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[8-[Bis(carboxymethyl)aminomethyl]-6-bromo-7-hydroxycoumarin-4-yl]methyl Moieties as Photoremovable Protecting Groups for Compounds with COOH, NH_2, OH, and C=O Functions

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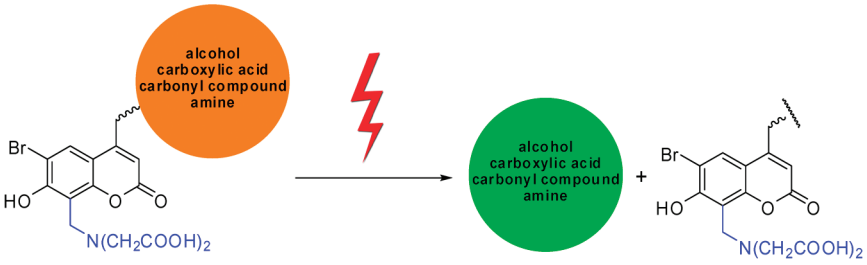
Received February 26, 2010



We introduce a variant of coumarin-based photoactivatable protecting groups and use it exemplarily for caging of a carboxylic acid, an amine, a phenol, and a carbonyl compound. The caged compounds are efficiently photolyzed at long-wavelength UV/vis irradiation. Compared to the corresponding (6-bromo-7-hydroxycoumarin-4-yl)methyl (Bhc) derivatives, the novel coumarin-type caged compounds are distinguished by (i) dramatically increased solubilities in aqueous buffers, (ii) lower pK_a values of the C7 hydroxyl of the coumarin chromophore, thus permitting efficient photorelease at lower pH, and (iii) higher photolysis quantum yields in the case of photoprotected carbonyl compounds. The primary step of the photocleavages occurs with rate constants of about 10^9 s^{-1}.

Introduction

Photoactivatable protecting groups have numerous applications in chemistry.[1,2] Their special advantage results from the fact that their removal only requires light, and light-induced reactions are usually very fast. Photoactivatable (caged) derivatives of biomolecules allow to control, both spatially and temporally, biological activity with light and represent powerful tools for studies of mechanisms and the kinetics of cellular processes.[3−6] When caged, the biomolecule is rendered biologically inactive by covalent attachment of the protecting group (caging group) to the key pharmacophoric functionality. Photolysis with a flash of light cleaves the modifying group and rapidly activates the molecule. Caging groups should be removable very fast in high yields and should exhibit high light sensitivity at long wavelengths (>350 nm). Furthermore, a high solubility of the caged molecule in aqueous buffer is frequently important.

Recently, coumarin-type caging groups such as (6-bromo-7-hydroxycoumarin-4-yl)methyl (Bhc)[7−11] and [7-[bis(carboxymethyl)amino]coumarin-4-yl]methyl (BCMACM)[12−15] have been introduced and have been applied to protect carboxylates,

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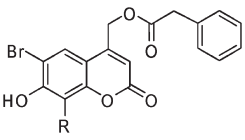
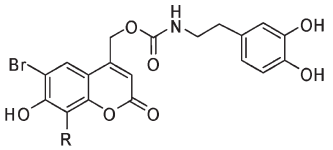
Published on Web 03/31/2010 DOI: 10.1021/jo100368w

phosphates, amines, alcohols, and also carbonyl compounds. The two caging groups show favorable photochemical and photophysical properties, but the application of Bhc-caged compounds is in the case of hydrophobic biomolecules restricted by their low solubility in aqueous buffer and that of the BCMACM derivatives by relatively low quantum yields in the case of poor leaving groups.

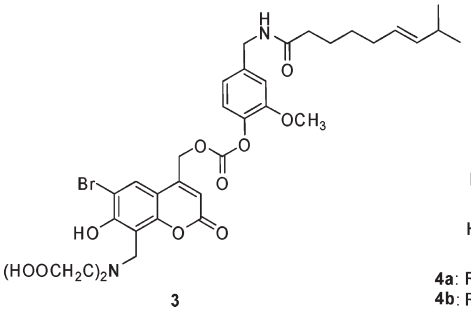
Herein, we describe the [8-[bis(carboxymethyl)aminomethyl]-6-bromo-7-hydroxycoumarin-4-yl]methyl (BBHCM) ester **1a**, the BBHCM carbamate **2a**, the BBHCM carbonate **3**, and the [8-[bis(carboxymethyl)aminomethyl]-6-bromo-7-hydroxycoumarin-4-yl] (BBHC)-substituted cyclic acetal **4a** and discuss their photophysical and photochemical properties in comparison with those of the corresponding Bhc-caged compounds **1b**, **2b** and the already known derivative **4b**.[10] The BBHCM chromophore, its [8-[bis(carboxymethyl)aminomethyl]-6-bromo-7-hydroxycoumarin-4-yl]-methoxycarbonyl (BBHCMOC) variant, and also BBHC-substituted 1,3-dioxolanes have not yet been reported.



1a: R = $CH_2N(CH_2COOH)_2$
1b: R = H



2a: R = $CH_2N(CH_2COOH)_2$
2b: R = H



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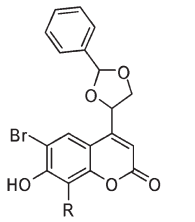
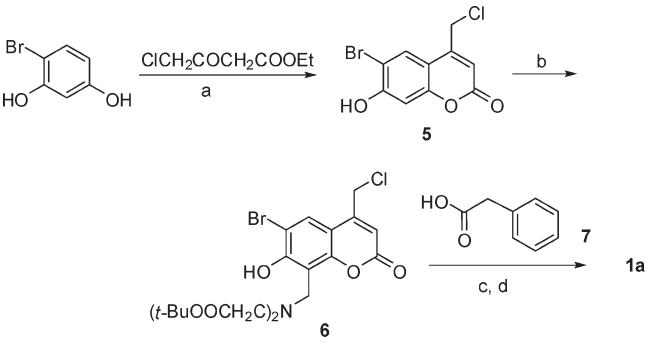


4a: R = $CH_2N(CH_2COOH)_2$
4b: R = H

Results and Discussion

Synthetic Studies. The syntheses are based on coumarinylmethyl caging chemistry.[7,9,10,12,14,15] The introduction of the methyleneiminodiacetic acid group in position 8 of the coumarin chromophore has been achieved by the Mannich reaction as previously described for 7-hydroxycoumarins.[16,17] The Mannich reaction can be directly carried out with the complete caged Bhc derivatives or with the introduced Bhc building blocks **5**[7] and **8**[7] leading to the new key compounds **6** and **9**, respectively. (Schemes 1 and 2). The described synthesis procedure of **5** has been improved

SCHEME 1. Synthesis of Compound 1a



[a]Reagents and conditions: (a) methanesulfonic acid, rt, 2 h, 91%; (b) paraformaldehyde, di-*tert*-butyl iminodiacetate, KOH, EtOH, reflux, 3 h, 62%; (c) DBU, benzene, reflux, 2 h, 69% (*t*-Bu-protected **1a**); (d) TFA/CH_2Cl_2/H_2O (75:24:1), rt, 1 h, 40 °C, 0.5 h, 95%.

by replacing sulfuric acid with methane sulfonic acid as acid catalyst and solvent. This modification resulted in a drastic reduction of the reaction time from 6 days to 2 h with simultaneous increase of the yield from 59% to 91%.

For synthesis of **1a**, phenylacetic acid (**7**) was esterified with **6** in the presence of 1,8-diazabicyclo[5.4.0]-7-undecene (DBU) and converted to **1a** by removal of the *tert*-butyl groups with trifluoroacetic acid (TFA) (Scheme 1). Preparation of **1b** succeeded analogously by coupling of **5** with **7**.

Compounds **2a** and **3** were prepared as shown in Scheme 2 by reaction of **9** with 4-nitrophenyl chloroformate to the activated carbonate **10**, reaction of **10** with the neurotransmitter dopamine (**11**), or the vanilloid receptor channel agonist capsaicin (**12**) in the presence of 4-(dimethylamino)-pyridine (DMAP) to yield the corresponding *tert*-butyl-protected derivatives, which were purified by flash chromatography and then deprotected with TFA to generate the carbamate **2a** and the carbonate **3**. Compound **2b** was synthesized using the same procedure starting from **8** by esterification with 4-nitrophenyl chloroformate and subsequent treatment of the activated carbonate with **11** in the presence of DMAP.

Synthesis of the caged benzaldehydes **4a** and **4b** is outlined in Scheme 3. The Bhc-ethanediol (Bhc-ED)-protected compound **4b** was prepared according to the literature procedure by acetalization of the aldehyde **14** with the diol **13** in the presence of pyridinium *p*-toluenesulfonate (PPTS) and $MgSO_4$.[10] Substitution at the 8-position of the coumarin nucleus of **4b** and formation of the derivative **15** was achieved by heating with freshly prepared Mannich reagent consisting of formaldehyde, dimethyl iminodiacetate, and KOH. Ester hydrolysis of **15** with LiOH in THF/H_2O produced the BBHC-ED-caged compound **4a**.

Properties. As expected, compounds **1a**, **2a**, **3**, and **4a** show, in contrast to the derivatives **1b**, **2b**, and **4b**, high solubilities in aqueous buffer (Table 1). This is important for administration of high concentrations of the phototriggers under physiological conditions.

The BBHC-ED- and Bhc-ED-protected aldehydes **4a** and **4b** are fully resistant to spontaneous hydrolysis in the dark. HPLC monitoring of the caged compounds in aqueous buffer, at pH 7.2, during a 24 h period revealed $<0.5\%$

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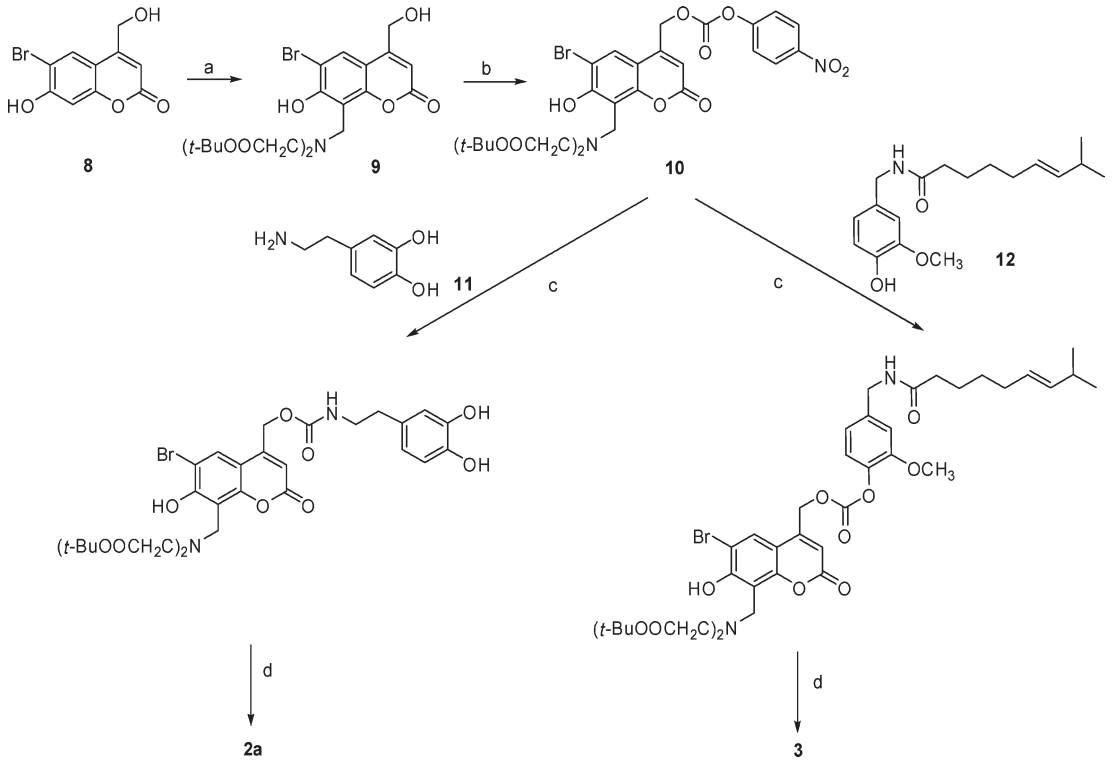
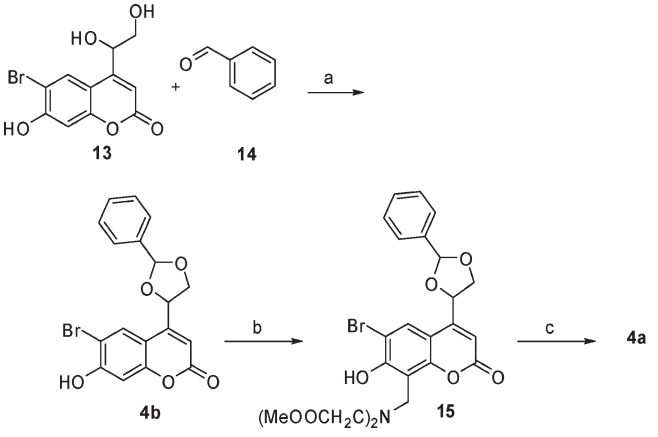
SCHEME 2. Synthesis of Compounds 2a and 3



[a]Reagents and conditions: (a) paraformaldehyde, di-*tert*-butyl iminodiacetate, KOH, EtOH, reflux, 9 h, 78%; (b) 4-nitrophenyl chloroformate, DIPEA, THF, rt, 4−5 h; (c) DMAP, DMF, rt, 1 h, 39% (precursor of **2a**) and 70% (precursor of **3**); (d) TFA/CH_2Cl_2/H_2O (75:24:1), rt, 1 h, 40 °C, 0.5 h, 100% (**2a**) and 95% (**3**).

SCHEME 3. Synthesis of Compound 4a



[a]Reagents and conditions: (a) PPTS, $MgSO_4$, toluene, reflux, 18 h, 42%; (b) paraformaldehyde, dimethyl iminodiacetate, KOH, EtOH, reflux, 7 h, 27%; (c) LiOH, THF/H_2O, rt, 24 h, 48%.

of the free uncaged compound **14**. More susceptibility to hydrolysis in the dark was observed in the case of the caged compounds **1a**, **2a**, and **3**. We found that approximately 7% of **2a** and 3% of **1a**, **1b**, or **3** were hydrolyzed in aqueous buffer, at pH 7.2, within 24 h. Nevertheless, BBHCM-caged carboxylic acids as well as BBHCMOC-caged amines and phenols should be sufficiently stable with respect to the time needed for physiological experiments.

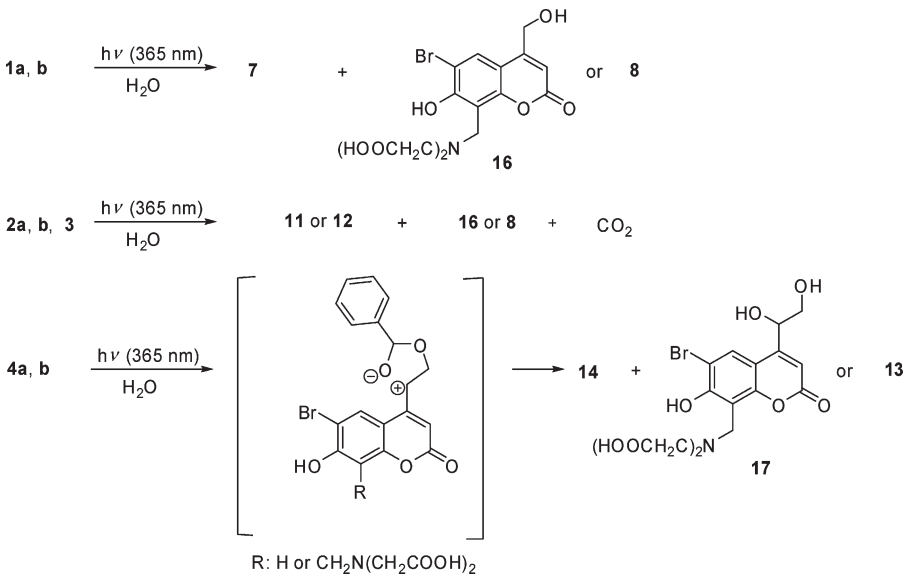
TABLE 1. Selected Photophysical and Chemical Properties of the Caged Compounds 1−4 and of 8 and 16 in CH_3CN/HEPES−KCl Buffer (5:95), pH 7.2

compd	λ_{abs}^{max}[a] (nm)	ε^{max}[b] (M^{-1} cm^{-1})	c_s[c] (μM)	ϕ_{chem}[d]	λ_f^{max}[e] (nm)	ϕ_f[f]	τ_f[g] (ns)
1a	375	17600	> 2000	0.10	472	0.08	0.66
1b	375	17200	21	0.10	476	0.04	0.44
2a[h]	372	18000	> 10000	0.12	470	0.12	1.37
2b[h]	372	17400	100	0.10	471	0.11	1.04
3	376	17000	500	0.06	468	0.03	n.d.
4a	372	18500	≫500	0.14	470	0.36	n.d.
4b	371	18300	62	0.06[i]	476	0.58	4.37
8	367	17600	n.d.		471	0.60	4.21
16	369	18200	n.d.		465	0.57	4.72

[a]Long-wavelength absorption maxima. [b]Extinction coefficients at the absorption maxima. [c]Concentration at saturation. [d]Photochemical quantum yields for disappearance of the caged compound upon 365 nm irradiation. [e]Fluorescence maxima. [f]Fluorescence quantum yields. [g]Fluorescence lifetimes of the lowest excited singlet state. [h]Measured in CH_3CN/phosphate buffer (5:95), pH 7.2. [i]Literature value 0.06.[10] n.d. = not determined.

Irradiation of all prepared caged derivatives **1**−**4** with light of wavelengths of 333−420 nm leads to photodeprotection of the compounds (Scheme 4). Photoactivation of **1a** and **1b** gives directly **7** and 8-[bis(carboxymethyl)aminomethyl]-6-bromo-7-hydroxy-4-(hydroxymethyl)coumarin (**16**) and compound **8**, respectively. Upon irradiation with light, the phototriggers **2a**,**b** and **3** release **16** or **8** as well as unstable carbamate or carbonate intermediates of the biomolecules **11** or **12**. Compounds **11** and **12** are formed in a second step by decarboxylation of the unstable

SCHEME 4. Photolysis of the Phototriggers 1−4



intermediates. Photolysis of **4a**,**b** leads to **14** and the diols **17** or **13**. Here, the aldehyde formation should proceed via a zwitterionic hemiacetal.[10] The photoreactions of the caged compounds **1**−**3** are efficient and clean and generate about 85−95% of the biomolecules. The extent of benzaldehyde release from **4a** and **4b** is lower. We found about 45−50% for both caged compounds. Other benzaldehyde derivatives were not detected. This observation is consistent with the result obtained with the Bhc derivative **4b**.[10]

We assume that the photochemical conversion proceeds in analogy to the photocleavage of other coumarinylmethyl derivatives via a photochemical S_N1 mechanism.[10,18−20] This is supported by the fact that photocleavages are more efficient in aqueous buffer than in nonpolar solvents (data not shown) and that the primary reaction steps are very fast. Time-resolved fluorescence measurements upon single-pulse excitation (0.5 ns half-width, 337 nm) of the compounds **1a**,**b** and **2a**,**b** revealed fluorescence contributions from the alcohols **8** or **16**. Because pure solutions of the phototriggers were irradiated, **8** and **16** must have been formed and excited during the photolysis pulse. Inserting the ϕ_{chem} values and the fluorescence quantum yields ϕ_f of **1a**,**b**, **2a**,**b**, and **8** or **16** in the kinetic scheme developed by us for other coumarinylmethyl esters[19,20] yields rate constants of about $1-3 \times 10^9$ s^{-1} for the heterolytic bond cleavage of **1a**,**b** or **2a**,**b**. Differences between Bhc and BBHC derivatives were not observed. For compound **3**, we assume a similar rate constant. The rate constants for the zwitterionic hemiacetal formations of **4a** and **4b** should correspond to that of Bhc-ED-caged progesterone[15] which is in the order of approximately 1×10^8 s^{-1}. However, we note that in the case of the phototriggers **2**−**4** the experiments do not allow conclusions regarding the rate of the secondary reaction, the release of **11**, **12**, or **14**. Compounds **11** and **12** were formed upon decarboxylation that is rate limiting. On the basis of the measured decarboxylation rates upon photolysis of other carbamate-caged amines and carbonate-caged phenols, the release of **11** and 12 should occur with rate constants of about > 100 s^{-1}.[21−24] The rate of the hemiacetal cleavage with release of **14** is at present unknown, and further work will be required to determine it.

Table 1 lists photophysical and photochemical data of caged compounds **1**−**4** and the alcohols **8** and **16** in aqueous buffer (pH 7.2). The absorption spectra show long-wavelength maxima at 367−376 nm, and the extinction coefficients are high. This allows uncaging within cells under nondamaging light conditions at this pH. However, absorptions are pH dependent. Only the deprotonated phenolate form of the coumarin chromophore gives the long-wavelength absorption. Decreasing the pK_a of the C7 hydroxyl group leads to an increase in the molar fraction of the ionized form and thus permits photorelease at lower pH values. The pK_a of the C7 hydroxyl of the Bhc chromophore in H_2O is 6.2,[11] and we estimated for the C7 hydroxyl group of the bis(carboxymethyl)aminomethyl-substituted derivative **16** a pK_a of 4.9. Consequently, only 50% of the Bhc chromophore is deprotonated at pH 6.0, whereas from the BBHC chromophore at pH 6.0 90% exists in the ionized (phenolate) form. Thus, introduction of the bis(carboxymethyl)aminomethyl substituent in position 8 permits effective photocleavage also at the lower end of the physiological pH range. Lowering of the pK_a of the coumarin phenolic proton is due to the aminomethyl group that can form an intramolecular hydrogen bond with the phenolic hydroxyl group.[17]

The single-photon photochemical quantum yields for disappearance of **1**−**4**, ϕ_{chem}, are reported in Table 1. The values were measured at conversion between 5 and 10% to avoid internal filter effects. They are relatively high. The

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uncaging quantum yields of the BBHC derivatives **1a** and **2a** are comparable with those of the Bhc compounds **1b** and **2b**. Taking into account that the product $\varepsilon\phi$ is proportional to the amount of the product release for a given photon exposure, the compounds have practically useful $\varepsilon\phi$ values due to their acceptable quantum yields and their high extinction coefficients. Remarkable is the photolysis quantum yield of the caged aldehyde **4a**, which corresponds to a 130% increase over that of **4b**. We also observed a clear increase of the quantum yields at introduction of an additional carboxymethylaminomethyl substituent in the Bhc chromophore with other photoprotected carbonyl compounds.[25]

The fluorescence quantum yields, ϕ_f, of **1**−**3** are significantly smaller than those of **4a** and **4b** and those of the photoreleased alcohols **8**, **16** (Table 1), and **17**.[25] Thus, fluorescence spectroscopic visualization of the progress of the photorelease from Bhc- and BBHCM-caged carboxylic acids, as well as Bhc-methoxycarbonyl- and BBHCMOC-caged amines and alcohols, within cells becomes possible, as previously demonstrated by using [6,7-[bis(carboxymethyl)-amino]coumarin-4-yl]-caged cyclic nucleotides.[26] Fluorescence decay times of the lowest singlet excited states of the compounds, τ_f, are about 0.5−1.5 ns for the caged compounds **1** and **2** and are somewhat longer for the caged aldehyde **4b** as well as for the alcohols **8** and **16** (Table 1). The comparison of the values of **1a**, **2a**, or **8** with those of the in position 8 unsubstituted analogues **1b**, **2b**, and **16** shows only small differences.

Some 7-hydroxy-8-(*N*-alkylaminomethyl)coumarins have strong chelation properties to metal ions.[18] We found exemplarily with compound **16** at pH values between 5 and 12 no or only small shifts in its excitation and/or emission spectrum upon addition of an excess of Ca^{2+} ions (data not shown) indicating that no disturbances are expected in biological studies with BBHCM-caged biomolecules which include Ca^{2+} ions release.

Conclusions

We evaluated the usefulness of the novel BBHCM/BBHCMOC/BBHC-ED caging moieties for the designing of phototriggers for biomolecules. Our results demonstrate that the moieties are very useful protecting groups. They allow caging and uncaging of the most important functional groups of biological relevance as shown exemplarily for the derivatives **1a**, **2a**, **3**, and **4a**. The phototriggers can be easily synthesized and possess favorable properties. They are sufficiently resistant to hydrolysis, are well soluble in aqueous buffer, and permit efficient photorelease at lower pH than the corresponding Bhc derivatives. Photocleavage is efficient upon long-wavelength UV/vis excitation. Compared to the introduced Bhc-ED caging group the novel BBHC-ED moiety shows besides their strongly increased water solubility higher photochemical quantum yields. The primary step of the photocleavages is very fast that leads us to assume that photocleavages of Bhc- and BBHCM-protected molecules proceed as previously described for other coumarinylmethyl-caged compounds.[18−20,27]

Experimental Methods

Materials. All starting materials were commercially available and used without further purification. Thin-layer chromatography (TLC) was performed using silica gel 60 F_{254} precoated plates. Flash chromatography was performed using silica gel 60 (30−60 μm). CH_3CN was HPLC grade. Water was purified with a Milli-Q-Plus system. 6-Bromo-7-hydroxy-4-(hydroxymethyl)-coumarin (**8**) was prepared using the procedure described by Furuta et al.[7] (6-Bromo-7-hydroxycoumarin-4-yl)ethane-1,2-diol (Bhc-ED, **13**) and 4-(6-bromo-7-hydroxycoumarin-4-yl)-2-phenyl-1,3-dioxolane (Bhc-ED-caged benzaldehyde, **4b**) were synthesized as reported by Lu et al.[10]

Instrumentation. ^{1}H NMR and ^{13}C NMR spectra were recorded on NMR spectrometers operating at 300 and 75.5 MHz, respectively. Chemical shifts (δ) are given in parts per million (ppm) using the residue solvent peaks as reference relative to TMS. *J* values are given in Hz. High-resolution mass spectra (HRMS) were recorded using electrospray ionization (ESI) mass spectrometry in the positive or negative ionization mode. Analytical reversed-phase HPLC (RP-HPLC) was carried out on an HPLC system (flow rate: 1 mL min^{-1}) equipped with a DAD-UV detector and a fluorescence detector (λ_{exc} = 380 nm, λ_{em} = 495 nm) using a Nucleodur 100-5 C18 ec column (100 Å, 5 μm, 250 mm × 4 mm). Preparative RP-HPLC was run on an HPLC system (flow rate: 10 mL min^{-1}) with a UV detector (λ_{exc} = 254 nm) over a Nucleodur 100-5 C18 ec column (100 Å, 5 μm, 250 mm × 21 mm). One-photon photolysis of all synthesized photoprotected compounds in solution was performed by using a high-pressure mercury lamp (HBO 500, O) with controlled light intensity and metal interference transmission filter (365 nm). For all experiments, UV and fluorescence quartz cuvettes with a path length of 1 cm and a cross-sectional area of 1 cm^2 were used. During irradiation, the solutions in the cuvettes were mixed by a magnetic stirrer. All synthetic and analytical procedures were performed under N_2 in darkness or under yellow light provided by sodium vapor lamps. The melting points are uncorrected.

6-Bromo-4-(chloromethyl)-7-hydroxycoumarin (5). To 4-bromoresorcinol (10 g, 52.9 mmol) in methanesulfonic acid (80 mL) was added ethyl 4-chloroacetoacetate (10.8 mL, 79.4 mmol). The mixture was stirred for 2 h at room temperature, poured into ice−water, and stirred for 1 h. The precipitate was filtered off, washed with cold water, and dried under vacuum to yield **5** as a colorless solid (13.8 g, 91%): mp 158−159 °C; TLC R_f=0.47 (*n*-hexane/AcOEt/AcOH, 49.5:49.5:1 v/v); t_R = 14.08 min (analytical HPLC, 5−95% B in A in 20 min, eluent A, H_2O/0.1%TFA; eluent B, CH_3CN); ^{1}H NMR (300 MHz, DMSO-d_6) δ = 4.99 (s, 2H), 6.47 (s, 1H), 6.92 (s, 1H), 7.99 (s, 1H), 11.53 (s, 1H); ^{13}C NMR (75.5 MHz, DMSO-d_6) δ = 41.2, 103.3, 106.1, 110.7, 112.1, 129.0, 150.1, 154.0, 157.5, 159.6; HRMS (ESI) $C_{10}H_6BrClO_3$, *m*/*z* $[M + H]^+$ calcd 288.9267, found 288.9261.

8-[Bis(*tert*-butoxycarbonylmethyl)aminomethyl]-6-bromo-4-(chloromethyl)-7-hydroxycoumarin (6). Paraformaldehyde (450 mg, 15 mmol CH_2O) and KOH (30 mg, 0.53 mmol) were dissolved in EtOH (3 mL) and cooled to 0 °C, and then di-*tert*-butyl iminodiacetate (3.68 g, 15 mmol) was added. The reaction mixture was stirred for 1 h at room temperature and then added dropwise to a solution of **5** (869 mg, 3 mmol) in EtOH (12 mL). The reaction mixture was refluxed for 3 h. After cooling, the precipitated solid was filtered off, washed with cold EtOH, recrystallized from EtOH, and dried under vacuum over P_2O_5.

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Compound **6** was obtained as a colorless solid (1.14 g, 62.3%): mp 74−75 °C; TLC R_f = 0.69 ($CHCl_3$/MeOH, 9:1 v/v); t_R = 16.41 min (analytical HPLC, 20−95% B in A in 20 min, eluent A, H_2O/0.1%TFA; eluent B, CH_3CN); ^1H NMR (300 MHz, DMSO-d_6) δ = 1.42 (s, 18H), 3.46 (s, 4H), 4.17 (s, 2H), 5.00 (s, 2H), 6.49 (s, 1H), 7.99 (s, 1H), 11.99 (bs, 1H); ^{13}C NMR (75.5 MHz, DMSO-d_6) δ = 27.7 (6C), 41.2, 48.0, 54.7 (2C), 81.4 (2C), 106.0, 110.2, 110.4, 112.0, 127.8, 150.4, 151.9, 157.3, 159.1, 169.8 (2C); HRMS (ESI) $C_{23}H_{29}BrClNO_7$, m/z [M + H]$^+$ calcd 546.0889, found 546.0888.

8-[Bis(*tert*-butoxycarbonylmethyl)aminomethyl]-6-bromo-7-hydroxy-4-(hydroxymethyl)coumarin (9). Paraformaldehyde (300 mg, 10 mmol CH_2O) and KOH (20 mg, 0.36 mmol) were dissolved in EtOH (2 mL) and cooled to 0 °C, and then di-*tert*-butyl iminodiacetate (2.45 g, 10 mmol) was added. The reaction mixture was stirred for 1 h at room temperature and then added dropwise to a solution of **8** (869 mg, 3 mmol) in EtOH (8 mL). The reaction mixture was refluxed for 2 h, the solvent was evaporated, and the residue was purified by flash chromatography. Elution using *n*-hexane/AcOEt (9:1 to 3:1, v/v) yielded **9** (963 mg, 65%) as a colorless solid: mp 158−159 °C; TLC R_f = 0.49 (*n*-hexane/AcOEt, 1:1 v/v); t_R = 16.58 min (analytical HPLC, 20−95% B in A in 20 min, eluent A, H_2O/0.1% TFA; eluent B, CH_3CN); ^1H NMR (300 MHz, DMSO-d_6) δ = 1.42 (s, 18H), 3.46 (s, 4H), 4.16 (s, 2H), 4.70 (s, 2H), 5.61 (s, 1H), 6.28 (s, 1H), 7.83 (s, 1H), 11.74 (bs, 1H); ^{13}C NMR (75.5 MHz, DMSO-d_6) δ = 27.7 (6C), 47.8, 54.6 (2C), 59.1, 81.4 (2C), 105.9, 107.6, 110.0, 110.8, 127.0, 151.5, 156.2, 156.9, 159.6, 169.8 (2C); HRMS (ESI) $C_{23}H_{30}BrNO_8$, m/z [M + H]$^+$ calcd 528.1228, found 528.1236.

8-[Bis(carboxymethyl)aminomethyl]-6-bromo-7-hydroxy-4-(hydroxymethyl)coumarin (16). Compound **9** (38.8 mg, 0.073 mmol) was stirred in a mixture (4 mL) of TFA/CH_2Cl_2/H_2O (75:24:1) for 1 h at 40 °C. The solvents were evaporated, and the residue was purified by preparative RP-HPLC using a linear gradient of 5−50% B in A over 40 min (eluent A, H_2O/0.1% TFA; eluent B CH_3CN, t_R = 28.3 min). Lyophilization gave the desired product (14 mg, 46%) as colorless solid: mp > 219 °C dec; t_R = 8.93 min (analytical HPLC, 5−50% B in A in 20 min, eluent A, H_2O/0.1% TFA; eluent B, CH_3CN); ^1H NMR (300 MHz, DMSO-d_6) δ = 3.50 (s, 4H), 4.19 (s, 2H), 4.70 (s, 2H), 5.60 (s, 1H), 6.28 (s, 1H), 7.82 (s, 1H), 12.61 (s, 2H); ^{13}C NMR (75.5 MHz, DMSO-d_6) δ = 47.9, 53.5 (2C), 59.1, 105.9, 107.6, 110.0, 110.7, 126.9, 151.6, 156.2, 157.1, 159.7, 172.2 (2C); HRMS (ESI) $C_{15}H_{14}BrNO_8$, m/z [M + H]$^+$ calcd 415.9976, found 415.9977.

[8-[Bis(*tert*-butoxycarbonylmethyl)aminomethyl]-6-bromo-7-hydroxycoumarin-4-yl]methyl Phenylacetate. 1,8-Diazabicyclo-[5.4.0]-7-undecene (DBU) (75 μL, 0.5 mmol) and phenylacetic acid (47.7 mg, 0.35 mmol) were added to a stirred solution of compound **6** (54.7 mg, 0.1 mmol) in benzene (1 mL). The reaction mixture was refluxed for 2 h, evaporated, and purified by preparative RP-HPLC using a linear gradient 50−95% B in A in 60 min (eluent A, H_2O/0.1% TFA; eluent B, CH_3CN, t_R = 54.9 min). Lyophilization gave the desired product (44.7 mg, 69%) as a colorless solid: mp 91−94 °C; TLC R_f = 0.88 (*n*-hexane/THF, 1:1 v/v); t_R = 16.70 min (analytical HPLC, 50−95% B in A in 20 min, eluent A, H_2O/0.1% TFA; eluent B, CH_3CN); ^1H NMR (300 MHz, DMSO-d_6) δ = 1.42 (s, 18H), 3.46 (s, 4H), 3.86 (s, 2H), 4.16 (s, 2H), 5.37 (s, 2H), 6.17 (s, 1H), 7.25−7.37 (m, 5H), 7.85 (s, 1H), 11.90 (bs, 1H); ^{13}C NMR (75.5 MHz, DMSO-d_6) δ = 27.7 (6C), 40.1, 47.9, 54.6 (2C), 61.4, 81.5 (2C), 106.1, 108.9, 110.0, 110.2, 127.0, 127.4, 128.4 (2C), 129.4 (2C), 134.0, 150.0, 151.6, 157.3, 159.0, 169.8 (2C)), 170.6; HRMS (ESI) $C_{31}H_{37}BrNO_9$, m/z [M + H]$^+$ calcd 646.1646, found 646.1631.

[8-[Bis(carboxymethyl)aminomethyl]-6-bromo-7-hydroxycoumarin-4-yl]methyl Phenylacetate (1a). The bis-*tert*-butyl ester (60 mg, 0.09 mmol) described above was stirred in a mixture (3 mL) of TFA/CH_2Cl_2/H_2O (75:24:1) for 1 h at room temperature and 0.5 h at 40 °C. The solvents were evaporated, and the residue was coevaporated two times with diethyl ether, dissolved in CH_3CN/H_2O, and lyophilized to give **1a** (17.2 mg, 95%) as yellow solid: mp > 192 °C dec; t_R = 4.4 min (analytical HPLC, 50−95% B in A in 20 min, eluent A, H_2O/0.1% TFA; eluent B, CH_3CN); ^1H NMR (300 MHz, DMSO-d_6) δ = 3.50 (s, 4H), 3.87 (s, 2H), 4.18 (s, 2H), 5.37 (s, 2H), 6.16 (s, 1H), 7.26−7.37 (m, 5H), 7.85 (s, 1H), 12.71 (s, 2H); ^{13}C NMR (75.5 MHz, DMSO-d_6) δ = 40.0, 47.9, 53.5 (2C), 61.5, 106.2, 108.8, 110.1, 110.2, 127.0, 127.3, 128.5 (2C), 129.4 (2C), 134.1, 150.1, 151.7, 157.5, 159.2, 170.7, 172.3 (2C); HRMS (ESI) $C_{23}H_{21}BrNO_9$, m/z [M + H]$^+$ calcd 534.0394, found 534.0389.

(6-Bromo-7-hydroxycoumarin-4-yl)methyl Phenylacetate (1b). DBU (75 μL, 0.5 mmol) and phenylacetic acid (47.7 mg, 0.35 mmol) were added to a stirred solution of compound **5** (29 mg, 0.1 mmol) in benzene (1 mL). The reaction mixture was refluxed for 4 h, evaporated, and purified by preparative RP-HPLC using a linear gradient 20−95% B in A in 60 min (eluent A, H_2O/0.1% TFA; eluent B, CH_3CN, t_R = 42.6 min). Lyophilization gave the desired product (34 mg, 87.4%) as a colorless solid: mp 179−182 °C; TLC R_f = 0.67 (*n*-hexane/AcOEt, 1:1 v/v); t_R = 13.50 min (analytical HPLC, 20−95% B in A in 20 min, eluent A, H_2O/0.1% TFA; eluent B, CH_3CN); ^1H NMR (300 MHz, DMSO-d_6) δ = 3.86 (s, 2H), 5.36 (s, 2H), 6.15 (s, 1H), 6.90 (s, 1H), 7.25−7.37 (m, 5H), 7.86 (s, 1H), 11.48 (s, 1H); ^{13}C NMR (75.5 MHz, DMSO-d_6) δ = 40.0, 61.3, 103.1, 106.2, 108.9, 110.4, 127.0, 128.4 (2C), 128.5, 129.4 (2C), 134.0, 149.7, 153.7, 157.4, 159.5, 170.6; HRMS (ESI): $C_{18}H_{13}BrO_5$, m/z [M + H]$^+$ calcd 389.0019, found 389.0020.

***N*-[[8-[Bis(*tert*-butoxycarbonylmethyl)aminomethyl]-6-bromo-7-hydroxycoumarin-4-yl]methoxycarbonyl]dopamine.** DIPEA (40 μL, 0.23 mmol) and 4-nitrophenyl chloroformate (46.2 mg, 0.23 mmol) were added to a stirred solution of **9** (101 mg, 0.19 mmol) in THF (15 mL). The reaction mixture was stirred for 1.5 h, filtered, and evaporated. The residue containing the intermediately formed activated carbonate **10** was dissolved in DMF (20 mL), after which dopamine (**11**, 43.6 mg, 0.23 mmol) and DMAP (56 mg, 0.46 mmol) were added. The reaction mixture was stirred for 1 h at room temperature, evaporated, and purified by preparative RP-HPLC using a linear gradient 50−95% A in B in 40 min (eluent A, H_2O/0.1% TFA; eluent B, CH_3CN, t_R = 22.95 min). Lyophilization gave the desired product (52.4 mg, 39%) as a colorless solid: mp 81−82 °C; TLC R_f = 0.43 (CH_2Cl_2/MeOH, 19:1 v/v); t_R = 16.58 min (analytical HPLC, 20−95% B in A in 20 min, eluent A, H_2O/0.1% TFA; eluent B, CH_3CN); ^1H NMR (300 MHz, DMSO-d_6) δ = 1.43 (s, 18H), 2.56 (t, J = 7.7, 2H), 3.15−3.20 (m, 2H), 3.46 (s, 4H), 4.17 (s, 2H), 5.26 (s, 2H), 6.20 (s, 1H), 6.43 (d, J = 6.9, 1H), 6.58 (s, 1H), 6.63 (d, J = 7.8, 1H), 7.57 (t, J = 5.6, 1H), 7.87 (s, 1H), 8.62 (s, 1H), 8.72 (s, 1H); ^{13}C NMR (75.5 MHz, DMSO-d_6): δ = 27.7 (6C), 34.8, 42.4, 47.9, 54.6 (2C), 60.8, 81.5 (2C), 106.1, 108.4, 110.1, 110.3, 115.5, 115.9, 119.2, 127.2, 129.9, 143.6, 145.1, 151.5, 151.6, 155.2, 157.3, 159.2, 169.8 (2C); HRMS (ESI) $C_{32}H_{39}BrN_2O_{11}$, m/z [M + H]$^+$ calcd 707.1815, found 707.1830.

***N*-[[8-[Bis(carboxymethyl)aminomethyl]-6-bromo-7-hydroxycoumarin-4-yl]methoxycarbonyl]dopamine (BBHCMOC-Caged Dopamine, 2a).** The bis-*tert*-butyl ester (60 mg, 0.09 mmol) described above was stirred in a mixture (4 mL) of TFA/CH_2Cl_2/H_2O (75:24:1) for 12 h at 40 °C. The solvents were evaporated, and the residue was purified by preparative RP-HPLC using a linear gradient 20−95% B in A in 40 min (eluent A, H_2O/0.1% TFA; eluent B, CH_3CN, t_R = 13.8 min). Lyophilization gave the desired product (46.1 mg, 86%) as colorless solid: mp 200−201 °C dec; t_R = 6.76 min (analytical HPLC, 20−95% B in A in 20 min, eluent A, H_2O/0.1% TFA; eluent B, CH_3CN); ^1H NMR (300 MHz, DMSO-d_6) δ = 2.55 (t, J = 7.5,

1H), 3.13−3.20 (m, 2H), 3.51 (s, 4H), 4.20 (s, 2H), 5.26 (s, 2H), 6.20 (s, 1H), 6.44 (d, J = 7.5, 1H), 6.58 (s, 1H), 6.63 (d, J = 7.8, 1H), 7.57 (t, J = 5.4, 1H), 7.85 (s, 1H), 8.63 (bs, 1H), 8.71 (bs, 1H), 12.67 (bs, 2H); ^{13}C NMR (75.5 MHz, DMSO-d_6) δ = 34.8, 42.4, 47.9, 53.5 (2C), 60.8, 106.1, 108.3, 110.2 (2C), 115.5, 115.9, 119.2, 127.1, 129.9, 143.6, 145.1, 151.5, 151.7, 155.2, 157.4, 159.3, 172.2 (2C); HRMS (ESI) $C_{24}H_{23}BrN_2O_{11}$, m/z [M + H]$^+$ calcd 595.0558, found 595.0547.

***N*-[(6-Bromo-7-hydroxycoumarin-4-yl)methoxycarbonyl]-dopamine (2b).** Compound **8** (1.19 g, 4.40 mmol), 4-nitrophenyl chloroformate (1.06 g, 5.28 mmol), and DIPEA (919 μL, 5.28 mmol) in THF (15 mL) were stirred for 1 h at room temperature, and then the solvent was evaporated. The formed (6-bromo-7-hydroxycoumarin-4-yl)methyl 4-nitrophenyl carbonate, dopamine (**11**, 1.0 g, 5.28 mmol), and DMAP (1.29 g, 10.55 mmol) were dissolved in DMF (20 mL), and the mixture was stirred for 8 h at room temperature. The solvent was evaporated and the residue purified by flash chromatography. Elution using CH_2Cl_2/MeOH (50:1 to 10:1, v/v) yielded **2b** (897 mg, 45%) as a colorless solid: mp 183−184 °C; TLC R_f = 0.13 (*n*-hexane/AcOEt, 1:1, v/v); t_R = 8.89 min (analytical HPLC, 20−95% B in A in 20 min, eluent A, H_2O/0.1% TFA; eluent B, CH_3CN); ^{1}H NMR (300 MHz, DMSO-d_6) δ = 2.55 (t, J = 7.2, 2H), 3.16 (dt, J = 7.0 and 6.4, 2H), 5.25 (s, 2H), 6.19 (s, 1H), 6.43 (d, J = 7.8, 1H), 6.58 (s, 1H), 6.63 (d, J = 8.0, 1H), 6.92 (s, 1H), 7.57 (t, J = 5.4, 1H), 7.87 (s, 1H), 8.62 (s, 1H), 8.71 (s, 1H), 11.48 (bs, 1H); ^{13}C NMR (75.5 MHz, DMSO-d_6) δ = 34.8, 42.4, 60.8, 103.2, 106.2, 108.4, 110.5, 115.5, 115.9, 119.2, 128.4, 129.9, 143.6, 145.1, 151.2, 153.8, 155.2, 157.5, 159.7; HRMS (ESI) $C_{19}H_{16}BrNO_7$, m/z [M + H]$^+$ calcd 450.0183, found 450.0181.

(*E*)-[8-[Bis(*tert*-butoxycarbonylmethyl)aminomethyl]-6-bromo-7-hydroxycoumarin-4-yl]methyl 2-Methoxy-4-[(8-methylnon-6-enamido)methyl]phenyl Carbonate. The activated carbonate **10** was prepared from **9** (264.2 mg, 0.5 mmol), 4-nitrophenyl chloroformate (121 mg, 0.6 mmol), and DIPEA (171.2 μL, 1.0 mmol) in THF (10 mL) by stirring for 4.5 h and workup as described for the bis-*tert*-butyl ester of **2a**. Dissolution of **10** in CH_2Cl_2 (10 mL), addition of DMAP (73.3 mg, 0.6 mmol) and capsaicin (**12**, 183.3 mg, 0.6 mmol), stirring overnight at room temperature, evaporation, and purification by flash chromatography (*n*-hexane/AcOEt 4:1 to 2:3, v/v) gave the desired product (300 mg, 69.8%) as a colorless solid: mp 67−70 °C; TLC R_f = 0.54 (*n*-hexane/THF, 1:1, v/v); t_R = 18.66 min (analytical HPLC, 50−95% B in A in 20 min, eluent A, H_2O/0.1% TFA; eluent B, CH_3CN); ^{1}H NMR (300 MHz, DMSO-d_6) δ = 0.92 (d, J = 6.7, 6H), 1.33 (quintet, J = 7.4, 2H), 1.43 (s, 18H), 1.52 (quintet, J = 7.4, 2H), 1.94 (q, J = 7.0, 2H), 2.07−2.25 (m, 3H), 3.47 (s, 4H), 3.78 (s, 3H), 4.18 (s, 2H), 4.26 (d, J = 5.8, 2H), 5.26−5.41 (m, 2H), 5.55 (s, 2H), 6.27 (s, 1H), 6.84 (dd, J = 8.1 and 2.2, 1H), 7.04 (s, 1H), 7.20 (d, J = 8.2, 1H), 7.91 (s, 1H), 8.31 (t, J = 5.9, 1H); ^{13}C NMR (75.5 MHz, DMSO-d_6) δ = 22.5 (2C), 24.8, 27.7 (6C), 28.7, 30.3, 31.6, 35.2, 41.7, 47.9, 54.6 (2C), 55.7, 65.2, 81.5 (2C), 106.2, 108.8, 110.0, 110.2, 111.8, 119.1, 121.8, 126.6, 127.4, 137.3, 138.1, 139.5, 149.3, 150.3, 151.7, 152.2, 157.4, 159.0, 169.8 (2C), 172.1; HRMS (ESI) $C_{42}H_{55}BrN_2O_{12}$ m/z [M + H]$^+$ calcd 859.3011, found 859.3013.

(*E*)-[8-[Bis(carboxymethyl)aminomethyl]-6-bromo-7-hydroxycoumarin-4-yl]methyl 2-Methoxy-4-[(8-methylnon-6-enamido)-methyl]phenyl Carbonate (BBHCMOC-Caged Capsaicin, 3). Compound **3** was prepared by deprotection of the bis-*tert*-butyl ester of **3** (100 mg, 0.116 mmol) with a mixture (15 mL) of TFA/CH_2Cl_2/H_2O (75:24:1) by stirring for 2 h at room temperature. Workup as described for **1a** gave **3** (82 mg, 95%) as a colorless solid: mp 175−177 °C; t_R = 14.6 min (analytical HPLC, 20−95% B in A in 20 min, eluent A, H_2O/0.1% TFA; eluent B, CH_3CN); ^{1}H NMR (300 MHz, DMSO-d_6) δ = 0.92 (d, J = 6.76, 6H), 1.33 (quintet, J = 7.4, 2H), 1.52 (quintet, J = 7.4, 2H), 1.94 (q, J = 7.0, 2H), 2.12−2.25 (m, 3H), 3.52 (s, 4H), 3,79 (s, 3H), 4.21 (s, 2H), 4.26 (d, J = 5.8, 2H), 5.26−5.41 (m, 2H), 5.55 (s, 2H), 6.26 (s, 1H), 6.84 (d, J = 8.1, 1H), 7.04 (s, 1H), 7.20 (d, J = 8.1, 1H), 7.90 (s, 1H), 8.31 (t, J = 5.8, 1H), 12.69 (bs, 2H); ^{13}C NMR (75.5 MHz, DMSO-d_6) δ = 22.5 (2C), 24.8, 28.7, 30.3, 31.7, 35.2, 41.7, 47.9, 53.5 (2C), 55.7, 65.2, 106.2, 108.7, 109.8, 110.3, 111.8, 119.1, 121.8, 126.6, 127.2, 137.3, 138.1, 139.5, 149.3, 150.3, 151.7, 152.2, 157.7, 159.2, 172.1, 172.2 (2C); HRMS (ESI): $C_{34}H_{39}BrN_2O_{12}$, m/z [M − H]$^-$ calcd 745.1614, found 745.1684.

4-[-8-[Bis(methoxycarbonylmethyl)aminomethyl]-6-bromo-7-hydroxycoumarin-4-yl]-2-phenyl-1,3-dioxolane (15). Paraformaldehyde (120 mg, 4.0 mmol CH_2O) and KOH (235 mg, 4.0 mmol) were dissolved in EtOH (10 mL) and cooled to 0 °C, and then dimethyl iminodiacetate hydrochloride (790 mg, 4.0 mmol) was added. The reaction mixture was stirred for 1 h at room temperature and then added dropwise to a solution of **4b** (200 mg, 0.66 mmol) in EtOH (10 mL). The reaction mixture was heated to reflux for 7 h, the solvent was evaporated, and the resulting crude product was purified by preparative RP-HPLC by using a linear gradient of 30−95% B in A in 60 min (eluent A, H_2O/0.1% TFA; eluent B, CH_3CN). The main fraction with t_R = 58.45−62.00 min was collected, evaporated, redissolved in CH_3CN/H_2O, and lyophilized to give the desired product (colorless solid, 100 mg, 27%) as diastereomer mixture: mp > 120 °C dec; TLC R_f = 0.37 (*n*-hexane/AcOEt 2:1 v/v); t_R = 18.32 and 18.54 min (analytical HPLC: 5−95% B in A in 20 min, eluent A, H_2O/0.1% TFA; eluent B, CH_3CN); ^{1}H NMR (300 MHz, DMSO-d_6) δ = 3.63 (s, 4H), 3.66 (s, 6H), 3.73−3.78/4.02−4.08[a] (m, 1H), 4.18/4.19[a] (s, 2H), 4.56/4.79[a] (t, J = 8.0, 1H), 5.64−5.73 (m, 1H), 5.96/6.12[a] (s, 1H), 6.22/6.45[a] (s, 1H), 7.44−7.49 (m, 3H), 7.55−7.60 (m, 2H), 7.90 (s, 1H), 11.77 (s, 1H); ^{13}C NMR (75.5 MHz, DMSO-d$_6$) δ = 47.9, 51.8 (2C), 53.6 (2C), 70.0/70.2[a], 72.9/73.0[a], 103.8/103.9[a], 106.3, 107.0, 107.9, 110.4, 110.5, 126.8 (2C), 127.3, 128.3, 128.6, 129.5/129.7[a], 136.3/137.4[a], 152.0, 154.2/154.3[a], 157.1/157.2[a], 159.4/159.5[a], 171.2 (2C); HRMS (ESI) $C_{25}H_{24}BrNO_9$ m/z [M + H]$^+$ calcd 562.0713, found 562.0704. [a]Diastereomers gave varying single signals.

4-[8-[Bis(carboxymethyl)aminomethyl]-6-bromo-7-hydroxycoumarin-4-yl]-2-phenyl-1,3-dioxolane (BBHC-ED-Caged Benzaldehyde, 4a). Compound **15** (100 mg, 0.18 mmol) was dissolved in THF/H_2O (5 mL, 1:1 v/v). LiOH × H_2O (15.1 mg, 0.36 mmol) was added, and the solution was stirred for 24 h at room temperature. The solvent was evaporated, and the resulting crude product was purified by preparative RP-HPLC using a linear gradient of 5−95% B in A in 60 min (eluent A, H_2O/0.1% TFA; eluent B, CH_3CN). The main fraction with t_R = 35.37−41.72 min was collected, evaporated, redissolved in CH_3CN/H_2O, and lyophilized to give the desired diastereomer mixture (46 mg, 48%) as a colorless solid: mp > 200 °C dec; t_R = 14.45 and 14.67 min (analytical HPLC: 5−95% B in A in 20 min, eluent A, H_2O/0.1% TFA; eluent B, CH_3CN); ^{1}H NMR (300 MHz, DMSO-d_6) δ = 3.52 (s, 4H) 3.74−3.79/4.02−4.06[a] (m, 1H), 4.21/4.22[a] (s, 2H), 4.56/4.79[a] (t, J = 8.0, 1H), 5.64−5.72 (m, 1H), 5.96/6.12[a] (s, 1H), 6.22/6.44[a] (s, 1H), 7.44−7.48 (m, 3H), 7.55−7.57 (m, 2H), 7.89 (s, 1H), 12.67 (bs, 2H); ^{13}C NMR (75.5 MHz, DMSO-d_6) δ = 48.0, 53.5 (2C), 70.0/70.2[a], 72.9/73.0[a], 103.8/103.9[a], 106.3, 106.9, 107.7, 110.2, 110.3, 126.7 (2C), 127.1, 128.3, 128.5, 129.4/129.7[a], 136.3/137.4[a], 152.0, 154.1/154.2[a], 157.4/157.5[a], 159.4/159.5[a], 172.1 (2C); HRMS (ESI) $C_{23}H_{20}BrNO_9$ m/z [M − 3H + 2Na]$^-$ calcd 575.9888, found 575.9921. [a]Diastereomers gave varying single signals.

Solubility. Saturated solutions of compounds **1**−**4** in CH_3CN/HEPES buffer (10 mM HEPES, 120 mM KCl adjusted to pH 7.2 with 2 N KOH) (5:95 v/v) were analyzed by analytical RP-HPLC at room temperature.

Hydrolytic Stability. Freshly prepared solutions of **1**−**4** in CH_3CN/HEPES buffer (5:95 v/v), pH 7.2, were left in the dark at room temperature and monitored over a period of 24 h by using analytical RP-HPLC.

Photochemical Quantum Yields. The differential photochemical quantum yields, ϕ_{chem}, were determined for **1**−**4** at 365 nm in CH_3CN/HEPES buffer (5:95), pH 7.2, by the relative method as previously described[28] using (6,7-dimethoxycoumarin-4-yl)-methyldiethyl phosphate ($\phi_{chem} = 0.08$)[29] as a standard. Identical absorbances for the references and **1**−**4** were used during photolysis. For kinetic investigations the irradiated solutions of **1**−**4** and of (6,7-dimethoxycoumarin-4-yl)methyl diethyl phosphate were analyzed by using analytical HPLC.

Fluorescence Quantum Yields. The fluorescence quantum yields, ϕ_f, of **1**−**4** were determined at 25 °C in CH_3CN/HEPES buffer (5:95 v/v), pH 7.2, by the relative method[30] versus quinine sulfate in 0.1 N H_2SO_4 as a standard ($\phi_f = 0.545$). At the excitation wavelength used, the absorbance values of the standard and the investigated compound were identical.

Time-Resolved Fluorescence Spectroscopy. Time-resolved fluorescence spectroscopy was carried out in CH_3CN/HEPES buffer (5:95), pH 7.2, at 23 °C. Fluorescence rise and decay curves were recorded in right-angle arrangement. We used an MSC 1600 N_2 laser from LTB (337 nm, pulse width 0.5 ns, maximum pulse energy 0.7 mJ) as excitation source. Details of the equipment and the deconvolution procedure of the decay curves are described elsewhere.[20]

pK_a Value Estimation. The pK_a of compound **16** was measured from its absorption spectra in H_2O at different pH values.[31]

Acknowledgment. This work was supported by the Deutsche Forschungsgemeinschaft (HA 2694/3-2) and the Fonds der Chemischen Industrie.

Supporting Information Available: ^{1}H NMR and ^{13}C NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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