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{7-[Bis(carboxymethyl)amino]coumarin-4-yl}methoxycarbonyl Derivatives for Photorelease of Carboxylic Acids, Alcohols/Phenols, Thioalcohols/ Thiophenols, and Amines

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Abstract: Light-induced release of biomolecules from inactive precursor molecules represents a powerful method to study cellular processes with high temporal and spatial resolution. Here we report the synthesis and photochemistry of a series of {7-[bis(carboxymethyl)amino]coumarin-4-yl}methyl carboxylates, carbonates, carbamates, and thiocarbonates as potential phototriggers for compounds with COOH, OH, NH_2, and SH functions. The compounds are soluble in aqueous buffer, show low fluorescence, and are efficiently photolysed by irradiation with UV/Vis or IR light to release carboxylates, alcohols, phenols, amines, thioalcohols, or thiophenols.

Keywords: coumarins · photoactivatable compounds · photochemistry · photolysis · protecting groups

Introduction

Photolabile protecting groups have numerous applications in chemistry. Their removal only requires light and allows a very mild deprotection of sensitive molecules. The protection of biological signaling molecules by conversion into inactive light-sensitive derivatives is also very attractive and photoactivatable or caged biomolecules represent a powerful tool for cell biology, because they permit the controlled delivery of bioactive molecules without physically disturbing within organised biological systems with high temporal and spatial precision.[1–3] When caged, the biomolecule is rendered biologically inactive by covalent attachment of a photochemically removable protecting group (caging group) to the key pharmacophoric functionality. Flash photolysis by using light of a specific wavelength cleaves the modifying group, that is, it triggers the uncaging and rapidly activates the molecule. Among the available different caging groups the coumarinylmethyl moieties have received increased attention in recent years. They are members of arylalkyl-type photo-removable protecting groups and photolysis of their caged derivatives produces upon cleavage of a C–O bond an anion of the leaving group and a solvent-trapped coumarin as a photo by-product.

Herein, we describe the caging and uncaging of different molecules with COOH, OH, NH_2, and SH functionalities (Scheme 1) by using our recently developed {7-[bis(carboxymethyl)amino]coumarin-4-yl}methyl (BCMACM) chromophore,[4] and its {7-[bis(carboxymethyl)amino]coumarin-4-yl}methoxycarbonyl (BCMACMOC) variant, respectively. The BCMACM protecting group is a water-soluble version of the earlier introduced 7-(dialkylamino)coumarinylmethyl caging groups.[5–7] Caging of carboxylic acids, alcohols, and amines by using coumarinylmethyl or coumarinylmethoxycarbonyl protecting groups has already been described.[4,8–19] Coumarinylmethoxycarbonyl protected thioalcohols and thiophenols have not yet been reported.

The special features of the BCMACM or BCMACMOC protecting groups are the efficient photochemical release upon irradiation in the visible wavelength region due to large long-wavelength absorptivities, the sensitivity to two-photon excitation, and the good solubility in aqueous buffer by virtue of their anionic centres. The simultaneous appearance of these properties is unique. Long-wavelength absorptions allow uncaging to occur under non-damaging light conditions to cells. Two-photon excitation is also favourable for a less-damaging release of biomolecules, gives significantly deeper tissue penetration, and allows more localised photorelease. Caging of biomolecules frequently leads to very hy-

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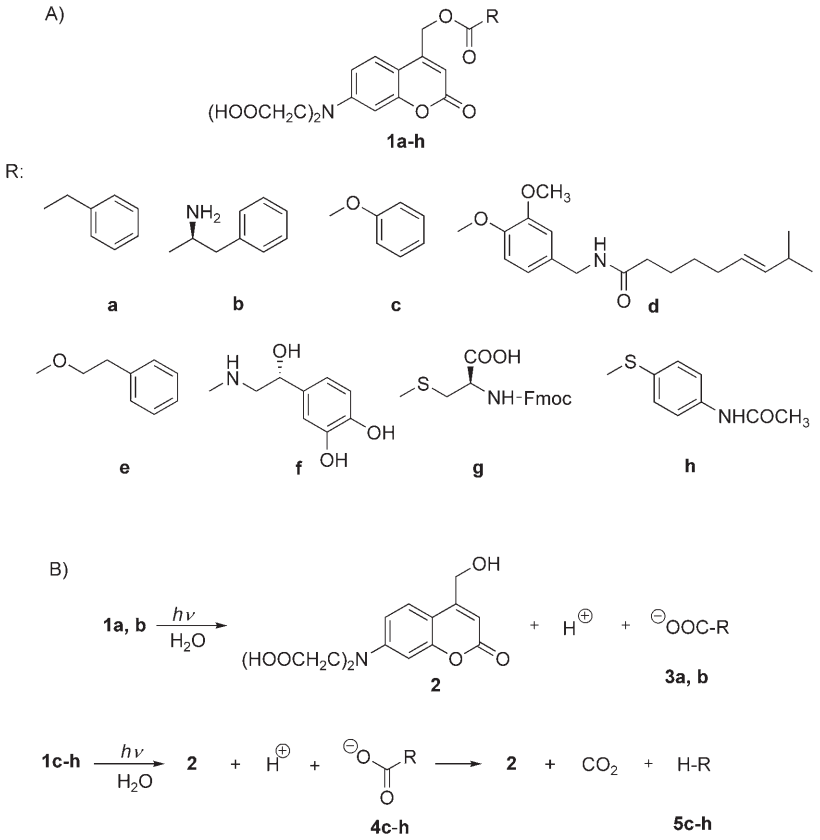


Scheme 1. A) Structures and B) photolysis of the phototriggers **1a**–**h**.

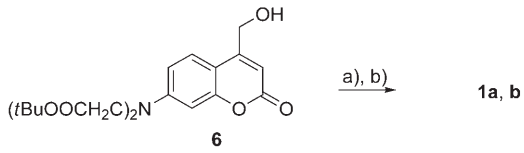
drophobic derivatives and therefore a water-soluble protecting group is favourable in most cases.

Results and Discussion

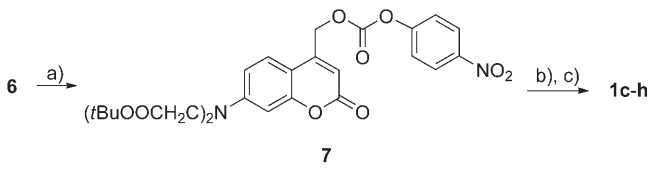
Caged model compounds **1a**,**c**,**e**,**h**, and the caged bioactive molecules **1b**,**d**,**f**,**g** (see Scheme 1)—compounds **1a** and **1d** we have already recently introduced[4,18]—were synthesised starting from the key building block (caging agent) 7-[bis-(*tert*-butoxycarbonylmethyl)amino]-4-(hydroxymethyl)coumarin (**6**).[4] For synthesis of **1a**,**b** phenylacetic acid or the Boc-protected amino acid L-phenylalanine were esterified with **6** in the presence of dimethylaminopyridine (DMAP) and *N*,*N*′-dicyclohexylcarbodiimide (DCC) and upon purification by using flash chromatography or preparative reverse-phase HPLC converted to **1a**,**b** by removal of the *tert*-butyl groups and, in the case of **1b**, also of the *tert*-butoxycarbonyl (Boc) group by using trifluoroacetic acid (TFA) (Scheme 2).

Compounds **1c**–**h** were prepared as shown in Scheme 3 by reaction of **6** with 4-nitrophenyl chloroformate to the carbonate **7**, reaction of **7** with phenol (**5c**), the vanilloid receptor channel agonist capsaicin (**5d**), 2-phenylethanol (**5e**), the α-adrenergic receptor ligand L-norepinephrine (**5f**), the *N*-(9-fluorenylmethoxycarbonyl) (Fmoc)-protected amino acid L-cysteine (**5g**), or 4-acetamidothiophenol (**5h**) in the presence of DMAP to yield the corresponding *tert*-butyl-protected derivatives, which were purified by flash chromatography, or by reverse-phase HPLC on a preparative scale and then deprotected with TFA to generate the carbonates **1c**–**e**, the carbamate **1f**, and the thiocarbonates **1g** and **1h**.

The BCMACM ester **1a**, the BCMACMOC-caged alcohol **1e**, the BCMACMOC-caged amine **1f**, and the BCMACMOC-caged thioalcohol **1g** are resistant to spontaneous hydrolysis in the dark. HPLC monitoring of the caged compounds in aqueous HEPES buffer, at pH 7.2, during a 24-hour period revealed $<0.5\%$ of the free uncaged compounds at room temperature (HEPES = 2-[4-(2-hydroxyethyl)-1-piperazinyl]-ethanesulfonic acid). More susceptibility to hydrolysis in the dark was observed in the case of the phenyl carbonates **1c**,**d** and the phenyl thiocarbonate



Scheme 2. Synthesis of **1a**,**b**: a) **3a**,**b**, DMAP, DCC, EtOAc, room temperature, 4 h; b) TFA/CH_2Cl_2/H_2O (75:24:1).



Scheme 3. Synthesis of **1c**–**h**: a) 4-Nitrophenyl chloroformate, DMAP or *i*Pr_2EtN, room temperature, 1 h; b) **5c**–**h**, DMAP or *i*Pr_2EtN, room temperature, 1–20 h; c) TFA/CH_2Cl_2/H_2O (75:24:1), room temperature, 30 min.

1h. We found that approximately 7.5% of **1c**, 3% of **1d** and 10% of **1h** were hydrolysed in HEPES buffer, at pH 7.2, within 24 h. For **1h** the estimated half-life time was about 140 h. Nevertheless, BCMACMOC-caged phenols and thiophenols should be sufficiently stable with respect to the time needed for electrophysiological experiments. Only **1b** was

found to a large extent to be unstable in the dark in aqueous HEPES buffer at pH 7.2. The percentages of **1b** remaining after one and six hours in the dark were 95 and 75%, respectively. Hydrolysis of **1b** is dependent upon pH. As expected[20] the compound is more stable at lower pH. We found that 17% of **1b** was hydrolysed in citrate/phosphate buffer at pH 4.0 within 24 h. Because hydrolysis in the dark is often a serious problem it seems that **1b** is less suitable as caged compound and requires some care to minimize contamination by free amino acid. Spontaneous hydrolysis was also described for the L-glutamic acid γ-(6-bromo-7-hydroxy−coumarin-4-yl)methyl ester in aqueous buffer.[9]

As expected, compounds **1a–h** were readily soluble in aqueous buffer at pH 7.2. The limit of aqueous solubility was not determined, but all the compounds were soluble to at least 1 mM concentrations without any problem. This is important for the administration of high concentrations of the phototriggers under physiological conditions.

Photoactivation of the (coumarin-4-yl)methyl esters **1a**,**b** by irradiation with light of the wavelengths of 334–436 nm in aqueous buffer leads to 7-[bis(carboxymethyl)amino]-4-(hydroxymethyl)coumarin (**2**), a proton, and the phenylacetic acid anion **3a** and phenylalanine anion **3b**, respectively (Scheme 1). Upon irradiation at 334–436 nm, the phototriggers **1c–h** release **2**, CO_2, and **5c–h** (Scheme 1). As we have shown earlier for other coumarinylmethyl-caged compounds[8,21,22] we assume that the photochemical conversion proceeds by means of a photochemical S_N1 mechanism (solvent-assisted photoheterolysis). Such a photolysis pathway implies that the formation of **2** and, in the case of the photocleavage of the compounds **1a**,**b**, also the formation of **3a** and **3b** is very fast. Using the linear free energy relationships found for 7-(dimethylamino)coumarinylmethyl esters between the rate of photocleavage and the pK_a values of the acids released during the photolysis,[22] we estimate that **1a–h** are photolysed within the nanosecond time-scale. However, photolytic cleavage of the carbonates **1c–d**, the carbamate **1f**, and the thiocarbonates **1g** and **1h** does not liberate the compounds **5c–h** directly, but unstable carbonate, carbamate, or thiocarbonate salt intermediates (**4c–h**) are formed, which undergo decarboxylation in a thermal hydrolysis step to yield **5c–h**. The decarboxylation is rate-limiting and results in a marked slowing of product release after photolysis.[12] On the basis of the measured decarboxylation rates upon photolysis of other carbonate-caged phenols,[23,24] carbonate-caged alcohols,[26] and carbamate-caged amines,[27,28] the formation of **5c**,**d**,**f** should occur with rate constants of $>100\ s^{-1}$ and that of **5e** with a rate constant of about $0.4\ s^{-1}$. Product-release rates from thiocarbonate intermediates upon photolysis have not yet been determined. The relatively slow kinetics in comparison to those of the esters **1a**,**b** and of phosphates will not be a problem in some applications, but of course can be a potential drawback that limits the applicability of these caged compounds.

The photochemical characteristics of the compounds **1a–h** in HEPES buffer (pH 7.2) are summarised in Table 1. The absorption spectra show maxima at about 380 nm and the extinction coefficients, ε, are high. As shown exemplarily in Figure 1 for compound **1f** the phototriggers **1a–h** absorb light up to 450 nm allowing uncaging at wavelengths >430 nm under non-damaging light conditions. The single-photon photochemical quantum yields for disappearance of **1a** and **1e–g**, ϕ_{chem}, are significantly lower than those of the phosphates[4] probably because the corresponding carboxylic, carbamic, or thiocarbonic acid anions **3a** and **4e–h** have lower leaving-group abilities. It appears that the strength of the photoreleased acid influences the efficiency of product formation. The photochemical quantum yields of disappearance of **1c**,**d**,**h** are comparatively high clearly due to the resonance stabilization of the leaving group by the phenyl moiety. However, the compounds **1a** and **1e–g** allow efficient uncaging too, because the product $\varepsilon\phi$ is proportional to the amount of the product release for a given photon exposure and the compounds have practically useful $\varepsilon\phi$ values due to their high extinction coefficients. BCMACM- and

Table 1. Long-wavelength absorption maxima, λ_{abs}^{max}, extinction coefficients at the absorption maxima and at 430 nm, ε^{max} and ε^{430}, photochemical quantum yields, ϕ_{chem}, fluorescence maxima, λ_f^{max}, fluorescence quantum yields, ϕ_f, of the BCMACM/BCMACMOC-caged compounds **1a–1h** and of **2** in CH_3CN/HEPES-KCl buffer (5:95), pH 7.2.

Compound	λ_{abs}^{max} [nm]	ε^{max} [$M^{-1}\,cm^{-1}$]	ε^{430} [$M^{-1}\,cm^{-1}$]	ϕ_{hem} [a]	λ_f^{max} [nm]	ϕ_f [b]
1a	381	20000	2100	0.02	488	0.040
1b	383	20000	3100	n.d.	n.d.	n.d.
1c	381	19200	2300	0.15	498	0.038
1d	383	18750	2300	0.12	478	0.021
1e	382	19800	2200	0.08	500	0.041
1f	380	18300	1600	0.01	503	0.087
1g	383	18500	2000	0.06	480	0.052
1h	383	18700	2700	0.09	497	0.011
2	376	18400	–	–	479	0.170

Estimated average uncertainties: [a] 15%. [b] 8%. n.d. = not determined.

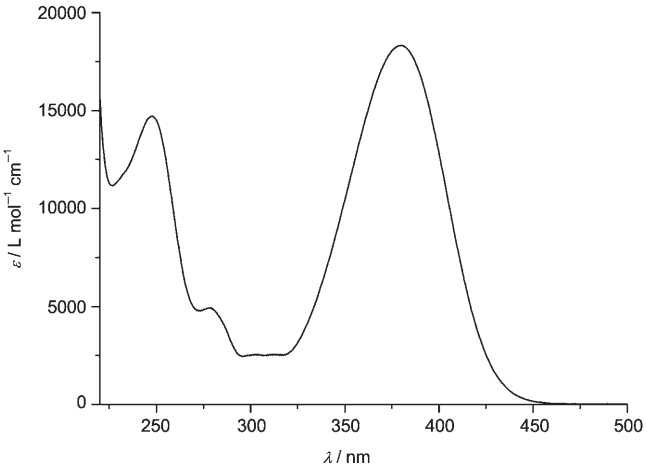


Figure 1. UV/Vis spectrum of **1f** in CH_3CN/HEPES-KCl buffer (5:95), pH 7.2.

BCMACMOC-caged compounds are sensitive not only to one-photon, but also to two-photon excitation, that means UV absorption can be replaced by the simultaneous absorption of two infrared (IR) photons. Two-photon uncaging allows more localised photorelease with excellent three-dimensional resolution, and IR light gives significantly deeper tissue penetration than UV/Vis light. Direct comparison of the time course for two-photon uncaging of **1a** and **1d**,**e** with that of *N*-[6-bromo-7-hydroxycoumarin-4-yl)methoxycarbonyl]-L-glutamic acid (Bhc-Glu)[9] by using a femtosecond-pulsed, mode-locked Ti-sapphire laser at 740 nm reveals that **1a** photodecomposes initially less efficiently (factor 0.4), whereas **1d** and **1e** photodecompose initially 2.6 and 1.5 times more efficiently than Bhc-Glu under identical conditions. Because Bhc-Glu displays a two-photon uncaging action cross section, δ_u, at 740 nm of about one Goeppert–Mayer unit (GM; $1\,GM = 10^{-50}\,cm^4\,s\,photon^{-1}$), the two-photon action cross section of **1a** should be lower and those of **1d,e** larger than 1 GM.

The fluorescence quantum yields, ϕ_i, of **1a**–**h** are significantly smaller than that of the photoreleased alcohol **2** (Table 1). Alcohol **2** also fluoresces inside cells.[4] Thus, fluorescence spectroscopic visualization of the progress of the photorelease from BCMACM- or BCMACMOC-caged compounds within cells becomes possible, as earlier demonstrated by using {6,7-[bis(carboxymethyl)amino]coumarin-4-yl}-caged cyclic nucleotides.[29]

We have very recently described useful applications of the caged capsaicin **1d** as a research tool in pain physiology.[18] The compound did not display measurable biological activity up to concentrations of 10 μM and also had high photosensitivity under physiological conditions. Compared with other caged vanilloid ligands,[24,25,30] **1d** shows a lot of advantages, only the relatively slow photorelease kinetics is not optimal. Compound **1h** and its *tert*-butyl-protected precursor are useful as active esters for the effective introduction of the BCMACMOC protecting group to SH or NH_2 functions of peptides. Applications by using **1f**,**g** are in progress.

Conclusion

We evaluated the usefulness of the BCMACM/BCMACMOC-caging moiety for the design of phototriggers for biomolecules with different classes of functional groups. Our results demonstrate that BCMACM and BCMACMOC are very useful caging groups. They allow caging and uncaging of the most important functional groups of biological relevance as shown exemplarily for the derivatives **1a**–**h**. The phototriggers possess favourable properties. They are, with the exception of **1b**, sufficiently resistant to hydrolysis and are soluble in aqueous buffer. Photocleavage is efficient upon long-wavelength UV/Vis excitation, as well as, exposure to IR light.

Other applications, for instance, in organic chemistry are conceivable, because hydrophilic photochemical protecting groups that allow efficient release of carboxylic acid, alcohol/phenol, amine, and thioalcohol/thiophenol leaving groups by using visible light in aqueous media are unique.

Experimental Section

Materials: *N*-Boc-L-phenylalanine (acid form of **3b**), phenol (**5c**), 2-phenylethanol (**5e**), and L-norepinephrine (**5f**) were purchased from Acros Organics (Belgium). *N*-Fmoc-(*S*)-trityl-L-cysteine, 4-nitrophenyl chloroformate, 4-acetamidothiophenol (**5h**), 4-dimethylaminopyridine (DMAP), *N*,*N*′-dicyclohexylcarbodiimide (DCC), and *i*Pr_2EtN were obtained from Fluka (Germany). Trifluoroacetic acid (TFA) was from Lancaster (UK). Capsaicin (**5d**) was obtained from Sigma (Germany). The remaining chemicals were of the highest grade commercially available and were used without further purification. 7-[Bis(carboxymethyl)-amino]-4-(hydroxymethyl)coumarin (**2**), 7-[bis(*tert*-butoxycarbonylmethyl)amino]-4-(hydroxymethyl)coumarin (**6**), and {7-[bis(carboxymethyl)-amino]coumarin-4-yl}methyl phenylacetate (**1a**) were prepared as described previously.[4] TLC plates (silica gel 60 F_{254}) were purchased from E. Merck (Germany). Silica gel for flash chromatography was from J. T. Baker (The Netherlands). CH_3CN from Riedel-de Haën (Germany) was of HPLC grade. Water was purified with a Milli-Q-Plus system (Millipore, Germany). All reactions were carried out under N_2. The synthetic and analytical procedures with caged compounds were performed under yellow light provided by sodium vapor lamps.

Instrumentation: ^1H and ^{13}C NMR spectra were recorded by using a Bruker AV 300 or a Bruker DRX 600 spectrometer. Chemical shifts are given in parts per million (ppm) by using the residue solvent peaks as reference relative to TMS. The *J* values are given in Hz. Mass spectra were measured by using electrospray ionization (ESI) mass spectrometry in the positive ionization mode by using an Agilent 6210 ESI-TOF spectrometer (Agilent Technologies, USA). UV/Vis spectra were recorded by using a UV/Vis spectrophotometer Lambda 9 (Perkin–Elmer). Fluorescence spectra were recorded by using a Jasco FP-6500 spectrometer. Analytical reverse-phase HPLC (RP-HPLC) was carried out by using a Shimadzu LC-20 system (flow rate: 1 mL min^{-1}) equipped with a DAD-UV detector and a fluorescence detector ($\lambda_{exc} = 380$ nm, $\lambda_{em} = 495$ nm) by using a PLRP-S column, 300 Å, 8 μm, 250 × 4.6 mm from Polymer Laboratories (UK) or a Nucleodur 100-5 C18 ec column, 100 Å, 5 μm, 250 × 4 mm, Macherey–Nagel (Germany). Preparative RP-HPLC was conducted by using a Shimadzu LC-8 A system (flow rate: 10 mL min^{-1}) with a UV/Vis detector (SPD-6 AV, $\lambda_{exc} = 254$ nm) over a Nucleogel RP 100-10 (100 Å, 10 μm, 300 × 25 mm) or a Nucleodur 100-5 C18 ec column (100 Å, 5 μm, 250 × 21 mm) from Macherey–Nagel (Germany). One-photon photolysis of all synthesised photoprotected compounds in solution was performed by using a high-pressure mercury lamp (HBO 500, Oriel, USA) with controlled light intensity and metal interference transmission filters (365 or 436 nm, Oriel, USA). 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 in darkness or under yellow light provided by sodium vapor lamps. The melting points were uncorrected.

***N*-Boc-L-phenylalanine {7-[bis(*tert*-butoxycarbonylmethyl)amino]coumarin-4-yl}methyl ester**: Compound **6** (64.3 mg, 0.15 mmol) was dissolved in EtOAc (3 mL), cooled to 10 °C, and then *N*-Boc-L-phenylalanine (39.8 mg, 0.15 mmol), some DMAP, and DCC (38.8 mg, 0.19 mmol) were added with stirring. The mixture was allowed to warm to room temperature, stirred for 4 h, filtered, evaporated, and the residue purified by preparative RP-HPLC. The desired product was eluted by use of a linear gradient of 40–95 % B in 60 min (eluent A, H_2O; eluent B, CH_3CN). The fraction with a retention time of 59 min was collected, evaporated in vacuo, redissolved in CH_3CN/H_2O, and lyophilised to give the desired product (45 mg, 45 %) as a pale yellow solid. M.p. 76–78 °C (decomp); TLC: $R_f = 0.53$ (THF/hexane 1:2 *v*/*v*); $t_R = 19.45$ min (analytical HPLC, 20–95 % B in A in 20 min, eluent A, H_2O; eluent B, CH_3CN); ^1H NMR (300 MHz, [D_6]DMSO, 27 °C, TMS): $\delta = 1.33$ (s, 9H), 1.42 (s, 18H), 2.89–

3.09 (m, 2H), 4.19 (s, 4H), 4.27–4.35 (m, 1H), 5.26–5.38 (m, 2H), 6.12 (s, 1H), 6.47 (d, $J=1.9$ Hz, 1H), 6.55 (dd, $J=9.0$ and 1.9 Hz, 1H), 7.20–7.30 (m, 5H), 7.47 ppm (d, $J=9.0$ Hz, 1H); ^{13}C NMR (75.5 MHz, [D$_6$]DMSO, 27°C): $\delta=27.7$, 28.1, 36.2, 53.5, 55.4, 61.6, 78.5, 81.1, 98.1, 106.7, 106.9, 109.1, 125.3, 126.5, 128.2, 129.1, 137.3, 150.0, 151.3, 155.0, 155.5, 160.2, 168.8, 171.6 ppm; HRMS (ESI): m/z: calcd for $C_{36}H_{46}N_2O_{10}$ $[M+Na]^+$: 689.3050; found: 689.3041.

L-Phenylalanine {7-[bis(carboxymethyl)amino]coumarin-4-yl}methyl ester trifluoroacetate, (*O*-BCMACM-caged phenylalanine, 1b): A sample (40 mg, 0.06 mmol) of the product described above was stirred in a mixture (5 mL) of TFA/CH_2Cl_2/H_2O (75:24:1) at room temperature for 30 min. The solvents were evaporated and the residue purified by preparative RP-HPLC (PLRP-S). Compound **1b** was eluted by use of a linear gradient of 5–95% B in 75 min (eluent A, H_2O/0.1% TFA; eluent B, CH_3CN). The fraction with a retention time of 35.4 min was collected, evaporated in vacuo, redissolved in CH_3CN/H_2O, and lyophilised to give **1b** (16 mg, 58.3%) as a pale-yellow solid. M.p. >110°C (decomp); $[\alpha]_D^{25}=+3.6$ ($c=0.67$ in DMSO); $t_R=11.8$ min (analytical HPLC, PLRP-S, 5–95% B in A in 20 min, eluent A, H_2O/0.1% TFA; eluent B, CH_3CN); ^{1}H NMR (300 MHz, [D$_6$]DMSO, 27°C, TMS): $\delta=3.08$–3.22 (m, 2H), 4.21 (s, 4H), 4.52 (t, $J=6.8$ Hz, 1H) 5.29 (d, $J=15.2$ Hz, 1H), 5.43 (d, $J=15.2$ Hz, 1H), 6.07 (s, 1H), 6.46 (s, 1H), 6.54 (d, $J=9.0$ Hz, 1H), 7.23–7.34 (m, 5H), 7.40 (d, $J=8.9$ Hz, 1H), 8.53 ppm (br s, 2H); ^{13}C NMR (75.5 MHz, [D$_6$]DMSO, 27°C): $\delta=36.1$, 53.2, 53.5, 62.6, 97.9, 106.6, 107.1, 109.0, 125.4, 127.4, 128.6, 129.3, 134.4, 148.9, 151.3, 155.1, 160.2, 168.6, 171.5 ppm; ^{19}F NMR (282.5 MHz, [D$_6$]DMSO, 27°C, with neat CF_3COOH as external reference relative to neat $CFCl_3$): $\delta=-73.9$ ppm; HRMS (ESI): m/z: calcd for $C_{23}H_{22}N_2O_8$ $[M+H]^+$: 455.1454; found 455.1468; elemental analysis calcd (%) for $C_{23}H_{22}N_2O_8\cdot 1\,H_2O\cdot CF_3COOH$ (586.48): C 51.20, H 4.30, N 4.73; found: C 50.92, H 4.10, N 4.98.

{7-[Bis(*tert*-butoxycarbonylmethyl)amino]coumarin-4-yl}methyl phenyl carbonate: DMAP (36.7 mg, 0.3 mmol), and 4-nitrophenyl chloroformate (60.5 mg, 0.3 mmol) were added with stirring to a solution of **6** (103 mg, 0.24 mmol) in THF (5 mL). The reaction mixture was stirred at room temperature for 1.0 h, filtered, and evaporated. The residue containing the intermediately formed active ester **7** was dissolved in CH_2Cl_2 (5 mL), after which DMAP (36.7 mg, 0.3 mmol) and phenol (**5c**, 28.2 mg, 0.3 mmol) were added. The reaction mixture was stirred for 3.5 h, evaporated, and purified by preparative RP-HPLC (Nucleogel 100-10). The desired product was eluted by using a linear gradient 50–95% B in 80 min (eluent A, H_2O; eluent B, CH_3CN). The main fraction with a retention time of 54.3 min was collected, evaporated in vacuo, redissolved in CH_3CN/H_2O, and lyophilised to give the product (65 mg, 50%) as pale yellow crystals. M.p. 79–81°C; TLC: $R_f=0.16$ (hexane/EtOAc, 16:6 v/v); $t_R=14.97$ min (analytical HPLC, PLRP-S, 5–95% B in A in 20 min, eluent A, H_2O; eluent B, CH_3CN); ^{1}H NMR (600 MHz, [D$_6$]DMSO, 27°C, TMS): $\delta=1.42$ (s, 18H), 4.20 (s, 4H), 5.49 (s, 2H), 6.17 (s, 1H), 6.49 (d, $J=2.1$ Hz, 1H), 6.61 (dd, $J=8.9$ and 2.2 Hz, 1H), 7.28–7.33 (m, 3H), 7.43–7.48 (m, 2H), 7.59 ppm (d, $J=8.9$ Hz, 1H); ^{13}C NMR (75.5 MHz [D$_6$]DMSO, 27°C): $\delta=27.7$, 53.5, 65.4, 81.1, 98.2, 106.9, 107.4, 109.2, 121.2, 125.5, 126.3, 129.6, 149.3, 150.7, 151.5, 152.5, 155.1, 160.2, 168.7 ppm; HRMS (ESI): m/z: calcd for $C_{29}H_{33}N_1O_9$ $[M+H]^+$: 540.2234; found: 540.2226.

{7-[Bis(carboxymethyl)amino]coumarin-4-yl}methyl phenyl carbonate (1c): The bis-*tert*-butyl ester of **1c** (54.0 mg, 0.1 mmol) was stirred in a mixture (5 mL) of TFA/CH_2Cl_2/H_2O (74:25:1) at room temperature for 20 min. The solvents were evaporated, and the residue was coevaporated two times with diethyl ether, dissolved in CH_3CN/H_2O, and lyophilised to give **1c** (40.6 mg, 93.5%) as a pale yellow solid. M.p. >110°C (decomp); TLC: $R_f=0.34$ ($CHCl_3$/MeOH/1% TFA, 95:5:1 v/v); $t_R=8.10$ min (analytical HPLC, PLRP-S, 30–95% B in A in 20 min, eluent A, 0.1% TFA/H_2O; eluent B, CH_3CN); ^{1}H NMR (600 MHz, [D$_6$]DMSO, 27°C, TMS): $\delta=4.24$ (s, 4H), 5.48 (s, 2H), 6.17 (s, 1H), 6.50 (d, $J=2.2$ Hz, 1H), 6.63 (dd, $J=9.0$ and 2.2 Hz, 1H), 7.30–7.33 (m, 3H), 7.43–7.48 (m, 2H), 7.57 (d, $J=9.0$ Hz, 1H), 12.93 ppm (s, 2H); ^{13}C NMR (75.5 MHz [D$_6$]DMSO, 27°C): $\delta=52.9$, 65.4, 98.1, 106.7, 107.3, 109.2, 121.2, 125.6, 126.4, 129.7, 149.3, 150.7, 151.5, 152.5, 155.2, 160.2, 171.3 ppm; HRMS (ESI): m/z: calcd for $C_{21}H_{17}N_1O_9$ $[M+H]^+$: 428.0982; found: 428.0991; elemental analysis calcd (%) for $C_{21}H_{17}N_1O_9\cdot 0.5\,H_2O$ (436.38): C 57.80, H 4.16, N 3.21; found: C 57.44, H 4.42, N 3.12.

(*E*)-{7-[Bis(*tert*-butoxycarbonylmethyl)amino]coumarin-4-yl}methyl 2-methoxy-4-[(8-methylnon-6-enamido)methyl]phenyl carbonate: This compound was prepared by modification of our previously reported procedure[18] by stirring of **6** (103 mg, 0.24 mmol), DMAP (36.7 mg, 0.3 mmol), and 4-nitrophenyl chloroformate (60.5 mg, 0.3 mmol) in THF (5 mL) for 1 h, filtration, evaporation of the filtrate, and reaction of the formed **7** with capsaicin (91.6 mg, 0.3 mmol) in CH_2Cl_2 (5 mL) in the presence of DMAP (36.7 mg, 0.3 mmol) for 3.5 h. Working up as described gave the pure product (65 mg, 36%).

(*E*)-{7-[Bis(carboxymethyl)amino]coumarin-4-yl}methyl 2-methoxy-4-[(8-methylnon-6-enamido)methyl]phenyl carbonate (BCMACMOC-caged capsaicin, 1d): Compound **1d** was prepared by deprotection of the bis-*tert*-butyl ester of **1d** (60 mg, 0.08 mmol) with a mixture (10 mL) of TFA/CH_2Cl_2/H_2O (50:50:1) by stirring at room temperature for 20 min. Working up as described[18] gave the pure product (54 mg, 100%). The spectral and analytical data for **1d** were in accordance with those earlier described.[18]

{7-[Bis(*tert*-butoxycarbonylmethyl)amino]coumarin-4-yl}methyl 2-phenylethyl carbonate: The active ester **7** was prepared from **6** (103 mg, 0.24 mmol) and 4-nitrophenyl chloroformate (60.5 mg, 0.3 mmol) in THF (5 mL) in the presence of DMAP (36.7 mg, 0.3 mmol) as described for the bis-*tert*-butyl ester of **1c**. Dissolution of **7** in CH_2Cl_2 (5 mL), addition of DMAP (36.7 mg, 0.3 mmol) and 2-phenylethanol (**5e**, 36.7 mg, 0.3 mmol), stirring at room temperature for 3.5 h, evaporation, purification by preparative RP-HPLC (PLRP-S) by using a linear gradient 50–95% B in 80 min (eluent A, H_2O; eluent B, CH_3CN, retention time = 56.9 min), and lyophilization gave the desired product (50 mg, 37%) as a pale yellow solid. M.p. 45–47°C; TLC: $R_f=0.75$ (THF/hexane, 1:1 v/v); $t_R=16.5$ min (analytical HPLC, PLRP-S, 50–95% B in A in 22 min, eluent A, H_2O; eluent B, CH_3CN); ^{1}H NMR (300 MHz, [D$_6$]DMSO, 27°C, TMS): $\delta=1.42$ (s, 18H), 2.95 (t, $J=6.7$ Hz, 2H), 4.20 (s, 4H), 4.37 (t, $J=6.7$ Hz, 2H), 5.33 (s, 2H), 6.01 (s, 1H), 6.48 (d, $J=1.9$ Hz, 1H), 6.58 (dd, $J=9.0$ and 1.9 Hz, 1H), 7.21–7.32 (m, 5H), 7.50 ppm (d, $J=9.0$ Hz, 1H); ^{13}C NMR (75.5 MHz [D$_6$]DMSO, 27°C): $\delta=27.7$, 34.2, 53.5, 64.4, 68.5, 81.1, 98.2, 106.9, 107.0, 109.2, 125.4, 126.4, 128.4, 128.8, 137.4, 149.9, 151.4, 153.9, 155.1, 160.2, 168.7 ppm; HRMS (ESI): m/z: calcd for $C_{31}H_{37}N_1O_9$ $[M+Na]^+$: 590.2366; found: 590.2360.

{7-[Bis(carboxymethyl)amino]coumarin-4-yl}methyl 2-phenylethyl carbonate (1e): The bis-*tert*-butyl ester of **1e** (43 mg, 0.075 mmol) was stirred in a mixture (7.5 mL) of TFA/CH_2Cl_2/H_2O (74:25:1) at room temperature for 20 min. The solvents were evaporated, and the residue was co-evaporated two times with diethyl ether, dissolved in CH_3CN/H_2O, and lyophilised to give **1e** (35 mg, 100%). Because the product was not absolutely pure it was purified by preparative RP-HPLC (Nucleodur 100-5). The desired product was eluted by use of a linear gradient of 30–95% B in 70 min (eluent A, H_2O/0.1% TFA; eluent B, CH_3CN). The fraction with a retention time of 32.4 min was collected, evaporated in vacuo, redissolved in CH_3CN/H_2O, and lyophilised to give pure **1e** (25 mg, 72%) as a pale yellow solid. M.p. >75°C (decomp); $t_R=14.2$ min (analytical HPLC, Nucleodur 100-5, 30–95% B in A in 20 min, eluent A, 0.1% TFA/H_2O; eluent B, CH_3CN); ^{1}H NMR (300 MHz, [D$_6$]DMSO, 27°C, TMS): $\delta=2.95$ (t, $J=6.7$ Hz, 2H), 4.23 (s, 4H), 4.37 (t, $J=6.7$ Hz, 2H), 5.33 (s, 2H), 6.01 (s, 1H), 6.48 (d, $J=2.4$ Hz, 1H), 6.60 (dd, $J=9.2$ and 2.4 Hz, 1H), 7.18–7.32 (m, 5H), 7.48 (d, $J=8.9$ Hz, 1H), 13.00 ppm (br s, 2H); ^{13}C NMR (75.5 MHz [D$_6$]DMSO, 27°C): $\delta=34.2$, 52.8, 64.5, 68.5, 98.1, 106.7, 106.9, 109.2, 125.4, 126.5, 128.4, 128.8, 137.5, 149.9, 151.5, 154.0, 155.1, 160.2, 171.3 ppm; HRMS (ESI): m/z: calcd for $C_{23}H_{21}N_1O_9$ $[M+H]^+$: 456.1295; found: 456.1291; elemental analysis calcd (%) for $C_{23}H_{21}N_1O_9\cdot 0.5\,H_2O$ (464.43): C 59.48, H 4.77, N 3.02; found: C 59.09, H 4.84, N 3.31.

***N*-{[7-[Bis(*tert*-butoxycarbonylmethyl)amino]coumarin-4-yl]methoxycarbonyl}-L-norepinephrine**: The active ester **7** was prepared from **6** (103 mg, 0.24 mmol) and 4-nitrophenyl chloroformate (60.5 mg, 0.3 mmol) in THF (5 mL) in the presence of DMAP (36.7 mg, 0.3 mmol) as described for the bis-*tert*-butyl ester of **1c**. Dissolution of **7** in DMF

(4 mL), addition of DMAP (36.7 mg, 0.3 mmol) and L-norepinephrine hydrochloride (**5f**, 51.4 mg, 0.25 mmol), again addition of DMAP (36.7 mg, 0.3 mmol) stirring at room temperature for 3.5 h, evaporation, purification by preparative RP-HPLC (Nucleogel 100-10) by using a linear gradient 30–95% B in 60 min (eluent A, H_2O; eluent B, CH_3CN, retention time = 37.2 min) and lyophilization gave the desired product (70 mg, 47.4%) as a pale-yellow solid. M.p. 115–116°C; TLC: $R_f = 0.38$ (THF/hexane, 1:1 v/v); $t_R = 11.2$ min (analytical HPLC, PLRP-S, 30–95% B in A in 20 min, eluent A, H_2O; eluent B, CH_3CN); ^{1}H NMR (300 MHz, [D$_6$]DMSO, 27°C, TMS): $\delta = 1.42$ (s, 18H) 3.04–3.15 (m, 2H), 4.19 (s, 4H), 4.41–4.45 (m, 1H), 5.19–5.22 (m, 3H), 6.10 (s, 1H), 6.47 (d, J = 1.9 Hz, 1H), 6.56 (m, 2H), 6.66 (d, J = 8.0 Hz, 1H), 6.74 (s, 1H), 7.46–7.52 (m, 2H), 8.67 (s, 1H), 8.78 ppm (s, 1H); ^{13}C NMR (75.5 MHz [D$_6$]DMSO, 27°C): $\delta = 27.7$, 48.7, 53.5, 60.8, 71.1, 81.1, 98.2, 105.9, 107.0, 109.1, 113.4, 115.1, 116.9, 125.1, 134.5, 144.3, 144.9, 151.3, 151.9, 155.0, 155.4, 160.5, 168.8 ppm; HRMS (ESI): m/z: calcd for $C_{31}H_{38}N_2O_{11}$ $[M+Na]^+$: 637.2373; found: 615.2361.

***N*-{{7-[Bis(carboxymethyl)amino]coumarin-4-yl}methoxycarbonyl}-L-norepinephrine (*N*-BCMACMOC-caged L-norepinephrine, 1f)**: The bis-*tert*-butyl ester of **1f** (45 mg, 0.073 mmol) was stirred in a mixture (15 mL) of TFA/CH_2Cl_2/H_2O (50:50:1) at room temperature for 10 min. The solvents were evaporated, and the residue was co-evaporated two times with diethyl ether, dissolved in CH_3CN/H_2O, and lyophilised to give 38.7 mg (0.073 mmol, 100%) of **1f**. Because the product was not absolutely pure it was purified by preparative RP-HPLC (Nucleogel 100-10). The desired product was eluted by use of a linear gradient of 5–60% B in 70 min (eluent A, H_2O/0.1% TFA; eluent B, CH_3CN). The fraction with a retention time of 35.5 min was collected, evaporated in vacuo, redissolved in CH_3CN/H_2O, and lyophilised to give pure **1f** (29.8 mg, 77%) as pale-yellow solid. M.p. >155°C (decomp); $[\alpha]_D^{25} = +29.4$ ($c = 0.67$ in DMSO); $t_R = 10.5$ min (analytical HPLC, (PLRP-S), 5–60% B in A in 20 min, eluent A, 0.1% TFA/H_2O; eluent B, CH_3CN); ^{1}H NMR (300 MHz, [D$_6$]DMSO, 27°C, TMS): $\delta = 3.04$–3.12 (m, 2H), 4.22 (s, 4H), 4.42 (t, J = 6.1 Hz, 1H), 5.22 (s, 3H), 6.09 (s, 1H), 6.47 (d, J = 1.7 Hz, 1H), 6.53–6.61 (m, 2H), 6.69 (dd, J = 7.1 and J = 1.7 Hz, 1H), 6.73 (d, J = 1.2 Hz, 1H), 7.48–7.53 (m, 2H), 8.71 (s, 1H), 8.82 (s, 1H), 12.89 ppm (brs, 2H); ^{13}C NMR (75.5 MHz [D$_6$]DMSO, 27°C): $\delta = 48.7$, 52.7, 60.8, 71.1, 98.0, 105.9, 106.8, 109.1, 113.4, 115.1, 116.9, 125.2, 134.5, 144.3, 144.9, 151.4, 151.8, 155.0, 155.5, 160.5, 171.3 ppm; HRMS (ESI): m/z: calcd for $C_{23}H_{22}N_2O_{11}$ $[M+Na]^+$: 525.1121; found: 525.1147; elemental analysis calcd (%) for $C_{23}H_{22}N_2O_{11}\cdot 1.5\,H_2O$ (464.43): C 52.18, H 4.76, N 5.29; found: C 52.19, H 4.98, N 5.42.

(*S*)-{{7-[Bis(*tert*-butoxycarbonylmethyl)amino]coumarin-4-yl}methoxycarbonyl}-*N*-Fmoc-L-cysteine: The active ester **7** was prepared by following the procedure described for the bis-*tert*-butyl ester of **1c** from **6** (154.3 mg, 0.36 mmol) and 4-nitrophenyl chloroformate (86 mg, 0.43 mmol) in CH_2Cl_2 (5 mL) in the presence of DMAP (52 mg, 0.3 mmol). To the solution of **7** were added a solution of *N*-Fmoc-L-cysteine (148 mg, 0.43 mmol; prepared by treatment of *N*-Fmoc-(*S*)-trityl-L-cysteine (252 mg, 0.43 mmol) with a mixture (4 mL) of TFA/CH_2Cl_2/H_2O (74:25:1) in the presence of triisopropylsilane (0.088 mL, 0.43 mmol) at room temperature, for 30 min, and evaporation) in CH_2Cl_2 (5 mL) and DMAP (150 mg, 1.23 mmol). The mixture was stirred at room temperature for 20 h, was evaporated, and purified by preparative RP-HPLC (Nucleodur 100-5) by using a linear gradient 30–95% B in 60 min (eluent A, 0.1% TFA/H_2O; eluent B, CH_3CN; retention time = 54.1 min). Lyophilization gave the desired product (49 mg, 17%) as a pale-yellow solid. M.p. 108–110°C (decomp); TLC: $R_f = 0.22$ (CH_2Cl_2/CH_3OH, 9:1 v/v); $t_R = 18.37$ min (analytical HPLC, Nucleodur 100-5, 30–95% B in A in 20 min, eluent A, H_2O; eluent B, CH_3CN); ^{1}H NMR (300 MHz, [D$_6$]DMSO, 27°C, TMS): $\delta = 1.41$ (s, 18H), 3.08–3.15 (m, 1H), 3.41–3.47 (m, 1H), 4.19 (s, 4H), 4.21–4.34 (m, 4H), 5.48 (s, 2H), 6.05 (s, 1H), 6.47 (d, J = 1.9 Hz, 1H), 6.58 (dd, J = 9.0 and J = 2.3 Hz, 1H), 7.31 (t, J = 7.3 Hz, 2H), 7.40 (t, J = 7.4 Hz, 2H), 7.47 (d, J = 8.9 Hz, 1H), 7.71 (d, J = 7.3 Hz, 2H), 7.87 (d, J = 8.0 Hz, 1H), 7.89 (d, J = 7.3 Hz, 2H), 12.88 ppm (brs, 1H); ^{13}C NMR (75 MHz [D$_6$]DMSO, 27°C): $\delta = 27.7$, 32.3, 46.6, 53.3, 53.5, 64.3, 65.8, 81.1, 98.2, 106.8, 107.2, 109.2, 120.1, 125.2, 125.4, 127.0, 127.6, 140.7, 143.7, 149.5, 151.5, 155.1, 156.0, 160.2, 168.7, 169.6, 171.5 ppm; HRMS (ESI): m/z: calcd for $C_{41}H_{44}N_2O_{12}S_1$ $[M+H]^+$: 789.2693; found: 789.2697; elemental analysis calcd (%) for $C_{41}H_{44}N_2O_{12}S_1$ (788.26): C 62.42, H 5.62, N 3.55, S 4.06; found: C 62.27, H 5.91, N 3.76, S 4.04.

(*S*)-{{7-[Bis(carboxymethyl)amino]coumarin-4-yl}methoxycarbonyl}-*N*-Fmoc-L-cysteine ((*S*)-BCMACMOC-caged *N*-Fmoc-L-cysteine, 1g): The bis-*tert*-butyl ester of **1g** (19.9 mg, 0.025 mmol) was stirred in a mixture (4 mL) of TFA/CH_2Cl_2/H_2O (75:24:1) at room temperature for 30 min. The solvents were evaporated, and the residue was coevaporated two times with diethyl ether, dissolved in CH_3CN/H_2O, and purified by preparative RP-HPLC (Nucleogel RP 100-10). The desired product was eluted by use of a linear gradient of 30–95% B in 60 min (eluent A, H_2O/0.1% TFA; eluent B, CH_3CN). The fraction with a retention time of 35.1 min was collected, evaporated in vacuo, redissolved in CH_3CN/H_2O, and lyophilised to give pure **1g** (16.9 mg, 98.6%) as a pale-yellow solid. M.p. >157°C (decomp); $[\alpha]_D^{25} = -35.7$ ($c = 0.67$ in DMSO); $t_R = 10.93$ min (analytical HPLC, PLRP-S, 30–95% B in A in 20 min, eluent A, 0.1% TFA/H_2O; eluent B, CH_3CN); ^{1}H NMR (300 MHz, [D$_6$]DMSO, 27°C, TMS): $\delta = 3.10$–3.14 (m, 1H), 3.42–3.45 (m, 1H), 4.23 (s, 4H), 4.23–4.35 (m, 4H), 5.47 (s, 2H), 6.04 (s, 1H), 6.48 (d, J = 1.9 Hz, 1H), 6.58 (dd, J = 9.0 and J = 2.0 Hz, 1H), 7.32 (t, J = 7.3 Hz, 2H), 7.40–7.41 (m, 2H), 7.46 (d, J = 8.9 Hz, 1H), 7.71–7.72 (m, 2H), 7.87 (d, J = 8.0 Hz, 1H) 7.89 (d, J = 7.2 Hz, 2H), 12.88 ppm (brs, 3H); ^{13}C NMR (75.5 MHz [D$_6$]DMSO, 27°C): $\delta = 32.2$, 46.6, 52.7, 53.3, 64.2, 65.7, 98.1, 106.6, 107.1, 109.2, 120.1, 125.2, 125.4, 127.0, 127.6, 140.7, 143.7, 149.5, 151.5, 155.1, 156.0, 160.2, 169.6, 171.2, 171.5 ppm; HRMS (ESI): m/z: calcd for $C_{33}H_{28}N_2O_{12}S_1$ $[M+H]^+$: 677.1451; found: 677.1450; elemental analysis calcd (%) for $C_{33}H_{28}N_2O_{12}S_1\cdot 0.5\,H_2O$ (685.66): C 57.81, H 4.26, N 4.09, S 4.68; found: C 57.78, H 4.27, N 4.14, S 4.49.

***O*-{{7-[Bis(carboxymethyl)amino]coumarin-4-yl}methyl} (*S*)-(4-acetamidophenyl) thiocarbonate (BCMACMOC-caged 4-acetamidothiophenol, 1h)**: The active ester **7** was prepared following the procedure described for the bis-*tert*-butyl ester of **1c** from **6** (103 mg, 0.24 mmol) and 4-nitrophenyl chloroformate (75 mg, 0.38 mmol) in CH_2Cl_2 (5 mL) in the presence of iPr$_2$EtN (48 mg, 0.38 mmol). To the solution of **7** were added a solution of 4-acetamidothiophenol (62 mg, 0.38 mmol) in CH_2Cl_2 (5 mL) and iPr$_2$EtN (48 mg, 0.38 mmol). The mixture was stirred at room temperature for 1 h and the formed *O*-{{7-[bis(*tert*-butoxycarbonylmethyl)-amino]coumarin-4-yl}methyl} (*S*)-(4-acetamidophenyl) thiocarbonate deprotected without separation by treatment with TFA (3 mL) and stirring for 30 min. Compound **1h** was precipitated by addition of Et_2O and purified by preparative RP-HPLC (Nucleodur 100-5) by using a linear gradient 10–50% B in 60 min (eluent A, 0.1% TFA/H_2O; eluent B, CH_3CN; retention time = 50.6 min). Lyophilization gave **1h** (61 mg, 49%) as a pale-yellow solid. M.p. >135°C (decomp); $t_R = 6.54$ min (analytical HPLC, Nucleodur 100-5, 30–95% B in A in 20 min, eluent A, 0.1% TFA/H_2O; eluent B, CH_3CN); ^{1}H NMR (300 MHz, [D$_6$]DMSO, 27°C, TMS): $\delta = 2.07$ (s, 3H), 4.23 (s, 4H), 5.48 (s, 2H), 6.01 (s, 1H), 6.48 (d, J = 2.0 Hz, 1H), 6.61 (dd, J = 9.0 and J = 2.0 Hz, 1H), 7.46 (d, J = 8.9 Hz, 1H), 7.51 (d, J = 8.7 Hz, 1H), 7.68 (d, J = 8.6 Hz, 2H), 10.17 (s, 1H), 12.78 ppm (brs, 2H); ^{13}C NMR (75.5 MHz, [D$_6$]DMSO, 27°C): $\delta = 24.1$, 52.7, 64.6, 98.1, 106.7, 107.2, 109.2, 119.3, 119.5, 125.5, 135.7, 141.1, 149.5, 151.5, 155.1, 160.2, 168.7, 171.2 ppm; HRMS (ESI): m/z: calcd for $C_{23}H_{20}N_2O_9S_1$ $[M+H]^+$: 501.0968; found: 501.0978; elemental analysis calcd (%) for $C_{23}H_{20}N_2O_9S_1\cdot H_2O$ (518.50): C 53.28, H 4.28, N 5.40, S 6.18; found: C 52.90, H 4.25, N 5.51, S 6.00.

Solubility: The solubilities of **1a–h** in HEPES buffer (10 mM HEPES and 120 mM KCl adjusted to pH 7.2 with 2 N KOH) were estimated by using analytical RP-HPLC at room temperature.

Hydrolytic stability: Freshly prepared solutions of **1a–h** in HEPES buffer, pH 7.2, and of **1b** in citrate/phosphate buffer, pH 4.0, 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 **1a** and **1c–h** at 365 nm in 5% CH_3CN/0.01 M HEPES/KOH buffer (pH 7.2) by the relative method as previously described[31] by using (6,7-dimethoxycoumarin-4-yl)methyl diethyl phosphate ($\phi_{chem} = 0.08$)[32] in 5% CH_3CN/0.01 M HEPES/KOH buffer (pH 7.2) as standard. Identical absorbances for the references and **1a** or

1c–h were used during photolysis. For kinetic investigations the irradiated solutions of **1a**, **1c–h**, and (6,7-dimethoxycoumarin-4-yl)methyl diethyl phosphate were analysed by using analytical HPLC.

Fluorescence quantum yields: The fluorescence quantum yields (ϕ_f) of **1a**, **1c–h**, and of **6** were determined at 25 °C in CH_3CN/HEPES buffer (5:95), pH 7.2, by the relative method[33] 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 compounds were identical.

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