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# Coumarin-4-ylmethoxycarbonyls as Phototriggers for Alcohols and Phenols

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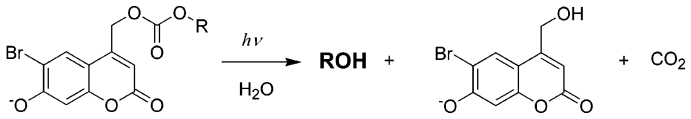
## ABSTRACT




**Caged compounds can be used to regulate the spatial and temporal dynamics of signaling molecules in live cells. Photochemical properties of coumarin-4-ylmethoxy carbonates (1a–d) are investigated to construct caged compounds of hydroxy-containing molecules. All the compounds possess desired properties as phototriggers for alcohols and phenols. The 6-bromo-7-hydroxycoumarin-4-ylmethoxycarbonyl (Bhcmoc) group has the highest photochemical efficiency and is applied to make caged compounds of 1,2-dioctanoylglycerol (di$C_8$), Tyr-OMe, and adenosine.**

Caged compounds are synthetic molecules whose biological activities are masked by covalent attachment of a photochemically removable protecting group ("caging group" or "phototrigger") to a functional group, which is of critical importance to an activity. Intracellular distribution of signaling molecules can be controlled using the chemistry of caged compounds with high spacial and temporal resolution.[1] Coumarin-4-ylmethyls are newly developed phototriggers. They have higher photolysis efficiencies than others such as 2-nitrobenzyls, so that the incident light intensity for the uncaging reaction could be lowered, leading to minimization of cell damage while maintaining higher spatial resolution. From recent investigations, the coumarin-type cages, including MCM[2], HCM (and ACM)[3], DMCM,[4] BCMCM,[5] DMACM (and DEACM),[5,6] and Bhc[7] have been successfully applied to protect phosphates,[2−7] amines,[7a,b,d] carboxylates,[2b,7] diols,[7e] and carbonyl compounds.[7f] Furthermore, the Bhc group was found to be photolyzed under two-photon excitation conditions with practically useful absorption cross-sections.[7]

We investigated photolabile protecting groups for hydroxy functionality to construct caged compounds of sugars, amino acids, lipid mediators, and other effector molecules. In a previous report, we designed and synthesized four arylmethyl carbonate-type protecting groups, among which the anthraquinone-2-ylmethoxycarbonyl (Aqmoc) was found to offer a unique property as a potential phototrigger for

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**Scheme 1.** Photolysis of Coumarin-4-ylmethoxycarbonyl Galactose Derivatives.

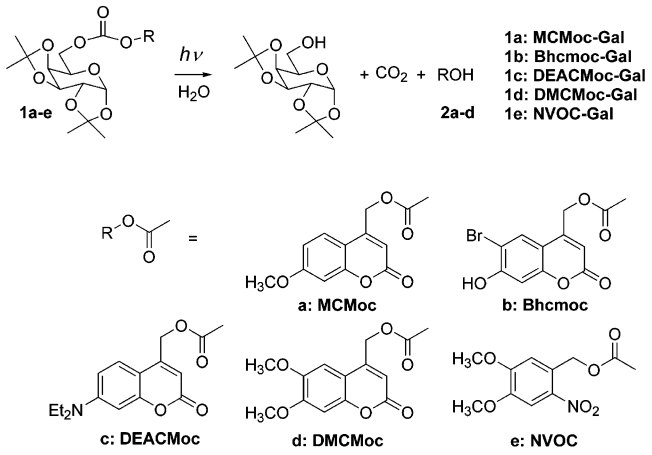


**Table 1.** Selected Photophysical and Chemical Properties of Coumarin-4-ylmethoxycarbonyl Phototriggers

| | $\lambda_{max}$ ($\epsilon$)[a] | $\epsilon_{350}$ | $\Phi_{350}$[b] | $\Phi\epsilon_{350}$ | solvent[c] |
|---|---|---|---|---|---|
| **1a** | 322 (12 100) | 4100 | 0.020 | 84 | A |
| | 323 (13 100) | 3400 | $2.9 \times 10^{-3}$ | 10 | C |
| **1b** | 374 (15 000) | 11 500 | 0.015 | 173 | A |
| | 376 (14 000) | 10 000 | $8.3 \times 10^{-3}$ | 83 | B |
| **1c** | 396 (17 300) | 6300 | $5.8 \times 10^{-3}$ | 37 | B |
| **1d** | 344 (10 800) | 10 400 | $6.5 \times 10^{-3}$ | 68 | A |
| **1e** | 344 (5400) | 5300 | $3.5 \times 10^{-4}$ | 2 | A |
| **3** | 372 (17 900) | 13 700 | 0.067 | 918 | A |

[a] Extinction coefficient ($cm^{-1}\ M^{-1}$). [b] Quantum yields for disappearance of starting materials upon 350 nm irradiation. [c] Solvent A: KMOPS (pH 7.2) containing 0.1% DMSO. Solvent B: KMOPS (pH 7.2) containing 25% acetonitrile. Solvent C: $H_2O$ containing 50% THF.

alcohols.[8] The present study is intended to evaluate whether the carbonate derivative of coumarin-based photoremovable protecting groups can serve as a potential phototrigger for hydroxy-containing molecules. Toward that end, we compared photochemical properties of the coumarin-4-ylmethyl carbonates and found that the 6-bromo-7-hydroxycoumarin-4-ylmethoxycarbonyl (Bhcmoc) group is the most suitable candidate for producing caged compounds of alcohols and phenols.[9]

One important criterion to be considered in seeking a new photoremovable protecting group for biological applications is photolysis efficiency. Relatively intense UV light is required for caged compounds with lower photolysis efficiency to release a substantial amount of biological messengers, thereby causing not only desired biological responses but also undesired cell damage. We have used the product of the photolysis quantum yield ($\Phi$) and molar absorptivity ($\epsilon$) to compare the overall efficiency of an uncaging reaction quantitatively.[10] Previous investigations revealed that the $\Phi\epsilon$ values of the coumarin-4-ylmethyl groups, MCM, HCM, and Bhc, introduced into phosphates (as esters),[2,3,7] carboxylates (as esters),[7] and amines (as carbamates)[7] were sufficiently high ($\Phi\epsilon$ 300−1200) for application to caging chemistry. However, photolysis efficiency of MCM carbonate (MCMoc) is much lower than we expected from results of other coumarin-type caged compounds. For example, typical $\Phi\epsilon$ values of phosphate esters of the MCM group are 600−800, whereas that of MCM carbonate was only 10 when photolysis was conducted in 50% THF−$H_2O$.[8]

First, we need to know whether the observed low photolysis efficiency of the MCMoc group is attributable to the inherent reactivity of all coumarin-4-ylmethyl carbonates or an introduction of appropriate substituents on coumarins can improve efficiency. We chose three types of coumarin cages: 6-bromo-7-hydroxycoumarin-4-ylmethyl (Bhc), 7-diethylaminocoumarin-4-ylmethyl (DEACM), and 6,7-dimethoxycoumarin-4-ylmethyl (DMCM). Their phosphate esters provide favorable photochemical properties as caged compounds. Using them, we prepared MCMoc-, Bhcmoc-, DEACMoc-, and DMCMoc-protected galactose derivatives (**1a**−**d**, respectively). Irradiation of the compounds with Rayonet 350 nm lamps under simulated physiological conditions (pH 7.2 KMOPS buffer) unmasked the parent galactose derivative (Scheme 1).

Absorption properties ($\lambda$ and $\epsilon$) and the photolysis quantum yields ($\Phi$) for disappearance of **1a**−**d** were measured and compared with NVOC-Gal (**1e**) having a 2-nitrobenzyl phototrigger. Table 1 shows some notable findings from the data. First, the photolytic efficiency of **1a** depends strongly on the nature of the solvent, which is consistent with the reactivity of the MCM ester of diethyl phosphate reported by Hagen and Bendig.[2b] The quantum yield of **1a** in solvent A (10 mM KMops, pH 7.2 containing 0.1% DMSO) was about 7 times larger than that in solvent C (50% THF−$H_2O$), indicating that the photolytic efficiency of the MCM group is less favorable when the reaction is performed in a solvent system with a lower polarity or in the presence of an organic solvent. This was further supported by the fact that the quantum yield of **1b** in solvent B (10 mM KMops, pH 7.2 containing 25% acetonitrile) was almost half of that in solvent A. Second, under a simulated physiological environment, all tested coumarin-4-ylmethyl carbonates (**1a**−**d**) have practically useful $\Phi\epsilon$ values that were more than an order of magnitude larger than that of NVOC-Gal (**1e**). Third, the Bhcmoc group (**1b**) has the largest $\Phi\epsilon$ value among the coumarin-4-ylmethoxycarbonyl phototriggers tested. The observed $\Phi\epsilon$ value of 173 was as large as the other Bhc-caged compounds protected as Bhc carboxylates and carbamates.

Furthermore, the Bhcmoc phototrigger can be applied to protect the phenolic hydroxy group. Thus, Bhcmoc-phenol (**3**) was synthesized from phenyl chloroformate and photolyzed to unmask the parent phenol with a $\Phi\epsilon$ value of 918, about 5 times better than that of aliphatic carbonates (Scheme

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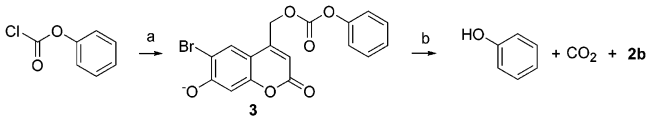
(9) Part of this work was presented at the International Chemical Congress of Pacific Basin Societies (Pacifichem 2000), Honolulu, Hawaii, Dec 14−19, 2000; Paper no. 80925280.

(10) Lester, H. A.; Nerbonne, J. M. *Annu. Rev. Biophys. Bioeng.* **1982**, *11*, 151−175.

**Scheme 2.** Synthesis and Photolysis of Bhcmoc-phenol[a]




[a] Reagents and conditions: (a) **2b**/DIEA/$CH_3CN$ (60%); (b) 350 nm/solvent A.

2). From the results, we inferred that the coumarin-type photochemically removable protecting groups, especially with 6-bromo and 7-hydroxy substituents (Bhcmoc), must serve as a potential phototrigger of hydroxy-containing molecules, both aliphatic and aromatic, with improved photolytic efficiency.

Next, we chose three types of biologically relevant molecules as model compounds to establish methods for introducing the Bhcmoc group to hydroxyl moieties and to investigate their chemical and physical properties: di$C_8$, a nonpolar aliphatic alcohol; Tyr-OMe, a phenol derivative; and adenosine, a polar alcohol with a free aromatic amino group.

The precursor molecules we designed were 6-bromo-7-methoxymethoxycoumarin-4-ylmethyl chloroformate (**5**, MOM-Bhcmoc-Cl) and 6-bromo-7-methoxymethoxy-coumarin-4-ylmethyl 4′-nitrophenyl carbonate (**6**, MOM-Bhcmoc-ONp) (Scheme 3). Selective protection of the phenol moiety of **2b** was achieved by MOMCl and DIEA in dichloromethane to yield **4** (85%). We synthesized MOM-Bhcmoc-Cl (**5**) by reaction of **4** and phosgene (generated from triphosgene and Aliquat$^R$ 336) in 78% isolated yield. Only a trace amount of the corresponding chloroformate was obtained when the reaction was performed using unprotected **2b**. The MOM protection of 7-OH of coumarin was also necessary to achieve a better yield of the *p*-nitrophenyl carbonate precursor (**6**).

In our previous report, Bhc *p*-nitrophenyl carbonate was used to introduce a Bhcmoc group into amines. We first attempted to protect the free hydroxy in diC8 with the *p*-nitrophenyl carbonate **6**. However, the isolated yield of MOM-Bhcmoc-diC8 did not exceed 50%. In contrast, an almost quantitative isolated yield was obtained with chloroformate **5**. Deprotection of the MOM group with TFA gave Bhcmoc-diC8 (**7**) in 91% overall yield.[11] As shown in Scheme 3, Tyr(Bhcmoc)-OMe (**8**) was synthesized from Boc-Tyr-OMe in two steps with quantitative yield, indicating that chloroformate **5** also serves as a precursor to introduce a Bhcmoc group into phenol. Synthesis of 5′-Bhcmoc-adenosine (**9**) was achieved via MOM-Bhcmoc-ONp (**6**) starting from 2′,3′-*O*-isopropylideneadenosine with 26% overall yield. A chemoselective protection of primary alcohol (5′-OH of ribose) over aromatic primary amine (N-6 of adenine) was also accomplished with other *p*-nitrophenyl carbonates.[8] In contrast, the use of the chloroformate **5** resulted in the introduction of a MOM-Bhcmoc group into both 5′-OH and N-6.

Photolysis reaction proceeds with the single-exponential decay of the concentration of the starting material; otherwise, photolysis byproducts or caged compounds themselves would interfere with the reaction. The time course for the consumption of **8** and **9** (shown in Figures 1 and 2 in Supporting Information) indicates that photolysis of **8** and **9** in a simulated physiological environment follows single exponential decays and proceeds with no remarkable interference by the released "Bhc" phototrigger or undesired side-reactions. However, the photolysis quantum yield of **7** markedly decreased (by an order of magnitude) and the time course for the consumption did not follow exponential decay when the reaction progressed to over 50% conversion (Figure 3 in Supporting Information). Quantum yields ($\Phi$) of disappearance were determined as 0.014 for **7**,[12] 0.022 for **8**, and 0.012 for **9**, respectively. The aliphatic carbonates, **1b**, **7**, and **9**, have similar quantum yields (0.012−0.015), which is approximately half that of aromatic carbonates, 0.064 for **3** and 0.022 for **8**. The remarkable difference in $\Phi$ values between the aliphatic and the aromatic carbonates probably results from the difference in acidity of the carbonic acids. Photoexcitation of the Bhc group must be followed by cleavage between the C4 methylene carbon of the Bhc group and the carbonate oxygen to produce the coumarin-4-ylmethyl cation and the corresponding carbonate anions.[13] Therefore, a difference in photolysis quantum yield ($\Phi$) should depend on the thermodynamic stability of the carbonate anions, which is predictable from the strength of their conjugate acids. The acid strength of the carbonic acid derived from an aliphatic alcohol is weaker than that from a phenol. Therefore, the photolysis quantum yields of the aliphatic carbonates are smaller than those of the aromatic ones. The observed decrease in quantum yield of **7** with the progress of reaction also accounted for formation of the ion pair intermediate. In an aqueous solution, the produced diacylglycerol tends to form hydrophobic aggregates, providing the Bhcmoc group with a less polar environment in which the formation of the ion pair must be less favorable.[14]

Quantitative production of the parent molecule concomitant with the progress of photolysis is important for recovery of the original activity. When the 2-nitrobenzyl photocleavers are employed, we sometimes encounter difficulty in obtaining 100% recovery of the original molecule even after photolysis is completed. This is probably the result of unwanted side reactions resulting from production of highly reactive nitroso-carbonyl byproducts. Thus, for photolysis of **8** and **9**, production of the parent molecules was quantified by HPLC analysis of aliquots taken from the photolysis mixture (Figures 1 and 2 in Supporting Information). Quantitative production of Tyr-OMe and adenosine was observed concomitant with consumption of the starting materials. The hydrolytic stability of caged compounds is often an issue in biological applications. The Bhcmoc-caged compounds **7**−**9**

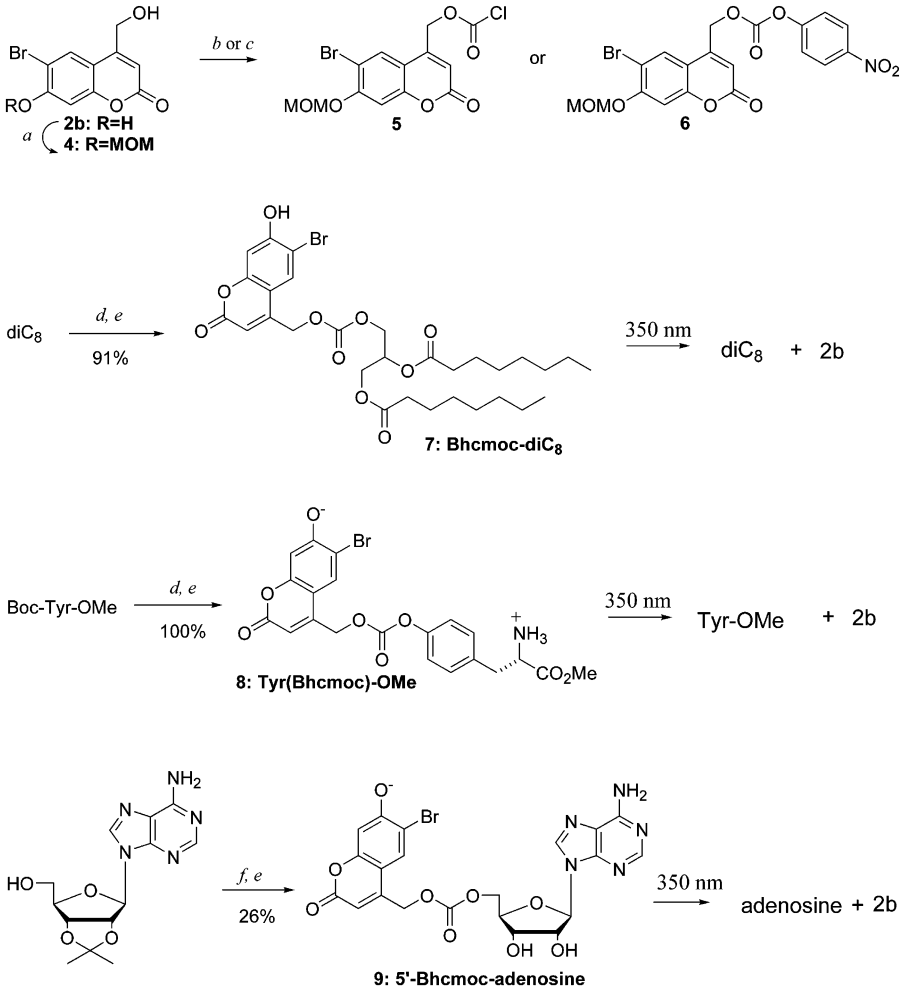
(11) J. W. Walker's group reported a preliminary synthetic study and a cell biological application of this compound. Robu, V. G.; Pfeiffer, E. S.; Robia, S. L.; Balijepalli, R. C.; Pi, Y.; Kamp, T. J.; Walker, J. W. *J. Biol. Chem.* **2003**, in press.

(12) Determined from the data within 50% conversion.

(13) Coumarin-4-ylmethyl cations are possible intermediates as suggested by Bendig et al. in ref 6a, Lawrence et al. in ref 7e, and Dore et al. in ref 7f.

(14) This would not be a severe drawback when the compound is subjected to live cell applications because the photolysis is performed in a small volume and the complete conversion into the product is not necessary.

**Scheme 3.** Synthesis and Photolysis of the Bhcmoc-Protected Hydroxyl-Containing Compounds[a]




[a] Reagents and conditions: (a) MOMCl/DIEA/$CH_2Cl_2$ (85%); (b) phosgene (78%); (c) 4-nitrophenyl-chloroformate/DMAP; (d) **5**/DMAP/$CH_3CN$; (e) TFA; (f) **6**/DMAP/$CH_3CN$.

show modest to good hydrolytic stabilities (see the half-lives in the dark ($t_{1/2}$) shown in Table 2).

In summary, we characterized photochemical properties of coumarin-4-ylmethyl carbonates (**1a**−**d**) as phototriggers for alcohols. Photolysis efficiencies ($\Phi\epsilon$ value) of **1a**−**d** were sufficiently high to allow application to caging chemistry. Practical methods of introducing the Bhcmoc group, which has the highest $\Phi\epsilon$ values, into alcohols and phenols have been developed and applied to synthesis of caged compounds of biologically relevant molecules (**7**−**9**). Observed chemical and physical properties of the compounds suggest that the Bhcmoc group would serve as a "phototrigger" for hydroxy-containing molecules, sugars, amino acids, and lipid mediators. Further study, including cell biological applications of the compounds, are currently under way.

**Table 2.** Selected Photophysical and Chemical Properties of Bhcmoc-Protected Molecules[a]

| | $\lambda_{max}$ ($\epsilon$)[b] | $\Phi_{dis}$[c] | $\Phi\epsilon_{dis}$ | $\Phi_{app}$[d] | $\Phi\epsilon_{app}$ | $t_{1/2}$[e] |
|---|---|---|---|---|---|---|
| **7** | 343 (11 600) | 0.014 | 160 | nd[f] | nd | 37 |
| **8** | 372 (13 900) | 0.022 | 300 | 0.020 | 270 | 38 |
| **9** | 373 (13 700) | 0.012 | 140 | 0.010 | 120 | 467 |

[a] All experiments were done in a simulated physiological saline solution (KMOPS (pH 7.2) containing 0.1% DMSO). [b] Extinction coefficient ($cm^{-1}$ $M^{-1}$). [c] Quantum yields for disappearance of the starting materials upon 350 nm irradiation. [d] Quantum yields for appearance of the products upon 350 nm irradiation. [e] Half-life (h) in the dark. [f] Not determined.

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

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## Synthesis

**1,2,3,4-Di-*O*-isopropylidene-D-galactopyranosyl 7-methoxycoumarin-4-ylmethoxycarbonate (1a).**

To a stirred solution of 82.9 mg (0.318 mmol) of 1,2,3,4-di-*O*-isopropylidene-D-galactopyranose in $CH_2Cl_2$ (2 mL) and $CH_3CN$ (2.5 mL) was added 49.3 mg (0.404 mmol) of 4-dimethylaminopyridine and 104.0 mg (0.387 mmol) of 7-methoxycoumarin-4-ylmethoxycarbonyl chloride. The reaction mixture was stirred at room temperature for 45 min. The solvents were removed with rotovap. Purification by flash column chromatography (15 g of $SiO_2$, $CH_2Cl_2/CH_3OH$ = 25/1) gave 123.9 mg (0.237 mmol. 94% yield) of **1a.**

**1a.** $^1$H NMR ($CDCl_3$) $\delta$ 1.34 (3H, s), 1.35 (3H, s), 1.46 (3H, s), 1.52 (3H, s), 3.88 (3H, s), 4.09 (1H, ddd, J=1.6, 5.5 & 8.0 Hz), 4.26 (1H, dd, J= 1.6 & 7.8 Hz), 4.31-4.43 (3H, m), 4.64 (1H, dd, J=2.4 & 7.8 Hz), 5.27 (1H, d, J=14 Hz), 5.34 (1H, d, J=14 Hz), 5.55 (1H, d, J=4.9 Hz), 6.39 (1H, s), 6.85 (1H, s), 6.87 (1H, d, J= 8.4 Hz), 7.41 (1H, d, J=8.4 Hz); $^{13}$C NMR ($CDCl_3$/TMS) $\delta$ 24.49, 24.91, 25.95, 26.03, 55.80, 64.53, 65.67, 67.32, 70.37, 70.68, 70.82, 96.25, 101.23, 108.88, 109.79, 110.33, 110.44, 112.67, 124.43, 148.37, 154.48, 155.58, 160.71, 162.90; UV $\lambda_{max}/nm$ ($\varepsilon$) solvent A: 322 (12,100), solvent C: 323 (13,100).

**1,2,3,4-Di-*O*-isopropylidene-D-galactopyranosyl 6-bromo-7-hydroxycoumarin-4-ylmethoxycarbonate (1b).**

To a stirred solution of 325.8 mg (1.202 mmol) of 6-bromo-7-hydroxy-4-hydroxymethylcoumarin (**2b**) in $CH_3CN$ (5 mL) was added 353.2 mg (2.89 mmol) of 4-dimethylaminopyridine and 264.0 mg (1.31 mmol) of 4-nitrophenyl chloroformate. The reaction mixture was stirred for 6 hr at room temperature. Then, another 152.5 mg (1.25 mmol) of 4-dimethylaminopyridine and 515 mg (1.99 mmol) of 1,2,3,4-di-*O*-isopropylidene-D-galactopyranose were added to the reaction mixture. After 24 hr, the mixture was diluted with $CHCl_3$, washed with 0.5 M citric acid and sat. NaCl, and dried over $MgSO_4$. Purification by column chromatography (50 g of $SiO_2$, n-Hexane/EtOAc = 3/2) gave 221.9 mg (0.398 mmol. 33% yield) of **1b**.

**1b.** $^1$H NMR ($CDCl_3$/TMS) $\delta$ 1.34 (3H, s), 1.35 (3H, s), 1.47 (3H, s), 1.53 (3H, s), 4.08-4.13 (2H, m), 4.25 (1H, dd, J=1.5, 7.8 Hz), 4.33-4.37 (3H, m), 4.63 (1H, dd, J=2.3, 7.8 Hz), 5.24 (1H, d, J=14 Hz), 5.27 (1H, d, J=14 Hz), 5.55 (1H, d, J=5 Hz), 6.41 (1H, s), 7.04 (1H, s), 7.62 (1H, s); $^{13}$C NMR ($CDCl_3$/TMS) $\delta$ 24.45, 24.86, 25.90, 25.99, 64.19, 65.67, 67.43, 70.32, 70.64, 70.78, 96.21, 104.37, 106.86, 108.91, 109.80, 111.24, 111.72, 126.84, 147.57, 154.36, 154.41, 155.83, 160.29; UV $\lambda_{max}$/*nm* ($\varepsilon$) solvent A: 374 (15,000), solvent B: 376 (14,000).

**1,2,3,4-Di-*O*-isopropylidene-D-galactopyranosyl 7-diethylaminocoumarin-4-ylmethoxycarbonate (1c).**

To a stirred suspension of 271.6 mg (1.04 mmol) of 7-diethylamino-4-hydroxymethylcoumarin in $CH_2Cl_2$ (7 mL) was added 141.7 mg (1.16 mmol) of 4-dimethylaminopyridine and232.0 mg (1.15 mmol) of 4-nitrophenyl chloroformate. The reaction mixture was stirred for 10 hr at room temperature. Thin layer chromatography indicated that the formation of intermediate 4-nitrophenyl carbonate completed. Then, another 142.2 mg (1.16 mmol) of 4-dimethylaminopyridine and 265.9 mg (1.02 mmol) of 1,2,3,4-di-*O*-isopropylidene-D-galactopyranose were added to the reaction mixture. After 20 hr, the mixture was diluted with $CH_2Cl_2$, washed with sat. $NaHCO_3$ and sat. NaCl, and dried over $MgSO_4$. Purification by flash column chromatography (23 g of $SiO_2$, $CH_2Cl_2/CH_3OH$ = 85/1 then $CH_2Cl_2/CH_3OH$ = 50/1) gave 440.9 mg (0.826 mmol. 81% yield) of **1c**.

**1c.** $^1$H NMR ($CDCl_3$/TMS) $\delta$ 1.21(6H, t, J=7.3 Hz), 1.34 (3H, s), 1.35 (3H, s), 1.46 (3H, s), 1.52 (3H, s), 3.41 (4H, q, J=7.3 Hz), 4.08 (1H, ddd, J=1.5, 5 & 8.5 Hz), 4.27 (1H, dd, J=1.5, 7.8 Hz), 4.33-4.37 (3H, m), 4.63 (1H, dd, J=2.3, 7.8 Hz), 5.23 (1H, d, J=14 Hz), 5.30 (1H, d, J=14 Hz), 5.55 (1H, d, J=4.9 Hz), 6.17 (1H, s), 6.50 (1H, d, J=2.4 Hz), 6.57 (1H, dd, J=2.4, 8.9 Hz), 7.28 (1H, d, J=8.9 Hz); $^{13}$C NMR ($CDCl_3$/TMS) $\delta$ 12.42(x 2), 24.49, 24.92, 25.95, 26.04, 44.77(x 2), 64.73, 65.65, 67.13, 70.39, 70.66, 70.81, 96.24, 97.87, 105.86, 106.78, 108.69, 108.88, 109.74, 124.40, 148.60, 150.64, 154.58, 156.28, 161.71; UV $\lambda_{max}$/*nm* ($\varepsilon$) solvent B:

250 (11,400), 396 (17,300).

**1,2,3,4-Di-*O*-isopropylidene-D-galactopyranosyl 6,7-dimethoxycoumarin-4-ylmethoxycarbonate (1d).**

To a stirred solution of 65.4 mg (0.251 mmol) of 1,2,3,4-di-*O*-isopropylidene-D-galactopyranose in $CH_2Cl_2$ (5 mL) and $CH_3CN$ (2 mL) was added 39.0 (0.32 mmol) of 4-dimethylaminopyridine and 90.8 mg (0.304 mmol) of 6,7-dimethoxycoumarin-4-ylmethoxycarbonylchloride. The reaction mixture was stirred at room temperature for 2 hr. The solvent was removed with rotovap. Purification by flash column chromatography (20 g of $SiO_2$, $CH_2Cl_2/CH_3OH$ = 80/1 then $CH_2Cl_2/CH_3OH$ = 50/1) gave 123.9 mg (0.237 mmol. 94% yield) of **1d.**

**1d.** $^1$H NMR ($CDCl_3$) $\delta$ 1.34 (3H, s), 1.35 (3H, s), 1.46 (3H, s), 1.52 (3H, s), 3.93 (3H, s), 3.95 (3H, s), 4.09 (1H, ddd, J=1.5, 5.5 & 8.0 Hz), 4.26 (1H, dd, J=1.5 & 7.8 Hz), 4.31-4.44 (3H, m), 4.64 (1H, dd, J= 2.4 & 7.8 Hz), 5.28 (1H, d, J=15 Hz), 5.34 (1H, d, J=15 Hz), 5.54 (1H, d, J= 4.9 Hz), 6.43 (1H, s), 6.84 (1H, s), 6.87 (1H, s); $^{13}$C NMR ($CDCl_3$/TMS) $\delta$ 24.48, 24.90, 25.94, 26.02, 56.38, 56.53, 64.67, 65.68, 67.36, 70.36, 70.67, 70.82, 96.24, 100.35, 103.91, 108.88, 109.36, 109.79, 110.80, 146.41, 148.12, 149.77, 153.01, 154.52, 160.89; UV $\lambda_{max}$/*nm* ($\varepsilon$) solvent A: 290 (4,500), 344 (10,800).

**1,2,3,4-Di-*O*-isopropylidene-D-galactopyranosyl 6-nitroveratryloxycarbonate (1e).**

To a stirred solution of 143.8 mg (0.552 mmol) of 1,2,3,4-di-*O*-isopropylidene-D-galactopyranose in $CH_2Cl_2$ (3.5 mL) and $CH_3CN$ (1 mL) was added 84.3 mg (0.69 mmol) of 4-dimethylaminopyridine and 180.5 mg (0.655 mmol) of 6-nitroveratryloxycarbonyl chloride. The reaction mixture was stirred at room temperature for 3 hr. The solvents were removed with rotovap. Purification by flash column chromatography (20 g of $SiO_2$, $CH_2Cl_2/CH_3OH$ = 98/1 then $CH_2Cl_2/CH_3OH$ = 25/1) gave 169.1 mg (0.339 mmol. 61% yield) of **1e.**

**1e.** $^1$H NMR ($CDCl_3$) $\delta$ 1.32 (3H, s), 1.34 (3H, s), 1.46 (6H, s), 3.96 (3H, s), 4.01 (3H, s), 4.11 (1H, ddd, J= 1.0, 5.5 & 7.0 Hz), 4.26 (1H, dd, J=1.0 & 8.0 Hz), 4.32-4.37 (3H, m), 4.62 (1H, dd, J=2.2 & 8.0 Hz), 5.53 (1H, d, J=4.9 Hz), 5.59 (1H, d, J=14 Hz), 5.63 (1H, d, J=14 Hz), 7.09 (1H, s), 7.74 (1H, s); $^{13}$C NMR ($CDCl_3$/TMS) $\delta$ 24.47, 24.91, 25.92 (X 2), 56.40, 56.58, 65.71, 66.30, 67.10, 70.35, 70.67, 70.88, 96.25, 108.09, 108.82, 109.47, 109.75, 127.12, 139.41, 148.16, 153.81, 154.67; UV $\lambda_{max}$/*nm* ($\varepsilon$)

solvent A: 344 (5,400).

**Phenyl 6-Bromo-7-hydroxycoumarin-4-ylmethoxycarbonate (3).**

To a stirred suspension of 134.9 mg (0.498 mmol) of **2b** in $CH_3CN$ (3.5 mL) was added 147 μL (0.84 mmol) of diisopropyl ethylamine and 63 μL (0.50 mmol) of phenyl chloroformate. The reaction mixture was stirred at room temperature for 3.5 hr. The solvents were removed with rotovap. Purification by flash column chromatography (17g of $SiO_2$, n-Hexane/EtOAc = 5/2) gave 117.7mg (0.301 mmol. 60% yield) of **3.**

**3.** $^1$H NMR ($CDCl_3$/TMS) $\delta$ 5.37 (2H, s), 6.49 (1H, s), 7.05 (1H, s), 7.10-7.15 (3H, m), 7.40-7.45 (2H, m), 7.67 (1H, s); $^{13}$C NMR ($CDCl_3$/TMS) $\delta$ 65.28, 103.20, 106.32, 109.90, 110.35, 121.22, 126.40, 128.74, 129.69, 148.60, 150369, 152.43, 153.89, 157.59, 159.52; UV $\lambda_{max}$/*nm* ($\varepsilon$) solvent A: 372 (17,900).

**6-Bromo-7-methoxymethoxy-4-hydroxymethylcoumarin (4).**

To a stirred suspension of 1.0846 g (4.001 mmol) of **2b** in $CH_2Cl_2$ (10 mL) was added 836 μL (4.80 mmol) of diisopropyl ethylamine and 365 μL (4.81 mmol) of chloromethyl methyl ether. The reaction mixture was stirred at room temperature for 40 min. The reaction mixture was diluted with $CHCl_3$, washed with 0.5 M citric acid and dried over $MgSO_4$. The solvents were removed with rotovap and high vacuum rotovap to give 1.065 g (3.380 mmol, 85% yield) of **4**.

**4.** $^1$H NMR ($CDCl_3$/TMS) $\delta$ 7.70 (s, 1H), 7.16 (s, 1H), 5.32 (s, 2H), 3.52 (s, 3H), 6.52 (t, 1H, J = 1.3 Hz), 4.86 (dd, 2H, J = 5.6, 1.3 Hz), 2.07 (t, 1H, J = 5.6 Hz); $^{13}$C NMR (DMSO-$d_6$/TMS) δ 56.23 (q), 59.06 (t), 94.80 (t), 103.51 (d), 107.49 (s), 108.96 (d), 112.75 (s), 128.19 (d), 153.51 (s), 155.13 (s), 155.76 (s), 159.93 (s); IR (ATR) 3508, 1703, 1604, 1267, 1158, 1095, 1083, 1032, 1009, 974, 894, 842 cm$^{-1}$.

**6-Bromo-7-methoxymethoxycoumarin-4-ylmethoxycarbonyl chloride (5).**

In a glass pressure bottle (Hyperglasstor, THG-A2, TAIATSU Techno) 1.9834 g (6.684 mmol) of triphogene was placed. To this

195.4 μL (0.483 mmol) of aliquat$^{R}$ 336 in n-hexane (4 mL) was added. The mixture was stirrer at room temperature for 19 hr to generate phosgene. To a stirred solution of 321.7 mg (1.021 mmol) of 4 in toluene (2 mL) and THF (3 mL) was added the phosgene solution (4 mL). The reaction mixture was stirred at 0°C for 3.5 hr. The solvents were removed under reduced pressure. The residual solid was washed with $CHCl_3$ and n-hexane (1/1 v/v) twice to give 352.0 mg (0.932 mmol, 91% yield) of **5**.

**5.** $^{1}$H NMR ($CDCl_3$/TMS) δ 3.53 (3H, s, MOM), 5.33 (2H, s, MOM), 5.42 (2H, d, J=1.5, H-4), 6.43 (1H, s, H-3), 7.19 (1H, s, H-8), 7.64 (1H, s, H-5); $^{13}$C NMR ($CDCl_3$/TMS) δ 56.73(q), 67.08(t), 95.17(t), 104.19(d), 108.82(s), 111.77(d), 112.58(s), 127.27(s), 145.28(s), 150.62(s), 154.27(s), 156.74(s), 159.49(s); IR (ATR) 1756, 1719, 1603, 1273, 1164, 1151 cm$^{-1}$.

**3-(6-Bromo-7-methoxymethoxycoumarin-4-ylmethoxycarbonyl)-1,2-dioctanoyl glycerol.**

To a stirred solution of 169.1 mg (0.491 mmol) of 1,2-dioctanoyl glycerol ($diC_8$) in $CH_3CN$ (4 mL) were added 115.3 mg (0.944 mmol) of 4-dimethylaminopyridine and 192.3 mg (0.509 mmol) of **5**. The reaction mixture was stirred at room temperature for 40 min. The solvents were removed with rotovap. Purification by flash column chromatography (35 g of $SiO_2$, n-hexane/AcOEt = 35/10) gave 321.1 mg (0.468 mmol. 95% yield) of the title compound.

$^{1}$H NMR ($CDCl_3$) δ 7.68 (s, 1H), 7.17 (s, 1H), 6.42 (t, 1H, J = 1.0 Hz), 5.32 (s, 1H), 5.30 (m, 1H), 5.28 (d, 1H, J = 1.0 Hz), 4.45 (dd, 1H, J = 11.9, 4.0 Hz), 4.35 (dd, 1H, J = 11.5, 4.6 Hz), 4.30 (dd, 1H, J = 11.5, 5.6 Hz), 4.19 (dd, 1H, J = 11.9, 5.6 Hz), 3.52 (s, 3H), 2.35 (t, 2H, J = 7.6 Hz), 2.33 (t, 2H, J = 7.6 Hz), 1.62 (4H, m), 1.28 (16H, m), 0.87 (6H, m); $^{13}$C NMR ($CDCl_3$/TMS) δ 173.2 (s), 172.9 (s), 159.8 (s), 156.5 (s), 154.2 (s), 147.0 (s), 127.4 (d), 112.1 (s), 111.8 (d), 108.6 (s), 104.1 (d), 95.1 (t), 68.4 (s), 66.6 (t), 64.4 (t), 61.6 (t), 56.7 (q), 34.1 (t), 34.0 (t), 31.6 (t), 29.0 (t), 28.9 (t), 24.8 (t), 22.5 (t), 14.0 (q); IR (ATR) 1735, 1606, 1441, 1277, 1202, 1160 cm$^{-1;}$ MS (ESI) m/z 707.10 ($C_{32}H_{45}{}^{79}BrO_{11}$+$Na^{+}$), 709.90 ($C_{32}H_{45}{}^{81}BrO_{11}$+$Na^{+}$) .

**3-(6-Bromo-7-hydroxy-4-ylmethoxycarbonyl)-1,2-dioctanoyl glycerol (7).**

A solution of 79.9 mg (0.117 mmol) of 3-(6-Bromo-7-methoxymethoxycoumarin-4-ylmethoxycarbonyl)-1,2-dioctanoyl glycerol in trifluoroacetic acid (2 mL) was stirred at room temperature for 15 min. The solvent was evaporated by rotovap and high vacuum rotovap to give 71.9 mg (0.112 mmol. 96% yield) of **7**.

**7.** $^{1}$H NMR ($CDCl_3$) δ 7.63 (s, 1H), 7.05 (s, 1H), 6.40 (t, 1H, J = 1.3 Hz), 5.33 (m, 1H), 5.28 (d, 2H, J = 1.3 Hz), 4.45 (dd, 1H, J = 11.9, 4.0 Hz), 4.35 (dd, 1H, J = 11.5, 4.3 Hz), 4.31 (dd, 1H, J = 11.5, 5.3 Hz), 4.20 (dd, 1H, J = 11.9, 5.6 Hz), 2.35 (t, 2H, J = 7.6 Hz), 2.34 (t, 2H, J = 7.6 Hz), 1.62 (m, 4H), 1.28 (m, 16H), 0.87 (m, 6H); $^{13}$C NMR ($CDCl_3$/TMS) δ 173.4 (s), 173.0 (s), 160.2 (s), 155.9 (s), 154.4 (s), 154.2 (s), 147.4 (s), 126.8 (d), 111.6 (s), 111.3 (d), 106.9 (s), 104.4 (d), 68.5 (s), 66.6 (t), 64.4 (t), 61.7 (t), 34.1 (t), 34.0 (t), 31.6 (t), 29.0 (t), 28.9 (t), 24.8 (t), 22.6 (t), 14.0 (q); IR (ATR) 3375 (broad), 1724, 1607, 1441, 1409, 1280, 1218, 1160 $cm^{-1;}$ MS (ESI) m/z 663.00 ($C_{30}H_{41}{}^{79}BrO_{10}$+$Na^+$), 664.80 ($C_{30}H_{41}{}^{81}BrO_{10}$+$Na^+$).

***O*-(6-Bromo-7-methoxymethoxycoumarin-4-ylmethoxycarbonyl)-*N-tert*-butoxycarbonyl-L-tyrosine methyl ester.**

To a stirred solution of 236.8 mg (0.802 mmol) of *N-tert*-butoxycarbonyl-L-tyrosine methyl ester in $CH_3CN$ (6 mL) were added 184.4 mg (1.51 mmol) of 4-dimethylaminopyridine and 268.8 mg (0.71 mmol) of **5**. The reaction mixture was stirred at room temperature for 15 min. The solvents were removed with rotovap. Purification by flash column chromatography (30 g of $SiO_2$, $CH_2Cl_2$/$CH_3OH$ = 100/1 then $CH_2Cl_2$/$CH_3OH$ = 80/1) gave 455.1 mg (0.71 mmol. 100% yield) of the title compound.

$^{1}$H NMR ($CDCl_3$) δ 7.72 (s, 1H), 7.20□7.12 (m, 5H), 6.47 (s, 1H), 5.38 (s, 2H), 5.32 (s, 2H), 5.17 (d, 1H, J = 7.9 Hz), 4.59 (m, 1H), 3.72 (s, 3H), 3.52 (s, 3H), 3.15 (dd, 1H, J = 13.9, 5.6 Hz), 3.04 (dd, 1H, J = 13.9, 6.3 Hz), 1.42 (s, 9H); $^{13}$C NMR ($CDCl_3$/TMS) δ 171.9 (s), 159.6 (s), 156.2 (s), 154.8 (s), 154.0 (s), 152.7 (s), 149.6 (s), 146.9 (s), 134.3 (s), 130.3 (d), 127.3 (d), 120.6 (d), 111.9 (s), 111.6 (d), 108.4 (s), 103.8 (d), 94.9 (t), 79.7 (s), 64.5 (t), 56.5 (q), 54.1 (d), 52.1 (q), 37.4 (t), 28.0 (q); IR (ATR) 1735, 1605, 1507, 1366, 1243, 1218, 1156 $cm^{-1;}$ MS (ESI) m/z 657.95 ($C_{28}H_{30}{}^{79}BrNO_{11}$+$Na^+$), 659.75 ($C_{28}H_{30}{}^{81}BrNO_{11}$+$Na^+$) .

***O*-(6-Bromo-7-hydroxycoumarin-4-ylmethoxycarbonyl)-L-tyrosine methyl ester hydrochloride (8).**

Hydrogen chloride gas was introduced into a stirred solution of 62.6 mg (0.098 mmol) of *O*-(6-Bromo-7-methoxymethoxycoumarin-4-ylmethoxycarbonyl)-*N-tert*-butoxycarbonyl-L-tyrosine methyl ester in $CH_2Cl_2$ (4 mL) at room trremperature for 2 min. The reaction mixture was stirred at room temperature for 3 hr. The solvent and the excess HCl were removed by rotovap and high vacuum rotovap to give 52.0 mg (0.098 mmol 100% yield) of **8**.

**8 (HCl salt).** $^{1}$H NMR ($CDCl_3$) δ 7.83 (s, 1H), 7.36-7.25 (m, 4H), 6.84 (s, 1H), 6.32 (s, 1H), 5.47 (s, 2H), 4.36 (dd, 1H, J = 7.3, 6.3 Hz), 3.82 (s, 3H), 3.31 (dd, 1H, J = 14.2, 6.3 Hz), 3.20 (dd, 1H, J = 14.2, 7.3 Hz); $^{13}$C NMR ($CDCl_3$/TMS) δ 170.3 (s), 162.3 (s), 159.3 (s), 155.7 (s), 154.5 (s), 152.2 (s), 150.3 (s), 133.7 (s), 131.8 (d), 129.5 (d), 122.8 (d), 112.0 (s), 110.8 (d), 108.1 (s), 104.4 (d), 66.4 (t), 55.1 (q), 53.7 (d), 36.7 (t); IR (ATR) 3383, 1175, 1736, 1608, 14006, 1272, 1249, 1235 cm$^{-1;}$ MS (ESI) m/z 491.90 ($C_{21}H_{18}{}^{79}BrNO_8$+$H^+$), 493.75 ($C_{21}H_{18}{}^{81}BrNO_8$+$H^+$).

**5'-(6-Bromo-7-methoxymethoxycoumarin-4-ylmethoxycarbonyl)-2',3'-O-isopropylideneadenosine.**

To a stirred suspension of 248.4 mg (0.757 mmol) of **4** in $CH_3CN$ (6 mL) were added 194.8 mg (1.60 mmol) of 4-dimethylaminopyridine and 167.5 mg (0.814 mmol) of 4-nitrophenyl chloroformate at room temperature. After 1.5 hr, another 110.3 mg (0.903 mmol) of 4-dimethylaminopyridine and 253.9 mg (0.826 mmol) of 2',3'-isopropylideneadenosine were added. The reaction mixture was stirred at room temperature for 3.5 hr. The solvents were removed with rotovap. The residue was diluted with $CHCl_3$ (30 mL), and washed with 0.5 M citric acid and sat. NaCl. The organic layer was dried over $MgSO_4$, and the solvent was removed by rotovap and high vacuum rotovap. Purification by flash column chromatography (35 g of $SiO_2$, $CH_2Cl_2$/$CH_3OH$ = 30/1 then $CH_2Cl_2$/$CH_3OH$ = 21/1) gave 307.4 mg (0.474 mmol. 63% yield) of the product.

$^{1}$H NMR ($CDCl_3$/TMS) δ 1.42 (s, 3H), 1.63 (s, 3H), 3.51 (s, 3H), 4.41 (dd, 1H, J = 11.2, 5.9 Hz), 4.51 (dd, 1H, J = 11.2, 4.0 Hz), 4.56 (m, 1H), 5.16 (dd, 1H, J = 6.3, 3.0 Hz), 5.22 (d, 2H, J = 1.7 Hz), 5.31 (s, 2H), 5.51 (dd, 1H, J = 6.3, 2.0 Hz), 6.19 (d, 1H, J = 2.0 Hz), 6.31 (br, 2H), 6.44 (t, 1H, J = 1.7 Hz), 7.12 (s, 1H), 7.60 (s, 1H), 7.94 (s, 1H), 8.32 (s, 1H); $^{13}$C NMR ($CDCl_3$/TMS) δ 25.3 (q), 27.0 (q), 56.6 (q), 64.2 (t), 68.1 (t), 81.4 (d), 84.3 (d), 84.8 (d), 90.7 (d), 95.0 (t), 103.9 (d), 108.5 (s), 111.5 (d), 112.0 (s), 114.5 (s), 120.0 (s), 127.2 (d), 139.5 (d), 147.0 (s), 149.1 (s), 153.1 (d), 153.9 (s), 154.0 (s), 155.8 (s), 156.3 (s),159.9 (s); IR (ATR) 1731, 1637, 1604, 1266, 1214, 1155 cm$^{-1}$; MS (ESI) m/z 647.95 ($C_{26}H_{26}{}^{79}BrN_5O_{10}$+$H^+$), 649.75 ($C_{26}H_{26}{}^{81}BrN_5O_{10}$+$H^+$).

**5'-(6-Bromo-7-hydroxy-4-ylmethoxycarbonyl)adenosine (9).**

To a stirred solution of 181.9 mg (0.281 mmol) of 5'-(6-Bromo-7-methoxymethoxycoumarin-4-ylmethoxycarbonyl)-2',3'-O-isopropylideneadenosine in trifluoroacetic acid (2 mL) was added 80 μL of water at room temperature. After 20 hr, the solvents were evaporated by rotovap and high vacuum rotovap. Purification by flash column chromatography (15 g of $SiO_2$,

$CH_2Cl_2/CH_3OH$ = 15/1 then $CH_3OH$) gave 92.5 mg (0.117 mmol. 42% yield) of **9**·2TFA.

**9·(2TFA salt).** $^1$H NMR ($CDCl_3$/TMS) δ 8.31 (s, 1H), 8.15 (s, 1H), 7.84 (s, 1H), 7.29 (s, 2H), 6.81 (s, 1H), 6.14 (s, 1H), 5.93 (d, 1H, J = 4.9 Hz), 5.60 (d, 1H, J = 5.6 Hz), 5.44 (d, 1H, J = 5.3 Hz), 5.38 (s, 2H), 4.67 (m, 1H), 4.47 (dd, 1H, J = 11.2, 3.6 Hz), 4.38 (dd, 1H, J = 11.2, 6.2 Hz), 4.26 (m, 1H), 4.14 (m, 1H); $^{13}$C NMR ($CDCl_3$/TMS) δ 159.8 (s), 156.1 (s), 154.2 (s), 153.9 (s), 152.7 (d), 152.4 (s), 149.4 (s), 149.2 (s), 139.7 (d), 128.2 (d), 119.2 (s), 109.3 (s), 108.3 (d), 107.3 (s), 103.4 (d), 87.8 (d), 81.3 (d), 72.9 (d), 70.3 (d), 68.2 (t), 64.6 (t); IR (ATR) 3337 (broad), 3184 (broad), 1685, 1646, 1604, 1406, 1271, 1249, 1206, 1139 $cm^{-1}$; MS (ESI) m/z 563.90 ($C_{21}H_{18}{}^{79}BrN_5O_9+H^+$), 565.75 ($C_{21}H_{18}{}^{81}BrN_5O_9+H^+$).

**Quantum efficiency measurement.**

Into a pyrex test tube of 12 mm diameter was placed 2 mL of 10 μM substrate solution in K-MOPS solution (pH 7.2) containing 1% DMSO. The solution was irradiated at 350 nm using either two or four RPR 350 nm lamps. Aliquots of 10 μL were removed periodically and analyzed by HPLC. The light output for the quantum efficiencies measurement was performed using ferrioxalate actinometry.

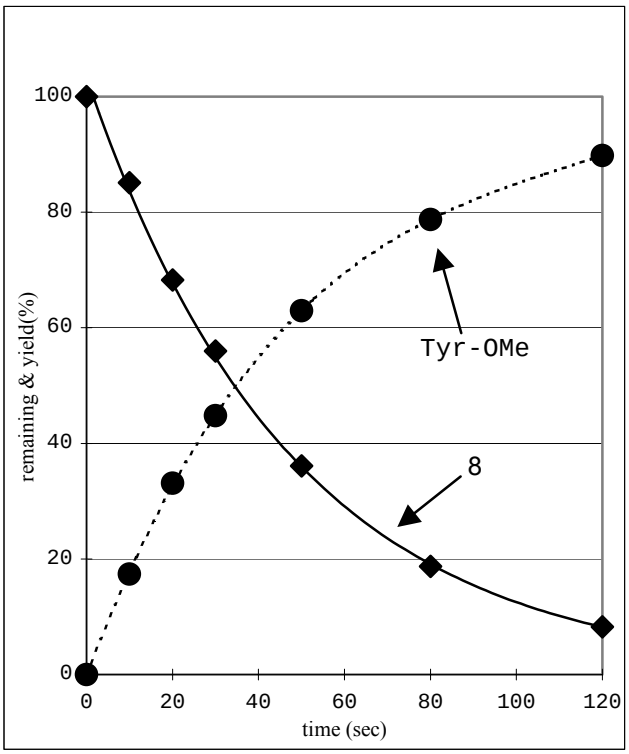


Figure 1. Time course of photolysis of Bhcmoc-Tyr-OMe ( 8) in solvent A upon 350 nm irradiation (two RPR 350 nm lamps). Concentrations of 8 and Tyr-OMe were quantified by HPLC analysis.

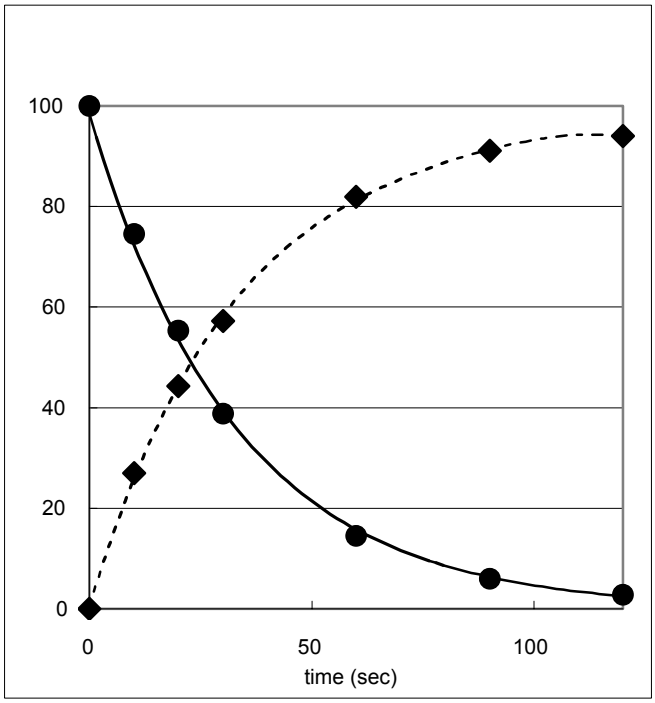


Figure 2. Time course of photolysis of 5'-Bhcmoc-adenosine ( 9) in solvent A upon 350 nm irradiation (four RPR 350 nm lamps). Concentrations of 9 and adenosine were quantified by HPLC analysis.

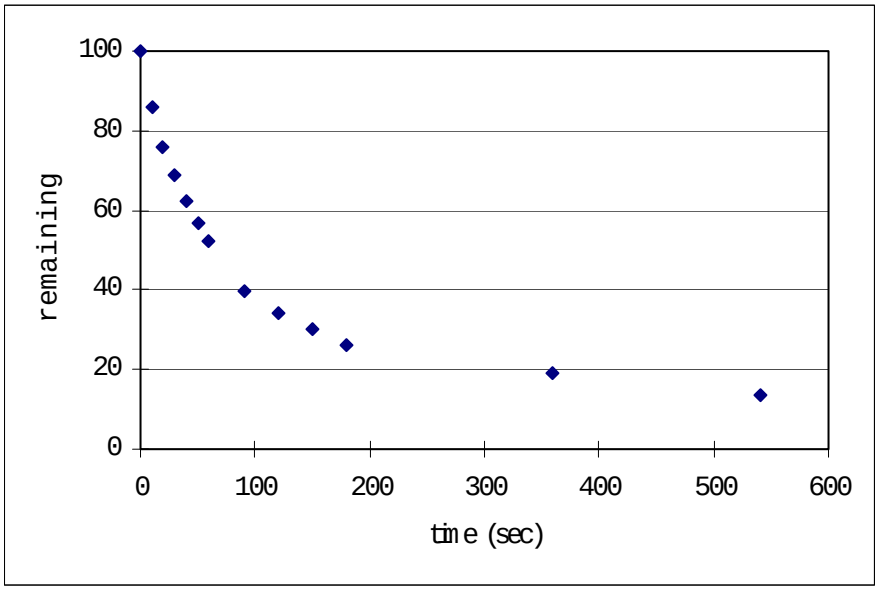


Figure 3. Time course of photolysis of Bhcmoc-dic8 (7) in solvent A upon 350 nm irradiation (two RPR 350 nm lamps). Concentrations of 7 were quantified by HPLC analysis.