Supplementary Information (SI) for Journal of Materials Chemistry B.

Supporting Information

for

Photosensitizer-Pendant Biotinylated Polyester as Nanocarrier for Targeted Photodynamic Therapy

Chhandita Chakraborty,[a‡] Subhendu Biswas [a‡] and Anindita Das* [a]

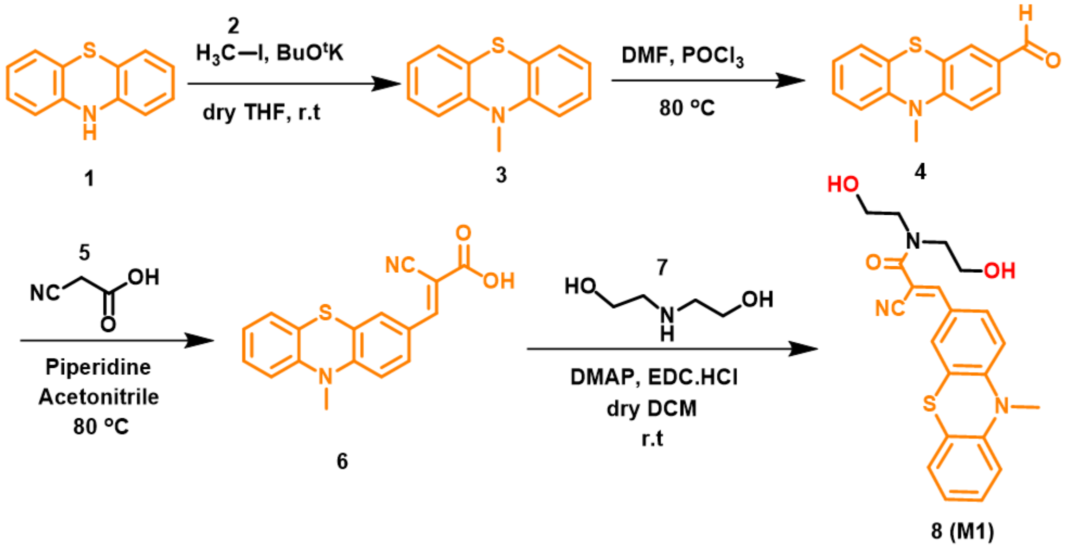
‡These authors contributed equally

[a]School of Applied and Interdisciplinary Sciences, Indian Association for the Cultivation of Science,
2A & 2B, Raja S. C. Mullick Road, Jadavpur, Kolkata-700032, India.

*Corresponding author; Email: psuad2@iacs.res.in

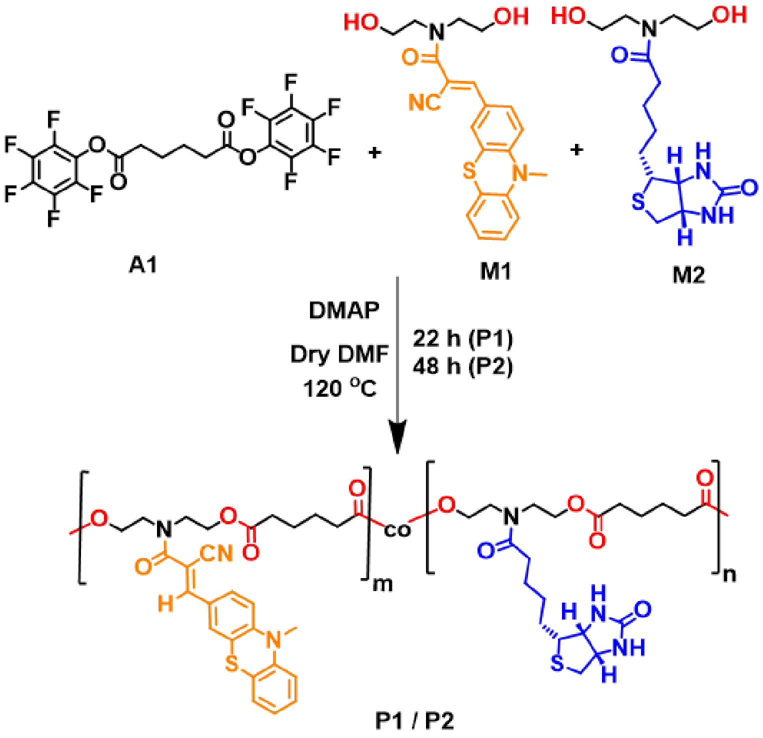
Synthesis and Characterization

Monomer **M1** was synthesized following the synthetic strategy given in Scheme S1. Synthesis of compounds **3**, **4** and **6** were reported by us elsewhere.[1]

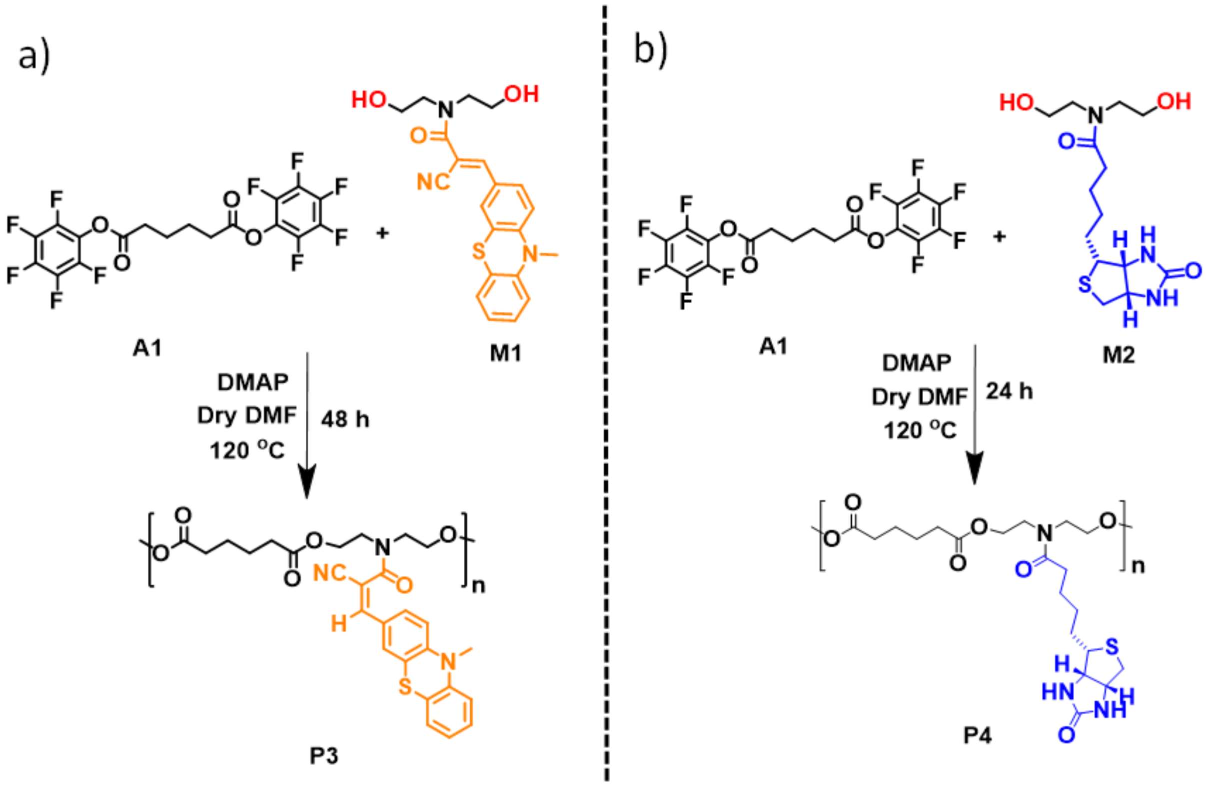


Scheme S1: Synthetic Scheme for monomer **M1** (compound **8**).

P1 was synthesized by polycondensation reaction of **A1** with **M1** and **M2.** Synthesis of **A1**[2] and **M2**[3] were reported elsewhere.[2,3]



Scheme S2: Synthetic scheme for polymers **P1** (m:n = 1:50) and **P2** (m:n = 1:10).



Scheme S3: Synthetic scheme for polymer (a) **P3** and (b) **P4**.

Synthesis of monomer M1: Compound **6** (200 mg, 0.65 mmol) and Dimethylaminopyridine (DMAP) (397 mg, 3.25 mmol) were taken in dry CH_2Cl_2 and stirred for 15 minutes at 0 °C and subsequently, 1-ethyl-3-(3- dimethylaminopropyl) carbodiimide hydrochloride (EDC.HCl) (249 mg, 1.3 mmol) was added to the reaction mixture. After 30 minutes of stirring, diethanol amine (200 μL, 1.95 mmol) was added to the reaction vessel and kept for stirring 48 h at room temperature in inert atmosphere. After that, the solution was dried in vacuum and the crude was purified by column chromatography using silica gel (100-200 mesh) as a stationary phase and 20% ethyl acetate in CH_2Cl_2 as eluent to obtain the desired product **M1** (compound **8**) as orange sticky solid (100 mg, yield: 40%). ^{1}H NMR (500 MHz, Chloroform-*d*): δ 8.08 (s, 1H), 7.85 (d, *J* = 8.7 Hz, 1H), 7.72 (s, 1H), 7.21-7.18 (m, 1H), 7.11 (d, *J* = 7.7 Hz, 1H), 7.01-6.98 (m, 1H), 6.86-6.83 (m, 2H), 4.44 (t, *J* = 5.8 Hz, 2H), 3.81 (t, *J* = 4.8 Hz, 2H), 3.61 (t, *J* = 5.4 Hz, 2H), 3.48 (t, *J* = 4.9 Hz, 2H), 3.44 (s, 3H), 3.26 (t, *J* = 5.8 Hz, 2H); HRMS (ESI): *m/z* calcd. 395.1337 $[M + H]^+$, found 395.1321 $[M + H]^+$.

Synthesis of A1 and **M2** were reported by us previously.[2,3]

Synthesis of the polymer P1

A1 (168.8 mg, 0.3525 mmol) was dissolved in 100 μL of dry DMF in a polymer vessel. This solution was kept in a water bath at 50 °C for 5 minutes to ensure complete dissolution. To this solution, DMAP

(8.6 mg, 0.0705 mmol) were added with stirring after which **M1** (27.9 mg, 0.0705 mmol) and **M2** (93.5 mg, 0.282 mmol) were added. The whole mixture was then degassed by purging dried argon gas for 20 minutes before stirring it at 120 °C in an oil bath for 22 h. The crude polymer obtained was dissolved in methanol and purified by precipitation from cold ether and collected after drying under reduced pressure to yield 110 mg of the pure polymer as orange sticky film. ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.31 (s, 1H), 8.15 (d, J = 8.7 Hz, 1H), 7.95 (s, 1H), 7.23-7.17 (m, 2H), 7.01-6.99 (m, 3H), 6.42 (s, 1H), 6.35 (s, 1H), 4.80 (m, 1H), 4.63-4.07 (m, 5H), 3.57-3.49 (m, 4H), 3.09 (s, 1H), 2.93-2.82 (m, 2H), 2.30 (m, 6H), 1.62-1.29 (m, 10H). Experimental M_n from SEC analysis = 23,000 g/mol, $Đ$ = 1.52 with respect to poly(methyl methacrylate) (PMMA) standard and DMF as eluent.

$$\mathrm{m} = \frac{xMn}{xMa+Na} \qquad \text{———} \qquad (1)$$

$$\mathrm{n} = \frac{Mn}{xMa+Na} \qquad \text{———} \qquad (2)$$

Where, x represents the integration ratio of the characteristic proton signals of the two monomers, **M1** and **M2** obtained from the ^{1}H NMR spectrum of **P1**

M_a and N_a denote the molecular weights of the two repeating segments

M_n is the number-average molecular weight of **P1**, as determined by SEC

Using equations (1) and (2), the values obtained for the degree of polymerization are "m" = 1 and "n" = 51 for **P1** polymer.

Synthesis of the polymer P2

A1 (90 mg, 0.1882 mmol) was dissolved in 100 µL of dry DMF in a polymer vessel. This solution was kept in a water bath at 50 °C for 5 minutes to ensure complete dissolution. To this solution, DMAP (4.6 mg, 0.03764 mmol) were added with stirring after which **M1** (15.3 mg, 0.0386 mmol) and **M2** (51.2 mg, 0.1543 mmol) were added. The whole mixture was then degassed by purging dried argon gas for 20 minutes before stirring it at 120 °C in an oil bath for 48 h. The crude polymer obtained was dissolved in methanol and purified by precipitation from cold ether and collected after drying under reduced pressure to yield 60 mg of the pure polymer as orange sticky film. ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.40 (s, 1H), 8.19 (d, J = 8.7 Hz, 1H), 7.99 (s, 1H), 7.15-6.92 (m, 5H), 6.42 (s, 1H), 6.36 (s, 1H), 4.31-4.29 (m, 1H), 4.14-4.08 (m, 5H), 3.77 (m, 4H), 3.58 (s, 3H), 3.15 (s, 1H), 2.94 (m, 2H), 2.28 (m, 4H), 2.2 (m, 2H), 1.61-1.33 (m, 10H). Experimental M_n from SEC analysis = 26,500 g/mol, $Đ$ = 1.07 with respect to poly(methyl methacrylate) (PMMA) standard and DMF as eluent.

Using equations (1) and (2), the values obtained for the degree of polymerization are "m" = 6 and "n" = 54 for **P2** polymer.

Synthesis of the polymer P3

A1 (50 mg, 0.11 mmol) was dissolved in 100 μL of dry DMF in a polymer vessel. This solution was kept in a water bath at 50 °C for 5 minutes to ensure complete dissolution. To this solution, DMAP (2.68 mg, 0.022 mmol) were added with stirring after which **M1** (44.6 mg, 0.113 mmol) was added. The whole mixture was then degassed by purging dried argon gas for 20 minutes before stirring it at 120 °C in an oil bath for 48 h. The crude polymer obtained was dissolved in methanol and purified by precipitation from cold ether and collected after drying under reduced pressure to yield 40 mg of the pure polymer as brownish solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.39 (s, 1H), 8.28 (d, J = 8.2 Hz, 1H), 8.15 (s, 1H), 7.18-6.80 (m, 5H), 3.21 (s, 1H), 2.12 (m, 4H), 1.23 (m, 4H). Experimental M_n from SEC analysis = 21,000 g/mol, $Đ$ = 1.07 with respect to poly(methyl methacrylate) (PMMA) standard and DMF as eluent.

Synthesis of the polymer P4

A1 (140 mg, 0.293 mmol) was dissolved in 100 μL of dry DMF in a polymer vessel. This solution was kept in a water bath at 50 °C for 5 minutes to ensure complete dissolution. To this solution, DMAP (7.16 mg, 0.0586 mmol) were added with stirring after which **M2** (99.4 mg, 0.300 mmol) was added. The whole mixture was then degassed by purging dried argon gas for 20 minutes before stirring it at 120 °C in an oil bath for 24 h. The crude polymer obtained was dissolved in methanol and purified by precipitation from cold ether and collected after drying under reduced pressure to yield 110 mg of the pure polymer as orange sticky film. ^{1}H NMR (500 MHz, DMSO-d_6): δ 6.41 (s, 1H), 6.35 (s, 1H), 4.30 (m, 1H), 4.14-4.12 (m, 5H), 3.76 (m, 4H), 3.15 (s, 1H), 2.82-2.79 (m, 2H), 2.30-2.27 (m, 4H), 2.15 (m, 2H) 1.61-1.32 (m, 10H). Experimental M_n from SEC analysis = 30,000 g/mol, $Đ$ = 1.06 with respect to poly(methyl methacrylate) (PMMA) standard and DMF as eluent.

Additional Figures

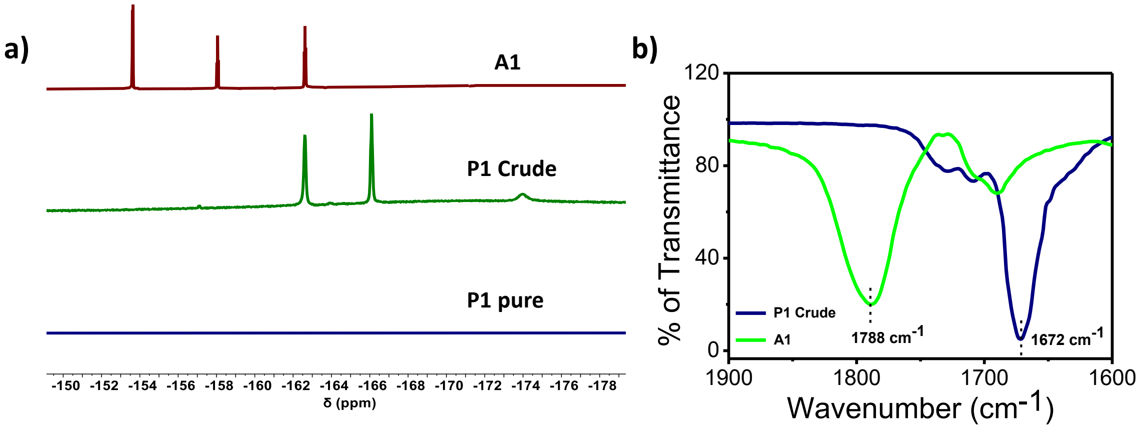


Figure S1: (a) Compared ^{19}F NMR spectra of crude and purified **P1** polymer with **A1** monomer in DMSO-d_6 solvent; After purification, **P1** shows no signal for the activated ester or released pentafluorophenol, suggesting near complete monomer consumption. (b) Selected region in the FTIR spectra of **P1** and **A1** monomer. The crude polymer shows a strong signal at 1672 cm^{-1} corresponding to the newly formed backbone carbonyl ester overlapping with the other carbonyl signals from the two incorporated **M1** and **M2** monomers. The complete disappearance of the PFP-ester signal of **A1** in the crude polymer is in agreement with the ^{19}F NMR data.

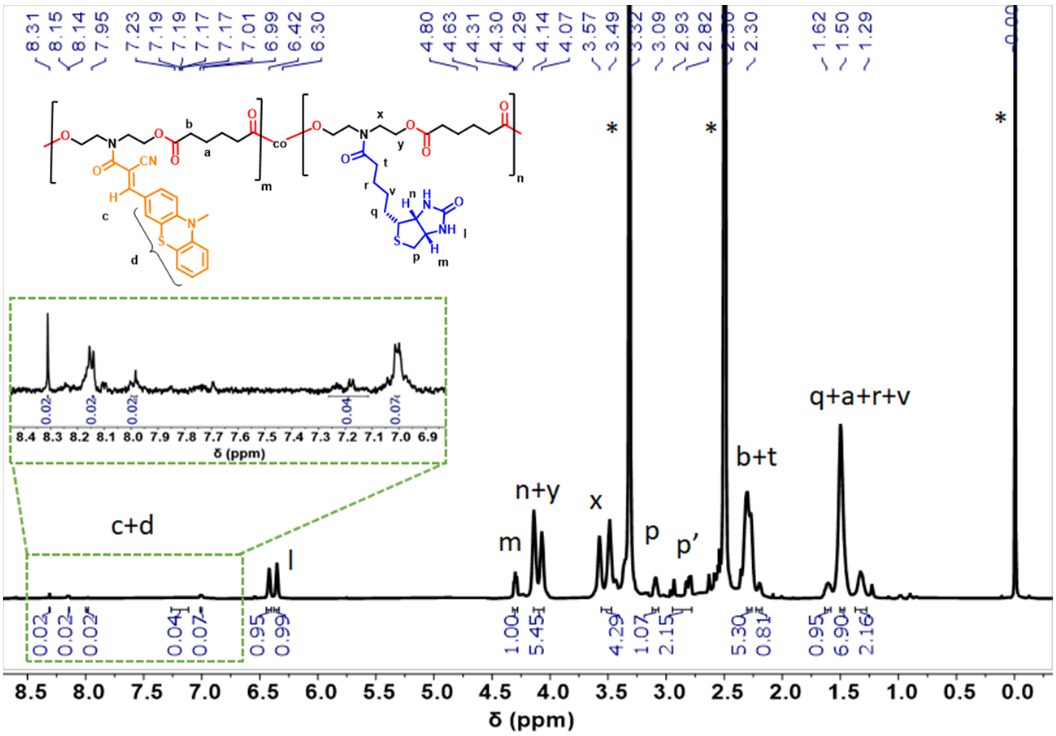


Figure S2: ^{1}H NMR spectrum of pure **P1** (m:n = 1:50) in DMSO-d_6. (*) indicates residual solvent peaks.

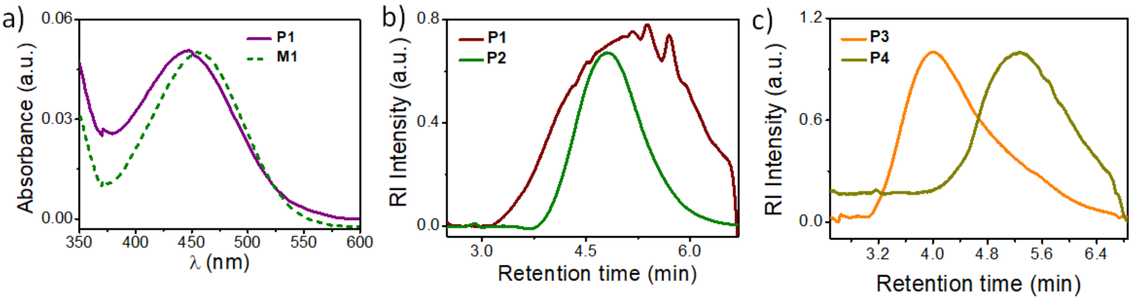


Figure S3: (a) UV-vis spectra of **P1** (C = 0.1 mg/mL) and **M1** (C = 7 μM) in $CHCl_3$; SEC plots of polyesters (b) **P1** and **P2** and (c) **P3** and **P4** with DMF as the eluent.

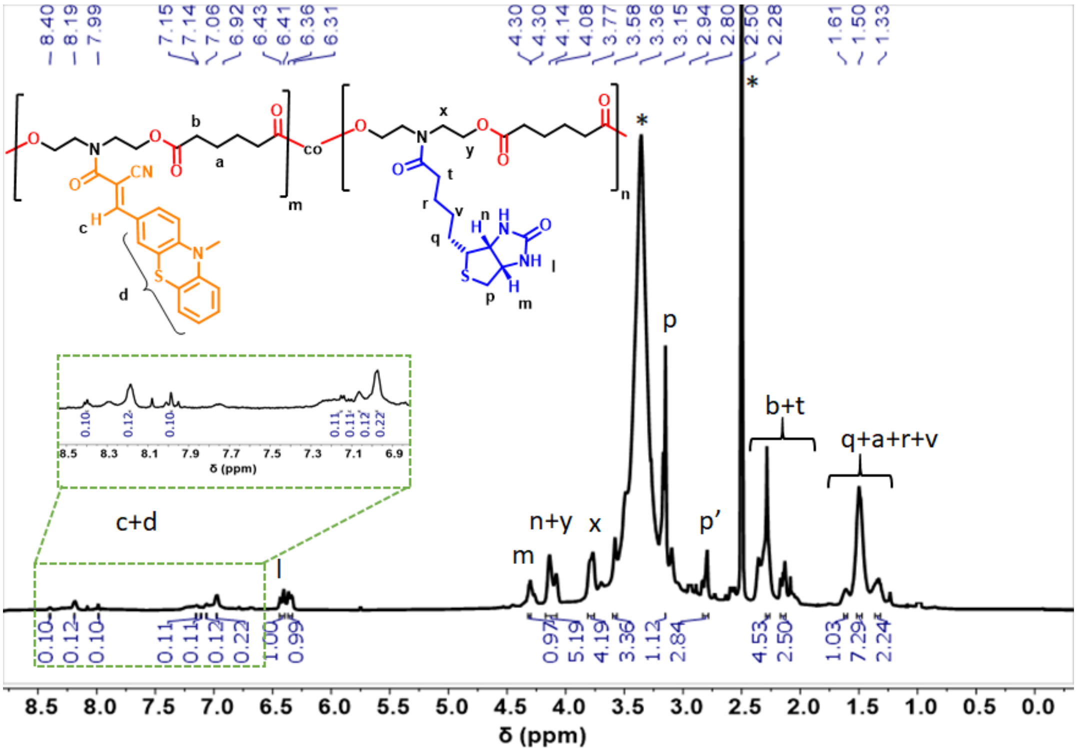


Figure S4: ^{1}H NMR spectrum of pure **P2** (m:n = 1:10) in DMSO-d_6. (*) indicates residual solvent peaks.

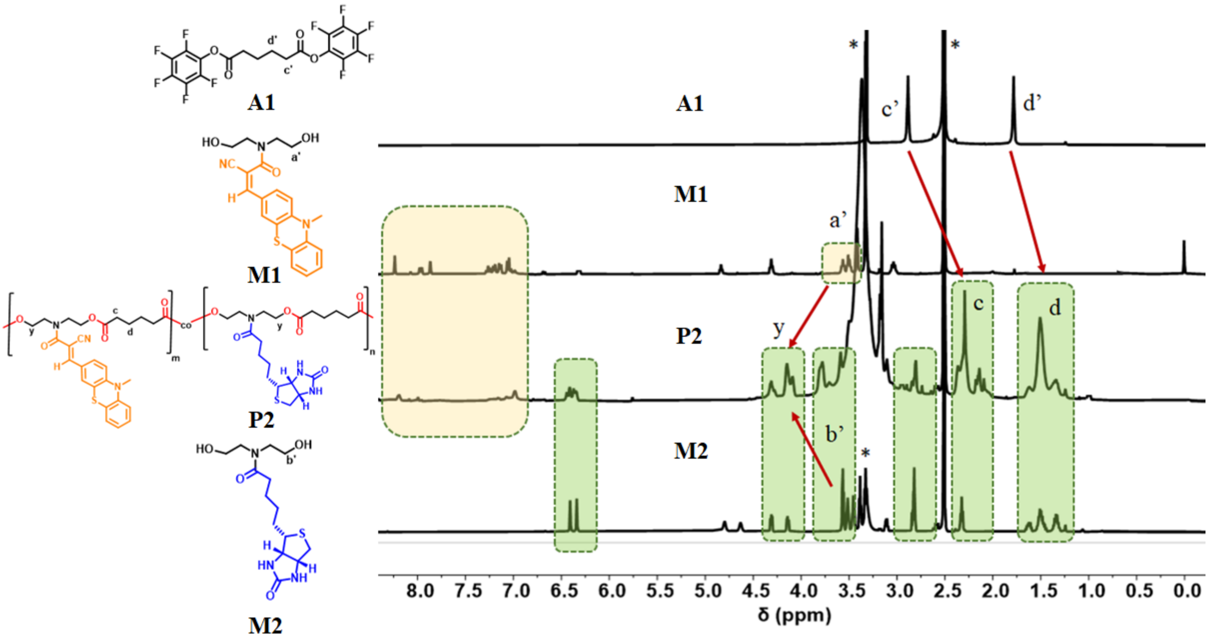


Figure S5: Stacked ^{1}H NMR of pure **P2** polymer with **A1** and **M1** and **M2** in DMSO-d_6 solvent. (*) indicates residual solvent peaks.

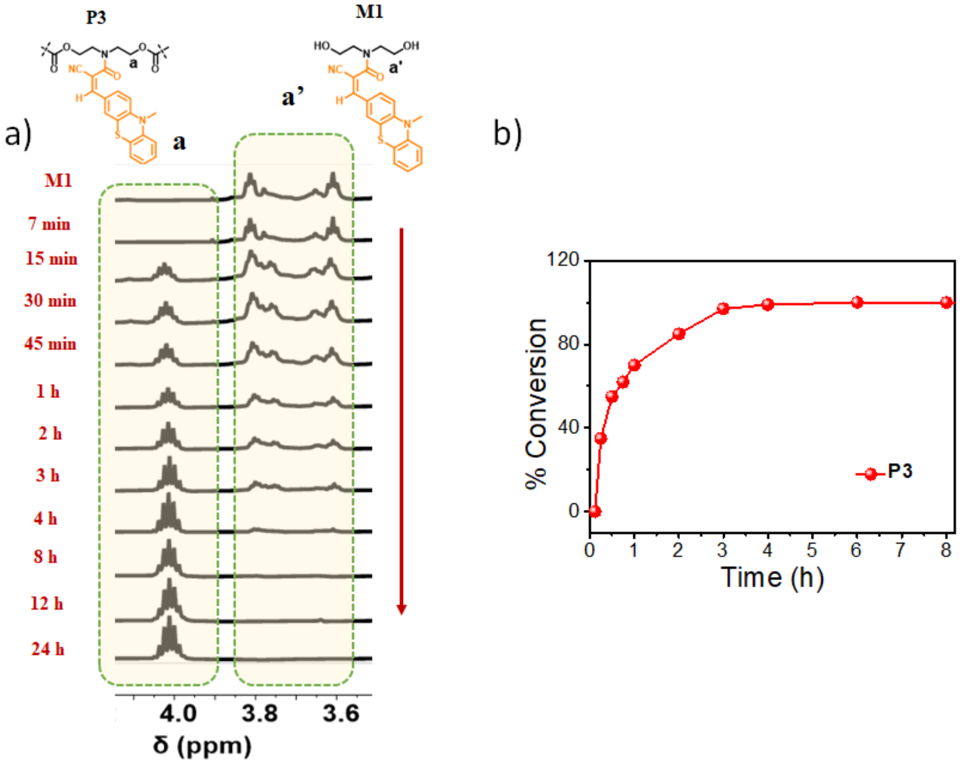


Figure S6: Progress of the polymerization reaction of **P3** as a function of time. Stacked ^{1}H NMR spectra of (a) crude **P3** polymer with **M1** in CDCl$_3$ monitored at different intervals of time; (b) % Conversion obtained from figure (a).

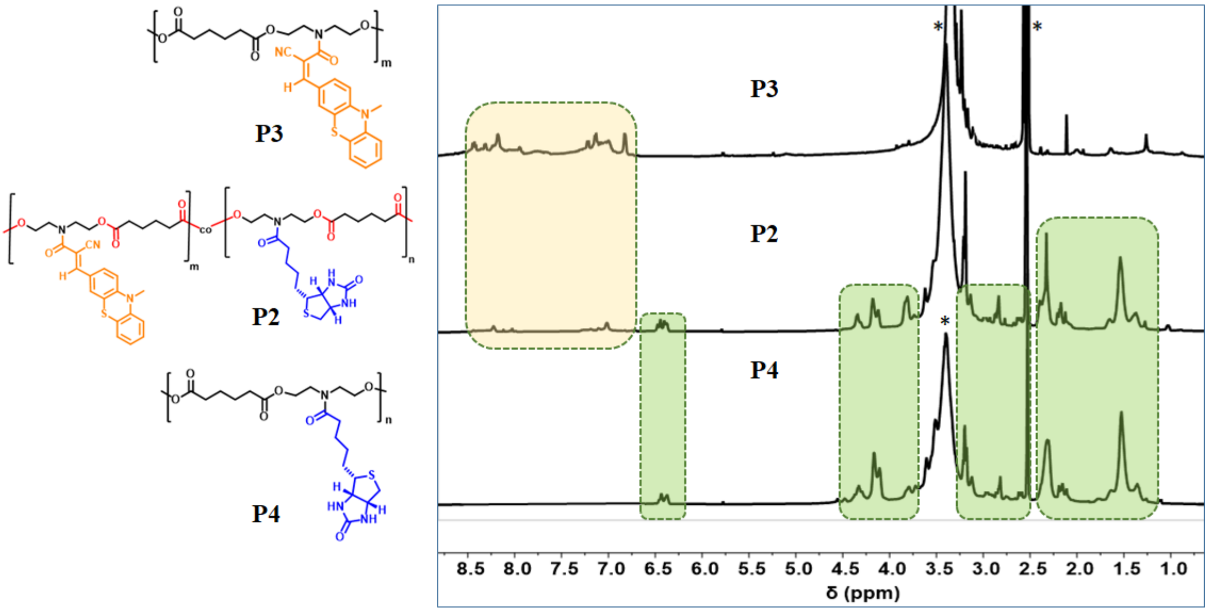


Figure S7: Stacked ^{1}H NMR spectra of pure **P2** polymer with **P3** and **P4** in DMSO-d_6 solvent. (*) indicates residual solvent peaks.

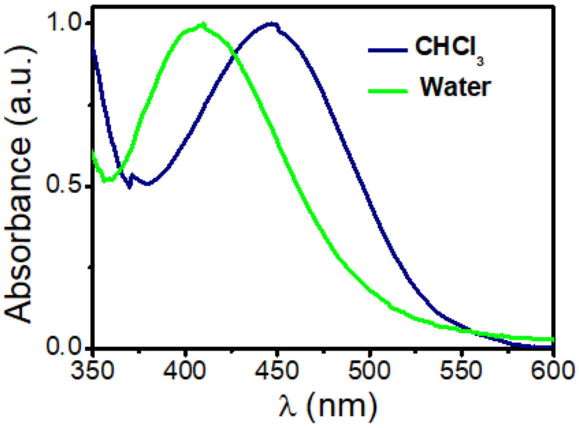


Figure S8: UV-vis spectra of **P1** (C = 0.1 mg/mL) in a non-aggregating solvent ($CHCl_3$) and an aggregating solvent (Water).

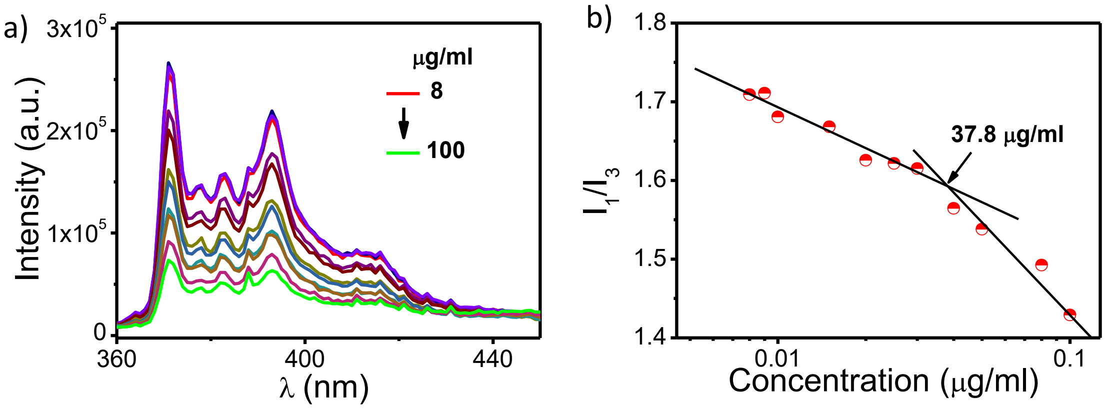


Figure S9: (a) Emission spectra of Pyrene ($C = 10^{-6}$ M) encapsulated in various concentrations of **P1** polyester in water (λ_{exc} = 337 nm); (b) Plot of Intensity ratio (I_1/I_3) of Pyrene versus concentrations of **P1** derived from Figure S9a for the determination of CAC.

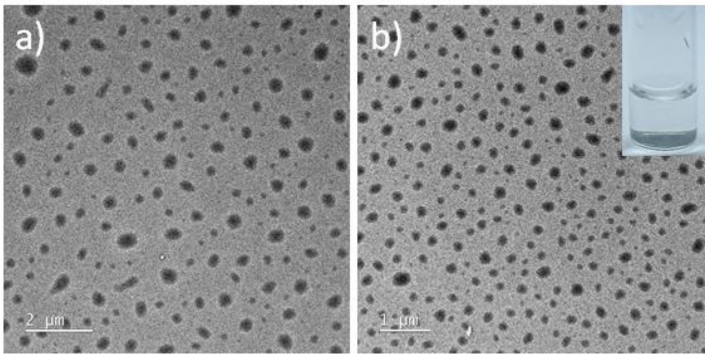


Figure S10: (a) and (b) TEM image of **P4** (C = 0.1 mg/mL) in water (inset: 0.1 mg/mL aqueous dispersion of **P4**).

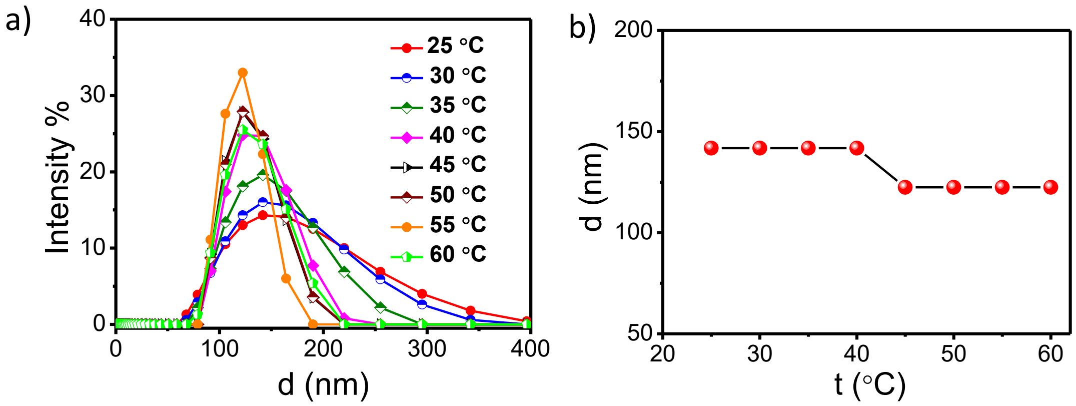


Figure S11: (a) Variable-temperature DLS plot of **P1** polymer in water from 25 ºC to 60 ºC; (b) Plot of variation of hydrodynamic diameter of **P1** with increasing temperature derived from Figure S11a.

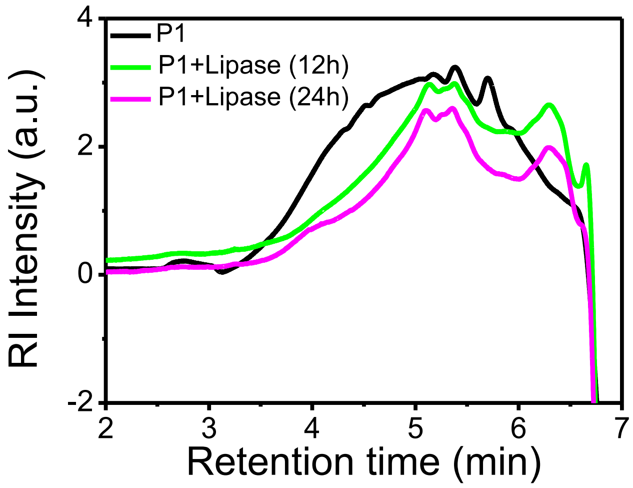


Figure S12: SEC plots of **P1** polyester before and after incubation with Lipase B from *Pseudomonas cepacia* for 12 h and 24 h with DMF as the eluent.

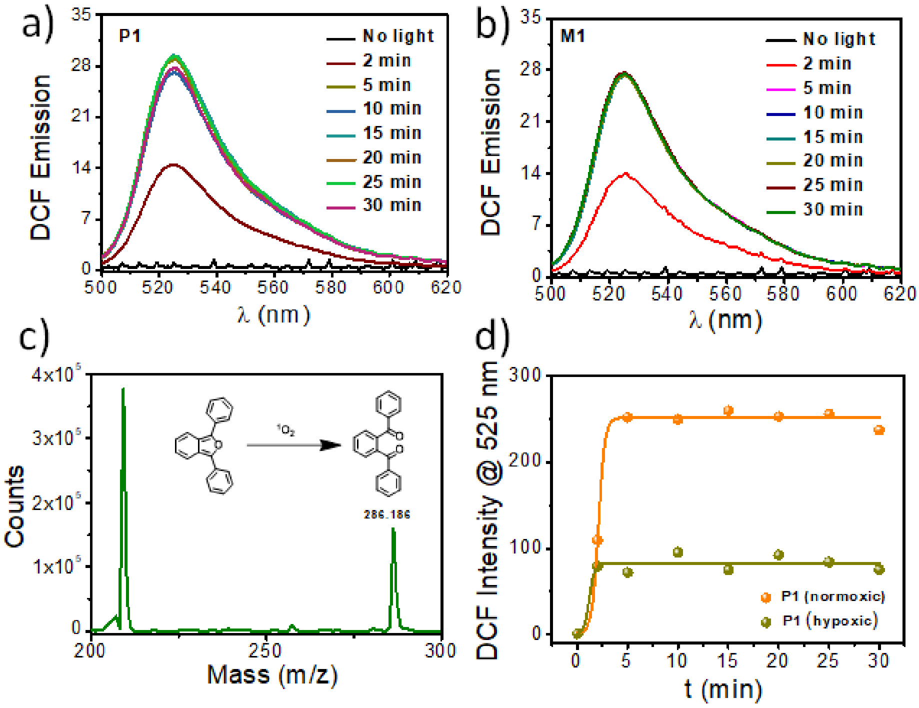


Figure S13: DCFH assay: Time-dependent normalized emission spectra of in situ generated DCF from an aqueous solution of (a) **P1** (*C* = 0.4 mg/mL) and (b) **M1** (*C* = 28 mM, which is equivalent to the concentration of photosensitizer present in 0.4 mg/mL of **P1**; (c) GC-MS spectrum showing a prominent mass peak at 286.186 corresponding to 1, 2-dibenzoyl benzene, upon photoirradiation of **P1** in the presence of Diphenylisobenzofuran (DPBF) by 427 nm light for 5 minutes; (d) Plot of DCF emission @ 525 nm at various time intervals upon irradiation of DCFH-treated **P1** in normoxic (aerial atmosphere) and hypoxic (inert atmosphere) conditions (*C* = 0.4 mg/mL).

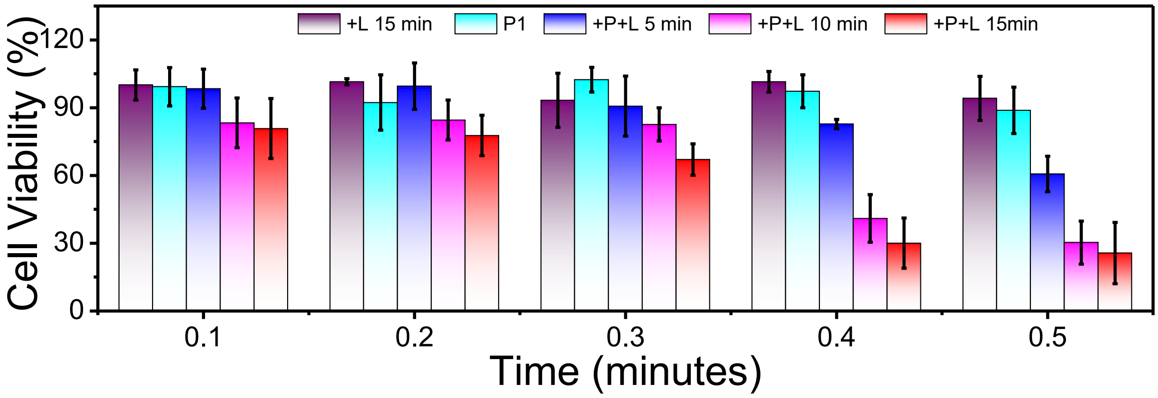


Figure S14: MTT assay of **P1** incubated with HeLa cells after different irradiation times at varying **P1** concentrations from 0.1 to 0.5 mg/mL (+L: cells irradiated with light in the absence of **P1**).

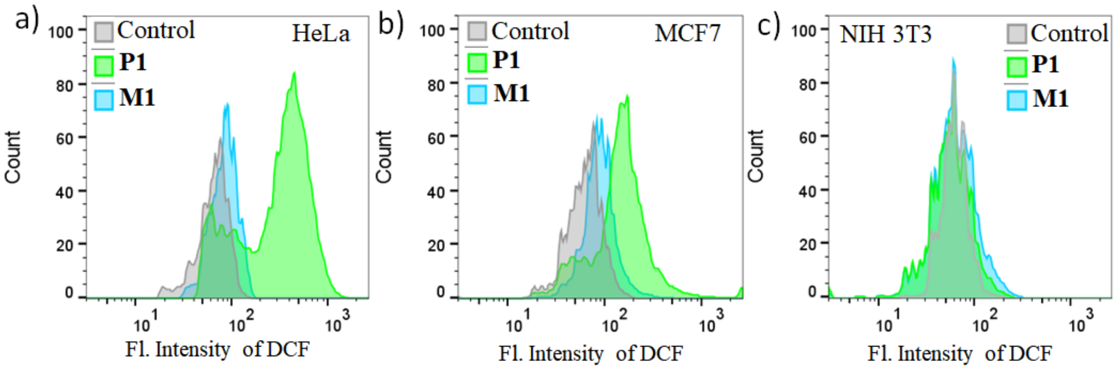


Figure S15: FACS analysis showing relative fluorescence intensity variation of DCF in different cell lines (a) HeLa; (b) MCF7 and (c) NIH 3T3 for **P1** and **M1** upon photoirradiation for 10 minutes (λ = 427 nm) (Concentration of DCFH-DA added is 20 μM).

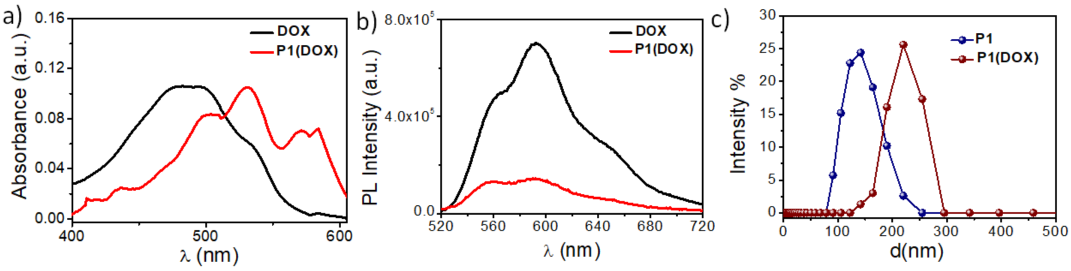


Figure S16: (a) UV-vis absorbance of free DOX (black) and **P1** encapsulated DOX (red); (b) Absorbance-normalized emission spectra of **P1** and free DOX ($\lambda_{ex.}$= 480 nm) in water; (c) Comparison of DLS spectra of **P1** and DOX-encapsulated **P1** in water.

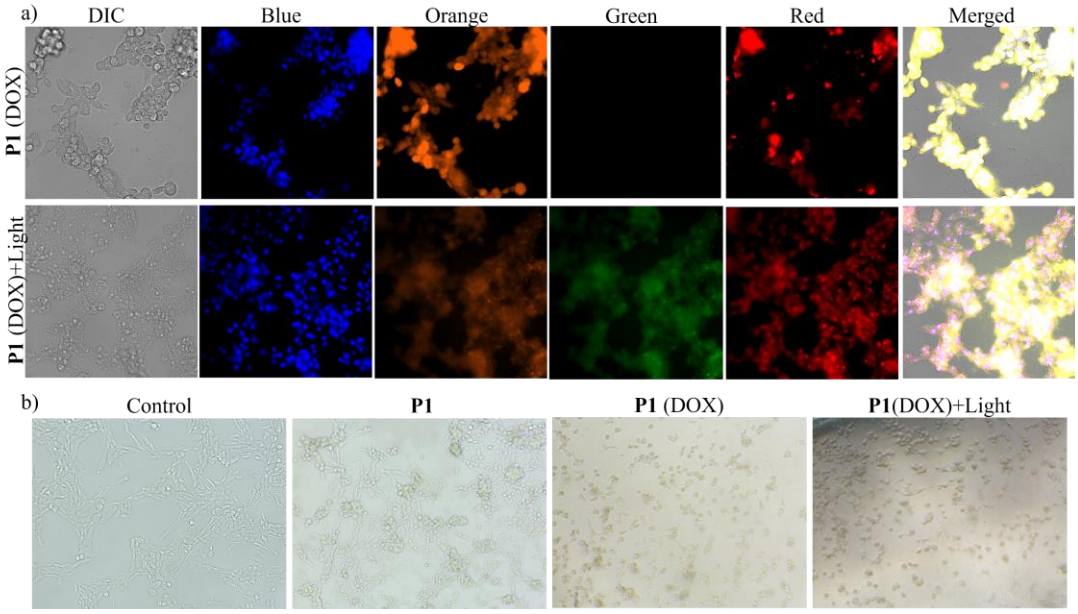


Figure S17: (a) Fluorescence microscopy images of MCF7 cells after incubation with **P1** for 24 h at 37 °C followed by 10 minutes of light irradiation (λ = 427 nm); Left to right images: differential interference contrast (DIC); blue-channel emission for the nuclei staining, Hoechst 33342; orange emission for **P1** polymer; green-channel emission for DCF dye; red-channel emission for the DOX emission and their overlay in the merged images; (b) Optical microscopy images showing loss of membrane integrity of MCF7 cell line in the presence of **P1** (DOX) and light irradiated **P1** (DOX).

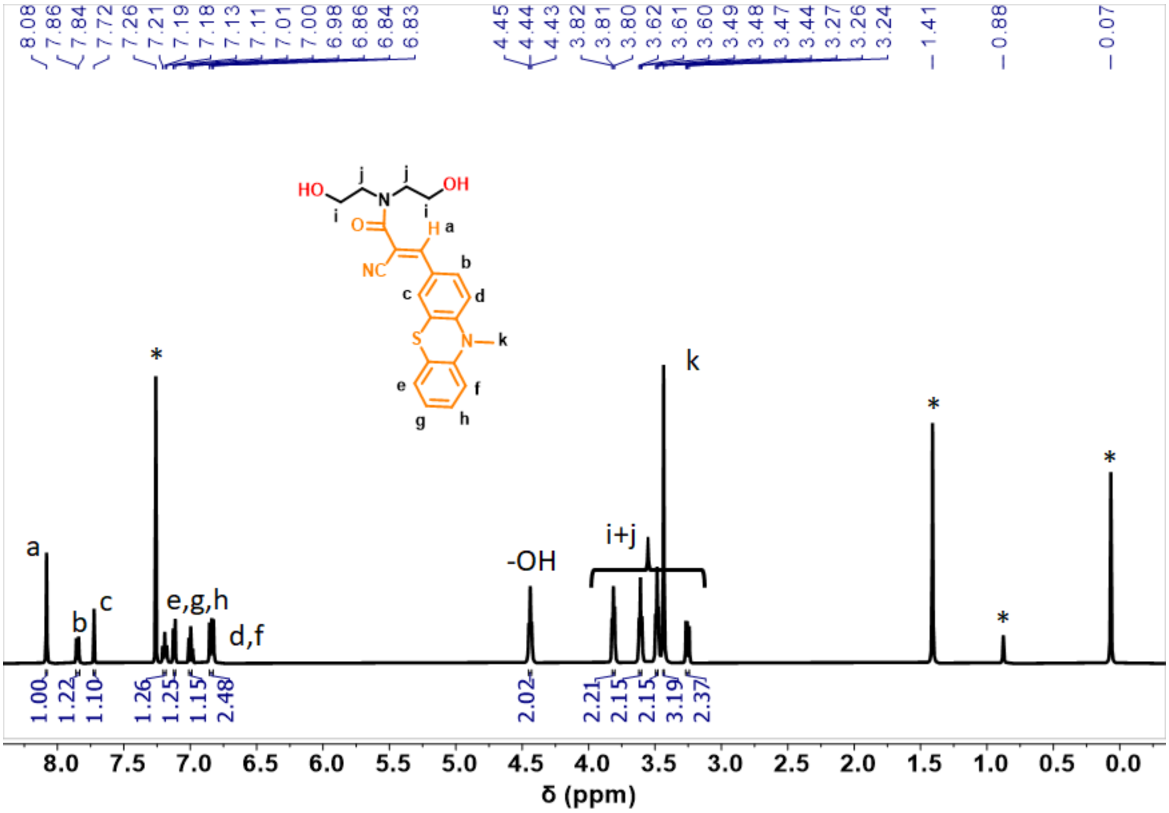


Figure S18: ^{1}H NMR spectrum of **M1** in $CDCl_3$. (*) indicates residual solvent peaks.

References

1) C. Chakraborty, A. Rajak and A. Das, *Nanoscale*, 2024, **16**, 13019.
2) S. Biswas and A. Das, *Chem. – A Eur. J.*, 2023, **29**, e202203849.
3) a) P. S. Pramod, Ruchira Shah and M. Jayakannan, *Nanoscale*, 2015,**7**, 6636; b) S. Biswas, P. Rajdev, A. Banerjee and A. Das, *Biomacromolecules,* 2025, **26**, 4661.