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Utilization of the Heavy Atom Effect for the Development of a Photosensitive 8-Azacoumarin-Type Photolabile Protecting Group

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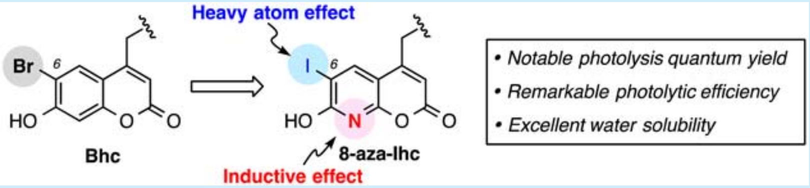
Supporting Information



ABSTRACT: A remarkable improvement of the photochemical properties of coumarin-type photolabile protecting groups was achieved by iodine substitution. The newly identified 7-hydroxy-6-iodo-8-azacoumarin (8-aza-Ihc)-caged acetate showed excellent photolytic efficiency, significantly higher than that of the corresponding bromine-containing coumarin- and azacoumarin-type caging groups. The results provide a solid approach to improving the photosensitivity of photolabile protecting groups.

Photolabile protecting groups (PPGs),[1] whose removal is performed by photoirradiation alone, are attractive protecting groups because their deprotection is generally very fast and can proceed even in aqueous media. PPGs have been used in various fields such as organic synthesis[2] and cell biology.[3] They are suitable for the spatiotemporal dynamic analysis of biologically active molecules by "caging" a functional group important for bioactivity. The inherent bioactivity of the molecules in physiological processes can be controlled by photoinduced deprotection (uncaging) of the PPG.[4] For clear analysis of the dynamics of bioactive molecules in living organisms, photoirradiation times should be as short as possible because light causes potentially harmful damage to cells or tissues.

The photolytic efficiency of PPGs is calculated as the product of the molar absorptivity (ε) and the photolysis quantum yield (Φ_{chem}) and allows a quantitative estimate of the overall efficiency of a photolysis reaction. There are, in general, two approaches to improving the photolytic efficiency. The first is to enhance the molar absorptivity at longer wavelengths ($\lambda >$ 350 nm) as shown with 8-cyano-7-hydroxyquinoline (CyHQ)[5] and nitrodibenzofuran (NDBF).[6] The second is to increase the photolysis quantum yield (Φ_{chem}) as shown in the case of 2-hydroxymethylene-8-dimethylaminoquinoline (8-DMAQ),[7] 4-carboxymethoxy-5,7-dinitroindoline (CDNI),[8] and 6-bromo-7-hydroxy-coumarin-4-ylmethyl (Bhc).[9] However, the lack of adequate models and general rules that predict structure-related photolytic efficiency have hampered the development of caging groups with improved photophysical and photochemical profiles. In this context, the strategy in the case of Bhc to improve the photolysis quantum yield is rational. This takes advantage of the heavy atom effect by introducing a bromine atom that can promote the rate of intersystem crossing ($S_1 \rightarrow T_1$) through spin−orbital coupling and suppress fluorescence emissions.[10] While there are several PPGs containing bromine,[11] to our knowledge, the application of an iodine atom, the heaviest of the halogens, has not been reported. However, the iodine modification has the potential to considerably improve the photolysis quantum yield of PPGs.

In this paper, we report the development of a novel 8-azacoumarin-type PPG with significant photolytic efficiency by introducing an iodine atom in anticipation of the heavy atom effect. Compared to 8-aza-6-bromo-7-hydroxycoumarin-4-yl-methyl acetate (8-aza-Bhc-CH_2OAc), the newly synthesized 8-aza-7-hydroxy-6-iodocoumarin-4-yl-methyl acetate (8-aza-Ihc-CH_2OAc) produces a dramatically faster photoreaction when irradiated at 365 nm and has distinguishing photochemical properties: the photolytic efficiency ($\varepsilon \cdot \Phi_{\text{chem}}$) of the 8-aza-Ihc-$CH_2OAc$ represents a 370% increase over that of the 8-aza-Bhc derivative.

We previously developed the azacoumarin chromophore, which was designed by the isosteric replacement of the enol double bond of Bhc with a polar and hydrophilic amide bond to increase the hydrophilicity.[12] With its use of highly hydrophilic PPGs, this is a promising chromophore for caging chemistry. In the Bhc group reported by Furuta et al.,[9] a bromine substituent was introduced to improve the photolysis quantum yield by the

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promotion of intersystem crossing,[13] and also to lower the pK_a value of the hydroxyl group at the C7-position by the inductive effect. This contributes to improvement of the quantum yield because the deprotonated form has more favorable photophysical and photochemical properties. Although the introduction of a bromine atom to C6 of the 8-azacoumarin chromophore also supports the increase in the photolytic efficiency by lowering the pK_a value of the chromophore, the photolytic efficiency of the 8-aza-Bhc group was slightly lower than that of the parent Bhc group. However, the 8-azacoumarin derivatives have lower pK_a values than the corresponding Bhc group (**1**) even without additional electron-withdrawing groups such as the bromine atom: the pK_a of 8-aza-7-hydroxycoumarin (8-aza-hc, **2**) is 5.67, lower than that of Bhc (**1**), which is 5.88 (Figure 1). These findings prompted us to investigate the introduction of a heavier iodine substituent in the 8-azacoumarin chromophore, it being thought that this would enable an improvement in the photochemical properties by more efficiently promoting intersystem crossing. Accordingly, we synthesized the 8-aza-Ihc-caged acetate (**3**) to evaluate its hydrophilic, photophysical, and photochemical properties in comparison with those of the corresponding Bhc- and 8-aza-Bhc-caged derivatives.

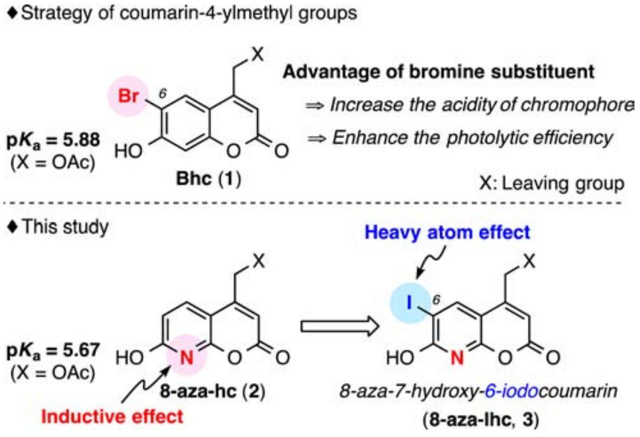
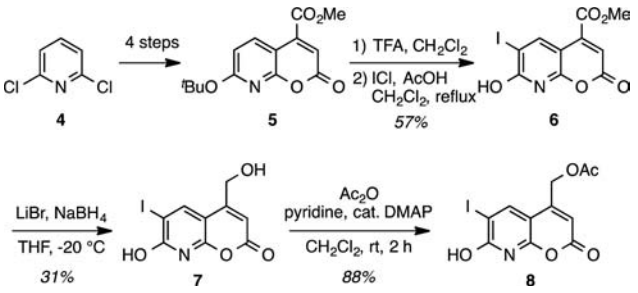


Figure 1. Design of a 6-iodinated 8-azacoumarin derivative **3**.

An 8-aza-Ihc-CH_2OAc (**8**) with an iodine atom at the C6-position was synthesized through the 8-azacoumarin derivative (**5**) with a methyl ester group at the C4-position (Scheme 1), which was prepared in four steps from 2,6-dichloropyridine (**4**) as described previously.[12] Iodination of **5** after the removal of the *tert*-butyl protecting group was performed with iodine monochloride in the presence of acetic acid to give the desired 6-iodinated 8-azacoumarin derivative (**6**) as the major product in 57% yield, accompanied by a 38% yield of a non-iodinated 8-azacoumarin lacking the *tert*-butyl protecting group, as a minor product. Subsequently, chemoselective reduction of the methyl ester group using $LiBH_4$ prepared *in situ*, followed by acetylation of the alcohol, produced the desired 8-aza-Ihc-CH_2OAc (**8**).

Scheme 1. Synthesis of 8-aza-Ihc-CH2OAc 8

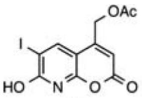


The hydrophilic properties of PPGs are critical to the utility of caged molecules, since the insolubility in water of caged molecules would induce irregular aggregation, leading to the decrease in the photolytic efficiency.[14] Thus, the aqueous solubility of 8-aza-Ihc-CH_2OAc (**8**) was evaluated by measuring the concentration (C_s) of a saturated solution in phosphate-buffered saline (PBS), and the pK_a value was determined using citric/phosphate buffer in the pH range 2.6–7.0 because the saturated concentration is correlated with the pK_a value of the chromophore (Table 1). The pK_a value of **8** was shown to be 4.49, which is lower than those of **9** and **11** but higher than that of 8-aza-Bhc-CH_2OAc (**10**).[12] As predicted, the C_s value of **8** was lower than that of the corresponding 6-brominated azacoumarin derivative (8-aza-Bhc-CH_2OAc, **10**), but the C_s value of **8** was higher than those of the reference compounds Bhc-CH_2OAc (**9**) and 8-aza-3-Bhc-CH_2OAc (**11**), possibly due to its lower pK_a value. We assumed that the low pK_a value of 8-aza-Ihc-CH_2OAc (**8**) stems from the inductive effects not only of the nitrogen atom but also of the iodine atom. The inductive effects could be correlated with the difference of electronegativity between iodine and bromine. These results indicated that 8-aza-Ihc-CH_2OAc (**8**) can be present in a deprotonated form under physiological conditions and has the potential to facilitate the photoreactions of high concentrations of the caged molecules.

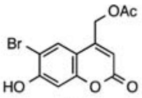
Table 1. Hydrophilic Properties of an Iodoazacoumarin Derivative (8), a Coumarin Derivative (9), and Azacoumarin Derivatives (10, 11)

compd	C_s[a] (μM)	pK_a
8	5014	4.49
9	602[b]	5.88[b]
10	10 832[b]	4.22[b]
11	3260[b]	5.07[b]

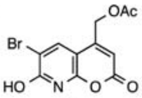
[a] Saturated concentration in PBS. [b] Values were derived from previous reports.[12,15]



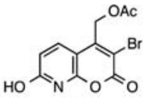
8



9
(Bhc-CH_2OAc)



10
(8-aza-Bhc-CH_2OAc)



11
(8-aza-3-Bhc-CH_2OAc)

The photosensitivity of 8-aza-Ihc-CH_2OAc (**8**) was evaluated by subjecting it to irradiation at 365 nm (0.743 mJ/cm^2 s) under quasi-physiological conditions (5 μM KMOPS buffer: 10 mM 4-morpholinepropane-1-sulfonic acid (MOPS), and 100 mM KCl, containing 0.1% DMSO: pH 7.14). HPLC analyses of the photolysis reaction of 8-aza-Ihc-CH_2OAc (**8**) showed that the peak corresponding to **8** disappeared with increasing irradiation time and, in contrast, the peak of 8-aza-Ihc-CH_2OH (**7**) developed at the same time (Figure 2). The HPLC analyses indicated that the photolysis of **8** proceeded quantitatively without generation of other byproducts such as the deiodinated compound.[16] The time courses of the photolysis of compounds **8**–**11** are shown in Figure 3, which tend toward a single-exponential decay as a trendline. The time to reach 90%

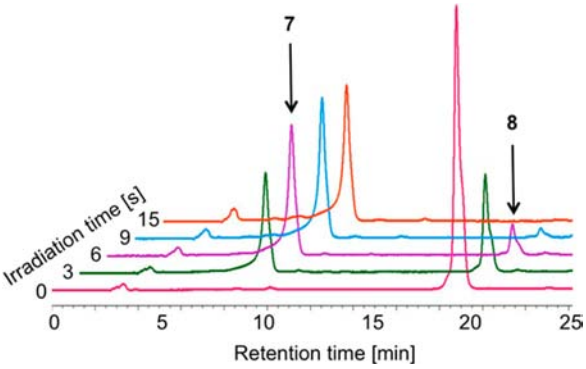


Figure 2. HPLC analyses for the photolysis reaction of **8** in KMOPS buffer (pH 7.14, containing 0.1% DMSO). Conditions: 50 mL of 5 mM sample irradiated at 365 nm for an arbitrary time was injected onto a reversed-phase HPLC; gradient: 0 min, 15% CH_3CN−85% H_2O (containing 0.1% TFA); 20 min, 35% CH_3CN−65% H_2O (containing 0.1% TFA).

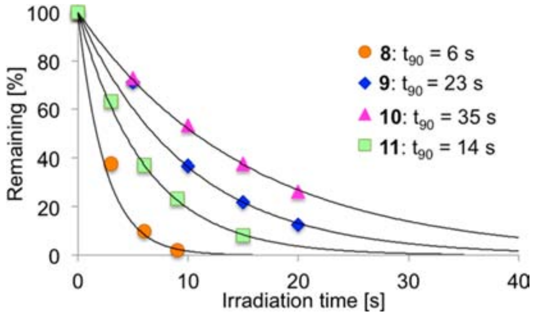


Figure 3. Time courses of the photolysis reaction of **8**−**11**.

conversion (t_{90}) in the photolysis of **8** is only 6 s, which is much faster than the photolysis of the 6-brominated coumarin and azacoumarin derivatives **9** and **10** (t_{90} = 23 s for **9** and 35 s for **10**). 8-Aza-Ihc-CH_2OAc (**8**) was photolyzed twice as efficiently at 365 nm as 8-aza-3-Bhc-CH_2OAc (**11**). In addition, the assessment of sensitivity to spontaneous hydrolysis under dark conditions showed satisfactory results: the hydrolysis of **8** in KMOPS buffer at room temperature was assessed as <5% over 12 h (Figure 4), indicating that 8-aza-Ihc-caged ester can be sufficiently stable in the physiological experiments. Whereas 8-aza-3-Bhc-CH_2OAc (**11**) was sensitive not only to the photolysis reaction but also to hydrolysis in PBS (pH 7.4, $t_{1/2}$ = 31 h), 8-aza-Ihc-CH_2OAc (**8**) was sensitive only to the photolysis reaction and maintained high hydrolytic stability in the dark ($t_{1/2}$ = 97 h in PBS). Thus, the C6-iodination of the azacoumarin chromophore is superior to the C3-bromination with respect not only to the photosensitivity but also to the hydrolytic stability in the dark.

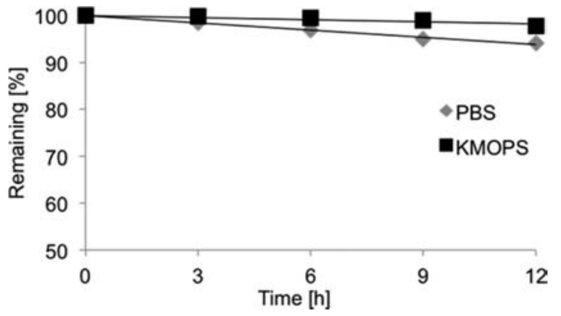


Figure 4. Evaluation of stability of **8** in the dark, in buffer (PBS: pH 7.4, KMOPS: pH 7.14).

Table 2. Photophysical and Photochemical Properties of Compounds 8, 9, 10, 11, *o*NB, and *p*HP

compd	λ_{max}[c] (nm)	ε_{365}[d] (M^{-1} cm^{-1})	t_{90}[e] (s)	Φ_{chem}[f]	$\varepsilon_{365}\cdot\Phi_{chem}$[g]
8	365	22 731	6	0.52	11 820
9	370	17 753	23	0.17	2942
10	362	22 070	35	0.11	2406
11	378	23 828	14	0.20	4766
***o*NB**[a]	265	228	3213	0.13	26
***p*HP**[b]	329	1408	1055	0.058	82

[a] *o*-Nitrobenzyl-OAc. [b] *p*-Hydroxyphenacyl-OAc. [c] Wavelength of maximum absorption. [d] Molar absorptivity at 365 nm. [e] Time to reach 90% conversion. [f] Quantum yield for photoreaction irradiated at 365 nm. [g] Photolytic efficiency.

Table 2 shows the photophysical and photochemical properties of all test compounds. In comparative experiments, the same photolysis reaction was carried out with two typical PPGs, *o*-nitrobenzyl (*o*NB)- and *p*-hydroxyphenacyl (*p*HP)-caged acetates, because the absolute values of the photochemical properties are different in each experimental system. The wavelength of the maximum absorption of 8-aza-Ihc-CH_2OAc (**8**) is 365 nm, which is comparable to that of 8-aza-Bhc-CH_2OAc (**10**) but slightly shifted to a shorter wavelength from that of 8-aza-3-Bhc-CH_2OAc (**11**) (378 nm). This blue shift is due to the destabilization of both the HOMO and LUMO energy levels and results from changing the position of the electron-withdrawing group from the C3 to C6.[17] The molar absorptivity of **8** at 365 nm was 22731 M^{-1} cm^{-1}, which is high, but comparable to that of **10** (ε_{365} = 22 070 M^{-1} cm^{-1}).

The photolytic efficiencies of the synthesized compounds are also reported in Table 2. In general, the value of $\varepsilon\cdot\Phi_{chem}$ should be over 100 if caged molecules are to have practical utility in biological applications. The photolysis quantum yield is calculated from the equation $\Phi_{chem} = 1/(I \times 10^3 \varepsilon t_{90})$ reported by Tsien,[18] where I is radiation intensity determined by actinometry with potassium ferrioxalate[19] and t_{90} is the time to reach 90% conversion. The value of t_{90} is determined by a single-exponential decay, which is obtained by a photolysis reaction. Notably, the photolysis quantum yield of 8-aza-Ihc-CH_2OAc (**8**) was 0.52, which was the highest value among the reported coumarin-4-ylmethyl-type PPGs. The value of the product $\varepsilon_{365}\cdot\Phi_{chem}$ is 11 820, which is well in excess of the practical standard value for caged molecules and is 2.5- to 5.0-fold higher than those with the coumarin platform and 144- to 455-fold higher than those of the other typical PPGs. Furthermore, the fluorescence quantum yield of **8** competing with the photolysis reaction is found to be 0.15, which is smaller than that for **9** (0.22), **10** (0.29), and **11** (0.25).[17] These results suggest that the energy for emitting fluorescence shifts can cause photolysis. Thus, the observed improvement of the photolytic efficiency of **8** can be explained by the iodine-derived heavy atom effect which promotes the rate of intersystem crossing. This in turn suggests that the photochemical cleavage of the azacoumarin-type PPGs could occur via the triplet state, similar to the photolysis reaction of other coumarinylmethyl types.[9a,20] Further study would be necessary however to reveal the photochemical mechanism of azacoumarin chromophores in detail.

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In summary, we have succeeded in improving the photolytic efficiency of 8-azacoumarin-type photolabile protecting groups by introducing an iodine atom to the C6-position of the 8-azacoumarin chromophore. The newly identified 8-aza-Ihc-CH$_2$OAc (**8**) has sufficient aqueous solubility and excellent photolytic efficiency ($\varepsilon_{365}\cdot\Phi_{\text{chem}}$ = 11 820), which is notably higher than those of the corresponding 6-brominated cumarin- and azacoumarin-type PPGs and the other typical PPGs. This is thought to be a consequence of the iodine-derived heavy atom effect. To our knowledge, this is the first case in which a substituent iodine atom has been used to improve the photolytic efficiency of PPGs. These results indicate that, in PPGs, 6-iodinated 8-azacoumarins will have minimal impact on cells or tissues because some photodamage is abated by shortening the photoirradiation time upon uncaging and that the 8-azacoumarin chromophore might proceed via a triplet state, although clear evidence for this has not been obtained to date. Ongoing studies are attempting to elucidate whether the photoreaction mechanism proceeds through a triplet state and to evaluate the two-photon sensitivity of the 8-azacoumarin-type PPGs with NIR/IR light.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.5b02720.

> Experimental procedures, synthesis and characterization date for new compound (PDF)

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Notes

The authors declare no competing financial interest.

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(16) An uncharacterized compound having a molecular weight corresponding to dimerization of 8-aza-Ihc-CH$_2$OH. See Supporting Information for details.

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