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Sequential Uncaging with Green Light can be Achieved by Fine-Tuning the Structure of a Dicyanocoumarin Chromophore

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We report the synthesis and photochemical properties of a series of dicyanocoumarinylmethyl (DEAdcCM)- and dicyanocoumarinylethyl (DEAdcCE)-based photocages of carboxylic acids and amines with absorption maximum around 500 nm. Photolysis studies with green light have demonstrated that the structure of the coumarin chromophore as well as the nature of the leaving group and the type of bond to be photocleaved (ester or carbamate) have a strong influence on the rate and efficiency of the uncaging process. These experimental observations were also supported by DFT calculations. Such differences in deprotection kinetics have been exploited to sequentially photolyze two dicyanocoumarin-caged model compounds (e.g., benzoic acid and ethylamine), and open the way to increasing the number of functional levels that can be addressed with light in a single system, particularly when combining dicyanocoumarin caging groups with other photocleavable protecting groups, which remain intact under green light irradiation.

1. Introduction

Light is an excellent non-invasive stimulus for controlling the outcome of molecular processes with high spatiotemporal precision and without causing contamination of the sample.[1] Owing to these extraordinary properties and to the high versatility and efficiency of light, the use of photocleavable protecting groups (PPGs; also commonly referred to as caging groups) have found widespread applications in several fields, from organic synthesis to materials science.[2] In recent years, an exquisite control of complex biological processes has also been achieved by using caged analogs of bioactive compounds (for example, peptides, proteins, oligonucleotides, neurotransmitters, antibiotics, agrochemicals),[3] which can be prepared by modifying an essential functionality with the appropriate PPG. Therefore, activation of the resulting caged compound requires irradiation with light of the appropriate wavelength and intensity to trigger the unmasking of such functionality and, for instance, the recovery of the biological activity of the parent compound.

Among the large number of PPGs reported to date,[2] coumarin derivatives fulfill some of the criteria for an ideal caging group: they are relatively easy to synthesize from commercially available precursors and amenable to structural modifications both to facilitate the attachment of the compound to be caged through different types of bonds (for example, ester, amide, carbamate, or carbonate) and to improve their photophysical and photochemical properties. The latter are important issues as most reported caging groups require UV irradiation for photoactivation, which is toxic and has a poor capacity of penetration in biological tissues. Among the coumarin-based PPGs described to date, those possessing an *N*,*N*-dialkylamino group at position 7 have larger molar extinction coefficients at longer wavelengths than their analogs with hydroxy or methoxy substituents at that position and exhibit moderate to high photochemical quantum yields. Moreover, the replacement of such *N*,*N*-dialkyamino substituents at the 7-position by azetidine, aziridine, or monoalkylated cyclic amines can be used to improve the photophysical properties of coumarin-based fluorophores.[4] Recently, the modification of the carbonyl group of the lactone in the *N*,*N*-diethylamino(coumarin 4-yl)-methyl platform has been proved to be a valuable way to fur-

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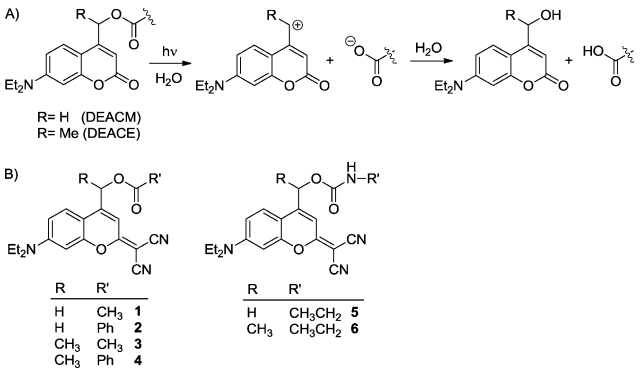
ther shift the wavelength of maximum absorption, which can be exploited to trigger uncaging with blue, cyan, or green light.[5] Indeed, dicyanomethylenecoumarin derivatives, which are obtained by conjugating two nitrile groups through position 2 of the coumarin moiety, are particularly attractive as they exhibit a maximum absorption around 500 nm in the 7-diethylamino series (487 nm in a coumarin derivative containing a benzyloxymethyl group at position 4 described by Jullien and co-workers[5c] and 475 nm when this position was occupied by a methyl as described by Granberg and colleagues[6]). Uncaging of dicyanocoumarin-caged cyclic morpholino oligonucleotides has been exploited in the context of gene silencing by Deiters' and Chen's group,[5a] and very recently we have described the synthesis of a dicyanocoumarin-caged RGD peptide for photocontrolled-targeted drug delivery.[5e] Nevertheless, such modifications of the lactone in the *N*,*N*-diethylamino(coumarin 4-yl)methyl platform usually led to a significant reduction of the uncaging quantum yield compared with the parent oxygenated coumarin or its thionated analog,[5c] which could suppose a limitation for future applications of dicyanocoumarins as PPGs.

The mechanism of photocleavage of coumarinylmethyl esters has been well established for the classical 7-(*N*,*N*-diethylamino)coumarin-4-yl)methyl (DEACM) caging group (R = H; see Scheme 1 A).[7] Upon electronic excitation, a solvent-assisted photoheterolytic bond cleavage produces the free carboxylic acid and the corresponding coumarinylmethyl alcohol, which is generated by reaction of the cationic intermediate with water followed by a fast deprotonation. Previous studies have demonstrated that stabilization of this intermediate (by incorporation of electron-donating substituents at the coumarin skeleton) and of the released carboxylate (by decreasing its basicity) have a positive effect on the photocleavage of coumarinylmethyl esters.[7] Recently, del Campo and collaborators have investigated the incorporation of a ethyl group at position 4 of the DEACM moiety (R = Me, see Scheme 1 A) for increasing the stability of the corresponding caged aspartic acid monomer during Fmoc-*t*Bu solid-phase peptide synthesis.[8] Despite the fact that this new caging group, 7-(*N*,*N*-diethylamino)coumarin-4-yl)ethyl (DEACE), generates a secondary cationic intermediate upon the photo S_N1 reaction of the corresponding DEACE-protected Asp derivative, no significant differences were found with respect to the DEACM analog regarding the photochemical properties, particularly the uncaging quantum yield at 360 nm. By contrast, incorporation of this modification in dicyanomethylene-coumarin-caged Asp monomers led to a faster uncaging process with green light (505 nm), which was attributed to the higher stability of the carbocation intermediate.[5e]



Scheme 1. General mechanism of the photolysis of a DEACM- or DEACE-caged carboxylic acid (A) and structure of the DEAdcCM- and DEAdcCE-caged model compounds synthesized in this work (B).

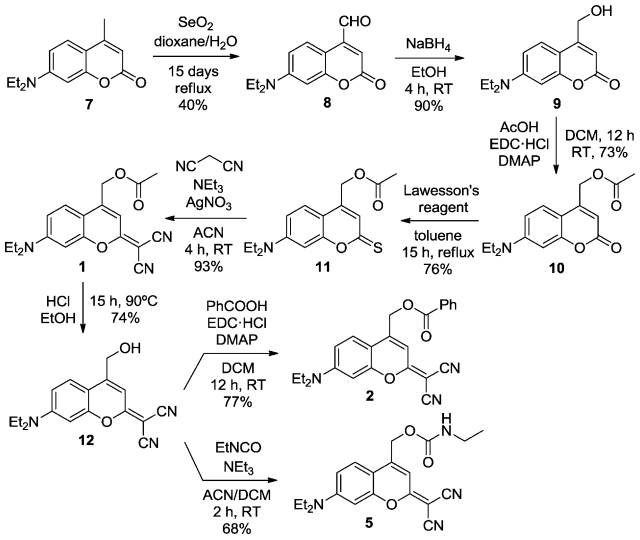
Taking into account the potential of dicyanocoumarin derivatives as PPGs, in this work we have synthesized a series of *N*,*N*-diethylamino-dicyanocoumarinylmethyl (DEAdcCM)- and dicyanocoumarinylethyl (DEAdcCE)-caged model compounds (**1**–**6**, Scheme 1 B) with the aim of studying their photophysical and photochemical properties, particularly how uncaging is influenced both by the chemical structure of the coumarin chromophore (R = H or Me) and by the nature of the leaving group (that is, aliphatic or aromatic carboxylic acid or amine). Photolysis studies with the corresponding caged model compounds upon irradiation with green light (505 nm) as well as DFT computational calculations have shown that both the structure of the dicyanocoumarin caging group and of the leaving group have a strong influence on the rate and efficiency of the photolysis process. Interestingly, by selecting the appropriate combination of dicyanocoumarin-based caging groups, we have demonstrated that carboxylic acid and amine functionalities can be sequentially released from the corresponding caged compounds by irradiation at 505 nm.

2. Results and Discussion

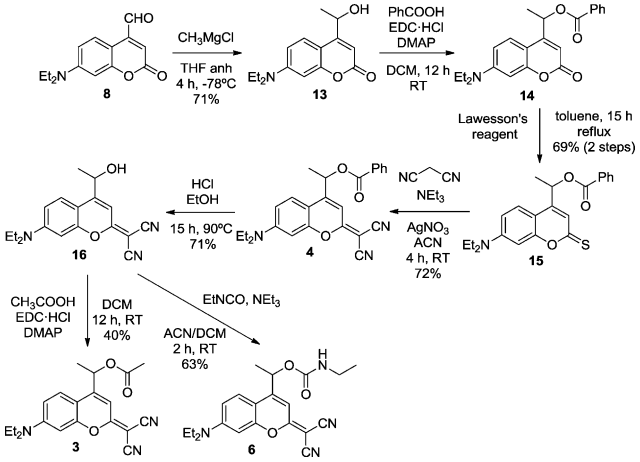
2.1. Synthesis of DEAdcCM- and DEAdcCE-Caged Model Compounds

As shown in Scheme 2, the synthesis of the DEAdcCM-caged model compounds (**1**, **2**, and **5**) was planned from 4-(acetoxymethyl)-7-(*N*,*N*-diethylamino)-2-thiocoumarin (**11**),[5c] a key intermediate that can be prepared from the commercially available precursor **7** in four steps.[5c,e] Condensation of the thionated coumarin with malononitrile in the presence of triethylamine and silver nitrate afforded 4-(acetoxymethyl)-2-(dicyanomethylene)-7-(*N*,*N*-diethylamino)-coumarin (**1**) in 93% yield after column chromatography. Hydrolysis of the acetyl group with HCl followed by esterification with benzoic acid afforded coumarin derivative **2**. Reaction of the alcohol intermediate **12** with ethyl isocyanate in the presence of triethylamine afforded compound **5** as a model for the protection of primary amines with DEAdcCM through a carbamate linkage. All the compounds were fully characterized by ESI mass spectrometry and ^1H and ^{13}C NMR spectroscopy.

The synthesis of the DEAdcCE-caged model compounds (**3**, **4**, and **6**) is summarized in Scheme 3. First, reaction of the aldehyde **8** with CH_3MgCl in anhydrous THF at low temperature (−78 °C) afforded coumarin alcohol **13** as a racemic mixture.[5e] After esterification with benzoic acid followed by thionation with Lawesson's reagent and condensation with malononitrile,



Scheme 2. Synthesis of dicyanocoumarinylmethyl-caged compounds (**1**, **2**, and **5**).



Scheme 3. Synthesis of dicyanocoumarinylethyl-caged compounds (**3**, **4**, and **6**).

the DEAdcCE-caged benzoic acid **4** was obtained. Hydrolysis of **4** followed by esterification with acetic acid or by reaction with ethyl isocyanate, afforded the two remaining DEAdcCE-caged model compounds (**3** and **6**, respectively).

2.2. Absorption and Emission Properties of Dicyanocoumarin Derivatives

The photophysical characterization of the dicyanocoumarin-caged model compounds (**1**–**6**) was carried out in a degassed 1:1 (v/v) mixture of Tris buffer (20 mM, pH 7.5) and MeCN. The UV/Vis absorption and emission spectra of the compounds are shown in Figure 1, and their photophysical properties are reported in Table 1. The maximum absorption wavelength, the shape of the absorption curve, and the molar extinction coefficients of the ester (**1**–**4**) and carbamate (**5**–**6**) derivatives were very similar, and consistent with the values previously reported

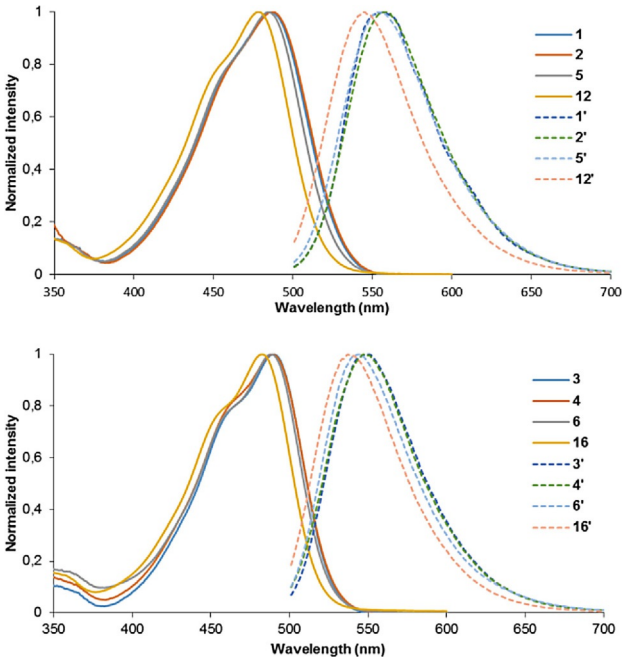


Figure 1. Comparison of the absorption (solid lines) and fluorescence (dotted lines) spectra of DEAdcCM- (top) and DEAdcCE-caged (bottom) model compounds with those of the corresponding dicyanocoumarin alcohols (**12** and **16**, respectively). Solvent: Tris buffer (20 mM, pH 7.5)/MeCN 1:1 (v/v).

Table 1. Photophysical parameters for dicyanocoumarin-caged model compounds (**1**–**6**) and for the corresponding photoreleased coumarin alcohol derivatives (**12** and **16**).[a]

Compound[a]	Absorption		Emission		
	λ_{max} [nm][b]	$\varepsilon(\lambda_{max})$ [$mM^{-1}cm^{-1}$][c]	λ_{Em} [nm][d]	$\Delta\nu$ [cm^{-1}][e]	ϕ_{Em}[f]
1	486	30.4	556	2633	0.17
2	487	32.5	558	2613	0.15
3	489	31.2	549	2193	0.11
4	489	32.0	548	2202	0.11
5	485	32.8	555	2600	0.15
6	488	33.0	544	2067	0.13
12	478	32.5	545	2572	0.17
16	482	33.0	539	2194	0.14

[a] Absorption and emission spectra were recorded in a 1:1 (v/v) mixture of Tris buffer (20 mM, pH 7.5) and MeCN at 25 °C. [b] Wavelength of the absorption maximum. [c] Molar absorption coefficient at λ_{max}. [d] Wavelength of the emission maximum upon excitation at 460 nm. [e] Stokes' shift. [f] Fluorescence quantum yield.

for **2**.[5c] As shown in Table 1, esterification either with benzoic acid or with acetic acid caused a slight redshift (about 5–11 nm) with respect to the parent coumarin alcohol derivatives (**12** or **16**), and in all cases the presence of the methyl group on the coumarin skeleton caused an additional redshift compared with non-methylated analogs (compare **1** and **3**). A similar bathochromic effect was observed for the emission wavelength of the caged compounds upon excitation at 460 nm when compared with the corresponding coumarin alcohol de-

Table 2. Photochemical properties of compounds **1**–**6**.[a]

Caged compound	R	Leaving compound	Uncaging % Caged compound at irradiation time 2 min	5 min	10 min	20 min	k_u [min^{-1}]	ε(505 nm) [mM^{-1}cm^{-1}]	$10^2\times\phi_{Ph}$[b]	ε(505 nm)$\times\phi_{Ph}$ [M^{-1}cm^{-1}]
1	H	AcOH	90	62	22	0	0.125	21.4	0.09 ± 0.03	19
2	H	PhCOOH	55	20	0	0	0.314	23.4	0.15 ± 0.01	34
3	Me	AcOH	28	2	0	0	0.644	22.0	0.28 ± 0.03	62
4	Me	PhCOOH	15	0	0	0	0.948	22.6	0.33 ± 0.00	75
5	H	$EtNH_2$	97	90	79	45	0.036	19.9	0.03 ± 0.01	5
6	Me	$EtNH_2$	71	36	7	0	0.208	21.3	0.12 ± 0.02	25

[a] Data presented: Percent of the starting caged compound at each irradiation time, uncaging first-order rate constant, molar extinction coefficient at the irradiation wavelength, uncaging quantum yield, and uncaging efficiency at 505 nm. [b] Results are the mean ± SDs from two independent experiments.

rivatives. However, the emission maximum was blueshifted in the DEAdcCE compounds with respect to the DEAdcCM counterparts (compare λ_{em} for **1** and **3**). The relative fluorescence quantum yields were calculated by using fluorescein as the standard ($\phi_{Em}=0.92$ in 0.1 M NaOH in water). All the compounds exhibited good quantum yields ($0.11<\phi_F<0.17$) and Stokes' shifts around 2600 cm^{-1} for DEAdcCM derivatives and of 2000–2200 cm^{-1} for the DEAdcCM analogs.

2.3. Photolysis Studies of Dicyanocoumarin-Caged Compounds

From the comparison of the molar extinction coefficients of DEAdcCM- and DEAdcCE-caged model compounds at their respective λ_{max} and at 505 nm (for example, for **1**: $\varepsilon=$ 30.4 mM^{-1}cm^{-1} and 21.4 mM^{-1}cm^{-1}, respectively; see Table 1 and Table 2), we decided to explore the use of green light to carry out the photoactivation studies. Green light is more suitable for biological applications than UV or blue light owing to its reduced photocytotoxicity[9] and deeper optical penetration in tissues.[10] A 505 nm LED source was used for irradiation of solutions of compounds **1**–**6** in a 1:1 (v/v) mixture of Tris buffer (20 mM, pH 7.5) and MeCN at 37 °C, and the course of the uncaging process was monitored by reversed-phase HPLC-ESI MS (see the Experimental Section for further details). As shown in Figure 2, the concentration of all the compounds decreased gradually with time upon irradiation with green light. In the case of DEAdcCM-caged compounds, coumarin alcohol **12** was released as the main photolytic product (Scheme 4). Conversely, photoactivation of DEAdcCE-caged compounds gave coumarin alcohol **16** together with other minor photolytic byproducts, particularly vinyl coumarin **17** (Scheme 4). The formation of this alkene can be explained by a β-elimination reaction from the secondary carbocation intermediate generated upon photoheterolysis of the ester bond (see Scheme 1).

Overall, the results from the photolysis experiments revealed the influence of 1) the structure of the dicyanocoumarin caging group, 2) the nature of the leaving group, and 3) the type of bond photocleaved (ester or carbamate), on the time necessary to release the carboxylic acid or the amine from the corresponding caged compounds. First, it is worth noting that photolysis of DEAdcCE-caged compounds was much faster than that of DEAdcCM (see Figure 2 and Table 2); this result

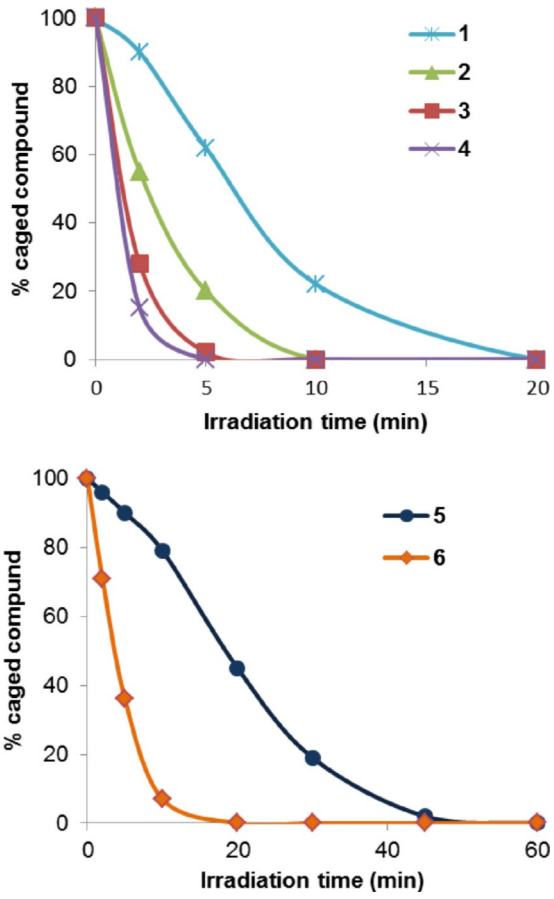
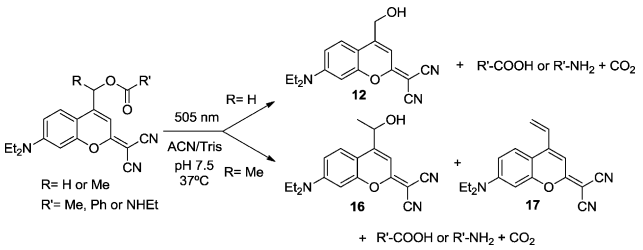


Figure 2. Plot of the temporal evolution of the amount of caged compounds **1**–**4** (top) and **5**–**6** (bottom) in a 1:1 (v/v) mixture of Tris buffer (20 mM, pH 7.5) and MeCN at 37 °C. Irradiation was performed at 505 nm with continuous stirring.

can be attributed to the different stability of the carbocation intermediates (primary or secondary) generated during photoheterolysis of the ester or carbamate bonds in the dicyanocoumarin-caged model compounds. For example, the release of acetic acid from **3** was almost complete after 5 min of irradiation whereas it required 20 min to completely uncage compound **1** ($k_u=0.125$ min^{-1} for **1** vs. $k_u=0.644$ min^{-1} for **3**). Similar trends were found when comparing **2** and **4**, and **5** and **6** (Table 2), in agreement with previous observations with (cou-



Scheme 4. Photolysis of DEAdcCM- and DEAdcCE-caged model compounds.

marin-4-yl)methyl esters (DEACM).[7] For these compounds, the rate of the overall uncaging process was found to be influenced by the rate constant of the initial heterolytic bond cleavage (see Scheme 1 A), which was higher when the primary coumarinyl carbocation intermediate was stabilized though the incorporation of substituents with strong electron-donating ability (alkoxy or dialkylamino).[7] In our case, the same tendency was observed by shifting the nature of the carbocation intermediate from primary to secondary through the incorporation of a methyl group in the coumarin moiety in a position adjacent to the bond to be photocleaved.

Second, the photocleavage process was also faster with good leaving groups on the molecule to be caged (compare benzoate vs. acetate in Table 2). Again, this can be attributed to the stabilization of the second component of the carbocation–carboxylate ion pair and, consequently, to an increase of the rate constant of the first bond cleavage.[7] As expected, the best combination in terms of uncaging rate ($k_u = 0.948\ min^{-1}$) was obtained for compound **4** as the release of benzoate is accompanied by the formation of a secondary carbocation intermediate. Third, the photolysis of carbamate-coumarin caged amines (**5** and **6**) was much slower than that of the ester analogs (**1** and **2**, respectively), which can be attributed to the lower leaving-group ability of the carbamic acid anion compared with carboxylate. This tendency has been found in other carbonate-, carbamate-, and thiocarbonate-coumarin caged compounds in which the final uncaged product is released after thermal decarboxylation of the intermediate.[11] Nevertheless, our results point out that the introduction of an additional methyl group in the skeleton of other coumarin derivatives could be used to increase the deprotection rate of carbamate-caged amines as well as that of carbonate-caged alcohols and phenols, where photolysis is usually much slower compared with uncaging of ester-caged carboxylic acids.

As shown in Table 2, the uncaging quantum yield (ϕ_{Ph}) of the caged model compounds was also determined upon irradiation at 505 nm (see the Experimental Section for further details). The product of the extinction coefficient at the irradiation wavelength and the quantum yield can be considered as an efficiency parameter of the process ($\varepsilon \times \phi_{Phot}$), for quantifying the uncaging process rate as a function of the photolysis time.[2b,12] In good agreement with results from photolysis studies, the quantum yields for DEAdcCE derivatives were higher than those of the DEAdcCM analogs (compare **1** and **3**, and **2** and **4**), resulting in a high product $\varepsilon \times \phi_{Phot}$.

2.4. Computational Studies

Having stablished how the photolysis rate of the dicyanocoumarin-caged model compounds is influenced by both the introduction of a methyl group in the coumarin moiety next to the bond to be photocleaved and the nature of the leaving group, we decided to get insight into these experimental observations from a theoretical point of view. DFT calculations were carried out with the Gaussian 09 (G09) software package (see the Experimental Section for further details). As previously stated, photocleavage of coumarin-caged compounds proceeds through a photo S_N1 mechanism and the stability of the two components of the resulting ion pair (coumarinyl carbocation and carboxylate) has a strong influence on the rate of the first photocleavage step and, consequently, on the overall rate of the uncaging process.[7] For this reason, we calculated the energy differences between the caged compounds (**1**–**4**) and their corresponding intermediate species. Consistent with the experimental data, DFT calculations predicted that photolysis of DEAdcCE-caged compounds (**3**) gives a small stabilization of the ion pair intermediate ($\Delta E = 1.2\ kcal\,mol^{-1}$) compared with the DEAdcCM (**1**) analogs with the same leaving group (Figure 3, top panel). A similar trend was obtained when comparing **2** and **4** (see Figure S11 in the Supporting Information). On the other hand, the calculations also supported the experimental observations regarding the nature of the leaving group: stabilization of the ion pair generated upon cleavage of

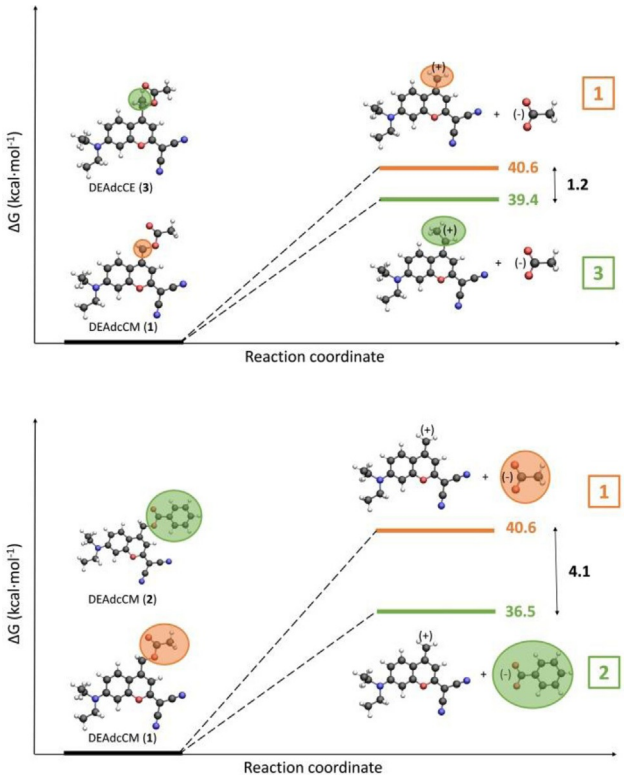


Figure 3. Free energy differences for the transformation of dicyanocoumarin-caged compounds in their respective intermediates (primary or secondary dicyanocoumarinyl carbocation + acetate or benzoate): **1** vs. **3** (top) and **1** vs. **2** (bottom).

the ester bond in **2** was about 4 kcal mol^{-1} higher compared with compound **1** (Figure 3, bottom panel), which agrees with the increased acidity of benzoic acid (pK_a = 4.2) compared with acetic acid (pK_a = 4.76). A similar value was obtained when comparing **3** and **4** (Figure S11 in the Supporting Information).

2.5. Sequential Uncaging Studies

The kinetics of a photodeprotection process depend both on the spectral properties of the PPG (λ_{max} and ε) and on the photolysis quantum yield (ϕ_{Ph}). As found by us and others, the reaction quantum yield can be fine-tuned through the modification of several parameters, including the structure of the chromophore, the nature of the connecting atom in the molecule to be caged, the type of photocleavable bond (ester, carbamate, etc.), and the basicity of the leaving group (aromatic or aliphatic carboxylate).[2b,3n,13] With the aim of controlling with light multiple functionalities in a single system, two main approaches have been explored to selectively address several uncaging processes. The first approach relies on using a set of different caging groups, which can be sequentially photolyzed at different wavelengths of light; this method requires the use of two or more PPGs with significantly different spectral properties (λ_{max}, ε, and ϕ_{Ph}). A second approach is well exemplified by the work of Heckel and collaborators,[3n] who demonstrated that selective uncaging can be achieved by irradiation at a single wavelength of light by exploiting the differences in quantum yield (and for instance of deprotection kinetics) of the same PPG when attached to the molecule to be caged through a different connecting atom.

On the basis of the photolysis studies and of the differences in the uncaging quantum yield between DEAdcCM- and DEAdcCE-caged compounds (Table 2), we wondered if sequential uncaging of at least two dicyanocoumarin-protected functionalities in the same reaction mixture could be achieved by irradiating with the same green light source. First, an equimolar mixture of compound **1** (DEAdcCM-caged acetic acid) and **4** (DEAdcCE-caged benzoic acid) as dicyanocoumarin-caged models of aliphatic or aromatic carboxylic acids, respectively, in the presence of uracil as an internal standard, was irradiated at 505 nm. Unfortunately, HPLC analysis revealed (Figure S12 in the Supporting Information) that despite the fact that **4** was completely uncaged in 4 min, about 40% of **1** was also deprotected. However, irradiation at 505 nm of a mixture of **4** (DEAdcCE-caged benzoic acid) and **5** (DEAdcCM-caged ethylamine) demonstrated that a carboxylic acid and an amine can be sequentially uncaged upon irradiation with the same wavelength of light by exploiting the different deprotection kinetics provided by the two dicyanocoumarin caging groups when connected to the caged functionalities through ester or carbamate linkages: **4** was completely uncaged in 3 min leaving >90% of **5** intact (Figure S13 in the Supporting Information). Such as promising results, led us to include compound **10** (see Scheme 2) as a model for the protection of a carboxylic acid with the classical DEACM coumarin, whose absorption at 505 nm is negligible. As shown in Figure 4 and Figure S14 (Supporting Information), upon irradiation at 505 nm of a mixture of **4**, **5**, and **10**, dicyanocoumarin-caged compounds (**4** and **5**) were sequentially uncaged whereas DEACM-caged acetic acid (**10**) remained intact, even after 1 h irradiating at 505 nm (Scheme 5). Hence, the combination of two structurally related dicyanocoumarin caging groups (DEAdcCM and DEAdcCE) with other PPGs with distinct absorption properties (for example, removable with UV or blue light) allows us to increase the number of levels of selectivity that can be addressed with light.

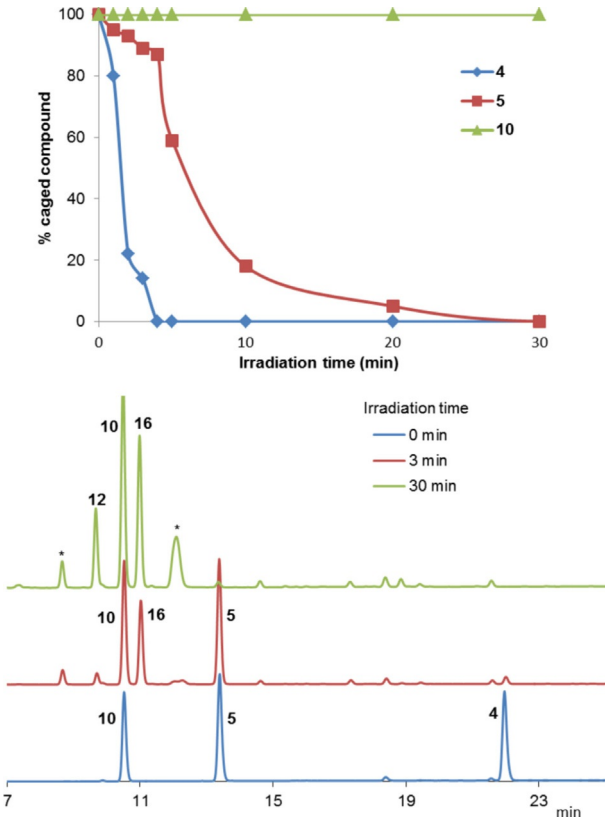
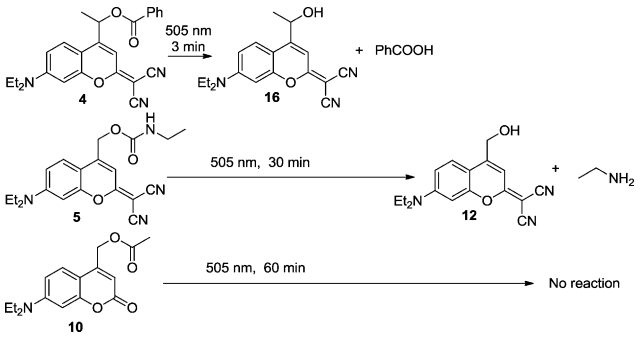


Figure 4. Plot of the temporal evolution of the amount of caged compounds **4**, **5**, and **10** (top) and HPLC chromatograms at a different time (bottom) after irradiation at 505 nm in a 1:1 (v/v) mixture of Tris buffer (20 mM, pH 7.5) and MeCN at 37 °C. *Unidentified coumarin side products.



Scheme 5. Schematic representation of the sequential uncaging with green light of two dicyanocoumarin-caged compounds (**4** and **5**) in the presence of DEACM-caged acetic acid (**10**).

3. Conclusions

We have described the synthesis and photophysical properties of novel DEAdcCM- and DEAdcCE-based photocleavable protecting groups and their suitability for caging carboxylic acids and amines through ester and carbamate bonds, respectively. Such chromophores have a maximum absorption around 500 nm, which facilitates uncaging with the biologically compatible green light, which is non-toxic and penetrates deeper into tissues than UV or blue light. Photolysis studies of the corresponding dicyanocoumarin-caged model compounds at 505 nm and DFT calculations have demonstrated that the structure of the coumarin chromophore as well as the nature of the leaving group strongly influence the rate and efficiency of the uncaging process, which has been exploited to sequentially photolyze two dicyanocoumarin-caged model compounds (benzoic acid and ethylamine) in the presence of DEACM-caged acetic acid. Such significant differences in the deprotection kinetics open the way to increasing the number of functional levels that can be addressed with light in a single system, particularly when combining dicyanocoumarin caging groups with other PPGs that remain stable upon irradiation at 505 nm. Finally, our findings on the impact of the stabilization of the carbocation intermediate generated during photoheterolysis of ester or carbamate bonds in dicyanocoumarin-based photocages could be applied to other coumarin-based systems to improve their photochemical properties. Overall, this would increase the potential of known coumarin-based caging groups for designing wavelength-selective systems in which multiple functionalities can be independently addressed by the different wavelengths of light. Work is in progress to extend this approach to other coumarin-based chromophores with improved redshifted properties.

Experimental Section

Materials and Methods

Common chemicals and solvents (HPLC grade or reagent grade quality) were purchased from commercial sources and used without further purification. Aluminium plates coated with a 0.2 mm thick layer of silica gel 60 F_{254} were used for thin-layer chromatography analyses (TLC), whereas flash column chromatography purification was carried out by using silica gel 60 (230–400 mesh). Reversed-phase high-performance liquid chromatography (HPLC) analyses were carried out with a Jupiter Proteo C_{18} column (250× 4.6 mm, 90 Å, 4 μm, flow rate: 1 mL min^{-1}) by using linear gradients of 0.045% trifluoroacetic acid (TFA) in H_2O (A) and 0.036% TFA in MeCN (B). NMR spectra were recorded at 25 °C with a 400 MHz spectrometer by using deuterated solvents. Tetramethylsilane (TMS) was used as an internal reference (0 ppm) for ^1H spectra recorded in $CDCl_3$ and the residual signal of the solvent (77.16 ppm) was used for ^{13}C spectra. Chemical shifts are reported in parts per million (ppm) in the δ scale, coupling constants in Hz, and multiplicity as follows: s (singlet), d (doublet), t (triplet), q (quadruplet), qt (quintuplet), m (multiplet), dd (doublet of doublets), td (doublet of triplets), ddd (doublet of doublet of doublets), br (broad signal). Electrospray ionization mass spectra (ESI-MS) were recorded with an instrument equipped with single quadrupole detector coupled to an HPLC, and high-resolution (HR) ESI-MS on a LC/MS-TOF instrument.

Synthesis of DEAdcCM-Caged Model Compounds

Compound 1: Malononitrile (1.1 g, 16.7 mmol) was added to a solution of **11** (1.01 g, 3.32 mmol; see the Supporting Information) in anhydrous MeCN (100 mL). After addition of NEt_3 (9.1 mL, 65.5 mmol) under Ar atmosphere and protection from light, the reaction mixture, which acquired an intense red color, was stirred for 20 min. Then, silver nitrate (1.13 g, 6.66 mmol) was added and stirred at room temperature for 4 h under an Ar atmosphere and protection from light. Finally, the solvent was removed under vacuum and the crude was purified by column chromatography (silica gel, 50–100% CH_2Cl_2 in hexanes, then from CH_2Cl_2 to 0.5% MeOH in CH_2Cl_2), to give 1.04 g (93% yield) of an orange solid. Characterization: TLC: R_f (2.5% MeOH in CH_2Cl_2): 0.60. ^1H NMR (400 MHz, $CDCl_3$): δ = 7.33 (1H, d, H5, J = 9.2 Hz), 6.75 (1H, br s, H3), 6.66 (1H, dd, H6, J = 9.2, 2.4 Hz), 6.60 (1H, d, H8, J = 2.4 Hz), 5.24 (2H, d, CH_2, J = 1.0 Hz), 3.45 (4H, q, CH_2 NEt_2, J = 7.2 Hz), 2.21 (3H, s, CH_3), 1.24 ppm (6H, t, CH_3 NEt_2, J = 7.2 Hz); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 171.8, 170.2, 155.0, 151.7, 146.1, 124.9, 114.6, 113.8, 110.6, 106.9, 105.9, 97.4, 61.1, 55.5, 45.0, 20.8, 12.4 ppm; HR ESI-MS (positive mode): m/z = 338.1491 (calcd mass for $C_{19}H_{20}N_3O_3$ $[M+H]^+$: 338.1505).

Compound 12: A solution of HCl in 1,4-dioxane (4 M, 1.5 mL, 6 mmol) was added to **1** (0.80 g, 2.37 mmol) in absolute ethanol (60 mL). The reaction mixture was heated at 90 °C for 12 h. Once the reaction was finished, the solvent was removed under reduced pressure and purified by chromatography column (silica gel, 50–100% of CH_2Cl_2 in hexane, then 0–0.8% of MeOH in CH_2Cl_2) to give 0.52 g of a yellow-orange solid (74% yield). Characterization: TLC: R_f (2.5% MeOH in CH_2Cl_2): 0.62; ^1H NMR (400 MHz, $CDCl_3$): δ = 7.34 (1H, d, H5, J = 9.2 Hz), 6.93 (1H, br t), 6.65 (1H, dd, H6, J = 9.2, 2.4 Hz), 6.60 (1H, d, H8, J = 2.4 Hz), 4.88 (2H, d, CH_2, J = 5.8 Hz), 3.45 (4H, q, CH_2 NEt_2, J = 7.2 Hz), 2.06 (1H, t, OH, J = 5.8 Hz), 1.23 ppm (6H, t, CH_3 NEt_2, J = 7.2 Hz); ESI-MS (positive mode): m/z = 295.75 (calcd mass for $C_{17}H_{18}N_3O_2$ $[M+H]^+$: 296.14).

Compound 2: 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC)·HCl (51 mg, 0.33 mmol), coumarin **12** (75 mg, 0.25 mmol), 4-dimethylaminopyridine (DMAP; 40 mg, 0.33 mmol), and benzoic acid (45 mg, 0.37 mmol) were dissolved in dry CH_2Cl_2 (2.5 mL) at 0 °C under an Ar atmosphere. After 10 min at 0 °C, the reaction mixture was stirred at room temperature for 4 h in the dark and under Ar. After evaporation under reduced pressure and purification by column chromatography (silica gel, CH_2Cl_2), 78 mg of an orange solid were obtained (77% yield). Characterization: TLC: R_f (5% MeOH in CH_2Cl_2): 0.86; ^1H NMR (400 MHz, $CDCl_3$): δ = 8.10 (2H, m, Ph), 7.62 (1H, m, Ph), 7.49 (2H, t, Ph, J = 7.6 Hz), 7.43 (1H, d, H5, J = 9.2 Hz), 6.89 (1H, br s, H3), 6.69 (1H, dd, H6, J = 9.2, 2.4 Hz), 6.62 (1H, d, H8, J = 2.4 Hz), 5.47 (2H, d, CH_2, J = 0.8 Hz), 3.46 (4H, q, CH_2 Et, J = 7.2 Hz), 1.24 ppm (6H, t, CH_3 Et, J = 7.2 Hz); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 171.8, 165.8, 155.1, 151.7, 146.0, 133.8, 129.8, 128.9, 128.8, 125.1, 114.5, 113.8, 110.7, 107.1, 106.4, 97.4, 61.7, 55.6, 45.0, 12.4 ppm; HR ESI-MS (positive mode): m/z = 400.1643 (calcd mass for $C_{24}H_{22}N_3O_3$ $[M+H]^+$: 400.1661).

Compound 5: NEt_3 (95 μL, 0.70 mmol) and ethyl isocyanate (107 μL, 1.35 mmol) were added to a solution of **12** (101 mg, 0.34 mmol) in a mixture of dry MeCN (3 mL) and CH_2Cl_2 (1.8 mL) under an Ar atmosphere. Once the reaction was finished (typically

2–3 h according to TLC), the solvent was removed under reduced pressure and purified by chromatography column (silica gel, 0–0.35% of MeOH in CH_2Cl_2) to give 85 mg of an orange solid (68% yield). Characterization: TLC: R_f (2% MeOH in CH_2Cl_2): 0.50; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.33 (1H, d, H5, J = 9.2 Hz), 6.75 (1H, br s), 6.65 (1H, dd, H6, J = 9.2, 2.8 Hz), 6.59 (1H, d, H8, J = 2.8 Hz), 5.26 (2H, br s, CH_2), 4.97 (1H, br s, NH), 3.45 (4H, q, CH_2 NEt_2, J = 7.2 Hz), 3.29 (2H, qt, CH_2 Et, J = 7.2 Hz), 1.23 (6H, t, CH_3 NEt_2, J = 7.2 Hz), 1.20 ppm (3H, t, CH_3 Et, J = 7.2 Hz); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 172.0, 155.1, 154.9, 151.6, 147.3, 124.7, 114.8, 113.9, 110.6, 106.9, 105.1, 97.3, 61.3, 55.2, 44.9, 36.2, 15.1, 12.4 ppm; HR ESI-MS (positive mode): m/z = 367.1752 (calcd mass for $C_{20}H_{23}N_4O_3$ $[M+H]^+$: 367.1770).

Synthesis of DEAdcCE-Caged Model Compounds (3, 4, 6)

Compound 15: EDC·HCl (1.6 g, 10.6 mmol), coumarin **13** (2.52 g, 9.6 mmol),[5e] DMAP (1.3 g, 10.6 mmol), and benzoic acid (1.4 g, 11.6 mmol) were dissolved in dry CH_2Cl_2 (40 mL) at 0 °C under an Ar atmosphere. After 10 min at 0 °C, the reaction mixture was stirred at room temperature for 17 h in the dark and under Ar. Subsequently, the organic solution was diluted with CH_2Cl_2 (60 mL) and washed with saturated NH_4Cl (25 mL), 5% aqueous $NaHCO_3$ (2×25 mL), water (25 mL), and dried over anhydrous Na_2SO_4. After filtration and evaporation under vacuum, the desired product (**14**) was obtained and used without further purification in the next step. Lawesson's reagent (2.5 g, 6.8 mmol) was added to a solution of **14** in toluene (50 mL). The mixture was stirred at 105 °C overnight under an Ar atmosphere and protected from light. Then, the solvent was evaporated under vacuum. After purification by column chromatography (silica gel, 50–65% CH_2Cl_2 in hexane), 2.53 g of an orange solid (69% yield from **13**) was obtained. Characterization: TLC: R_f (5% MeOH in CH_2Cl_2): 0.28; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.10 (2H, m, Ph), 7.62 (1H, m, Ph), 7.55 (1H, d, H5, J = 10 Hz), 7.48 (2H, t, Ph, J = 7.6 Hz), 7.16 (1H, s, H3), 6.68 (2H, m, H6 + H8), 6.32 (1H, q, CH-CH_3, J = 6.8 Hz), 3.44 (4H, q, CH_2 NEt_2, J = 7.2 Hz), 1.71 (3H, d, CH-CH_3, J = 6.8 Hz), 1.23 ppm (6H, t, CH_3 NEt_2, J = 7.2 Hz); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 197.4, 165.5, 159.5, 150.9, 147.9, 133.5, 129.8, 129.5, 128.6, 124.9, 119.2, 110.4, 108.0, 97.6, 67.6, 45.0, 21.0, 12.4 ppm. ESI-MS (positive mode): m/z = 382.66 (calcd mass for $C_{22}H_{24}NO_3S$ $[M+H]^+$: 382.15).

Compound 4: A solution of malononitrile (52 mg, 0.79 mmol) and NEt_3 (0.2 mL, 1.54 mmol) in anhydrous MeCN (2 mL) was added to a solution of **15** (202 mg, 0.53 mmol) in MeCN (8 mL) under an Ar atmosphere. The reaction mixture was stirred in the dark for 2 h. Then, $AgNO_3$ (196 mg, 1.15 mmol) was added and stirring was maintained for 2 h. After filtration and removal of the solvent under reduced pressure, the product was purified by column chromatography (silica gel, 0–100% CH_2Cl_2 in hexane) to give 158 mg (72% yield) of an orange solid. Characterization: TLC: R_f (5% MeOH in CH_2Cl_2): 0.63; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.09 (2H, m, Ph), 7.61 (1H, m, Ph), 7.54 (1H, d, H5, J = 9.2 Hz), 7.49 (2H, t, Ph, J = 7.6 Hz), 6.85 (1H, s, H3), 6.69 (1H, dd, H6, J = 9.2, 2.6 Hz), 6.61 (1H, d, H8, J = 2.6 Hz), 6.33 (1H, q, CH-CH_3, J = 6.8 Hz), 3.45 (4H, q, CH_2 NEt_2, J = 7.2 Hz), 1.73 (3H, d, CH-CH_3, J = 6.8 Hz), 1.24 ppm (6H, t, CH_3 NEt_2, J = 7.2 Hz); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 172.1, 165.5, 155.4, 152.2, 151.6, 133.7, 129.7, 129.2, 128.7, 125.3, 114.7, 113.9, 110.7, 106.8, 104.6, 97.6, 67.9, 55.3, 45.0, 21.1, 12.5 ppm. HR ESI-MS (positive mode): m/z = 414.1805 (calcd mass for $C_{25}H_{24}N_3O_3$ $[M+H]^+$: 414.1818).

Compound 16: A solution of aqueous HCl (37%, 50 mL, 0.58 mol) was added to **4** (0.24 g, 0.58 mmol) in MeOH (50 mL). The reaction mixture was heated at 90 °C for 15 h. Once the reaction was finished, the solvent was removed under reduced pressure and purified by chromatography column (silica gel, 0–3% of MeOH in CH_2Cl_2) to give 0.13 g of an orange solid (71% yield). Characterization: TLC: R_f (5% MeOH in CH_2Cl_2): 0.40; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.46 (1H, d, H5, J = 9.2 Hz), 6.91 (1H, s, H3), 6.66 (1H, dd, H6, J = 9.2, 2.6 Hz), 6.59 (1H, d, H8, J = 2.6 Hz), 5.19 (1H, m, CH-CH_3), 2.10 (1H, d, OH, J = 3.6 Hz), 3.45 (4H, q, CH_2 NEt_2, J = 7.2 Hz), 1.57 (3H, d, CH-CH_3, J = 6.8 Hz), 1.24 ppm (6H, t, CH_3 NEt_2, J = 7.2 Hz); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 172.4, 156.1, 155.2, 151.4, 125.3, 114.9, 114.2, 110.5, 107.0, 104.2, 97.4, 66.0, 54.7, 44.9, 24.0, 12.5 ppm; HR ESI-MS (positive mode): m/z = 310.1542 (calcd mass for $C_{18}H_{20}N_3O_2$ $[M+H]^+$: 310.1556).

Compound 3: Acetic anhydride (85 μL, 0.77 mmol) and DMAP (94 mg, 0.77 mmol) were added to a solution of **16** (47.6 mg, 0.15 mmol) in anhydrous CH_2Cl_2 (4 mL) under an Ar atmosphere. The reaction mixture was stirred in the dark overnight. After removal of the solvent under reduced pressure, the product was purified by column chromatography (silica gel, 0–0.1% MeOH in CH_2Cl_2) to give 21 mg (40% yield) of an orange solid. Characterization: TLC: R_f (1% MeOH in CH_2Cl_2): 0.66; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.43 (1H, d, H5, J = 9.2 Hz), 6.72 (1H, s, H3), 6.67 (1H, dd, H6, J = 9.2, 2.4 Hz), 6.60 (1H, d, H8, J = 2.4 Hz), 6.08 (1H, q, CH-CH_3, J = 6.8 Hz), 3.45 (4H, q, CH_2 NEt_2, J = 7.2 Hz), 2.16 (3H, s, CH_3CO), 1.58 (3H, d, CH-CH_3, J = 6.8 Hz), 1.24 ppm (6H, t, CH_3 NEt_2, J = 7.2 Hz); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 172.1, 169.9, 155.3, 152.3, 151.6, 125.2, 114.8, 113.9, 110.7, 106.7, 104.3, 97.6, 67.2, 55.2, 45.0, 21.1, 12.5 ppm; HR ESI-MS (positive mode): m/z = 352.1648 (calcd mass for $C_{20}H_{22}N_3O_3$ $[M+H]^+$: 352.1661).

Compound 6: NEt_3 (600 μL, 4 mmol) and ethyl isocyanate (380 μL, 4.8 mmol) were added to a solution of **16** (62 mg, 0.20 mmol) in a mixture of dry MeCN (2.5 mL) and CH_2Cl_2 (1.5 mL) under an Ar atmosphere. Once the reaction was finished (24 h according to TLC), the solvent was removed under reduced pressure and purified by chromatography column (silica gel, 0–0.75% of MeOH in CH_2Cl_2) to give 42 mg of an orange solid (63% yield). Characterization: TLC: R_f (2% MeOH in CH_2Cl_2): 0.46; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.44 (1H, d, H5, J = 9.2 Hz), 6.73 (1H, s, H3), 6.67 (1H, dd, H6, J = 9.2, 2.4 Hz), 6.59 (1H, d, H8, J = 2.4 Hz), 6.03 (1H, q, CH-CH_3, J = 6.8 Hz), 4.90 (1H, br s, NH), 3.45 (4H, q, CH_2 NEt_2, J = 7.2 Hz), 3.24 (2H, qt, NH-CH_2-CH_3, J = 7.2 Hz), 1.55 (3H, d, CH-CH_3, J = 6.8 Hz), 1.23 (6H, t, CH_3 NEt_2, J = 7.2 Hz), 1.17 ppm (3H, t, NH-CH_2-CH_3, J = 7.2 Hz); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 172.3, 155.3, 154.8, 153.5, 151.6, 125.2, 115.0, 114.1, 110.6, 106.8, 104.0, 97.5, 67.2, 54.7, 44.9, 36.1, 15.1, 12.5 ppm; HR ESI-MS (positive mode): m/z = 381.1922 (calcd mass for $C_{21}H_{25}N_4O_3$ $[M+H]^+$: 381.1927).

Photophysical Properties

Absorption spectra were recorded with a Varian Cary 500 UV/Vis/NIR spectrophotometer at 25 °C. Emission spectra were registered in a Photon Technology International (PTI) fluorimeter. Solutions for emission measurements were adjusted to concentrations such that the absorption was around 0.04 at the excitation wavelength (λ_{Ex} = 460 nm). Fluorescence quantum yields, Φ_F, were calculated from Equation (1):

$$\Phi_F = \Phi_F^s \frac{Abs^s}{Abs} \frac{A}{A^s} \frac{n^2}{n_s^2} \quad (1)$$

in which the superscript "s" stands for standard samples, Abs is the absorbance at the excitation wavelength (λ_{Ex}), A is the integrated area of the corresponding emission spectrum, and n is the refractive index of the solvent used.[14] The uncertainty in the experimental value of Φ_F has been estimated to be approximately 10%. The standard fluorophore used for the determination of the fluorescence quantum yield was fluorescein dissolved in aqueous sodium hydroxide (0.1 M) with a reported absolute fluorescence quantum yield, Φ_F, of 0.92.[15] All samples were measured in 1×1 cm quartz cuvettes (Hellma).

Irradiation Experiments

Photolysis studies were performed at 37 °C in a custom-built irradiation setup from Microbeam including a cuvette, thermostated cuvette holder, and a mounted high power LED of 505 nm (100 mW cm^{-2}). In a typical experiment, the irradiation samples contained the dicyanocoumarin-caged model compound (20 μM) and 12 μL of a 30 μM solution of uracil (internal standard) in a 1:1 (v/v) mixture of Tris buffer pH 7.5 and MeCN. After irradiation, the samples were analyzed by reversed-phase HPLC-ESI MS with a Jupiter Proteo C_{18} column (250×4.6 mm, 90 Å, 4 μm, flow rate: 1 mL min^{-1}) by using linear gradients of 0.1% formic acid in H_2O (A) and 0.1% formic acid in MeCN (B).

Uncaging Quantum Yield Determination

The uncaging quantum yield for coumarin derivatives **1**–**6** upon illumination at 505 nm was estimated by comparing the initial rate of photo-uncaging with the rate of photobleaching of (*E*)-2-[1-(2,5-dimethyl-3-furyl-9-ethylidene]-3-isopropylidene succinic anhydride (Aberchrome 540), a well-known chemical actinometer.[16,17] Indeed, the ring-opening back reaction of this particular organic chromophore can be easily exploited for actinometry in the visible region of the electromagnetic spectrum by measuring the decrease in absorbance at 494 nm of a known volume of the red toluene solution, which was previously obtained by irradiating the ring-opened isomer of Aberchrome 540 with UV light (Philips high pressure mercury lamp, 500 W, 320 nm $< \lambda_{Irrad} <$ 390 nm) for 5 min.

The photon flow onto the sample was calculated by using Equation (2):

$$\frac{Nh\nu}{t} = \frac{\Delta A \times V}{\Phi_\lambda \times \varepsilon \times \Delta t} \quad (2)$$

where ΔA is the decrease in absorbance at 494 nm, Δt is the irradiation time, V is the volume of the irradiated solution (3 mL), Φ_λ is the quantum yield for the ring-opening reaction, and ε is the molar absorption coefficient of the ring-closed isomer of Aberchrome 540 at 494 nm (8200 M^{-1} cm^{-1}). The quantum yield for the photobleaching reaction in toluene at 21 °C, was calculated by the following expression [Eq. (3)]:

$$\Phi_\lambda = 0.178 - 2.4 \times 10 - 4\,\lambda_{Ac} \quad (3)$$

where λ_{Ac} is the activation wavelength (in this instance, λ_{Ac} = 505 nm). The photon flow thus determined was 1.05× 10^{-7} Einstein s^{-1}.

The kinetic traces for the uncaging step were fitted to a monoexponential decreasing function and the initial slope was determined by differentiation. The uncaging quantum yield, Φ_{Ph}, was determined by calculating the ratio between the initial slope and the number of absorbed photons by the sample as determined by actinometry.[18]

Computational Calculations

According to the Bell–Evans–Polanyi principle, it can be considered that the changes in the exothermicity of the reaction for two similar dicyanocoumarin-caged compounds is proportional to the differences in reaction activation energies. Thus, we computed the free energy changes associated with the transformation of each compound in its corresponding ion pair intermediate species and compared the results for each pair of similar compounds (that is, differing either in the type of carbocation or anion released in the reaction). All calculations were carried out with the Gaussian 09 (G09) software package.[19] The electronic structures were computed by Density Functional Theory (DFT)[20] by using the B3LYP functional[21] and the 6-31G** basis set.[22] The most stable enantiomers of all chiral centers were chosen for structural optimizations, which were performed in an implicit water solvent (SMD solvation model[23]).

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Conflict of Interest

The authors declare no conflict of interest.

Keywords: caged compounds · coumarins · photolysis · protecting groups · UV/Vis spectroscopy

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