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Brominated Hydroxyquinoline as a Photolabile Protecting Group with Sensitivity to Multiphoton Excitation

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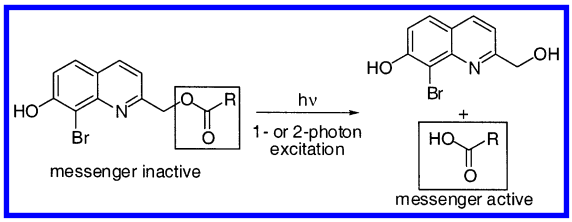
ABSTRACT



The synthesis and photochemistry of a new photolabile protecting group for carboxylic acids based on 8-bromo-7-hydroxyquinoline (BHQ) is described. BHQ possesses a greater single photon quantum efficiency than 4,5-dimethoxy-4-nitrobenzyl ester (DMNB) and 6-bromo-7-hydroxycoumarin-4-ylmethyl (Bhc), and it has sufficient sensitivity to multiphoton-induced photolysis for use in vivo. Its increased solubility and low fluorescence make it quite useful as a caging group for biological messengers.

Photolabile protecting groups and linkers[1] for a variety of functional groups have become important in combinatorial chemistry[2] and cell biology,[3] and a number of molecular structures have been utilized, such as 2-nitrobenzyl, benzoin, 7-nitroindoline, phenacyls, coumarinylmethyl, and anthraquinon-2-ylmethoxycarbonyl.[4] Photoremovable protecting groups for controlling and manipulating cell physiology are valuable because of their ability to inactivate or "cage" a physiologically active messenger and then release or "uncage" it with a flash of light. This is an excellent way to achieve temporal control over messenger release and examine the fast kinetics or spatial heterogeneity of biochemical responses in cell or tissue culture.

To be useful in biological experiments, a caging group must undergo photolysis rapidly, in high yield, and at wavelengths that are not detrimental to the biological system. It should not interfere with the methods used to measure the response of the system, and the post-photolysis remains of the caging group should not interact with the physiological processes under study. Ideally, the "caged" compound will exhibit satisfactory water solubility and hydrolytic stability in the dark.

Most caging groups require ultraviolet (UV) light, which is damaging to cells. A less damaging approach utilizes infrared (IR) light and multiphoton excitation,[5] which confines the messenger activation to the focus of the laser beam, ~1 (μm)^3 (Figure 1). In single-photon uncaging processes (UV wavelengths), any molecules of the caged

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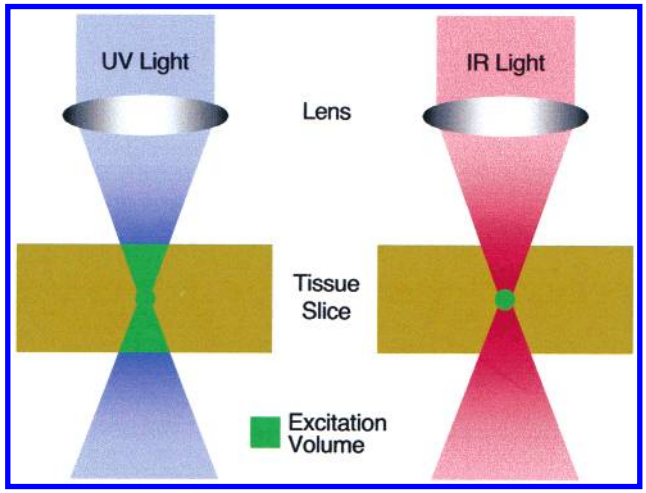


Figure 1. Three-dimensional selectivity of single vs multiphoton excitation.

compound exposed to the beam of light are uncaged, severely limiting the three-dimensional spatial resolution of messenger release. In a multiphoton or two-photon process, the chromophore simultaneously absorbs (i.e., within 1 fs) two IR photons from an fs-pulsed and tightly focused laser beam. At very high intensities, two IR photons exploit the metastable virtual state and have the same effect as one photon of half the wavelength. Uncaging occurs only at the focus of the laser beam, and because cells are relatively transparent to IR light, photodamage to the tissue, light absorption, and scattering are minimized, allowing much deeper and more accurate penetration into complex tissue samples than can be achieved with UV light. Thus, multiphoton uncaging provides an excellent method for controlling the temporal *and spatial* release of biological effectors in real time and on living tissue.

Photolabile protecting groups possessing large two-photon absorbance cross-sections are rare, but 6-bromo-7-hydroxycoumarin-4-ylmethyl (**2**, Bhc, Figure 2) was introduced as a multiphoton sensitive caging agent for neurotransmitters,[6] DNA and RNA,[7] diols,[8] and an inhibitor of nitric oxide synthase.[9] Bhc has fast photolytic uncaging kinetics but limited water solubility, and fluorescence limits its use in conjunction with fluorescent indicators. MNI-glutamate (**3**) has recently been reported to release glutamate upon two-photon excitation in sufficient quantities to be useful for investigating the function of glutamate receptors.[10]

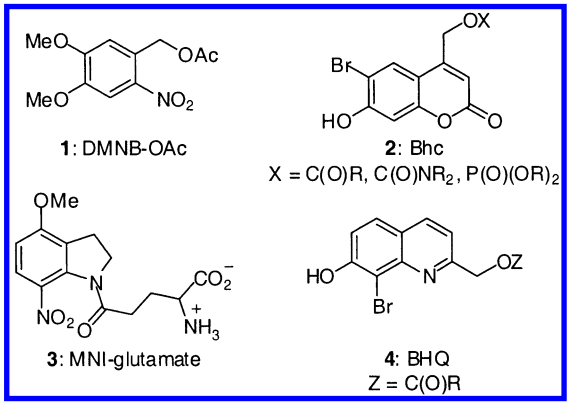
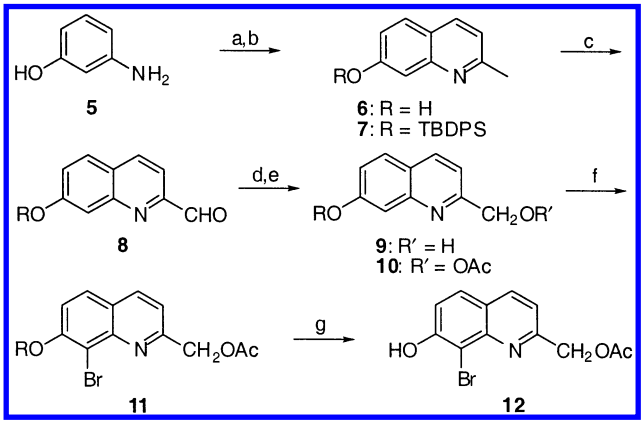


Figure 2. Photolabile protecting groups for carboxylic acids.

We report the synthesis and some photochemical properties of a new photoremovable protecting group for carboxylic acids based on 8-bromo-7-hydroxyquinoline **4** (BHQ, Figure 2) and compare it to two known caging groups, 4,5-dimethoxy-4-nitrobenzyl ester (**1**, DMNB) and Bhc (**2**). Quinoline and its derivatives show interesting photochemical properties, and several of them are used as fluorescent probes for different metals and ions.[11] Although the photochemical and photophysical properties of 7-hydroxyquinoline have been studied,[12] its derivatives have not been used as caging groups.

Our test compound, 8-bromo-7-hydroxyquinoline-2-ylmethyl acetyl ester (**12**, BHQ-OAc), was prepared from *m*-aminophenol (**5**) and crotonaldehyde in seven steps (Scheme 1). A Skraup reaction using concentrated hydrochloric acid and tetrachloro-1,4-benzoquinone (*p*-chloranil)

Scheme 1. Synthesis of BHQ-OAc[a]



[a] Reagents and conditions: (a) $CH_3CH{=}CHCHO$, 12 N HCl, *p*-chloranil, *n*-BuOH, 105 °C, 1 h, 44%; (b) TBDPSCl, imid CH_3CN, rt, 14 h, 80%; (c) SeO_2, *m*-xylene, 135 °C, 17 h, 84%; (d) $NaBH_4$, EtOH, rt, 6 h, 87%; (e) Ac_2O, pyr, rt, 4 h, 86%; (f) Br_2, AcOH, rt, 5 h, 79%; (g) TBAF, THF, rt, 4 h, 65%.

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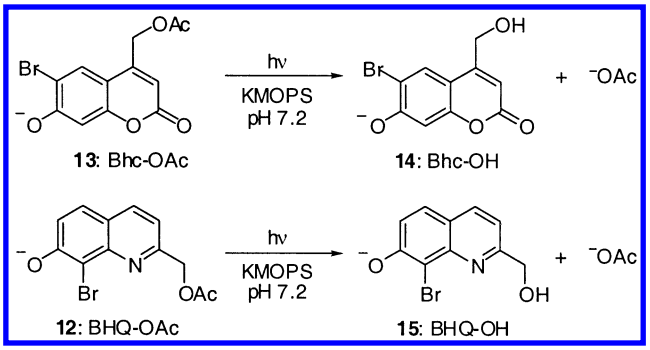
provided 7-hydroxyquinaldine (**6**),[13] whose hydroxy group was subsequently protected as the *tert*-butyldiphenylsilyl (TBDPS) ether. The 2-methyl group was oxidized with selenium dioxide, and the resulting aldehyde **7** was reduced to the alcohol **9** using sodium borohydride in ethanol. Treatment with acetic anhydride in pyridine provided acetate **9**, which, like other 7-hydroxy quinolines,[14] was brominated in the 8-position.[15] Removal of the TBDPS protecting group using tetrabutylammonium fluoride in THF provided BHQ-OAc (**12**). Bhc-OAc (**13**) was synthesized according to the published procedure.[6]

Upon photolysis with 365 nm light under simulated physiological conditions in KMOPS (100 mM KCl, 10 mM MOPS, pH 7.2), Bhc-OAc (**13**) and BHQ-OAc (**12**) are each converted to their respective hydroxy derivative, Bhc-OH (**14**) or BHQ-OH (**15**), and acetate (Scheme 2). A comparison

Scheme 2. Photolysis of Bhc-OAc and BHQ-OAc[18]



of the time courses for these reactions, obtained from HPLC analysis of aliquots taken at periodic intervals, shows that BHQ-OAc photodecomposes more rapidly than Bhc-OAc (Figure 3). From these data, single-photon uncaging quantum efficiencies, Q_{u1}, were determined from the relationship $Q_{u1} = (I\sigma t_{90\%})^{-1}$,[16] where I is the irradiation intensity in ein·cm^{-2}·s^{-1} (determined by potassium ferrioxalate actinometry[17]), σ is the decadic extinction coefficient (10^3 times ϵ, molar extinction coefficient) in cm^2·mol^{-1}, and $t_{90\%}$ is the irradiation time in seconds for 90% conversion to product.

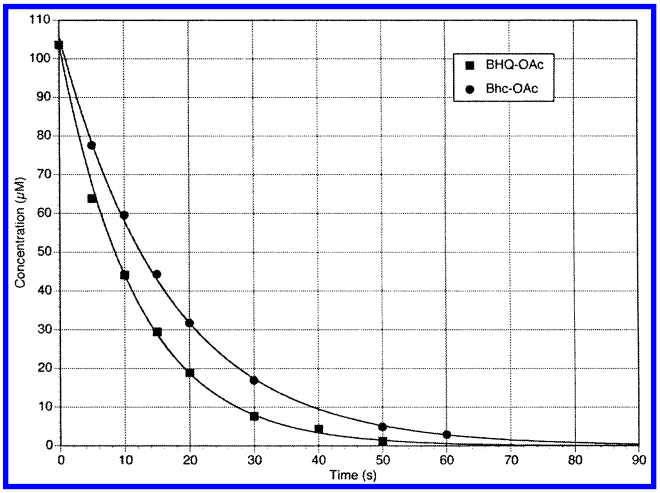


Figure 3. Time course of single photon photolysis of BHQ-OAc (**12**) and Bhc-OAc (**13**) at 365 nm. Concentrations of BHQ-OAc and Bhc-OAc were determined by HPLC and are the average of 4 runs (see the Supporting Information). Solid lines are least-squares fits of simple decaying exponentials, which gave time constants $\tau = 11.7$ and 16.6 s and coefficients of determination $R^2 = 0.99$ and 1.00, for BHQ-OAc and Bhc-OAc, respectively.

These data are summarized in Table 1 along with selected absorption data.

Table 1. Photochemical Properties of DMNB-OAc, Bhc-OAc, and BHQ-OAc

	λ_{max} (nm)	ϵ (M^{-1}·cm^{-1})	ϵ_{365} (M^{-1}·cm^{-1})	Q_{u1} (mol/ein)	δ_u (740 nm)	δ_u (780 nm)
1[a]	346	6100	5200	0.005	0.03	0.01
12	369	2600	2580	0.29	0.59	0.087
13	370	15 000[a]	14 800[a]	0.036	0.72	0.21

[a] Values taken from ref 6.

The sensitivity to two-photon photolysis of potential caged compounds is quantified as the uncaging action cross-section δ_u. This value is the product of the two-photon absorbance cross-section δ_a and the uncaging quantum yield Q_{u2}, and to be biologically useful, δ_u should exceed 0.1 Goeppert−Mayer (GM), where 1 GM is defined as 10^{-50} cm^4·s/photon.[6] Two-photon uncaging cross-sections were measured using a fs-pulsed, mode-locked Ti:sapphire laser with fluorescein as an external standard because its two-photon fluorescence cross-section has been well-characterized.[19] The progress of the uncaging reaction was measured by HPLC and graphed as a function of time (Figure 4). The initial rate of photolysis was used to determine N_p, the number of molecules formed per second, which is related to δ_u by the equation

$$\delta_u = \frac{N_p \phi Q_{f2} \delta_{aF} C_F}{\langle F(t) \rangle Cs}$$

where ϕ is the collection efficiency of the detector, Q_{f2} is

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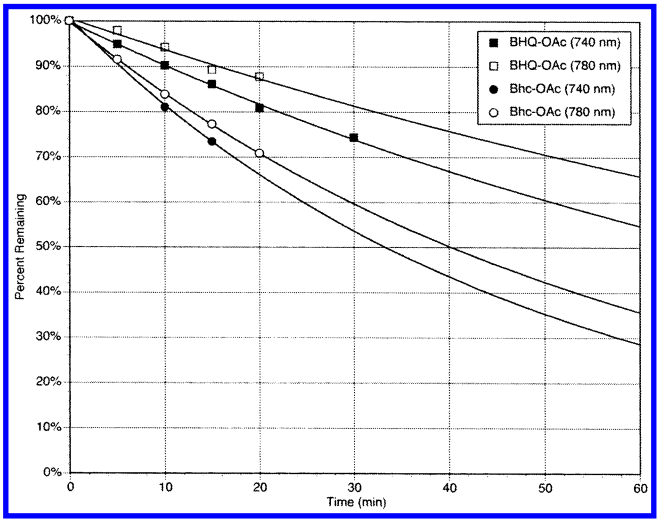

Figure 4. Time course of two-photon photolysis of BHQ-OAc (**12**) and Bhc-OAc (**13**) at 740 and 780 nm (average power 345 and 380 mW exiting the cuvette, respectively; 155 fs pulse width). The concentrations of BHQ-OAc and Bhc-OAc were determined by HPLC and are the average of three runs (see the Supporting Information). For comparison, the concentrations have been normalized and reported as a percent. Solid lines are least-squares fits of simple decaying exponentials, which gave time constants τ = 100 and 48 min and coefficients of determination R^2 = 0.997 and 0.997 for BHQ-OAc and Bhc-OAc at 740 nm, respectively. At 780 nm, τ = 142 and 58 min and R^2 = 0.974 and 1.00 for BHQ-OAc and Bhc-OAc, respectively.

the two-photon fluorescence quantum yield of fluorescein, δ_{aF} is the fluorescein absorbance cross-section, C_F is the concentration of fluorescein, $\langle F(t)\rangle$ is the time-averaged fluorescent photon flux, and C_S is the initial concentration of caged substrate. The two-photon uncaging cross-section δ_u for BHQ-OAc was found to be 0.59 GM at 740 nm, compared to 0.72 GM for Bhc-OAc. We attribute our lower measured value of δ_u for Bhc-OAc, compared to that reported in the literature,[6] to the reduced average power used in our experiments (345 vs 530 mW). Clearly, BHQ-OAc has a sufficiently high two-photon uncaging cross section at 740 nm to be physiologically useful.

Ideally, "caged" compounds should be hydrolytically stable in aqueous buffer solution in the absence of light. Dark hydrolytic stability of BHQ-OAc was established in KMOPS at pH 7.2. The hydrolysis was monitored by HPLC as a function of time, looking for disappearance of starting material and appearance of the remnant of the photochemical reaction, BHQ-OH (**15**) or Bhc-OH (**14**).[20] The results indicate that the BHQ-OAc hydrolysis followed a simple decaying exponential curve with a time constant τ = 70.9 h. While it is more susceptible to dark hydrolysis than Bhc-OAc (τ = 517 h), BHQ-OAc is sufficiently stable for use in physiological experiments.

The successful use of "caged" compounds in cellular studies requires adequate solubility in aqueous solutions of moderately high ionic strength. The solubility of BHQ-OAc is much higher than Bhc-OAc. For example, a 0.78 mM solution of BHQ-OAc could be made in KMOPS with 2% MeOH as a cosolvent, whereas to prepare just a 0.32 mM solution of Bhc-OAc required 33% MeOH to be used.

BHQ-OAc exhibits lower levels of fluorescence when excited at 365 nm compared to Bhc-OAc (Figure 5), which

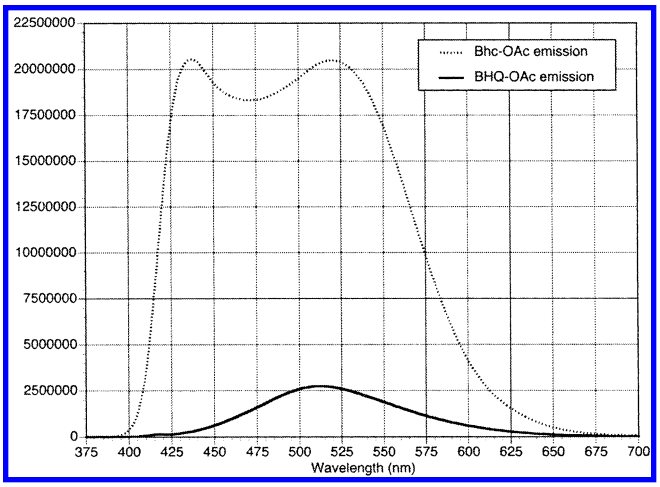

Figure 5. Fluorescence spectra of BHQ-OAc (**12**) and Bhc-OAc (**13**) (100 mM in KMOPS, λ_{ex} = 365 nm).

should facilitate its use in conjunction with fluorescent indicators. Lowered fluorescence probably explains the higher quantum efficiency of BHQ-OAc over Bhc-OAc in that the photochemical processes compete better with the photophysical processes.

The results presented in this paper indicate that BHQ can be used as a photolabile protecting group to "cage" biologically active messengers containing a carboxylate functionality. The major advantages of BHQ-OAc are its high quantum efficiency of one-photon photolysis, increased solubility in aqueous buffers, and very low fluorescence. Further investigation into the generality, mechanism, and usefulness in biological systems is underway.

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Supporting Information Available: Experimental procedures and characterization data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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(18) UV spectra of Bhc-OAc and BHQ-OAc show that the predominant form of these molecules at pH 7.2 is the phenolate ion ($\lambda_{max} \approx$ 370 nm) and not the phenol ($\lambda_{max} \approx$ 320 nm). The pK_a of BHQ-OAc is 6.8.

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(20) The identity of **15** or **14** in the reaction mixture was confirmed by comparison with the HPLC retention times of authentic samples and LC/MS analysis.