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Phototriggers for Nucleobases with Improved Photochemical Properties

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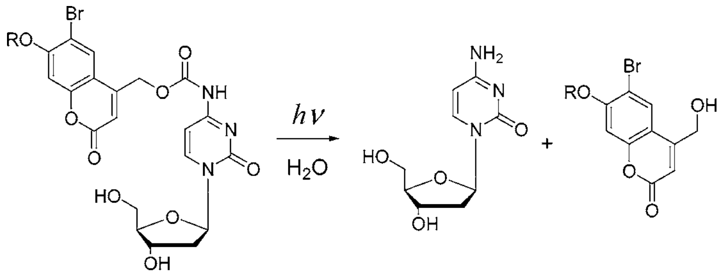
ABSTRACT



Bhcmoc-dC (R=H) $\Phi\varepsilon$ = 900 at 350 nm, δ_u = 0.35 GM at 740 nm
Bmcmoc-dC (R=CH_3) $\Phi\varepsilon$ = 1600 at 350 nm

Synthesis and photochemical properties of new photoremovable protecting groups for nucleobases are described. Four caged 2′-deoxycytidines (dCs) were synthesized, and their photochemical properties were measured under simulated physiological conditions. Two new coumarin-caged dCs show better photochemical and photophysical properties than those of the caged dCs having previously reported caging groups.

Oligonucleotides and their synthetic analogues have been recognized as tools that can control and manipulate gene functions. Antisense oligos, antigene molecules, aptamers, miRNAs, siRNAs, and non-coding RNAs are examples. A specific method for controlling function of these regulatory oligonucleotides is desirable to realize conditional activation or knockdown of genes of interest. These regulatory oligos recognize their target sequences of DNAs or RNAs and give a perturbation to their physiological functions. The process is mostly initiated by a complementary double-strand formation via base-to-base recognition.

Caged compounds are molecules designed so that their biological functions will be temporarily masked and reactivated by applying external triggers such as ultraviolet light.[1] Caged compounds of oligonucleotides would serve as new functional molecules that can be activated by applying external triggers, thereby enabling manipulation of gene expression, transcription activation, and translation inhibition with high spatio−temporal resolution.

Results of a previous study show that nucleobases,[2] internucleotide phosphates,[3] or hydroxyl groups on ribose

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(1) See, for example: Goeldner, M., Givens, R. S., Eds. *Dynamic Studies in Biology: Phototriggers, Photoswitches and Caged Biomolecules*; Wiley-VCH: New York, 2005.

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rings[4] can be modified using photolabile protecting groups. Protection of nucleobases is advantageous when the complementary double-strand formation must be controlled. The collection of photoremovable protective groups designed for preparing caged compounds has been growing,[5] but reported caging groups available for caged nucleobases are of only two types: *o*-nitrobenzyl (NB-type) and 2-(*o*-nitrophenyl)-ethyl (2-NPE-type) groups.[2]

Although the reported compounds are useful for photocontrol of DNA or RNA structures and functions, NB- and 2-NPE-type phototriggers present inherent drawbacks. Both groups have their absorption maxima at around 290 nm, which results in poor absorption of uncaging light, typically of 330−385 nm. To overcome this shortage, introducing alkoxy substituents into the phenyl ring can give a longer absorption maximum with higher molar absorptivity. The introduction of alkoxy substitutions somehow reduces the photolysis quantum yield; for that reason, the overall photolysis efficiency of the alkoxylated *o*-nitrobenzyl group is almost identical to that of the parent compounds. Furthermore, both NB- and 2-NPE-types need introduction of substituents on the carbon atom α or β to the leaving groups to achieve improved photolysis quantum yields. Consequently, the carbon atoms form an asymmetric center and produce a pair of diastereomers when the group is used to produce a caged compound of a chiral molecule. Chromatographic separation of the diastreomers is necessary to avoid the resultant complexity.[3]

The purpose of this study is to develop new caged compounds of nucleobases having improved physical and chemical properties. To achieve that goal, we investigated the utility of coumarin-type protecting groups as phototriggers for nucleobases. Among the coumarin-based caging groups reported[6] were chosen (6-bromo-7-hydroxycoumarin-4-yl)methyl (Bhc) group (R^1 = Br, R^2 = OH in Figure 1)[7] for the following reasons. First, Bhc group has its absorption maximum at around 370 nm with large molar absorptivity (ϵ_{max} = 17,000−19,000 $M^{-1}cm^{-1}$) under a simulated physiological solution (pH 7.2). Second, results of a previous study showed that the photolysis quantum yield of the Bhc group was larger than that of other coumarin cages including the (7-diethylaminocoumarin-4-yl)methyl (DEACM) group (R^1 = H, R^2 = Et_2N in Figure 1).[8a] Third, the Bhc group has no stereocenters. In addition, we have reported Bhc-caged compounds of neurotransmitters,[7] second messengers,[8] and mRNAs.[3c,d,9] All have improved photochemical properties for both one- and two-photon excitation conditions; they were used in either live cells, brain slices, or the whole body. We added Bmc group, which is the 7-methoxy analogue of the Bhc group, into an entry to see if free hydroxyl group in the Bhc group is necessary for photolysis efficiency. In addition, if we were able to add a substituent in this position without sacrificing any photolysis efficiency, the synthetic scheme would be simplified, and an additional function would be tethered.

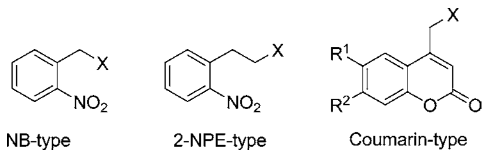

Figure 1. Phototriggers for caged compounds. X denotes a leaving group.

For this study, three types of caging groups were introduced into C-4 exocyclic amine in deoxycytidine as carbamates to give four caged dCs (Figure 2). Photochemical properties of the caged dCs were measured under the same reaction conditions, so that a direct comparison of their photolysis reactivity could be made.

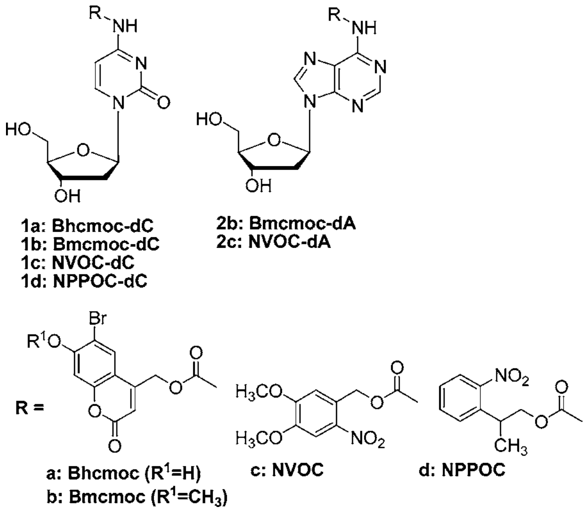

Figure 2. Structures of the caged nucleosides tested in this study.

Two coumarin-type protecting groups, (6-bromo-7-hydroxycoumarin-4-yl)methoxycarbonyl (Bhcmoc) and (6-bromo-7-methyoxycoumarin-4-yl)methoxycarbonyl (Bmcmoc), were introduced into 2′-deoxycytidine (dC) using the temporal

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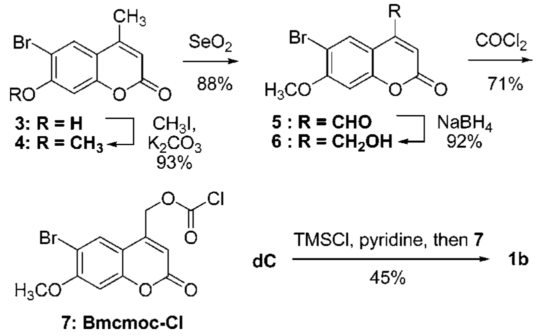
protection protocol[2a,10] via the corresponding chloroformates to yield two new caged dCs: Bhcmoc-dC (**1a**) and Bmcmoc-dC (**1b**) (Scheme 1). Similarly, 4-*N*-(6-nitrovertryloxycarbonyl)-dC, NVOC-dC (**1c**)[2a] and 4-*N*-[2-(2-nitrophenyl)-propoxy]carbonyl-dC, NPPOC-dC (**1d**) were synthesized. Since the coumarin-type caging groups have no stereogenic center, both **1a** and **1b** were obtained as a single diastereomer, whereas **1d** was obtained as a mixture of two diastereomers and was subsequently used as a mixture.

Scheme 1. Synthesis of Bmcmoc-dC **1b**



The absorption properties of the caged dCs are summarized in Table 1. The $\pi-\pi^*$ absorption bands that are responsible for the photolysis reaction lie at 370 nm for **1a** and 329 nm for **1b**, indicating that alkylation of 7-OH on coumarin ring engenders a nearly 40-nm blue-shift of the band. All caged dCs studied were photolyzed to yield the parent dC and photobyproducts upon 350-nm irradiation under a simulated physiological condition (aqueous buffer solution at pH 7.2). For example, the HPLC traces shown in Figure 3 indicate the almost quantitative production of dC and (6-bromo-7-hydroxycoumarin-4-yl)methanol as a photo-byproduct. The photolytic consumption of each of the caged dCs can be approximated by single-exponential decay (Figure 4), suggesting no undesired secondary effect that interferes with photolysis throughout the reaction (see also Figure S2 in Supporting Information). Therefore, we can calculate photolytic quantum yields from the decay curves using the equation $\Phi = 1/(I \times 10^3 \epsilon t_{90\%})$, in which I is the incident light intensity in einstein cm^{-2} s^{-1}, ϵ is the molar absorptivity of each substrate at irradiated wavelength in M^{-1} cm^{-1}, and $t_{90\%}$ is the time to reach 90% conversion.[11]

Table 1. Selected Photophysical and Chemical Properties of Caged Nucleosides

	λ_{max} (nm)	ϵ_{max} ($\times 10^4$)[a]	Φ_{350}[b]	$\Phi\epsilon_{350}$[c]	δ_{u740}[d]
1a	292, 370	1.00, 1.40	0.084	900	0.35
1b	292, 329	1.13, 0.99	0.30	1600	
1c	293, 346	1.33, 0.60	1.0×10^{-3}	6	
1d	290	1.07	0.38	150	
2b	269, 330	1.41, 0.96	0.24	1200	
2c	269, 363	1.55, 0.49	1.1×10^{-3}	5	

[a] Molar absorptivity (M^{-1} cm^{-1}). [b] Quantum yields for disappearance of starting materials upon 350-nm irradiation in KMOPS (pH 7.2) containing 0.1% DMSO. [c] Product of the photolysis quantum yield and molar absorptivity at 350 nm. [d] Two-photon uncaging action cross section at 740 nm.

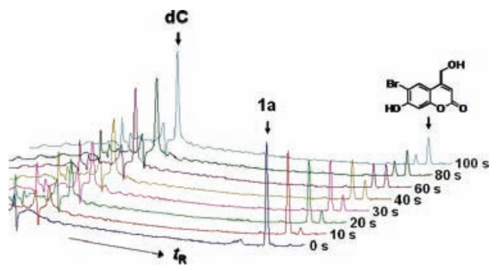


Figure 3. HPLC traces for the photolysis of **1a** (measured at 260 nm). Samples were analyzed after the specified irradiation time.

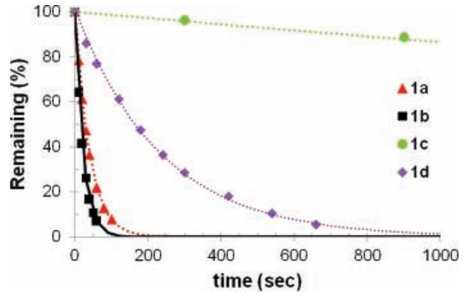


Figure 4. Time course for photolysis of the caged cytidines. Samples (10^{-5} M) were irradiated at 350 nm (10 mJ/s) under simulated physiological conditions. **1a** (red, ▲), **1b** (black, ■), **1c** (green, ●), **1d** (purple, ◆).

Because the samples were irradiated continuously with constant light intensity, the decay curves reflect the amount of photolyzed caged cytosines during the specified irradiation time. The faster decay curves represent higher photolysis efficiency. Interestingly, the photolysis quantum yield of **1b** is 4 times greater than that of **1a**; consequently the overall photolysis efficiency $\Phi\epsilon_{350}$, the product of photolysis quantum yield and molar absorptivity at the irradiated wavelength (350 nm in this experiment) of **1b** is 2× as large as that of **1a** (Table 1). Although the reason for the difference in quantum yields between **1a** and **1b** remains unclear, similar enhancement was observed for (7-methoxycoumarin-4-yl)-methyl (MCM) (R^1 = H, R^2 = OCH_3 in Figure 1) ester of cAMP (MCM-cAMP); $\Phi = 0.12$ for MCM-cAMP and 0.062 for its 7-hydroxyl counterpart (HCM-cAMP).[12] The $\Phi\epsilon$ values of the coumarin-caged dCs (**1a** and **1b**) were markedly

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larger than those of other caged dCs (**1c** and **1d**) at 350 nm, which is advantageous for cell biological applications because cell damage caused by UV irradiation must be diminished.

Although wavelengths of 350 nm and longer are desirable as an uncaging light source, it would be fair to make a comparison to other wavelength ranges. All compounds show two absorption maxima: one at around 290 nm and one at a longer wavelength (except for **1d**). The shorter one at 290 nm corresponds to the cytosine base; therefore, the longer ones absorbed by the protective groups must be responsible for the photolysis reaction. Assuming that photolysis quantum yields are independent of the irradiated wavelength as long as the same absorption bands are excited, we can estimate action spectra of uncaging efficiency $\Phi\epsilon$ at the longer absorption bands (Figure 5). The photoreactivity of **1b** is far better than that of **1c** or **1d** throughout the wavelength ranges depicted in Figure 5. For wavelengths of 360 nm and longer, the $\Phi\epsilon$ values of **1a** became larger than those of **1b**. For example, the calculated $\Phi\epsilon$ values at 400 nm are 450 for **1a**, 30 for **1b**, 1 for **1c**, and 0 for **1d**. The values showed good agreement with observed photolysis reactivity at 400 nm: 19% conversion for **1a** (10^{-5} M in KMOPS), 1% for **1b**, and no detectable conversion for **1c** and **1d** after 90-s irradiation. From the treatment described here, we can estimate the photoreactivity of the compounds at the given wavelength to make a quantitative comparison.

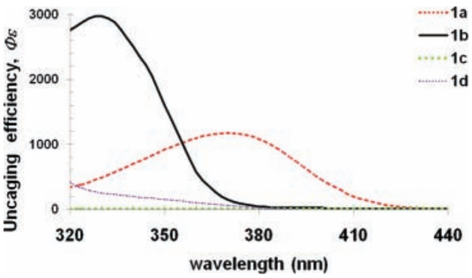

Figure 5. Calculated uncaging action spectra of the caged cytidines.

Next, two-photon uncaging action cross sections (δ_u) of **1a** were measured as 0.35 GM (Goeppert−Meyer, 1 GM = 10^{-50} cm^4 s photon^{-1}) at 740 nm, 0.19 GM at 760 nm, 0.18 GM at 780 nm, and 0.16 GM at 800 nm. The values measured here are similar to those of other Bhc-caged compounds reported by our group,[7,8b] and are sufficiently high to be useful for cell biological applications.

Finally, we tested whether the observed advantages of the coumarin cage in photochemical properties hold for purinergic nucleosides. Therefore, we synthesized caged adenosines Bmcmoc-dA (**2b**) and NVOC-dA (**2c**).[2a] The overall photolysis efficiency of **2b** summarized in Table 1 was as large as that of **1b** and more than 200 times larger than that of **2c** for 350-nm irradiation.

Results of this study demonstrate that photolysis quantum yields of caged cytidines were determined under the same reaction condition, which enables us to make quantitative comparisons of their photochemical properties. The photolytic efficiency of coumarin-caged nucleosides having Bhcmoc and Bmcmoc groups on their nucleobases was higher than those of NB- and 2-NPE-type caged compounds, meaning that we can reduce the uncaging light intensity with the coumarin-caged nucleosides to minimize cell damage caused by UV irradiation. The Bhcmoc-caged nucleosides can be uncaged under a two-photon excitation condition with practically high uncaging action cross section. In addition, the coumarin-type caging groups have no stereo centers. Consequently, when chiral molecules are caged, complexity arising from diastereomer formation is avoided. Data reported here should provide clues to facilitate selection of appropriate caged compounds of nucleobases.

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Supporting Information Available: Procedures for the preparation and photolysis and spectroscopic data for compounds **1a**−**d**, **2b**, and **2c** are provided. This material is available free of charge via the Internet at http://pubs.acs.org.

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