Tetrahedron 66 (2010) 9798–9807

Contents lists available at ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

1-Acetylpyrene with dual functions as an environment-sensitive fluorophore and fluorescent photoremovable protecting group

Avijit Jana, Sanghamitra Atta, Sujan K. Sarkar, N.D. Pradeep Singh *

Department of Chemistry, Indian Institute of Technology Kharagpur, Kharagpur 721302, West Bengal, India

ARTICLE INFO

Article history:
Received 2 July 2010
Received in revised form 21 October 2010
Accepted 31 October 2010
Available online 5 November 2010

Keywords:
1-Acetylpyrene
Fluorescence
Photocleavage
Photoremovable protecting group

ABSTRACT

A series of new fluorescent ester conjugates of carboxylic acids including amino acids was synthesized by coupling with an environment-sensitive fluorophore 1-acetylpyrene. Interestingly, the fluorescence properties of the ester conjugates and 1-acetylpyrene were found to be highly sensitive to its surrounding environment. The results obtained from the photolysis of the ester conjugates indicated that various factors like solvent, irradiation wavelength, and the structure of the conjugates govern the rate of the photocleavage.

1. Introduction

Photoremovable protecting groups (PRPGs) for various functional groups are of great interest since they have demonstrated potential applications in synthetic organic chemistry,[1] biochemistry,[2] and materials science.[2a,3] To date several PRPGs have been developed to mask different functional groups including carboxylic acids,[4] alcohols,[5] amines,[6] phosphates,[7] aldehydes,[8] and ketones.[8] The carboxyl group containing molecules can be effectively protected by a variety of PRPGs, such as 2-(dimethylamino)-5-nitrophenol,[9] 3-nitro-2-naphthalenemethanol,[10] α-carboxy nitrobenzyl,[11] *p*-hydroxyphenacyl,[12] α-keto amides,[13] 1-acyl-nitroindolines,[14] anthracene-9-methanol,[15] and derivatives of quinoline[16] as well as coumarin.[17]

Among the aforementioned PRPGs some groups are fluorescent and have greater advantage over non-fluorescent protecting groups since they not only release molecules of interest at desired location for a specific period of time, but also allow us to visualize, quantify and follow the spatial distribution, localization, and depletion of the released molecules.[18] The above strategy of using fluorescent PRPGs has been successfully employed for temporal and spatially controlled delivery of bioactive molecules in the study of numerous processes in biological[19] and medical research field.[20] In particular, labeling of amino acids by fluorescent PRPGs have offered great benefits. Firstly, it allows detection of small aliphatic amino acids with neither fluorescent nor strong absorption in the UV/vis region by a far more sensitive technique than common UV absorption.[21] Secondly, it helps us to visualize the amino acids involved in biological process.[22] Finally, since the amino acids are tagged by fluorescent PRPG, they can be released for a specific period of time at a desired location. For example, release of neuroactive amino acids (e.g., γ-aminobutyric acid, glycine, glutamic acid, etc.) by using fluorescent PRPG in the treatment of neuropsychiatric diseases has been reported.[23]

Although fluorescent PRPGs are of great utility, only limited number of PRPG of above class have been reported. In recent years PRPGs of polycyclic aromatic compounds namely anthraquinone,[24] pyrene,[24,25] phenanthrene,[24] anthracene,[15] and coumarins[18a,b] moieties have been targeted as fluorescent photolabile protecting groups. Among them use of pyren-1-yl methyl as fluorescent photolabile protecting group for alcohols,[24] carboxylic acids,[26] phosphates,[27] and amines[28] has been demonstrated. Although, pyrene is a well known fluorophore, to the best of our knowledge, pyren-1-yl methyl is the only fluorescent PRPG derived from the pyrene skeleton. Hence a search of another set of pyrene derivatives with improved photophysical and photochemical properties led us to investigate, 1-acylpyrenes.

1-Acylpyrenes are used as photoprobes[29,30] since their fluorescence properties are highly sensitive to medium polarity and the hydrogen bonding of the microenvironment. For example, 1-heptanoylpyrene[29] exhibits very low fluorescence in non-polar solvents like hexane or diethyl ether. But as we move to polar solvents like methanol and acetonitrile the fluorescence efficiency of 1-heptanoylpyrene increases and becomes almost identical to

* Corresponding author. Tel.: +91 3222 282324; fax: +91 3222 282252; e-mail address: ndpradeep@chem.iitkgp.ernet.in (N.D.P. Singh).

0040-4020/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2010.10.090

1-heptylpyrene and also the fluorescence emission band shifts from violet to blue. More interestingly, 1-heptanoylpyrene showed further increase in the fluorescence efficiency and red shift of the fluorescence emission band in binary aqueous mixtures of acetonitrile and methanol. The above fluorescence behavior of 1-heptanoylpyrene is due to their close lying ($\pi-\pi^*$) and ($n-\pi^*$) singlet excited states. Similar fluorescence trends were also observed in 1-octanoylpyrene[30] and pyrene-1-carbaldehyde.[31] Considering 1-acylpyrenes' high fluorescence efficiency, environment-sensitive emission properties and absorption maximum greater than 350 nm prompted us to develop one of its derivatives to act both as an environment-sensitive fluorophore and as a fluorescent PRPG.

In this paper we report a novel environment-sensitive fluorophore namely 1-acetylpyrene (**1**) as a photocage for carboxylic acids and amino acids. The synthesis and the characterization of 1-acetylpyrenyl ester conjugates were discussed. The absorption and emission properties of 1-acetylpyrene and its ester conjugates were also measured. The photorelease ability of 1-acetylpyrene was studied by irradiating the ester conjugates at different UV wavelengths (254, 350, and 410 nm). Further the effect of solvent on the rate of photorelease was also investigated.

2. Results and discussion

2.1. Synthesis of ester conjugates (4a–n)

A series of carboxylic acids including amino acids were protected by 1-(Bromoacetyl) pyrene (**2**) in the form of esters (**4a**–**n**) as outlined in Scheme 1. 1-(Bromoacetyl) pyrene (**2**) was readily prepared from the commercially available 1-acetylpyrene (**1**) by reaction with cupric bromide. Protection of carboxylic acids (**3a**–**g**) was straightforward. Treatment of carboxylic acids with 1 equiv of **2** in the presence of K_2CO_3/KI in dry *N*,*N*-Dimethylformamide (DMF) at 50 °C for a period of 8–12 h afforded the corresponding esters in good to excellent yield (Table 1, entries **4a**–**g**). However, for protecting amino acids (**3h**–**n**) we followed slightly different protocol. Boc-protected amino acids were treated with **2** using 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU) as catalyst at 0 °C in 1,4-dioxan, which provided high yield of protection (Table 1, entries **4h**–**n**).

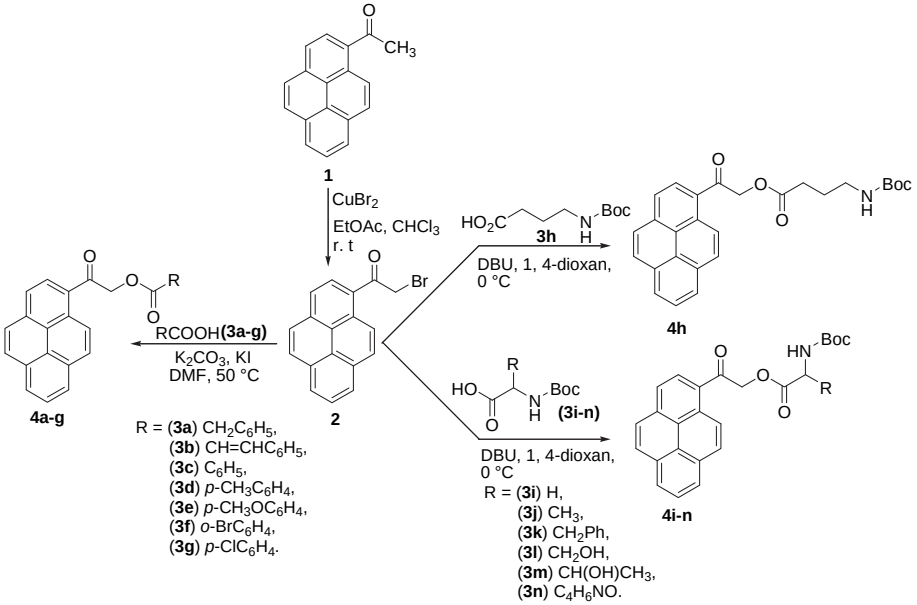
All ester conjugates were characterized by IR, ^{1}H, ^{13}C NMR, and mass spectral analysis. The IR spectra of conjugates **4a**–**n** showed a band at around 1730 cm^{-1} due to the stretching vibration of the newly formed ester carbonyl group. In addition the spectra also showed the carbonyl band of the 1-acetylpyrene protecting group in the range of 1650–1700 cm^{-1}. The confirmation of the presence of the newly formed ester group was further supported by ^{13}C NMR spectra, which showed the ester carbonyl at δ 171 ppm in addition to carbonyl signal of the 1-acetylpyrene at δ 196 ppm.

2.2. Photophyiscal properties of ester conjugates (4a–n)

The photophysical properties of all the ester conjugates and its fluorophore 1-acetylpyrene were investigated. The UV/vis absorption and emission spectra of degassed 2×10^{-6} M solution of esters (**4a**–**n**) and 1-acetylpyrene (**1**) in absolute ethanol (EtOH) were recorded. The absorption and emission maxima, molar absorptivities, and fluorescence quantum yield of the above esters along with 1-acetylpyrene (**1**) are summarized in Table 1. Fluorescence quantum yields were calculated using anthracene as standard (Φ=0.27 in ethanol).[32]

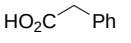
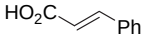
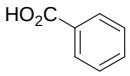
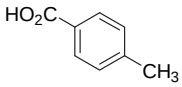
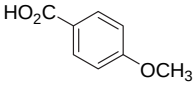
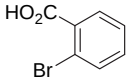
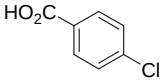
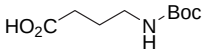
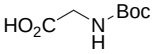
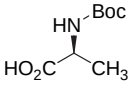
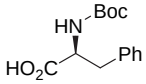
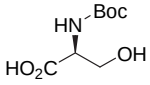
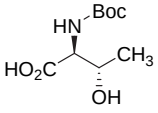
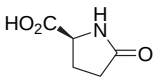
Fig. 1a shows the normalized absorption and the emission spectra of GABA conjugate **4h** in ethanol. The absorption spectrum of **4h** shows an intense band centered at 354 nm with ε 20.7×10^3 mol^{-1} L cm^{-1} while in the emission spectrum the emission maxima was red shifted to about 440 nm. We observed similar absorption and emission maxima for all the other ester conjugates (Table 1), which clearly suggest that 1-acetylpyrene moiety only dictates the position of the absorption and emission maxima ruling out the influence of its counterpart carboxylic acids. The Stokes' shift has been calculated from the difference in the absorption and the emission maxima and the magnitude of the Stokes' shift of all the conjugates varies between 86 and 98 nm. Further the conjugated esters also showed moderate fluorescence quantum yield ($0.028<\Phi<0.056$).

*2.2.1. Fluorescence spectra of ester conjugate (**4h**) in neat solvents.* The GABA ester conjugate **4h** (2×10^{-6} M) was excited at 356 nm and the emission spectra were recorded in various solvents. Fig. 1b indicates the fluorescence of conjugate **4h** is due to the monomeric excited singlet state and displays strong solvent dependence like other 1-acyl derivatives of pyrene.[30,31] The fluorescence solvatochromism of the conjugate **4h** is due to their closely lying $^1\pi-\pi^*$ and $^1n-\pi^*$ single states. In non-polar solvent (e.g.,



Scheme 1. Synthesis of 1-acetylpyrene-carboxylic acid ester conjugates.

Table 1
Synthetic yields, UV/vis and fluorescence data for the ester conjugates (**4a**–**n**) and its precursor 1-acetylpyrene (**1**) in absolute ethanol

Compound	Carboxylic acid	Synthetic yield (%)[a]	UV/vis		Fluorescence		
			λ_{max} (nm)[b]	$\varepsilon\times10^3$ (mol^{-1} L cm^{-1})[c]	λ_{max} (nm)[d]	Stokes' shift (nm)[e]	Φ_F[f]
4a		80	356	20.5	450	94	0.032
4b		76	355	20.2	451	96	0.035
4c		88	355	22.4	453	98	0.028
4d		91	356	20.9	453	97	0.030
4e		92	356	21.0	453	97	0.035
4f		70	356	20.4	451	95	0.044
4g		83	356	20.9	449	93	0.033
4h		80	354	20.7	440	86	0.030
4i		76	355	20.4	450	95	0.037
4j		82	356	20.4	451	95	0.035
4k		75	356	20.5	452	96	0.034
4l		79	357	21.2	450	93	0.041
4m		82	356	21.1	451	95	0.056
4n		86	357	20.9	450	93	0.040
1	–	–	359	15.0	439	80	0.023

[a] Based on isolated yield.
[b] Maximum absorption wavelength.
[c] Molar absorption coefficient (in mol^{-1} L cm^{-1}) 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%).

n-hexane) the fluorescence spectrum of conjugate **4h** is structured with peaks around 385–430 nm with weak emission. This particular fluorescence is from the non-fluorescent lowest $^1n-\pi^*$ state. However in the weakly or moderately interacting polar solvents the energy of the $^1\pi-\pi^*$ is brought below the $^1n-\pi^*$ by solvent relaxation during the lifetime of the excited state.[31c] Thus in the polar solvent the emissive $^1\pi-\pi^*$ becomes the LUMO and results in increased intensity of fluorescence.

Further, we also observed strong red shift of the fluorescence emission band in hydroxylic polar solvents similar to pyrene-1-carbaldehyde, which can be attributed due to geometric rearrangement of the molecule or of the solvation shell in the

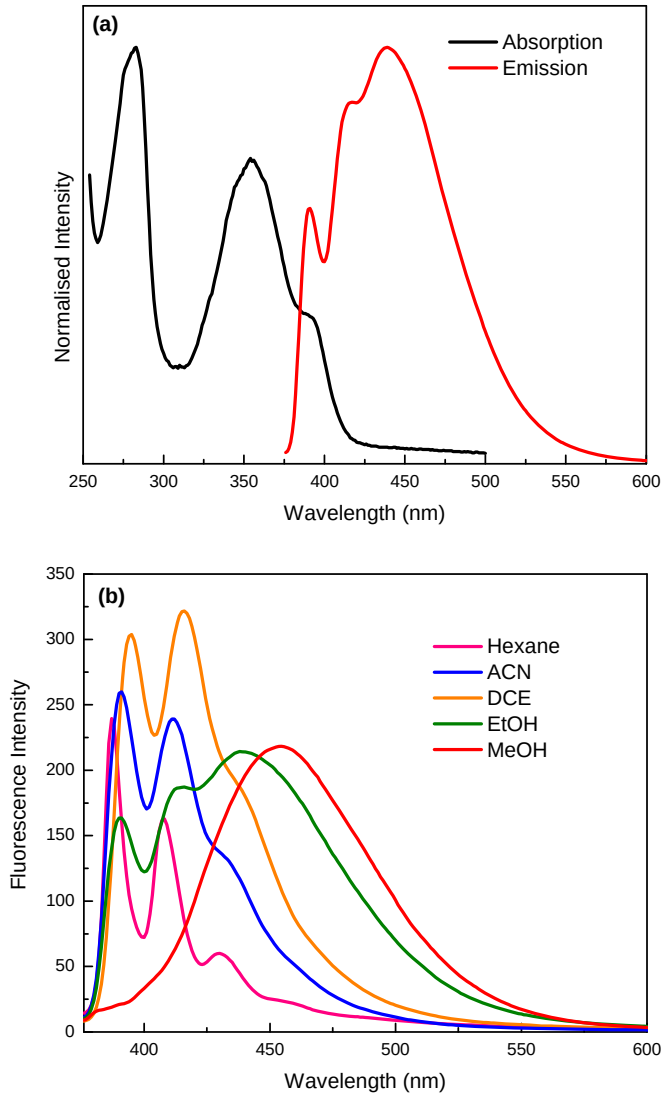


Fig. 1. a) Normalized UV/vis absorption spectrum (black line) and emission spectrum (red line) of ester **4h** in EtOH (2.0×10^{-6} M). (b) Corrected fluorescence spectra of the ester **4h** in Hexane, ACN, Dichloroethane (DCE), EtOH, and MeOH (2.0×10^{-6} M).

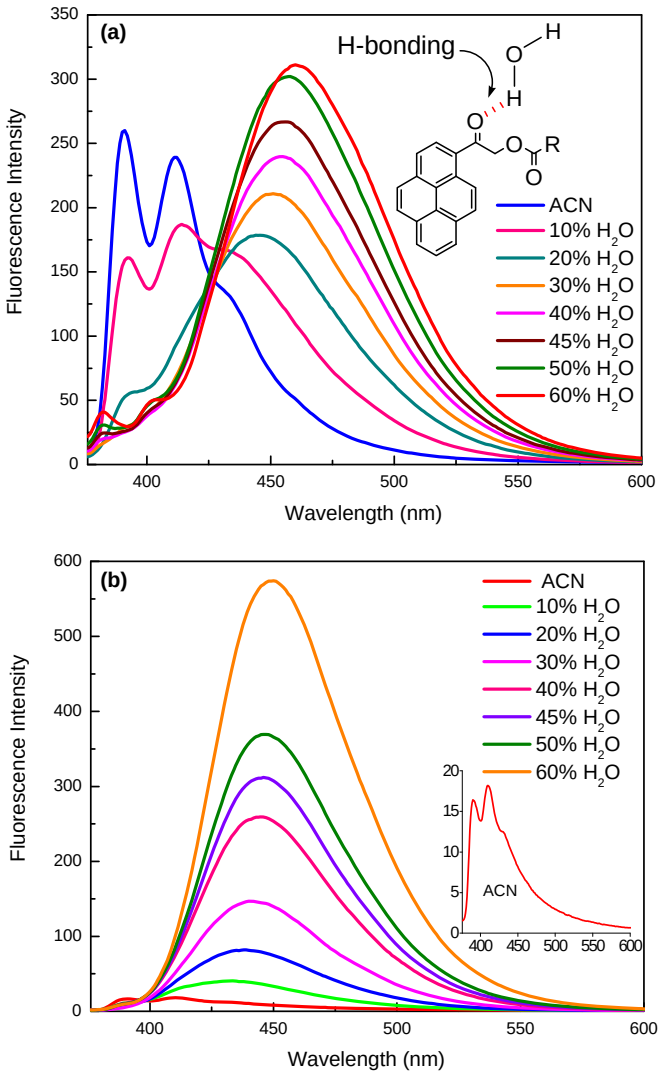


Fig. 2. a) Corrected fluorescence spectra of the ester **4h** in ACN and in increasing percentage of water in ACN (2.0×10^{-6} M). (b) Corrected fluorescence spectra of 1-acetylpyrene (**1**) in ACN and in increasing percentage of water in ACN (2.0×10^{-6} M).

fluorescent $^{1}\pi-\pi^{*}$ excited state.[29] Moreover the red shift is also accompanied by a loss of fine structure and an increase of band width, which indicates the occurrence of an intramolecular charge transfer (amine group is an electron donor and the carbonyl moiety of 1-acetylpyrene is electron deficient) allowing the involvement of an excited state with intramolecular charge transfer (ICT) character.[33]

*2.2.2. Fluorescence spectra of ester conjugate (**4h**) in aqueous binary mixtures.* In order to understand the effect of hydrogen bonding interaction on the fluorescence spectra, we measured the emission spectra of **4h** in acetonitrile/water mixtures. In similar to 1-heptanoylpyrene, we also noted that upon addition of increasing amount of water the fluorescence spectra of GABA conjugate (**4h**) is red shifted with a concomitant increase in fluorescence intensity (Fig. 2a). This can be attributed to the fact that hydrogen bonding raises the energy of the triplet $^{3}n-\pi^{*}$ (which was found in the vicinity of lowest singlet $^{1}\pi-\pi^{*}$excited states) above the lowest singlet $^{1}\pi-\pi^{*}$excited states thereby reducing the efficiency of the intersystem crossing.[29]

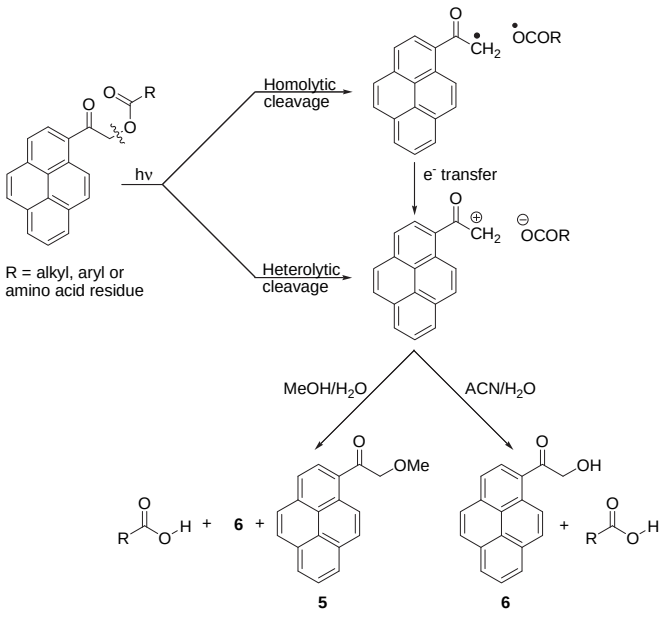
Further we also observed isoemmisive point at λ=427 nm, which indicates existence of an equilibrium between the conjugate **4h** and its hydrogen bonded complex. Similar phenomenon was also manifested in the emission spectra (Fig. 2b) of 1-acetylpyrene (**1**) in acetonitrile/water mixtures.

The photophysical studies showed that 1-acetylpyrene (**1**) and its ester conjugates exhibited strong fluorescence, large Stokes' shift, moderate fluorescence quantum yield, and moreover their fluorescence properties were sensitive to its surrounding environment, thus makes 1-acetylpyrene to be an environment-sensitive fluorophore.

2.3. Photolysis of ester conjugates (4a–n)

Considering our main interest, we investigated the application of 1-acetylpyrene as a fluorescent PRPG for various carboxylic acids including amino acids. Photolysis of conjugates (**4a**–**n**) in ACN/4-(2-hydroxyethyl)piperazine-1-ethanesulfonicacid (HEPES) buffer solution (50:50) using 125 W medium pressure Hg lamp at different irradiation wavelengths (254, 350, and 410 nm) were studied in order to determine the best photocleavage condition. The course of the photocleavage reaction was monitored by ^{1}H NMR spectroscopy. A known amount of photolysate was taken at regular intervals of time, then solvent was evaporated under

vacuum and redissolved in $CDCl_3$ with 1,2-dichloroethane as the internal standard and the ^1H NMR was recorded. The photocleavage of the conjugate esters were then quantified by comparing the integration of the α-CH_2 signal to that of the internal standard. The reaction was followed until the consumption of the starting material is less than 5% of the initial area. In all cases ^1H NMR spectra showed clean photocleavage of the ester conjugates to produce their corresponding carboxylic acids (Scheme 2). In several examples as indicated in Table 2 the photoproducts (carboxylic acids and 1-(hydroxyl acetyl) pyrene) were confirmed by isolating and comparing their spectra with corresponding authentic samples. The quantum yield was calculated using potassium ferrioxalate as an actinometer.[34]



Scheme 2. Possible photocleavage pathway for the photolysis of 1-acetylpyrene-carboxylic acid ester conjugates.

life was calculated for all the conjugates and the results are tabulated in Table 2.

The course of photolysis of conjugated esters was also monitored by fluorescence spectroscopy (As a representative example fluorescence spectra of **4h** at regular interval of time of irradiation is presented in Supplementary data Fig. S3).

2.4. Effect of the conjugate structure on photorelease

Like pyrene-1-ylmethyl,[20] we also observed similar influence of the structure of amino acids on the rate of photocleavage. Conjugates of amino acids with polar side chain (**4l** and **4m**) and amino acids with longer chain length (**4h**) had lower quantum yield for photocleavage (Table 2). Further, in the case of aromatic carboxylic acids we found that electron donating substituent like *p*-methyl (**4d**) and *p*-methoxy (**4e**) were efficiently released in higher quantum yield compared to halogen substituted carboxylic acids (**4f**–**g**).

2.5. Effect of irradiation wavelength on photorelease

The influence of the irradiation wavelength on the extent of photocleavage of conjugates (**4a**–**n**) in acetonitrile/HEPES buffer (50:50) is shown in Table 3. The time required for photocleavage of conjugates increases as the irradiation wavelength increases. Since C–O bond disassociation energy is 86 kcal/mol and as we increase the irradiation wavelength from 254 nm to 410 nm, the excitation energy decreases from 112 kcal/mol to 70 kcal/mol. Hence, GABA conjugate **4h** was fully depleted at 254 nm after 26 min of irradiation, while it required 65 min at 350 nm and 304 min at 410 nm (Table 3, entry 4h). The incident photon flux (I_0) at 254, 350, and 410 nm is 1.8×10^{17}, 1.55×10^{17}, and 2.13×10^{16} photons $s^{-1}cm^{-2}$, respectively.

2.6. Solvent effect on photorelease

To understand the role of the solvent on the rate of photocleavage, we carried out the photolysis of GABA conjugate **4h** in aqueous mixtures of methanol, acetonitrile, and THF and the results are included in Table 4. The photocleavage of **4h** in methanol/H_2O (50:50) found to be more efficient compared to similar system of acetonitrile (though the cleavage in methanol is efficient the solubility of the conjugates is not as good as compared to acetonitrile). This can be attributed not only due to the formation of ionic intermediates (Scheme 2) but also depends on the hydrogen bonding

Table 2
Photolytic data of ester conjugates (**4a**–**n**) at different irradiation wavelengths in acetonitrile/HEPES (50:50) solution

Ester	254 nm			350 nm			410 nm		
	$\varepsilon\times10^3$ (mol^{-1} L cm^{-1})[a]	$t_{1/2}$ (min)[b]	Φ[c]	$\varepsilon\times10^3$ (mol^{-1} L cm^{-1})[a]	$t_{1/2}$ (min)[b]	Φ[c]	$\varepsilon.10^3$ (mol^{-1} L cm^{-1})[a]	$t_{1/2}$ (min)[b]	Φ[c]
4a[d]	19.2	2.7	0.62	22.8	6.9	0.42	3.9	55.5	0.39
4b	18.6	3.5	0.51	22.4	9.7	0.30	3.7	58.8	0.38
4c[d]	17.6	4.1	0.41	21.5	11.6	0.25	3.3	64.1	0.35
4d[d]	17.8	3.8	0.45	22.3	7.4	0.40	3.6	57.6	0.39
4e[d]	18.4	3.0	0.56	22.8	5.8	0.51	3.8	55.4	0.41
4f	17.5	4.3	0.40	21.4	9.3	0.32	3.0	75.7	0.29
4g[d]	17.2	4.6	0.37	18.7	10.4	0.28	2.8	78.7	0.28
4h[d]	18.0	6.1	0.28	20.7	15.0	0.19	3.2	70.4	0.32
4i	17.8	3.8	0.45	22.5	9.3	0.32	3.6	60.8	0.36
4j[d]	17.4	5.3	0.32	21.1	9.9	0.29	2.8	61.3	0.36
4k[d]	17.8	5.6	0.30	18.0	12.9	0.23	2.9	61.1	0.36
4l	18.4	6.4	0.27	18.4	12.7	0.23	3.9	74.0	0.30
4m	17.6	6.4	0.27	17.2	12.0	0.24	3.3	72.9	0.30
4n[d]	19.1	5.5	0.31	17.7	11.1	0.26	3.1	72.4	0.31

[a] Molar absorption coefficient (in mol^{-1} L cm^{-1}) at the irradiation wavelength.
[b] Half-life under photolytic conditions (in minutes).
[c] Photochemical quantum yield (error limit within ±5%).
[d] Esters conjugates for which the photoproducts were isolated and compared with authentic samples.

Based on ^1H NMR data for each compound, the natural logarithm of the concentration of ester conjugate (lnC) vs irradiation time was plotted (see Fig. S2 in Supplementary data) and we observed a linear correlation for the disappearance of the starting material, which suggested a first order reaction kinetics. The half-

Table 3
Irradiation time required for photolysis of ester conjugates (**4a**–**n**) at different wavelengths in acetonitrile/HEPES (50:50) solution

Compound	Time required for photocleavage of (**4a**–**n**)		
	254 nm (min)	350 nm (min)	410 nm (min)
4a	12	30	240
4b	15	42	254
4c	18	50	277
4d	16	32	250
4e	13	25	240
4f	19	40	327
4g	20	45	340
4h	26	65	304
4i	17	40	263
4j	24	43	265
4k	24	56	264
4l	28	55	320
4m	28	52	315
4n	24	48	313

and polarity of the solvent since they have influence on the excited states.

Further to mimic the biological condition, we also carried out the photolysis of **4h** in acetonitrile/HEPES mixtures. We noticed the efficiency of the photoreaction of conjugate **4h** increases continuously with increasing amount of water (For example, efficiency of photocleavage of conjugate **4h** increased by a factor of two times (350 nm) as we move from 10% to 50% of HEPES in acetonitrile), which is in contrast to its fluorescence behavior, which increases at the beginning and slows down after reaching a maximum (Fig. 2a). The interpretation of the increase of quantum yield of photochemistry is due to the better stabilization of the newly generated ion-pair (Scheme 2) by solvation, which leads to the acceleration of the rate of photolysis thereby reducing the fluorescence efficiency, the similar phenomenon is also observed in caged coumarins.[18a]

The stability of the ester conjugates was also tested by keeping them in the dark in an aqueous solvent for a period of 15 days. The rate constant for the hydrolysis of the ester conjugates was calculated using ^{1}H NMR, and were found to be in the range of 2.28×10^{-3} day^{-1} to 9.28×10^{-3} day^{-1} (see Table S1 in the Supplementary data for the hydrolysis constant of all the ester conjugates).

2.7. Mechanism of the photorelease

The solvent study indicates that the photocleavage of the ester conjugates proceeds through an ionic mechanism. The initial photochemical step involves excitation of the 1-acetylpyrene chromophore to its singlet excited state, which then undergoes rapid singlet–triplet intersystem crossing. Subsequently, from the triplet excited state it then undergoes homolytic cleavage of the C–O bond followed by an electron transfer[15] to produce an ion-pair of 1-pyrene-1-yl-ethanoyl carbocation and carboxylate anion or concurrently, the heterolytic cleavage[20] of the C–O bond could directly afford the already mentioned ion-pair. After ion-pair separation in polar solvent, the newly formed carbocation undergoes nucleophilic attack by the solvent molecule to yield 1-(hydroxyacetyl)pyrene (**6**) (while in aqueous methanolic solvent we observed both 1-hydroxy and 1-(methoxyacetyl)pyrene (**5**)).

3. Conclusion

Carboxylic acids including amino acids were protected by 1-acetylpyrene to give their corresponding ester conjugates in good to excellent yields using simple procedure. Photophysical studies revealed that 1-acetylpyrene and its ester conjugates showed good fluorescence properties and more interestingly their fluorescence properties were found to be highly sensitive to its surrounding environment. Further we also demonstrated the ability of the 1-acetylpyrene to release carboxylic acids and amino acids in aqueous organic solvents using visible light. In conclusion, we have showed that 1-acetylpyrene can act as an environment-sensitive fluorophore as well as a fluorescent photoremovable protecting group.

4. Experimental section

4.1. General

^{1}H NMR (200 MHz) spectra were recorded on a BRUKER-AC 200 MHz spectrometer. Chemical shifts are reported in parts per million from tetramethylsilane with the solvent resonance as the internal standard (deuterochloroform: 7.26 ppm). Data are reported as follows: chemical shifts, multiplicity (s=singlet, d=doublet, t=triplet, m=multiplet), coupling constant (Hz). ^{13}C NMR (50 MHz) spectra were recorded on a BRUKER-AC 200 MHz Spectrometer with complete proton decoupling. Chemical shifts are reported in parts per million from tetramethylsilane with the solvent resonance as the internal standard (deuterochloroform: 77.0 ppm). UV/vis absorption spectra were recorded on a Shimadzu UV-2450 UV/vis spectrophotometer, fluorescence emission spectra were recorded on a Hitachi F-7000 fluorescence spectrophotometer, FTIR spectra were recorded on a Perkin–Elmer RXI spectrometer and HRMS spectra were recorded on a JEOL-AccuTOF JMS-T100L mass spectrometer. Photolysis of all the ester conjugates were carried out using 125 W medium pressure mercury lamp supplied by SAIC (India). Chromatographic purification was done with 60–120 mesh silica gel (Merck). For reaction monitoring, precoated silica gel 60 F_{254} TLC sheets (Merck) were used.

Table 4
Photolytic data of ester conjugate **4h** at different irradiation wavelengths in different solvent systems

Solvent system	350 nm			410 nm		
	$\varepsilon\times10^3$ (mol^{-1} L cm^{-1})[a]	$t_{1/2}$ (min)[b]	Φ_{phot}[c]	$\varepsilon\times10^3$ (mol^{-1} L cm^{-1})[a]	$t_{1/2}$ (min)[b]	Φ_{phot}[c]
MeOH	20.7	32.2	0.09	3.9	263.4	0.08
MeOH/H_2O (50:50)	20.7	11.4	0.26	3.9	40.9	0.54
ACN	20.7	40.1	0.07	3.9	341.0	0.07
ACN/H_2O (50:50)	20.7	15.0	0.19	3.9	70.4	0.32
THF	20.6	55.0	0.05	3.9	542.2	0.04
THF/H_2O (50:50)	20.7	21.1	0.14	3.7	80.1	0.28
ACN/HEPES (90:10)	20.6	30.7	0.10	3.6	86.0	0.26
ACN/HEPES (70:30)	20.7	20.4	0.14	3.4	79.4	0.28
ACN/HEPES (50:50)	20.7	15.0	0.19	3.2	70.4	0.30

[a] Molar absorption coefficient (in mol^{-1} L cm^{-1}) at the irradiation wavelength.
[b] Half-life under photolytic conditions (in minutes).
[c] Photochemical quantum yield (error limit within ±5%).

4.2. General procedure for the synthesis of the ester conjugates 4a–g

1-(Bromoacetyl)pyrene (1 equiv) was dissolved in dry *N*,*N*-Dimethylformamide (DMF) (2 mL), potassium iodide (1.2 equiv), potassium carbonate (1.2 equiv), and the corresponding carboxylic acid **3a**–**g** (1 equiv) were added. The reaction mixture was stirred at 50 °C for 8–12 h. The solvent was removed by rotary evaporation under reduced pressure and the crude residue was purified by column chromatography using ethylacetate (EtOAC) in pet. ether.

*4.2.1. 2-Oxo-2-(pyren-3-yl)ethyl 2-phenylacetate (**4a**).* 1-(Bromoacetyl)pyrene (0.100 g, 0.29 mmol), potassium iodide (0.057 g, 0.34 mmol), potassium carbonate (0.048 g, 0.34 mmol), and phenyl acetic acid (0.056 g, 0.29 mmol) were used. The reaction mixture was stirred for 10 h. The crude residue was purified by column chromatography with 20% EtOAc in pet. ether to give the title compound (**4a**) (0.088 g, 80%) as a yellow solid, mp: 135 °C; TLC R_f 0.43 (20% EtOAc in pet. ether); ^{1}H NMR ($CDCl_3$, 200 MHz): δ=9.04 (d, *J*=9.6 Hz, 1H), 8.28 (m, 8H), 7.25 (s, 5H), 5.49 (s, 2H), 3.85 (s, 2H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ=196.5, 171.5, 134.5, 133.8, 131.2, 130.7, 130.3, 130.2, 129.6 (2C), 128.8 (2C), 127.4, 127.2, 126.7 (2C), 126.5 (2C), 125.9, 125.1, 124.7, 124.3, 124.1 (2C), 68.4, 41.1; FTIR (KBr) ν_{max} (cm^{-1}): 1737 (OCO), 1636 (CO); UV/vis (EtOH): λ_{max} (ε) 356 (20.5×10^3), 400 (6.3×10^3); LCMS: *m*/*z* (%): 401.1(100) $[M+Na]^+$, 379.1(5), 301.1(10); HRMS (ES^+) *m*/*z* calcd for $C_{26}H_{19}O_3$ $[M+H]^+$: 379.1334; found: 379.1328.

*4.2.2. 2-Oxo-2-(pyren-3-yl)ethyl cinnamate (**4b**).* 1-(Bromoacetyl) pyrene (0.100 g, 0.29 mmol), potassium iodide (0.057 g, 0.34 mmol), potassium carbonate (0.048 g, 0.34 mmol), and cinnamic acid (0.043 g, 0.29 mmol) were used. The reaction mixture was stirred for 8 h. Purification of the crude residue by column chromatography, using EtOAc/pet. ether mixtures of increasing polarity as the eluent gave compound **4b** as a yellow solid (0.086 g, 76%), mp: 161 °C; TLC R_f 0.45 (20% EtOAc in pet. ether); ^{1}H NMR ($CDCl_3$, 200 MHz): δ=9.02 (d, *J*=9.4 Hz, 1H), 8.31 (d, *J*=8.2 Hz, 1H), 8.26–8.00 (m, 7H), 7.86 (d, *J*=16.0 Hz, 1H), 7.56 (s, 2H), 7.34 (s, 3H), 6.65 (d, *J*=16.0 Hz, 1H), 5.62 (s, 2H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ=196.3, 166.5, 146.3, 134.4, 134.3, 130.9, 130.6, 130.2, 130.0, 128.9 (2C), 128.5 (2C), 128.3 (2C), 127.0, 126.6 (2C), 126.4, 125.8, 125.0, 124.6, 123.9 (3C), 117.0, 76.9; FTIR (KBr) ν_{max} (cm^{-1}): 1720 (OCO), 1684 (CO); UV/vis (EtOH): λ_{max} (ε) 355 (20.2×10^3), 400 (6.2×10^3); LCMS: *m*/*z* (%): 391.2 (100) $[M+H]^+$, 377.2 (75), 229.1(20); HRMS (ES^+) *m*/*z* calcd for $C_{27}H_{19}O_3$ $[M+H]^+$: 391.1334; found 391.1350.

*4.2.3. 2-Oxo-2-(pyren-3-yl)ethyl benzoate (**4c**).* 1-(Bromoacetyl) pyrene(0.100 g, 0.29 mmol), benzoic acid (0.035 g, 0.29 mmol), potassium carbonate (0.048 g, 0.34 mmol), and potassium iodide (0.057 g, 0.34 mmol) were used and the reaction mixture was stirred for 10 h. A reddish precipitate was obtained, which on purification by column chromatography, using EtOAc/pet. ether mixtures of increasing polarity as the eluent gave compound **4c** (0.093 g, 88%) as a brown solid, mp: 130 °C; TLC R_f 0.48 (20% EtOAc in pet. ether); ^{1}H NMR ($CDCl_3$, 200 MHz): δ=9.02 (d, *J*=9.4 Hz, 1H), 8.33 (d, *J*=8.2 Hz, 1H), 8.22–7.99 (m, 9H), 7.56 (t, *J*=7.2 Hz, 1H), 7.56–7.43 (m, 2H), 5.71 (s, 2H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ=196.0, 166.2, 134.4, 133.3, 130.9, 130.5, 130.1, 130.0 (3C), 129.5, 128.5 (3C), 127.0, 126.5 (2C), 126.3, 125.8, 124.9, 124.5, 124.0, 123.9 (2C), 68.2; FTIR (KBr) ν_{max} (cm^{-1}): 1721 (OCO), 1640 (CO); UV/vis (EtOH): λ_{max} (ε) 355 (22.4×10^3), 400 (7.2×10^3); LCMS: *m*/*z* (%): 365.4 (100) $[M+H]^+$, 229.2 (8), 163.2 (14); HRMS (ES^+) *m*/*z* calcd for $C_{25}H_{17}O_3$: 365.1178 $[M+H]^+$; found: 365.1190.

*4.2.4. 2-Oxo-2-(pyren-3-yl)ethyl 4-methylbenzoate (**4d**).* 1-(Bromoacetyl)pyrene(0.100 g, 0.29 mmol), 4-methyl benzoic acid (0.039 g, 0.29 mmol), potassium carbonate (0.048 g, 0.34 mmol), and potassium iodide (0.057 g, 0.34 mmol) were used. The reaction mixture was stirred for 8 h. A brown residue was obtained, which on purification using 20% EtOAc in pet. ether affords compound **4d** (0.100 g, 91%) as a yellow solid, mp: 165 °C; TLC R_f 0.48 (20% EtOAc in pet. ether); ^{1}H NMR ($CDCl_3$, 200 MHz): δ=9.02 (d, *J*=9.4 Hz, 1H), 8.36 (d, *J*=8.2 Hz, 1H), 8.26–8.14 (m, 5H), 8.08–8.01 (m, 4H), 7.27 (d, *J*=8.0 Hz, 2H), 5.69 (s, 2H), 2.42 (s, 3H); ^{13}C NMR (CDCl3, 50 MHz): δ=196.3, 166.3, 144.1, 134.3, 131.0, 130.5, 130.0 (2C), 129.9, 129.2 (2C), 128.6, 127.0, 126.7, 126.5 (3C), 126.3, 125.8, 125.0, 124.6, 124.1 (2C), 123.9, 68.1, 21.7; FTIR (KBr) ν_{max} (cm^{-1}): 1719 (OCO), 1637 (CO); UV/vis (EtOH): λ_{max} (ε) 356 (20.9×10^3), 400 (6.2×10^3); LCMS: *m*/*z* (%): 379.4 (100) $[M+H]^+$, 177.4 (15), 119.2 (17); HRMS (ES^+) *m*/*z* calcd for $C_{26}H_{19}O_3$ $[M+H]^+$: 379.1334; found 379.1334.

*4.2.5. 2-Oxo-2-(pyren-3-yl)ethyl 4-methoxybenzoate (**4e**).* 1-(Bromoacetyl)pyrene (0.100 g, 0.29 mmol), potassium iodide (0.057 g, 0.34 mmol), potassium carbonate (0.048 g, 0.34 mmol), and 4-methoxy benzoic acid (0.044 g, 0.29 mmol) were used. The reaction mixture was stirred for 8 h. A reddish-brown residue was obtained, which on purification using 25% EtOAc in pet. ether gave compound **4e** (0.105 g, 92%) as a yellow solid, mp: 137 °C; TLC R_f 0.45 (20% EtOAc in pet. ether); ^{1}H NMR ($CDCl_3$, 200 MHz): δ=9.02 (d, *J*=9.4 Hz, 1H), 8.37 (d, *J*=8.2 Hz, 1H), 8.27–7.90 (m, 9H), 6.95 (d, *J*=8.8 Hz, 2H), 5.68 (s, 2H), 3.87 (s, 3H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ=196.5, 165.9, 163.7, 134.3, 132.1 (2C), 131.0, 130.5, 130.0, 129.9, 128.6, 127.0, 126.5 (2C), 126.3, 125.8, 125.0, 124.6, 124.1, 123.9 (2C), 121.8, 113.8 (2C), 68.0, 55.4; FTIR (KBr) ν_{max} (cm^{-1}): 1717 (OCO), 1638 (CO); UV/vis (EtOH): λ_{max} (ε) 356 (21.0×10^3), 400 (6.3×10^3); LCMS: *m*/*z* (%): 395.1 (100) $[M+H]^+$, 193.1 (12), 135.0(18); HRMS (ES^+) *m*/*z* calcd for $C_{26}H_{19}O_4$ $[M+H]^+$: 395.1283; found 395.1274.

*4.2.6. 2-Oxo-2-(pyren-3-yl)ethyl 2-bromobenzoate (**4f**).* 1-(Bromoacetyl)pyrene (0.100 g, 0.29 mmol), potassium iodide (0.057 g, 0.34 mmol), potassium carbonate (0.048 g, 0.34 mmol), and 2-bromo benzoic acid (0.058 g, 0.29 mmol) were used. The reaction mixture was stirred for 12 h. Purification of the crude residue by column chromatography, using EtOAc/pet. ether mixtures of increasing polarity as the eluent gave compound **4f** (0.089 g, 70%) as a yellow solid, mp: 128 °C; TLC R_f 0.50 (20% EtOAc in pet. ether); ^{1}H NMR ($CDCl_3$, 200 MHz): δ=9.03 (d, *J*=9.4 Hz, 1H), 8.37 (d, *J*=8.2 Hz, 1H), 8.29–8.04 (m, 8H), 7.71 (t, *J*=7.2 Hz, 1H), 7.47–7.29 (m, 2H), 5.76 (s, 2H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ=195.6, 165.6, 134.4 (2C), 132.95, 132.0, 131.4, 131.0, 130.5, 130.2, 130.1, 129.6, 128.3, 127.2, 127.0, 126.5, 126.4, 125.8, 125.0, 124.5, 124.1, 123.9 (2C), 122.1, 68.4; FTIR (KBr) ν_{max} (cm^{-1}): 1734 (OCO), 1677 (CO); UV/vis (EtOH): λ_{max} (ε) 360 (20.4×10^3), 400 (6.7×10^3); LCMS: *m*/*z* (%): 445.2 (98), 443.1 (100) $[M+H]^+$, 229.1 (36), 242.8 (8), 240.8 (5); HRMS (ES^+) *m*/*z* calcd for $C_{25}H_{16}BrO_3$ $[M+H]^+$: 443.0283; found 443.0291.

*4.2.7. 2-Oxo-2-(pyren-3-yl)ethyl 4-chlorobenzoate (**4g**).* 1-(Bromoacetyl)pyrene(0.100 g, 0.29 mmol), 4-chloro benzoic acid (0.039 g, 0.29 mmol), potassium carbonate (0.048 g, 0.34 mmol), and potassium iodide (0.057 g, 0.34 mmol) were used. The reaction mixture was stirred for 10 h. A brown residue was obtained, which on purification using 20% EtOAc in pet. ether affords compound **4g** (0.096 g, 83%) as a yellow solid, mp: 125 °C; TLC R_f 0.55 (20% EtOAc in pet. ether); ^{1}H NMR ($CDCl_3$, 200 MHz): δ=9.05 (d, *J*=9.4 Hz, 1H), 8.36 (d, *J*=8.2 Hz, 1H), 8.29–7.96 (m, 9H), 7.38 (d, *J*=6.2 Hz, 2H), 5.74 (s, 2H); ^{13}C NMR (CDCl3, 50 MHz): δ=195.7, 165.4, 139.8, 134.4, 131.4 (2C), 130.9, 130.4, 130.2, 130.0, 129.2, 128.8 (2C), 128.2, 127.9, 126.9, 126.6, 126.5, 126.4, 125.8, 125.0, 124.5, 124.0, 123.9, 68.3; FTIR (KBr) ν_{max} (cm^{-1}): 1723 (OCO), 1701 (CO); UV/vis (EtOH): λ_{max} (ε) 356 (20.9×10^3), 400 (6.7×10^3); LCMS: *m*/*z* (%): 401.6 (44), 399.4 (100)

$[M+H]^+$, 229.4 (43), 197.2 (14), 141.0 (9); HRMS (ES^+) *m/z* calcd for $C_{25}H_{16}ClO_3$ $[M+H]^+$: 399.0788; found 399.0787.

4.3. General procedure for the synthesis of compound 4h–n

1-(Bromoacetyl)pyrene (1 equiv) was dissolved in dry dioxan and the corresponding Boc-protected amino acid **3h–n** (1 equiv) was added followed by 1.2 equiv of 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU) at 0 °C. The reaction mixture was stirred at room temperature for 8–12 h. The solvent was removed by rotary evaporation under reduced pressure and the crude residue was purified by column chromatography with EtOAc in pet. ether as an eluent.

*4.3.1. tert-Butyl 3-((2-oxo-2-(pyren-3-yl)ethoxy)carbonyl)propylcarbamate (**4h**).* 1-(Bromoacetyl) pyrene (0.100 g, 0.29 mmol), 4-(*tert*-butoxycarbonylamino)butyric acid (0.059 g, 0.29 mmol), and DBU (0.053 g, 0.35 mmol) were used. The reaction mixture was stirred for 10 h. A deep brown semi-solid residue obtained, which on purification by column chromatography using 30% EtOAc in pet. ether gave the ester conjugate **4h** (0.103 g, 80%) as a pale yellow solid, mp: 148 °C; TLC R_f 0.31 (25% EtOAc in pet. ether); ^1H NMR ($CDCl_3$, 200 MHz): δ=8.96 (d, *J*=9.4 Hz, 1H), 8.29–8.02 (m, 8H), 5.49 (s, 2H), 4.81 (s, NH), 3.24 (q, *J*=6.4 Hz, 2H), 2.59 (t, *J*=7.4 Hz, 2H), 1.93 (m, 2H), 1.45 (s, 9H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ=196.3, 173.0, 155.9, 134.5, 131.1, 130.6, 130.3, 130.2 (2C), 128.5, 127.1, 126.7, 126.5, 125.9, 125.0, 124.6, 124.2 (2C), 124.1, 79.9, 67.9, 40.0, 31.3, 28.6 (3C), 25.4; FTIR (KBr) ν_{max} (cm^{-1}): 3365 (NH), 1737 (OCO), 1684 (CO), 1653 (CONH); UV/vis (EtOH): λ_{max} (ε) 354 (20.7×10^3), 400 (7.0×10^3); LCMS: *m/z* (%): 446.2 (56) $[M+H]^+$, 390.4 (18), 346.4 (100), 261.2 (53); HRMS (ES^+) *m/z* calcd for $C_{27}H_{27}NO_5$ $[M]^+$: 445.1889; found: 445.1908.

*4.3.2. tert-Butyl ((2-oxo-2-(pyren-3-yl)ethoxy)carbonyl)methylcarbamate (**4i**).* 1-(Bromoacetyl) pyrene (0.100 g. 0.29 mmol), *N*-(*tert*-butoxycarbonyl)glycine (0.051 g, 0.29 mmol), and DBU (0.053 g, 0.35 mmol) were used. The reaction mixture was stirred for 10 h. Crude reaction mixture was purified by column chromatography using 35% EtOAc in pet. ether to give compound **4i** (0.092 mg, 76%) as a light yellow solid, mp: 141 °C; TLC R_f 0.32 (25% EtOAc in pet. ether); ^1H NMR ($CDCl_3$, 200 MHz): δ=8.92 (d, *J*=9.4 Hz, 1H), 8.19–7.89 (m, 8H), 5.51 (s, 2H), 5.214 (s, NH), 4.21 (d, *J*=5.4 Hz, 2H), 1.51 (s, 9H); ^{13}C NMR (CDCl3, 50 MHz): δ=195.2, 170.3, 155.8, 134.5, 130.9, 130.4, 130.2, 130.1 (2C), 127.7, 126.9, 126.7, 126.6, 126.4, 125.7, 124.9, 124.4, 123.9 (2C), 80.2, 68.2, 42.4, 18.4 (3C); FTIR (KBr) ν_{max} (cm^{-1}): 3364 (NH), 1733 (OCO), 1695 (CO); UV/vis (EtOH): λ_{max} (ε) 355 (20.4×10^3), 400 (6.7×10^3); LCMS: *m/z* (%): 440.1 (100) $[M+Na]^+$, 385.0 (6), 384.1 (25), 362.1 (12), 318.0 (11), 261.1 (11), 130.0 (5); HRMS (ES^+) *m/z* calcd for $C_{25}H_{23}NO_5$ $[M]^+$: 417.1576; found: 417.1583.

*4.3.3. tert-Butyl (S)-1-((2-oxo-2-(pyren-3-yl)ethoxy)carbonyl)ethylcarbamate (**4j**).* 1-(Bromoacetyl) pyrene (0.100 g, 0.29 mmol), *N*-(*tert*-butoxycarbonyl)-L-alanine, and DBU (0.053 g, 0.35 mmol) were used. The reaction mixture was stirred for 12 h. A semi-solid residue obtained, which on purification by column chromatography using 30% EtOAc in pet. ether gave compound **4j** (0.103 mg, 82%) as a brown solid, mp: 129 °C; TLC R_f 0.46 (25% EtOAc in pet. ether); ^1H NMR ($CDCl_3$, 200 MHz): δ 8.98 (d, *J*=9.4 Hz, 1H), 8.32–8.05 (m, 8H), 5.68 (d, *J*=16.4 Hz, 1H), 5.42 (d, *J*=16.2 Hz, 1H), 5.09 (s, 1NH), 4.54 (m, 1H), 1.50 (d, *J*=9.0 Hz, 3H), 1.46 (s, 9H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ=195.4, 173.2, 155.2, 134.4, 130.9, 130.4, 130.2, 130.1, 128.0, 126.9, 126.6, 126.5, 126.4, 125.7, 124.9, 124.4, 124.0, 123.9 (2C), 79.9, 68.1, 49.3, 28.4 (3C), 18.8; FTIR (KBr) ν_{max} (cm^{-1}): 3387 (NH), 1761 (OCO), 1717 (CO), 1683 (CONH); UV/vis (EtOH): λ_{max} (ε) 356 (20.4×10^3), 400 (6.3×10^3); LCMS: *m/z* (%): 432.2 (55) $[M+H]^+$, 376.4 (91), 332.2 (100), 261.2 (35); HRMS (ES^+) *m/z* calcd for $C_{26}H_{25}NO_5$ $[M]^+$: 431.1733; found: 431.1741.

*4.3.4. tert-Butyl(S)-1-((2-oxo-2-(pyren-3-yl)ethoxy)carbonyl)-2-phenylethyl carbamate (**4k**).* 1-(Bromoacetyl)pyrene (0.100 g. 0.29 mmol), *N*-(*tert*-butoxycarbonyl)-L-phenylalanine (0.147 g, 0.29 mmol), and DBU (0.053 g, 0.35 mmol) were used. The reaction mixture was stirred for 12 h. The crude reaction mixture was purified by column chromatography using 25% EtOAc in pet. ether to give ester conjugate **4k** (0.110 mg, 75%) as a deep yellow solid, mp: 139 °C; TLC R_f 0. 54 (25% EtOAc in pet. ether); ^1H NMR ($CDCl_3$, 400 MHz): δ=8.97 (d, *J*=9.4 Hz, 1H), 8.21–8.14 (m, 4H), 8.09–7.95 (m, 4H), 7.306 (s, 5H), 5.63 (d, *J*=16.4 Hz, 1H), 5.43 (d, *J*=16.4 Hz, 1H), 5.09 (d, *J*=8.2 Hz, 1H), 4.84 (m, 1H), 3.42 (dd, *J*=14.0 Hz, 5.2 Hz, 1H), 3.19 (dd, *J*=14.0 Hz, 7.2 Hz, 1H), 1.43 (s, 9H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ=195.5, 171.9, 155.4, 136.3, 134.6, 131.1, 130.6, 130.4, 130.2, 129.7 (2C), 128.7 (2C), 128.1, 127.1, 127.0, 126.8, 126.7, 126.6, 125.9, 125.0, 124.5, 124.2, 124.0 (2C), 80.2, 68.3, 54.6, 38.4, 28.5 (3C); FTIR (KBr) ν_{max} (cm^{-1}): 3371 (NH), 1756 (OCO), 1684 (CO), 1595 (CONH); UV/vis (EtOH): λ_{max} (ε) 356 (20.5×10^3), 400 (6.5×10^3); LCMS: *m/z* (%): 530.1 (100) $[M+Na]^+$, 474.1 (12), 302.0 (16), 292.0 (8), 260.9 (6), 130.1 (12), 102.0 (11); HRMS (ES^+) *m/z* calcd for $C_{32}H_{29}NO_5$ $[M]^+$: 507.2046; found: 507.2050.

*4.3.5. tert-Butyl(S)-1-((2-oxo-2-(pyren-3-yl)ethoxy)carbonyl)-2-hydroxyethyl carbamate (**4l**).* 1-(Bromoacetyl)pyrene (0.100 g. 0.29 mmol), *N*-(*tert*-butoxycarbonyl)-L-serine (0.059 g, 0.29 mmol), and DBU (0.053 g, 0.35 mmol) were used. The reaction mixture was stirred for 10 h. A dark brown liquid obtained, which on purification by column chromatography using 50% EtOAc in pet. ether gave ester conjugate **4l** (0.102 mg, 79%) as a brown viscous liquid, which on keeping in refrigerator for 24 h gives a brown solid, mp: 99 °C; TLC R_f 0.57 (50% EtOAc in pet. ether); ^1H NMR ($CDCl_3$, 200 MHz): δ=8.97 (d, *J*=9.4 Hz, 1H), 8.22–7.85 (m, 8H), 5.89 (d, *H*=16.8 Hz, 1H), 5.82 (d, *J*=9.4 Hz, 1H), 5.36(d, *J*=12.0 Hz, 1H), 4.48 (d, *J*=10.0 Hz, NH), 4.16 (d, *J*=7.2 Hz, 1H), 4.04 (dd, *J*=11.8 Hz, 3.2 Hz, 1H), 3.65 (s, OH), 1.55 (s, 9H); ^{13}C NMR ($CDCl_3$, 50 MHz): 196.0, 171.1, 155.9, 135.0, 130.9, 130.7, 130.6, 130.4, 130.3, 127.0, 126.9, 126.8, 126.4, 126.2, 124.8, 124.4, 123.9 (2C), 123.8, 80.4, 68.3, 64.5, 56.5, 28.6 (3C); FTIR (KBr) ν_{max} (cm^{-1}): 3443 (OH), 2923 (NH), 1752 (OCO), 1690 (CO), 1622 (CONH); UV/vis (EtOH): λ_{max} (ε) 357 (21.2×10^3), 400 (8.4×10^3); LCMS: *m/z* (%): 470.1 (100) $[M+Na]^+$, 414.1 (28), 403.1 (21), 370.1 (14), 302.2 (24), 288.2 (31), 284.2 (26), 215.2 (25); HRMS (ES^+) *m/z* calcd for $C_{25}H_{23}NO_5$ $[M]^+$: 417.1576; found: 417.1576; HRMS (ES^+) *m/z* calcd for $C_{26}H_{25}NO_6$ $[M]^+$: 447.1682; found: 447.1676.

*4.3.6. tert-Butyl (1S,2S)-1-((2-oxo-2-(pyren-3-yl)ethoxy)carbonyl)-2-hydroxypropyl carbamate (**4m**).* 1-(Bromoacetyl)pyrene (0.100 g. 0.29 mmol), *N*-(*tert*-butoxycarbonyl)-L-threonine (0.064 g, 0.29 mmol), and DBU (0.053 g, 0.35 mmol) were used. The reaction mixture was stirred for 8 h. A dark yellowish solid obtained, which on purification by column chromatography using 40% EtOAc in pet. ether gave ester conjugate **4m** (0.110 mg, 82%) as a yellow solid, mp: 76 °C; TLC R_f 0.67 (50% EtOAc in pet. ether); ^1H NMR ($CDCl_3$, 200 MHz): δ=8.87 (d, *J*=9.4 Hz, 1H), 8.12–7.87 (m, 6H), 7.71–7.64 (m, 2H), 5.75 (d, *J*=16.0 Hz, 1H), 5.35 (d, *J*=16.4 Hz, 1H), 5.30 (s, NH), 4.74 (q, *J*=6.0 Hz, 1H), 4.58 (d, *J*=9.6 Hz, 1H), 3.79 (s, OH), 1.52 (s, 9H), 1.42 (d, *J*=6.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ=195.8, 171.4, 156.4, 134.7, 130.7, 130.4, 130.3, 130.1, 126.8, 126.7, 126.5 (3C), 125.9, 124.5, 124.2, 123.6 (2C), 123. 5, 79.9, 68.6, 68.0, 59.5, 28.4 (3C), 18.9; FTIR (KBr) ν_{max} (cm^{-1}): 3449 (OH), 3353 (NH), 1755 (OCO), 1689 (CO), 1624 (CONH); UV/vis (EtOH): λ_{max} (ε) 356 (21.0×10^3), 400 (8.4×10^3); LCMS: *m/z* (%): 462.2 (53) $[M+H]^+$, 406.2 (53), 362.4

(100), 261.2 (9); HRMS (ES^+) m/z calcd for $C_{27}H_{27}NO_6$ $[M]^+$: 461.1838; found: 461.1842.

*4.3.7. 2-Oxo-2-(pyren-3-yl)ethyl 5-oxopyrrolidine-2-carboxylate (**4n**).* 1-(Bromoacetyl)pyrene (0.100 g. 0.29 mmol), pyroglutamic acid (0.037 g, 0.29 mmol), and DBU (0.053 g, 0.35 mmol) were used. The reaction mixture was stirred for 12 h. Crude reaction mixture was purified by column chromatography using 80% EtOAc in pet. ether to give the ester conjugate **4n** (0.092 mg, 86%) as a dark yellow solid, mp: 139 °C; TLC R_f 0.55 (100% EtOAc in pet. ether); ^1H NMR ($CDCl_3$, 200 MHz): δ=8.88 (d, J=9.4 Hz, 1H), 8.23–7.81 (m, 8H), 6.90 (s, NH), 5.56 (d, J=16.2 Hz, 1H), 5.44 (d, J=16.2 Hz, 1H), 4.50 (t, J=5.2 Hz, 1H), 2.61–2.36 (m, 4H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ=194.8, 178.8, 172.1, 134.5, 130.8, 130.3, 130.1 (2C), 130.0, 127.4, 126.9, 126.7, 126.6, 126.5, 125.7, 124.8, 124.3, 123.9 (2C), 68.2, 55.6, 29.3, 25.2; FTIR (KBr) ν_{max} (cm^{-1}): 3222 (NH), 1746 (OCO), 1695 (CO), 1595 (CONH); UV/vis (EtOH): λ_{max} (ε) 357 (20.9×10^3), 400 (8.1×10^3); LCMS: m/z (%): 372.4 (100) $[M+H]^+$, 354.2 (20), 261.0 (31), 243.2 (9), 170.2 (16); HRMS (ES^+) m/z calcd for $C_{23}H_{18}NO_4$ $[M+H]^+$: 372.1236; found: 372.1233.

4.4. Deprotection photolysis

A solution of 10^{-5} M of the conjugate esters (**4a**–**n**) was prepared in acetonitrile/HEPES buffer (50:50, pH=7.5). Half of the solution was kept in dark and to the remaining half nitrogen was passed and irradiated using a 125 W medium pressure Hg lamp filtered by suitable filter with continuous stirring. The photolysate at regular interval of time was taken, then solvent was evaporated under vacuum and redissolved in $CDCl_3$ with 1,2-dichloroethane as the internal standard and ^1H NMR spectra were recorded. The photocleavage of esters were determined by ^1H NMR integration of the ester methylene peak relative to the internal standard. The estimated error is ±5%. The quantum yield of disappearance of the starting ester was analyzed employing potassium ferrioxalate as an actinometer.[32]

4.5. Preparative photolysis

A solution of conjugate esters (indicated in Table 2) (0.05 mmol) in acetonitrile/HEPES (50:50, pH=7.5) was irradiated using a 125 W medium pressure Hg lamp filtered by suitable filter. The irradiation was monitored by TLC at regular interval of time. After completion of photolysis, solvent was removed under vacuum and photoproducts were isolated by column chromatography using EtOAc in hexane as an eluent to give the free carboxylic acid and 1-(hydroxyacetyl)pyrene(**6**). The photoproducts were then weighed, analyzed by ^1H NMR and compared with the authentic sample. When the above photolysis was carried out in methanloic solution we isolated both 1-(methoxyacetyl)pyrene(**5**) and 1-(hydroxyacetyl)pyrene(**6**).

4.5.1. 1-(Methoxyacetyl)pyrene[35]. TLC R_f 0.55 (50% EtOAc in pet. ether), mp 92 °C; ^1H NMR ($CDCl_3$, 200 MHz) 8.98 (d, J=9.2 Hz, 2H), 8.32–8.04 (m, 8H), 4.87 (s, 2H,), 3.59 (s, 3H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ=201.3, 135.2, 131.1, 130.8, 130.6 (2C), 127.2, 127.1 (2C), 126.8 (2C), 126.7, 126.3, 124.7, 124.3 (2C), 124.1, 68.2, 58.3.

4.5.2. 1-(Hydroxyacetyl)pyrene[36]. TLC R_f 0.45 (50% EtOAc in pet. ether), mp 87 °C; ^1H NMR ($CDCl_3$, 200 MHz) δ=9.26 (d, J=9.4 Hz, 1H), 8.05–8.31 (m, 8H), 5.09 (s, 2H, CH_2), 3.83 (s, 1H, OH); ^{13}C NMR ($CDCl_3$, 50 MHz) δ=201.3, 135.2, 131.1, 130.8, 130.6 (2C), 127.2, 127.1 (2C), 126.8 (2C), 126.7, 126.3, 124.7, 124.3 (2C), 124.1, 67.2.

Acknowledgements

We thank DST (SERC Fast Track Scheme) for financial support, DSTFIST for 400 MHz NMR, Central Drug Research Institute, Lucknow for HRMS analysis, Prof. Soumen Hazra for helping with LCMS analysis. We are also grateful to Prof. J. Dey and Prof. N. Sarkar for their valuable suggestions regarding the photophysical behavior of the ester conjugates. Avijit is thankful to CSIR (India) and Sanghamitra is grateful to UGC (India) for their research fellowship.

Supplementary data

Supplementary data related to this article can be found online, at doi:10.1016/j.tet.2010.10.090.

References and notes

1. (a) Pillai, V. N. R. *Synthesis* **1980**, *1*; (b) Greene, T. W.; Wuts, G. M. *Protective Groups in Organic Chemistry*, 3rd ed.; John Wiley: New York, NY, 1999.
2. (a) Abelson, J.; Simon, M.; Marriott, G. *Caged Compounds*; Academic: New York, NY–London, 1998; (b) Nerbonne, J. M. *Curr. Opin. Neurobiol.* **1996**, *6*, 379; (c) Shigeri, Y.; Tatsu, Y.; Yumoto, N. *Pharmacol. Ther.* **2001**, *91*, 85; (d) Pelliccioli, A. P.; Wirz, J. *Photochem. Photobiol. Sci.* **2002**, *1*, 441.
3. Bochet, C. G. *J. Chem. Soc., Perkin Trans. 1* **2002**, 125.
4. (a) Bochet, C. G. *Tetrahedron Lett.* **2000**, *41*, 6341; (b) Schaper, K.; Mobarekah, S. A. M.; Grewer, C. *Eur. J. Org. Chem.* **2002**, 1037.
5. Loudwig, S.; Goeldner, M. *Tetrahedron Lett.* **2001**, *42*, 7957.
6. Peng, L.; Wirz, J.; Goeldner, M. *Tetrahedron Lett.* **1997**, *38*, 2961.
7. (a) Dinkel, C.; Wichmann, O.; Schultz, C. *Tetrahedron Lett.* **2003**, *44*, 1153; (b) Dinkel, C.; Schultz, C. *Tetrahedron Lett.* **2003**, *44*, 1157; (c) Li, W.-H.; Llopis, J.; Whitney, M.; Zlokarnik, G.; Tsien, R. Y. *Nature* **1998**, *392*, 936.
8. (a) Lu, M.; Fedoryak, O. D.; Moister, B. R.; Dore, T. M. *Org. Lett.* **2003**, *5*, 2119; (b) Wang, P.; Hu, H.; Wang, Y. *Org. Lett.* **2007**, *9*, 1533; (c) Yu, J.-Y.; Tang, W.-J.; Wang, H.-B.; Song, Q.-H. *J. Photochem. Photobiol., A* **2007**, *185*, 101; (d) Kantevari, S.; Narasimhaji, C. V.; Mereyala, H. B. *Tetrahedron* **2005**, *61*, 5849.
9. Banerjee, A.; Grewer, C.; Ramakrishnan, L.; Jäger, J.; Gameiro, A.; Breitinger, H.-G. A.; Gee, K. R.; Carpenter, B. K.; Hess, G. P. *J. Org. Chem.* **2003**, *68*, 8361.
10. Singh, A. K.; Khade, P. K. *Tetrahedron* **2005**, *61*, 10007.
11. Grewer, C.; Jäger, J.; Carpenter, B. K.; Hess, G. P. *Biochemistry* **2000**, *39*, 2063.
12. Givens, R. S.; Weber, J. F. W.; Conrad, P. G.; Orosz, G.; Donahue, S. L.; Thayer, S. A. *J. Am. Chem. Soc.* **2000**, *122*, 2687.
13. Ma, C.; Steinmetz, M. G.; Kopatz, E. J.; Rathore, R. *J. Org. Chem.* **2005**, *70*, 4431.
14. Papageorgiou, G.; Ogden, D. C.; Barth, A.; Corrie, J. E. T. *J. Am. Chem. Soc.* **1999**, *121*, 6503.
15. Singh, A. K.; Khade, P. K. *Tetrahedron Lett.* **2005**, *46*, 5563.
16. (a) Zhu, Y.; Pavlos, C. M.; Toscano, J. P.; Dore, T. M. *J. Am. Chem. Soc.* **2006**, *128*, 4267; (b) Davis, M. J.; Kragor, C. H.; Reddie, K. G.; Wilson, H. C.; Zhu, Y.; Dore, T. M. *J. Org. Chem.* **2009**, *74*, 1721; (c) Li, Y. M.; Shi, J.; Cai, R.; Chen, X. Y.; Guo, Q. X.; Liu, L. *Tetrahedron Lett.* **2010**, *51*, 1609; (d) Li, Y. M.; Shi, J.; Caia, R.; Chen, X. Y.; Luo, Z. F.; Guo, Q. X. *J. Photochem. Photobiol., A* **2010**, *211*, 129.
17. (a) Shembekar, V. R.; Chen, Y.; Carpenter, B. K.; Hess, G. P. *Biochemistry* **2005**, *44*, 7107; (b) Hagen, V.; Dekowski, B.; Kotzur, N.; Lechler, R.; Wiesner, B.; Briand, B.; Beyermann, M. *Chem.—Eur. J.* **2008**, *14*, 1621; (c) Piloto, A. M.; Rovira, D.; Costa, S. P. G.; Gonçalves, M. S. T. *Tetrahedron* **2006**, *62*, 11955; (d) Fonseca, A. S. C.; Gonçalves, M. S. T.; Costa, S. P. G. *Tetrahedron* **2007**, *63*, 1353.
18. (a) Schade, B.; Hagen, V.; Schmidt, R.; Herbrich, R.; Krause, E.; Eckardt, T.; Bendig, J. *J. Org. Chem.* **1999**, *64*, 9109; (b) Hagen, V.; Frings, S.; Bendig, J.; Lorenz, D.; Wiesner, B.; Kaupp, U. B. *Angew. Chem., Int. Ed.* **2002**, *41*, 3625; (c) Welch, M. B.; Martinez, C. I.; Zhang, A. J.; Jin, S.; Gibbs, R.; Burgess, K. *Chem.—Eur. J.* **1999**, *5*, 951; (d) Eckardt, T.; Hagen, V.; Schade, B.; Schmidt, R.; Schweitzer, C.; Bending, J. *J. Org. Chem.* **2002**, *67*, 703; (e) Politz, J. C. *Trends Cell Biol.* **1999**, *9*, 284; (f) Muller, C.; Even, P.; Viriot, M.-L.; Carre, M.-C. *Helv. Chim. Acta* **2001**, *84*, 3753; (g) McGall, G. H.; Barone, A. D.; Diggelmann, M.; Fodor, S. P. A.; Gentalen, E.; Ngo, N. *J. Am. Chem. Soc.* **1997**, *119*, 5081.
19. (a) Muller, C.; Even, P.; Viriot, M.-L.; Carré, M.-C. *Helv. Chim. Acta* **2001**, *84*, 3735; (b) Tang, X. J.; Dmochowski, I. J. *Org. Lett.* **2005**, *7*, 279; (c) Vasquez, M. E.; Nitz, M.; Stehn, J.; Yaffe, M. B.; Imperiali, B. *J. Am. Chem. Soc.* **2003**, *125*, 10150.
20. (a) Canepari, M.; Nelson, L.; Papageorgiou, G.; Corrie, J. E. T.; Ogden, D. *J. Neurosci. Methods* **2001**, *112*, 29; (b) Curten, B.; Kullmann, H. M. P.; Bier, M. E.; Kandler, K.; Schmidt, B. F. *Photochem. Photobiol.* **2005**, *81*, 641.
21. Fernandes, M. J. G.; Goncalves, M. S. T.; Costa, S. P. G. *Tetrahedron* **2007**, *63*, 10133.
22. (a) Shah, A. J.; Crespi, F.; Heidbreder, C. *J. Chromatogr., B* **2002**, *781*, 151; (b) Schwartz, L. J.; Patterson, G. H. *Methods Cell Biol.* **2008**, *85*, 45.
23. Ravaglia, G.; Forti, P.; Maioli, F.; Bianchi, G.; Martelli, M.; Talerico, T.; Servadei, L.; Zoli, M.; Mariani, E. *Am. J. Clin. Nutr.* **2004**, *80*, 483.
24. Furuta, T.; Hirayama, Y.; Iwamura, M. *Org. Lett.* **2001**, *3*, 1809.
25. Iwamura, M.; Hodota, C.; Ishibashi, M. *Synlett* **1991**, 35.
26. Iwamura, M.; Ishikawa, T.; Koyama, Y.; Sakuma, K.; Iwamura, H. *Tetrahedron Lett.* **1987**, *28*, 679.
27. Furuta, T.; Torigai, H.; Osawa, T.; Iwamura, M. *Chem. Lett.* **1993**, 1179.

28. Okada, S.; Yamashita, S.; Furuta, T.; Iwamura, M. *Photochem. Photobiol.* **1995**, *61*, 431.
29. Armbruster, C.; Knapp, M.; Rechthaler, K.; Schamschule, R.; Parusel, A. B. J.; Köhler, G.; Wehrmann, W. *J. Photochem. Photobiol., A* **1999**, *125*, 29.
30. Szczubiałka, K.; Moczek, Ł.; Goliszek, A.; Nowakowska, M.; Kotzev, A.; Laschewsky, A. *J. Fluorine Chem.* **2005**, *126*, 1409.
31. (a) Bayliss, N. S.; McRae, E. G. *J. Phys. Chem.* **1954**, *58*, 1002; (b) Brealey, G. C.; Kasha, M. *J. Am. Chem. Soc.* **1955**, *77*, 4462; (c) Kalyanasundaram, K.; Thomas, J. K. *J. Phys. Chem.* **1977**, *81*, 2176.
32. Endo, A.; Suzuki, K.; Yoshihara, T.; Tobita, S.; Yahiro, M.; Adachi, C. *Chem. Phys. Lett.* **2008**, *155*, 460.
33. Bandyopadhyay, P.; Bharadwaj, P. K.; Basu Roy, M.; Dutta, R.; Ghosh, S. *Chem. Phys.* **2000**, *255*, 325.
34. Rabek, J. F. *Radiometry and Actinometry: Experimental Methods in Photochemistry and Photophysics*; Wiley: New York, NY, 1982; Vol. 2; p 944.
35. Magnus, P.; Roy, G. *Organometallics* **1982**, *1*, 553.
36. Wong, F. F.; Chang, P. W.; Lin, H. C.; You, B. J.; Huang, J. J.; Lin, S. K. *J. Organomet. Chem.* **2009**, *694*, 3452.