Supporting Information

Fluorescent Photoremovable precursors (acridin-9-ylmethyl)ester: Synthesis, Photophysical, Photochemical and Biological applications

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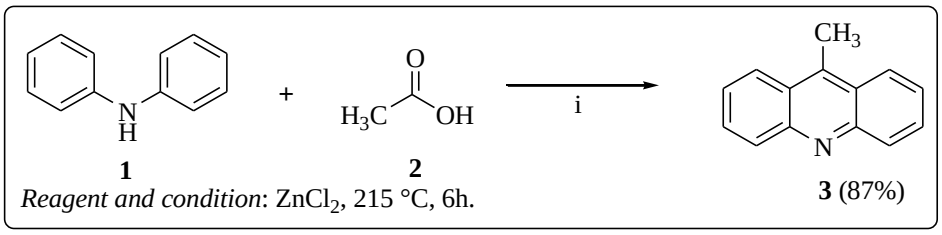
General information

^{1}H NMR (200 MHz and 400 MHz) spectra were recorded on a BRUKER–AC 200 MHz and BRUKER–AC 400 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 and 100 MHz) spectra were recorded on a BRUKER–AC 200 MHz and BRUKER–AC 400 MHz Spectrometer with complete proton decoupling. Chemical shifts are reported in parts per million from tetramethylsilane with the solvent resonance 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 PerkineElmer 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 F254 TLC sheets (Merck) were used.

Synthesis of fluorescent PRPG, 9–methylacridine

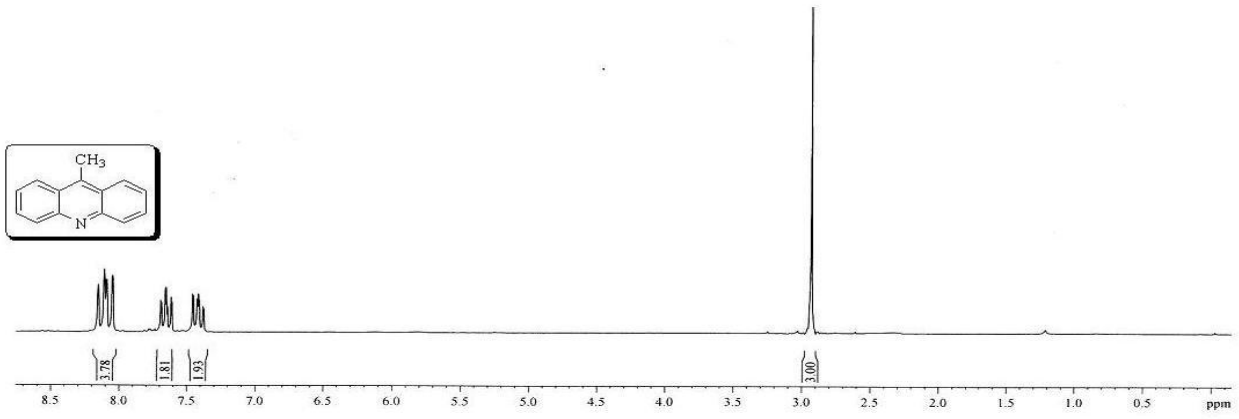
The protecting group 9–methylacridine was synthesized following the procedure as described by Suzuki et al.[1] Treatment of *N*,*N*–diphenyl amine with glacial acetic acid in presence of zinc chloride at 215 ˚C for 6 h afforded the protecting group 9–methylacridine with excellent yield as shown in **Scheme 1**.



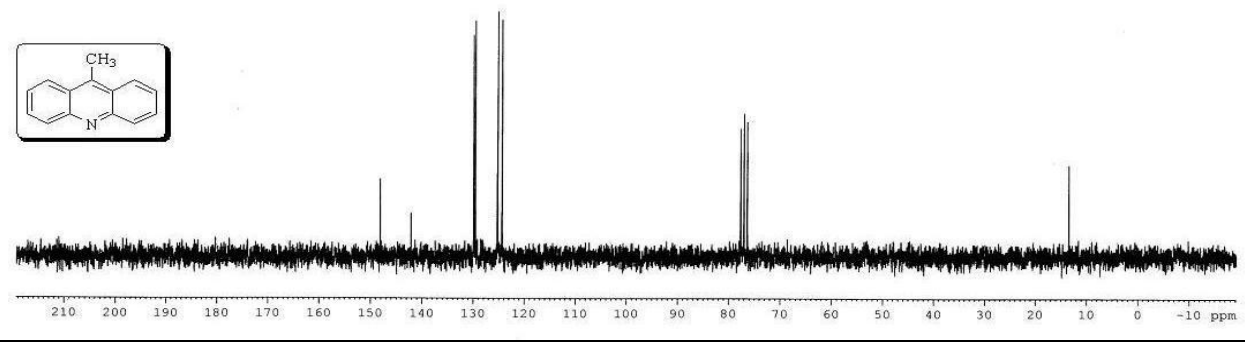
Scheme 1. Synthesis of 9–methylacridine

A mixture of diphenylamine (6.0g, 36 mmol), acetic acid (6.0 g, 100 mmol) and zinc chloride (25.6 g, 188 mmol) was heated up to 180 ˚C with continuous stirring. Then excess acetic acid was removed from the reaction mixture by distillation and the reaction mixture was heated at 220 ˚C for additional 5 h followed by addition of aqueous ammonia solution. The resulted yellow precipitates were collected by filtration. The residue was dissolved in chloroform and neutralized by washing with aqueous $NaHCO_3$ and dried over anhydrous Na_2SO_4. The organic solvent was then removed under reduced pressure to give 5.96 g (87 %) of crude product which was further purified by column chromatography using ethyl acetate in pet ether as an eluent.

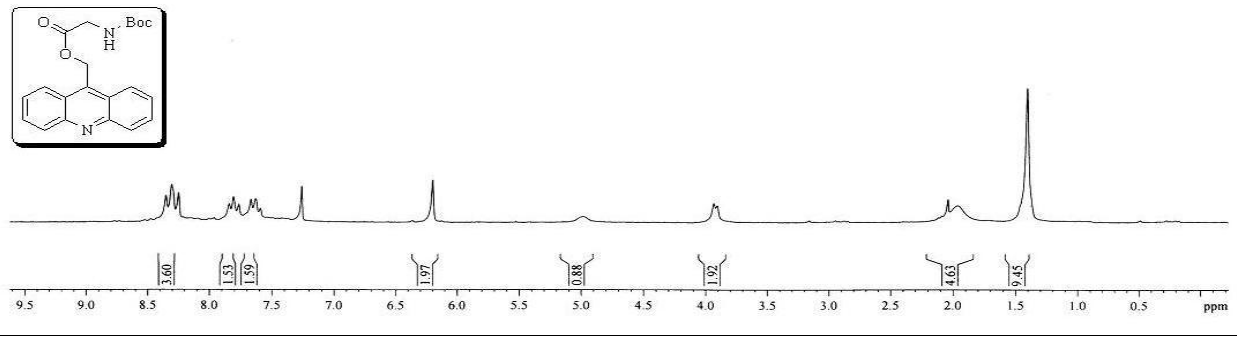
^{1}H NMR spectra of 1:



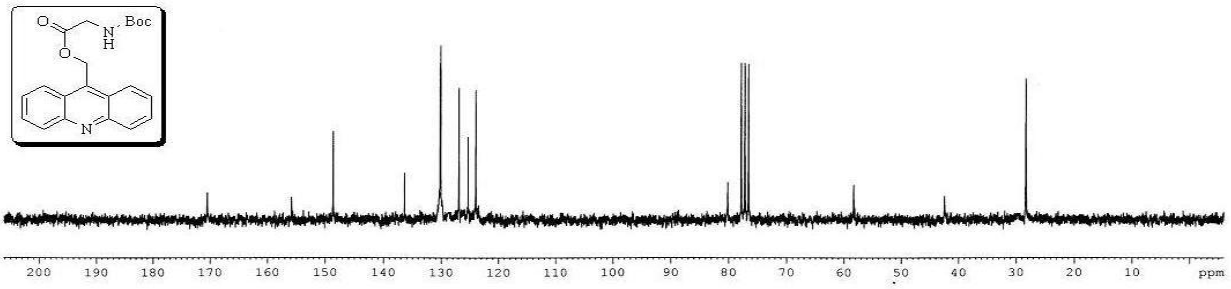
^{13}C NMR spectra of 1:



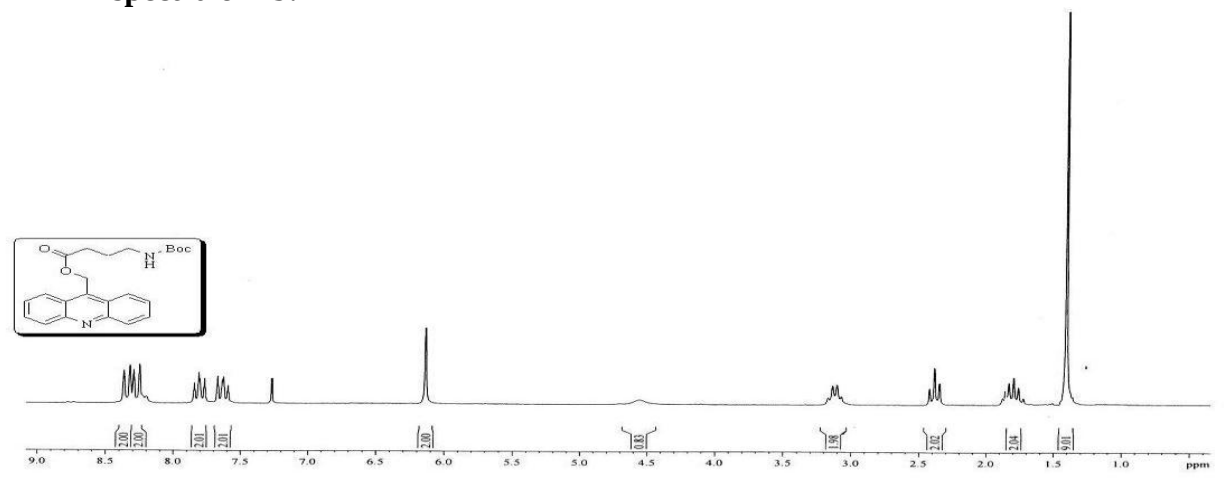
^{1}H NMR spectra of 4a:



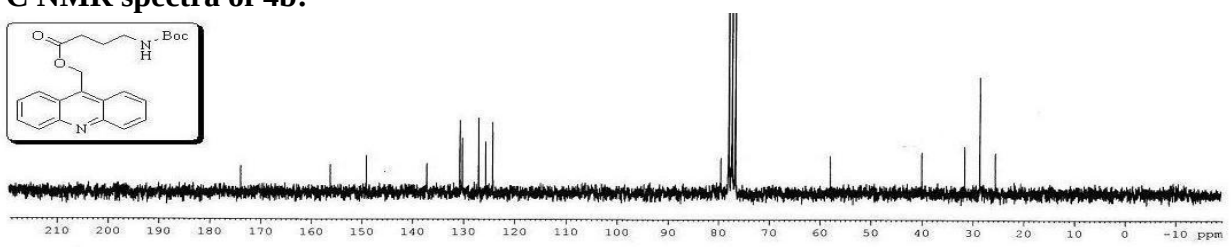
^{1}H NMR spectra of 4a:



^{1}H NMR spectra of 4b:



^{13}C NMR spectra of 4b:



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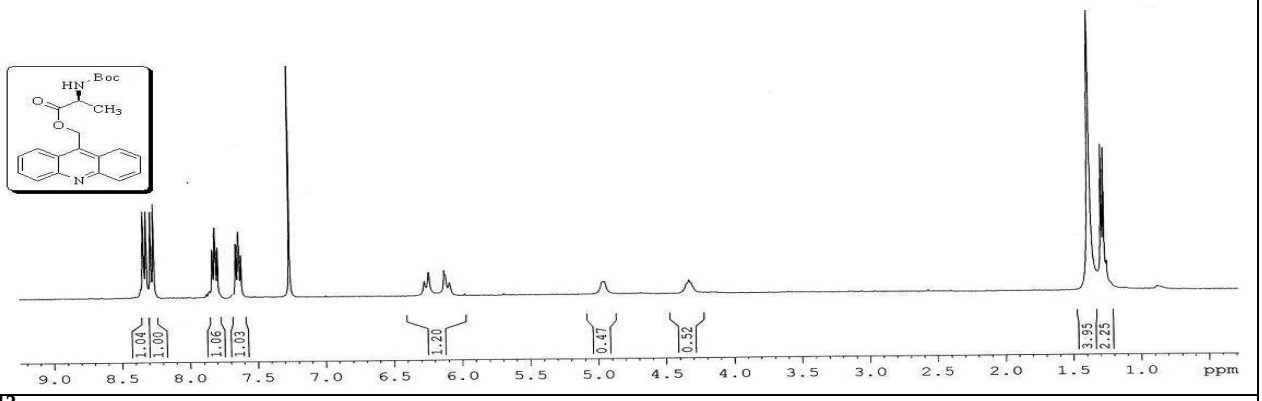
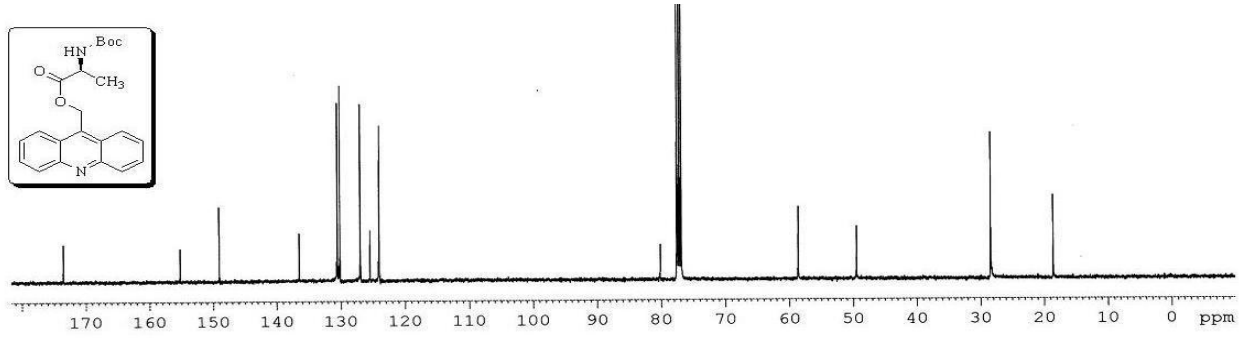


^{13}C NMR spectra of 4c:



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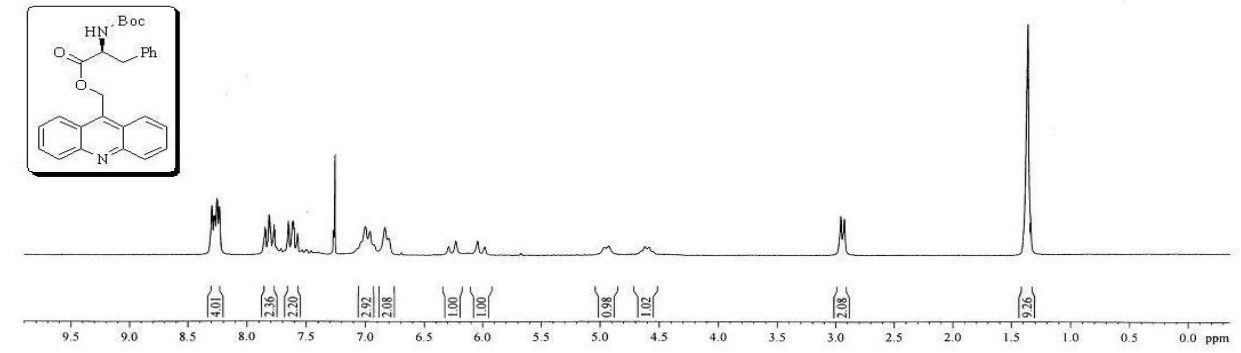
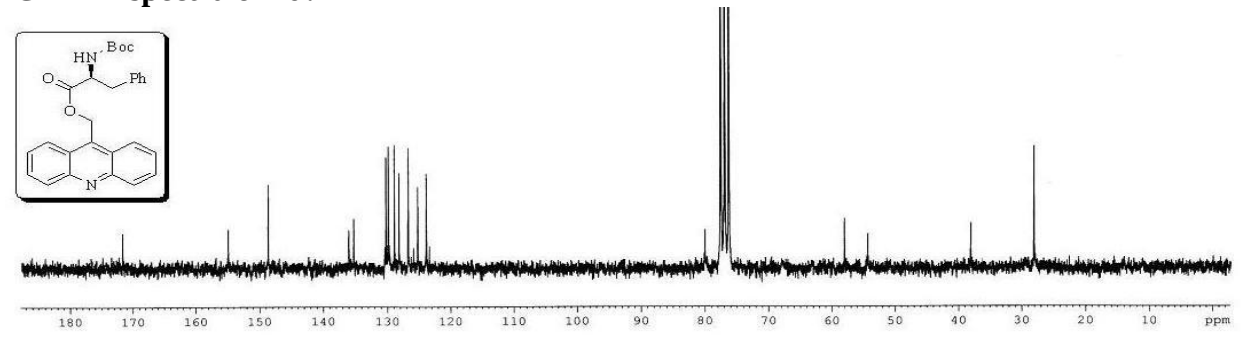
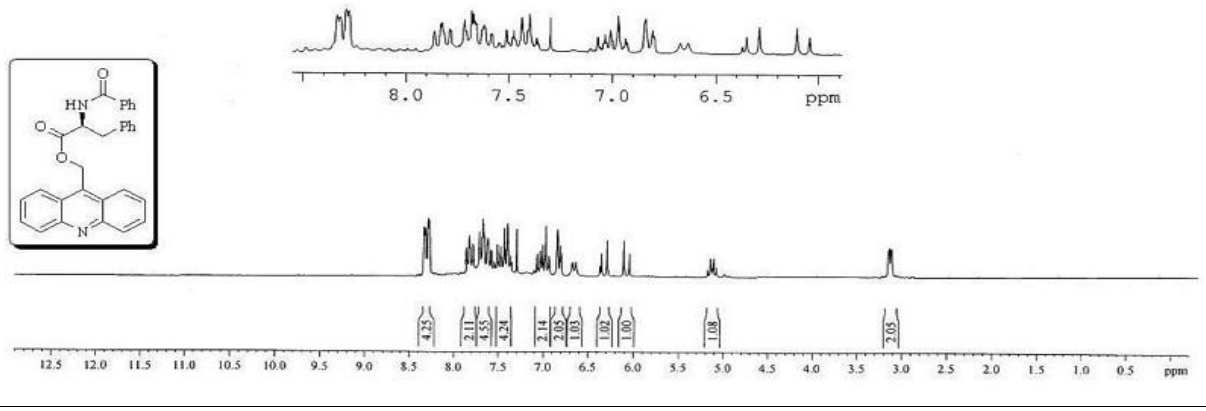


^{13}C NMR spectra of 4d:



^{1}H NMR spectra of 4e:



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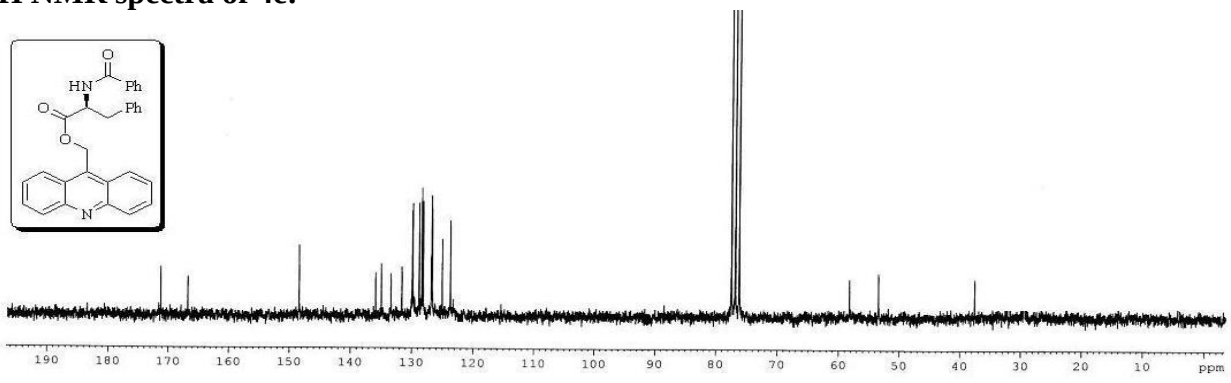
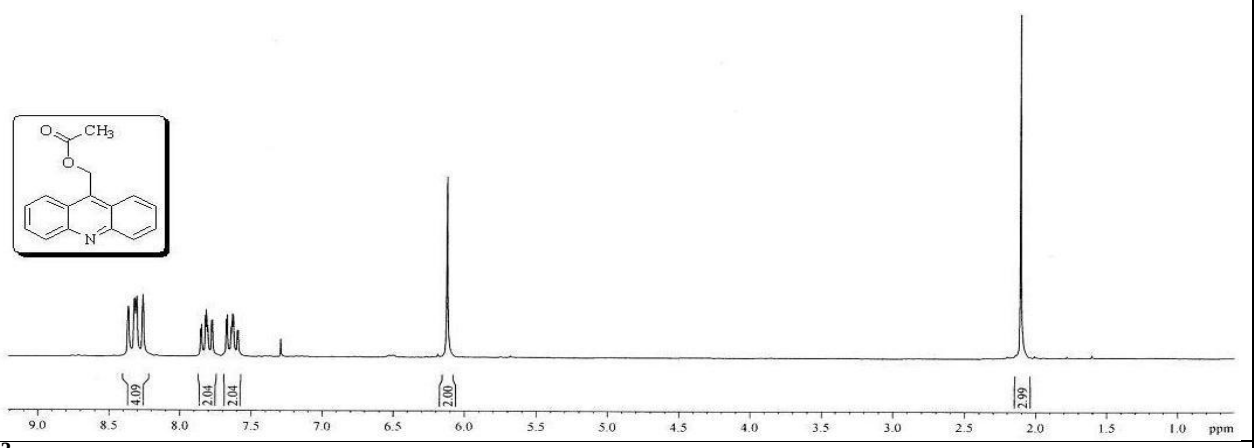
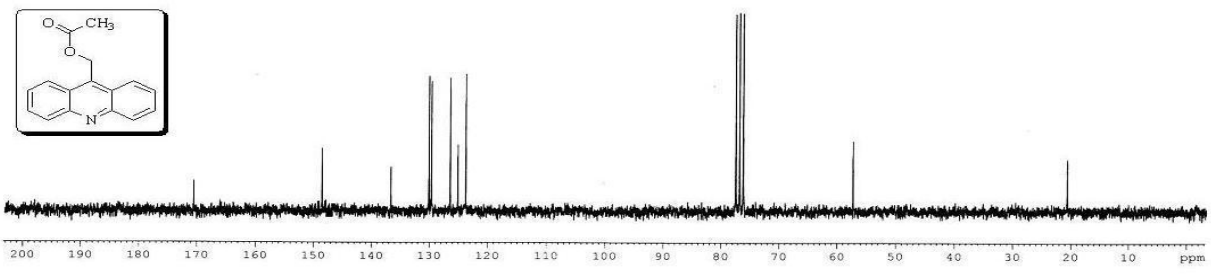


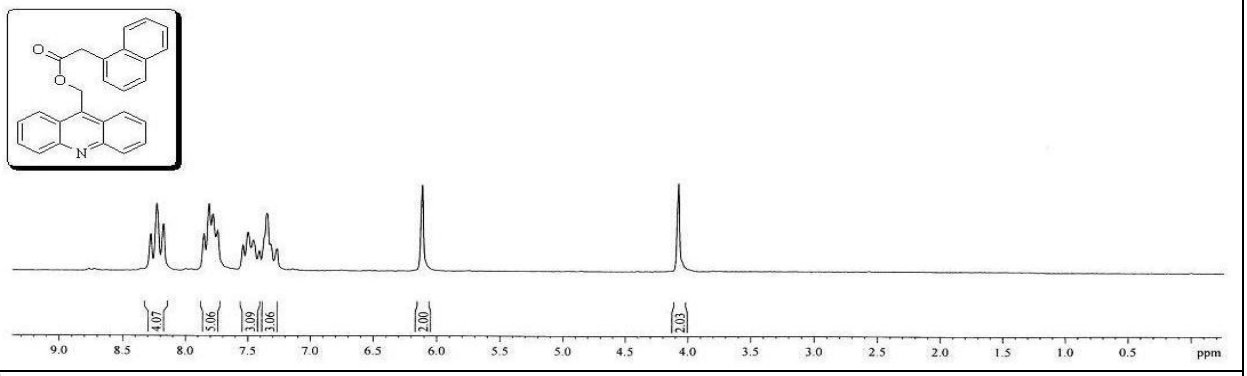
^{1}H NMR spectra of 4f:



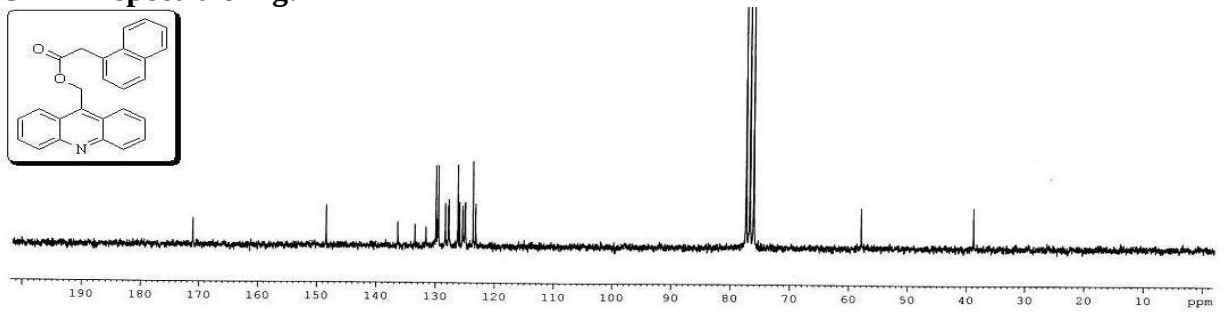
^{13}C NMR spectra of 4f:



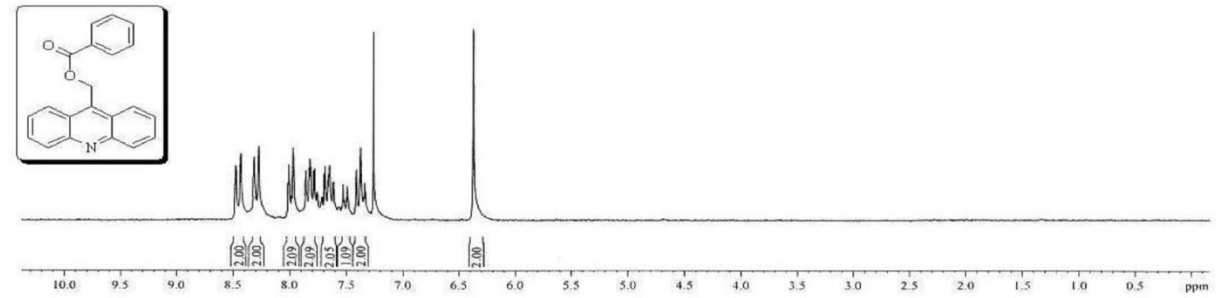
^{1}H NMR spectra of 4g:



^{13}C NMR spectra of 4g:



^{1}H NMR spectra of 4h:



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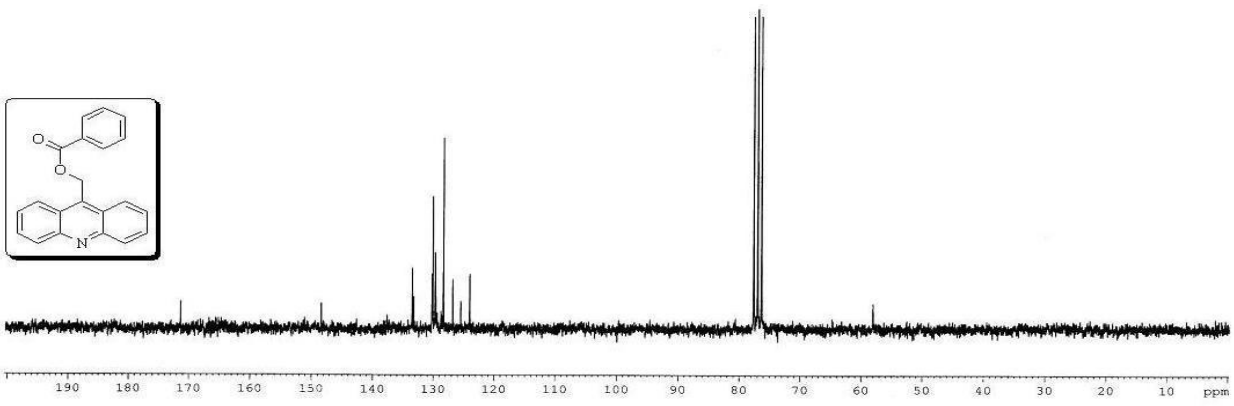
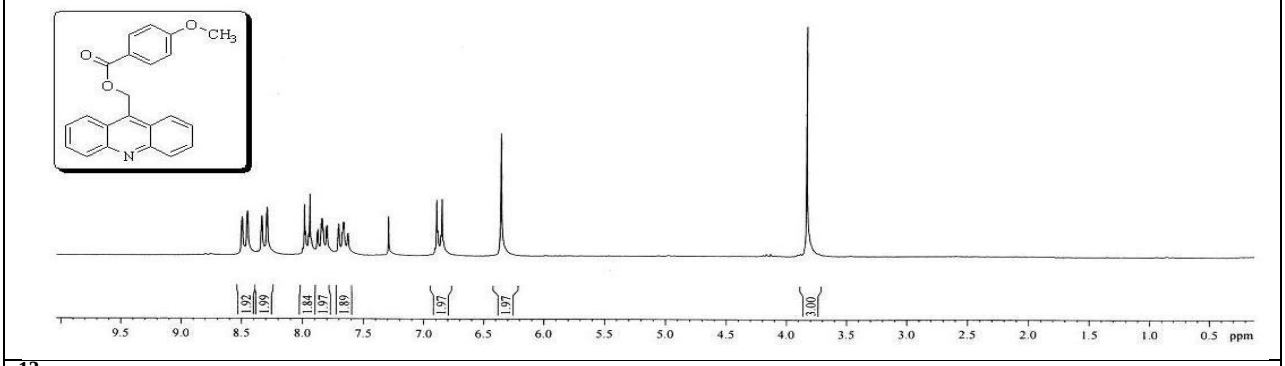


^{1}H NMR spectra of 4i:



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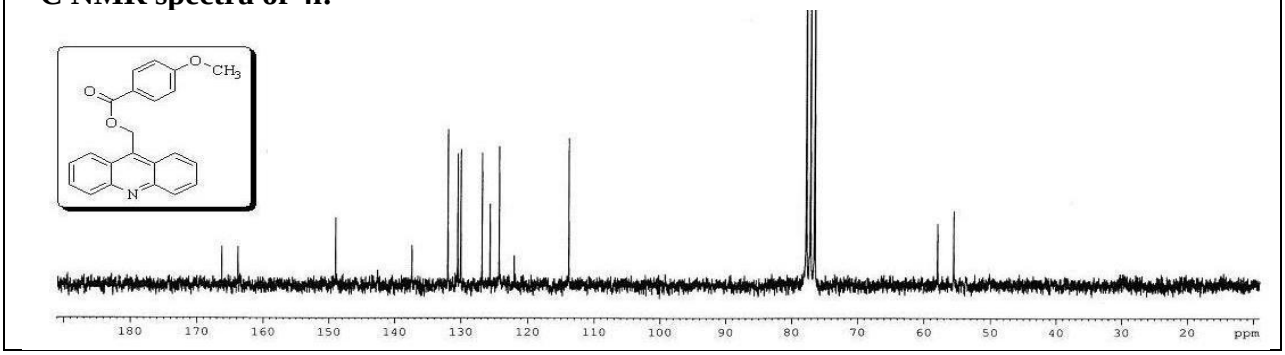
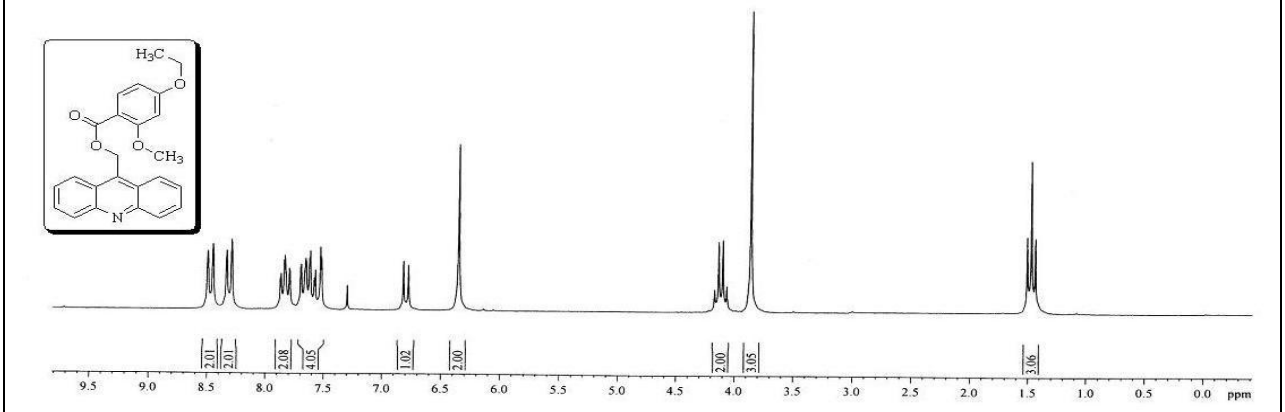
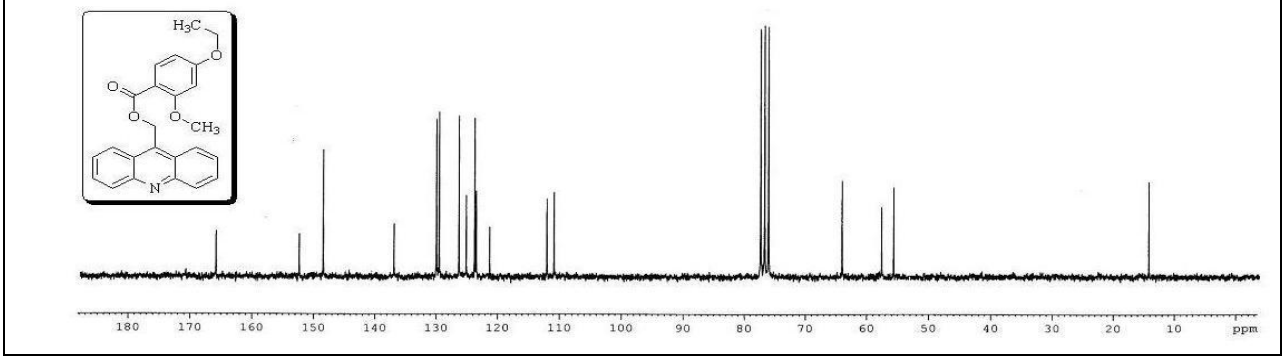


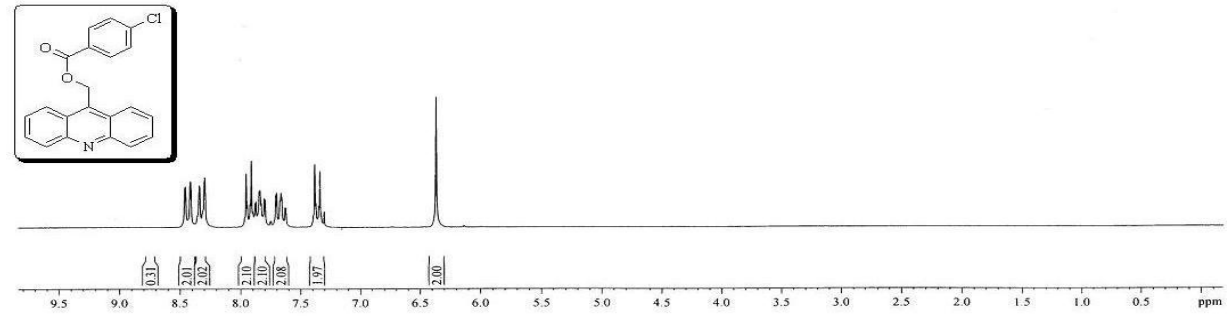
^{1}H NMR spectra of 4j:



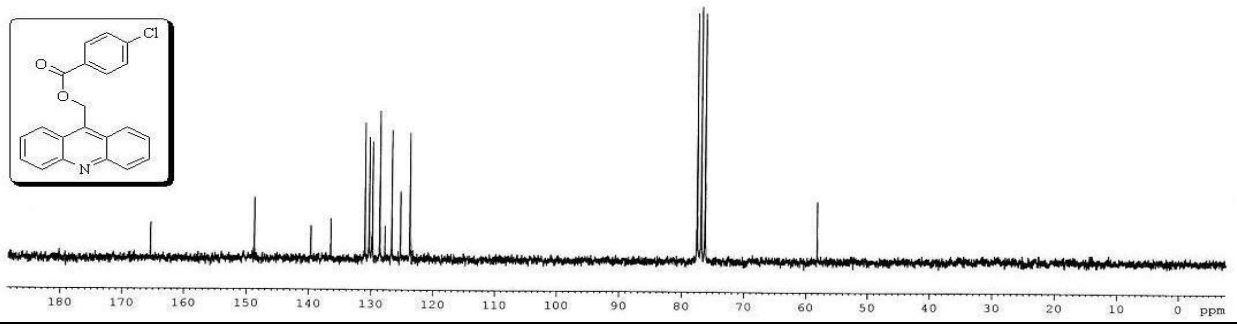
^{13}C NMR spectra of 4j:



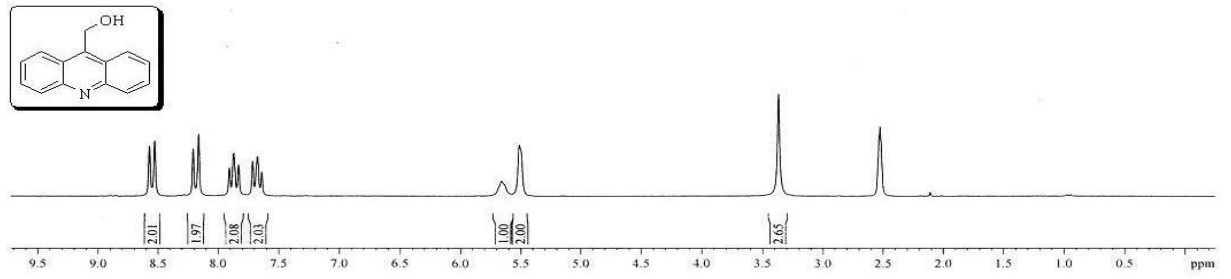
^{1}H NMR spectra of 4k:



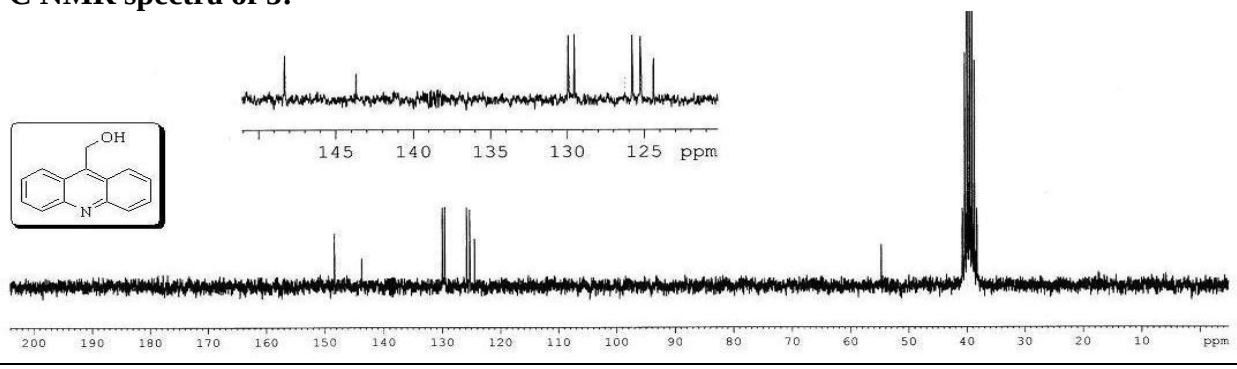
^{1}H NMR spectra of 4k:



^{1}H NMR spectra of 5:



^{13}C NMR spectra of 5:



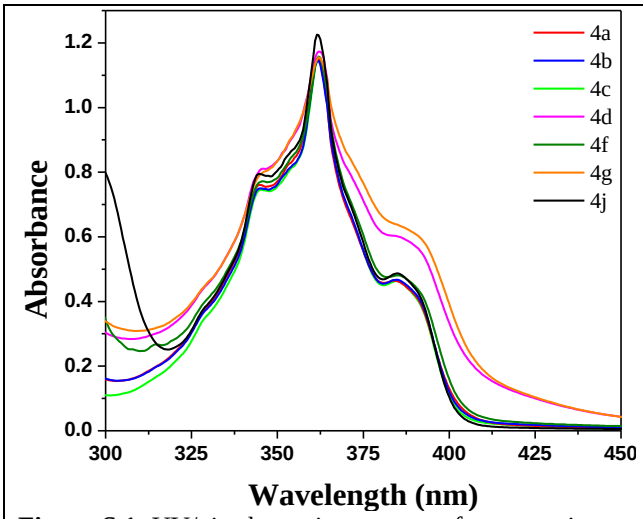


Figure S.1. UV/vis absorption spectra of ester conjugate **(4a–d, 4f–g, and 4j)**

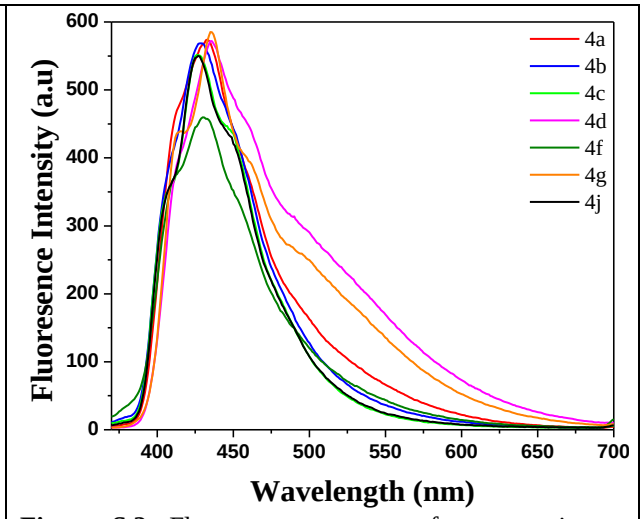


Figure S.2. Fluorescence spectra of ester conjugate **(4a–d, 4f–g, and 4j)**

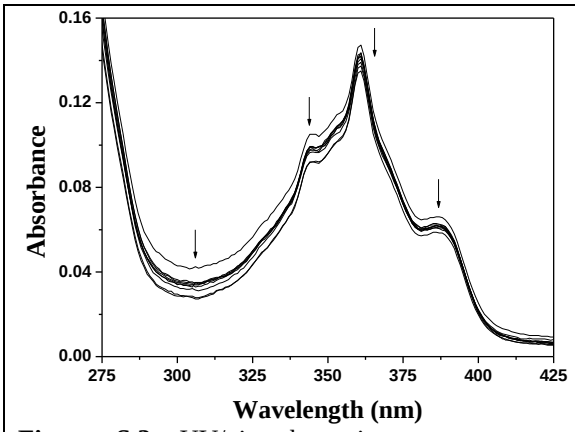


Figure S.3. UV/vis absorption spectra at regular intervals of photolysis of ester conjugate **4i** in ACN/H_2O (75/25) using an irradiation wavelength of ≥356 nm

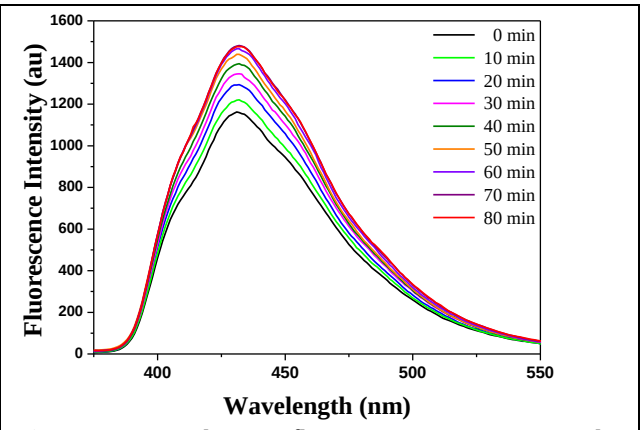


Figure S.4. Steady state fluorescence spectra at regular intervals of photolysis of ester conjugate **4i** in ACN/H_2O (75/25) using an irradiation wavelength of ≥356 nm

4.6. Hydrolytic stability of ester conjugates (4a–k)

The hydrolytic stability of ester conjugates were investigated by keeping the solution of ester conjugates **(4a–k)** individually in dark at three different initial pH values (4.5, 6, and 7.5) for a period of 30 days.

Table S.1. Hydrolytic stability data of ester conjugates (**4a–k**)

Caged ester	Time (day)	Hydrolytical stability data of caged carbonates (**6a–h**)		
		% depleted at pH 4.5 [a]	% depleted at pH 6 [a]	% depleted at pH 7.5[a]
4a	30	7	6	5
4b	30	10	5	6

4c	30	9	8	7
4d	30	10	8	7
4e	30	11	8	8
4f	30	11	9	8
4g	30	13	10	10
4h	30	15	10	9
4i	30	12	9	10
4j	30	13	7	9
4k	30	11	9	5

[a] % of decomposition of caged esters was calculated using HPLC.

The course of hydrolysis of all ester conjugate was followed by RP–HPLC. We observed only 7–15% (which was obtained from the HPLC peak area and is the average of three runs) decomposition for ester conjugates at three different pH values (**Table S.1**).

Cell cytotoxicity assay of caged conjugate (6b) and photoproduct acridin–9–ylmethanol (7)

The cytotoxicity in vitro was measured using the MTT[2] (3–(4,5–dimethylthiazol–2–yl)–2,5–diphenyltetrazolium bromide, a yellow tetrazole) assay in L929 cell line. Briefly, cells growing in log phase were seeded into 96–well cell–culture plate at 1×10^4 cells/ml. Different concentration of compound **5** and the caged ester **4b** were added in the wells with an equal volume of PBS in the control wells. The cells were then incubated for 72 h at 37 °C in 5% CO_2. Latter, fresh media containing 0.40 mg/ml MTT were added to the above 96–well plates and incubated at 37 °C for additional 6 h. Thereafter, the medium was removed, the formazan crystals formed were dissolved in DMSO and the absorbance was recorded at 595 nm. The cytotoxic effect of each treatment was expressed as percentage of cell viability relative to the untreated control cells defined as: [[OD 570 nm treated cells]/[OD 570 nm control cells]] x 100.