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Authors: Pradeep Singh, VENKATESH YARRA, Surajit Nandi, Maniklal Shee, Biswajit Saha, and Anakuthil Anoop

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Bis-Acetyl Carbazole: Photoremovable Protecting Group for Sequential Release of Two Different Functional Groups and its Application for Therapeutic Release

Yarra Venkatesh,[a] Surajit Nandi[a], Maniklal Shee, [a] Biswajit Saha,[b] Anakuthil Anoop*[a] and N. D. Pradeep Singh*[a]

Abstract: In this paper, we present fluorescent photoremovable protecting group (FPRPG) based on bis-acetyl carbazole for the release of two different functional groups like carboxylic acids, alcohols, thiols and amines in a sequential fashion. Dual arm caged bis-acetyl carbazoles with different combinations of two unlike functional groups were synthesized. Photophysical studies showed that caged bis-acetyl carbazoles are blue fluorescent and their emission properties are sensitive to the environment. Sequential photorelease of two different functional groups by bis-acetyl carbazole was analyzed by HPLC, UV and emission spectroscopy. The mechanism for the dual release by bis-acetyl carbazole was investigated and supported by TD-DFT calculations. To demonstrate the application of dual release ability of bis-acetyl carbazole FPRPG, we synthesized drug delivery system (DDS) in which one arm of bis-acetyl carbazole is linked to the carboxylic functional group of chlorambucil (CBL) and the other arm is attached to the hydroxyl group of ferulic acid ethyl ester (FAEE). In-vitro studies showed that our DDS presents excellent properties like photoregulated dual drug delivery, cellular uptake, and biocompatibility.

Introduction

Drug delivery systems (DDSs) which are capable of on-demand delivery of two or more drugs with spatial, temporal and dosage control efficiency have gained great interest. An easy way to achieve spatio-temporal control over drug delivery is to cage the active molecule by photoremovable protecting group (PRPG), which on excitation by external stimulus light can release the active molecule.[1-6] Recently, single chromophoric dual DDSs have become great advantageous over single DDSs particularly in cancer treatment since it can overcome toxicity and other side effects related with high doses of single drugs.[7, 8] However, to design DDSs for dual release, we require PRPG which can release two different active molecules sequentially on exposure to light. PRPGs based on single chromophore for dual release are less in number compared to single release PRPGs.[9-15] Known examples of single chromophore based PRPGs for dual release are thioacetal ortho-nitrobenzaldehyde (TNB),[16] 3-diethylaminobenzyl (DEABn),[17] perylene-3,4,9,10-tetrayltetramethyl,[18] and bimane.[19]

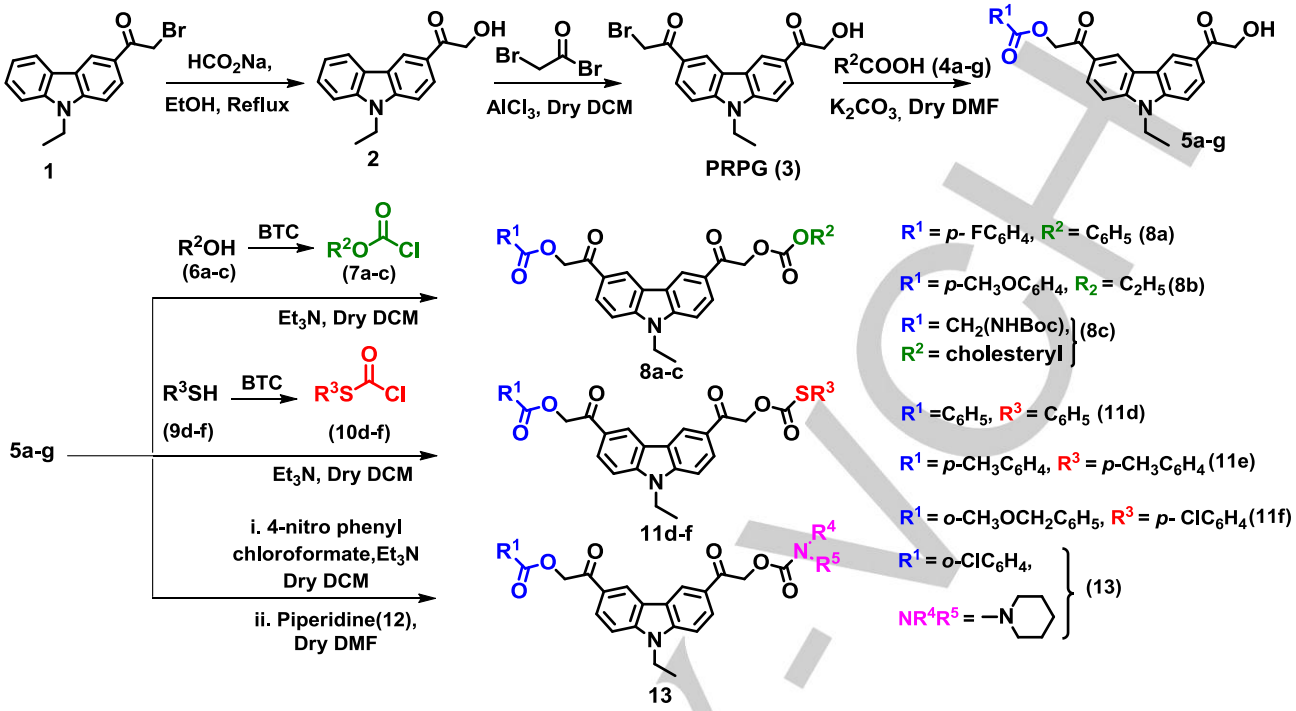
The aforementioned PRPGs are mostly used to cage two different molecules having same functional groups like carboxylic or alcoholic groups. However, there are several therapeutic agents with different end functionality e.g alcohols (paclitaxel, doxorubicin, gemcitabine, camptothecin), amines (5-fluorouracil, ciprofloxacin, dopamine), carboxylic acids (methotrexate, chlorambucil, melphalan, caffeic acid) and thiols (captopril) used in DDS. Hence, to develop photoresponsive DDS for any given two drugs, it is essential to have a PRPG which has the ability to cage two different functional groups like carboxylic acids, alcohols, thiols and amines.

Klan and coworkers developed single chromophore based on 4-acetyl-2-nitrobenzyl (ANB) moiety for the sequential release of two different functional groups.[20] The advantage of ANB protecting group is unique ability to release the functional groups in a selective and orthogonal manner upon irradiation. The limitations to utilize ANB chromophore in the area of drug release are i) it requires UV light <350 nm and ii) non-fluorescent in nature. Recently, our group demonstrated acetyl carbazole as a fluorescent PRPG (FPRPG) for the dual release of same functional group (carboxylic acids).[21,22] Herein, we present bis-acetyl carbazole as a FPRPG for the sequential release of two different functional groups like carboxylic acids, alcohols, thiols and amines on exposure to UV light (λ ≥ 365 nm). Dual arm caged bis-acetyl carbazoles with different combinations of two different functional groups were synthesized. Environment sensitive fluorescence behaviour of bis-acetyl carbazole and its caged compounds were studied. The mechanism for the photorelease was investigated and supported by DFT calculations. As proof of concept, we designed drug delivery system (DDS) having chlorambucil (carboxylic functionality) on one arm of bis-acetyl carbazole and ferulic acid ethyl ester (hydroxyl functionality) on the other arm. *In vitro* cellular imaging and cytotoxicity of our drug delivery system was also explored.

Results and Discussion

PRPG **3** and their dual arm caged compounds (combination of carboxylic and hydroxyl **8a-c**, carboxylic and thiol **11d-f**, and carboxylic and amine **13** functionalities**)** were synthesized following a sequence of chemical reactions as shown in **Scheme 1**. Compound **1** was synthesized according to a known procedure.[21] Next, **2** was obtained by refluxing **1** with sodium formate in ethanol. Friedel-Crafts acylation of **2** with bromoacetyl bromide in the presence of $AlCl_3$ yielded PRPG **3**. The single arm caged esters 5a-g of PRPG **3** was synthesized from their corresponding carboxylic acids **4a-g** by esterification reaction.

[a] Yarra Venkatesh, Surajit Nandi, Maniklal Shee, Dr. Anakuthil Anoop and Dr. N. D. Pradeep Singh
Department of Chemistry, Indian Institute of Technology Kharagpur, 721302, West Bengal, India.
E-mail:ndpradeep@chem.iitkgp.ernet.in
[b] Dr. Biswajit Saha.
Department of Biotechnology, Indian Institute of Technology Kharagpur, 721302, West Bengal,India .



Scheme 1. Synthesis of PRPG 3 and dual arm caged compounds (**8a-c**, **11d-f** and **13)**

Then, we synthesized dual arm caged compounds from their corresponding single arm caged esters. First, we converted alcohols (**6a-c**) and thiols (**9d-f**) into their corresponding chloroformates by treating with bis(trichloromethyl) carbonate (BTC) at 0 °C. Next, dual arm caged compounds **8a-c** and **11d-f** were synthesized by treating single arm caged esters (**5a-c)** with alcohol chloroformates (**7a-c)** and single arm caged esters **5d-f** with thiol chloroformates (**10d-f**), respectively. Finally, dual arm caged compound **13** was synthesized by following a chemoselective protocol as reported by Suzuki et al.[23] Treatment of single arm caged ester **5g** with 4-nitrophenyl chloroformate in the presence of Et_3N in DCM, followed by addition of piperidine (**12**) in dry dimethylformamide (DMF) afforded **13**. All the synthesized dual arm caged compounds were characterized by ^1H, ^{13}C and mass spectral analysis. (See **Figure S1-S17** in **SI**).

Figure. 1a shows the normalized UV absorption and emission spectra of dual arm caged compound **8a** (1×10^{-5} M) in absolute ethanol. The absorption spectrum of **8a** shows intense peak centered at 338 nm. In the emission spectrum, the emission maximum of **8a** was red shifted to about 456 nm. We noticed that the Stoke's shift for all the dual arm caged compounds varies between 118 and 120 nm **(Table 1)**. The absorption, emission maxima, Stoke' shifts and fluorescence quantum yield of all the caged compounds **(8a-c, 11d-f and 13)** are summarized in **Table 1**. The fluorescence quantum yields (Φ_f) of dual arm caged compounds in absolute ethanol (EtOH) at room temperature were in the range of $0.221 \le \Phi_f \le 0.275$ **(Table 1)**. The fluorescence quantum yields (Φ_f) of caged compounds were calculated using 9,10-diphenylanthracene as the standard (Φ_f = 0.95 in ethanol).

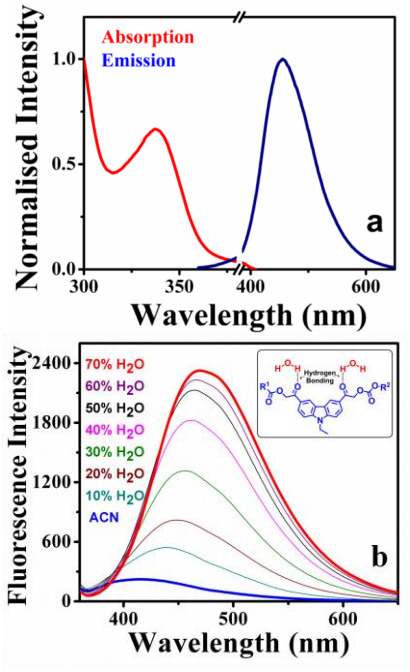
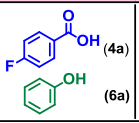
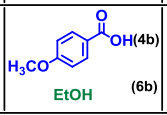
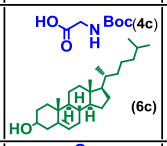
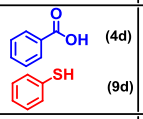
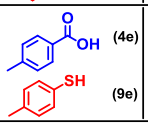
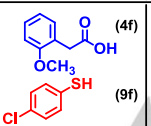
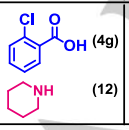


Figure 1. (a) UV absorption (red line) and emission spectra (blue line) in absolute ethanol (b) emission spectra in ACN/H_2O mixtures of dual arm caged compound **8a** (1×10^{-5} M). Excitation wavelength: 350 nm.

To check the environmentally sensitive property of our dual arm caged compound, emission spectra of **8a** were recorded in a different ratio of acetonitrile-water solvent systems **(Figure. 1b)**. The excitation of **8a** in pure acetonitrile at 350 nm produced emission maximum at around 410 nm which corresponds to n-π* transition. On addition of increasing amount of water (70% water), the emission maximum got red shifted to about 470 nm with an increase in fluorescence intensity. This red shift of the emission implies that the formation of a hydrogen bond between acetyl carbonyl group of carbazole and water. The increase in the intensity of the emission is because the hydrogen bonding raises the energy of ^{3}n-π* state (which was found in the vicinity of lowest ^{1}π-π* excited states) of bis-acetyl carbazole above the lowest ^{1}π-π* excited states, thereby reducing the efficiency of intersystem crossing.[24,25]

Table 1. Synthetic Yields and Photophysical Properties of Caged Compounds (8a-c, 11d-f and 13).

Caged Compounds	Carboxylic Acids, Alcohols, Thiols and Amines	Synthetic yield[a] %	Absorption		Fluorescence		
			λ_{max} (nm)[b]	$\varepsilon\times10^4$ (L mol^{-1}cm^{-1})[c]	λ_{max} (nm)[d]	Stoke's shift (nm)[e]	Φ_f[f]
8a	 (4a) (6a)	92	338	2.71	456	118	0.268
8b	 (4b) (6b)	89	337	2.78	457	120	0.275
8c	 (4c) (6c)	93	338	2.70	458	120	0.274
11d	 (4d) (9d)	87	338	2.54	458	120	0.221
11e	 (4e) (9e)	83	337	2.56	456	119	0.225
11f	 (4f) (9f)	88	337	2.58	457	120	0.229
13	 (4g) (12)	72	337	2.67	457	120	0.247

[a]Based on isolated yield. [b]Maximum absorption wavelength. [c]Molar absorption coefficient at maximum absorption wavelength. [d]Maximum emission wavelength. [e]Difference between maximum absorption wavelength and maximum emission wavelength. [f]Fluorescence quantum yield (error limit within ± 5%).

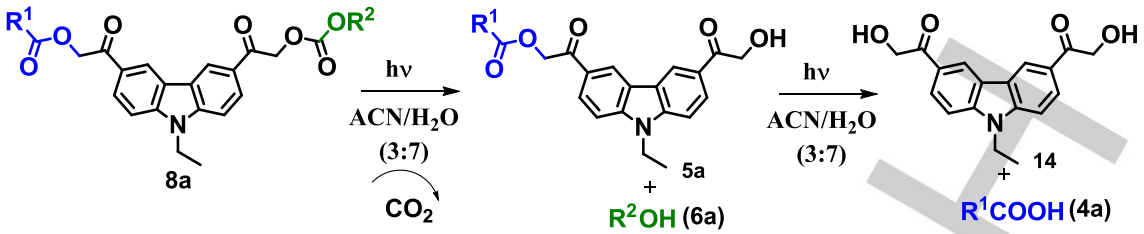
Dual arm caged compounds **8a-c**, **11d-f** and **13** (1.0×10^{-4} M) was dissolved in acetonitrile/water (ACN /H_2O) (3:7 v/v), individually, purged with argon for 15 min, and irradiated with a 125-W Hg medium-pressure UV lamp (λ ≥ 365 nm) through a 1M $CuSO_4$ solution as the UV cut–off filter. The course of photoreaction was followed by reverse phase (RP) HPLC, UV absorption and fluorescence spectroscopy. We found all the caged compounds **(8a-c, 11d-f and 13)** released their corresponding alcohols **(6a-c)**, thiols **(9d-f)** and amine **(12)** along with carboxylic acids in high chemical and good quantum yields (**Table 2**). In every case the release of alcohols, thioalcohols and amine ($0.133 \le \Phi_p \le 0.140$) by caged compounds was relatively faster than their counterpart carboxylic acids ($0.097 \le \Phi_p \le 0.102$). The photochemical quantum yield (Φ_p) was calculated using potassium ferrioxalate as an actinometer.[19]

For caged compounds **8b** and **13**, the % of carboxylic acids, alcohols, and amines released by caged compounds were calculated by ^{1}H NMR spectroscopy using 1,2-dichloroethane as an internal standard.

Table 2. Photochemical Data of Caged Compounds (8a-c, 11d-f, and 13) in ACN /H_2O (3:7)

caged compounds	% of deprotection[a]	photochemical quantum yield (Φ_p[b])
8a	97 (6a)	0.139
	94 (4a)	0.101
8b	95 (6b)	0.136
	93 (4b)	0.100
8c	96 (6c)	0.138
	91 (4c)	0.098
11d	97 (9d)	0.140
	92 (4d)	0.099
11e	95 (9e)	0.133
	94 (4e)	0.101
11f	96 (9f)	0.138
	93 (4f)	0.102
13	95 (12)	0.135
	90 (4g)	0.097

[a]% of the carboxylic acids, alcohols, thiols and amines released as determined by RP-HPLC as well as ^{1}H NMR, [b]Photochemical quantum yield for the carboxylic acids, alcohols, thiols and amines at λ ≥365 nm (error limit within ± 5%).



Scheme 2. Sequential Photorelease of Two Different Functional Groups from **8a**

To show the photorelease profile of dual arm caged compounds, we irradiated **8a** in ACN /H_2O (3:7 v/v) at regular intervals of time and monitored the photolysis by RP-HPLC (see **Figure 2** and **Scheme 2**). The HPLC profile shows gradual depletion of the peak at t_R 5.68 min by increasing irradiation time, indicating the photodecomposition of the caged compound **8a**. After 15 min of irradiation, we noted the appearance of four new peaks at t_R 5.98 min, 3.02 min, 3.69 min, and 5.02 min, corresponding to photorelease of phenol (**6a**), carboxylic acid (**4a)**, final photoproduct 1,1'-(9-ethyl-9H-carbazole-3,6-diyl)bis(2-hydroxyethanone) (**14)** and the intermediate single arm caged ester **5a** respectively. Further, we continued the photolysis up to 45 min resulted in complete depletion of the peak at t_R 5.68 min indicating the complete photodecomposition of the dual arm caged compound **8a** with the complete release of alcohol **6a**. Only after 60 min of irradiation, we noted complete decomposition of single arm caged ester **5a** with the release of carboxylic acid **4a.** The photoproducts were confirmed by injecting authentic samples and also by isolation and characterization by ^{1}H NMR spectroscopy. Next, we calculated the rate constants (k_p) for the release of **6a** and **4a** and found to be 5.6627x10^{-4} and 4.3816x10^{-4} respectively (see **Fig S24** in **SI**).

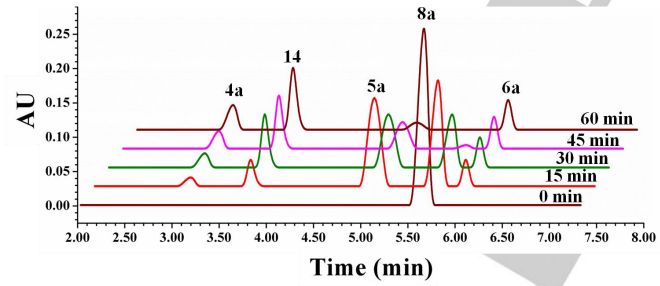


Figure 2. HPLC profile of caged compound **8a** (1 x 10^{-4} M) in ACN/H_2O (30:70 v/v) at regular time intervals of photolysis (λ ≥ 365 nm). The vertical axes were offset by 20 mAU units and the horizontal axes by 10 s to facilitate better visualization.

The above HPLC analysis indicates the sequential release of two different functional groups by dual arm caged compound **8a** upon irradiation. We also followed the photolysis of **8a** in ACN/H_2O (3:7 v/v) by UV absorption and emission spectroscopy (**Figure 3**).

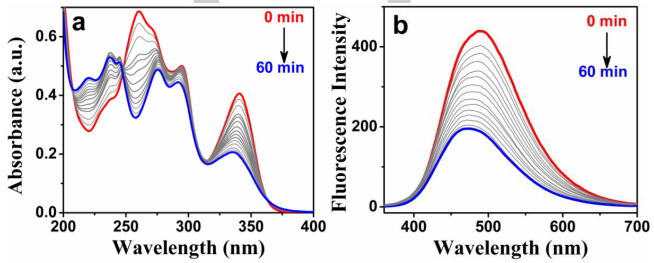


Figure 3. (a) UV absorption spectra (left) and (b) Emission spectra (right) of **8a** recorded during photolysis at regular interval of time (0-60 min). Excitation wavelength: 350 nm.

The hydrolytic stability of all the caged compounds (**8a-c, 11d-f** and **13**) was also tested by keeping them individually under dark in ACN/ PBS containing 10% fetal bovine serum (3:7) at pH = 7.4. The solution of caged compounds (1×10^{-4} M) was kept in ultrasonic for 10 min to make the solution homogeneous and stored at 37 °C in dark condition for 72 h. Then all the solutions were analyzed by reverse phase HPLC as well as ^{1}H NMR. We observed only 3−5% of carboxylic acids and 7-10% of alcohols, thiols, and amine depleted from our caged compounds (see **Table S1** in **SI**).

To understand sequential photorelease mechanism, we have carried out a computational study using Density Functional Theory calculations. A relaxed surface scan was carried out in the ground state (S0; gas phase) from caged compound **8b** to the released photoproduct **5b** by increasing the C–O bond distance from 1.312 Å to 2.312 Å with 0.1 Å step size. Then, for each point on the ground state potential energy surface (PES), we calculated excitation energy for the S1 and T1 states. The same procedure was followed for the second photoreleased step **5b** to **14**. The energy is monotonously increasing in the S0 and S1 states (**Figure 4**) while the T1 state has a barrier between the substrate and the product side, in both steps. Beyond the C-O bond length ~2.313 Å, the energy of the T1 state become less than the S0 state. The same scenario was found for the second photoreleased step.

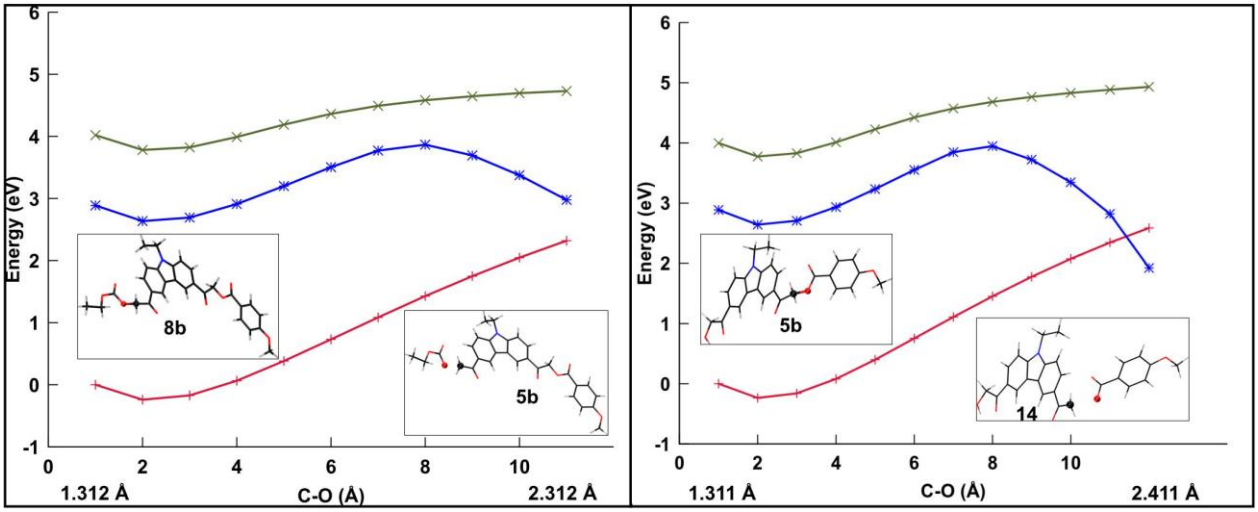
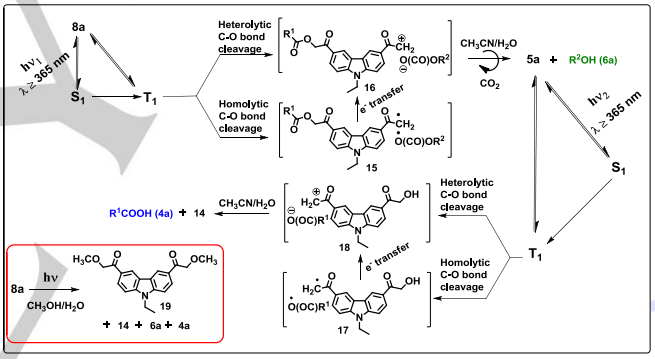


Figure 4. PES scan of the S0 (red), S1(green), and T1(blue) states calculated at CAM-B3LYP/cc-pVTZ //CAM-B3LYP/cc-pVTZ. Plot on the left indicates the photo-release mechanism in step 1 and the right side indicates the photo-release mechanism in step 2.

After observing maxima in the triplet state surface, we calculated the transition state in the T1 excited state at CAM-B3LYP/ccPVDZ level of theory for both the photo-dissociation steps. The C–O bond distance in TS is 2.043 Å for the first step and 2.010 Å for the second step. The activation energy ($\Delta E^{\ddagger}$) in the T1 state is 15.17 for the first photorelease step and 15.02 kcal/mol for the second step. To check the effect of solvent, we have carried out single point calculations of the substrate and the transition states in triplet excited state with polarizable continuum model (PCM).[26] We have used the ε value corresponding to 3:7 mixture of the acetonitrile-water mixture. The corresponding activation energies reduced to 6.34 kcal/mol and 7.31 kcal/mol for the first and second photorelease step. This indicates a relatively fast first photorelease step compared to the second step.

Based on our earlier studies,[21] DFT calculations and solvent study on photorelease, we suggested a possible mechanism for the photorelease of dual arm caged compounds (**8a−c**, **11d-f** and **13)** as shown in **Scheme 3**. The initial photochemical step involves excitation of the dual arm caged compound **8a** to its singlet excited state (S_1), which then undergoes intersystem crossing (ISC) to the triplet state (T_1). Initially cleavage of the C–O bond of carbonate ester of **8a** proceeds from the triplet excited state either by direct C–O bond heterolysis to generate an ion-pair intermediate **16** or homolysis of the C–O bond followed by single-electron transfer to generate **16**. This ion-pair intermediate **16** reacts with the polar solvent to yield photoproduct, single arm caged ester **5a** and phenol with the loss of CO_2 (**6a**). Further excitation of single arm caged ester **5a** yielded carboxylic acid (**4a**) and photoproduct 1,1'-(9-ethyl-9H-carbazole-3,6-diyl)bis(2-hydroxyethanone) (**14**) following the similar mechanism as described for the release of alcohol.

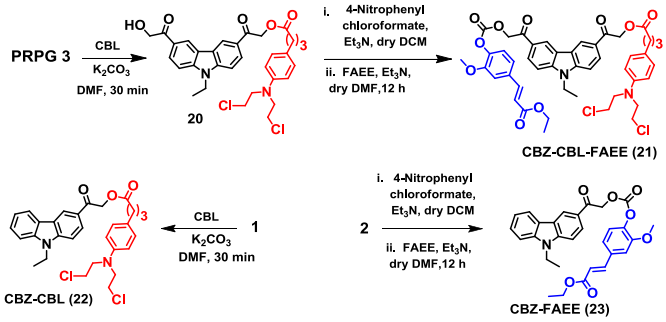


Scheme 3. Possible Photorelease Mechanism for the Dual Release

To confirm the formation of carbocation intermediates **16** and **18** we carried out the photolysis of **8a** in CH_3OH/H_2O (3:7 v/v) system for 60 min (see Inset of **Scheme 3**). We identified the photolysate at 60 min contained both the photoproducts 3,6-bis(methoxyacetyl) 9-ethyl 9*H* carbazole (**19**) and 3,6-bis(hydroxyacetyl) 9-Ethyl 9*H* Carbazole (**14**) (^{1}H NMR spectroscopy, See **Figure S23** in SI) and High-resolution mass spectrometry.

To demonstrate the application of dual release ability of bis-acetyl carbazole PRPG **3,** we designed a drug delivery system (DDS) in which one arm of bis-acetyl carbazole is stitched with chlorambucil (CBL) by ester linkage and the other arm is attached with ferulic acid ethyl ester (FAEE) by carbonate linkage. The DDS containing two different bio-active molecules (CBZ-CBL-FAEE) **(21)** was synthesized in two steps. First, single arm caged DDS (**20)** was prepared by the esterification reaction of CBL with PRPG **3**. Then, dual CBZ-CBL-FAEE (**21**) was synthesized by the treatment of **20** with 4-nitrophenyl

chloroformate in the presence of Et_3N in DCM, followed by addition of FAEE in dry DMF**.** To show the advantage of dual caged DDS over single caged DDS, we also synthesized single caged DDS, CBZ-CBL (**22**) and CBZ-FAEE (**23**) as shown in **Scheme 4**. The dual and single caged DDS were characterized by ^1H, ^{13}C, and mass spectral analysis (See **Fig S18-22** in SI).



Scheme 4. Synthesis of single and dual caged DDS

Dual release ability of our DDS was studied by carrying out the photolysis of CBZ-CBL-FAEE (**21**) in ACN/PBS buffer (3:7 v/v) solution. After 60 min of irradiation, the aliquot was collected and analyzed by RP-HPLC. From HPLC, the % of FAEE (96% and Φ_p=0.138) and CBL (93% and Φ_p=0.100) release was calculated.

To understand the cellular internalization behavior of dual arm caged DDS **(21)**, we performed cell imaging studies using cancerous HeLa cells. Cells were incubated with 10 µM of **21** in cell culture medium for 3 h and monitored by fluorescence microscopy. After 3 h of incubation, cells showed bright blue fluorescence confirming the cellular internalization of DDS (**Fig. 5ib** and **ic**).

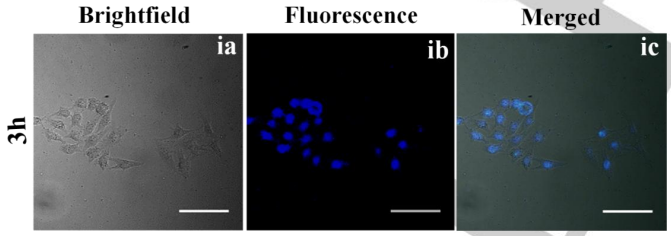


Figure 5. Confocal fluorescence and brightfield images of Hela cells after 3 h of incubation with dual arm caged DDS **21** exhibited blue fluorescence indicating successful internalization (ib and ic) (scale bar = 20 µm). Excitation at 330 nm [emission channel = 408 nm].

To check the biocompatibility of the DDS (21), we carried out in vitro cytotoxicity assay of **21** in normal cells (NIH 3T3). The result shows that at 100 µM concentration, our DDS exhibited 82% and 45% cell viability before and after photolysis, respectively (see **Fig S25** in **SI**).

Next, we investigated the anticancer activity of single caged DDSs (CBZ-CBL and CBZ-FAEE), the physical combination of two different single arms caged DDS (CBZ-CBL:CBZ-FAEE, 1:1 mixture), photoproduct **14** and a dual arm caged DDS (CBZ-CBL-FAEE). MTT assay was carried out on the Hela cells before and after photolysis for all DDSs and the results are shown in **Figure. 6**. It was observed that before photolysis cell viability remains more than 70% at concentration of 20 µM for CBZ-CBL, CBZ-FAEE, CBZ-CBL:CBZ-FAEE (1:1 mixture), photoproduct **14**, and CBZ-CBL-FAEE (**Figure 6a**). After photolysis, single arm caged DDSs (CBZ-CBL, CBZ-FAEE) and photoproduct **14** exhibited cell viability of above 60% (IC_{50} at 15 µM), 65% (IC_{50} at 15 µM) and 74% (IC_{50} at 15 µM) respectively. In the case of dual drug release, MTT assay results revealed that after photolysis dual arm caged DDS (CBZ-CBL-FAEE) expressed appreciably lower cell viability of 48% (IC_{50} at 10 µM) in comparison to combination of two different single arms caged DDS (CBZ-CBL:CBZ-FAEE, 1:1 mixture) having cell viability of 47 % (IC_{50} at 15 µM) (**Figure 6b**). This result demonstrates that dual arm caged DDS can exhibit efficient antitumor activity by sequential release of FAEE and CBL.

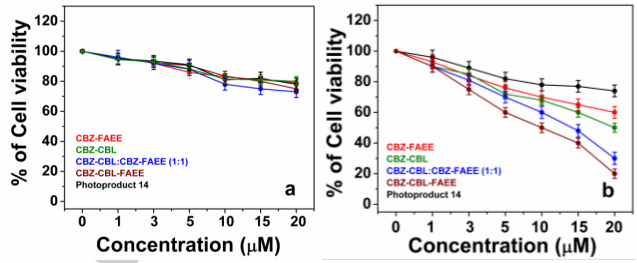


Figure 6. (a,b) Cell viability assay of single arm caged DDS (CBZ-CBL and CBZ-FAEE), combination of two different single arm caged DDS (CBZ-CBL: CBZ-FAEE, 1:1 mixture), dual arm caged DDS (CBZ-CBL-FAEE) and photoproduct 14 in Hela cell line: (a) before photolysis, (b) after photolysis. Values are presented as mean ± SD.

Conclusions

We have demonstrated bis-acetyl carbazole as a fluorescent PRPG for the dual release of different functional groups including carboxylic acids, alcohols, thiols, and amines. Dual arm caged compounds of bis-acetyl carbazole on exposure to UV light released both functional groups in high chemical yield. However, the release of alcohols, thioalcohols and amines by caged compounds was found to be relatively faster in comparison to carboxylic acids. The DFT calculations shows that there exists an energy barrier in the T1 surface for both the photorelease steps. Therefore, the rate of the photorelease steps would be governed by the energy barrier in the T1 state. Indeed, the activation energies ($\Delta E^{\ddagger}$) in the solvent medium (PCM solvation model) suggest that the first photorelease step is faster than the second photorelease step. Finally, our synthesized dual DDS CBZ-CBL-FAEE conjugate exhibited excellent properties like good biocompatibility, in vitro cellular imaging and efficient antitumor activity in comparison to single caged CBZ–CBL, CBZ–FAEE and CBZ-CBL:CBZ-FAEE (1:1) conjugates. In future, carbazole based monochromophoric

FPRPG for the orthogonal release of two different functional groups will be developed.

Experimental Section

General Information: All commercially available anhydrous solvents dimethylformamide (DMF), dichloromethane (DCM), petroleum ether (PE) and ethyl acetate (EA) and other chemicals were used without further purification. Acetonitrile and dichloromethane were distilled from CaH_2 before use. NMR spectra were recorded on a 600 and 400 MHz instrument. ^{1}H NMR chemical shifts were referenced to the tetramethylsilane signal (0 ppm), ^{13}C NMR chemical shifts were referenced to the solvent resonance (77.23 ppm, $CDCl_3$). Chemical shifts (δ) are reported in ppm, and spin−spin coupling constants (J) are given in Hz. The following abbreviations were used to explain multiplicities: s = singlet, d = doublet, t = triplet, q = quadruplet, m = multiplet. UV/vis absorption spectra were recorded on UV/vis spectrophotometer and fluorescence spectra were recorded on fluorescence spectrophotometer. High-resolution mass spectra (HRMS) were recorded on ESI-TOF (electrospray ionization-time-of-flight). Photolysis of all the ester conjugates was carried out using a 125 W medium pressure mercury lamp. RP-HPLC was taken using mobile phase acetonitrile/methanol (7:3), at a flow rate of 1mL / min (detection: UV 254 nm). Chromatographic purification was done with 60–120 mesh silica gel. For reaction monitoring, precoated silica gel 60 F254 TLC sheets were used.

2-bromo-1-(9-ethyl-9H-carbazol-3-yl)ethanone (1): 9-ethyl carbazole (0.300 g, 1.53 mmol) was dissolved in dry dichloromethane (DCM) (20 mL), followed by aluminium chloride ($AlCl_3$) (0.205 g, 1.53 mmol) was added and the solution of bromoacetyl bromide (0.310 g, 1.53 mmol) in DCM (10 mL) was added over 30 minutes while stirring at 0 °C. After completion of addition, stirring was done at 25 °C for additional 4 h. The reaction mixture was poured in 800 g of ice water and extracted with DCM (100 mL). The organic layer was dried over Na_2SO_4, the solvent was evaporated by rotary evaporator, the residue was purified by column chromatography (eluting agent: chloroform), recrystallized from ethyl acetate-hexane to obtain the product **2**. Light green crystals (0.349 g, 72%). mp: 99-100 ºC. ^{1}H NMR (600 MHz, chloroform-d) δ 8.74 (s, 1H), 8.23 – 8.02 (m, 2H), 7.53 (q, *J* = 6.2, 4.6 Hz, 1H), 7.44 (dd, *J* = 8.9, 3.6 Hz, 1H), 7.41 – 7.36 (m, 1H), 7.32 (t, *J* = 7.4 Hz, 1H), 4.57 (s, 2H), 4.36 (q, *J* = 7.1 Hz, 2H), 1.45 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 190.9, 143.2, 140.9, 127.1, 126.9, 125.4, 123.3, 123.1, 122.8, 120.9, 120.5, 109.3, 108.5, 38.1, 31.4, 14.0. HRMS (ESI$^+$) calcd for $C_{16}H_{14}BrNO$ [M + Na]$^+$, 338.0156; found: 338.0160.

1-(9-ethyl-9*H*-carbazol-6-yl)-2-hydroxyethanone (2): 2-bromo-1-(9-ethyl-9H-carbazol-3-yl)ethanone (0.500 g, 1.58 mmol) was dissolved in ethanol and sodium formate (0.430 g, 6.32 mmol) was added. The reaction mixture was refluxed for 4 h. After completion of reaction, the reaction mixture was poured in 300 mL water and extracted with ethyl acetate (100 mL). The organic layer was dried over Na_2SO_4, the solvent was evaporated by rotary evaporator, the residue was purified by column chromatography using 20% EtOAc in pet ether to give the product. Yellow solid (0.296 g, 74%). mp: 92-93 ºC. ^{1}H NMR (400 MHz, chloroform-d) δ 8.65 (s, 1H), 8.13 (d, *J* = 7.7 Hz, 1H), 8.03 (d, *J* = 8.6 Hz, 1H), 7.59 – 7.50 (m, 1H), 7.44 (d, *J* = 8.2 Hz, 1H), 7.39 (d, *J* = 8.7 Hz, 1H), 7.32 (t, *J* = 7.5 Hz, 1H), 4.99 (s, 2H), 4.35 (t, *J* = 7.2 Hz, 2H), 3.45 (s, 1H), 1.44 (t, *J* = 7.3 Hz, 3H).^{13}C NMR (101 MHz, chloroform-d) δ 197.5, 143.4, 140.8, 126.9, 125.7, 124.7, 123.1, 123.1, 121.4, 120.9, 120.4, 109.3, 108.6, 77.6, 77.2, 76.9, 65.3, 38.0, 14.0. HRMS (ESI$^+$) calcd for $C_{16}H_{15}NO_2$ [M + H]$^+$, 254.1181; found: 254.1169.

2-bromo-1-(9-ethyl-6-(2-hydroxyacetyl)-9*H*-carbazol-3-yl)ethanone (3): Compound **2** (0.300 g , 1.18 mmol) was dissolved in DCM (20 mL), followed by aluminium chloride ($AlCl_3$) (0.158 g, 1.18 mmol) was added and the solution of bromoacetyl bromide (0.239 g, 1.18 mmol) in DCM (10 mL) was added over 30 minutes while stirring at 0 °C. After completion of addition, stirring was done at 25 °C for additional 4 h. The reaction mixture was poured in 800 g of ice water and extracted with DCM (100 mL). The organic layer was dried over Na_2SO_4, the solvent was evaporated by rotary evaporator, the the residue was purified by column chromatography using 30% EtOAc in pet ether to give the product. Light brown solid (0.336 g, 76%). mp: 105-106 ºC. ^{1}H NMR (600 MHz, chloroform-d) δ 8.83 (s, 1H), 8.76 (s, 1H), 8.22 (d, *J* = 8.5 Hz, 1H), 8.14 (d, *J* = 8.6 Hz, 1H), 7.52 (d, *J* = 6.2 Hz, 2H), 5.04 (s, 2H), 4.59 (s, 2H), 4.45 (d, *J* = 7.4 Hz, 2H), 1.51 (d, *J* = 7.2 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 197.4, 190.7, 144.1, 143.9, 128.0, 126.7, 126.6, 125.9, 123.2, 123.0, 122.9, 121.6, 121.5, 109.3, 65.3, 38.4, 30.8, 13.9. HRMS (ESI$^+$) calcd for $C_{18}H_{16}BrNO_3$ [M + H]$^+$, 374.0392; found: 374.0381.

General Procedure for the Synthesis of Single arm Ester Conjugates (5a − g): 2-bromo-1-(9-ethyl-6-(2-hydroxyacetyl)-9H-carbazol-3-yl)ethanone (1 equiv) was dissolved in dry N,N-dimethylformamide (DMF) (2 mL), potassium carbonate (K_2CO_3) (1.2 equiv), and the corresponding carboxylic acids **4a−g** (1 equiv) were added. The reaction mixture was stirred at room temperature for 30 min. After completion of the reaction it was extracted with ethyl acetate and washed with saturated NaCl solution and dried over Na_2SO_4. The solvent was removed by rotary evaporation under reduced pressure and the crude residue was purified by column chromatography using ethyl acetate (EtOAC) in pet ether.

General Procedure for the Synthesis of chloroformates (7a-c and 10d-f):

Bis(trichloromethyl)carbonate (0.34 equiv) was dissolved in 30 mL DCM, and cooled in ice bath. **6a-c** and **9d-f** (1 equiv) were slowly added with stirring, and then mixed solution of triethylamine (1 equiv) and 10 mL DCM was dropped at 0-5 °C. After dropping, then stirred for 2 h at room temperature. The crude mixture was washed with water (3 × 40 mL), and then DCM layer was dried over anhydrous Na_2SO_4, filtrated and concentrated to give the corresponding compounds **7a-c** and **10d-f**. The reaction mixture was used as such in the next step without further treatment.

General Procedure for the Synthesis of Dual arm Caged Compounds (8a−c and **11d-f):** To a solution of **5a-g** (1 equiv) and triethylamine (Et_3N)(1.5 equiv) in dry DCM (10 mL), was added a solution of corresponding chloroformates (**7a-c** and **10d-f**) (1 equiv) in DCM dropwise at 0°C . After stirring at room temperature for 30 minutes the reaction mixture was diluted with DCM and washed with 1N HCl followed by saturated $NaHCO_3$ and finally with saturated NaCl solution. The collected organic layer dried over Na_2SO_4 and solvent was removed under vaccum. The crude product was purified by column chromatography using EtOAc in pet ether.

2-(9-ethyl-6-(2-((phenoxycarbonyl)oxy)acetyl)-9H-carbazol-3-yl)-2-oxoethyl 4-fluorobenzoate (8a): Treatment of **3** (0.150 g, 0.40 mmol) with 4-fluorobenzoic acid (4a) (0.056 g, 0.40 mmol) in

the presence of K_2CO_3 (0.066 g, 0.48 mmol) in dry DMF at room temperature for a period of 30 min. The crude conjugate was purified by column chromatography using 30% EtOAc in pet ether to give the product **5a**. To a solution of **5a** (0.100 g, 0.23 mmol) and Et_3N (0.035 g, 0.34 mmol) in dry DCM (10 mL), was added a solution of phenyl chloroformate (0.036 g, 0.23 mmol) in DCM dropwise at 0°C . After stirring at room temperature for 30 minutes the reaction mixture was diluted with DCM and washed with 1N HCl followed by saturated $NaHCO_3$ and finally with saturated NaCl solution. The collected organic layer dried over Na_2SO_4 and solvent was removed under vaccum. The crude product was purified by column chromatography using 40% EtOAc in pet ether to give the product. White solid (0.117 g, 92%). mp: 73-74 ºC. ^{1}H NMR (600 MHz, chloroform-d) δ 8.80 (s, 1H), 8.76 (s, 1H), 8.24 – 8.18 (m, 3H), 8.16 (d, *J* = 8.6 Hz, 1H), 7.53 (d, *J* = 8.6 Hz, 2H), 7.41 (t, *J* = 7.8 Hz, 3H), 7.31 – 7.27 (m, 2H), 7.16 (t, *J* = 8.4 Hz, 2H), 5.74 (s, 2H), 5.64 (s, 2H), 4.45 (q, *J* = 7.1 Hz, 2H), 1.51 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 191.3, 190.6, 166.2 (d, $^1J_{C-F}$ = 252.7 Hz), 165.4, 153.8, 151.4, 144.08, 144.0, 132.9, 132.8, 129.7, 127.04, 127.0, 126.8, 126.6, 126.4, 123.2, 123.1, 121.7, 121.7, 121.2, 115.9 (d, $^2J_{C-F}$ = 21.9 Hz), 109.5, 77.4, 77.2, 77.0, 69.4, 66.7, 38.5, 14.1. HRMS (ESI^+) calcd for $C_{32}H_{24}FNO_7$ $[M + H]^+$, 554.1615; found: 554.1634.

2-(6-(2-((ethoxycarbonyl)oxy)acetyl)-9-ethyl-9H-carbazol-3-yl)-2-oxoethyl 4-methoxybenzoate (8b): Treatment of **3** (0.150 g, 0.40 mmol) with *p*-anisic acid (4b) (0.060 g, 0.40 mmol) in the presence of K_2CO_3 (0.066 g, 0.48 mmol) in dry DMF at room temperature for a period of 30 min. The crude conjugate was purified by column chromatography using 30% EtOAc in pet ether to give the product **5b**. To a solution of **5b** (0.100 g, 0.22 mmol) and Et_3N (0.034 g, 0.33 mmol) in dry DCM (10 mL), was added a solution of ethyl chloroformate (0.024 g, 0.22 mmol) in DCM dropwise at 0°C . After stirring at room temperature for 30 minutes the reaction mixture was diluted with DCM and washed with 1N HCl followed by saturated $NaHCO_3$ and finally with saturated NaCl solution. The collected organic layer dried over Na_2SO_4 and solvent was removed under vaccum. The crude product was purified by column chromatography using 40% EtOAc in pet ether to give the product. White solid (0.103 g, 89%). mp: 64-65 ºC. ^{1}H NMR (600 MHz, chloroform-d) δ 8.80 (s, 1H), 8.74 (s, 1H), 8.20 (d, *J* = 8.5 Hz, 1H), 8.18 – 8.10 (m, 3H), 7.52 (dd, *J* = 8.6, 4.5 Hz, 2H), 6.97 (d, *J* = 8.8 Hz, 2H), 5.71 (s, 2H), 5.52 (s, 2H), 4.45 (q, *J* = 7.2 Hz, 2H), 4.31 (q, *J* = 7.1 Hz, 2H), 3.89 (s, 3H), 1.50 (t, *J* = 7.2 Hz, 3H), 1.38 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) 191.6, 191.0, 165.9, 163.7, 155.0, 143.8, 132.1, 127.0, 126.8, 126.6, 122.9, 121.9, 121.5, 121.4, 113.7, 109.2, 68.5, 66.3, 64.7, 55.5, 38.3, 14.3, 13.9. HRMS (ESI^+) calcd for $C_{29}H_{27}NO_8$ $[M + H]^+$, 518.1815; found: 518.1824.

2-(6-(2-(((((8S,9S,10R,13R,14S,17R)-10,13-dimethyl-17-((R)-6-methylheptan-2-yl)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl)oxy)carbonyl)oxy)acetyl)-9-ethyl-9H-carbazol-3-yl)-2-oxoethyl 2-((tert-butoxycarbonyl)amino)acetate (8c): Treatment of **3** (0.150 g, 0.40 mmol) with 2-((tert-butoxycarbonyl) amino) acetic acid (4c) (0.070 g, 0.40 mmol) in the presence of K_2CO_3 (0.066 g, 0.48 mmol) in dry DMF at room temperature for a period of 30 min. The crude conjugate was purified by column chromatography using 30% EtOAc in pet ether to give the product 5c. To a solution of 5c (0.100 g, 0.21 mmol) and Et_3N (0.032 g, 0.31 mmol) in dry DCM (10 mL), was added a solution of cholesteryl chloroformate (0.095 g, 0.21 mmol) in DCM dropwise at 0°C. After stirring at room temperature for 30 minutes the reaction mixture was diluted with DCM and washed with 1N HCl followed by saturated $NaHCO_3$ and finally with saturated NaCl solution. The collected organic layer dried over Na_2SO_4 and solvent was removed under vaccum. The crude product was purified by column chromatography using 40% EtOAc in pet ether to give the product. White solid (0.174 g, 93%). mp: 72-73 ºC. ^{1}H NMR (400 MHz, chloroform-d) δ 8.68 (s, 1H), 8.66 (s, 1H), 8.13 (t, *J* = 8.4 Hz, 2H), 7.48 (d, *J* = 8.8 Hz, 2H), 5.56 (s, 2H), 5.49 (s, 1H), 5.41 (d, *J*=4.0 Hz, 1H), 5.19 (s, 1H), 4.59-4.43 (m, 1H), 4.39 (q, J = 7.0 Hz, 2H), 4.18 (d, J = 5.3 Hz, 2H), 2.49-2.46 (m, 2H), 2.02-1.09 (m, 23H), 1.48 (s, 9H), 0.95 (s, 6H), 0.89-0.82 (m, 9H), 0.63 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 191.2, 190.8, 170.4, 156.0, 154.6, 144.0, 143.97, 139.5, 126.9, 126.8, 126.7, 123.2, 123.2, 123.1, 121.6, 109.5, 80.2, 78.9, 68.6, 66.7, 56.9, 56.3, 50.2, 42.5, 39.9, 39.7, 38.5, 38.1, 37.1, 36.8, 36.4, 36.0, 32.1, 32.0, 28.5, 28.4, 28.2, 27.8, 24.5, 24.0, 23.0, 22.8, 21.3, 19.5, 18.9, 14.1, 12.1. HRMS (ESI^+) calcd for $C_{53}H_{72}N_2O_9$ $[M + Na]^+$, 903.5136; found: 903.5138.

2-(9-ethyl-6-(2-(((phenylthio)carbonyl)oxy)acetyl)-9H-carbazol-3-yl)-2-oxoethyl benzoate (11d): Treatment of **3** (0.150 g, 0.40 mmol) with benzoic acid (4d) (0.048 g, 0.40 mmol) in the presence of K_2CO_3 (0.066 g, 0.48 mmol) in dry DMF at room temperature for a period of 30 min. The crude conjugate was purified by column chromatography using 30% EtOAc in pet ether to give the product **5d.** To a solution of **5d** (0.100 g, 0.24 mmol) and Et_3N (0.036 g, 0.36 mmol) in dry DCM (10 mL), was added a solution of S-phenyl carbonochloridothioate (0.041 g, 0.24 mmol) in DCM dropwise at 0°C. After stirring at room temperature for 30 minutes the reaction mixture was diluted with DCM and washed with 1N HCl followed by saturated $NaHCO_3$ and finally with saturated NaCl solution. The collected organic layer dried over Na_2SO_4 and solvent was removed under vaccum. The crude product was purified by column chromatography using 40% EtOAc in pet ether to give the product. White solid (0.115 g, 87%). mp: 70-71 ºC. ^{1}H NMR (600 MHz, chloroform-d) δ 8.78 (s, 1H), 8.71 (s, 1H), 8.19 (d, *J* = 7.3 Hz, 3H), 8.12 (d, *J* = 8.7 Hz, 1H), 7.64 – 7.58 (m, 3H), 7.50 (q, *J* = 9.4, 8.0 Hz, 4H), 7.45 – 7.38 (m, 3H), 7.26 (s, 1H), 5.74 (s, 2H), 5.61 (s, 2H) 4.43 (q, *J* = 6.7, 6.3 Hz, 2H), 1.49 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 191.5, 190.6, 170.2, 166.4, 144.1, 144.0, 135.1, 133.6, 130.2, 129.9, 129.7, 129.4, 128.7, 127.6, 127.2, 127.0, 126.9 126.7, 123.2, 123.18, 121.8, 109.5, 68.7, 66.7, 38.5, 14.1. HRMS (ESI^+) calcd for $C_{32}H_{25}NO_6S$ $[M + H]^+$, 552.1481; found: 552.1481.

2-(9-ethyl-6-(2-(((p-tolylthio)carbonyl)oxy)acetyl)-9H-carbazol-3-yl)-2-oxoethyl 4-methylbenzoate (11e): Treatment of **3** (0.150 g, 0.40 mmol) with *p*-toluic acid (4e) (0.054 g, 0.40 mmol) in the presence of K_2CO_3 (0.066 g, 0.48 mmol) in dry DMF at room temperature for a period of 30 min. The crude conjugate was purified by column chromatography using 30% EtOAc in pet ether to give the product **5e.** To a solution of **5e** (0.100 g, 0.23 mmol) and Et_3N (0.035 g, 0.34 mmol) in dry DCM (10 mL), was added a solution of S-p-tolyl carbonochloridothioate (0.043 g, 0.23 mmol) in DCM dropwise at 0°C. After stirring at room temperature for 30 minutes the reaction mixture was diluted with DCM and washed with 1N HCl followed by saturated $NaHCO_3$ and finally with saturated NaCl solution. The collected organic layer dried over Na_2SO_4 and solvent was removed under vaccum. The crude product was purified by column chromatography using 40% EtOAc in pet ether to give the product. White solid (0.112 g, 83%). mp: 74-75 ºC. ^{1}H NMR (600 MHz, chloroform-d) δ 8.79 (s, 1H), 8.71 (s, 1H), 8.19 (d, *J* = 8.5 Hz, 2H), 8.12 (d, *J* = 8.6 Hz, 2H), 8.07 (d, *J* = 7.9 Hz, 2H), 7.52 – 7.47 (m, 4H), 7.28 (d, *J* = 7.9 Hz, 2H), 7.21 (d, *J* = 7.8 Hz, 2H), 5.71 (s, 2H), 5.59 (s, 2H), 4.43 (q, *J* = 7.2 Hz, 2H), 2.44 (s, 3H), 2.36 (s, 3H), 1.49 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 191.5, 190.5, 170.3, 166.3, 144.1, 143.8, 143.8, 140.1,

134.9, 130.1, 129.2, 127.0, 126.8, 126.8, 126.7, 126.5, 123.8, 123.0, 122.9, 121.5, 121.5, 109.3, 68.5, 66.4, 38.3, 21.8, 21.3, 14.1. HRMS (ESI^+) calcd for $C_{34}H_{29}NO_6S$ $[M + H]^+$, 580.1794; found: 580.1813.

2-(6-(2-((((4-chlorophenyl)thio)carbonyl)oxy)acetyl)-9-ethyl-9H-carbazol-3-yl)-2-oxoethyl 2-(2-methoxyphenyl)acetate (11f): Treatment of **3** (0.150 g, 0.40 mmol) with *o*-methoxyphenylacetic acid (4f) (0.066 g, 0.40 mmol) in the presence of K_2CO_3 (0.066 g, 0.48 mmol) in dry DMF at room temperature for a period of 30 min. The crude conjugate was purified by column chromatography using 30% EtOAc in pet ether to give the product **5f.** To a solution of **5f** (0.100 g, 0.21 mmol) and Et_3N (0.033 g, 0.31 mmol) in dry DCM (10 mL), was added a solution of S-(4-chlorophenyl) carbonochloridothioate (0.045 g, 0.21 mmol) in DCM dropwise at 0°C. After stirring at room temperature for 30 minutes the reaction mixture was diluted with DCM and washed with 1N HCl followed by saturated $NaHCO_3$ and finally with saturated NaCl solution. The collected organic layer dried over Na_2SO_4 and solvent was removed under vaccum. The crude product was purified by column chromatography using 40% EtOAc in pet ether to give the product. White solid (0.121 g, 88%). mp: 75-76 ºC. ^{1}H NMR (600 MHz, chloroform-d) δ 8.71 (s, 1H), 8.67 (s, 1H), 8.12 (dt, *J* = 8.4, 4.0 Hz, 2H), 7.55 (d, *J* = 8.5 Hz, 2H), 7.51 – 7.46 (m, 2H), 7.38 (d, *J* = 8.4 Hz, 2H), 7.30 (d, *J* = 7.3 Hz, 1H), 7.25 (d, *J* = 6.8 Hz, 1H), 6.94 (t, *J* = 7.4 Hz, 1H), 6.88 (d, *J* = 8.2 Hz, 1H), 5.61 (s, 2H), 5.50 (s, 2H), 4.42 (q, *J* = 6.6, 6.0 Hz, 2H), 3.87 (s, 2H), 3.84 (s, 3H), 1.48 (t, *J* = 7.2 Hz, 3H).^{13}C NMR (151 MHz, $CDCl_3$) δ 191.4, 190.2, 171.5, 169.4, 157.6, 143.9, 143.7, 136.2, 136.1, 131.1, 129.5, 128.7, 127.0, 126.8, 126.7, 126.4, 125.9, 123.0, 122.9, 122.7, 121.5, 120.6, 110.5, 109.3, 109.2, 68.7, 66.3, 55.5, 38.3, 35.5, 13.9. HRMS (ESI^+) calcd for $C_{34}H_{28}ClNO_7S$ $[M + H]^+$, 630.1353; found: 630.1375.

2-(6-(2-((2-chlorobenzoyl)oxy)acetyl)-9-ethyl-9H-carbazol-3-yl)-2-oxoethyl piperidine-1-carboxylate (13): Treatment of **3** (0.150 g, 0.40 mmol) with *o*-chlorobenzoic acid (4f) (0.062 g, 0.40 mmol) in the presence of K_2CO_3 (0.066 g, 0.48 mmol) in dry DMF at room temperature for a period of 30 min. The crude conjugate was purified by column chromatography using 30% EtOAc in pet ether to give the product **5g.** To a solution of **5g** (0.100 g, 0.22 mmol) and Et_3N (0.033 g, 0.33 mmol) in dry DCM (20 mL) at 0 °C, was added a solution of 4-nitrophenyl chloroformate (0.044 g, 0.22 mmol) in DCM (20 mL) dropwise. After stirring at room temperature for 30 minutes the solvent was evaporated to give the product as yellow crystals (0.115 g, 85%) and in situ reaction with piperidine (0.023 g, 0.28 mmol) in dry DMF at 0 °C, and the reaction mixture was stirred for 4 h. After completion of the reaction, it was concentrated under reduced pressure. The residue was extracted with EtOAc and washed with saturated NaCl solution and dried over Na_2SO_4. The solvent was removed by rotary evaporation under reduced pressure followed by column chromatography using 40% EtOAc in pet ether to give the product. White solid (0.075 g, 72%). mp: 70-71 ºC. ^{1}H NMR (600 MHz, Chloroform-*d*) δ 8.79 (s, 1H), 8.76 (s, 1H), 8.19-8.16 (m, 2H), 8.10 (d, *J* = 7.8 Hz, 1H), 7.52-7.45 (m, 4H), 7.38 (t, *J* = 8.0 Hz, 1H), 5.75 (s, 2H), 5.49 (s, 2H), 4.44 (q, *J* = 7.2 Hz, 2H), 3.60-3.50 (m, 4H), 1.63-1.55 (m, 6H), 1.50 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 193.0, 191.1, 165.3, 155.1, 144.0, 143.9, 134.3, 133.1, 132.2, 131.3, 127.4, 126.9, 126.9, 123.3, 123.1, 121.7, 121.6, 109.4, 109.4, 67.0, 66.9, 45.4, 38.5, 29.9, 24.6, 14.1. HRMS (ESI^+) calcd for $C_{31}H_{29}ClN_2O_6$ $[M + H]^+$, 561.1792; found: 561.1776.

(E)-2-(6-(2-(((4-(3-ethoxy-3-oxoprop-1-en-1-yl)-2-methoxyphenoxy)carbonyl)oxy)acetyl)-9-ethyl-9H-carbazol-3-yl)-2-oxoethyl 4-(4-(bis(2-chloroethyl)amino)phenyl)butanoate (21): Treatment of **3** (0.150 g, 0.40 mmol) with chlorambucil (0.121 g, 0.40 mmol) in the presence of K_2CO_3 (0.066 g, 0.48 mmol) in dry DMF at room temperature for a period of 30 min. The crude conjugate was purified by column chromatography using 30% EtOAc in pet ether to give the product **18**. To a solution of **18** (0.100 g, 0.167 mmol) and Et_3N (0.025 g, 0.25 mmol) in dry DCM (20 mL) at 0 °C, was added a solution of 4-nitrophenyl chloroformate (0.033 g, 0.167 mmol) in DCM (20 mL) dropwise. After stirring at room temperature for 30 minutes the solvent was evaporated to give the product as yellow crystals (0.108 g, 85%) and in situ reaction with (E)-ethyl 3-(4-hydroxy-3-methoxyphenyl)acrylate (FAEE) (0.031 g, 0.141 mmol) in the presence of Et_3N (0.021 g, 0.212 mmol) in dry DMF (2 mL) at 0 °C and then stirred at room temperature overnight. After completion of the reaction it was extracted with EtOAc and washed with saturated NaCl solution and dried over Na_2SO_4. The solvent was removed by rotary evaporation under reduced pressure The crude product was purified by column chromatography using 40% EtOAc in pet ether to give the product. White solid (0.083 g, 70%). mp: 62-63 ºC. ^{1}H NMR (600 MHz, Chloroform-*d*) δ 8.76 (s, 2H), 8.17 (dd, *J* = 6.9, 1.7 Hz, 2H), 7.66 (d, *J* = 16.0 Hz, 1H), 7.57 – 7.48 (m, 2H), 7.30 (d, *J* = 8.1 Hz, 1H), 7.15 (t, *J* = 10.6 Hz, 4H), 6.66 (d, *J* = 8.5 Hz, 2H), 6.41 (d, *J* = 16.0 Hz, 1H), 5.64 (s, 2H), 5.52 (s, 2H), 4.46 (q, *J* = 5.9, 4.9 Hz, 2H), 4.29 (q, *J* = 7.1 Hz, 2H), 3.95 (s, 3H), 3.73 (t, *J* = 7.0 Hz, 4H), 3.65 (t, *J* = 7.1 Hz, 4H), 2.68 (t *J* = 7.5 Hz, 2H), 2.57 (t, *J* = 7.4 Hz, 2H), 2.05 (p, *J* = 7.4 Hz, 2H), 1.52 (t, *J* = 7.2 Hz, 3H), 1.37 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (151 MHz $CDCl_3$) δ 191.3, 190.2, 173.1, 166.7, 152.9, 151.5, 144.3, 143.8, 143.7, 143.7, 141.6, 133.8, 129.8, 126.9, 126.7, 126.6, 126.4, 123.0 122.9, 121.4, 121.1, 118.8, 112.4, 111.6, 109.3, 77.2, 77.0, 76.8, 69.4, 65.9, 60.6, 56.1, 53.7, 40.5, 38.3, 33.9, 33.3, 26.8, 14.3, 13.8. HRMS (ESI^+) calcd for $C_{45}H_{46}Cl_2N_2O_{10}$ $[M+H]^+$, 845.2608; found: 845.2583.

2-(9-Ethyl-9H-carbazol-3-yl)-2-oxoethyl 4-(4-(bis(2-chloroethyl)-amino)phenyl)butanoate (22): Treatment of **1** (0.100 g, 0.31mmol) with chlorambucil (0.096 g, 0.31 mmol) in the presence of K_2CO_3 (0.052 g, 0.37 mmol) in dry DMF at room temperature for a period of 30 min. The crude conjugate was purified by column chromatography using 20% EtOAc in pet ether to give the product 12. Light yellow solid (0.150 g, 88%). mp: 150−152 °C. ^{1}H NMR (600 MHz, chloroform-d) δ 8.70 (s, 1H), 8.16 (d, J = 7.7 Hz, 1H), 8.08 (dd, J = 8.8, 1.7 Hz, 1H), 7.62−7.44 (m, 2H), 7.42 (d, J = 8.6 Hz, 1H), 7.35 (t, J = 7.3 Hz, 1H), 7.21−7.10 (m, 2H), 6.71−6.63 (m, 2H), 5.52 (s, 2H), 4.37 (q, J = 7.3 Hz, 2H), 3.72 (t, J = 7.4 Hz, 4H), 3.65 (t, J = 6.7 Hz, 4H), 2.69 (t, J = 7.6 Hz, 2H), 2.58 (t, J = 7.4 Hz, 2H), 2.07 (p, J = 7.5 Hz, 2H), 1.46 (t, J = 7.3 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 191.5, 173.2, 144.3, 143.0, 140.6, 130.8, 129.8, 126.7, 125.7, 125.6, 123.1, 122.8, 121.3, 120.8, 120.2, 112.2, 109.1 108.4, 66.0, 53.6, 40.6, 37.9, 33.9, 33.3, 26.9, 13.8. HRMS (ESI^+) calcd for $C_{30}H_{32}Cl_2N_2O_3$ $[M+Na]^+$, 561.1688; found: 561.1688.

(E)-ethyl 3-(4-(((2-(9-ethyl-9H-carbazol-3-yl)-2-oxoethoxy)carbonyl)oxy)-3-methoxyphenyl)acrylate (23): To a solution of **2** (0.100 g, 0.39 mmol) and Et_3N (0.059 g, 0.58 mmol) in dry DCM (20 mL) at 0 °C, was added a solution of 4-nitrophenyl chloroformate (0.079 g, 0.39 mmol) in DCM (20 mL) dropwise. After stirring at room temperature for 30 minutes the solvent was evaporated to give the product (0.140 g, 85%) and in situ reaction with (E)-ethyl 3-(4-hydroxy-3-methoxyphenyl)acrylate (FAEE) (0.074 g, 0.334 mmol) in the presence of Et_3N (0.050 g, 0.501 mmol) in dry DMF (2 mL) and then stirred at room temperature overnight. After completion of the reaction it was extracted with ethyl acetate and washed with saturated NaCl solution and dried

over Na_2SO_4. The solvent was removed by rotary evaporation under reduced pressure. The crude product was purified by column chromatography using 30% EtOAc in pet ether to give the product. White solid (0.120 g, 72%). mp: 72-73 ºC. ^{1}H NMR (600 MHz, Chloroform-d) δ 8.70 (s, 1H), 8.11 (dd, J = 42.8, 8.2 Hz, 2H), 7.64 (d, J = 16.0 Hz, 1H), 7.54 (t, J = 7.6 Hz, 1H), 7.48 – 7.41 (m, 2H), 7.32 (t, J = 7.4 Hz, 1H), 7.27 (d, J = 8.1 Hz, 1H), 7.13 (d, J = 10.4 Hz, 2H), 6.45 – 6.33 (m, 1H), 5.62 (s, 2H), 4.39 (d, J = 3.8 Hz, 2H), 4.27 (q, J = 7.1 Hz, 2H), 3.91 (s, 3H), 1.45 (d, J = 7.3 Hz, 3H), 1.34 (t, J = 7.1 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 190.2, 166.7, 153.0, 151.5, 143.7, 143.1, 141.7, 140.7, 133.7, 126.8, 125.7, 125.2, 123.0, 122.9, 121.3, 121.1, 120.8, 120.3, 118.7, 111.6, 109.1, 108.5, 69.4, 60.6, 56.1, 37.9, 14.3, 13.8. HRMS (ESI$^+$) calcd for $C_{29}H_{27}NO_7$ $[M+H]^+$, 502.1866; found: 502.1853.

Characterization of Photoproducts:

3,6-Bis(hydroxyacetyl) 9-Ethyl 9H Carbazole: ^{1}H NMR (600 MHz, Chloroform-d) δ 8.74 (s, 2H), 8.14 (d, J = 8.6 Hz, 2H), 7.52 (d, J = 8.6 Hz, 2H), 5.03 (s, 4H), 4.45 (q, J = 7.2 Hz, 2H), 1.49 (d, J = 7.2 Hz, 3H). HRMS (ESI$^+$) calcd for $C_{18}H_{17}NO_4$ $[M+H]^+$, 312.1236; found: 312.1245.

3,6-Bis(Methoxyacetyl) 9-Ethyl 9H Carbazole: ^{1}H NMR (600 MHz, Chloroform-d) δ 8.80 (s, 2H), 8.20 (d, J = 8.6 Hz, 2H), 7.51 (d, J = 8.6 Hz, 2H), 4.87 (s, 4H), 4.43 (q, J = 7.2 Hz, 2H), 3.59 (s, 3H), 1.50 (d, J = 7.2 Hz, 3H). HRMS (ESI$^{+)}$ calcd for $C_{20}H_{21}NO_4$ $[M+H]^+$, 340.1549; found: 340.1541.

Computational Method: All the optimizations were carried out with CAM-B3LYP-D2/cc-pVDZ[27] level of theory. Frequency was calculated for the stationary points and absence of imaginary frequency confirmed a true minima and presence of only one imaginary frequency confirmed the transition states. The PES scan was also carried out at the same level. After completion of the scan, we performed single points at each point in the PES at CAM-B3LYP-D2/cc-pVTZ level of theory for the S0, S1, and T1 state. All the calculations were carried out using Gaussian09 software package.[28]

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Keywords: Bis-Acetyl Carbazole • Photoremovable Protecting Group • Release of Two Different Functional Groups • DFT calculations • Sequential Drug Delivery

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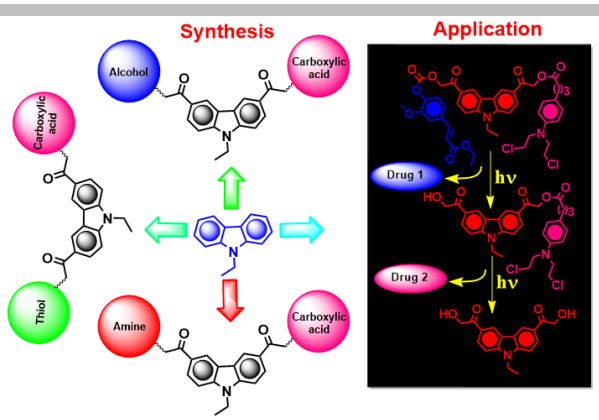

Yarra Venkatesh,[a] Surajit Nandi[a], Maniklal Shee,[a] Biswajit Saha,[b] Anakuthil Anoop[a] and N. D. Pradeep Singh*[a]*

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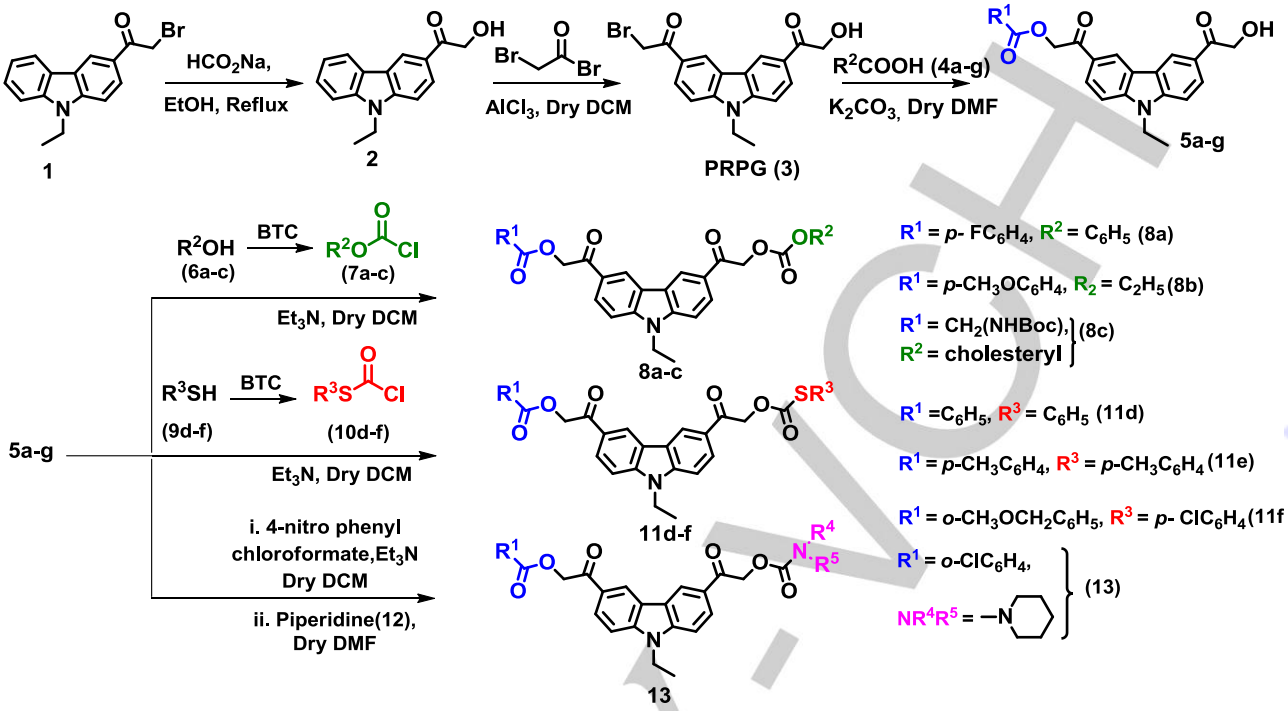
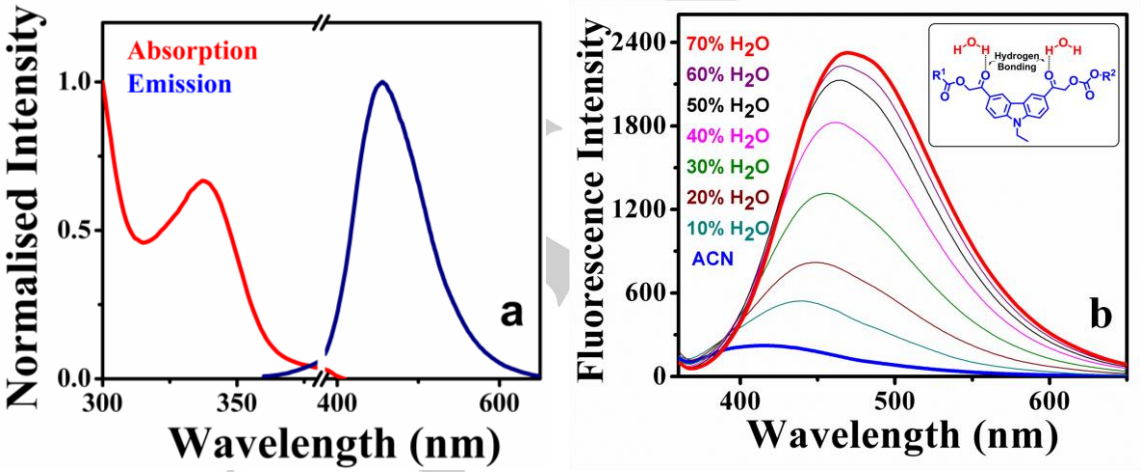
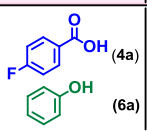
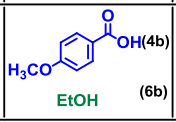
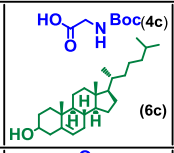
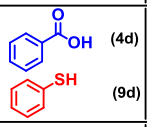
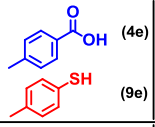
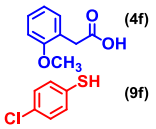
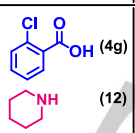
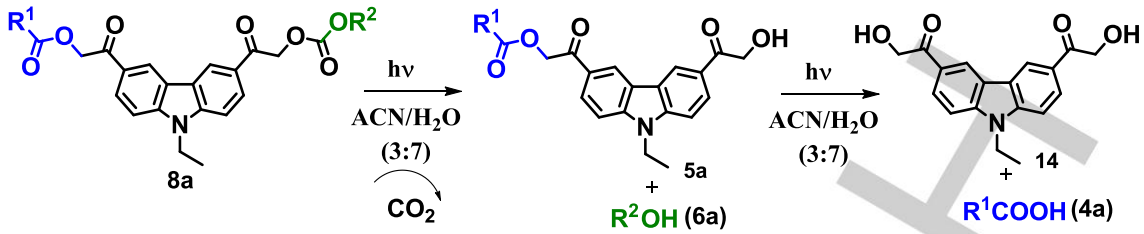
Bis-Acetyl Carbazole: Photoremovable Protecting Group for Sequential Release of Two Different Functional Groups and its Application for Therapeutic Release

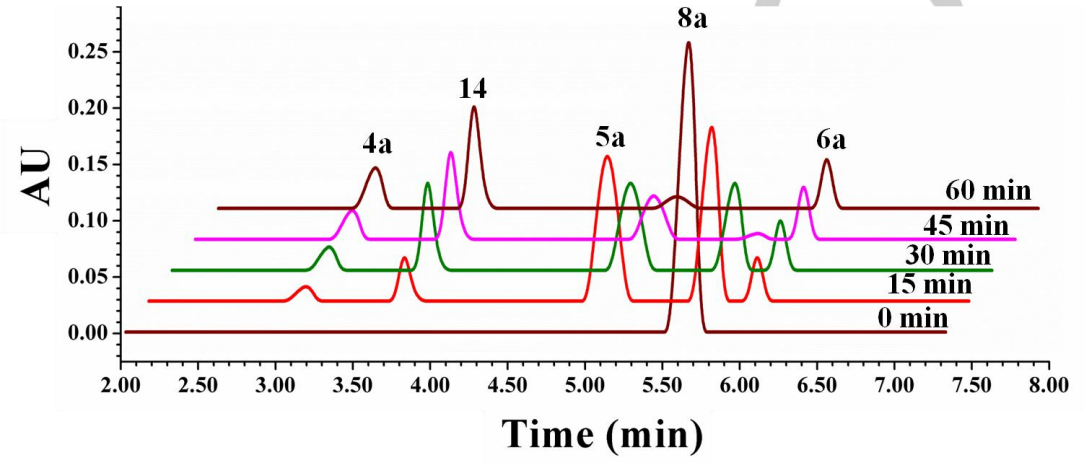


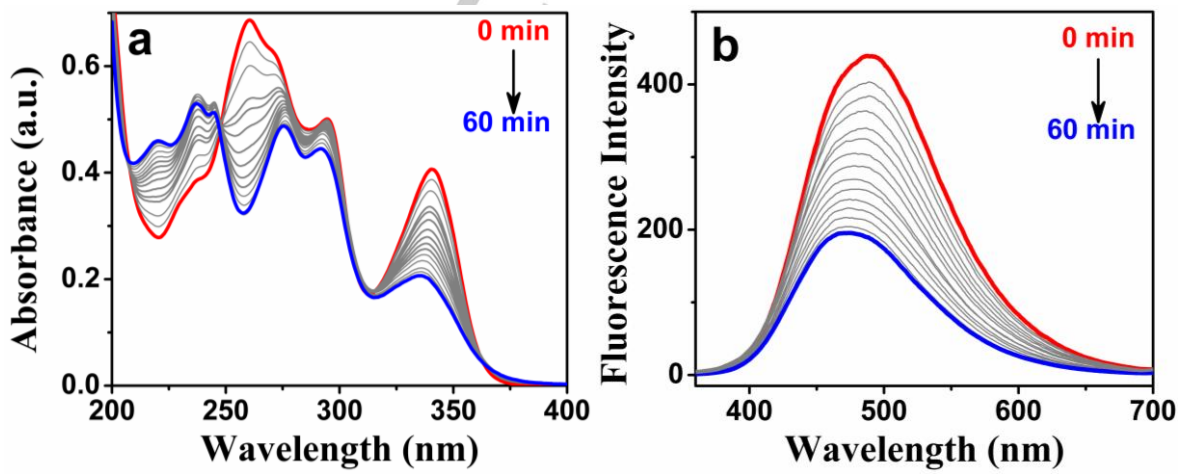


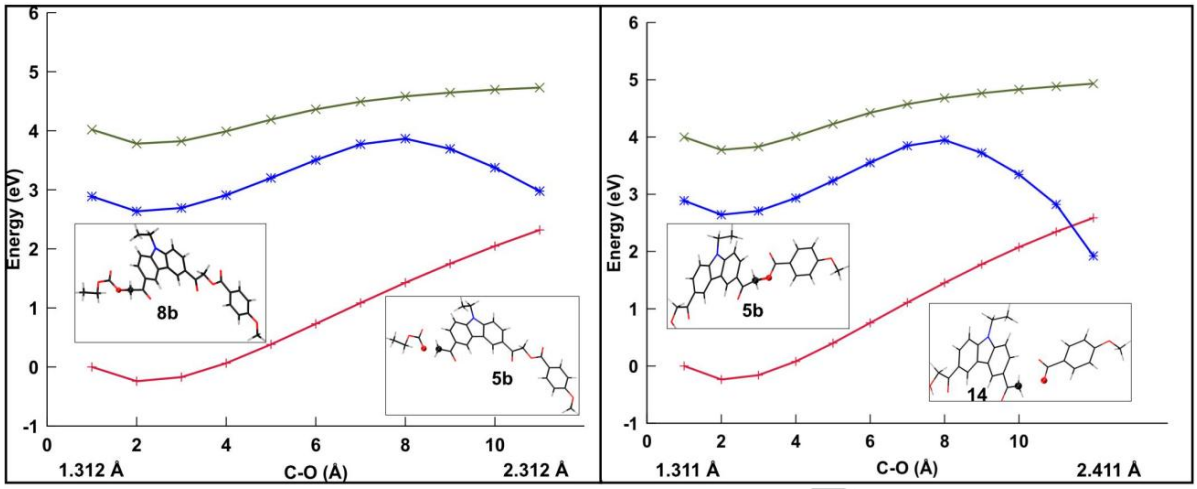
Caged Compounds	Carboxylic Acids, Alcohols, Thiols and Amines	Synthetic yield[a] %	Absorption		Fluorescence		
			λ_{max} (nm)[b]	$\varepsilon \times 10^4$ (L mol^{-1}cm^{-1})[c]	λ_{max} (nm)[d]	Stoke's shift (nm)[e]	Φ_f[f]
8a	 (4a) (6a)	92	338	2.71	456	118	0.268
8b	 (4b) (6b)	89	337	2.78	457	120	0.275
8c	 (4c) (6c)	93	338	2.70	458	120	0.274
11d	 (4d) (9d)	87	338	2.54	458	120	0.221
11e	 (4e) (9e)	83	337	2.56	456	119	0.225
11f	 (4f) (9f)	88	337	2.58	457	120	0.229
13	 (4g) (12)	72	337	2.67	457	120	0.247

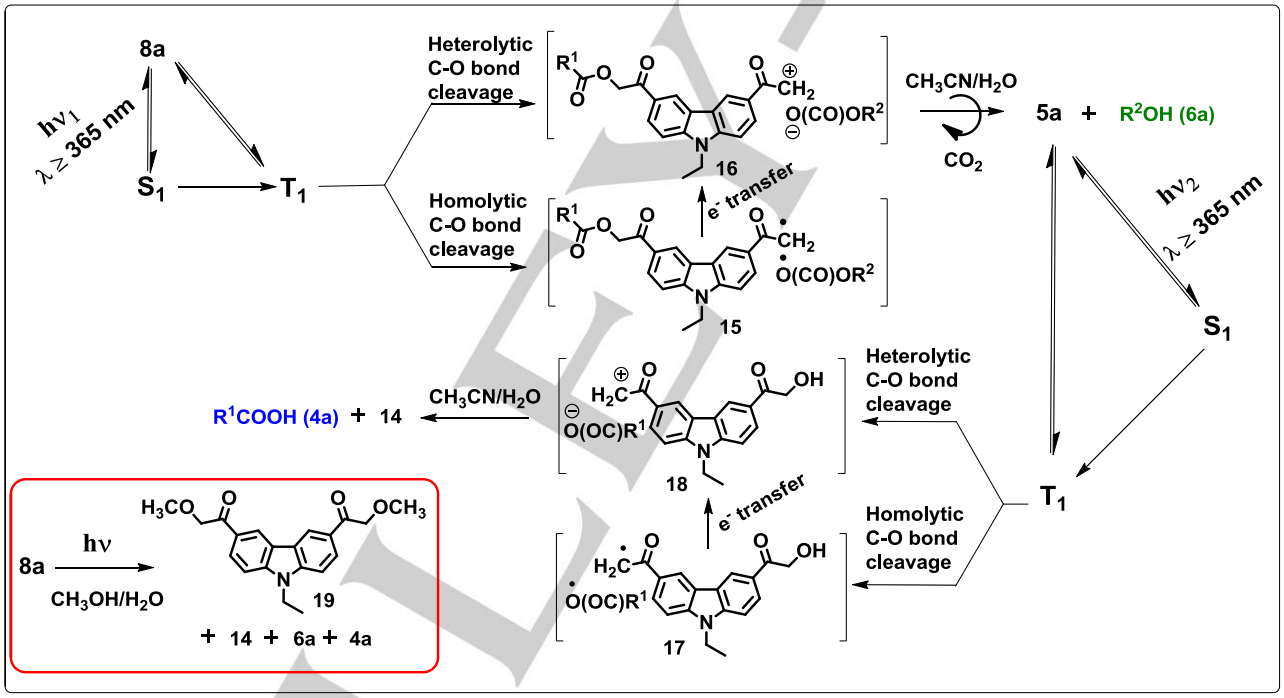
caged compounds	% of deprotection[a]	photochemical quantum yield (Φ_p[b])
8a	97 (6a)	0.139
	94 (4a)	0.101
8b	95 (6b)	0.136
	93 (4b)	0.100
8c	96 (6c)	0.138
	91 (4c)	0.098
11d	97 (9d)	0.140
	92 (4d)	0.099
11e	95 (9e)	0.133
	94 (4e)	0.101
11f	96 (9f)	0.138
	93 (4f)	0.102
13	95 (12)	0.135
	90 (4g)	0.097

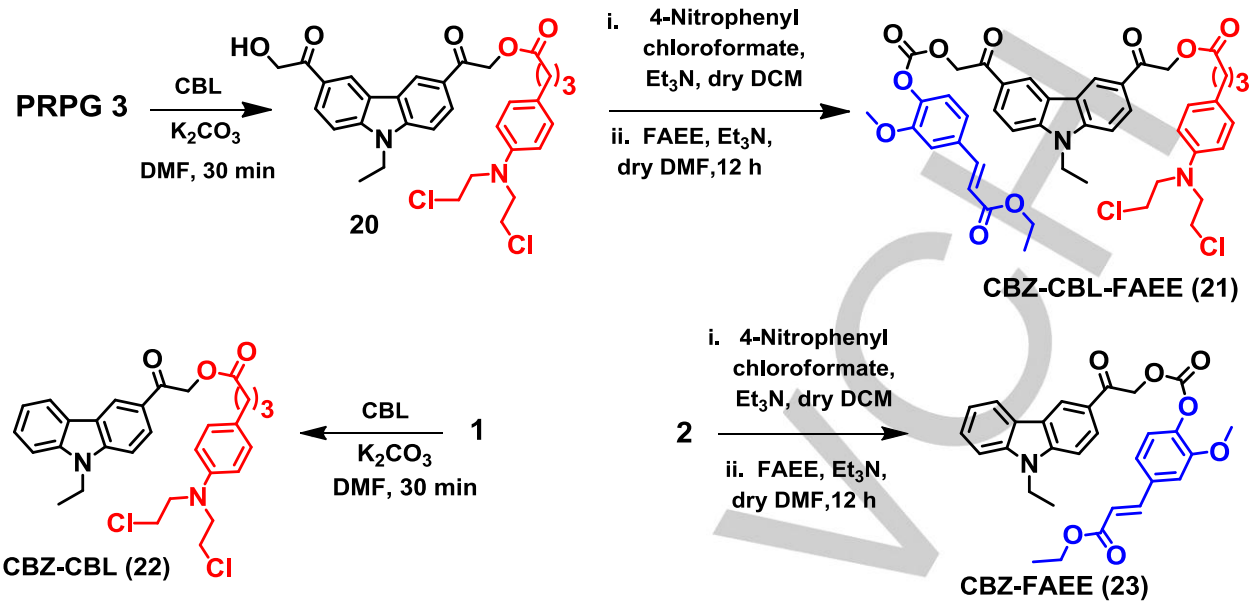


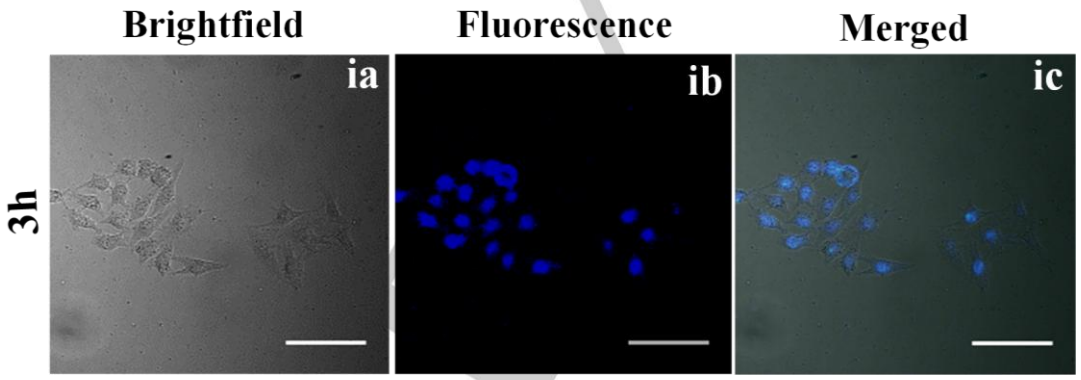


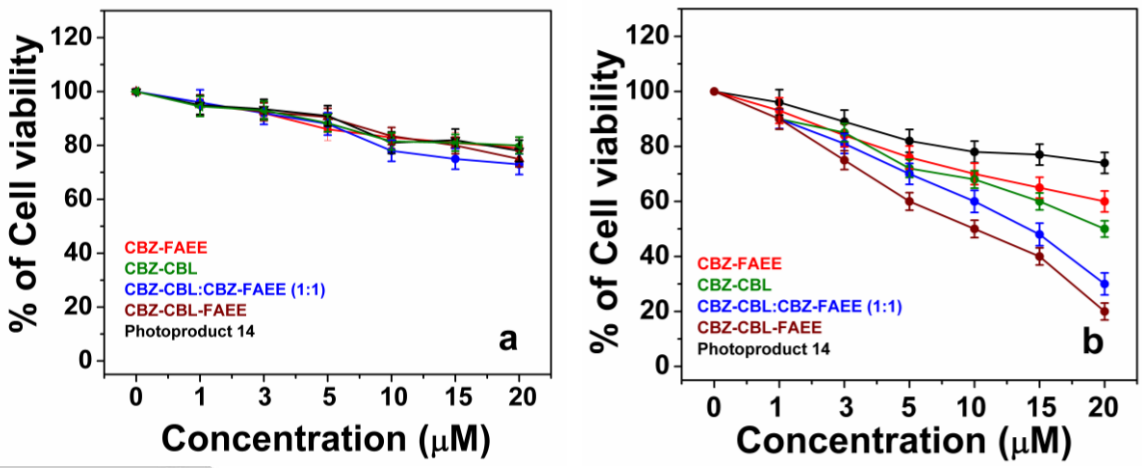












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