ORGANIC LETTERS

2012
Vol. 14, No. 2
572–575

Styryl Conjugated Coumarin Caged Alcohol: Efficient Photorelease by Either One-Photon Long Wavelength or Two-Photon NIR Excitation

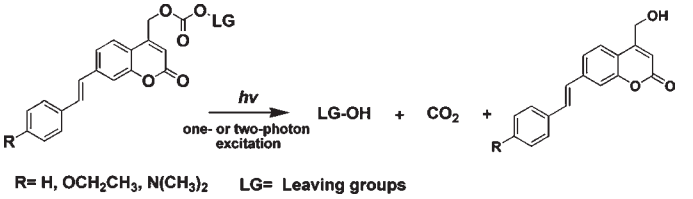
Chunyan Bao,[†] Guanshui Fan,[†] Qiuning Lin,[†] Bo Li,[‡] Shuiyu Cheng,[†] Qi Huang,[†] and Linyong Zhu*,[†]

Shanghai Key Laboratory of Functional Materials Chemistry, Institute of Fine Chemicals, East China University of Science and Technology, Shanghai, 200237, P. R. China, and Key Laboratory for Polar Materials and Devices, Ministry of Education, East China Normal University, Shanghai, 200241, P. R. China

Linyongzhu@ecust.edu.cn

Received November 29, 2011

ABSTRACT



The synthesis and photorelease properties of a new phototrigger for alcohols are described. Compared to ester 4 caged by the reported [7-(diethylamino)coumarin-4-yl]methoxycarbonyl (DEACM) phototrigger, the caged ester 3 shows an efficient single-photon photolysis efficiency upon irradiation of long wavelength light (λ = 475 nm) and a stronger two-photon photolysis sensitivity with 800 nm laser light. Its promising properties and the efficient photorelease of adenosine make it very useful as a caging group for biological applications.

Phototriggers (or caging groups) for a variety of functional groups have numerous applications in biotechnology and cell biology.[1−3] In contrast to most other protecting strategies, the removal of this category only requires light and allows a very mild activation of sensitive molecules, which permits functional molecules to evolve to intracellular distribution with high special and temporal precision. To date, most phototriggers need ultraviolet (UV) light to achieve cleavage, which is a major drawback because the presence of UV light is damaging to cells and it also provides poor penetration due to light scattering and absorbance by intrinsic biological chromophores.[4] To promote the application in biological areas, two approaches are effective in designing biologically suitable phototriggers: one method is to create new phototriggers that can absorb at longer wavelengths,[5] or modify the existing phototriggers by different substitutions.[6] However this approach always encounters difficulties in synthesizing and elongating the conjugation of the phototriggers without adversely affecting the bond cleavage rates and selectivity. Another method is to use two-photon excitation with near-infrared (NIR) light that is more suitable for biomedical application because of the minimization of cell damage while maintaining higher spatial resolution. However, to the best of our

[†] East China University of Science and Technology.
[‡] East China Normal University.

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

(2) (a) Mayer, G.; Heckel, A. *Angew. Chem., Int. Ed.* **2006**, *45*, 4900–4921. (b) Ellis-Davis, G. C. R. *Nat. Methods* **2007**, *4*, 619–628.

(3) Rai, P.; Mallide, S.; Zheng, X.; Rahmanzadeh, R.; Mir, Y.; Elrington, S.; Khurshid, A.; Hasan, T. *Adv. Drug Delivery Rev.* **2010**, *62*, 1094–1124.

(4) Schwarz, A.; Ständer, S.; Berneburg, M.; Böhm, M.; Kulms, D.; Steeg, H.; Grosse-Heitmeyer, K.; Krutmann, J.; Schwarz, T. *Nat. Cell Biol.* **2002**, *4*, 26–31.

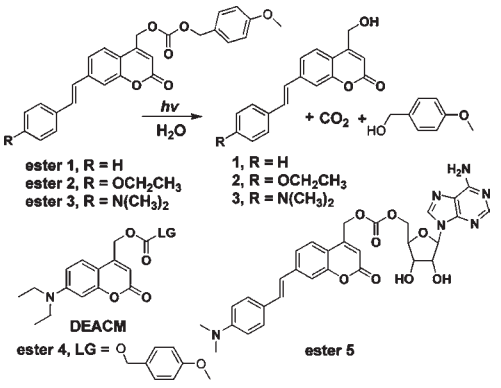
(5) (a) Chen, Y.; Steinmetz, M. G. *Org. Lett.* **2005**, *7*, 3729–3732. (b) Tucker, M. J.; Courter, J. R.; Chen, J.; Atasoylu, O.; Smith, O. B., III; Hochstrasser, R. M. *Angew. Chem., Int. Ed.* **2010**, *49*, 3612–3616.

(6) Aujard, I.; Benbrahim, C.; Gouget, M.; Ruel, O.; Baudin, J.-B.; Neveu, P.; Jullien, L. *Chem.—Eur. J.* **2006**, *12*, 6865–687.

10.1021/ol203188h
Published on Web 12/27/2011

knowledge, most phototriggers' sensitivity to two-photon excitation is low due to inefficient two-photon uncaging cross-section δ_u.[6,7] Thus, development of new phototriggers that are sensitive to long wavelength or NIR light appears to be an essential step toward biological applications.

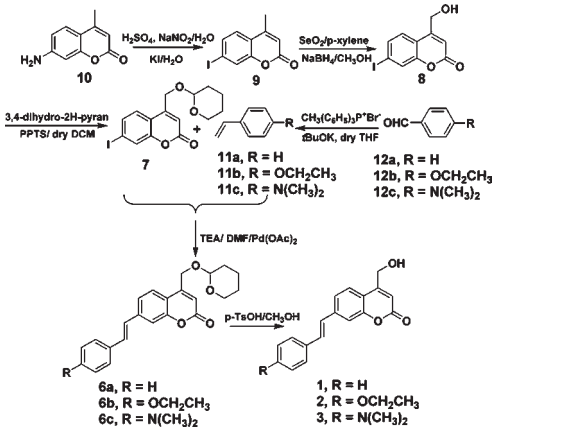
Scheme 1. Structures of Esters **1**–**5** and the Photolysis Mechanism for Esters



Among the developed phototriggers (such as 2-nitrobenzyl,[6,8] benzoin,[9] 7-nitroindoline,[10] phenacyls[11]), coumarin-based phototriggers arouse more interest and have been applied to cage carboxylates, phosphates, amines, and also alcohols compounds.[12–14] Compared with the most popular 4,5-dimethoxy 2-nitrobenzyl phototriggers with absorbance normally located at ca. 350 nm and δ_u reaching only ca. 0.01 GM within the 700–750 nm range,[7b,15] coumarin-based phototriggers have longer wavelength absorption and larger δ_u.[12c,13c,14b] The mechanism for the photorelease has been extensively investigated; they are members of arylalkyl-type photoremovable protecting groups, and the photolysis upon cleavage of a C–O bond produces a leaving group and a solvent-trapped coumarin as a photo-byproduct (as shown in Scheme 1).[13c] Recently, we reported a substituted 7-amino coumarin as a phototrigger for a controlled drug release system and anticancer therapy.[12a] This paper illustrates development of a coumarin platform to design new phototriggers with efficient photorelease by either one-photon long wavelength or two-photon NIR excitation.

During the process, para-substituted styryl was introduced to the 7-position of coumarin-based phototriggers, with the expectation that the electron-donating substitution and elongated π-conjugation could make the absorbance of the phototriggers red shift and enlarge their molar absorption coefficient. Beyond the anticipated red shift, the introduction of electron-donating conjugated chromophores (D-π-A backbone formation) is expected to exhibit an improved cross section for two-photon absorption (TPA) δ_a (Scheme 1).[16] Thus, the caged model compounds (esters **1**–**3**) were synthesized by the protecting of 4-methoxy -benzyl alcohol. The corresponding photorelease properties were examined and compared to those of ester **4** from the known [7-(diethylamino)coumarin-4-yl]methoxycarbonyl (DEACM) phototrigger.[12b] The photophysical examination shows that **3** provides prospective long wavelength absorption with a maximum at 407 nm (ca. 30 nm red shift) and large TPA cross section with 309 GM (increased

Scheme 2. Synthesis of Photosensitive Protecting Groups **1**–**3**



(7) (a) Neveu, P.; Aujard, I.; Benbrahim, C.; Saux, T. L.; Allemand, J.; Vriz, S.; Bensimon, D.; Jullien, L. *Angew. Chem., Int. Ed.* **2008**, *47*, 3744–3746. (b) 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. (c) Buckup, T.; South, A.; Kim, H.-C.; Hampp, N.; Motzkus, M. *J. Photochem. Photobiol. A: Chem.* **2010**, *210*, 188–192.

(8) (a) Ibsen, S.; Zahavy, E.; Wrasdilo, W.; Berns, M.; Chan, M.; Esener, S. *Pharm. Res.* **2010**, *27*, 1848–1860. (b) Álvarez, M.; Best, A.; Unger, A.; Alonso, J. M.; Campo, A.; Schmelzeisen, M.; Koynov, K.; Kreiter, M. *Adv. Funct. Mater.* **2010**, *20*, 4265–4272. (c) Choi, S. K.; Thomas, T. P.; Mthta, C. B.; Baker, J. R., Jr. *Chem. Commun.* **2010**, *46*, 2632–2634.

(9) McCoy, C. P.; Rooney, C.; Edwards, C. R.; Jones, D. S.; Gorman, S. P. *J. Am. Chem. Soc.* **2007**, *129*, 9572–9573.

(10) Papageorgiou, G.; Ogden, D. C.; Barth, A.; Corrie, J. E. T. *J. Am. Chem. Soc.* **1999**, *121*, 6503–6504.

(11) (a) Givens, R. S.; Jung, A.; Park, C.-H.; Weber, J.; Bartlett, W. *J. Am. Chem. Soc.* **1997**, *119*, 8369–8370. (b) Orth, R.; Sieber, S. A. *J. Org. Chem.* **2009**, *74*, 8476–8479.

(12) (a) Lin, Q.; Huang, Q.; Li, C.; Bao, C.; Liu, Z.; Li, F.; Zhu, L. *J. Am. Chem. Soc.* **2010**, *132*, 10645–10647. (b) Babinm, J.; Pelletier, M.; Lepage, M.; Allard, J.; Morris, D.; Zhao, Y. *Angew. Chem., Int. Ed.* **2009**, *48*, 3329–3332. (c) Lu, M.; Fedoryak, O. D.; Moister, B. R.; Dore, T. M. *Org. Lett.* **2003**, *5*, 2119–2122.

(13) (a) Hagen, V.; Kilic, F.; Schaal, J.; Dekowski, B.; Schmidt, R.; Kotzur, N. *J. Org. Chem.* **2010**, *75*, 2790–2797. (b) Hagen, V.; Dekowski, B.; Kotzur, N.; Lechler, R.; Wiesner, B.; Briand, B.; Beyermann, M. *Chem.—Eur. J.* **2008**, *14*, 1621–1627. (c) 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.

(14) (a) Mizukami, S.; Hosoda, M.; Satake, T.; Okada, S.; Hori, Y.; Furuta, T.; Kikuchi, K. *J. Am. Chem. Soc.* **2010**, *132*, 9524–9525. (b) Furuta, T.; Watanabe, T.; Tanabe, S.; Sakyo, J.; Matsuba, C. *Org. Lett.* **2007**, *9*, 4717–4720. (c) Nomura, W.; Narumi, T.; Ohashi, N.; Serizawa, Y.; Lewin, N. E.; Blumberg, P. M.; Furuta, T.; Tamamura, H. *ChemBioChem* **2011**, *12*, 535–539.

(15) Brown, E. B.; Shear, J. B.; Adams, S. R.; Tsien, R. Y.; Webb, W. W. *Biophys. J.* **1999**, *76*, 489–499.

(16) Gug, S.; Bolze, F.; Specht, A.; Bourgogne, C.; Goeldner, M.; Nicoud, J. *Angew. Chem., Int. Ed.* **2008**, *47*, 9525–9529.

ca. 150 times). In the end, the controlled photochemical release of bioactive adenosine caged by the phototrigger **3** was successfully performed, which validated the potential application in biological systems.

Esters **1**–**4** were prepared from compounds **1**–**3** and DEACM alcohol respectively via two steps: first, the alcohols were activated by coupling to 4-nitrophenyl chloroformate in the presence of DMAP (4-(dimethylamino)-pyridine),[7b] and then the intermediate products were allowed to react with 4-methoxybenzyl alcohol to yield final esters. Compounds **1**–**3**, the photosensitive protective groups, were prepared from commercially available 7-amino-4-methyl coumarin (**10**) in five steps by (1) diazotation with $NaNO_2$ followed by nucleophilic substitution with iodide to obtain **9**;[17] (2) oxidation by SeO_2 and then reduction by $NaBH_4$ to yield **8**; (3) protection of benzyl hydroxyl by 3,4-dihydro-2*H*-pyran;[18] (4) Heck coupling reaction with corresponding styrenes; and (5) deprotection of benzyl hydroxyl to obtain compounds **1**–**3** (Scheme 2).[18]

The absorption spectra of the esters are showed in Figure 1. Ester **3** shows the biggest red shift of maximum absorption, and its absorption band extends up to 500 nm, which suggests that the donating power of the substituted

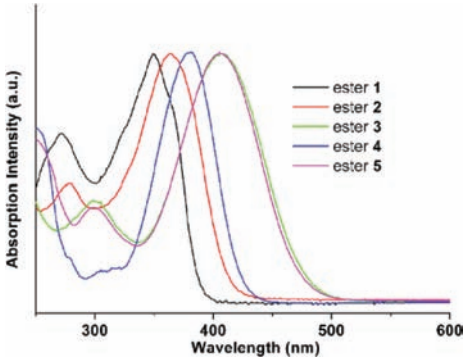


Figure 1. Comparison of UV–vis spectra for esters **1**–**5**.

groups has a great effect on the absorption of the phototriggers. Compared to that of ester **4**, the above results confirm our design intention that the introduction of electron-donating substitution and elongated conjugation will make the absorbance of the phototriggers red-shift as well as enlarge their molar absorption coefficient.

To demonstrate these new designed phototriggers with improved photochemical properties, the photolysis experiments were examined. First, the hydrolytic stability of these esters under simulated physiological conditions were checked, which shows that all of the remaining rates of the esters under dark conditions are higher than 93.5%. It indicates that these phototriggers are stable enough for biological application. Second, the photolysis of ester **3** was selected to be performed to compare with ester **4** under the same photolysis conditions (as shown in Table 1). Although the photolysis quantum yield of ester **3** is smaller than that of ester **4**, the overall photolysis efficiency $\Phi_{chem}\varepsilon$, the photolysis quantum yield and molar absorptivity, exhibits the benefit along with the red shift of the irradiation wavelength for ester **3** (more than three times larger than that of ester **4** at 430 nm). Ester **3** also shows efficient photolysis even upon 475 nm irradiation. The concrete photolysis process was also performed and analyzed by HPLC. As shown in Figure 2, ester **3** photodecomposes gradually with

Table 1. Selected Photophysical and Chemical Properties of Esters **1**–**5**[a]

ester	λ_{max} (nm)	ε_{max} ($\times 10^{-4}$)[b]	Φ_{chem} ($\times 10^{3}$)[c]	$\Phi_{chem}\varepsilon$	δ_{800nm} (GM)
1	349	5.77	—	—	45.4[f]
2	366	2.75	—	—	85.5[f]
3	407	2.88	0.83	8.28[d]	309.5[f]
				2.46[e]	0.26[g]
4	377	1.24	45.0	2.29[d]	2.3[f]
				—[e]	0.12[g]
5	407	2.97	0.79	8.18[d]	303.5[f]
				2.36[e]	0.24[g]

[a] All one-photon experiments were done in CH_3CN:H_2O (9:1) solution. [b] Molar absorptivity at maximum absorption wavelength (cm^{-1} M^{-1}). [c] Photolysis quantum yields for consumption of esters upon irradiation of 420 nm light. [d] Photolysis quantum yield and molar absorption at 430 nm. [e] Photolysis quantum yield and molar absorption at 475 nm. [f] TPA (δ_a) cross section at 800 nm (0.60 mJ·cm^{-2} per pulse). [g] Two-photon uncaging (δ_u) cross section at 800 nm (0.60 mJ·cm^{-2} per pulse).

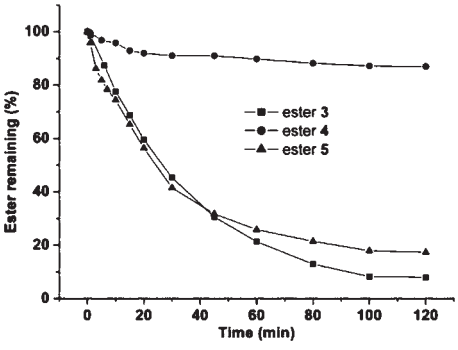


Figure 2. Time course of single photon photolysis of esters **3**, **4**, and **5** at 475 nm (28 mW/cm^2). Samples (10^{-4} M) were irradiated in CH_3CN/H_2O (9:1) solution.

the elongation of irradiation time to release 4-methoxybenzyl alcohol. The photolysis curve for ester **3** shows approximate single-exponential decay, suggesting no undesired secondary effect that interferes with photolysis throughout the reaction.[14b] However, for ester **4**, there is almost no

(17) Mao, W.; Wang, T.; Zeng, H.; Wang, Z.; Chen, J.; Shen, J. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 4570–4573.
(18) Blay, G.; Hernández-Olmos, V.; Pedro, J. *Org. Lett.* **2010**, *12*, 3058–3061.

photolysis due to nonirradiation wavelength excitation at 475 nm. The remarkable photolysis upon irradiation of long wavelength light for ester **3** exhibits an advantage especially for some biological systems that are hardly sensitive to UV light. The incomplete photolysis for the ester **3** may be attributed to an internal filtering effect from the photo-byproduct (compound **3**).

Two-photon uncaging cross sections (δ_u) of the esters were measured using a fs-pulsed Ti:sapphire laser at 800 nm with fluorescein as an external standard.[19] As summarized in Table 1, ester **3** shows notably large δ_a at 800 nm and is ~150 times higher than that of ester **4** (~309 GM, the largest for phototriggers as known), which confirms the introduction of a D-π-A backbone exhibits improved a cross section for TPA. The progress of photolysis was

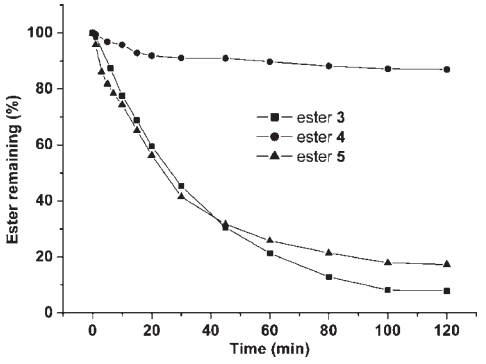


Figure 3. Time course of two-photon photolysis of esters **3**, **4**, and **5** at 800 nm (0.60 mJ·cm^{-2} per pulse). Samples (10^{-4} M) were irradiated in CH_3CN/H_2O (9:1) solution.

measured by HPLC and graphed as a function of time (Figure 3). It shows that the photolysis of ester **3** is much faster than that of ester **4**, and the value of δ_u arrives at 0.26 GM, which is more than twice that of ester **4**. It suggests that ester **3** provides more efficient two-photon sensitivity at 800 nm and is sufficiently high for physiological use. Compared with δ_a, the relative smaller increase of δ_u for ester **3** is attributed to the decreased photolysis quantum yield accompanying the elongation of the conjugation.[6] Photolysis measurements with varying two-photon excitation density were also performed for esters **3** and **4** under the same conditions. Their photolytic consumption rates (K) (Table 2) indicate that the two-photon photolysis rate of ester **3** is about two times faster than that of ester **4**, which is coincident with their δ_u value. The approximate

(19) Xu, C.; Webb, W. W. *J. Opt. Soc. Am. B* **1996**, *13*, 481–491.

Table 2. Two-Photon Photolytic Consumption Rates (K) at Different Excitation Density for Esters **3** and **4**[a]

esters	K_1	K_2	K_3	K_4
3	0.01572	0.00814	0.00785	0.00387
4	0.00761	–	0.00396	0.00189

[a] The two-photon excitation density for K_1-K_4 was 0.60, 0.5, 0.4, and 0.3 mJ·cm^{-2} per pulse, respectively. The unit for K is nmol/min. The values were taken from the HPLC analysis by taking a small aliquot (100 μL) of the photolysis solution after certain time intervals.

quadratic dependence of the uncaging rate K as a function of the incident laser intensity confirms that the photorelease is truly induced by the two-photon excitation.[20] These results indicate that the two-photon photolysis efficiency is improved substantially for our designed phototrigger that will enhance the feasibility for biological application.

Last, a caged purine nucleoside (ester **5**) using {7-[(4-(dimethylamino)styryl]coumarin-4-yl}methoxycarbonyl as a phototrigger was synthesized, and the synthetic route was similar to that for ester **3** (see Supporting Information). The photorelease properties are also summarized in Table 1, which reveals similar results to those for ester **3**. Upon irradiation, either one-photon (λ = 475 nm, 28 mJ/s) or two-photon excitation (λ = 800 nm, 0.6 mJ/cm^{-2}), free adenosine is released gradually by controlling the irradiation time (see Figures 2–3).

In conclusion, we successfully prepared a new phototrigger based on {7-[(4-(dimethylamino)styryl]coumarin-4-yl}methoxycarbonyl for alcohols, which are confirmed to regulate the release of the caged alcohols under irradiation of long wavelength light (475 nm) or two-photon NIR light (800 nm). Its salient advantages of long wavelength excitation and efficient two-photon sensitivity increase its usefulness for caging biologically active molecules containing a hydroxyl functionality.

Acknowledgment. We thank the NSFC(20903039, 21073062), "Chen Guang" project (09CG25), Shanghai Rising-star program "10QA1401600", and Fundamental research funds for the central university for financial support.

Supporting Information Available. Synthesis and experimental procedures, and characterization data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

(20) (a) Brown, E. B.; Shear, J. B.; Adams, S. R.; Tsien, R. Y.; Webb, W. W. *Biophy. J.* **1999**, *76*, 489–499. (b) Gug, S.; Charon, S.; Specht, A.; Alarcon, K.; Ogden, D.; Zietz, B.; Léonard, J.; Haacke, S.; Bolze, F.; Nicoud, J.; Goeldner, M. *ChemBioChem* **2008**, *9*, 1303–1307.