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# Isostere-Based Design of 8-Azacoumarin-Type Photolabile Protecting Groups: A Hydrophilicity-Increasing Strategy for Coumarin-4-ylmethyls

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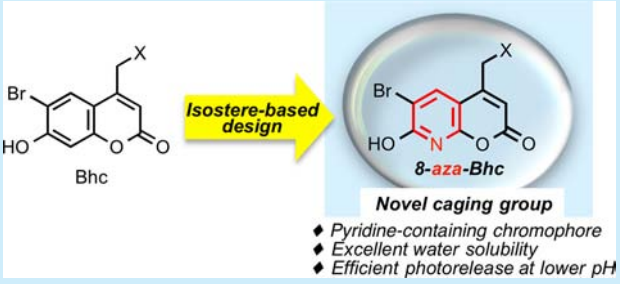
Supporting Information

**ABSTRACT:** Described is the development of 8-azacoumarin-4-ylmethyl groups as aqueous photolabile protecting groups. A key feature of the strategy is the isosteric replacement of the C7−C8 enol double bond of the Bhc derivative with an amide bond, resulting in conversion of the chromophore from coumarin to 8-azacoumarin. This strategy makes dramatically enhanced water solubility and facile photocleavage possible.




Chemical processes mediated by photolabile protecting groups find numerous utilities in synthetic organic chemistry,[1] chemical biology, and cell biology.[2] The exceptional utilities of photolabile protecting groups include their mild conditions associated with the photocleavage that can proceed smoothly and quickly even in aqueous conditions and their potential as photoactivatable molecules or caged compounds that enable spatial and temporal control of their biological functions.[3] Among various caging groups,[4−7] coumarins have had widespread applications to caging chemistry in recent years. In particular, the potential of two-photon photolysis with practically useful absorption cross sections (720−900 nm) is among the outstanding advantages of coumarin types such as the 6-bromo-7-hydroxycoumarin-4-ylmethyl (Bhc) group.[7d] However, one of the drawbacks of coumarin types is their low aqueous solubility. Aqueous solubility is critical for the utility of caged compounds, since hydrophobic caged compounds will be aggregated in physiological conditions and the photocleavage would be plagued by sluggish reactivity.[7h]

There are a few methods for increasing the aqueous solubility of coumarin chromophores. One successful example is the introduction of one or more hydrophilic carboxyl groups such as BCMACM,[8] BBHCM,[9] and DEAC450.[10] Although these approaches effectively achieve the increase of hydrophilicity of coumarin chromophores, the development of new strategies for increasing the hydrophilicity with high photosensitivity remains challenging.

In this report, we disclose a simple and powerful strategy based on the concept of the amide-alkene isosterism for increasing the hydrophilicity of coumarin-type photolabile protecting groups (Figure 1), leading to the development of novel 8-azacoumarin-type protecting groups. The newly designed 8-azacoumarin-type protecting groups have approximately 10- to 18-fold enhanced solubility in aqueous buffer compared to that of the parent Bhc group and possess photophysical and photochemical properties favorable for caging chemistry. Our presented strategy has the potential to provide new solutions for the development of caged compounds with enhanced hydrophilicity.

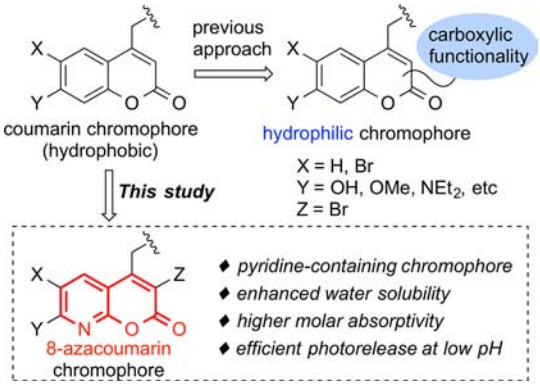



**Figure 1.** Strategies to increase the hydrophilicity of the coumarin chromophore.

Our studies started from the design of novel chromophores with enhanced hydrophilicity (Figure 2). Our approach to increasing hydrophilicity is based on the introduction of polar and hydrophilic amide functionality into the coumarin chromophore. As shown in Figure 2, the C7−C8 enol double

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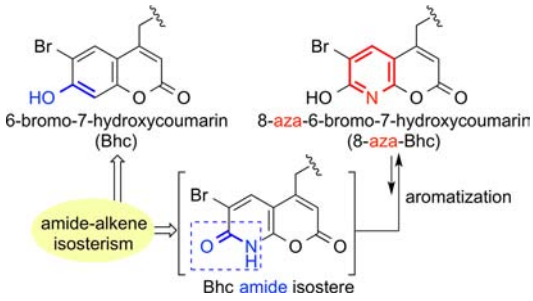



**Figure 2.** Isostere-based design of an 8-azacoumarin-type photolabile protecting group.

bond of the coumarin chromophore was replaced with an amide bond followed by aromatization of the lactam moiety to form a hydroxypyridine-containing 8-azacoumarin chromophore. This strategy relies on the concept of structural isosterism of amides and alkenes (enols), familiar in medicinal chemistry[11] and exemplified by alkene-type dipeptide isosteres,[12] which are regarded as ideal ground state mimetics of dipeptides and thus have been applied to many biologically active peptides.[13]

Our synthesis of 8-azacoumarins with a hydroxylmethyl group at the C4 position began with 2,6-dichloropyridine **3** (Scheme 1). Successive treatments of **3** with potassium *tert*-butoxide and sodium benzyl alkoxide followed by hydrogenolysis of the benzyl group afforded the hydroxypyridine derivative **4** in an excellent yield (91% in 3 steps). Reaction of **4** with dimethyl acetylenedicarboxylate (DMAD) in the presence of $PPh_3$ provided the desired 8-azacoumarin **5** with an ester functionality at the C4 position in 65% yield.[14] Chemoselective reduction was required for the conversion of this conjugated ester of **5** to the corresponding alcohol, since the lactone moiety of 8-azacoumarin is subject to cleavage by reducing agents. Screening of various reducing agents revealed that the use of $LiBH_4$ prepared *in situ* in THF at −20 °C afforded acceptable conversion giving the desired 8-azacoumarin derivative **6** with a hydroxylmethyl group at the C4 position, in 45% isolated yield. Compound **6** was subjected to acetylation of the alcohol followed by TFA treatment to give the desired 4-acetoxymethyl-8-aza-hc (8-aza-hc-$CH_2OAc$, **7**). Furthermore, in order to study the substituent (heavy atom) effects[7d] on the 8-azacoumarin chromophore, the 6-brominated derivative (8-aza-Bhc-$CH_2OAc$, **9**) was also synthesized in a similar manner (Scheme 1b).

**Scheme 1. Synthesis of Azacoumarin Derivatives 7 and 9**

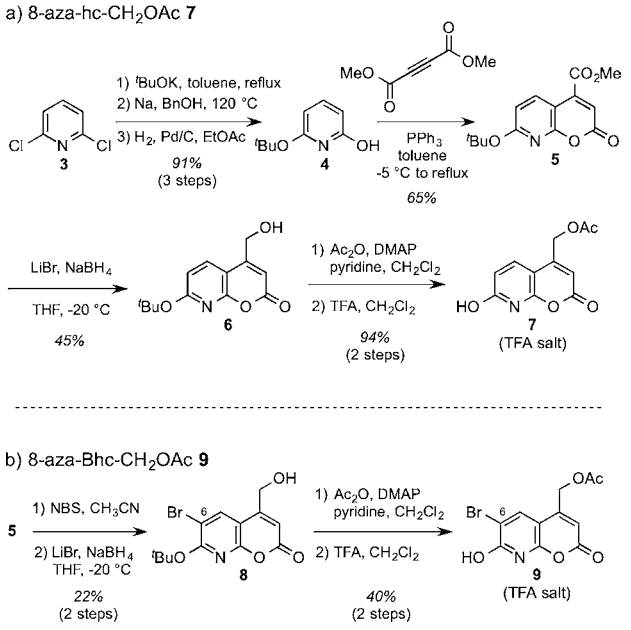
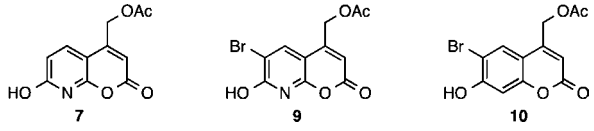



**Table 1. Hydrophilic Properties of 8-Azacoumarins 7 and 9 and Bhc Derivative 10**




| compd | $C_s$[a] ($\mu$M) | p$K_a$[b] |
|---|---|---|
| 7 | 6611 | 5.67 |
| 9 | 10832 | 4.22 |
| 10 | 602 | 5.88[c] |

[a]Concentration at saturation in PBS buffer (0.1% DMSO). [b]Determined using citric/phosphate buffer in the pH range 2.6−7.0. [c]Literature value = 6.2 in $H_2O$.[15]

The key underlying concept of our approach is the introduction of an amide functionality to the coumarin chromophore to increase the aqueous solubility. The aqueous solubility of 8-azacoumarin derivatives **7** and **9** in PBS (0.1% DMSO) was evaluated with the parent Bhc derivative **10** (Table 1). As expected, 8-azacoumarin derivatives showed hydrophilicity much higher than that of **10**; in particular, the saturated concentration ($C_s$) of 8-aza-Bhc-$CH_2OAc$ **9** was approximately 18-fold greater than that of **10**. These results indicate that the replacement of the chromophore involving the conversion of the coumarin into 8-azacoumarin enabled the enhancement of hydrophilicity of the coumarin chromophore. It is noteworthy that 8-aza-Bhc-$CH_2OAc$ **9** showed hydrophilicity higher than that of the nonbrominated compound **7** possibly due to the lower p$K_a$ value (4.22) of the chromophore. In addition, HPLC monitoring of **9** in PBS at room temperature showed that **9** was highly resistant to spontaneous hydrolysis in the dark and that only 2% of **9** was hydrolyzed in 12 h.[16]

The photochemical properties of azacoumarin derivatives **7**, **9**, and **10** in aqueous solutions were examined. 8-Aza-hc-$CH_2OAc$ **7** was subjected to photolysis in 5 $\mu$M KMOPS buffer (10 mM MOPS; 4-morpholinepropane-1-sulfonic acid, and 100 mM KCl) solution at pH 7.2 at 350 nm. Figure 3 shows the time courses of photolysis reactions of synthetic compounds in terms of the consumption of the starting materials and indicates that the photolytic reaction of **7** follows a single-exponential decay with the time to reach 50% conversion ($t_{50}$) for photolysis of **7** at 29 s. As expected, introduction of the bromo group resulted in the remarkable increase of the photochemical reactivity; the value of $t_{50}$ for photolysis of **9** is 13 s, which is slightly longer than that of **10** (9 s) but is about 2.3 times shorter than that of **7**. Although the photolytic mechanism of 8-azacoumarin derivatives **7** and **9** is not fully understood at this stage, these observations suggest the possibility of 8-azacoumarin-based chromophores working as photolabile protecting groups.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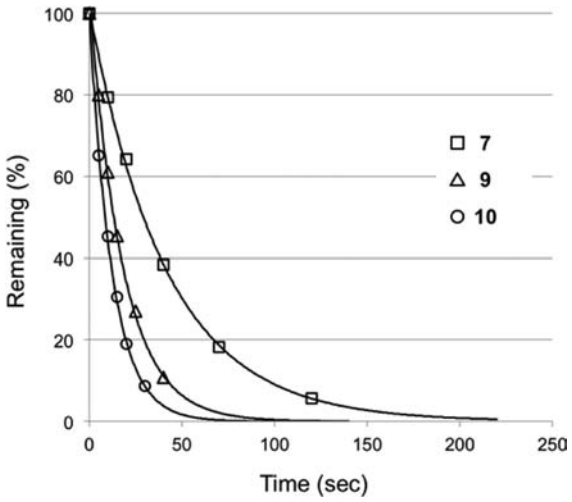


**Figure 3.** Time courses of photolysis reactions of **7**, **9**, and **10**. Samples were subjected to photolysis in 5 $\mu$M KMOPS buffer solution at pH 7.2 at 350 nm (10 mJ/s). All data are the mean values for at least two independent experiments.

Photophysical and photochemical properties of the 8-azacoumarin derivatives **7** and **9** and the Bhc derivative **10** are shown in Table 2. The absorption maxima shifted slightly to shorter wavelength, from 370 nm for **10** to 356 and 362 nm for **7** and **9**, respectively, indicating that like the Bhc group azacoumarin-based protecting groups can be cleaved under uncaging light conditions (330−385 nm). The molar absorptivities at 350 nm of **7** ($\varepsilon$ = 20175 $M^{-1}$ $cm^{-1}$) and **9** ($\varepsilon$ = 20583 $M^{-1}$ $cm^{-1}$) are higher than that of **10**. The photolysis quantum yields for disappearance of starting materials were calculated from the single decay curves using the equation $\Phi = 1/(I \times 10^3 \varepsilon t_{90})$ as reported by Tsien.[17] The quantum yields of disappearance were determined as 0.026 for **7** and 0.059 for **9**, respectively, which are 2−5 times lower than that of **10** (0.13) possibly due to the relatively strong fluorescence of the 8-azacoumarin chromophore.[18] An important factor in the development of new photolabile protecting groups is photolysis efficiency. The photolysis efficiency of caged compounds is evaluated with the product of the photolysis quantum yield ($\Phi_{chem}$) and molar absorptivity ($\varepsilon$) and allows quantitative comparison of the overall efficiency of a photolysis reaction.[19] The $\varepsilon_{350}\cdot\Phi_{chem}$ values of **7** and **9** are 526 and 1211, respectively, and that of **10** is 1806. The observed $\varepsilon_{350}\cdot\Phi_{chem}$ values of 8-azacoumarin derivatives **7** and **9** were sufficiently high to support practical applications to caging chemistry. Taking into account the excellent aqueous solubility of the 8-azacoumarin chromophore, 8-azacoumarin-based photolabile protecting groups promise to be useful for caging chemistry.

**Table 2. Selected Photophysical and Photochemical Properties of Compounds 7, 9, and 10**

| compd | $\lambda_{max}$ [a] (nm) | $\varepsilon_{max}$ [b] ($M^{-1}$ $cm^{-1}$) | $\varepsilon_{350}$ [c] ($M^{-1}$ $cm^{-1}$) | $\Phi_{chem}$ [d] | $\varepsilon_{350}\cdot\Phi_{chem}$ [e] |
|---|---|---|---|---|---|
| 7 | 356 | 20799 | 20175 | 0.026 | 526 |
| 9 | 362 | 23520 | 20583 | 0.059 | 1211 |
| 10 | 370 | 18071 | 13774 | 0.13 | 1806 |

[a]Long-wavelength absorption maxima. [b]Molar absorptivity at the absorption maxima. [c]Molar absorptivity at 350 nm. [d]Quantum yields for the disappearance of starting materials upon irradiation at 350 nm. [e]Product of the photolysis quantum yield and molar absorptivity.

In conclusion, we have designed and performed a simple and robust strategy for increasing the hydrophilicity of coumarin chromophores based on the concept of structural isosterism of amides and enols. Replacement of the C7−C8 enol double bond of the Bhc group with a polar and hydrophilic amide bond led to the development of novel 8-azacoumarin-type protecting groups, which can be removed photolytically and which showed markedly enhanced hydrophilicity and high photolysis efficiency supporting applications to caging chemistry. These studies provide the basis for future work including the development of novel hydrophilic molecules, to which it is difficult by standard approaches to introduce additional hydrophilic functionalities. Current efforts are aimed at expanding this strategy to other caging groups and functional molecules, which will offer highly effective methods for spatial and temporal control of biological activities.

## ■ ASSOCIATED CONTENT

### Supporting Information

Experimental detail, synthesis, and characterization data. This material is available free of charge via the Internet at http://pubs.acs.org.

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### Author Contributions

[#]T.N and H.T. contributed equally to this work.


### Notes

The authors declare no competing financial interest.

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## ■ REFERENCES

(1) (a) Pillai, V. N. R. *Synthesis* **1980**, 1−26. (b) Binkley, R. W.; Flechtner, T. W. In *Synthetic Organic Photochemistry*; Horspool, W. M., Ed.; Plenum: New York, 1984; pp 375−423. (c) Pillai, V. N. R. *Organic Photochemistry*; Padwa, A., Ed.; Marcel Dekker: New York, 1987; Vol. *9*, pp 225−323. (d) Bochet, C. G. *J. Chem. Soc., Perkin Trans. 1* **2002**, 125−142. (e) Bochet, C. G.; Blanc, A. In *CRC Handbook of Organic Photochemistry and Photobiology*; Griesbeck, A., Oelgemöller, M., Ghetti, F., Eds.; CRC: Boca Raton, 2012; Vol. *1*, pp 73−93. (f) Klán, P.; Šolomek, T.; Bochet, C. G.; Blanc, A.; Givens, R.; Rubina, M.; Popik, V.; Kostikov, A.; Wirz, J. *Chem. Rev.* **2013**, *113*, 119−191.

(2) (a) Adams, S. R.; Tsien, R. Y. *Annu. Rev. Physiol.* **1993**, *55*, 755−784. (b) Curley, K.; Lawrence, D. S. *Pharmacol. Ther.* **1999**, *82*, 347−354. (c) Dorman, G.; Prestwich, G. D. *Trends Biotechnol.* **2000**, *18*,

64−77. (d) Shigeri, Y.; Tatsu, Y.; Yumoto, N. *Pharmacol. Ther.* **2001**, *91*, 85−92.
(3) (a) Caged Compounds. In *Methods in Enzymology*; Marriot, G., Ed.; Academic Press: New York, 1998; Vol. *291*. (b) Mayer, G.; Heckel, A. *Angew. Chem., Int. Ed.* **2006**, *45*, 4900−4921. (c) Ellis-Davies, G. C. R. *Nat. Methods* **2007**, *4*, 619−628. (d) Lee, H. M.; Larson, D. R.; Lawrence, D. S. *ACS Chem. Biol.* **2009**, *4*, 409−427.
(4) Nitrobenzyl-type: (a) Engels, J.; Schlaeger, E.-J. *J. Med. Chem.* **1977**, *20*, 907−911. (b) Kaplan, J. H.; Forbush, B., III; Hoffman, J. F. *Biochemistry* **1978**, *17*, 1929−1935.
(5) Benzoin-type: (a) Sheehan, J. C.; Wilson, R. M. *J. Am. Chem. Soc.* **1964**, *86*, 5277−5281. (b) Givens, R. S.; Athey, P. S.; Kueper, L. W., III; Matuszewski, B.; Xue, J. *J. Am. Chem. Soc.* **1992**, *114*, 8708−8710. (c) Hansen, K. C.; Rock, R. S.; Larsen, R. W.; Chan, S. I. *J. Am. Chem. Soc.* **2000**, *122*, 11567−11568.
(6) Phenacyl-type: (a) Sheehan, J. C.; Umezawa, K. *J. Org. Chem.* **1973**, *38*, 3771−3774. (b) Givens, R. S.; Weber, J. F. W.; Jung, A. H.; Park, C.-H. *Methods Enzymol.* **1998**, *291*, 1−29. (c) Conrad, P. G., II; Givens, R. S.; Weber, J. F. W.; Kandler, K. *Org. Lett.* **2000**, *2*, 1545−1547.
(7) Coumarin-type: (a) Givens, R. S.; Matuszewski, B. *J. Am. Chem. Soc.* **1984**, *106*, 6860−6861. (b) Furuta, T.; Torigai, H.; Sugimoto, M.; Iwamura, M. *J. Org. Chem.* **1995**, *60*, 3953−3956. (c) Furuta, T.; Iwamura, M. *Methods Enzymol.* **1998**, *291*, 50−63. (d) Furuta, T.; Wang, S. S. H.; Dantzker, J. L.; Dore, T. M.; Bybee, W. J.; Callaway, E. M.; Denk, W.; Tsien, R. Y. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 1193−1200. (e) Hagen, V.; Bendig, J.; Fring, S.; Eckardt, T.; Helm, S.; Reuter, D.; Kaupp, U. B. *Angew. Chem., Int. Ed.* **2001**, *40*, 1045−1048. (f) Eckardt, T.; Hagen, V.; Schade, B.; Schmidt, R.; Schweitzer, C.; Bendig, J. *J. Org. Chem.* **2002**, *67*, 703−710. (g) Geisler, D.; Kresse, W.; Wiesner, B.; Bendig, J.; Kettenmann, H.; Hagen, V. *ChemBioChem* **2003**, *4*, 162−170. (h) Nomura, W.; Narumi, T.; Ohashi, N.; Serizawa, Y.; Lewin, N. E.; Blumberg, P. M.; Furuta, T.; Tamamura, H. *ChemBioChem* **2011**, *12*, 535−539.
(8) Hagen, V.; Dekowski, B.; Nache, V.; Schmidt, R.; Geissler, D.; Lorenz, D.; Eichhorst, J.; Keller, S.; Kaneko, H.; Benndorf, K.; Wiesner, B. *Angew. Chem., Int. Ed.* **2005**, *44*, 7887−7891.
(9) Hagen, V.; Kilic, F.; Schaal, J.; Dekowski, B.; Schmidt, V. R.; Kotzur, N. *J. Org. Chem.* **2010**, *75*, 2790−2797.
(10) Olson, J. P.; Kwon, H.-B.; Takasaki, K. T.; Chiu, C. Q.; Higley, M. J.; Sabatini, B. L.; Ellis-Davies, G. C. R. *J. Am. Chem. Soc.* **2013**, *135*, 5954−5957.
(11) (a) Kim, B. H.; Venkatsean, N. *Curr. Med Chem.* **2002**, *9*, 2243−2270. (b) Meanwell, N. A. *J. Med. Chem.* **2011**, *54*, 2529−2591.
(12) For some examples of unsubstituted alkene dipeptide isosteres, see: (a) Jenkins, C. L.; Vasbinder, M. M.; Miller, S. J.; Raines, R. T. *Org. Lett.* **2005**, *7*, 2619−2622. (d) Wiktelius, D.; Luthman, K. *Org. Biomol. Chem.* **2007**, *5*, 603−605. (e) Badur, N. G.; Harms, K.; Koert, U. *Synlett* **2007**, *1*, 99−102 and references therein.
(13) (a) Namanja, A.; Wang, X. J.; Xu, B.; Mercedes-Camacho, A. Y.; Wilson, B. D.; Wilson, K. A.; Etzkorn, F. A.; Peng, J. W. *J. Am. Chem. Soc.* **2010**, *132*, 5607−5609. (b) Tamamura, H.; Hiramatsu, K.; Ueda, S.; Wang, Z.; Kusano, S.; Terakubo, S.; Trent, J. O.; Peiper, S. C.; Yamamoto, N.; Nakashima, H.; Otaka, A.; Fujii, N. *J. Med. Chem.* **2005**, *48*, 380−391. (c) Narumi, T.; Hayashi, R.; Tomita, K.; Kobayashi, K.; Tanahara, N.; Ohno, H.; Naio, T.; Kodama, E.; Matsuoka, M.; Oishi, S.; Fujii, N. *Org. Biomol. Chem.* **2010**, *8*, 616−621 and references therein.
(14) Yavari, I.; Adib, M.; Hojabri, L. *Tetrahedron* **2002**, *58*, 6895−6899.
(15) Furuta, T.; Takeuchi, H.; Isozaki, M.; Takahashi, Y.; Kanehara, M.; Sugimoto, M.; Watanabe, T.; Noguchi, K.; Dore, T. M.; Kurahashi, T.; Iwamura, M.; Tsien, R. Y. *ChemBioChem* **2004**, *5*, 1119−1128.
(16) HPLC monitoring of the hydrolysis stability in PBS of azacoumarins revealed that compound **7** was more resistant than the parent Bhc **10**. See Supporting Information for details.
(17) Tsien, R. Y.; Zuker, R. S. *Biophys. J.* **1986**, *50*, 843−853.
(18) The quantum yields of fluorescence were determined as 0.39 for **7** and 0.29 for **9**, respectively.
(19) Brown, E. B.; Shear, J. B.; Adams, S. R.; Tsien, R. Y.; Webb, W. W. *Biophys. J.* **1999**, *76*, 489−499.