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Wavelength-Orthogonal Photocleavable Monochromophoric Linker for Sequential Release of Two Different Substrates

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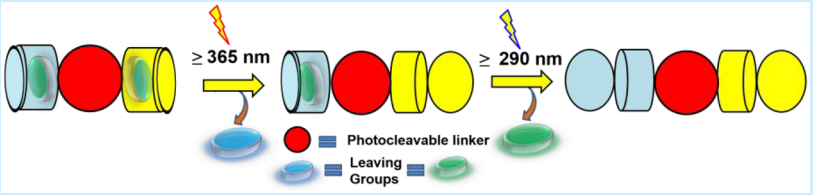
Supporting Information



ABSTRACT: A wavelength-orthogonal photocleavable monochromophoric linker was developed that is based on a 3-acetyl-9-ethyl-6-methylcarbazole (AEMC) moiety substituted at both the phenacyl and benzylic positions with different carboxylic acids. The different carboxylic acids were released sequentially upon irradiation with light of $\lambda \geq 365$ nm and $\lambda \geq 290$ nm, respectively.

Wavelength-orthogonal photorelease has emerged as a powerful strategy, particularly in the areas of solid-state synthesis, combinatorial drug delivery, photodeprotection of oligonucleotides, protein kinase activation, and surface lithography.[1−10] The Bochet group was the first to introduce the sequential release of two different substrates using wavelength-orthogonal photoremovable protecting groups (PRPGs).[11−14] They used a nitroveratryl carbamate (NVOC) and a benzoin derivative in the same solution and demonstrated the sequential release of different carboxylic acids by irradiation of the NVOC group with longer-wavelength light (≤420 nm) and the benzoin derivative with shorter-wavelength light (≥254 nm) (Figure 1a). Later, Wang and co-workers showed the sequential release of dicarboxylic acid by utilizing the different photochemical behaviors of 3-(diethylamino)benzyl (DEABn, $\lambda \geq 310$ nm) and 3,5-dimethoxybenzyl (DMBn, $\lambda \geq 279$ nm) PRPGs.[15−21] The above-mentioned strategies mainly employed bichromophoric systems for the sequential release of different substrates.

Recently, the use of monochromophoric systems has become a more attractive strategy for the release of two different bioactive molecules compared with the bichromophoric systems because it can reduce the number of released photoproducts, thereby decreasing the toxicity of the given system. Wong and our research group independently developed monochromophoric systems for the release of two different substrates based on thioacetal-*o*-nitrobenzaldehyde (TNB) and carbazole PRPGs, respectively.[22−24] The main limitation of the above systems is that the two substrates were released simultaneously at the same wavelength. In the literature there is only one example of a monochromophoric system for the sequential release of two different functional groups. It was developed by Klan et al. and is based on a 4-acetyl-2-nitrobenzyl moiety (a combination of 2-nitrobenzyl and phenacyl groups) (Figure 1b).[25] The limitations of this approach were that (i) the second release from the phenacyl group requires a hydrogen atom donor as a chemical activator along with light and (ii) the monochromophoric linker is nonfluorescent in nature and therefore lacks the ability to provide information regarding the uncaging process.

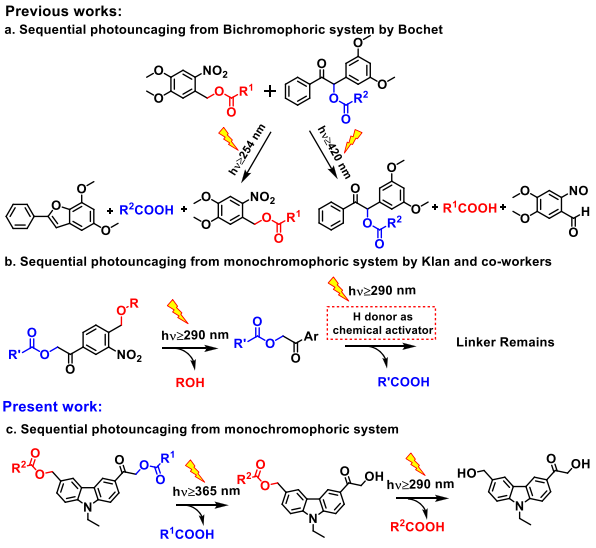


Figure 1. (a) Sequential photorelease from a bichromophoric system. (b) Sequential photorelease from a monochromophoric system. (c) Proposed sequential photorelease from a monochromophoric system with wavelength orthogonal.

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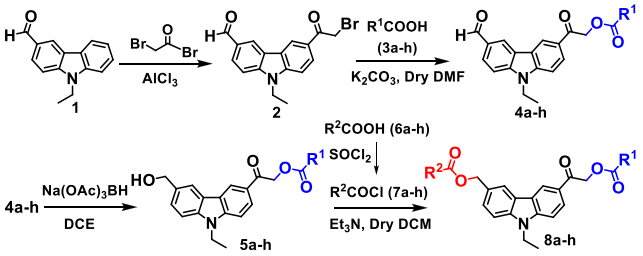
Thus, there is a need for the development of new photocleavable fluorescent monochromophoric systems that are capable of releasing two different substrates in a wavelength-orthogonal manner without using any external donors. In this work, we have designed a monochromophoric fluorescent photocleavable linker based on a carbazole chromophore, 3-acetyl-9-ethyl-6-methylcarbazole (AEMC), which has properties of two well-known photoremovable protecting groups (a combination of benzyl and phenacyl), from which the leaving groups (LGs) can be sequentially released in a wavelength-orthogonal manner (Figure 1c).

Dual caged esters (**8a**–**h**) were synthesized following a sequence of chemical reactions as shown in Scheme 1. First, 9-ethyl-9*H*-carbazole-3-carbaldehyde (**1**) was synthesized according to a known procedure.[26] Then compound **2** was obtained by Friedel–Crafts (FC) acylation of **1** with 1 equiv of bromoacetyl bromide. The corresponding single caged esters **4a**–**h** were synthesized from **2** by carrying out an esterification reaction with corresponding carboxylic acids (**3a**–**h**) in the presence of K_2CO_3. Then we synthesized the dual-arm caged esters (**8a**–**h**) from their respective single-arm caged esters (**4a**–**h**) in two steps. In the first step, the aldehydes were selectively reduced with sodium triacetoxyborohydride (Na-$(OAc)_3BH$) to afford alcohols **5a**–**h.** Finally, dual caged esters **8a**–**h** were obtained by an esterification reaction between alcohols **5a**–**h** and acid chlorides **7a**–**h** of the corresponding carboxylic acids **6a-h.** All of the synthesized dual caged esters were well-characterized by ^{1}H and ^{13}C NMR spectroscopy and mass spectral analysis (see Figures S1–S49).

Scheme 1. Synthesis of Dual Caged Esters (8a–h)



Next, the photophysical properties of synthesized dual caged esters **8a**–**h** were recorded in acetonitrile/water (ACN/H_2O) (3:7 v/v). Figure 2a and Figure 2b show the normalized absorption and emission spectra of **8a** (1×10^{-5} M), respectively. The absorption spectrum of **8a** shows three absorption bands at 290, 322, and 334 nm, which correspond to π–π^* transitions to a higher-energy excited singlet state (S_2) and a lower-energy excited singlet state (S_1) and an n–π^* transition, respectively.[24,27] In the case of the emission spectrum, the emission maximum of **8a** was red-shifted to 508 nm. The photophysical properties of **8a**–**h** and **9a** are summarized in Table 1 and Figures S50 and S51. Furthermore,

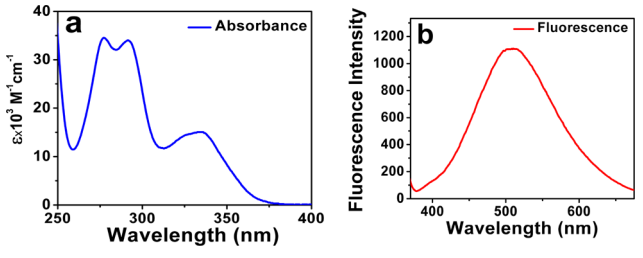
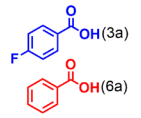
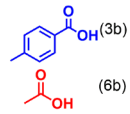
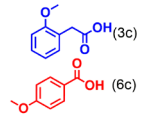
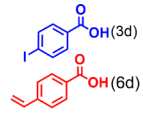
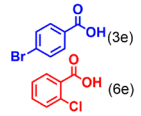
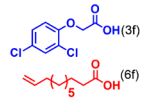
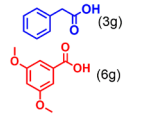
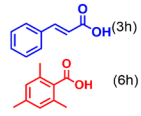


Figure 2. (a) UV absorption spectrum and (b) emission spectrum (λ_{ex} = 350 nm) of **8a**.

Table 1. Synthetic Yields and Photophysical Properties of 8a–h

caged ester	carboxylic acids	synthetic yield[a] %	absorption π–π* (nm)[b]	absorption n-π* (nm)[b]	fluorescence λ_{max} (nm)[c]	fluorescence Φ_f[d]
8a		93	290	332	506	0.121
8b		88	291	331	504	0.123
8c		95	290	330	508	0.119
8d		94	292	331	501	0.122
8e		94	291	331	507	0.12
8f		92	291	332	508	0.118
8g		91	293	330	506	0.124
8h		89	292	331	508	0.122

[a]Based on the isolated yield for the last step. [b]Maximum absorption wavelength. [c]Maximum emission wavelength. [d]Fluorescence quantum yield (error limit within ±5%).

the fluorescence quantum yields (Φ_f) of **8a**–**h** were calculated with 9,10-diphenylanthracene as a standard (Φ_f = 0.95 in ethanol) and were found to be in the range of $0.118 \leq \Phi_f \leq 0.124$.

Considering that our main interest was to study the wavelength-orthogonal release ability of our fluorescent monochromophoric linker, we irradiated a solution of dual caged esters **8a**–**h** (1.0×10^{-4} M) individually in ACN/H_2O (3:7 v/v) at different wavelengths using a 125 W medium-pressure Hg lamp as the UV light source. Here, we used 1 M $CuSO_4$ solution and Pyrex as the UV cutoff filters to produce light of $\lambda \geq 365$ nm and $\lambda \geq 290$ nm, respectively.

First, we irradiated dual caged ester **8a** with the light of $\lambda \geq 365$ nm at regular intervals of time, and the photolysis was monitored by reversed-phase (RP) HPLC (Figure 3). The HPLC chart shows gradual depletion of the peak at t_R = 7.96

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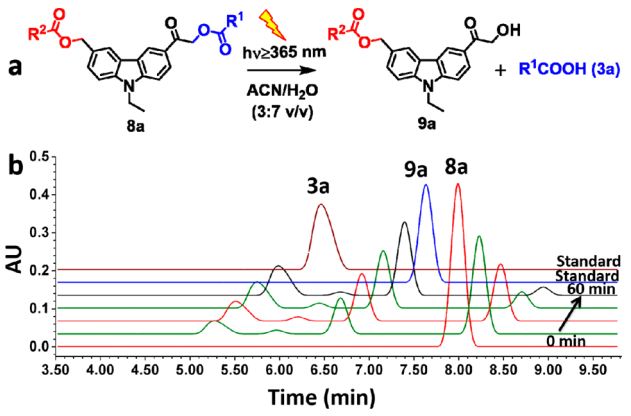

Figure 3. (a) Photorelease of carboxylic acid **3a** from **8a** using light of $\lambda \geq 365$ nm. (b) Overlay of HPLC chromatograms of **8a** at regular time intervals (0−60 min) of irradiation with UV light (≥365 nm). The *y* axes are offset by 10 mAU and the *x* axes by 15 s to facilitate visualization. AU = arbitrary units.

min with a gradual increase in the irradiation time, indicating the photodecomposition of **8a**. On the other hand, we also noted a gradual increase of two new major peaks at t_R = 6.43 and 5.02 min, corresponding to single caged ester **9a** and carboxylic acid **3a**, respectively. The newly formed photoproducts were isolated and confirmed by injection of authentic samples. Furthermore, we characterized the photoproduct **9a** by ^{1}H NMR, ^{13}C NMR, and mass spectral analysis (Figures S52−S54). Next, we also followed the photolysis of **8a** at $\lambda \geq$ 365 nm by fluorescence spectroscopy, and the results showed that the fluorescence intensity increases with blue-shifted emission with a gradual increase in irradiation time (Figure S55).

Next, we also investigated the photorelease ability of dual caged esters **8b−h** (1.0×10^{-4} M) individually in ACN/H_2O (3:7 v/v) using light of $\lambda \geq$ 365 nm. Irradiation of **8b−h** in ACN/H_2O (3:7 v/v) results in the release of the corresponding carboxylic acids in high chemical yields (88−94%), as shown in Table 2 and Figure S56. Furthermore, the photochemical quantum yields (Φ_p) for the release of carboxylic acids were calculated using potassium ferrioxalate as an actinometer and found to be 0.094−0.101, as shown in Table 2.

To check the orthogonal capability of the designed system, we continued the photolysis of **9a** at the same wavelength ($\lambda \geq$ 365) for up to 4 h. From the HPLC data, we found that there was no photodecomposition even after 4 h of photolysis (Figure S57).

After the first uncaging using light of $\lambda \geq$ 365 nm, we were interested to evaluate ability of caged ester **9a** to undergo the second photorelease using light of $\lambda \geq$ 290 nm. We irradiated caged ester **9a** with light of $\lambda \geq$ 290 nm at regular time intervals (Figure 4). The HPLC chart (Figure 4) shows that

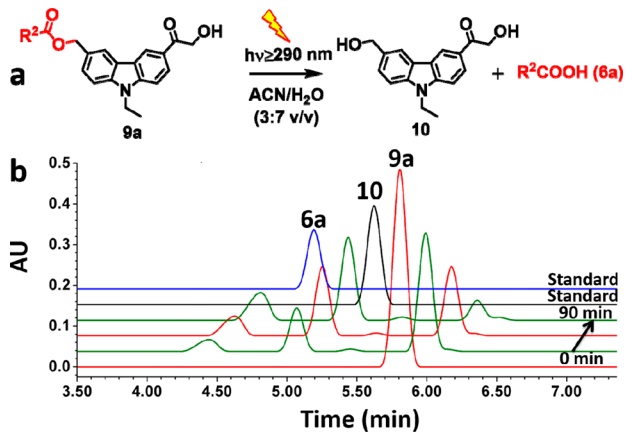

Figure 4. (a) Photorelease of carboxylic acid **6a** from **9a** using light of $\lambda \geq$ 290 nm. (b) Overlay of HPLC chromatograms of **9a** at regular time intervals (0−90 min) of irradiation with UV light (≥290 nm). The *y* axes are offset by 10 mAU and the *x* axes by 15 s to facilitate visualization. AU = arbitrary units.

with increasing irradiation time, carboxylic acid **6a** (t_R = 4.26 min) and the final photoproduct, 1-(9-ethyl-6-(hydroxymethyl)-9*H*-carbazol-3-yl)-2-hydroxyethanone (**10**) (t_R = 4.89 min), were released effectively by our caged ester **9a**. Furthermore, the photoproducts were isolated and confirmed by injection of an authentic sample, and the final photoproduct **10** was characterized by ^{1}H NMR spectroscopy and mass spectral analysis (Figures S58 and S59). Furthermore, we also followed the photolysis of **9a** at $\lambda \geq$ 290 nm by fluorescence spectroscopy (Figure S60) and found that the fluorescence intensity increased with a gradual increase in irradiation time.

Next, we also carried out the photolysis of caged esters **9b−h** in a similar manner using light of $\lambda \geq$ 290 nm, and the release of the corresponding carboxylic acids **6b−h** was followed by RP-HPLC (Figure S61). In the case of single caged esters **9b** and **9f**, the percentages of released carboxylic acids **6b** and **6f**, respectively, were calculated on the basis of

Table 2. Photochemical Properties of 8a−h and 9a−h

caged ester	% of acid release[a]	ε[b] (10^3 $M^{-1}cm^{-1}$)	Φ_u(≥365 nm)[c]	$\varepsilon\Phi_u$[d] ($M^{-1}cm^{-1}$)	caged ester	% of acid release[a]	ε[e] (10^3 M^{-1} cm^{-1})	Φ_u(≥290 nm)[f]	$\varepsilon\Phi_u$[g] (M^{-1} cm^{-1})
8a	93 (**3a**)	1.73	1.0×10^{-1}	173	**9a**	91 (**6a**)	33.76	1.55×10^{-2}	523.3
8b	88 (**3b**)	1.71	0.94×10^{-1}	160.7	**9b**	89[h] (**6b**)	33.69	1.51×10^{-2}	508.7
8c	91 (**3c**)	1.69	0.98×10^{-1}	165.6	**9c**	88 (**6c**)	33.76	1.5×10^{-2}	506.4
8d	89 (**3d**)	1.75	0.96×10^{-1}	168	**9d**	87 (**6d**)	33.64	1.48×10^{-2}	497.9
8e	92 (**3e**)	1.7	0.99×10^{-1}	168.3	**9e**	90 (**6e**)	33.68	1.53×10^{-2}	515.3
8f	89 (**3f**)	1.74	0.95×10^{-1}	165.3	**9f**	87[h] (**6f**)	33.67	1.49×10^{-2}	501.7
8g	90 (**3g**)	1.74	0.97×10^{-1}	168.8	**9g**	90 (**6g**)	33.73	1.52×10^{-2}	512.7
8h	94 (**3h**)	1.72	1.01×10^{-1}	173.7	**9h**	89 (**6h**)	33.77	1.51×10^{-2}	509.9

[a]% of the carboxylic acid as determined by HPLC. [b]Molar absorption coefficient at 365 nm. [c]Photochemical quantum yield for carboxylic acid release using light of $\lambda \geq$ 365 nm (error limit within ±10%). [d]Photosensitivity at 365 nm (error limit within ±5%). [e]Molar absorption coefficient at 290 nm. [f]Photochemical quantum yield for carboxylic acid release using light of $\lambda \geq$ 290 nm (error limit within ±10%). [g]Photosensitivity at 290 nm (error limit within ±5%). [h]% of the carboxylic acid released as determined on the basis of decomposition of the starting material.

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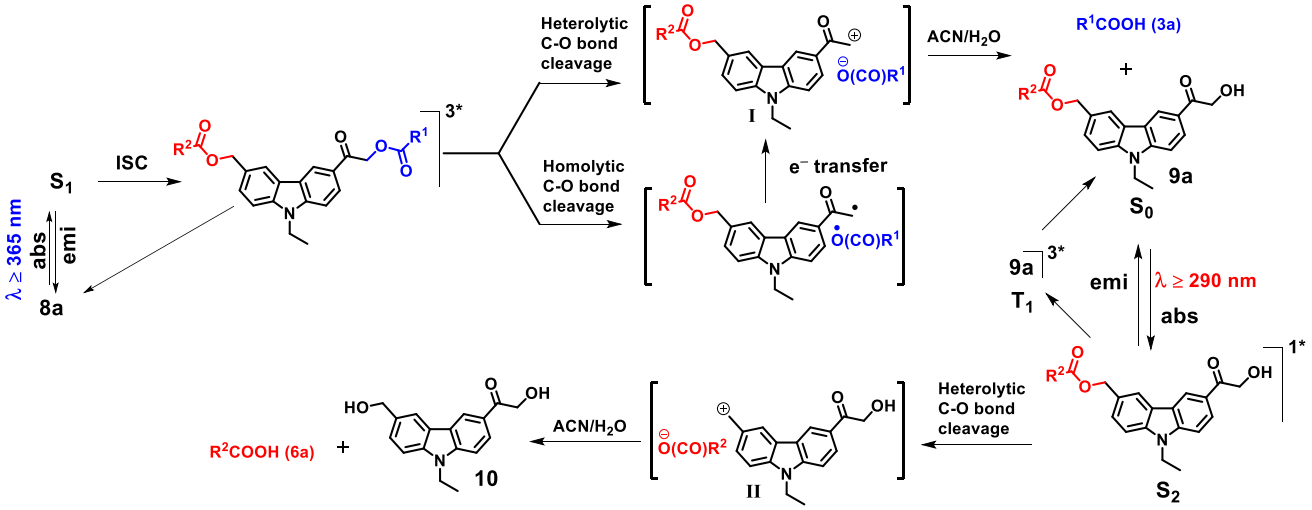
Scheme 2. Possible Photorelease Mechanism for Orthogonal Release



decomposition of the starting material. The photochemical properties of **9b**–**h** are shown in Table 2. Overall, the designed orthogonal monochromophore has the efficient ability to release carboxylic acids having electron-donating and -withdrawing substituents in a sequential manner.

On the basis of the literature and our earlier work,[24] we suggested a possible mechanism for the first release, as shown in Scheme 2. Irradiation of **8a** using light of $\lambda \geq 365$ in aqueous ACN leads to cleavage of the C–O bond of the phenacyl ester to form ion-pair intermediate **I**. Trapping of **I** by the polar solvent yields the single caged ester **9a** along with the released carboxylic acid **3a**.

To understand whether the photorelease mechanism for the second release goes by a singlet or triplet excited state, we performed sequential experiments like fluorescence quenching and triplet excited state quenching of **9a** using benzophenone (a triplet sensitizer) and potassium sorbate (PS), respectively. The results of the quenching experiments suggest that the photocleavage of **9a** occurs via the singlet excited state (Figures S62 and S63). Hence, we suggested a possible photorelease mechanism for the second release, as shown in Scheme 2. Irradiation of **9a** using light of $\lambda \geq 290$ in aqueous ACN leads to the higher-energy singlet excited state (S_2). From the singlet excited state, heterolytic cleavage of the C–O bond of the benzyl ester forms ion-pair intermediate **II**. Trapping of **II** by the polar solvent yields the final photoproduct **10** along with the released carboxylic acid **6a**.

In conclusion, we have demonstrated for the first time a wavelength-orthogonal fluorescent photocleavable monochromophoric linker based on the AEMC moiety for the sequential release of different carboxylic acids. We achieved the orthogonal release of different carboxylic acids in high chemical and moderate quantum yields upon irradiation using light of $\lambda \geq 365$ nm and $\lambda \geq 290$ nm, respectively. The current limitation of the application is the need to use UV light ($\lambda < 410$ nm). In the future, we intend to use our system to design a visible-light-activated monochromophoric linker for combinatorial drug delivery systems with real-time monitoring ability.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.orglett.9b04323.

Synthesis details, characterization data by ^{1}H and ^{13}C NMR and HRMS spectra of all synthesized compounds, photophysical properties, experimental details of photolysis, HPLC traces for the photolysis of all the caged compounds, and quenching experiments (PDF)

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Notes

The authors declare no competing financial interest.

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