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Anthraquinon-2-ylmethoxycarbonyl (Aqmoc): A New Photochemically Removable Protecting Group for Alcohols

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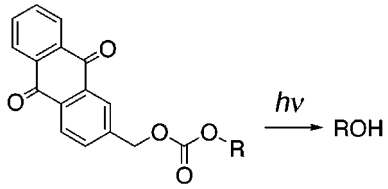
ABSTRACT



Synthesis and photochemistry of a new photochemically removable protecting group for alcohols is described. Four carbonates of galactose derivatives (1–4) were synthesized from the corresponding arylmethanols via 4-nitrophenyl carbonate intermediates. Among them, photolysis of anthraquinon-2-ylmethoxycarbonyl (Aqmoc) galactose (1) proceeded with overall photolysis efficiency of 150 (quantum yield 0.10, and molar absorptivity 1500 M^{-1} cm^{-1}) and rate constant of $\sim 10^6$ s^{-1}. To demonstrate its application to a biologically related molecule, 5′-Aqmoc-adenosine (5) was synthesized and photolyzed to yield adenosine in 91% yield.

Photochemically removable protecting groups for various functional groups have great potential in both synthetic[1] and biological chemistry. The importance of this type of protection has been growing because of recent advances in solid-phase synthesis in combinatorial chemistry[2] and the need for photochemically labile probe molecules known as caged compounds in cell biology.[3] There have been continuous efforts to exploit new photolabile protecting groups, such as benzoin-type,[4] phenacyl-type,[5] and coumarinylmethyl-type,[6] since 2-nitrobenzyl groups were introduced to make caged biologically important phosphates.[7] The functional groups to be masked appear to have mainly been phosphates or carboxylates. The photolabile hydroxy protecting groups, other than the 2-nitrobenzyl-type, that have been reported thus far are *o*-hydroxycinnamate,[8] hydroxystyrylsilyl,[9] 3,5-

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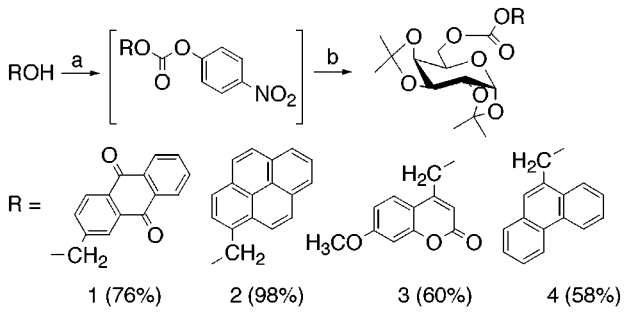
dimethoxybenzoin carbonate,[10] 2-benzoylbenzoic acid,[11] and 9-phenylxanthyl groups.[12] However, the application of these groups in caging chemistry has been somewhat limited.

In this study, we examined the synthesis and photochemistry of four photolabile protecting groups for alcohols and found that the anthraquinon-2-ylmethoxy-carbonyl (Aqmoc) group shows satisfactory and unique photoreactivity, which could be favorable for a wide range of applications.

It is well-known that appropriately functionalized arylmethyl esters of carboxylates are photolyzed to produce parent acids.[13] By a structural analogy, one can easily imagine that arylmethyl carbonates might be photolyzed to produce parent alcohols. Thus, we designed four candidates as photoremovable protecting groups for alcohols, [anthraquinon-2-ylmethoxycarbonyl (Aqmoc), 7-methoxycoumarin-4-ylmethoxy carbonyl (MCMoc), pyren-1-ylmethoxy carbonyl (Pmoc), and phenanthren-9-ylmethoxy carbonyl (Phmoc)], since anthraquinon-2-ylmethyl esters of carboxylate[14] and phosphate,[15] 7-methoxycoumarin-4-ylmethyl ester of phosphate,[6a] and pyren-1-ylmethyl esters of carboxylate[16] and phosphate[17] are known to be photolyzed.

Four carbonates of galactose derivatives were synthesized from the corresponding arylmethanols via 4-nitrophenyl carbonates (Scheme 1). Although the 4-nitrophenyl carbonate

Scheme 1. Synthesis of the Arylmethyl Carbonates (**1**−**4**)[a]



[a] (a) 4-Nitrophenylchloroformate/DMAP. (b) 1,2,3,4-di-*O*-isopropylidenegalactopyranoside/DMAP.

intermediates are stable enough to be isolated, we chose a one-pot sequential addition procedure because of the simplicity and achieved good to excellent yields to prepare the desired carbonates.

Since the goal of our project is to develop novel caged compounds having improved and unique photoreactivity that could be applied to caging chemistry, we focused our attention on the properties in aqueous solution. Thus, the resulting galactose derivatives (**1**−**4**) were subjected to photolysis in 50% THF−H_2O (100 μM) at 350 nm.

All compounds produced a parent galactose derivative[18] and photo byproducts, as indicated in Scheme 2. Several

Scheme 2. Photolysis of the Arylmethyl Carbonates

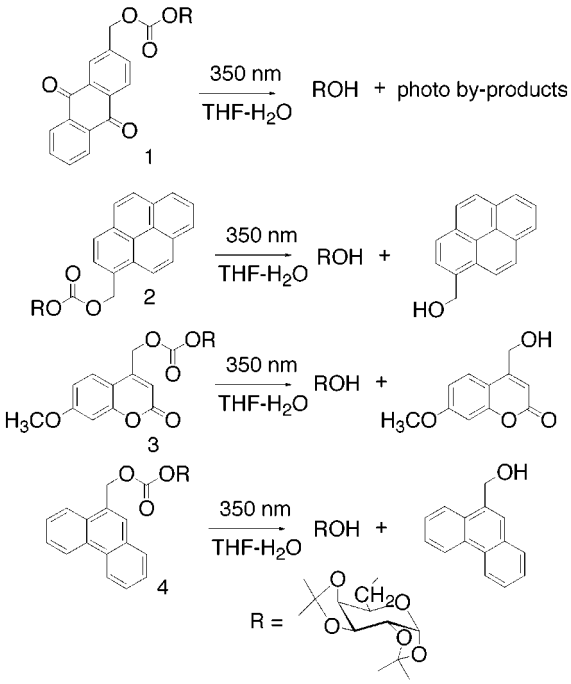


photo byproducts other than the parent alcohol were detected in the photolysis mixture of **1** in THF−H_2O. While we have not yet been able to isolate and thoroughly characterize all of the byproducts, one of the major byproducts that stems from the anthraquinone moiety was determined to be anthraquinon-2-ylmethanol tetrahydro-furanyl ether on the basis of LC-MS analysis (*m*/*z* 308).

Figure 1 shows that the time courses for the consumption of the starting materials follow single-exponential decay, suggesting that the photolysis reactions proceeded without any interference by photo byproducts.

The quantum yields for the disappearance of the starting materials were determined and are summarized in Table 1 with selected absorption data. A higher efficiency of photolysis at around 350 nm would be desirable, especially for cell biological applications, since we can reduce the light intensity in an uncaging reaction to minimize cell damage and chromophore bleaching. Among the four compounds tested in this study, the Aqmoc group showed a satisfactory high efficiency of photolysis. The $\Phi\epsilon$ value, which is a

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(18) Although we did not do any product isolation, the production of the parent galactose derivative and photo byproducts from each carbonate was checked by HPLC and thin-layer chromatography, respectively.

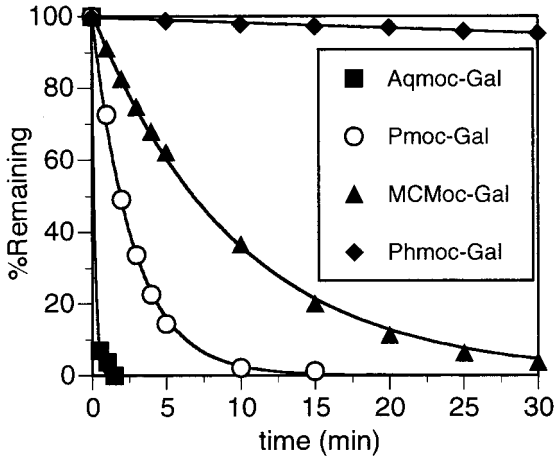


Figure 1. Time course for the consumption of the arylmethyl carbonates (**1**−**4**) in 50% THF−H_2O upon 350 nm irradiation (RPR-350 nm × 16).

product of the quantum yield and extinction coefficient, is an indication of the efficiency of photolysis: a high value reflects a high efficiency. Typical $\Phi\epsilon$ values for conventional 2-nitrobenzyl-type cages are 20−100, so a value of 150 for Aqmoc-Gal is high enough for its application in caging chemistry. Table 1 also shows the solubility in water containing 1% DMSO.

Table 1. Selected Physical and Chemical Properties

	$\lambda_{max}{}^a$ (ϵ^b)	$\epsilon_{350}{}^b$	$\Phi_{350}{}^c$	$\Phi\epsilon_{350}$	solubility^d
1	327 (4 100)	1 500	0.10	150	2×10^{-4}
2	323 (13 100)	3 400	2.9×10^{-3}	10	3×10^{-5}
3	343 (30 800)	6 200	6.7×10^{-3}	42	2×10^{-6}
4	297 (9 530)	190	8.9×10^{-4}	0.17	1×10^{-5}

[a] Absorption spectra were taken in 50% THF−H_2O. [b] Extinction coefficient (cm^{-1} M^{-1}). [c] Quantum yields for the disappearance of the starting materials upon 350 nm irradiation. [d] The molar concentration (mol dm^{-3}) of the saturated solution in deionized water containing 1% DMSO.

Next, we investigated the mechanism of the photolysis of Aqmoc-Gal and found that the photolytic consumption of the starting material was effectively quenched by *trans*-1,3-pentadiene (E_T = 247 kJ mol^{-1}) in a concentration-dependent manner, suggesting that photolysis would occur via the triplet excited state. On the basis of a Stern−Volmer analysis, the lifetime of the triplet excited state was estimated to be 21.8 ns ($k_q\tau$ = 218 M^{-1}, assuming $k_q = k_{diff} = 10^{10}$ s^{-1} M^{-1}), from which the rate constant of photolysis was calculated to be 4.6×10^6 s^{-1}.

Figure 2 shows the difference in the absorption spectrum of the Aqmoc moiety before and after photolysis. The disappearance of a relatively strong 330-nm peak, which is characteristic of the n-π^* transition of the anthraquinone chromophore, and the appearance of a weakly absorbing long-wavelength peak at around 380 nm are noteworthy. The

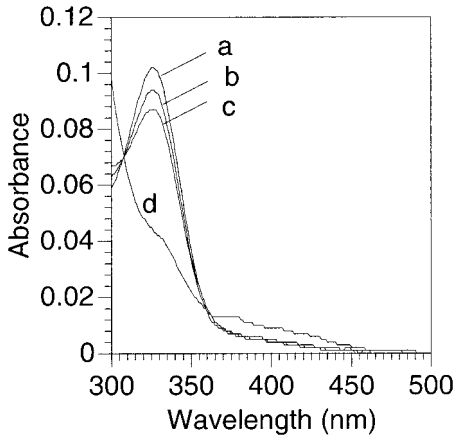
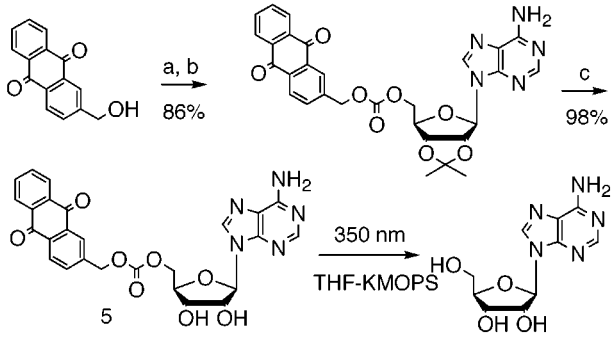


Figure 2. Absorption spectra of 2.5 μM Aqmoc-Gal (**1**) in THF−H_2O upon 350 nm irradiation (RPR-350 nm × 4): (a) 0 min; (b) 0.5 min; (c) 1 min; (d) 4 min of irradiation, respectively.

production of strongly absorbing photo byproducts often causes a problem known as a filter effect. Therefore, disappearance of the strong absorption band in the UV region concomitant with photolysis reflects an additional benefit of the Aqmoc group, especially for photolysis in thick samples, such as protein crystals and biological tissues.

The isolated yield of the deprotected alcohol from the more concentrated solution of **1** should be noted. The photolysis of 2 mM solution of **1** in 50% THF−H_2O gave the desired 1,2,3,4-di-*O*-isopropylidene-D-galactopyranose in 68% isolated yield together with bis(1,2,3,4-di-*O*-isopropylidene-D-galactopyranosyl) carbonate (16% yield), which might be the secondary product from the reaction between **1** and the deprotected alcohol. The observed isolated yield for the deprotection is not satisfactory from a synthetic viewpoint and seems to be the drawback of Aqmoc group. However, the trouble encounterd in the reaction is not so serious for our purposes, because we could minimize such secondary reactions by using more diluted solutions in caging chemistry and by attaching the substrate on a solid support in solid-phase synthetic chemistry.

Scheme 3. Synthesis and Photolysis of 5′-Aqmoc-Adenosine (**5**)[a]



[a] (a) 4-Nitrophenylchloroformate/DMAP. (b) 2′,3′-*O*-isopropylideneadenosine/DMAP. (c) TFA/THF−H_2O.

Since one important and potential application of photoremovable alcohol protecting groups is solid-phase oligonucleotide array synthesis,[19] we tested the Aqmoc group for nucleoside protection. Thus, 5′-Aqmoc-adenosine (**5**) was synthesized. Upon photolysis, free adenosine was obtained from **5** (10 μM solution in 50% THF−KMOPS buffer) in 91% yield.[20]

In conclusion, we synthesized four arylmethyl carbonate-type photolabile protecting groups and found that the Aqmoc group may be useful in caging chemistry. Although the Aqmoc group is still limited in its application to cell biology, i.e., lower photolytic efficiency without THF (data not shown), and the possible production of a reactive intermediate, the present concept of the combination of an arylmethyl group and a hydroxyl functionality as a carbonate enables us to make new photoremovable protecting groups with various photochemical properties.

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Supporting Information Available: Procedure for the preparation and photolysis and spectroscopic data for compounds **1**−**5** is provided. This material is available free of charge via the Internet at http://pubs.acs.org.

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