Supplementary Data

1-(Hydroxyacetyl)pyrene a new fluorescent phototrigger for cell imaging and caging of alcohols, phenol and adenosine

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1. General information

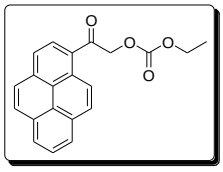
All reagents were purchased from Sigma Aldrich and used without further purification. Acetonitrile and dichloromethane were distilled from CaH_2 before use. ^1H NMR spectra were recorded on a BRUKER-AC 200 MHz spectrometer. Chemical shifts are reported in ppm from tetramethylsilane with the solvent resonance 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 ppm 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, FT-IR 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 caged carbonates were carried out using 125 W medium pressure Hg lamp supplied by SAIC (India). Chromatographic purification was done with 60-120 mesh silica gel (Merck). For reaction monitoring, precoated silica gel 60 F254 TLC sheets (Merck) were used. RP-HPLC was taken using mobile phase acetonitrile, at a flow rate of 1mL / min (detection: UV 254 nm).

2. General procedure for the synthesis of caged carbonates (5a-f):

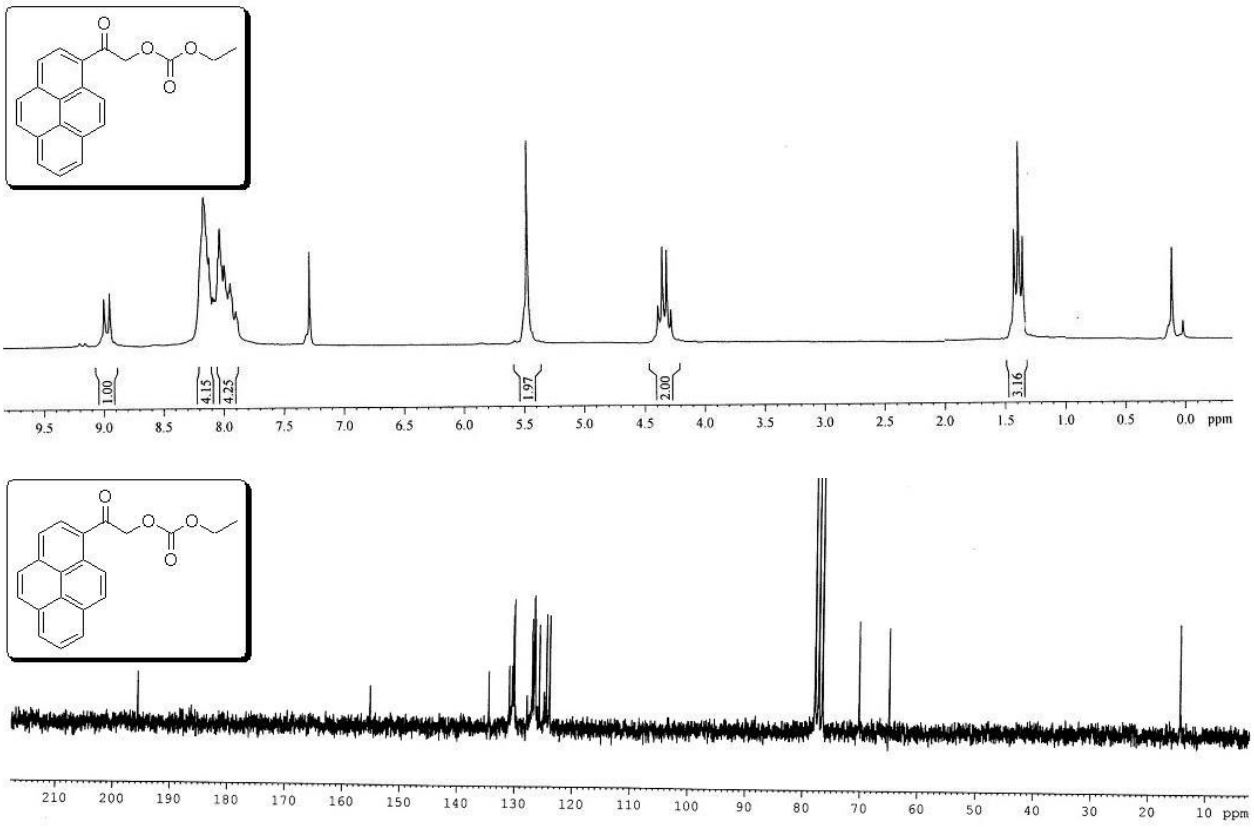
1-(Hydroxyacetyl)pyrene (1 equiv) was dissolved in dry DCM (5 mL), and to the solution corresponding alcohol-chloroformate (1 equiv) was added followed by 1.2 equiv of *N*,*N*-dimethylpyridin-4-amine (DMAP). The reaction mixture was stirred at room temperature for 8–10 hour. The solvent was removed by rotary evaporation under reduced pressure and the crude residue was purified by column chromatography with EtOAc in pet ether as an eluant.

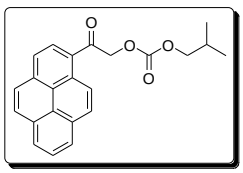
3. Characterization data for caged carbonates (5a-f)

Ethyl 2-oxo-2-(pyren-3-yl)ethyl carbonate (5a) : 1-(Hydroxyacetyl)pyrene (0.130 g, 0.50 mmol), ethyl chloroformate (0.054 g, 0.50 mmol) and DMAP (0.073 g, 0.60



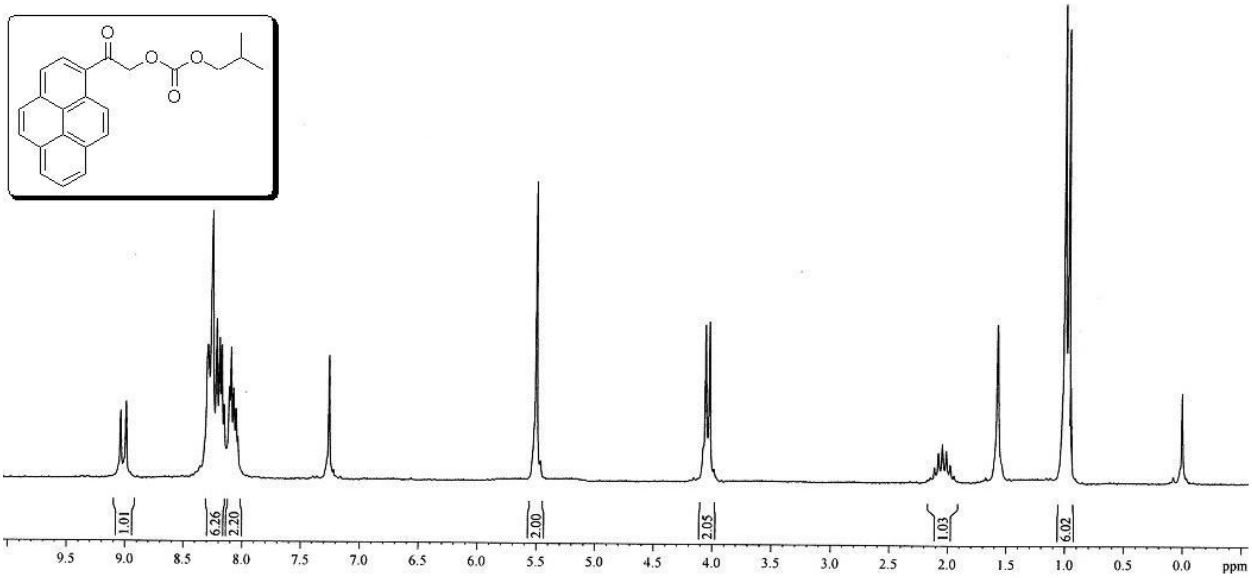
mmol) were used and the reaction mixture was stirred for 8 h at room temperature. The crude reaction mixture was purified by column chromatography using 10% EtOAc in pet ether to give the carbonate **5a** (0.148 g, 89%) as a yellow solid, mp: 104-107 °C; ^{1}H NMR ($CDCl_3$, 200 MHz): δ = 8.97 (d, J = 9.4 Hz, 1H), 8.17-7.90 (m, 8H), 5.48 (s, 2H), 4.39-4.28 (q, J = 7.2 Hz, 2H), 1.39 (t, J = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ = 196.0, 155.2, 134.5, 130.9, 130.5, 130.3, 130.1, 127.9, 127.0, 126.7, 126.6, 126.5, 125.8, 124.9, 124.5, 123.9, 70.2, 64.9, 14.4; $FTIR_{KBr}$ (cm^{-1}): 1750, 1683; HRMS cal. for $C_{21}H_{16}O_4$ = 332.1049, found = 332.1049.

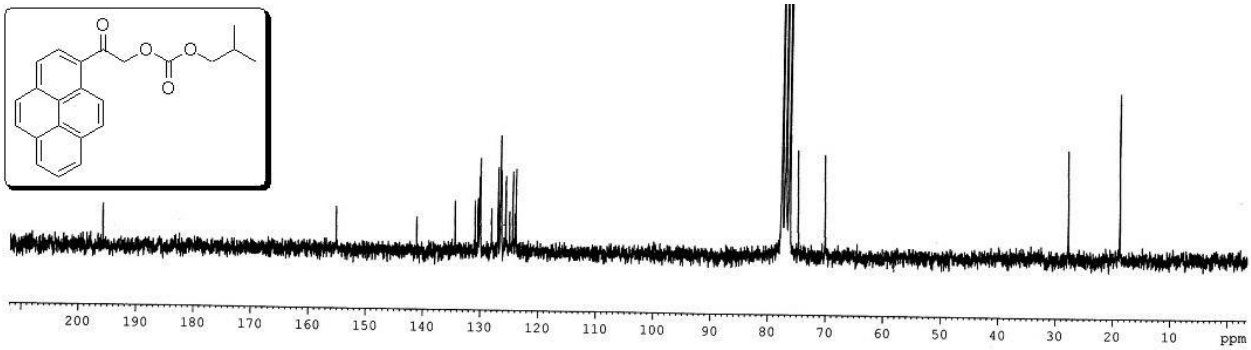


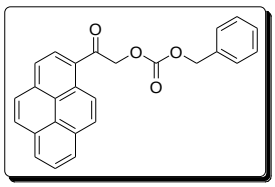


Isobutyl 2-oxo-2-(pyren-3-yl)ethyl carbonate(5b): 1-(Hydroxyacetyl)pyrene (0.130 g, 0.50 mmol), isobutyl chloroformate (0.068 g, 0.50 mmol) and DMAP (0.073 g, 0.60 mmol) were used and the reaction mixture was stirred for 7 h at room temperature. The crude reaction mixture was purified by column chromatography using 10% EtOAc in pet ether to give the carbonate

ester **5b** (0.162 g, 90%) as a yellow solid, mp: 135 °C; ^{1}H NMR ($CDCl_3$, 200 MHz): δ = 9.02 (d, J = 9.6 Hz, 1H), 8.29-8.04 (m, 8H), 5.51 (s, 2H), 4.04 (d, J = 6.6 Hz, 2H), 2.18-1.95 (m, 1H), 1.01 (s, 3H), 0.97 (s, 3H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ = 195.9, 155.3, 134.5, 131.0, 130.5, 130.3, 130.1, 128.2, 127.0, 126.6, 126.5, 125.7, 125.0, 124.5, 123.9, 74.8, 70.1, 27.9, 18.9; $FTIR_{KBr}$ (cm^{-1}): 1749, 1686; HRMS cal. for $C_{23}H_{20}O_4$ = 360.1362, found = 360.1362.

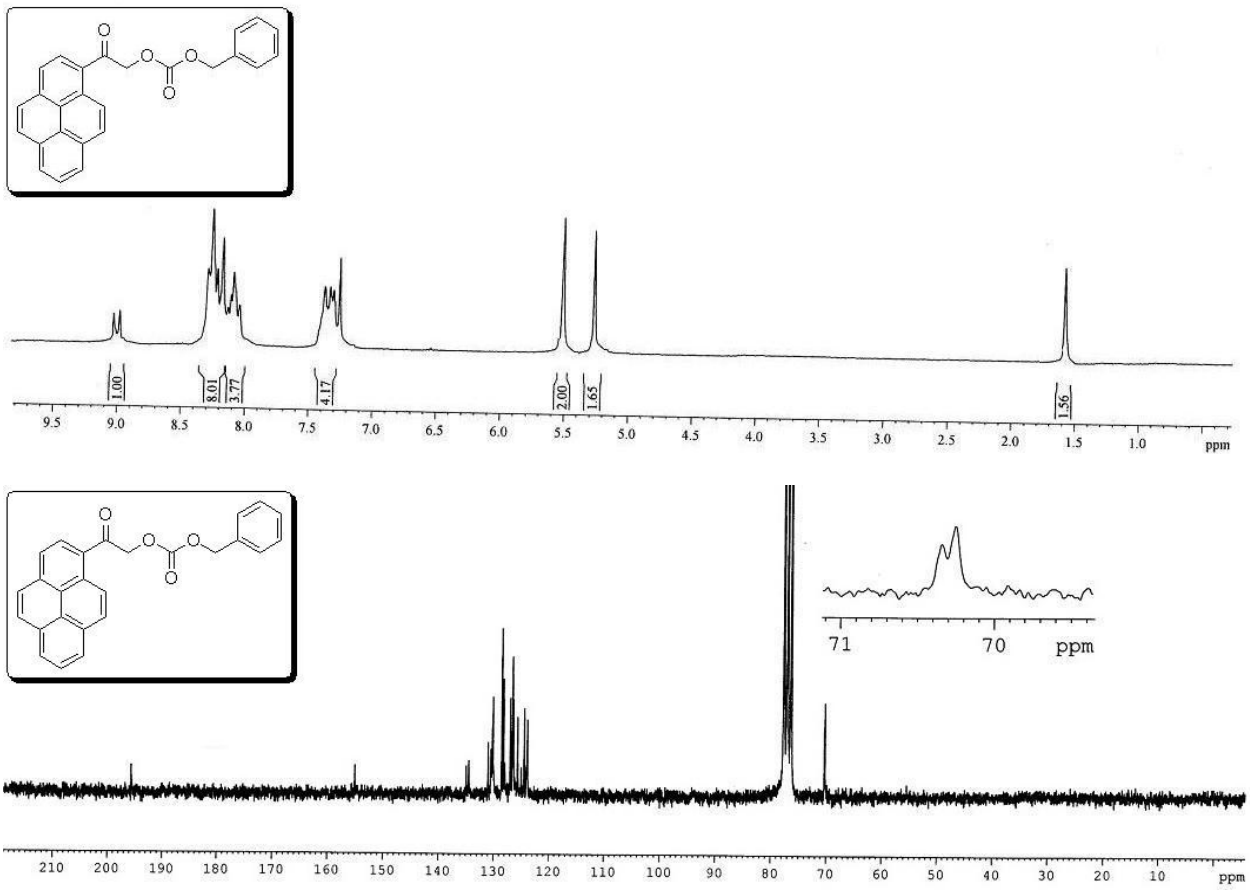


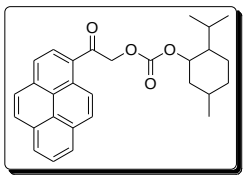




Benzyl 2-oxo-2-(pyren-3-yl)ethyl carbonate(5c): 1-(Hydroxyacetyl)pyrene (0.130 g, 0.50 mmol), benzyl chloroformate (0.085 g, 0.50 mmol) and DMAP (0.073 g, 0.60 mmol) were used and the reaction mixture was stirred for 8 h at room temperature. The crude reaction mixture was purified by column chromatography using 10% EtOAc in pet ether to give the carbonate ester **5c** (0.181 g, 92%) as a yellow solid, mp: 135 °C; ^{1}H NMR ($CDCl_3$, 200

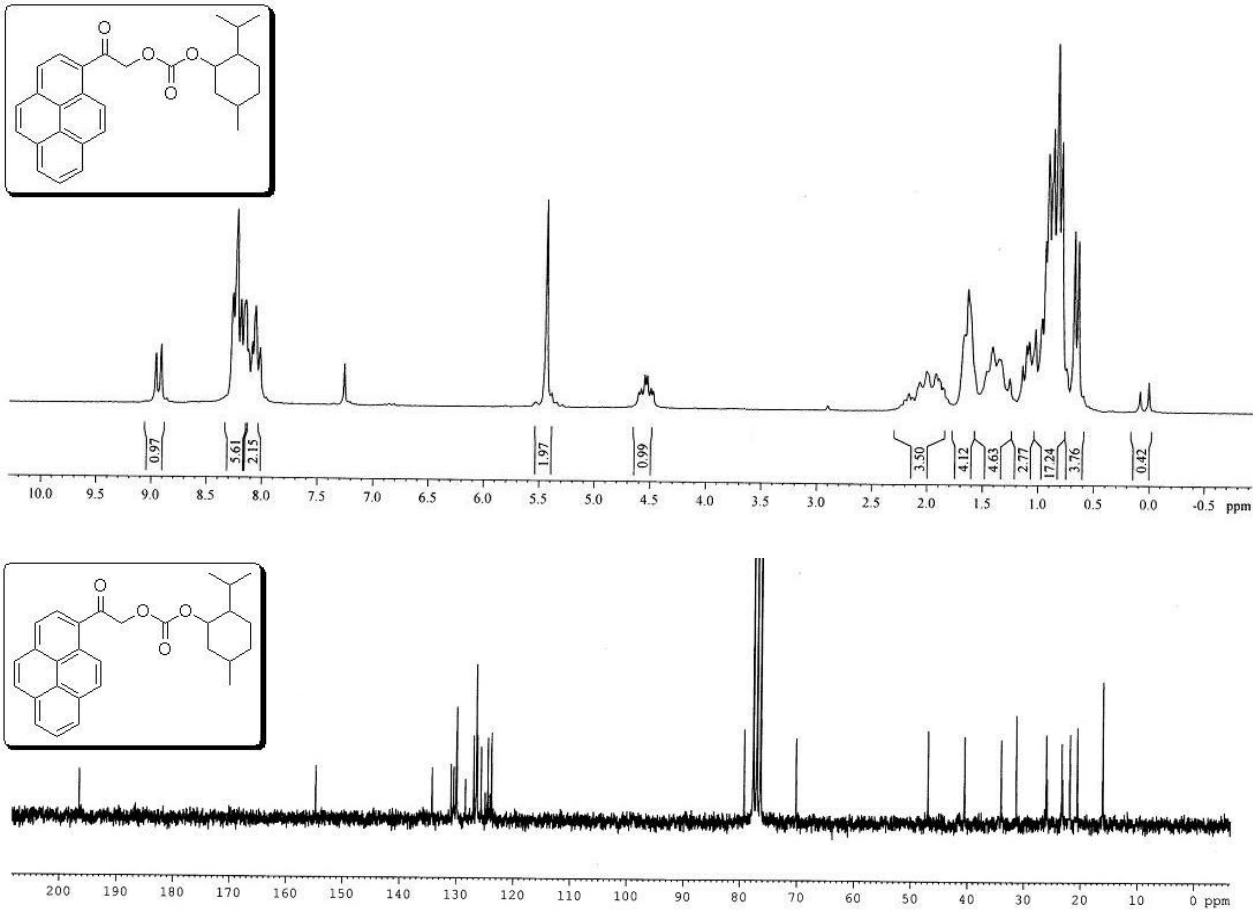
MHz): δ = 9.01 (d, J = 9.4 Hz, 1H), 8.29-8.045 (m, 9H), 7.38-7.31 (m, 4H), 5.51 (s, 2H), 5.27 (s, 2H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ = 195.7, 155.1, 134.9, 134.5, 131.0, 130.5, 130.3, 130.1, 128.6, 128.3, 128.0, 127.0, 126.6, 126.5, 125.7, 125.0, 124.5, 123.9, 70.4, 70.3; $FTIR_{KBr}$ (cm^{-1}): 1774, 1686; HRMS cal. for $C_{26}H_{18}O_4$ = 394.1205, found = 394.1205.

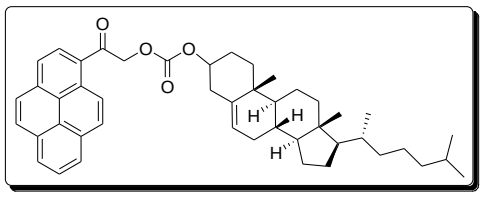




2-isopropyl-5-methylcyclohexyl 2-oxo-2-(pyren-3-yl)ethyl carbonate (5d): 1-(Hydroxyacetyl)pyrene (0.130 g, 0.50 mmol), menthol chloroformate (0.109 g, 0.50 mmol) and DMAP (0.073 g, 0.60 mmol) were used and the reaction mixture was stirred for 8 h at room temperature. The crude reaction mixture was purified by column chromatography using 10% EtOAc in pet ether to

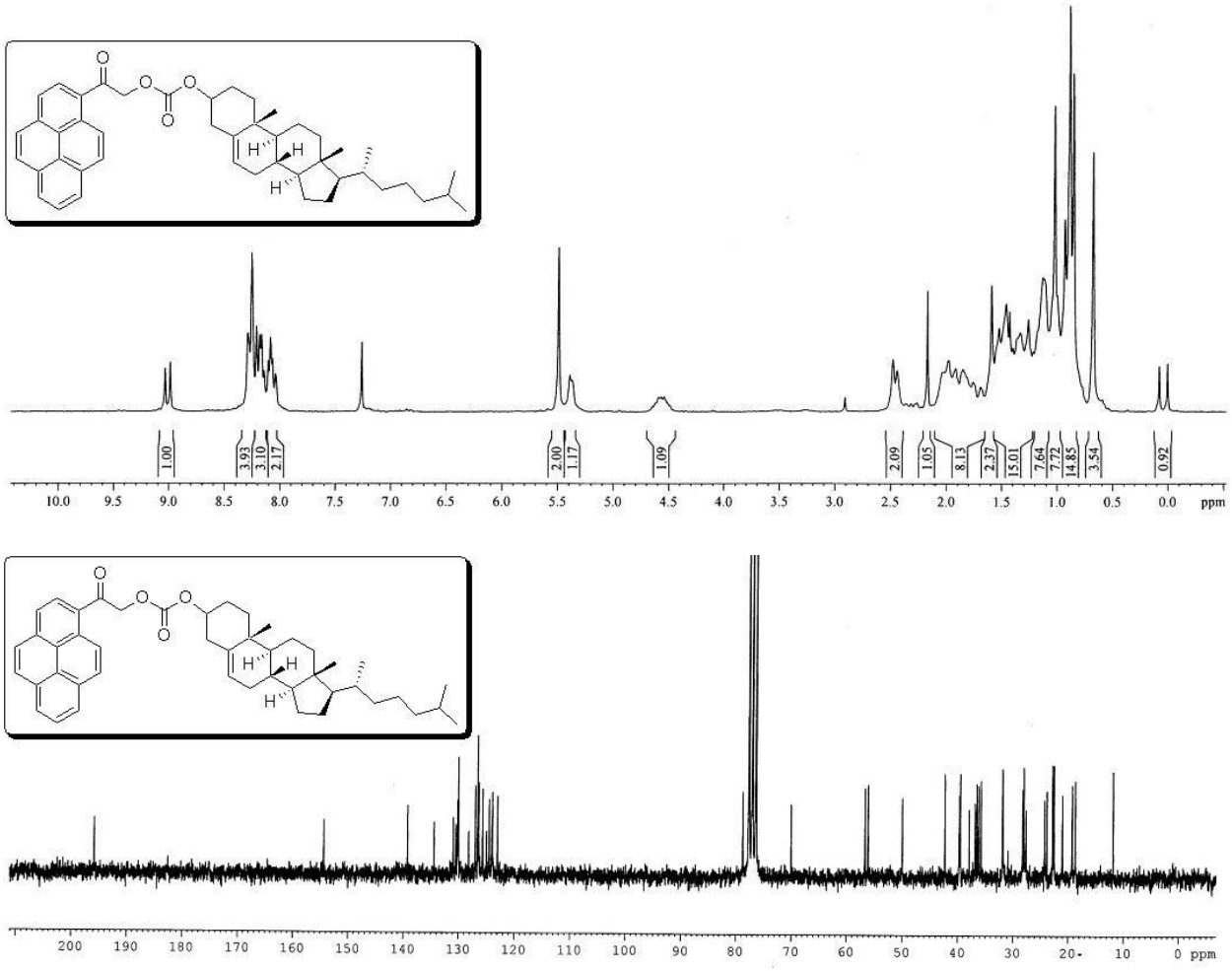
give the carbonate ester **5d** (0.214 g, 97%) as a yellow solid, mp: 125-128 °C; ^{1}H NMR ($CDCl_3$, 200 MHz): δ = 8.94 (d, J = 9.4 Hz, 1H), 8.27-8.02 (m, 8H), 5.44 (s, 2H), 4.60-4.48 (m, 1H), 2.07-0.68 (m, 15H), 0.62 (d, 10.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ = 196.6, 154.8, 134.3, 131.0, 130.5, 130.1, 129.9, 128.5, 127.0, 126.5, 126.4, 125.7, 125.0, 124.5, 124.1, 123.9, 79.2, 70.2, 46.9, 40.5, 34.1, 31.4, 26.0, 21.9, 20.6, 16.1; $FTIR_{KBr}$ (cm^{-1}): 1741, 1707; HRMS cal. for $C_{29}H_{30}O_4$ = 442.2144, found 442.2144.

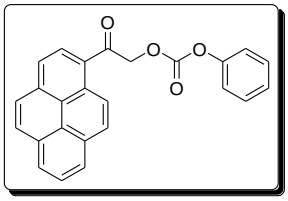




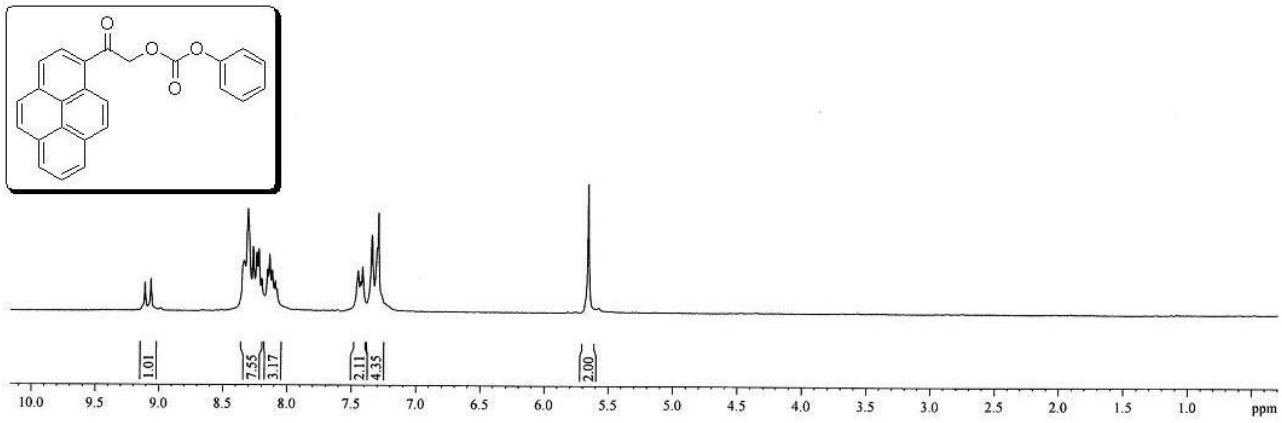
(8R,9R,10S,13S,14R,17S)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-10,13-dimethyl-17-((S)-6-methylheptan-2-yl)-1H-cyclopenta[a]phenanthren-3-yl 2-oxo-2-(pyren-3-yl)ethyl carbonate(5e): 1-(Hydroxyacetyl)pyrene (0.130 g, 0.50 mmol), cholesterol

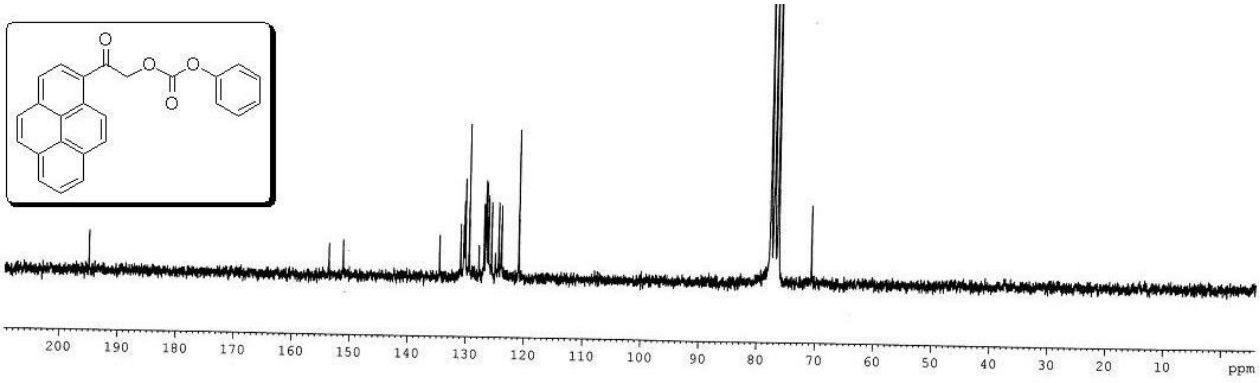
chloroformate (0.225 g, 0.50 mmol) and DMAP (0.073 g, 0.60 mmol) were used and the reaction mixture was stirred for 8 h at room temperature. The crude reaction mixture was purified by column chromatography using 10% EtOAc in pet ether to give the carbonate ester **5e** (0.348 g, 97%) as a yellow solid, mp: 155-158 $^{\circ}$C; ^{1}H NMR ($CDCl_3$, 200 MHz): δ = 9.01 (d, J = 9.4 Hz, 1H), 8.29-8.04 (m, 8H), 5.49 (s, 2H), 5.38 (d, J = 4.0 Hz, 1H), 4.65-4.54 (m, 1H), 2.46 (d, J = 7.4 Hz, 2H), 2.04-0.85 (m, 38H), 0.68 (s, 3H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ = 195.9, 154.4, 139.3, 134.5, 131.0, 130.5, 130.2, 130.1, 128.2, 127.0, 126.6, 126.4, 125.7, 125.0, 124.5, 124.1, 123.9, 123.0, 78.8, 70.1, 56.7, 56.2, 50.0, 42.3, 39.7, 39.5, 37.9, 36.9, 36.6, 36.2, 35.8, 31.9, 28.2, 28.0, 27.6, 24.3, 23.9, 22.8, 22.6, 21.1, 19.3, 18.7; $FTIR_{KBr}$ (cm^{-1}): 1750, 1691; HRMS cal. for $C_{46}H_{56}O_4$ = 672.4179, found 672.4179.





2-oxo-2-(pyren-3-yl)ethyl phenyl carbonate(5f): 1-(Hydroxyacetyl)pyrene (0.130 g, 0.50 mmol), phenyl chloroformate (0.078 g, 0.50 mmol) and DMAP (0.073 g, 0.60 mmol) were used and the reaction mixture was stirred for 8 h at room temperature. The crude reaction mixture was purified by column chromatography using 10% EtOAc in pet ether to give the carbonate ester **5f** (0.169 g, 89%) as a yellow solid, mp: 110-115 $^{\circ}$C; ^{1}H NMR ($CDCl_3$, 200 MHz): δ = 9.08 (d, J = 9.4 Hz, 1H), 8.34-8.08 (m, 9H), 7.45-7.34 (m, 4H), 5.66 (s, 2H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ = 195.0, 153.7, 151.2, 134.6, 131.0, 130.5, 130.4, 130.2, 129.5, 127.8, 127.0, 126.7, 126.6, 126.5, 126.2, 125.7, 125.1, 124.5, 124.0, 121.0, 70.7; $FTIR_{KBr}$ (cm^{-1}): 1774, 1691; HRMS cal. for $C_{25}H_{16}O_4$ = 380.1049, found 380.1049.





4.i. Photophysical properties of the phototrigger 3:

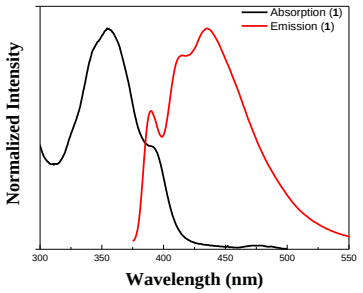


Fig. S.1a. Normalized absorption and emission spectra of the phototrigger **3**.

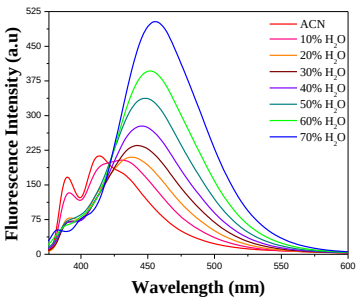


Fig. S.1b. Corrected fluorescence spectra of the phototrigger **3**(10^{-5} M).

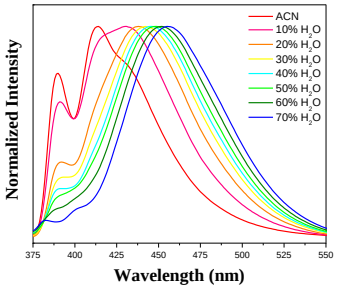


Fig. S.1c. Normalized fluorescence spectra of the phototrigger **3**(10^{-5} M).

4.ii. Photophysical properties of the caged carbonate (5a):

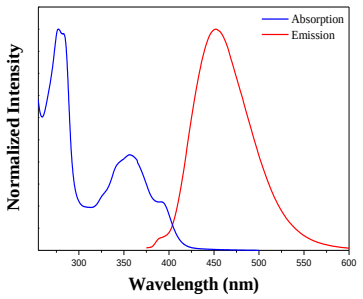


Fig. S.3a. Normalized absorption and fluorescence spectra of the caged carbonate **5a** (10^{-5} M).

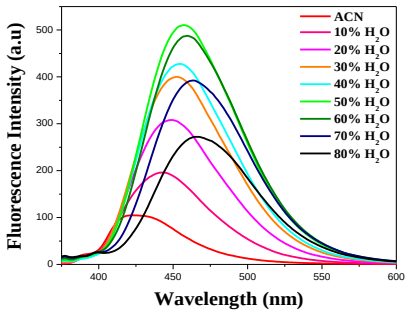


Fig. S.3b. Corrected fluorescence spectra of the caged carbonate **5a** (10^{-5} M).

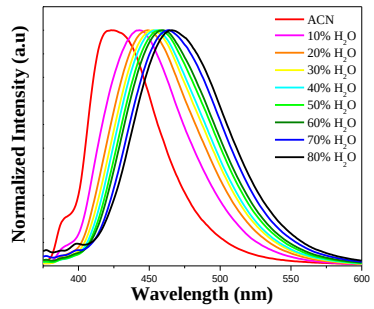


Fig. S.3c. Normalized fluorescence spectra of the caged carbonate **5a** (10^{-5} M).

4.iii. Photophysical properties of the caged carbonate (5c):

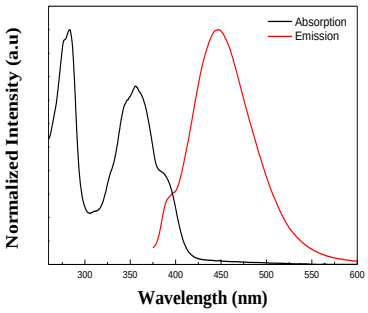


Fig. S.4a. Normalized absorption and fluorescence spectra of the caged carbonate **5c** (10^{-5} M).

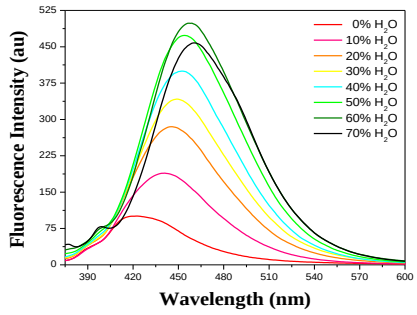


Fig. S.4b. Corrected fluorescence spectra of the caged carbonate **5c** (10^{-5}

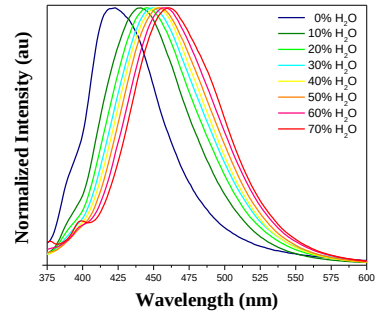


Fig. S.4c. Normalized fluorescence spectra of the caged carbonate **5c**

	M).	(10^{-5} M).

4.iv. Photophysical properties of the caged carbonate (5f):

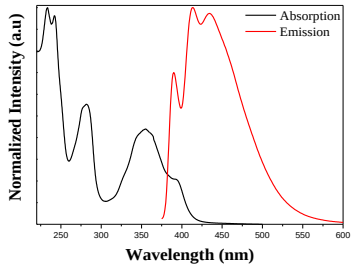


Fig. S.5a. Normalized absorption and fluorescence spectra of the caged carbonate **5f** (10^{-5} M).

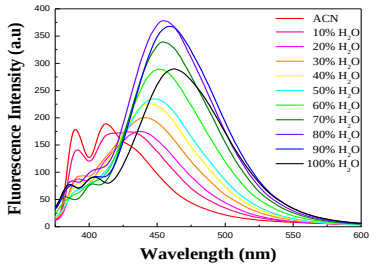


Fig. S.5b. Corrected fluorescence spectra of the carbonate **5f** (10^{-5} M).

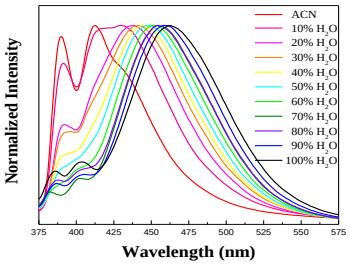


Fig. S.5c. Normalized fluorescence spectra of the carbonate **5f** (10^{-5} M).

4.v. Overlay of absorption and emission spectra of caged carbonates (5a-f):

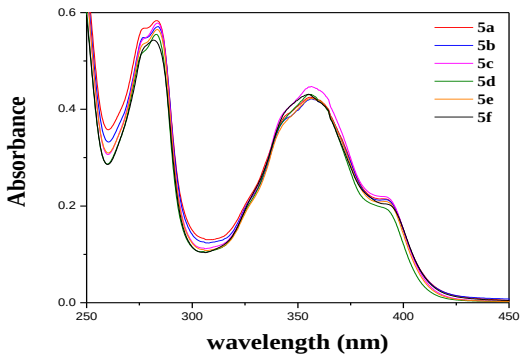


Fig. S.2a. UV/vis absorption spectra of caged carbonates (**5a-f**).

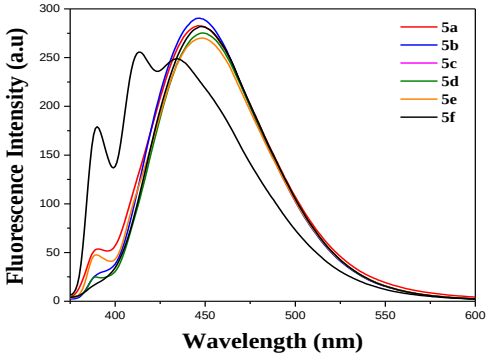


Fig. S.2b. Corrected fluorescence spectra of caged carbonates (**5a-f**).

5. Photolysis data for the caged carbonates 5f:

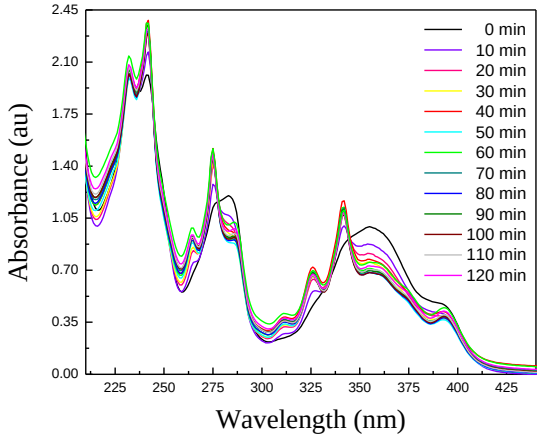


Fig. S.6a. UV/vis absorption spectra at regular intervals of photolysis of cage carbonate **5c** in ACN/H_2O (50:50).

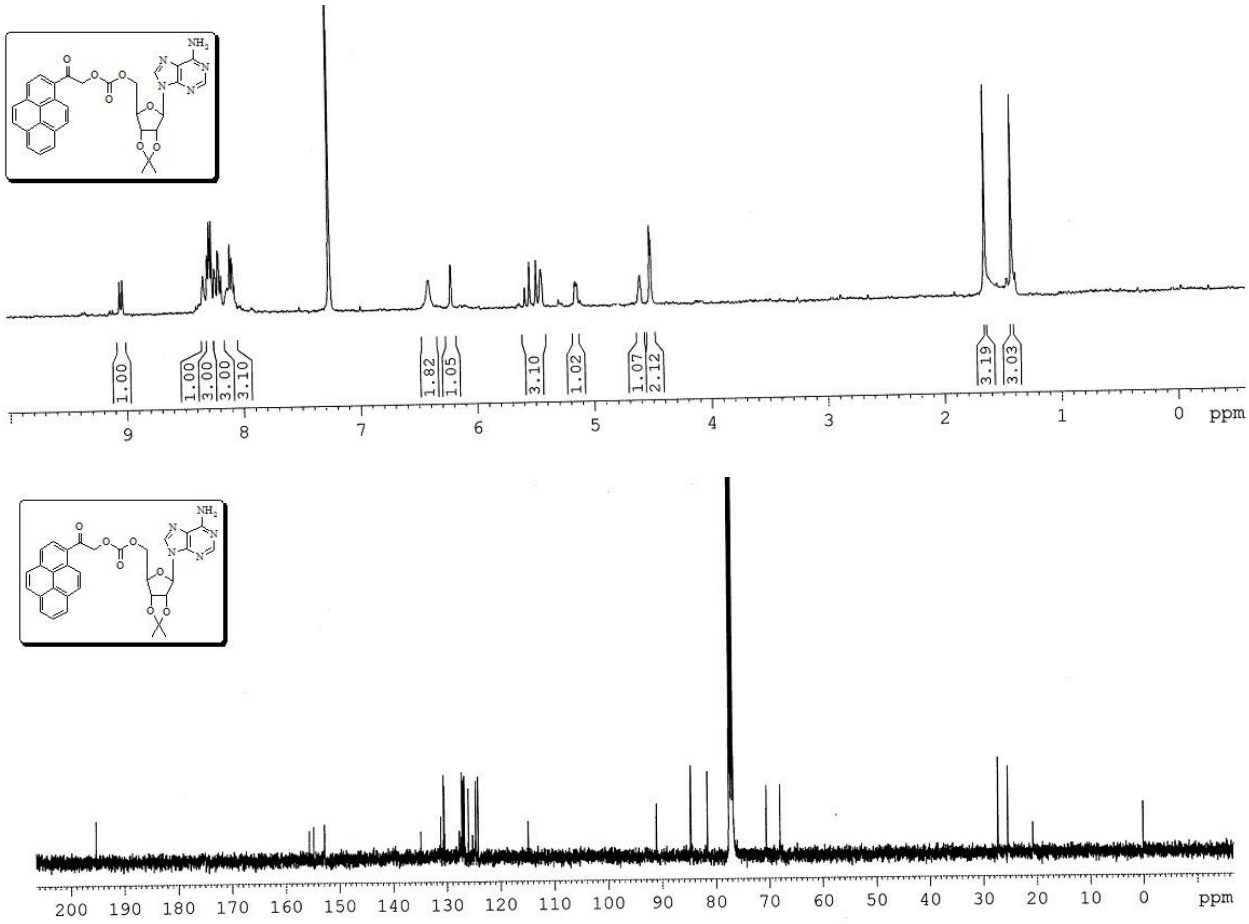
6.i. General procedure for the synthesis of caged adenosine 9:

1-(Hydroxyacetyl)pyrene (1 equiv) was dissolved in dry $CHCl_3$ (5 mL), and to the solution 4-nitro phenyl chloroformate (1 equiv) was added followed by 1.2 equiv of N,N-dimethylpyridin-4-amine (DMAP) and the reaction mixture was stirred at room temperature for 6 h. Then (4-(6-amino-9H-purin-9-yl)-tetrahydro-2,2-dimethylfuro[3,4-d][1,3]dioxol-6-yl)methanol (1 equiv) and 1.2 equiv of DMAP was added. The reaction mixture was refluxed for 36 h. The solvent was removed by rotary evaporation under reduced pressure and the crude residue was purified by column chromatography with $CHCl_3$/MeOH (70:30) as an eluant.

6.ii. Characterisation data of the caged adenosine 9:

*(4-(6-amino-9H-purin-9-yl)-tetrahydro-2,2-dimethylfuro[3,4-d][1,3]dioxol-6-yl)methyl 2-oxo-2-(pyren-3-yl)ethyl carbonate (**9**):* Yellow solid, mp: 138 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 9.04 (d, J = 9.6 Hz, 1H), 8.34 (s, 1H), 8.30-8.24 (m, 3H), 8.23-8.19 (m, 3H), 8.13-8.08 (m, 3H), 6.42 (broad s, 2H, NH), 6.22 (s, 1H), 5.59-5.45 (m, 3H), 5.15 (d, J = 3.6 Hz, 1H), 4.61 (d, J = 3.2 Hz, 1H), 4.51 (d, J = 4.0 Hz, 2H), 1.65 (s, 3H), 1.42 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ =

195.3, 155.7, 154.9, 152.9, 134.9, 131.2, 130.7, 130.5, 130.4, 127.7, 127.2, 127.0, 126.9, 126.8, 126.0, 125.2, 124.6, 124.2, 114.9, 91.1, 84.8, 84.7, 81.6, 70.6, 68.0, 27.3, 25.5; $FTIR_{KBr}$ (cm^{-1}): 1749, 1653; HRMS cal. for $C_{32}H_{28}N_5O_7$ [MH^+] = 594.1989, found 672.4179.



6.iii. Photolysis data for the caged adenosine 9:

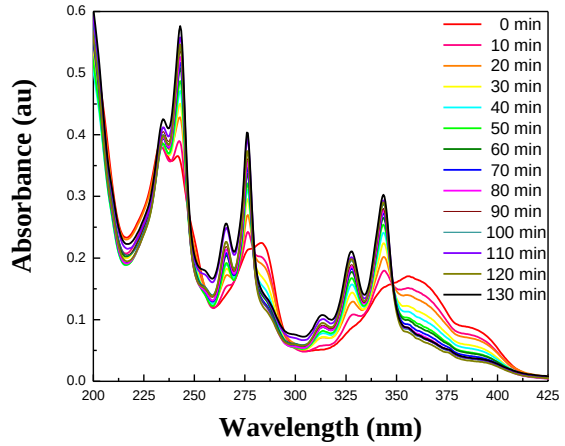


Fig. S.7a. UV/vis absorption spectra at regular intervals of photolysis of cage carbonate **9** in ACN/H_2O (50:50).

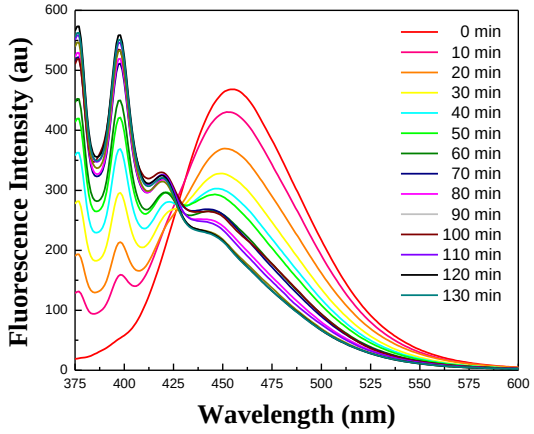


Fig. S.7b. Corrected fluorescence spectra at regular intervals of photolysis of cage carbonate **9** in ACN/H_2O (75:25).

7. Hydrolytical stability data of caged carbonates (5a-f) and 9

Table S1. Hydrolytical stability data of caged carbonates (5a-f) and 9

Carbonate	Time (day)	Hydrolytical stability data of caged carbonates (**5a-f**) and **9**		
		% of depeted [a](pH 4.5)	% of depeted [a](pH 6)	% of depeted [a](pH 7.5)
5a	15	3	2	5
5b	15	4	5	4
5c	15	4	7	7
5d	15	5	4	6
5e	15	6	5	3
5f	15	5	8	7
9	15	3	5	4

[a] % of decomposition of caged compounds was calculated using ^{1}H NMR/HPLC

8. Cell imaging data for phototrigger 3

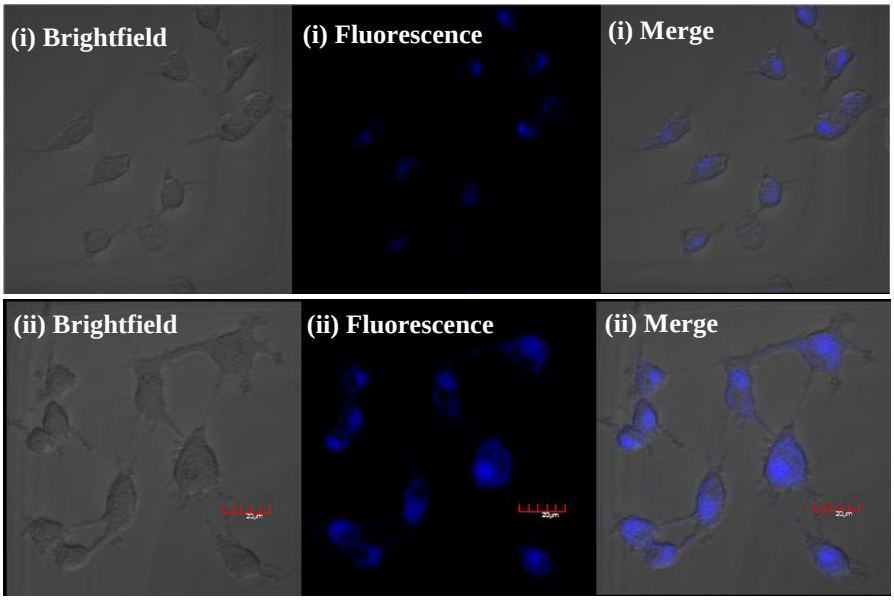


Fig. S.5 Confocal brightfield and fluorescence images of L929 cells incubated with the phototrigger **3** (i) (5×10^{-6} M), (ii) (5×10^{-5} M) (λ_{ex} 410 Cells were incubated separately with the phototrigger **3** for 6 h**.**

9. Cell imaging data for caged adenosine 9

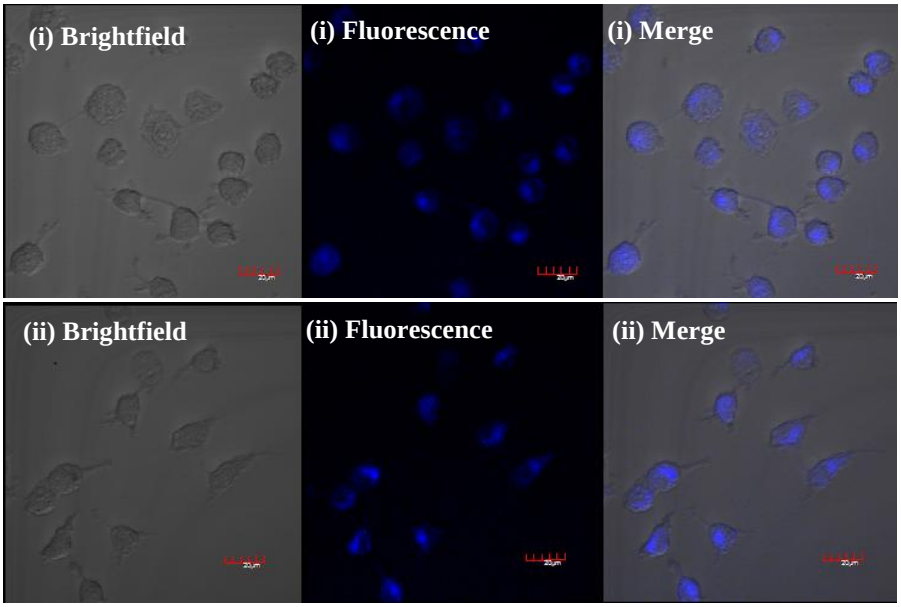


Fig. S.5 Confocal brightfield and fluorescence images of L929 cells incubated with the phototrigger **9** (i) (5×10^{-6} M), (ii) (5×10^{-5} M) (λ_{ex} 410 Cells were incubated separately with the phototrigger **9** for 6 h**.**

10. Cell viability data for the phototrigger 3

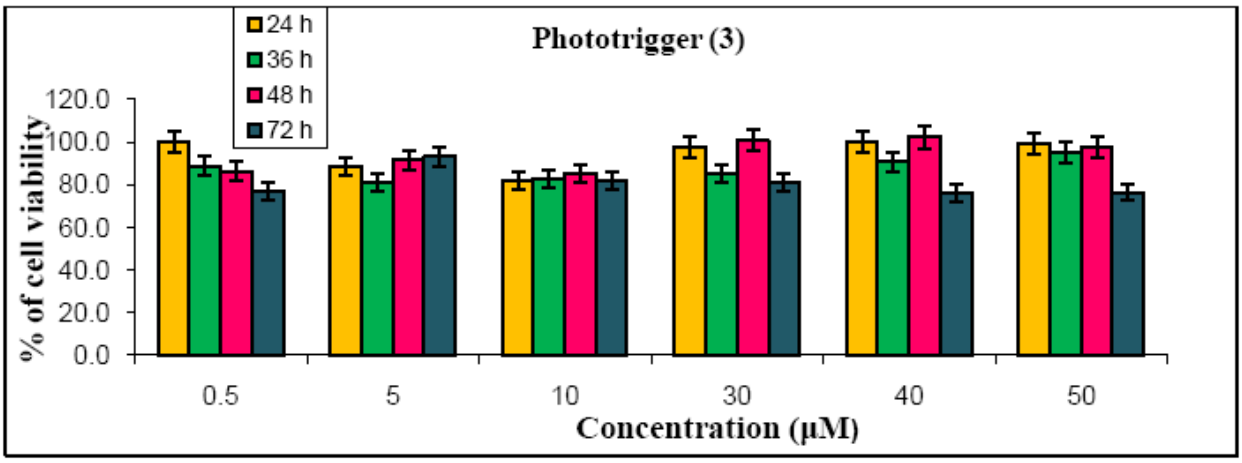


Fig. 6a Cell viability test for the phototrigger **3** against L929 cell line in different concentration of **3** after different incubation time (Values are presented as mean ±SD).