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Anthracene-9-methanol—a novel fluorescent phototrigger for biomolecular caging

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Abstract—Photoexcitation of a solution of anthracene-9-methanol derived esters at ~386 nm in CH_3CN/H_2O (3:2 v/v) results in fluorescence emission in the 380–480 nm range, with quantum yields of fluorescence (Φ_f) in the 0.01–0.09 range and releases of the carboxylic acids in good chemical yields (43–100%), with quantum yields of photoreaction (Φ_{PR}, i.e., the photodisappearance of the esters) in the 0.067–0.426 range.

Photochemical release of bioactive components from a 'caged molecule' is an important strategy in the study of numerous processes in biology, medicine and materials science.[1–3] The prerequisites for a chromophore to act as a phototrigger in caging applications include photoactivatability of the chromophore under physiological conditions and at biologically acceptable wavelengths, with a high rate and quantum yield of the photoreaction, etc. Many phototriggers such as 2-nitrobenzyl, benzoin, phenacyl, etc. have been synthesised and utilised in the caging of molecules.[1c] Recently, polycyclic aromatics have been found to act as good phototriggering groups for alcohols, amines, peptides, phosphates and carboxylic acids. Some of the known polycyclic aromatics are anthraquinon-2-ylmethoxycarbonyl[4] (Aqmoc, λ_{max} 327 nm), pyren-1-ylmethoxycarbonyl[5] (Pmoc, λ_{max} 323 nm), 7-methoxycoumarin-4-ylmethoxycarbonyl[6a,b] (Mmoc, λ_{max} 343 nm) and phenanthren-9-ylmethoxycarbonyl[4] (Phmoc, λ_{max} 297 nm). Attempts to synthesise either fluorescent phototriggers or to convert a photolabile protecting group into fluorescence phototriggers are limited.[5,6] Fluorescent phototriggers have a few advantages over photocleavable protecting groups. They may be useful in monitoring changes in concentration and thus allow tracing of the location of 'caged molecules' inside living cells by the fluorescent microscope technique.[6,7] Fluorescent phototriggers also allow visualisation of support grafting during in situ synthesis of oligonucleotides and peptides.[8] To the best of our knowledge, fluorescent phototriggers other than coumarin and pyrene are not known so far. However, the caging applications of these phototriggers are restricted as they have low quantum yields, require specific solvents for the photoreaction and generally the photolysis wavelength is not more than 350 nm. Herein, we report a new fluorescent phototrigger, namely anthracene-9-methanol (**1**), which shows improved photochemical properties useful in caging carboxylic acids.

In order to study the phototrigger properties of **1** for carboxylic acids, ester derivatives from five different carboxylic acids, viz *o*-chlorobenzoic acid, hippuric acid, *m*-nitrobenzoic acid, phenylacetic acid and acetic acid were prepared. Ester derivatives **1a–d** were prepared by DCC/DMAP mediated condensation of **1** (1 mmol) with the carboxylic acids in dichloromethane at ambient temperature (Scheme 1).[9] Ester **1e** was prepared by the reaction of **1** (1 mmol) with acetic anhydride (2 mmol) in the presence of DMAP (0.1 mmol) and pyridine (1 mmol) in dichloromethane. All ester derivatives **1a–e** were purified by column chromatography and characterised by melting point, FT-IR, ^1H NMR, ^{13}C NMR and mass spectral analysis.[10]

Solutions (1.0×10^{-3} M) of esters **1a–e** in $CH_3CN–H_2O$ (3:2) showed UV–vis absorptions in the 300–400 nm range with λ_{max} values at ca. 383–386 nm. The

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Keywords: Caging; Anthracene-9-methanol; Esters; Photochemistry; Phototrigger.

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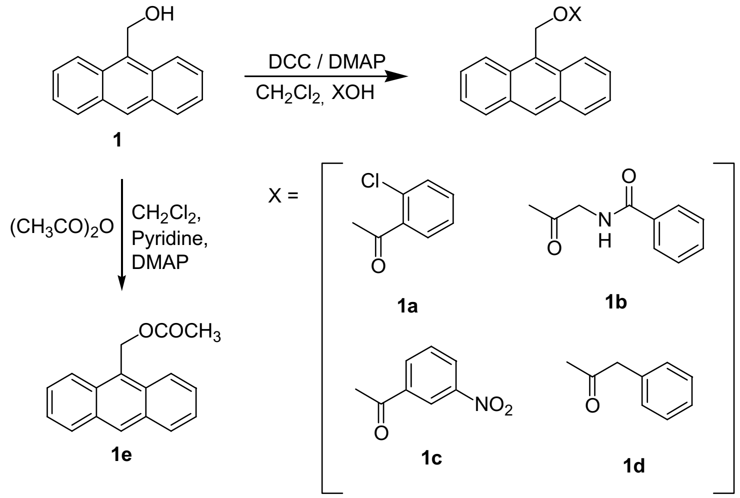
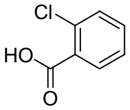
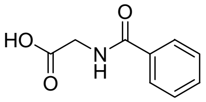
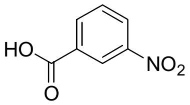
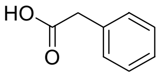
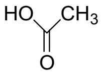


Scheme 1.

Table 1. Photochemical properties of esters **1a**–**e**

Ester	Carboxylic acid	ε (mol^{-1} cm^{-1} L) at 386 nm in 3:2 v/v $CH_3CN–H_2O$	Φ_f (λ_{ex} 386 nm)	Φ_{PR} ($\lambda_{h\upsilon}$ 386 nm)	$h\upsilon$ time[a] (min)	% Photorelease[b]
1a		7590	0.018	0.087	40	100
1b		6705	0.089	0.335	10	51
1c		8572	0.010	0.067	360	68
1d		8220	0.093	0.148	20	43
1e		7292	0.077	0.426	20	ND

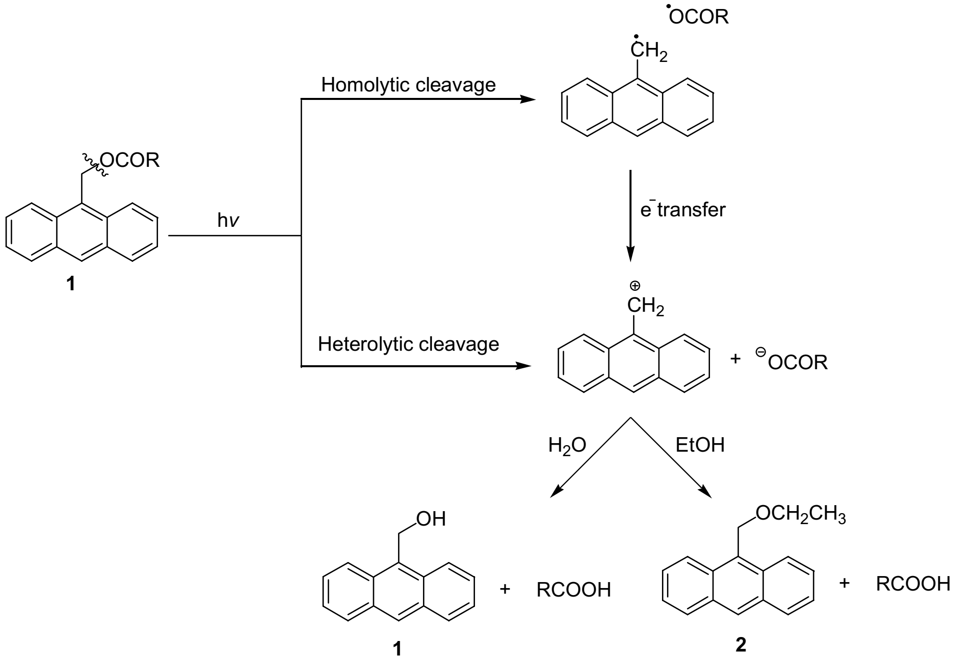
ND: Release of acetic acid could not be detected by HPLC.

[a] Time for complete disappearance of starting esters (1.0×10^{-3} M).

[b] Yield calculated using HPLC.

fluorescence emission spectra of the esters (1.0×10^{-6} M in $CH_3CN–H_2O$, 3:2) are typical of anthracene fluorescence emission with four bands located at 389, 411, 434 and 460 nm. The fluorescence quantum yield (Φ_f) of the esters changes with the nature of the carboxylic acid group.[11] In comparison to **1**(Φ_f, 0.036), the Φ_f is higher for **1b**, **1d** and **1e** and lower for **1a** and **1c** (Table 1). The low Φ_f for **1a** and **1c** could be due to intersystem crossing to the triplet state because of the presence of nitro- and chloro groups in the corresponding esters.

To examine the effect of solvent, a 1.0×10^{-3} M solution of ester **1a** was photolysed in various solvent systems including ethanol, 3:2 (v/v) THF–H_2O, 1,4-dioxane–H_2O and $CH_3CN–H_2O$.[11] Its photolysis in ethanol and $CH_3CN–H_2O$ resulted in 100% release of *o*-chlorobenzoic acid, with the time for complete photodisappearance of **1a** being 75 and 40 min in ethanol and $CH_3CN–H_2O$, respectively. In THF–H_2O and 1,4-dioxane–H_2O systems, the yields, however, were relatively low (95% and 89%, respectively) and the photolysis times were 90 and 60 min, respectively. Thus, the photorelease in $CH_3CN–H_2O$ (3:2 v/v) was found to be better than that found in other solvent systems. Therefore, the $CH_3CN–H_2O$ (3:2 v/v) solvent system was used to study the photochemical properties of esters **1b**–**1e**. The quantum yields of photoreaction (Φ_{PR}, that is, the photodisappearance of the esters **1a**–**e**) were in the range 0.067–0.426 (Table 1). Good photorelease of carboxylic acids was obtained in the cases of **1a** and **1c**. However, with **1b** and **1d**, the photorelease yields were less, which could be due to secondary photoreactions of the esters or of esters with the parent alcohol **1** generated during the photolysis. Minor photo-by-products were detected



Scheme 2.

by HPLC analysis of the photo-mixtures but these by-products could not be characterised. Attempts were made to determine the origin of these photo-by-products and it was found that the majority of the by-products originated from secondary photoreactions of the released parent alcohol **1**. To confirm the secondary photoreaction, photolysis of a 1.0×10^{-3} M solution of **1** in CH_3CN–H_2O (3:2) was performed and it was found that the photoproducts obtained were similar to those of the by-products which were formed during the photolysis of **1a**–**e**.

Chromophore **1** and the released *o*-chlorobenzoic acid were the major photoproducts in the photolysis of **1a** in the CH_3CN–H_2O (3:2) system. However, photolysis of **1a** in ethanol gave 10-(ethoxymethyl) anthracene (**2**) and *o*-chlorobenzoic acid as the major photoproducts. A plausible photochemical mechanism is shown in Scheme 2. Homolytic cleavage of the C–O bond followed by electron transfer can yield an anthryl-9-methylene carbocation, which on nucleophilic attack by the solvent molecule leads to the respective products. Another possible mechanism could involve heterolytic cleavage of the C–O bond, which can directly give the 9-anthryl-methylene carbocation.

Thus, anthracene-9-methanol (**1**) can be an efficient fluorescent phototrigger for molecules containing a carboxyl function. Furthermore, **1** can also be used for caging via other functional groups such as $-NH_2$, $-PO_3H$, –OH, etc.

Acknowledgements

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References and notes

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10. **1a**: Yield 98%; Mp: 122–124 °C; FTIR (KBr) ν_{max} (cm^{-1}): 1736 (OCO); ES-MS: m/z found: 369.091 (M$^+$+Na) calcd for $C_{22}H_{15}O_2Cl$ 369.810 (M$^+$+Na); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.54 (1H, s, Ar-H), 8.44 (d, $J = 8.8$ Hz, 2H, Ar-H), 8.04 (d, $J = 8.4$ Hz, 2H, Ar-H), 7.71 (d, $J = 7.6$ Hz, 1H, Ar-H), 7.60 (t, $J = 6.8$ Hz, 1H, Ar-H), 7.50 (t, $J = 7.6$ Hz, 1H, Ar-H), 7.40 (d, $J = 8$ Hz, 1H, Ar-H), 7.34 (t, $J = 8.8$ Hz, 2H, Ar-H), 7.18 (t, $J = 7.2$ Hz, 2H, Ar-H) and 6.41 (s, 2H, CH_2O); ^{13}C NMR (75 MHz): δ 165.9, 133.9, 132.7, 131.7, 131.4, 131.2, 131.1, 129.8, 129.5, 129.2, 126.8, 126.6, 125.8, 125.2, 124.0 and 59.9. **1b**: Yield 90%; Mp: 182–184 °C; FTIR (KBr) ν_{max} (cm^{-1}): 3329 (NH), 1738 (OCO) and 1632 (NHCO); ES-MS: m/z found:

392.1268 (M^++Na) calcd for $C_{24}H_{19}NO_3$ 392.1263 (M^++Na); ^1H NMR (400 MHz, $CDCl_3$): δ 8.54 (s, 1H, Ar-H), 8.36 (d, J = 10 Hz, 2H, Ar-H), 8.04 (d, J = 8.4 Hz, 2H, Ar-H), 7.77 (d, J = 7.2 Hz, 2H, Ar-H), 7.59 (t, J = 4.8 Hz, 2H, Ar-H), 7.50 (t, J = 7.6 Hz, 2H, Ar-H), 7.43 (t, J = 7.2 Hz, 2H, Ar-H), 6.62 (br s, 2H, NH), 6.30 (s, 2H, CH_2O) and 4.25 (d, J = 4.8 Hz, 2H, CH_2); ^{13}C NMR (100 MHz): δ 170.3, 167.6, 133.8, 131.9, 131.4, 131.1, 129.7, 129.3, 128.7, 127.1, 126.9, 125.3, 123.8, 60.1 and 41.9. **1c**: Yield 81%; Mp: 152–154 °C; FTIR (KBr) ν_{max} (cm^{-1}): 1723 (OCO), 1538 and 1354 (NO_2); GC–MS (% rel int): 380 (M^++Na, 11), 362 (12), 300 (100), 281 (42), 204 (23), 165 (17), 140 (33), 115 (41) and 91 (38); ^1H NMR (300 MHz, $CDCl_3$): δ 8.81 (t, J = 2.4 Hz, 1H, Ar-H), 8.56 (s, 1H, Ar-H), 8.42 (d, J = 9 Hz, 2H, Ar-H), 8.36–8.27 (m, 2H, Ar-H), 8.06 (d, J = 8.4 Hz, 2H, Ar-H), 7.64–7.49 (m, 5H, Ar-H) and 6.46 (s, 2H, CH_2O); ^{13}C NMR (75 MHz): δ 164.8, 135.5, 131.9, 131.5, 131.3, 129.7, 129.6, 129.3, 127.5, 127.0, 125.5, 125.3, 124.8, 123.8 and 60.4. **1d**: Yield 64%; Mp: 112–114 °C; FTIR (KBr) ν_{max} (cm^{-1}): 1744 (OCO); GC–MS (% rel int): 326 (M^+, 17), 191 (100), 165 (6) and 91 (12); ^1H NMR (400 MHz, $CDCl_3$): δ 8.48 (s, 1H, Ar-H), 8.27 (d, J = 8 Hz, 2H, Ar-H), 8.01 (d, J = 8.4 Hz, 2H, Ar-H), 7.78–7.45 (m, 4H, Ar-H), 7.32–7.23 (m, 5H, Ar-H), 6.15 (s, 2H, CH_2O) and 3.62 (s, 2H, CH_2); ^{13}C NMR (75 MHz): δ 171.9, 134.2, 133.9, 131.4, 131.1, 129.4, 129.3, 129.1, 128.6, 127.3, 127.2, 126.7, 126.1, 125.2, 124.0, 59.4 and 41.3. **1e**: Yield 50%; Mp: 108–110 °C; FTIR (KBr) ν_{max} (cm^{-1}): 1723 ($OCOCH_3$); GC–MS (% rel int): 250 (M^+, 33), 191 (100), 165 (7) and 94 (9); ^1H NMR (300 MHz, $CDCl_3$): 8.51 (s, 1H, Ar-H), 8.33 (d, J = 8.8 Hz, 2H, Ar-H), 8.03 (d, J = 8.4 Hz, 2H, Ar-H), 7.58 (t, J = 7.6 Hz, 2H, Ar-H), 7.49 (t, J = 7.5 Hz, 2H, Ar-H), 6.15 (s, 2H, OCH_2) and 2.09 (s, 3H, CH_3); ^{13}C NMR (75 MHz): δ 171.2, 131.3, 131.0, 129.2, 129.1, 126.6, 126.1, 125.1, 123.9, 58.9 and 21.2.

11. Rabek, J. F. Radiometry and Actinometry. In *Experimental Methods in Photochemistry and Photophysics*; Wiley: New York, 1982; Vol. 2, pp 944–946, The Φ_f against anthracene (Φ_f, 0.2 in ethanol) as a standard was determined at ambient temperature. The Φ_{PR} was calculated for ester disappearance at 386 nm, and the number of quanta absorbed were calculated by ferrioxalate actinometry. Photolyses were performed using a 400 W medium pressure Hg lamp and the desired wavelengths were obtained by using a monochromator/>370 nm glass filter having 47% and 96% transmittance at 370 and 420 nm, respectively. The distance between the sample and the lamp was 2 cm. HPLC analyses were performed on a Hitachi instrument consisting of a L-6250 intelligent pump and a U-2000 spectrophotometer under the following conditions: column, ALTEX ODS (5μ, 4.6 mm × 25 cm), solvent, $CH_3CN–H_2O$ (3:2 v/v), flow rate, 0.8 mL/min, detection λ 226 nm.