A Strategy for the Construction of Caged Diols Using a Photolabile Protecting Group

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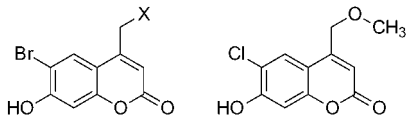
Abstract: Caged analogues of biologically active compounds have received widespread attention as temporally and spatially controlled probes of cell-based processes. Recently, a coumarin-4-ylmethyl derivative has been used to cage carboxylates, sulfonates, carbamates, and phosphates. We describe herein a synthetic strategy that furnishes photosensitive caged diols and provides an entry into the protection/photodeprotection of functionality with modest leaving group abilities.

The wide variety of roles played by metabolites, proteins, and genes in biological systems has been explored using approaches that range from simple inhibitory molecules to transgenic animals. However, these technologies are generally unable to assess the precise temporal and/or spatial influence exerted by specific biomolecules in the context of a particular biological event. For example, the role of a specific enzyme during the various stages of mitosis or at the onset of carcinogenesis in an adult animal is difficult to address with a simple dead-end inhibitor or a transgenic animal model. Photoactivatable ("caged") analogues of biomolecules furnish a simple means to control, both spatially and temporally, biological activity with light.[1] A wide variety of caged species have been described, including Ca^{2+}, NO, metal ion indicators, and peptides. More recently, caged enzymes, such as protein kinases, protein phosphatases, proteinases, and others, have been reported.[2] Unfortunately, the application of these caged species to living animals has been thwarted by the short wavelengths (<360 nm for the *o*-nitrobenzyl derivatives) required for the uncaging process. Light of this wavelength has poor tissue penetration due to the presence of both intra- and extracellular chromophores that absorb in this region of the electromagnetic spectrum. A photolabile-protecting group containing a large two-photon absorbance cross-section was recently introduced as a caging agent (**1**).[3]



1 **2**

Under conditions of high photon flux, the 7-hydroxycoumarin moiety can absorb and combine the energies of two long wavelength photons. Two-photon excitation is equivalent to the absorption of one photon at half the wavelength and possesses the inherent advantage of significantly deeper tissue penetration. Although the caging agent **1** holds significant promise for in vivo studies, it has been used to cage only a limited number of functional groups [e.g., carboxylate (**1a**, $X = -O_2CR$), phosphate [**1b**, $X = -O_2P(OR)_2$], and carbamate (**1c**, $X = -O_2CNHR$)]. By contrast, aliphatic alcohols fail to undergo photocleavage when caged with the coumarin moiety (e.g., **2**). One possible explanation for this behavior is that the photoliberation process proceeds via a mechanism that generates charged intermediates. For example, the 7-methoxy analogue of **1** photohydrolyzes via formation of an ion pair.[4] Such a mechanism would limit photodeprotection to leaving groups that could stabilize an initially formed anionic intermediate.[3,5] As part of our program to define the temporal role of signaling proteins in biochemical pathways, we required access to a caging agent for diols that also has the requisite physical stability to function in a living animal.

Coumarin derivatives have recently been shown to serve as caging agents for such biologically important molecules as cAMP,[5a,c,d,e] cGMP,[5c,d] cytidine diphosphate,[5b] and glutamic acid.[3] Bendig and his colleagues have recently reported that carboxylates, sulfonates, and phosphates caged with the 7-methoxy derivative of **1** are photoliberated via an S_N1 mechanism that generates an initially formed ion pair.[4] One might predict that such a mechanism would exclude functionality unable to stabilize the negatively charged intermediate produced during photoheterolysis. Indeed, we found that the coumarin ether derivative **2** is completely resistant to photocleavage. This observation is consistent with a previous report that coumarin caged alcohols are resistant to photodeprotection.[6] We reasoned that it should be possible to promote the photodeprotection of a coumarin-caged aliphatic alcohol if the latter were to lie adjacent to an electron-rich center that could assist the cleavage process. Consequently, we decided to investigate the possibility

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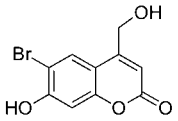
that aliphatic ether, if present as a component of an acetal, could undergo light-initiated cleavage.

The coumarin aldehyde **4** was prepared via oxidation (MnO_2/THF/90% yield) of the previously described coumarin alcohol **3**.[3] The aldehyde was subsequently used to convert 1,2-, 1,3-, and 1,4-diols to their corresponding acetals. All photolysis reactions were performed in a



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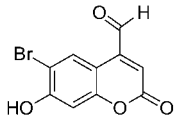

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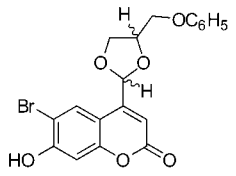
quartz cuvette irradiated with a 200 W Hg arc lamp. UV and IR filters were placed between the cuvette and the lamp to reduce exposure to short wavelength light (<348 nm) and heat, respectively. The 1,3-dioxolanes **5**−**8** (Chart 1), prepared from the 1,2-diols, experience rapid

Chart 1



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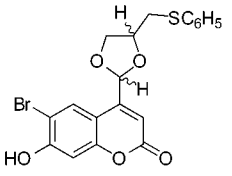

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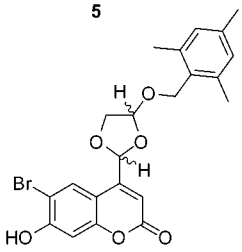

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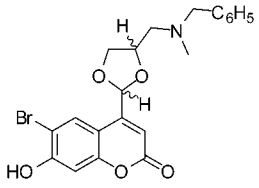

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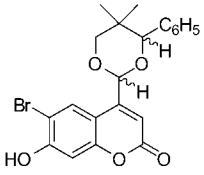

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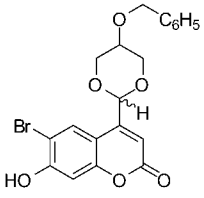

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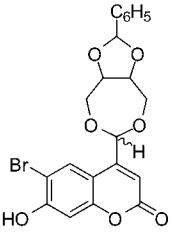

photoliberation to the desired free diol products. Starting material conversion and product formation were followed as a function of time using HPLC (Figures 1 and 2). For example, nearly 75% of compounds **6** and **7** are converted to the uncaged diols after irradiation for 10 min (Figure 1). An analogous conversion rate is displayed by the dioxolane **5** (data not shown). Quantum yields (ϕ) for the photocleavage process were determined by ferrioxalate actinometry.[7] The ϕ values for compounds **5**−**7** are between 0.004 and 0.005 (Table 1), which are analogous

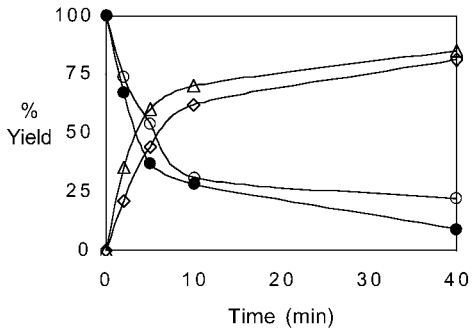

Figure 1. Photolytic dissociation of **6** (●) and **7** (○) and formation of diols from **6** (△) and **7** (◇), respectively, in 1:1 CH_3-OH/buffer (0.01 M HEPES, 0.12 M NaCl, 3 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, pH 7.4).

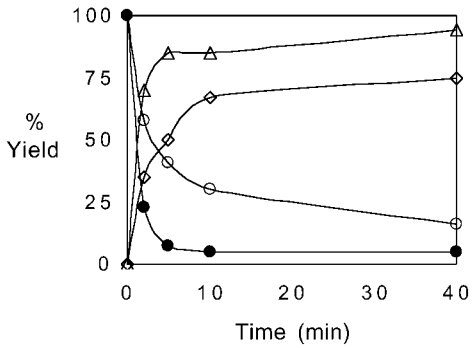

Figure 2. Photolytic dissociation of **8** (●) and **11** (○) and formation of diols from **8** (△) and **11** (◇), respectively, in 1:1 CH_3OH/buffer (0.01 M HEPES, 0.12 M NaCl, 3 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, pH 7.4).

Table 1. Photochemical Properties of Acetals 5−8 and 11

acetal	ϵ_{348}[a]	ϕ
5	9700	0.0041
6	8500	0.0053
7	9400	0.0051
8	8000	0.0278
11	7100	0.0052

[a] Extinction coefficient (M^{-1} cm^{-1}) at 348 nm.

to quantum yields reported for coumarin caged phosphates.[4] Interestingly, compound **8**, which contains a tertiary amine, is even more rapidly converted to the photocleaved product (85% conversion in 5 min) (Figure 2). The ϕ for the 1,3-dioxolane **8** is 7-fold greater than those obtained with the dioxolanes **5**−**7**. The tertiary amine in dioxolane **8** could potentially serve as a proton donor during acetal decomposition (Scheme 1) and thereby accelerate the overall rate of photouncaging.

In marked contrast, the 1,3-dioxanes **9** and **10**, which were prepared from 1,3-diols, are inert to photolysis. Indeed, even after 40 min of irradiation, we were unable to detect any observable free diol formation. This observation is consistent with the general observation that six-membered-ring acetals are thermodynamically more stable than their five-membered-ring counterparts.[8] For example, the acid-catalyzed rate of cyclic acetal hydrolysis is more rapid in less stable ring systems.[8] However, even given the unique stability commonly ascribed to the

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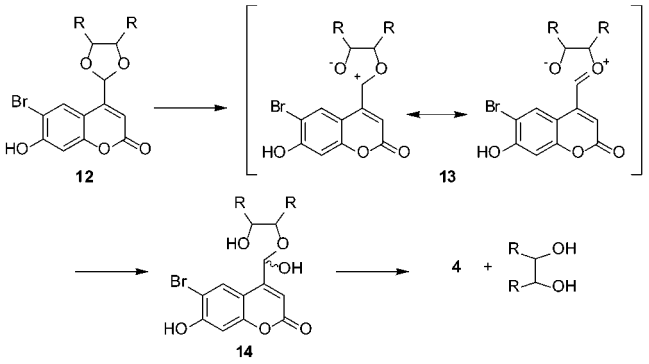
Scheme 1



six-membered acetal rings, it is surprising that the 1,3-dioxane ring displays properties so dramatically different from its 1,3-dioxolane counterpart.

We also examined the photouncaging properties of the 1,3-dioxepane ring contained in **11**. This species, like the 1,3-dioxolanes, undergoes substantial uncaging (~65%) within 10 min of irradiation. The ϕ (0.005) associated with the conversion of **11** to the free diol is essentially identical to those displayed by the 1,3-dioxolanes **5**−**7**. We note that the 10 min irradiation time required for the in vitro uncaging of the caged diols should be reduced to <10 s inside a cell. For example, we have found that a caged fluorescein dextran is uncaged >120 times faster in vivo than in vitro (data not shown). This is due to a dramatically enhanced laser-driven photon flux through a small cell volume (~2 pL).[9]

Although the mechanism of light-mediated coumarin acetal uncaging remains to be determined, a possible scenario is outlined in Scheme 1. Excitation of the coumarin chromophore in **12** should generate the *intramolecular* ion pair contained within the intermediate **13**. By comparison, an *intermolecular* ion pair serves as an early intermediate in the photodecomposition of coumarin caged carboxylates, carbamates, and phosphates (**1a**−**c**). In the latter instances, the positive charge produced following photoexcitation is dispersed into the aromatic ring system. Apparently, the leaving group potential of simple aliphatic alcohols (e.g., **2**) is not sufficient to generate the courmain-stabilized cationic intermediate. By contrast, the adjacent ether moiety of the acetal in **12** may serve as an electron-rich trigger that can assist the departure of the alkoxide and simultaneously further stabilize the subsequently formed electron deficient center (**13**). The intermediate **13**, when trapped by solvent (H_2O), should furnish the hemiacetal **14**, which can then decompose to provide the liberated diol and the aldehyde **4**. However, other mechanistic scenarios are also possible. For example, the triphenylpyrylium tetrafluoroborate sensitized photolysis of acetals may proceed via a radical cation intermediate.[10] Consequently, further work will be required to elucidate the mechanistic details of the photoliberation of coumarin-caged diols.

We note that the aldehyde **4** is a potent electrophile that could react with and therefore damage a variety of physiologically important biomolecules. Indeed, in general, photoliberation of caged species (e.g., *o*-nitrobenzyl derivatives) commonly produces byproducts structurally analogous to **4** that can potentially interfere with biological processes. However, the liberation of caged species in cells appears to proceed in a fashion that inflicts little or no damage. This may be due, in part, to the high intracellular concentration of glutathione. Indeed, sulfhydryl reagents have previously been shown to protect proteins from photoinitiated damage by scavenging reactive intermediates, such as aryl aldehydes.[11]

The utility of caged species in living animals will be dependent upon their hydrolytic stability in the dark. For example, an *o*-nitrobenzyl-caged derivative of glutamic acid displays a half-life of less than 20 h in the absence of light.[3] The corresponding coumarin-caged ester analogue of glutamic acid suffers 50% hydrolysis within 2 days.[3] These short half-lives seriously hamper the application of these caged derivatives to living organisms. By contrast, glutamic acid caged through a carbamate linkage to the coumarin shows no detectable hydrolysis after 35 h (pH 7.2 at room temperature).[3] With these features in mind, we examined the hydrolytic stability of compounds **5**−**8** and **11** in two different systems: a 1:1 mixture of CH_3OH/H_2O and a 1:1 mixture of CH_3OH/buffer (0.01 M HEPES, 0.12 M NaCl, 3 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, pH 7.4). The methanolic component of these solvent systems was required to solubilize the various acetal derivatives. In addition, the HEPES buffer system has been previously employed by others to mimic the intracellular environment.[4] We were unable to observe any hydrolysis of the 1,3-dioxolane and 1,3-dioxepane ring systems to the corresponding free diols after 2 weeks.

In summary, we have developed a strategy to cage diols using a photolabile protecting group. Surprisingly, although coumarin-caged 1,2- and 1,4-diols are rapidly photoliberated, the corresponding 1,3-derivatives appear to be completely resistant to photolysis. We note that photodeprotection of the coumain-acetals directly affords the free diol. This is in marked contrast to *o*-nitrobenzylidene acetals which, upon photodecomposition, yields hydroxy monobenzoates.[12] The strategy outlined herein should prove applicable to a wide variety of diol-substituted biologically active compounds, including species containing the ribose ring system, various steroids, and glycerol derivatives. Previous studies have demonstrated that the 7-hydroxycoumarin moiety **1** possesses a biologically useful cross-section for two photon photolysis.[3] Consequently, it may be possible to photoliberate the coumarin-caged diols described herein via multiphoton photolysis as well. These studies are currently in progress.

Experimental Section

General Methods. Analytical HPLC (monitored at 250 or 350 nm) employed Vydac C4 (250 mm × 3.0 mm) or Econosil SI (250 mm × 4.6 mm) columns: a linear gradient from 70% A (water) 30% B (methanol) to 0% A (water) 100% B (methanol) over 20 min with flow rate at 1 mL/min.

Synthesis of 6-Bromo-7-hydroxy-4-formylcoumarin (4). To a solution of 6-bromo-7-hydroxy-4-hydroxymethylcoumarin[2] (2.5 g, 9.2 mmol) in anhydrous THF (30 mL) under an Ar

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atmosphere was added manganese oxide (3.5 g, 40.2 mmol). After the suspension was stirred at room temperature overnight, the black solid was removed by filtration. The filtrate was evaporated under reduced pressure, and the resulting crude product was purified by flash chromatography on silica gel (CH_2Cl_2/CH_3OH = 18:1) to afford a yellow solid (2.2 g, 90% yield).

General Procedure for the Synthesis of 6-Bromo-7-hydroxycoumarin Acetals 5−11. Compound **4** (30 mg, 0.115 mmol) was dissolved in anhydrous toluene (5 mL) under an Ar atmosphere. After addition of a diol (1.3 equiv), pyridinium *p*-toluenesulfonate (1 equiv), and anhydrous $MgSO_4$ (100 mg), the reaction mixture was stirred at 110 °C overnight. The reaction was then diluted with EtOAc and washed with brine. The organic layer was separated and dried over $MgSO_4$. After evaporation of the solvent under reduced pressure, the crude product was purified by flash chromatography on silica gel (CH_2Cl_2/CH_3OH = 30:1).

General Procedure for Photolysis of 6-Bromo-7-hydroxycoumarin Acetals 5−11 and Quantum Yield Determinations. A 180 μL 1 mM solution of 6-bromo-7-hydroxycoumarin acetal in 1:1 CH_3OH/HEPES (0.01 M HEPES, 0.12 M NaCl, 3 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, pH 7.4) was placed in a quartz cuvette in ice. Photolysis was performed using an Oriel 200 W Hg arc lamp (model 6283) with a 348 nm UV filter (Oriel, lot no. 51260; 50% internal transmittance at 348 nm and cutoff at <325 nm) to remove short wavelength light and an IR filter (to remove heat). The spectral irradiance (at 0.5 m) of the lamp in the region of the coumarin λ_{max} (±50 nm) varies from 50 to 200 mW m^2 nm^{-1}. Aliquots of 10 μL were removed and analyzed by analytical HPLC. The photochemical quantum yields, ϕ, defined as the ratio of photolabile molecules converted to the amount of photons absorbed, were determined using 6 mM potassium ferrioxate actionmetry according to the equation $\phi = \Delta P/(It)$,[7] where t is the irradiation time. ΔP, the amount of photolabile converted, was determined using HPLC peak areas. The value of I, the light intensity, was measured by using chemical actionmetry.

Half-Life Determination for 6-Bromo-7-hydroxycoumarin Acetals. One millimolar solutions of 6-bromo-7-hydroxycoumarin acetals in 1:1 CH_3OH/HEPES (0.01 M HEPES, 0.12 M NaCl, 3 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, pH 7.4) (condition A) and in 1:1 CH_3OH/H_2O (deionized) (condition B) were placed in the dark (wrapped with aluminum foil) at room temperature. Aliquots of 10 μL were taken at various time intervals for HPLC analysis.

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Supporting Information Available: Experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

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