Preparation of a Water-Soluble "Cage" Based on 3′,5′-Dimethoxybenzoin

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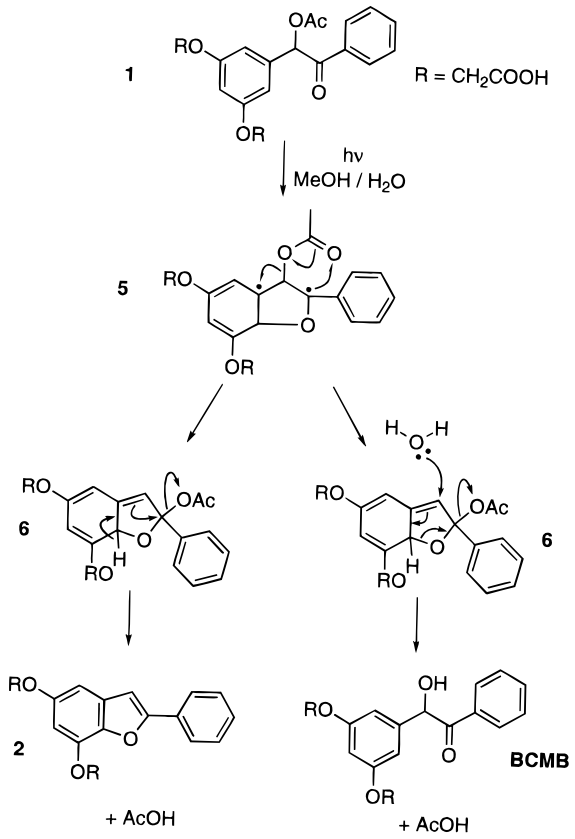
Photolabile protecting groups have been used in a wide variety of applications, including organic synthesis,[1−3] various forms of lithography,[4] and rapid manipulations of cellular chemistry.[5−9] For protection of carboxylates and phosphates, *o*-nitrobenzyl esters are the most commonly used derivatives.[5] However, several features of benzoinyl esters, particularly 3′,5′-dimethoxybenzoinyl (DMB) esters, have led to their further development.[10,11] The useful properties of DMB esters include extremely rapid photolysis, high quantum yields of conversion, and the generation of only a single inert photoproduct in addition to the free acid.[11] In contrast, *o*-nitrobenzyl esters exhibit photolysis on the millisecond time scale,[12] produce reactive nitroso photoproducts,[5] and will reduce metal centers in proteins instead of undergoing photolysis.[13] The advantages of DMB esters have led to their use as protecting groups for carboxylates,[11] phosphates,[14] alcohols,[15] and amines,[16] and in the development of lithographic synthesis,[17] muscle relaxation studies,[18] and photolabile linkers.[19] Biological applications, however, are strongly limited due to the insolubility of benzoyl caged compounds.[20] This difficulty becomes more significant upon photolysis, as the phenylbenzofuran photoproduct will precipitate out of aqueous solution even under conditions where the parent ester is soluble. Although organic cosolvents can alleviate this problem in a few cases, a more practical solution is to modify the DMB group to enhance its solubility. This approach is similar to the one used by Milburn et al. to introduce a negative charge on a nitrobenzyl caged carbamoylcholine.[21] In an analogous manner, charged functionalities have been introduced into the DMB group to produce 3′,5′-bis(carboxymethoxy)benzoin (BCMB).

Scheme 1



The BCMB protecting group was prepared in a manner similar to that previously reported for the benzoinyl linker.[19,22] Starting from 3,5-dihydroxybenzaldehyde, the hydroxyls were protected as TBDMS ethers. The benzaldehyde was condensed with phenyl dithiane lithium anion, and the TBDMS-protected hydroxyls were unmasked and alkylated with *tert*-butylbromoacetate in a single step. After hydrolysis of the dithiane, test compounds for photolysis were generated by acylation with acetic anhydride followed by hydrolysis of the 3′ and 5′ esters. The two carboxylates render both the caged acetate **1** and the resulting photoproduct **2** water-soluble in excess of 6 mM. In contrast, 5,7-methoxy-2-phenylbenzofuran, a photoproduct of DMB ester photolysis, is insoluble at micromolar concentrations.

Although photolysis of DMB phosphates has been observed under aqueous conditions,[14,18] nothing has yet been reported on the effect of water on the photolysis pathway. To clarify the photolysis of these substituted benzoinyl esters under aqueous conditions, the caged acetate **1** was examined (Scheme 1). When **1** is irradiated from 320 to 400 nm under aerobic conditions in methanol, the characteristic 300 nm band of the substituted phenylbenzofuran is observed. Under aqueous conditions, however, the same absorption band is produced, but only in about 30% overall yield (Figure 1). Overirradiation products were ruled out as an explanation for the reduced yield, as they form on a much longer time scale than that seen here. Also eliminated was the possibility that the phenylbenzofuran has a smaller extinction coefficient in water. Instead, the reduced benzofuran yield was due to the formation of a second photoproduct. Preparative scale photolysis and purification of the methanol photolysis solution

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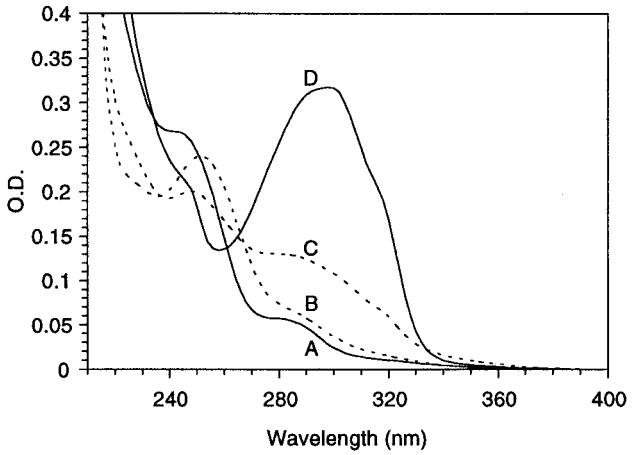


Figure 1. Steady-state photolysis of 59.6 μM *O*-acetyl-BCMB (**1**), irradiated from 320 to 400 nm using a filtered mercury vapor arc lamp, (A) in methanol, irradiated 0 s; (B) in buffer (100 mM sodium phosphate, pH 7.40), irradiated 0 s; (C) in buffer, irradiated 60 s (to completion); and (D) in methanol, irradiated 30 s (to completion).

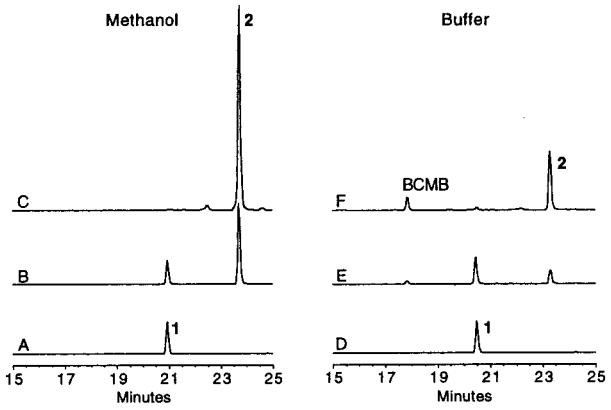
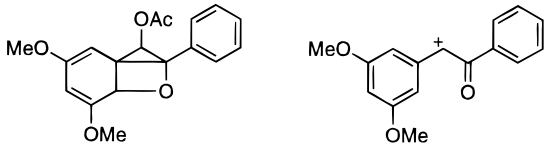


Figure 2. HPLC of **1** (59.6 μM) photolysis mixtures. Aliquots of **1** were dissolved in methanol or buffer and irradiated. Samples were analyzed by RP-HPLC after a total of 5 min in solution to prevent complications from spontaneous hydrolysis, which occurred with a half-life of ca. 24 h in the buffer solution. Irradiation time (s): (A) 0, (B) 5, (C) 75, (D) 0, (E) 5, and (F) 90. Detection at 290 nm. At this wavelength, the extinction coefficient of **2** is much larger than that of either BCMB or **1**. The yield of **2** in buffer was 29% of the yield in methanol, so approximately 70% of **1** will undergo photohydrolysis to BCMB under aqueous conditions. Products were identified by FAB-MS, and BCMB was further identified by ^{1}H NMR.

by reversed-phase HPLC revealed two components, the starting acetate **1** and the phenylbenzofuran **2**. Under aqueous photolysis conditions, an additional component was observed (Figure 2). The additional photoproduct turned out to be BCMB, as identified by both FAB-MS and ^{1}H NMR.

This particular photosolvolysis reaction has never been observed for DMB esters, perhaps for a pair of reasons. First, due to the fact that DMB acetates are not sufficiently soluble in aqueous solution, some have used organic cosolvents,[19,23] which would be expected to reduce the yield of BCMB (see below). Second, the most convenient wavelength for monitoring the progress of the reaction by chromatography is 290 nm, the ϵ_{max} of the phenylbenzofuran product, where the ϵ of **1** or BCMB is almost 15-fold lower. Thus, significant quantities of the nucleophilic trapping product can appear to be minor impurities (see Figure 2E and F). The fact that in aqueous solution the acetate is lost from **1** while cyclization is prevented in a significant fraction of the photoproduct has interesting implications for the photolysis pathway. These results show that the benzylic carbon must be accessible to nucleophilic attack by water at some point along the photolysis pathway. At first, this would seem to disfavor the oxetane intermediate **3**, postulated by Sheehan, in preference to intermediates such as the cation **4**, proposed by Givens[24,25] and others[16,26] for the photolysis of benzoinyl phosphates. However, an argument against the direct formation of



cation **4** is that such heterolytic cleavage is usually seen for π,π^* excited states of benzyl esters, not for the benzoyl n,π^* excited state that is responsible for this particular cyclization.[11] A modified version of Sheehan's original mechanism that can account for the product photochemistry from the carbonyl n,π^* state is shown in Scheme 1. Here, instead of the direct formation of the oxetane **3**, the first intermediate is the biradical **5**.[27] This biradical can undergo an acetoxy migration to generate **6**,[28] which can then rearomatize to give the benzofuran **2** or can undergo nucleophilic attack by water to produce BCMB. This pathway does not require the formation of the highly strained **3** and is, therefore, consistent with the rapid photolysis of alkoxybenzoin cages.[29]

The fact that cyclization intermediates of BCMB acetates may be trapped in a bimolecular process provides the first handle for determining the photolysis rates of methoxy substituted benzoinyl acetates. Photolysis of **1** was conducted in various ethanol/water mixtures, and the relative yield of **2** was determined. Kinetic competition analysis of the photocyclization yields provided a rough lower estimate of 5 ps for the lifetime of **6**, under the assumption that trapping by water is diffusion controlled. This lifetime is consistent with the previously described limits based on triplet quenching[11] as well as direct measurement.[18,19] It is important to note that, even though an additional photolysis pathway has been observed here, BCMB still functions as a cage, since both pathways yield the desired carboxylate.

The remarkable photolysis rate of the benzoinyl class of protecting groups makes them ideal for the phototriggering of rapid reactions. Water-soluble cages will extend this technique to biological systems that were previously inaccessible. Applications currently under development include the investigation of redox proteins using caged electron donors and dynamics studies of biopolymers.

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Supporting Information Available: Synthesis scheme for BCMB, photolysis methods, and rate data (5 pages, print/PDF). See any current masthead page for ordering information and Web access instructions.

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(28) Migration of the acetoxy group may occur to either radical center. Migration to the 2-position of the phenylbenzofuran is shown.

(29) This mechanism is also consistent with the one proposed by Shi et al.[22] In this case, hydrolysis of Shi's intermediate **3** would lead to ring opening and the formation of 3′, 5′-dimethoxybenzoin. Shi's mechanism, like the one proposed here, also proceeds from the more likely n,π^* manifold.