and 363.9768 (^{81}Br), found 361.9808 (^{79}Br) and 363.9782 (^{81}Br).

BHQ-Glycerol (OPh) (16). (7-Acetyl-8-bromoquinolin-2-yl)formaldehyde (**11**, 50 mg, 0.17 mmol), 1-phenoxypropane-2,3-diol (**14**, 86 mg, 0.51 mmol), and pyridinium *p*-toluenesulfonate (85 mg, 0.34 mmol) were dissolved in toluene (25 mL); $MgSO_4$ (50 mg) was added as a drying agent. The reaction mixture was heated at reflux for 48 h. After vacuum filtration, the filtrate was concentrated, and the resulting residue was purified by flash chromatography using EtOAc/hexane (3:7) to yield **16** as a white solid (41 mg, 0.1 mmol, 60% yield), mp = 210 °C (dec). The product exists as a pair of diastereomers (1:1), according to ^{1}H NMR: ^{1}H NMR ($CDCl_3$) δ [8.16 (d, J = 8.4 Hz), 8.15 (d, J = 8.4

Hz) (1H)], [7.72 (d, J = 9.2 Hz), 7.72 (d, J = 9.2 Hz) (1H)], [7.64 (dd, J = 8.0, 1.2 Hz), 7.59 (dd, J = 8.4, 1.2 Hz) (1H)], 7.31 (3H, m), 6.97 (3H, m), [6.24 (s), 6.12 (s) (1H)], [4.86 (m), 4.72 (m) (1H)], 4.55 (0.5H, m), [4.32 (d, J = 6.0 Hz), 4.31 (d, J = 6.0 Hz) (1H)], 4.26 (1H, m), 4.15 (1.5H, m); ^{13}C NMR ($CDCl_3$) δ (158.48, 158.37), 157.98, 154.18, 145.24, 137.39, 129.58, 128.22, (124.64, 124.52), (121.31, 121.25), (116.86, 116.74), 114.56, 108.29, (104.95, 104.89), (104.52, 104.46), 75.09, (68.41, 68.32), 68.07; FTIR (neat) 2924, 1599, 1496, 1442, 1374, 1241, 1102, 907, 842, 728 cm^{-1}; HR-MS (ESI) m/z calcd for $(C_{19}H_{16}BrNO_4 + H)^+$ 402.0335 (^{79}Br) and 404.0317 (^{81}Br), found 402.0350 (^{79}Br) and 404.0330 (^{81}Br).

BHQ-Glycerol (SPh) (17). (7-Acetyl-8-bromoquinolin-2-yl)formaldehyde (**11**, 50 mg, 0.17 mmol), 1-phenthioxypropane-2,3-diol (**15**, 94 mg, 0.51 mmol), pyridinium *p*-toluenesulfonate (85 mg, 0.34 mmol), and $MgSO_4$ (50 mg) were mixed in toluene (25 mL). After the same reaction and workup procedure as the synthesis of compound **16**, compound **17** was acquired as a pale yellow solid (39 mg, 0.093 mmol, 55% yield), mp = 205 °C (dec). The product exists as a pair of diastereomers (3:2), according to ^{1}H NMR: ^{1}H NMR ($CDCl_3$) δ [8.15 (d, J = 8.4 Hz), 8.13 (d, J = 8.0 Hz) (1H)], [7.72 (d, J = 9.2 Hz), 7.71 (d, J = 9.2 Hz) (1H)], [7.59 (d, J = 8.8 Hz), 7.51 (d, J = 8.4 Hz) (1H)], 7.42 (1.5H, m), 7.33 (4H, m), 7.23 (0.5H, m), [6.19 (s), 6.05 (s) (1H)], [4.62 (m), 4.50 (m) (1H)], [4.45 (m), 4.24 (m) (1H)], [4.13 (m), 3.94 (m) (1H)], [3.45 (m), 3.42 (m) (1H)], [3.20 (m), 3.10 (m) (1H)]; ^{13}C NMR ($CDCl_3$) δ 158.05, 154.19, 145.26, 137.41, (130.08, 129.91), 129.16, 128.23, (126.81, 126.71), 124.61, 118.38, (116.82, 116.65), 105.29, 104.91, 104.26, (75.95, 75.70), 70.57, 70.08; FTIR (neat) 2925, 2359, 1613, 1508, 1439, 1374, 1337, 1304, 1187, 1092, 908, 842, 734 cm^{-1}; HR-MS (ESI) m/z calcd for $(C_{19}H_{16}BrNO_3S + H)^+$ 418.0107 (^{79}Br) and 420.0088 (^{81}Br), found 418.0121 (^{79}Br) and 420.0103 (^{81}Br).

Determination of the Quantum Efficiency for One-Photon Photolysis. KMOPS-buffered solutions (3 mL) of the substrates (100 μM) in quartz cuvettes (21-Q-10, Starna, Atascadero, CA) were irradiated with 365-nm UV light from a mercury lamp (Spectroline SB-100P; Spectronics, Westbury, NY) with a light filter. The spectral output of the lamp after the glass filters (CS0-52 and CS7-60, Ace Glass, Vineland, NJ) has a band between 350 and 380 nm. The duration of each irradiation period ranged from 5 to 60 s. After each period of irradiation, a 20-μL aliquot of the solution was removed for analysis by HPLC, using an external standard method to determine concentrations. The analytes were eluted isocratically (flow rate of 1 mL/min) with acetonitrile and water containing 0.1% TFA. The solvent composition was 50% acetonitrile with 50% water/TFA for compounds **4**, **7**, **16**, and **17** and 40% acetonitrile and 60% water/TFA for compound **13**. Compounds **4**, **13**, **16**, and **17** were monitored at 330 nm, and compound **7** was monitored at 299 nm. The progress curves were plotted as simple decaying exponentials. Quantum efficiencies were calculated using eq 1,[30,31] where I is the irradiation intensity in ein·cm^{-2}·s^{-1}, σ is the decadic extinction coefficient ($10^3\epsilon$, the molar extinction coefficient) in cm^2·mol^{-1}, and $t_{90\%}$ is the irradiation time in s for 90% conversion to product. The UV intensity of the lamp I was measured by potassium ferrioxalate actinometry[32] in the same setup. Piperonylic acid and 1-phenthioxypropane-2,3-diol were monitored for their formation during the process and plotted as an exponential rise to max curve.

Stern−Volmer Triplet Quenching Experiment. Sodium 2-naphthalensulfonate or potassium sorbate was dissolved in methanol and diluted to generate a 10 mM solution in KMOPS buffer. A 30-, 60-, and 90-μL aliquot was added to each of 3 different KMOPS-buffered solutions of BHQ-OAc (3 mL, 100 μM). The quantum efficiencies were determined as described for one-photon photolysis.

Determination of the Dark Hydrolysis Rate. Substrates were dissolved in KMOPS and stored in the dark at room temperature. HPLC analysis was carried out periodically as described for one-photon photolysis. Data were fit to simple decaying exponentials from which the time constant, τ, was calculated.

Measurement of the Two-Photon Uncaging Cross Section. Measurements were carried out in microcuvettes (10 × 1 × 1 mm illuminated dimensions) with an effective filling volume of 20 μL (26.10F-Q-10, Starna, Atascadero, CA) using light from a fs-pulsed and mode-locked Ti:sapphire laser (Mira 900 pumped by a Verdi, Coherent, Santa Clara, CA) focused on the center of the cuvette chamber with a 25-mm focal length lens optimized for IR lasers (06LXP003/076, Melles-Griot, Irvine, CA). The pulse width of the laser was 144 fs, and the average powers exiting the apparatus were 330, 310, 285, 285, and 295 mW for **18a**−**c** and **21d**,**e**, respectively. The two-photon uncaging cross-section (δ_u) was estimated by referencing to fluorescein, a compound with a known fluorescence quantum yield (Q_{fF} = 0.9 mol/ein) and absorbance cross section (δ_{aF} = 30 GM at 740 nm),[33,34] using eq 2, where N_p is the number of product molecules formed/unit time (molecules/s, determined by HPLC analysis as in the one-photon photolysis), ϕ is the collection efficiency of the detector (SED033 on an IL-1700, International Light, Newburyport, MA) used to measure the fluorescence of fluorescein emitted at a right angle to the beam and passed though a 535/45 nm band-pass filter (Chroma Technologies, Brattleboro, VT), C_F is the concentration of the fluorescein standard (mol/L), $\langle F(t)\rangle$ 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 BHQ-protected substrate (mol/L).

Time-Resolved IR Experiments. TRIR experiments were conducted following the method of Hamaguchi and co-workers[40,41] as described previously.[42] Briefly, the broad-band output of a $MoSi_2$ IR source (JASCO) is crossed with excitation pulses from a Nd:YAG laser. Changes in IR intensity are monitored by an MCT photovoltaic IR detector (Kolmar Technologies, KMPV11-1-J1), amplified, and digitized with a Tektronix TDS520A oscilloscope. The experiment is conducted in the dispersive mode with a JASCO TRIR-1000 spectrometer at 16 cm^{-1} resolution.

Oxygen-18 Labeling Experiment. As a control, a 100-μM solution of BHQ-OAc in water was irradiated at 365 nm from a mercury lamp (Spectroline SB-100P; Spectronics, Westbury, NY) for 2.5 min as described for one-photon photolysis. The reaction was analyzed by LC-MS (Biobasic 4 column, 150 × 1 mm, 50 μm, 300 Å; gradient elution of acetonitrile and water/TFA, 0 min 0% acetonitrile, 8 min 40% acetonitrile, 40 min 70% acetonitrile, 45 min 100% acetonitrile; analysis at 214 nm), selecting the peak corresponding to BHQ-OH for mass analysis. A 100-μM solution of BHQ-OAc in ^{18}O-labeled water was irradiated for 2.5 min, and the reaction was analyzed by LC-MS as in the unlabeled control experiment.

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Supporting Information Available: MS data for the ^{18}O-labeling experiment, tables of optimized geometry and calculated and observed IR frequencies and intensities for BHQ-OAc and BHQ-phosphate, and ^{1}H NMR spectra of synthesized compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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