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Bhc-diol as a Photolabile Protecting Group for Aldehydes and Ketones

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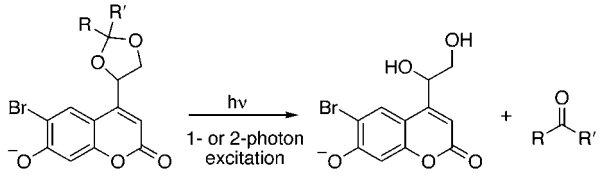
ABSTRACT



6-Bromo-4-(1,2-dihydroxyethyl)-7-hydroxycoumarin (Bhc-diol) can be used under simulated physiological conditions as a photoremovable protecting group for aldehydes and ketones. The single- and two-photon-induced release of benzaldehyde, piperonal, acetophenone, and cyclohexanone is demonstrated.

The use of photolabile protecting groups[1] to control the activity of biological effectors in a temporally and spatially dependent manner has become important in the investigation of cellular signal transduction pathways.[2] They have also enabled the use of photolithographic techniques for the construction of complex oligonucleotide, polypeptide, and other spatially addressable combinatorial libraries.[3] Many photoremovable protecting groups have been created for these purposes, including 2-nitrobenzyl,[4a] benzoin,[4b] 7-nitroindoline,[4c] phenacyls,[4d] coumarinylmethyl,[4e] and anthraquinon-2-ylmethoxycarbonyl.[4f]

The strategy employed for controlling the activity of a biological effector of interest is straightforward: a photoremovable protecting ("caging") group is covalently attached to the bioactive molecule, the chromophore−effector conjugate ("caged" compound) is introduced into the biological culture or animal, and then the active form of the effector is released at the desired time and location with a pulse of light ("uncaging"). 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 postphotolysis remains of the caging group must not interact with the physiological processes under study. The caged compound should exhibit good water solubility and hydrolytic stability in the dark.

Most uncaging protocols employ UV light, which due to the short wavelengths (<360 nm) required, is damaging to cells, lacks three-dimensional spatial resolution, and provides poor penetration due to light scattering and the presence of native chromophores that absorb at these wavelengths. A less damaging, more deeply penetrating, and more spatially

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selective approach utilizes IR light and multiphoton excitation (MPE),[5] which confines the messenger activation to the focus of the laser beam, ~1 (μm)^3. As an added advantage, chromophores with sensitivity to MPE tend to be highly sensitive to single-photon excitation, decreasing irradiation times and enabling the use of low-intensity light sources in conventional applications of caged compounds.

Photolabile protecting groups that possess sufficiently large two-photon absorbance cross-sections, δ_u, for biological applications exist.[6] For example, 6-bromo-7-hydroxycoumarin-4-ylmethyl (**1**, Bhc, Figure 1) has been utilized as a

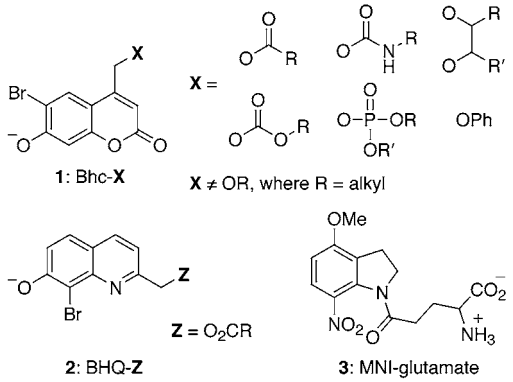
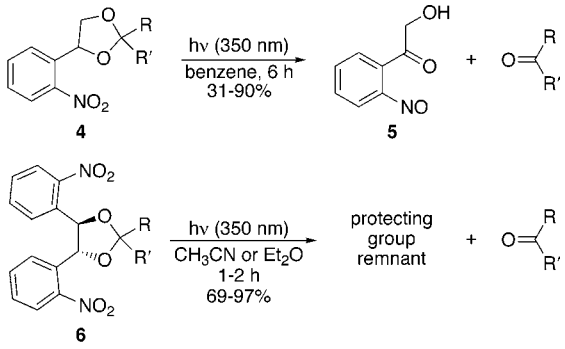


Figure 1. Two-Photon Releasing Groups with Biological Utility.

multiphoton-sensitive caging agent for neurotransmitters,[7] DNA and RNA,[8] diols,[9] and an inhibitor of nitric oxide synthase.[10] Recently, we reported the synthesis, photochemistry, and potential use of 8-bromo-7-hydroxyquinoline (**2**, BHQ) as a caging group for biological effectors possessing carboxylate groups.[11] MNI-glutamate (**3**) has been shown to release glutamate upon two-photon excitation in sufficient quantities to be useful for investigating the function of glutamate receptors.[12]

There exists a lack of physiologically useful caging groups for ketones and aldehydes, functional groups that are found in many biological effectors, especially drugs. Synthetically useful photoremovable protecting groups for carbonyls such as *N*,*N*-dimethylhydrazones[13] require the generation of singlet oxygen, while others require a triplet sensitizer, as in the case of dithioacetals.[14] Both methods would be incompatible with biological systems. *o*-Nitrophenylethylene glycol derivatives **4**[15] and **6**[16] release carbonyl compounds upon exposure to 350 nm light in organic solvents (Scheme 1).

Scheme 1. *o*-Nitrophenylethylene Glycols as Photoremovable Protecting Groups for Aldehydes and Ketones



Similarly, polymer-supported *o*-nitrophenylethylene glycols[17] offer photoremovable protection to aldehydes, releasing them after exposure to a visible-light mercury lamp for 7 h. These protecting groups require significant synthetic adaptation for physiological use, and they would still suffer from very poor sensitivity to MPE.

As part of our program to develop applications for two-photon-sensitive photoremovable protecting groups, we required a caging agent capable of releasing ketones inside living cells, tissues, and animals. We rationalized that acetals and ketals of 6-bromo-4-(1,2-dihydroxyethyl)-7-hydroxycoumarin (Bhc-diol-acetal/ketal, **7**) would be capable of liberating aldehydes and ketones upon single- or two-photon photolysis under simulated physiological conditions[18] (Scheme 2). Our success in this endeavor is somewhat surprising because saturated alcohols cannot be released directly from Bhc; **X** must be a good leaving group (Figure 1).[9,19] Apparently, trapping the zwitterion **8**, a possible intermediate suggested by Bendig et al.,[20] with solvent (H_2O or ^-OH) followed by dissociation to Bhc-diol (**9**) and the carbonyl

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(6) δ_u is a measure of the sensitivity of a chromophore to two-photon photolysis. To be biologically useful, it should exceed 0.1 GM (Goeppert−Mayer, 10^{-50} cm^4 s photon^{-1}). See ref 7.

(7) 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*, 1193−2000.

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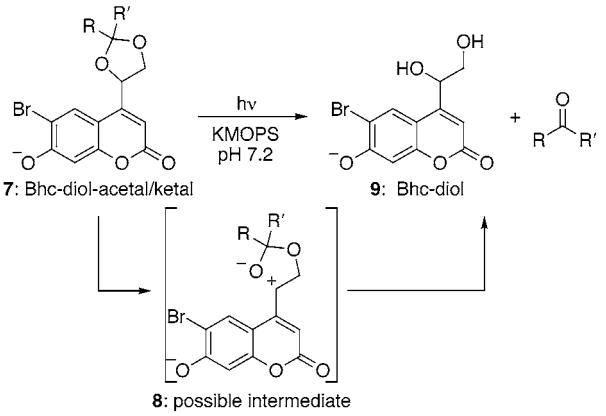
(18) KMOPS buffer: 100 mM KCl and 10 mM MOPS titrated to pH 7.2 with KOH.

(19) Schade, B.; Hagen, V.; Schmidt, R.; Herbich, R.; Krause, E.; Eckardt, T.; Bendig, J. *J. Org. Chem.* **1999**, *64*, 9109−9117.

(20) On the basis of studies of (7-methoxycoumarin-4-yl)methyl-caged phosphates, carboxylates, and sulfonates, Bendig et al. proposed a photo S_N1 reaction (solvent-assisted photoheterolysis) mechanism. See ref 19.

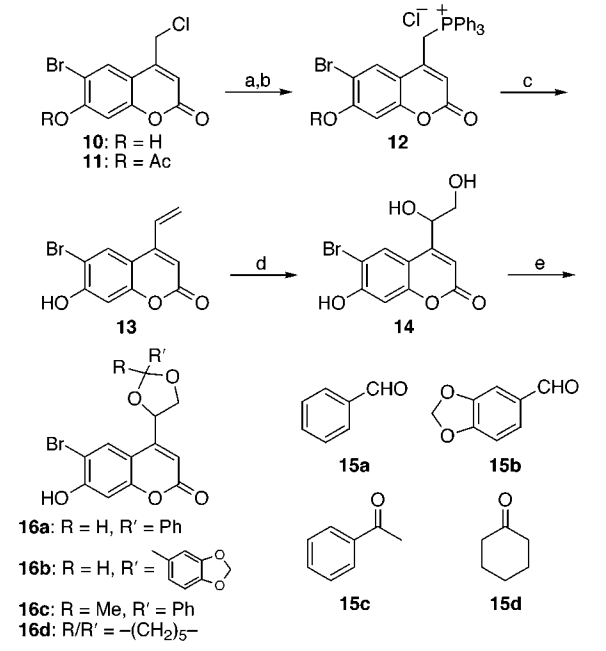
Scheme 2. Photolysis of Bhc-diol-Protected Aldehydes and Ketones



compound is competitive with recombination to regenerate the starting material **7**.

The Bhc-diol-protected aldehydes and ketones were synthesized as shown in Scheme 3. Coumarin **10** was prepared

Scheme 3. Synthesis of Bhc-diol-Protected Aldehydes and Ketones[a]



[a] Reagents and conditions: (a) Ac_2O, pyr, 85%; (b) PPh_3, CH_3CN, 85 °C, 82%; (c) 37% CH_2O, 15% Na_2CO_3, H_2O, 72%; (d) OsO_4, NMO, H_2O, acetone, 80%; (e) **15a**−**d**, PPTS, $MgSO_4$, toluene, BuOH, 110 °C, 57%, 28%, 22%, and 39% for **16a**−**d**, respectively.

in 90% yield by a Pechman condensation of 4-bromoresorcinol with ethyl 4-chloroacetoacetate in concentrated sulfuric acid,[7] followed by protection of the phenolic hydroxy group as the acetate. Formation of the triphenyl phosphonium salt **12** was accomplished by treating the chloride **11** with triphenylphosphine in acetonitrile at 85 °C. Olefination of compound **12** by a Wittig reaction with aqueous formaldehyde, using sodium carbonate as the base, proceeded with concomitant loss of the acetate to reveal alkene **13**.[21] Osmium tetroxide dihydroxylation provided Bhc-diol (**14**), which was acetalized or ketalized by refluxing in toluene with benzaldehyde (**15a**), piperonal (**15b**), acetophenone (**15c**), or cyclohexanone (**15d**) in the presence of pyridinium *p*-toluenesulfonate (PPTS) with some solid magnesium sulfate added to remove water. This afforded Bhc-diol-acetals **16a** and **16b** and Bhc-diol-ketals **16c** and **16d** as mixtures of diastereomers (in the case of **16a**−**c**) in modest yields.

Irradiation of Bhc-diol-acetals/ketals **16a**−**d** with 365 nm light in pH 7.2 KMOPS buffer unmasked the protected aldehydes and ketones (Figure 2). Comparing the time

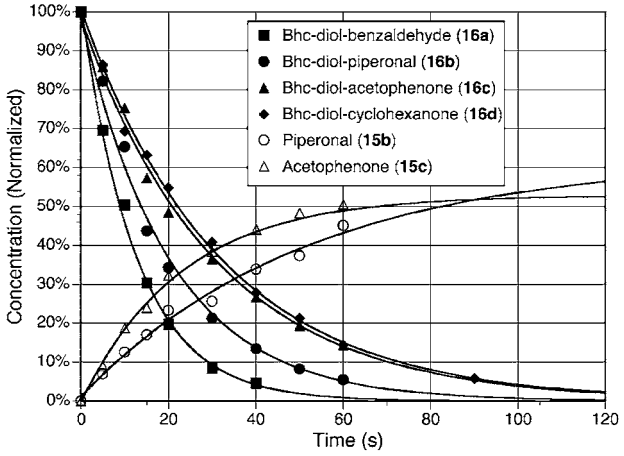


Figure 2. Time course of single-photon photolysis of Bhc-diol-protected aldehydes and ketones at 365 nm. Concentrations were determined by HPLC and are the averages of 3 runs (see Supporting Information). For comparison, the concentrations have been normalized and reported as a percentage. Solid lines through solid symbols are least-squares fits of simple decaying exponentials, which gave time constants τ = 12.5, 20.3, 30.8, and 31.6 s for **16a**, **16b**, **16c**, and **16d**, respectively. Fits for **16a**−**d** had coefficients of determination $R^2 \geq 0.996$. Solid lines through the open symbols are fits of an exponential rise to a maximum for the photolysis products **15b** and **15c**.

courses for these reactions, obtained from HPLC analysis of aliquots taken at periodic intervals, reveals that Bhc-diol-benzaldehyde and Bhc-diol-piperonal were photolyzed slightly more efficiently than the acetophenone and cyclohexanone derivatives. From these data, single-photon uncaging quantum efficiencies, Q_{u1}, were determined as previously described.[11,22,23] They are summarized in Table 1 along with selected absorption data. The quantum efficiencies for single-photon photolysis of Bhc-diol-protected benzaldehyde, pip-

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Table 1. Photochemical Properties of Bhc-diol-Protected Aldehydes and Ketones

	λ_{max} (nm)	ϵ_{365} (M^{-1} cm^{-1})	ϵ_{370} (M^{-1} cm^{-1})	Q_{u1} (mol/ein)	δ_u (740 nm)
16a	370	18 000	18 500	0.057	0.90
16b	370	19 500	19 800	0.035	0.60
16c	370	18 000	18 400	0.030	1.23
16d	370	12 600	12 800	0.032	0.51

eronal, acetophenone, and cyclohexanone are similar to those of other Bhc-protected compounds.[7−11]

Each of the Bhc-diol-protected compounds **16a**−**d** was tested for its resistance to spontaneous hydrolysis in the dark under simulated physiological conditions. No appreciable decomposition of the conjugate was observed after 7 days in pH 7.2 KMOPS buffer.

The extent of carbonyl compound release as measured by HPLC was half of what was expected. To explore the possibility of secondary photochemical degradation, KMOPS-buffered solutions of piperonal and acetophenone were each exposed to 365 nm light for 1 min, but neither compound showed any significant decomposition. Irradiation of a 1:1 mixture of Bhc-diol and piperonal or acetophenone in KMOPS buffer also showed no change in concentration of the carbonyl compounds. Degradation of the carbonyl compounds after uncaging is not the result of a direct or a Bhc-diol-mediated photochemical process.

Two-photon uncaging cross-sections, δ_u, of **16a**−**d** were measured using a femtosecond-pulsed, mode-locked Ti:sapphire laser with fluorescein as an external standard as previously described.[11] The values of δ_u determined for **16a**−**d** (Table 1) are similar to the values obtained for other Bhc-protected compounds.[7−11] Any discrepancies are probably a result of differences in laser power and optical setups employed. The longer time constants (min), as compared to the single-photon kinetics (s), are due to the small volume of the sample that is actually irradiated (~1 fL) relative to the bulk solution (20 μL). Sufficient quantities of the Bhc-diol-acetal/ketal for HPLC analysis must diffuse into the laser's focal volume, where uncaging efficiency is high, undergo photolysis, and then diffuse back out into the bulk solution.

The results presented in this paper indicate that Bhc-diol can be used as a photolabile protecting group to cage biologically active messengers containing a carbonyl functionality. Since Bhc has been used in biological systems, this represents the first example of a photolabile protecting group capable of releasing aldehydes and ketones by single- and two-photon excitation under physiological conditions. Further investigation into the mechanism of photolysis and usefulness in biological systems is underway.

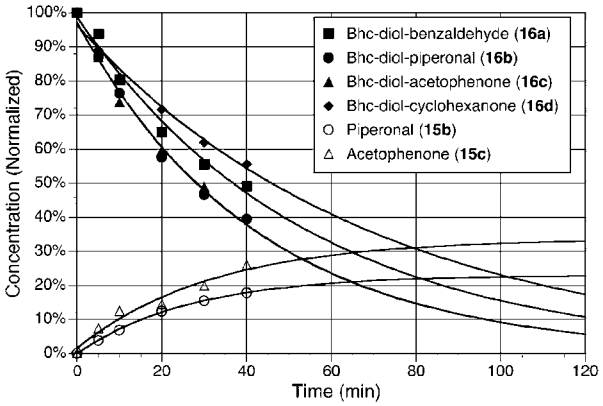

Figure 3. Time course of two-photon photolysis of Bhc-diol-protected aldehydes and ketones at 740 nm (average power 300−335 mW exiting the cuvette; 96−115 fs pulse width). Concentrations were determined by HPLC and are the averages of at least 3 runs (see Supporting Information). For comparison, the concentrations have been normalized and reported as a percentage. Solid lines are least-squares fits of simple decaying exponentials, which gave time constants τ = 54.1, 42.3, 42.4, and 70.0 min for **16a**, **16b**, **16c**, and **16d**, respectively. Fits for **16a**−**d** had coefficients of determination $R^2 \geq 0.984$. Solid lines through the open symbols are fits of an exponential rise to a maximum for the photolysis products **15b** and **15c**.

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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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