Supporting Information

for

Synthesis of Amphiphilic Cationic Polyesters and Their Antibacterial Activity

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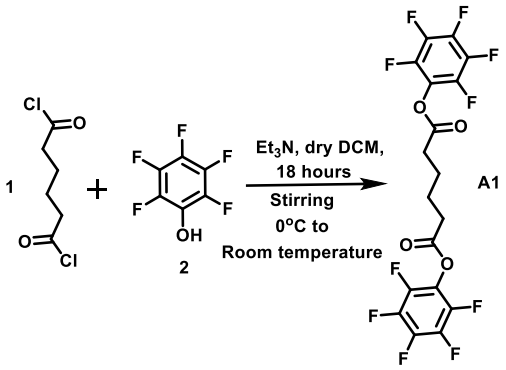
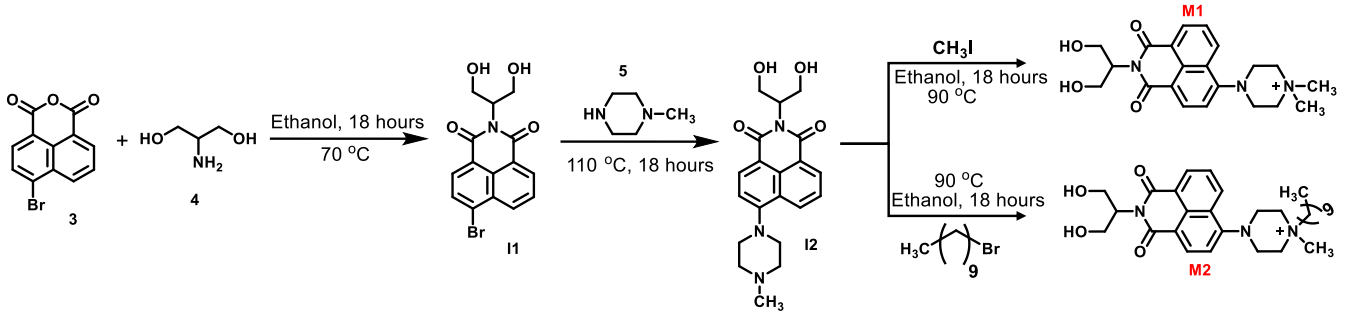
Materials and methods: All reagents were obtained from commercial suppliers and used without further purification unless otherwise mentioned. Enzyme lipase from *Pseudomonas cepacia* was purchased from Sigma Aldrich. 4-Bromo-1,8-naphthalic anhydride, Pentafluorophenol, Adipoyl chloride, 2-amino-1,3-propanediol, Diethanolamine, 2-Ethylhexyl acrylate, and 1-Methylpiperazine were purchased from TCI Chemicals. 4-Dimethylaminopyridine (DMAP) was purchased from Avra Synthesis Pvt. Ltd. 1-Bromodecane, Iodomethane, and Piperidine were procured from Spectrochem Chemicals. All the solvents were dried properly following standard procedures before setting up the reactions. ^{1}H NMR, ^{13}C NMR, and ^{19}F NMR spectra were recorded on a Bruker Ascend 600 MHz or 400 MHz spectrometer using deuterated solvents from Eurisotop. Chemical shifts (δ) are reported in a ppm unit with TMS as the internal standard. The coupling constants (J) are reported in hertz (Hz). Column chromatography was carried out on silica gel (100-200 mesh). Number average molecular weight (M_n) and dispersity ($Đ$) of the polymers were measured by size exclusion chromatography (SEC) using 0.5 wt% LiBr mixed *N*, *N*-Dimethylformamide (DMF) as an eluent at 75 °C with a flow rate of 0.5 mL/min, where the GPC instrument contained a Waters 515 HPLC pump, a Waters 2414 refractive index (RI) detector, one PolarGel-M guard column (5037.5 mm) and two Polar Gel-M analytical columns (30037.5 mm). FT-IR spectra were recorded in a PerkinElmer Spectrum 100 FT-IR Spectrometer. UV-Vis spectra were recorded in a JASCO V750 spectrophotometer. Fluorescence spectra were recorded in a FluoroMax-3 spectrophotometer, from Horiba Jobin Yvon. Spectroscopic grade solvents were used for UV-Vis and photoluminescence studies. Transmission Electron Microscopy (TEM) studies were captured in a JEOL-2010EX machine operating at an accelerating voltage of 200 kV. Dynamic light scattering (DLS) and Zeta potential measurements were carried out in the Malvern instrument. SEM images of bacteria samples were captured using JEOL-JSM-7500F field Emission scanning electron microscopy (SEM). HRMS were done on XEVO G2-XS Q Tof and Micromass Q-Tof Micro machine. The absorbance of the MTT assay at 570 nm and the Haemolysis assay at 414 nm were monitored by the microplate reader (VARIOSKAN, Thermo Fisher). Confocal laser scanning microscopy (CLSM) images were collected in the Leica TCS SP8 microscope.

Synthesis and Characterization



Scheme S1: Synthetic scheme for activated diester monomer **A1**.

Synthesis of Bis(perfluorophenyl) adipate (A1): The synthetic procedure has been followed from our earlier work. [1] To a solution of pentafluorophenol (compound **2**) (5.03 g, 0.027 mol) in 20 mL freshly dried DCM, 4.56 mL of triethyl amine (0.033 mol) was added and the mixture was stirred at the ice-cold condition for a few minutes. To this, 1.58 mL of the adipoyl chloride (compound **1**) (0.011 mol) dissolved in 20 mL freshly dried DCM was added dropwise. The whole mixture was kept stirring for 18 hours, initially at ice-cold conditions and then at room temperature. It was followed by a workup in DCM/water mixture (3 x 40 mL), from which the organic layer was collected and concentrated. The crude product was purified by column chromatography. The desired product was eluted with a 4% ethyl acetate/hexane mixture and was finally collected as a crystalline white solid in a 48% yield. ^{1}H NMR (400 MHz, Chloroform-*d*): δ 2.76 (m, 2H), 1.93 (m, 2H). ^{19}F NMR (377 MHz, Chloroform-*d*): δ -153.40 (d, J = 21.4 Hz), -158.44 (t, J = 21.6 Hz), -162.80 (t, J = 21.3 Hz). Melting Temperature (T_m): 82 °C. HRMS (ESI): m/z calculated for $[M+Na]^+$: 501.0161, Experimentally obtained: 501.0160.



Scheme S2: Synthetic scheme for monomers **M1** and **M2**.

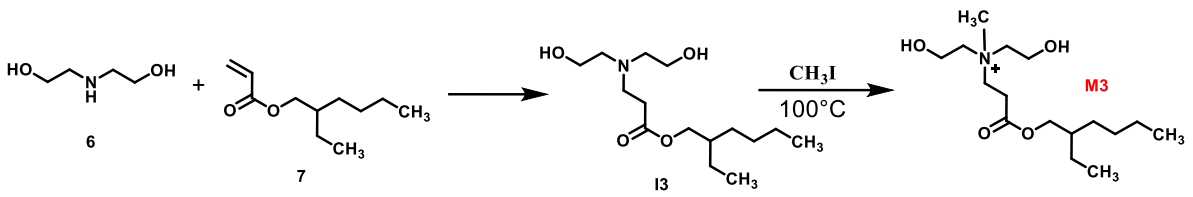
Synthesis of Compound I1: 2-amino-1,3-propanediol (compound **4**) (0.55 mL, 7.2 mmol) was added dropwise to a suspension of 4-bromo-1,8-naphthalic anhydride (compound **3**) (1 g, 3.6 mmol) dissolved in 14 mL EtOH with continuous stirring. The mixture was refluxed for 18 hours under an inert atmosphere.[2] Workup was done in an

ethyl acetate/water medium where the ethyl acetate part was collected and passed through sodium sulfate and was then concentrated to obtain a light yellowish-colored compound (1.2 g, yield: 96%). ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.59-8.56 (m, 1H), 8.38-8.33 (m, 1H), 8.25-8.23 (m, 1H), 8.03-8.0 (m, 1H), 5.24-5.19 (m, 1H), 4.81-4.79 (m, 2H), 3.98-3.93 (m, 2H), 3.83-3.78 (m, 2H). Melting Temperature (T_m): 242 °C. HRMS (ESI-TOF) m/z calculated for $[M+Na]^+$: 371.985; Experimentally obtained: 371.985.

Synthesis of Compound I2: Compound **I1** (650 mg, 1.86 mmol) was mixed with 1-methylpiperazine (compound **5**) (618 µL, 5.58 mmol) in a 10 mL round bottom flask and was stirred at 110 °C under reflux under an inert atmosphere for 18 hours. The solid product obtained was then purified by column chromatography using 5% methanol in dichloromethane as an eluent to obtain a yellow solid as the pure product (1.15 g, yield: 92%). ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.45 (d, J = 7.3 Hz, 1H), 8.43 (d, J = 8.3 Hz, 1H), 8.38 (d, J = 8.0 Hz, 1H), 7.82-7.80 (m, 1H), 7.34 (d, J =8.1 Hz, 1H), 5.24 – 5.19 (m, 1H), 4.77-4.75 (m, 2H), 3.95-3.91 (m, 2H), 3.83-3.79 (m, 2H), 3.28 – 3.19 (m, 4H), 2.71-2.66 (m, 4H), 2.31 (s, 3H). Melting Temperature (T_m): 310 °C. HRMS (ESI-TOF) m/z calculated for $[M+H]^+$ 370.177; Experimentally obtained: 370.177.

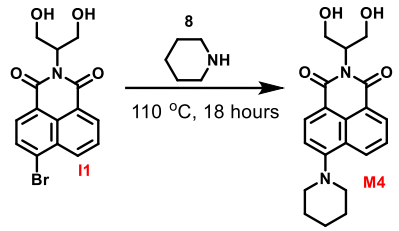
Synthesis of Monomer M1: Compound **I2** (250 mg, 0.677 mmol) was taken in a clean and dry polymer vessel, to which 500 µL of iodomethane was added into the vessel with stirring under an inert atmosphere for 24 hours at room temperature. The next day, another 500 µL was added to the mixture, and the reaction was run for another 24 hours. Workup was done in chloroform/water medium from where the aqueous part was collected and lyophilized to yield yellow colored solid compound (200 mg, yield: 77%). ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.50-8.48 (m, 2H), 8.42 (d, J = 8.0 Hz, 1H), 7.88-7.85 (m, 1H), 7.46 (d, J = 8.0 Hz, 1H), 5.24 – 5.21 (m, 1H), 3.94 – 3.92 (m, 2H), 3.83 – 3.80 (m, 2H), 3.71-3.63 (m, 4H), 3.51-3.38 (m, 6H), 3.25-3.17 (m, 4H). Melting Temperature (T_m): >350 °C HRMS (ESI-TOF) m/z calculated for $[M]^+$ 384.192; Experimentally obtained: 384.144.

Synthesis of Monomer M2: Compound **I2** (880 mg, 2.38 mmol), n-decyl bromide (1.48 mL, 7.15 mmol), and 5 mL ethanol were taken in a clean, dry polymer vessel and stirred under an inert atmosphere for 24 hours at 90 °C temperature. Workup was then done in chloroform/water medium and the aqueous part was collected and concentrated to yield a fluorescent, yellow-colored solid pure compound (1.2 g, yield: 98%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.52 – 8.41 (m, 3H), 7.88-7.84 (m, 1H), 7.51 – 7.45 (m, 1H), 5.24-5.21 (m, 1H), 4.78-4.75 (m, 2H), 3.97-3.91 (m, 2H), 3.85-3.71 (m, 6H), 3.65-3.52 (m, 6H), 3.22 (s, 3H), 1.78-1.72 (m, 2H), 1.36 – 1.24 (m, 14H), 0.88-0.85 (m, 3H). Melting Temperature (T_m): 310 °C; HRMS (ESI-TOF) m/z calculated for $[M]^+$ 510.333; Experimentally obtained: 510.333.



Scheme S3: Synthetic scheme for monomer **M3**.

Synthesis of Monomer M3: 2-Ethylhexyl Methacrylate (compound **7**) (1.127 mL, 5.426 mmol) was added to diethanol amine (compound **6**) (0.576 mL, 5.96 mmol) in a polymer vessel and stirred for 24 hours. Workup was done in DCM/water medium. A colourless liquid (Compound **I3**) was obtained after evaporating the DCM layer. In a sealed tube, the compound **I3** was taken (544.9 mg, 1.88 mmol), and to this, iodomethane (0.555 mL, 8.87 mmol) was added dropwise. The reaction was kept stirring for 3 days at 100 °C. A workup was then done in DCM/water medium and the aqueous part was collected and concentrated to obtain the product in 70% yield as a light oil. ^{1}H NMR (600 MHz, Chloroform-d): δ 4.19-4.16 (m, 4H), 4.05-3.98 (m, 2H), 3.92 (t, J = 7.7 Hz, 2H), 3.85 – 3.78 (m, 4H), 3.37 (s, 3H), 2.98 (t, J = 7.7 Hz, 2H), 1.62-1.58 (m, 1H), 1.43 – 1.23 (m, 8H), 0.91-0.87 (m, 6H). HRMS (ESI-TOF) m/z calculated for $[M]^+$ 304.248; Experimentally obtained: 304.283.



Scheme S4: Synthetic scheme for monomer **M4**.

Synthesis of Monomer M4: Compound **I1** (300 mg, 0.856 mmol) was added to piperidine (Compound **8**) (1.712 g, 20.105 mmol) in a 50 mL round bottom flask and was refluxed at 110 °C under an inert atmosphere for 18 hours. The solid product obtained was then purified by column chromatography using dichloromethane/methanol as an eluent to obtain a yellow solid as the pure product in 82% yield. ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.45 (d, J = 7.3 Hz, 1H), 8.41 (d, J = 8.4 Hz, 1H), 8.37 (d, J = 8.1 Hz, 1H), 7.81 (t, J = 7.8 Hz, 1H), 7.32 (d, J = 8.1 Hz, 1H), 5.22 (h, J = 6.3 Hz, 1H), 4.74 (t, J = 5.9 Hz, 2H), 3.95-3.91 (m, 2H), 3.83-3.79 (m, 2H), 3.21-3.17 (m, 4H), 1.85-1.82 (m, 4H), 1.69-1.65 (m, 2H). Melting Point: 178 °C. HRMS (ESI-TOF) m/z calculated for $[M+H]^+$ 355.166; Experimentally obtained: 355.203.

Synthesis of Polymer P1: A1 (124.42 mg, 0.260 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 (6.35 mg, 0.052 mmol) and **M1** (100 mg, 0.260 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 hours. The crude polymer obtained was dissolved in chloroform, purified by precipitation from the cold ether, and collected after drying under reduced pressure to yield 76 mg of the pure polymer as a sticky mass. ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.55-8.37 (b, 3H), 7.88-7.80 (m, 1H), 7.45-7.36 (m, 1H), 5.48-5.43 (m, 1H), 4.63-4.48 (b, 4H), 3.90-3.76 (m, 4H), 2.97 –2.62 (m, 6H), 2.24-1.93 (b, 4H), 1.43-1.16 (b, 4H). Experimental M_n from SEC analysis = 18,600 g/mol, $Đ$ = 1.29 with respect to the polystyrene standard and DMF as eluent with 0.5% LiBr at 75 °C, which appears to be overestimated due to the trailing in the column and probable aggregation in the DMF solvent during SEC analysis. Unavoidable trailing in the SEC profile did not allow accurate molecular weight determination.

Synthesis of Polymer P2 (P2a/P2b): A1 was dissolved in 100 µL of dry DMF in two separate polymer vessels as (93.63 mg, 0.1958 mmol) for **P2a** and (88.95 mg, 0.186 mmol) for **P2b**. These solutions were kept in a water bath at 50 °C for 5 minutes to ensure complete dissolution. To these solutions, DMAP (4.77 mg, 0.039 mmol) and **M1** (100 mg, 0.1958 mmol) were added. Those mixtures were then degassed with dried argon gas for 20 minutes before stirring it at 120 °C in oil bath for 48 hours. The crude polymers obtained were dissolved in chloroform, purified by precipitation from cold ether and collected after drying under reduced pressure to yield pure polymers **P2a** (83 mg) and **P2b** (64 mg) as sticky brown masses. ^{1}H NMR (**P2a**/**P2b**) (600 MHz, DMSO-*d*6): δ 8.59 – 8.38 (m, 3H), 7.87-7.81 (m, 1H), 7.51-7.46 (m, 1H), 5.47-5.42 (m, 1H), 4.67-4.46 (b, 4H), 3.86-1.73 (b, 17H), 1.45-0.78 (b, 4H). Experimental M_n for **P2a** from SEC analysis = 15,600 g/mol, $Đ$ = 1.23 with respect to the polystyrene standard and DMF as eluent with 0.5% LiBr at 75 °C. Experimental M_n for **P2b** from SEC analysis = 12,500 g/mol, $Đ$ = 1.27 with respect to the polystyrene standard and DMF as eluent with 0.5% LiBr at 75 °C. Theoretical M_n for **P2b** = 10,640 g/mol.[1] Unavoidable trailing in the column might lead to the deviation from the theoretical molecular weight.

Synthesis of Polymer P3: A1 (126 mg, 0.263 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 (6.4 mg, 0.052 mmol) and **M3** (100 mg, 0.260 mmol) were added. The whole mixture was then degassed with dried argon gas for 20 minutes before stirring it at 120 °C in an oil bath for 48 hours. The crude polymer obtained was dissolved in chloroform, purified by precipitation from the cold ether, and collected after drying under reduced pressure to yield 56 mg of the pure polymer as a sticky mass. ^{1}H NMR (600 MHz, $CDCl_3$): δ 4.69-4.55 (b, 4H), 4.03-4.00 (m, 1H), 3.37-2.93 (b, 11H), 2.52-2.33 (b, 4H), 1.37-1.20 (b, 12H), 0.93-0.82 (b, 6H). The poor solubility of these polymers in common organic solvents did not allow to determine the molecular weight by SEC analysis.

Synthesis of Polymer P4a: A1 (100 mg, 0.209 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 (5.1 mg, 0.042 mmol) and **M4** (75.95 mg, 0.209 mmol) were added. The whole mixture was then degassed with dried argon gas for 20 minutes before stirring it at 120 °C in an oil bath for 48 hours. The crude polymer

obtained was dissolved in chloroform, purified by precipitation from cold ether and collected after drying under reduced pressure to yield 89 mg of the pure polymer as sticky mass. ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.54-8.40 (m, 3H), 7.69-7.67 (m, 1H), 7.18 (s, 1H), 5.59 (b, 1H), 4.64 (b, 4H), 3.25 (b, 4H), 2.37 –1.28 (b, 14H). Experimental M_n for **P4a** from SEC = 9900 g/mol, $Đ$ = 1.28 with respect to polystyrene standard and DMF as eluent.

Synthesis of Polymer P4b: A1 (128.52 mg, 0.2687 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 (6.56 mg, 0.054 mmol) and **M4** (100 mg, 0.282 mmol) were added. The whole mixture was then degassed with dried argon gas for 20 minutes before stirring it at 120 °C in an oil bath for 48 hours. The crude polymer obtained was dissolved in chloroform, purified by precipitation from the cold ether, and collected after drying under reduced pressure to yield 76 mg of the pure polymer as sticky mass. ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.53-8.40 (m, 3H), 7.69-7.65 (m, 1H), 7.17 (s, 1H), 5.58 (b, 1H), 4.67 (b, 4H), 3.24 (b, 4H), 2.29 –1.28 (b, 14H). Experimental M_n for **P4b** from SEC analysis = 7600 g/mol, $Đ$ = 1.58 with respect to polystyrene standard and DMF as eluent. Theoretical M_n for **P2b** = 10,320 g/mol.[1]

Synthesis of Polymer P5: A1 (152.6 mg, 0.32 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 (13 mg, 0.106 mmol), **M3** (51.2 mg, 0.167 mmol), and **M4** (59.3 mg, 0.167 mmol) were added. The whole mixture was then degassed with dried argon gas for 20 minutes before stirring it at 120 °C in an oil bath for 48 hours. The crude polymer obtained was dissolved in chloroform, purified by precipitation from the cold ether, and collected after drying under reduced pressure to yield 52 mg of the pure polymer as a sticky mass. ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.62-8.33 (m, 3H), 7.74-7.64 (m, 1H), 7.23-7.14 (m, 1H), 5.64-5.54 (b, 1H), 4.69-4.55 (b, 4H), 4.31-4.15 (b, 2H), 3.31-3.26 (m, 4H), 2.71 –1.15 (m, 4H), 0.91-0.837 (m, 6H). The poor solubility of this polymer in common organic solvents did not allow for obtaining the SEC data.

Experimental Section

Solution Preparation for UV-Vis, PL, DLS and Zeta Potential Measurements: A measured amount of **M1**, **M2**, **P1, P2 (P2a/P2b), P3, P4a,** and **P5** was taken in a clean and dry glass vial, and to it a fixed volume of HPLC-grade methanol was added to make the stock solutions. From there, measured quantities were taken out into a vial and methanol was evaporated by heating the vial at 70 °C to obtain a thin film, which was then dissolved in a measured amount of HPLC-grade water to attain the required concentrations after subjecting them to heating at the boiling temperature of water, i.e., 100 °C, and cooling at room temperature of 25 °C to ensure proper dissolution of the film. These solutions were then used for spectroscopy (UV-vis, PL), DLS, and zeta potential measurements.

FT-IR Study: Samples were prepared by directly dissolving the given polymer in HPLC grade methanol. The given solutions were then placed between two CaF_2 windows (path length = 0.2 mm) and spectral measurements were

carried out with scan range = 4000−1000 cm^{-1}, resolution = 1.0 cm^{-1}, number of scans = 64, and T = 25 °C. For the enzymatic degradation study, the sample was prepared by lyophilizing the phosphate buffer solution of **P2a** pretreated with enzyme lipase.

Degradation Study: 1mg/mL solution of **P2a** polymer was prepared in H_2O: D_2O (3:1), to which 500 μL of enzymatic solution of lipase from *Pseudomonas cepacia* (6.6 mg/mL in phosphate buffer solution of pH = 7.4) was added. In another batch, 500 μL of phosphate buffer solution (pH=7.4) without any enzyme was added. Both the samples were incubated at 37 °C for 24 hours at 200 rpm before lyophilizing and recording their SEC traces.[1,3,4]

Bacterial Preparation: *S. aureus* (ATCC 25923, MTCC 96), *E. coli* (ATCC 25922), *E. coli* (Top10) (ATCC-PTA-10989), and *E. coli* (DH5α) (ATCC 68233) were grown aerobically in Luria−Bertani (LB) agar/broth at 37 °C. Bacteria were harvested at the logarithmic growth phase with optical densities (OD) from 0.6 to 1.0 at 600 nm.

Minimum Inhibitory Concentration (MIC) Determination: The lowest concentration of **P1**, **P2** series, **P3** and **P5** polyesters required to fully inhibit the growth of bacteria is defined as the MIC. It was determined by a broth microdilution assay.[5] Stock solutions of **P1**, **P2** (**P2a** and **P2b**), **P3,** and **P5** (*C* = 2.0 mg/mL) were prepared in aqueous medium following the procedure described before. Serial dilutions were made to get different concentrations of the sample in LB broth (50 μL), with the final addition of 50 μL bacterial solutions to each well of noncoated polystyrene 96-well plates with a final inoculum concentration of 0.5×10^6 CFU/mL. The plates were incubated at 37 °C for 18 hours with gentle shaking at 180 rpm. The MIC was taken as the lowest concentration of polyester at which there was a 100% reduction in growth. Broth alone and broth containing only bacterium cells were used as sterility control and growth control, respectively. All experiments were performed in triplicate.

Minimum Bactericidal Concentration (MBC) Determination: After the determination of MIC, solutions from different wells (MIC, 2MIC, 4MIC, 8MIC, 16MIC, control) were taken out and spread into LB agar plate, previously differentiated into separate regions. The plates were incubated aerobically at 37 °C overnight and the bacterial growths were observed through the naked eye. This experiment was also performed in triplicate.

Zone Inhibition Assay: 100 μL of bacterial suspension in LB broth ($c = 5x10^8$ CFU/ mL, $OD_{600} = 0.5$) was spread on an LB agar plate by a glass spreader to prepare bacterial lawn. The holes were then cut out on this agar plate and 100 μL of 2 mg/mL solutions of polymers and their respective dye concentration monomers were added carefully and aerobically kept at 37 °C overnight before taking the pictures.

Kinetic Growth Assay: A noncoated polystyrene 96-well plate was prepared exactly as described in the MIC determination. It was then subjected to incubation at 37 °C with gentle shaking at 180 rpm. Every hour, the absorbance values were measured in the microplate reader (VARIOSKAN, Thermo Fisher) at 600 nm. The final

readings were adjusted by subtracting the absorbance values of the polymers serially diluted with the LB broth, maintaining similar conditions in the absence of any bacteria.

Scanning Electron Microscopy (SEM): Bacterial [*E. coli* (ATCC 25922) and *S. aureus* (ATCC 25923)] suspensions in LB broth (500 μL, 10^9 CFU/mL) were incubated with 500 μL of polymer solution (concentration of stock = 8xMIC, and final concentration will be 4xMIC) for 2 h at 37 °C, and then the suspensions were centrifuged at 4000 rpm for 10 min. The precipitates were washed with 1mL of 0.85% NaCl solution twice. The pellets were dissolved in 1mL of 2.5% glutaraldehyde in PBS and incubated at rt for 30 min and then overnight at 4 °C. Next, the pellets were collected by centrifugation and washed with DI water twice. Subsequently, dehydration of the sample was performed in different ethanol grades (10, 30, 50, 70, 80, 90, and 100%, 15 min each step). Samples were drop-cast on glass plates, and images were recorded using a JEOL-JSM-7500F field-emission scanning electron microscope at 0.3 kV for *E. coli* (ATCC 25922) and 1 kV for *S. aureus* (ATCC 25923).

Live/Dead Assay Using Fluorescence Microscopy: *E. coli* (ATCC 25922), and *S. aureus* (ATCC 25923) were grown to the late-log phase ($OD_{600} \sim 1$) and then were collected by centrifugation at 4000 rpm for 10 min at room temperature. The cell pellet was resuspended in 0.85% NaCl followed by adjusting the OD_{600} in such a way that the concentration was 10^9 CFU/mL for both microbes. Subsequently, ten times serial dilution was done to get the bacterial suspension with a concentration of 10^8 CFU/mL. Then, 300 μL of this suspension was mixed with 300 μL of a polymer solution (Conc. of stock = 8× MIC and final Conc. = 4x MIC) and incubated for 2 hours at 37 °C. An aliquot of 10^8 CFU/mL of both bacteria was used as a live-cell control and incubated under the same conditions but in the absence of the polymer **P2a**. After incubation, 1.0 μL of a 1:1 (v/v) mixture of SYTO-9 (3.34 mM in DMSO) and propidium iodide (20 mM in DMSO) was added to each of the 200 μL bacterial suspension in 0.85% NaCl solution except the one where only propidium iodide (20 mM in DMSO) was used and no SYTO-9 was used. These were then incubated for 30 min in the dark. Subsequently, the extra dye was washed with 0.85% NaCl solution and then 10 μL of this stained bacterial suspension was drop-cast on a clean microscopic glass slide, covered with a glass lid, and examined under a confocal laser scanning microscope in Leica TCS SP8.

Cytotoxicity Assay Using MTT: Cytotoxicity assay was performed with the polymers **P1** and **P2a** and the monomers **M1** and **M2**. HeLa cells in complete DMEM medium were seeded in 96-well plates with a cell density of $1x10^4$ cells/well and incubated for 24 h at 37 °C in a 5 % CO_2 incubator. After 24 h of incubation, the medium was carefully replaced by 100 μL of the different concentrations of polymer and monomer solutions and the cell plates were incubated for 24 h. After that, polymer/monomer-containing media was removed and 100 μL DMEM medium was added, and then 50 μL of freshly prepared 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution (5 mg/mL in DMEM) was added into each well and incubated for 4 h at 37 °C. Next, the medium with the MTT solution was carefully removed and 100 μL of DMSO was added into each well, and the plate was gently shaken for 10 min at room temperature to dissolve all formed precipitates. The absorbance of MTT

at 570 nm was recorded by a microplate reader (VARIOSKAN, Thermo Fisher). Cell viability was calculated by the ratio of absolute absorbance of the cells incubated with the polymer/monomer solution to that of the cells incubated with only the culture medium.

Hemolysis Assay: This experiment was done as per the approved protocol of the Animal Ethics Committee of Indian Association for the Cultivation of Science (IACS), Kolkata. RBCs from a healthy 6-week-old BALB/c mice (1.0 mL) were suspended in 9 mL of PBS buffer (pH 7.4) and centrifuged at 2000 rpm for 5 min. The supernatant was removed by pipetting and RBCs were resuspended in PBS. This procedure was repeated twice. Finally, the RBC pellet was resuspended in 10 mL of PBS buffer and again diluted four times to get a stock solution where RBC was 2.5% v/v. Then, solutions of **M1**, **M2**, **P1,** and **P2** series polyesters in PBS buffer (50 μL) were prepared by serial dilution in a sterile 96-well flat-bottom polystyrene plate and the RBC suspension (150 μL) was added to each well and incubated at 37 °C with shaking at 180 rpm for 1 hour. Triton X-100 (0.1% v/v in water) was used as the positive lysis control, and PBS buffer was used as the negative control. Three columns containing the polyesters were diluted following the same conditions and instead of any RBC, 150 μL PBS was added to consider as control wells. The supernatant (100 μL) from each well was transferred to another sterile 96-well flat-bottom polypropylene plate. The absorbance of the released hemoglobin at 414 nm was measured using a Varioskan microplate reader (Thermo Fisher). Then the absorbance of the control wells was subtracted from the absorbance of the wells containing RBC and polymers/monomers to obtain the actual absorbance for the lysed RBC. Then the percentage of hemolysis was calculated relative to the positive control and negative control solvents after subtraction of the absorbance of the polyesters at 414 nm.

% of Hemolysis = [Absorbance of lysed RBC after treatment with polymer/monomer-Absorbance of only polymer/monomers – Absorbance of lysed RBC in PBS solution]/ [Absorbance of lysed RBC after treated with 0.1% triton X 100 – Absorbance of lysed RBC in PBS solution]x100.

Additional Figures

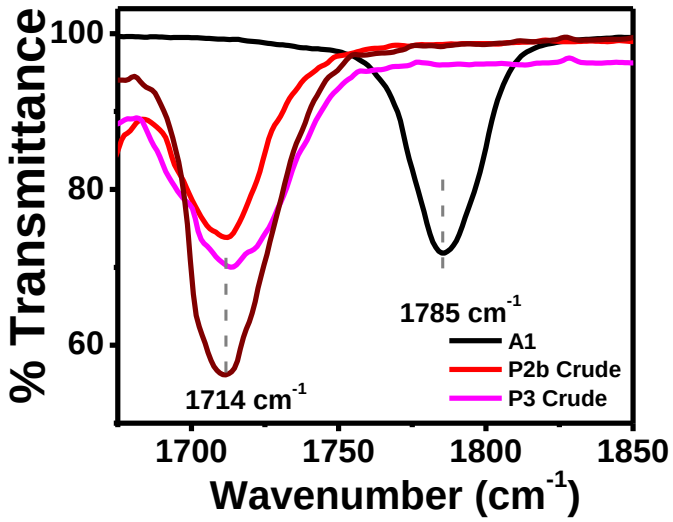


Figure S1: Comparison of the FT-IR spectra of crude **P2b** and crude **P3** with **A1** shows complete consumption of the activated ester peak (1785 cm^{-1}) and formation of new backbone esters (1714 cm^{-1}).

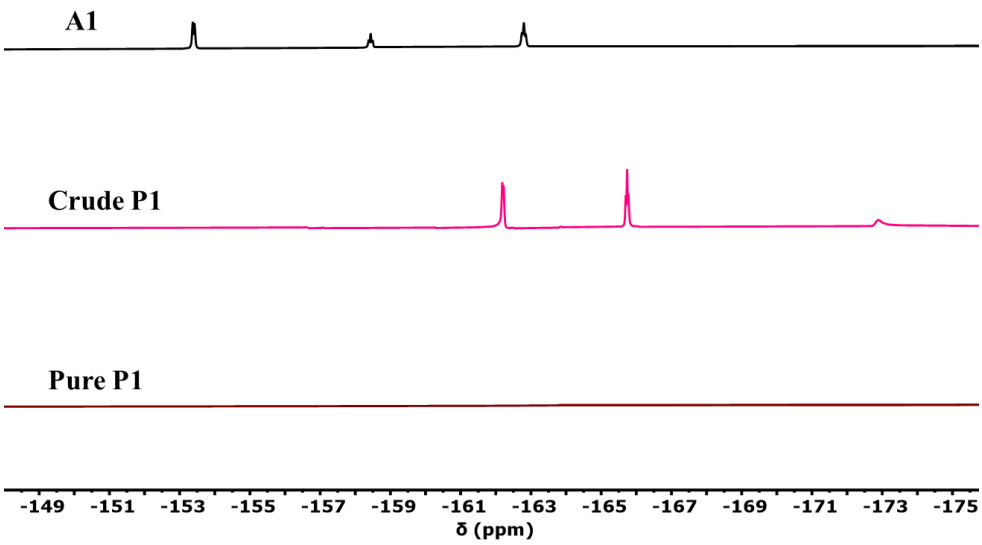


Figure S2: Stacked ^{19}F NMR spectra of crude and purified **P1** with **A1** in DMSO-d_6.

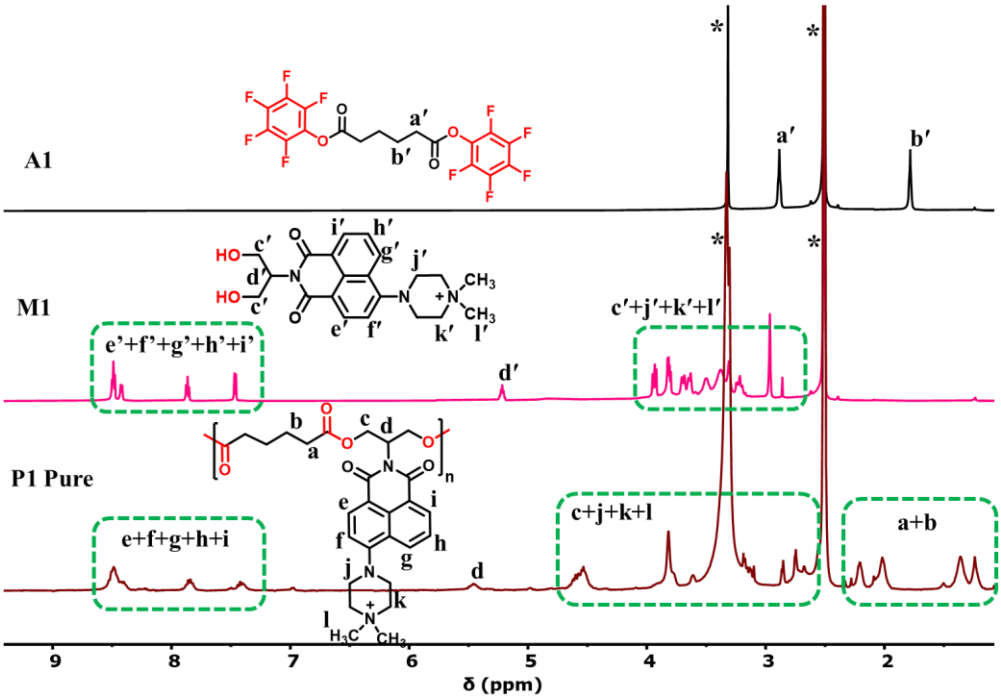


Figure S3: Stacked ^{1}H NMR spectra of purified **P1**, **A1** and **M1** in DMSO-d_6.

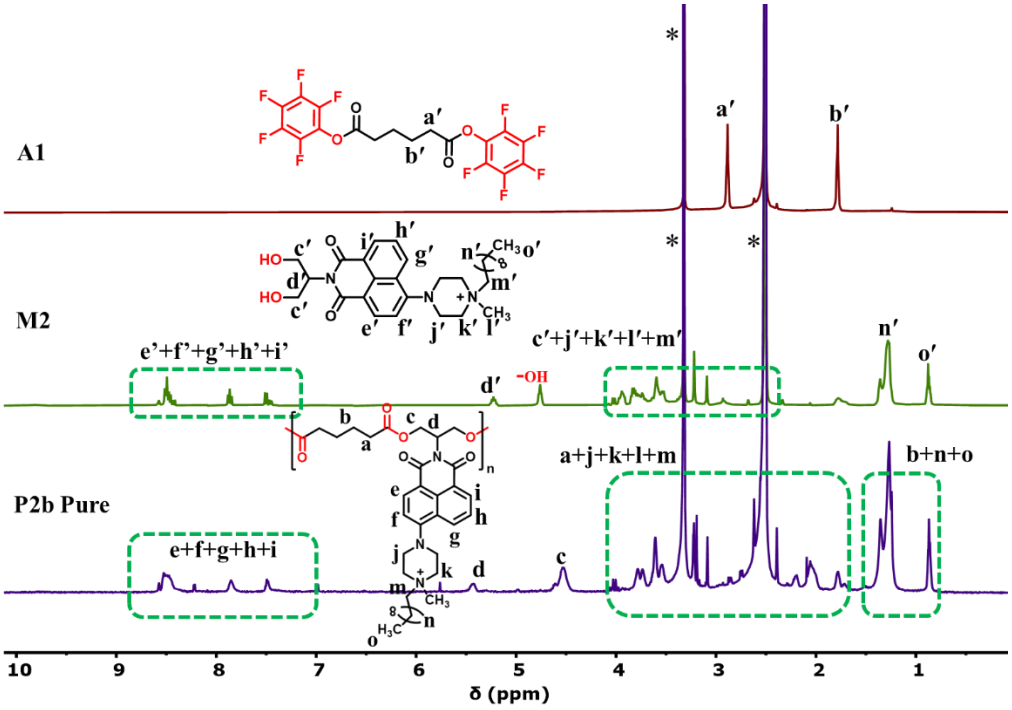


Figure S4: Stacked ^{1}H NMR spectra of purified **P2b**, **A1** and **M1** in DMSO-d_6.

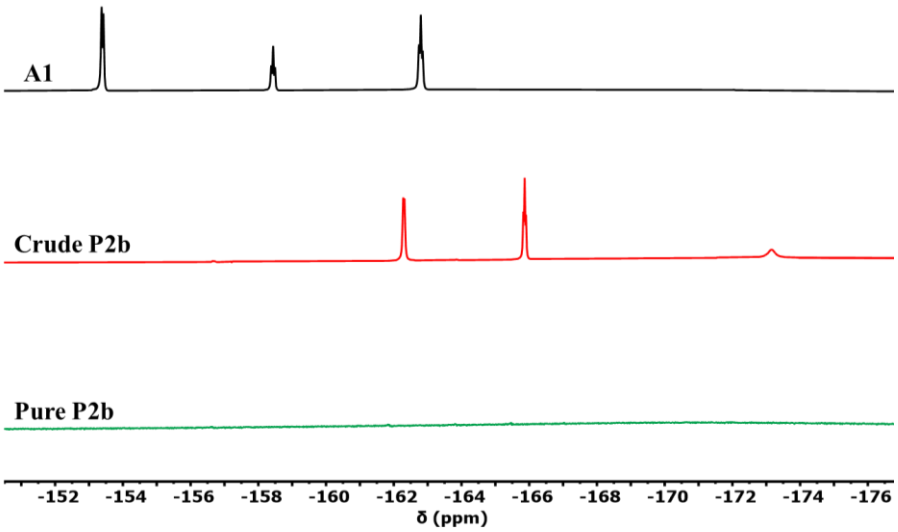


Figure S5: Stacked ^{19}F NMR spectra of crude and purified **P2b** with **A1** in DMSO-d_6.

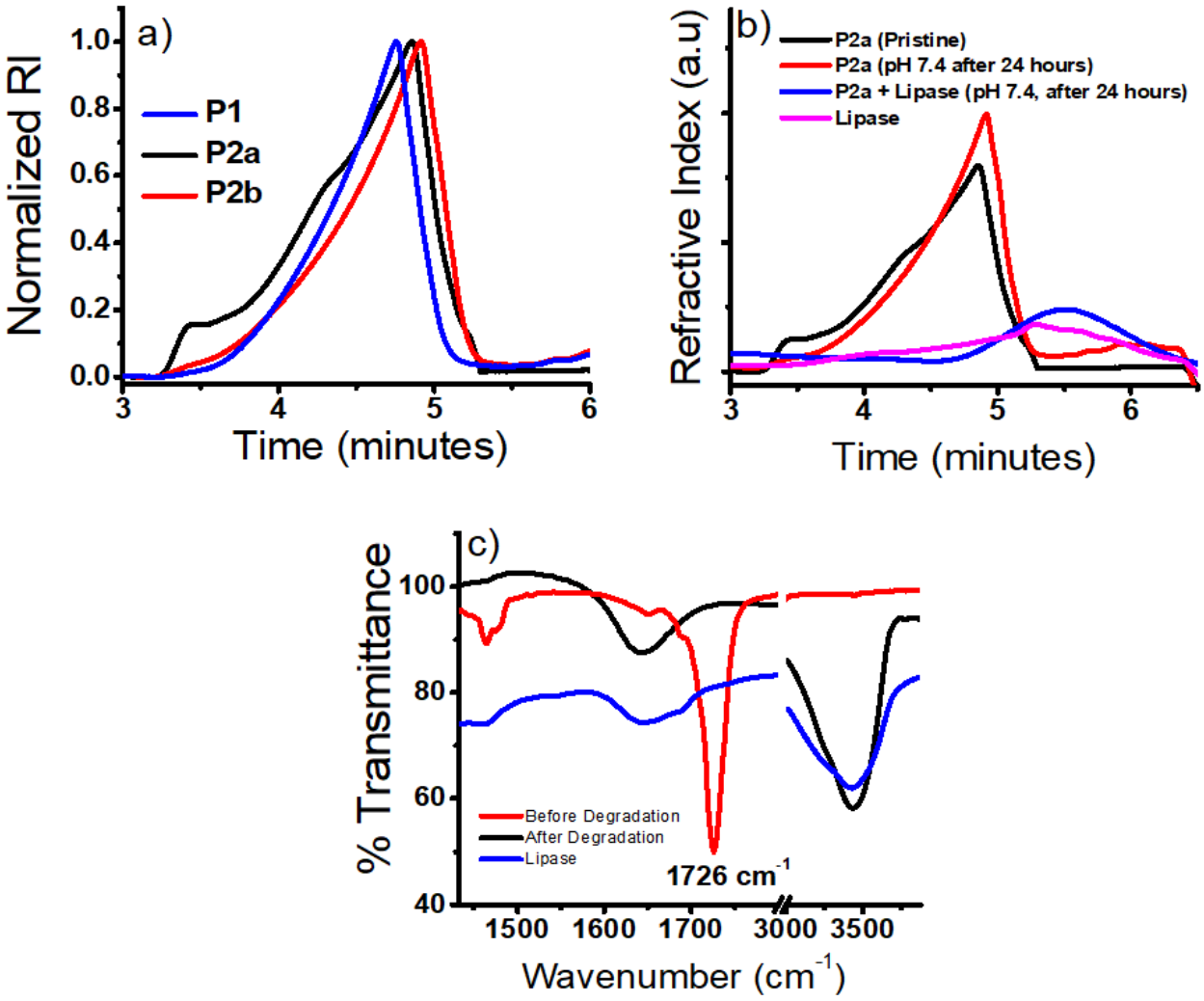


Figure S6: SEC traces of (a) **P1**, **P2a**, and **P2b** and (b) **P2a** before and after treatment with enzyme lipase for 24 h in phosphate buffer at pH = 7.4 compared with its pristine form. Eluent = 0.5% LiBr in DMF. (c) Compared FT-IR spectra of **P2a** before and after degradation with lipase showing complete disappearance of the carbonyl ester stretching frequency at 1726 cm^{-1}.

Note: Unavoidable trailing in the SEC profiles did not allow for accurate molecular weight estimation in the tested cationic polyesters.[6] SEC traces only provide qualitative information about molecular weight trends and enzymatic degradation.

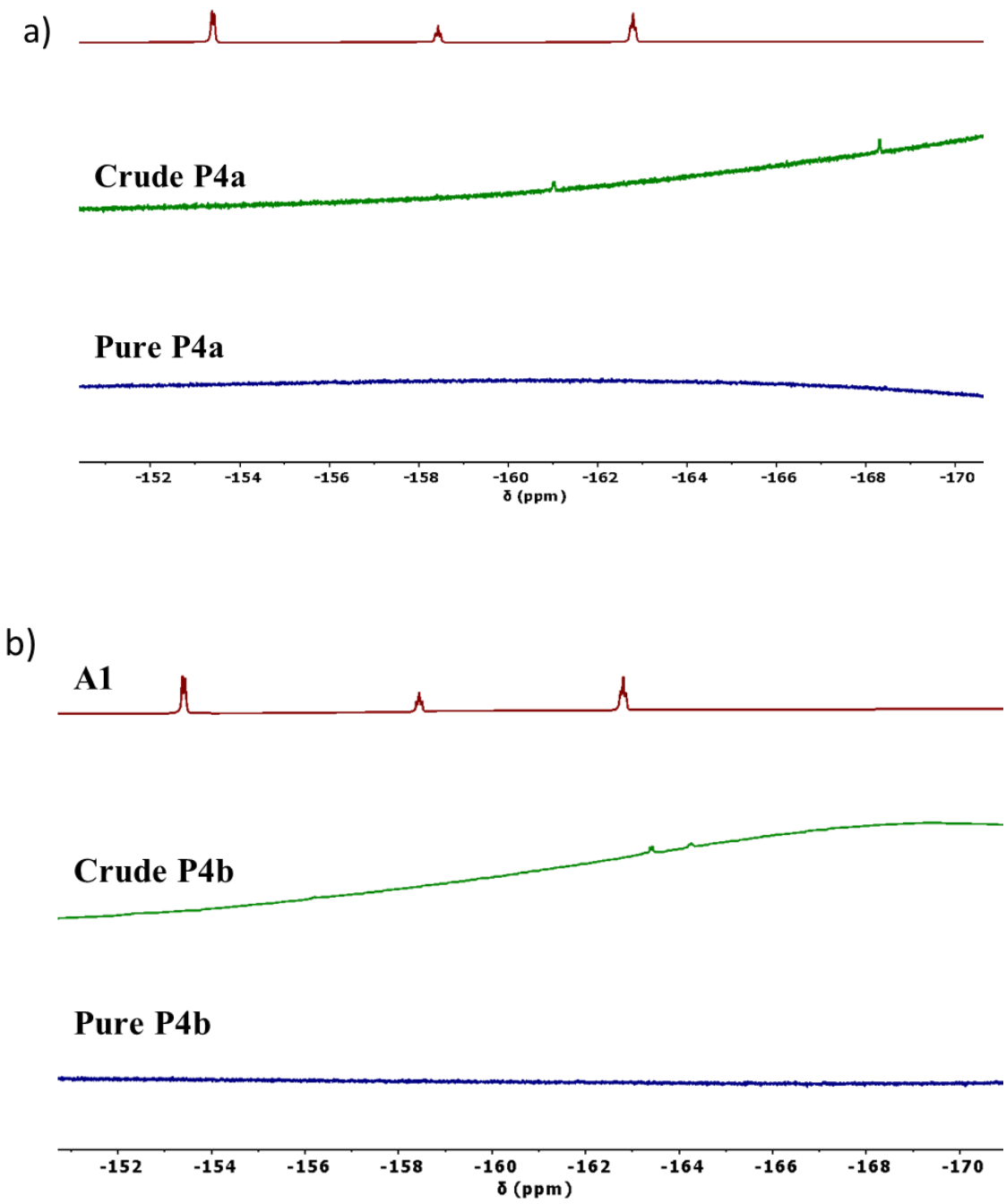


Figure S7: Stacked ^{19}F NMR spectra of crude and purified (a) **P4a** and (b) **P4b** with **A1** in $CDCl_3$.

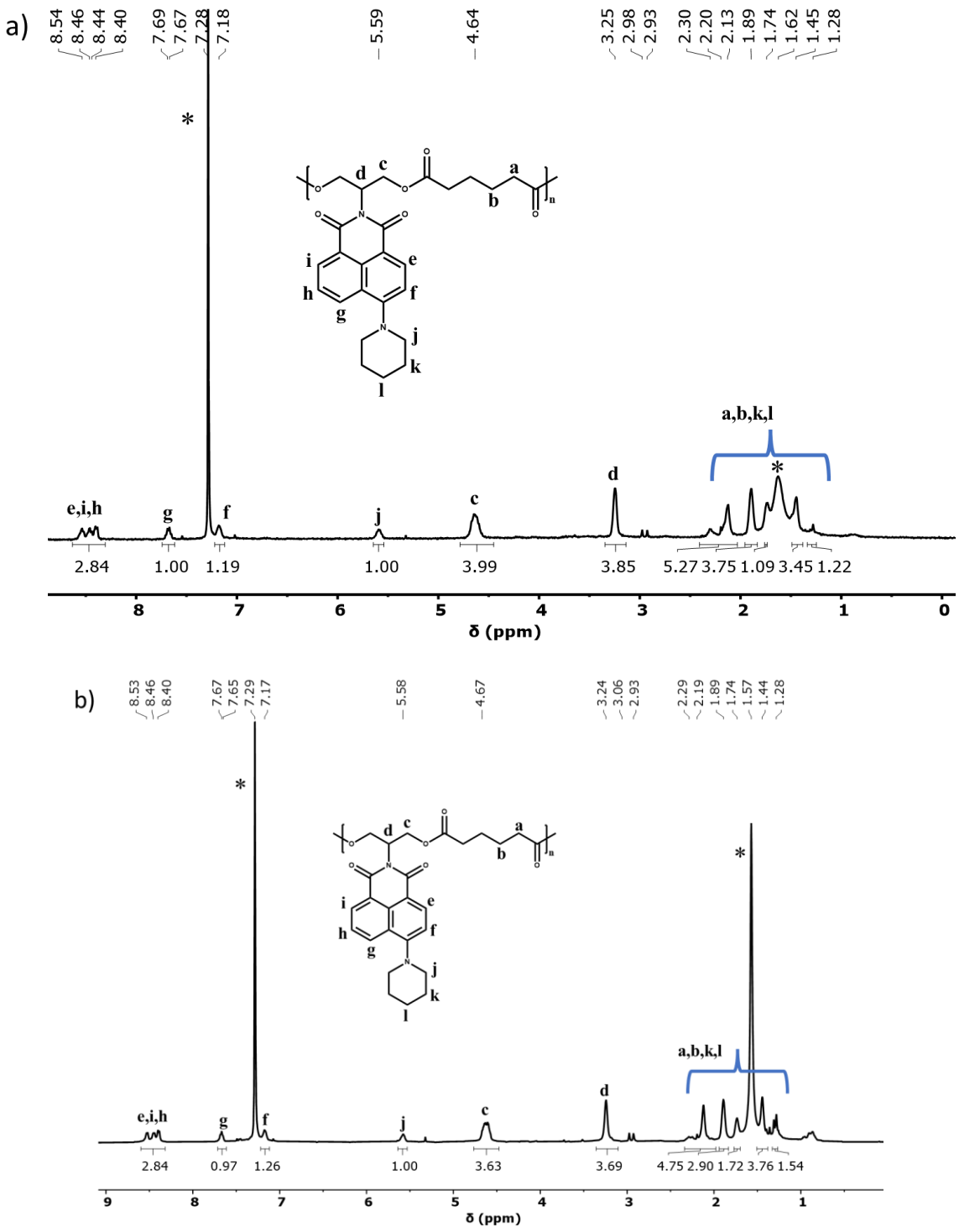


Figure S8: ^{1}H NMR spectrum of **(**a) **P4a** and (b) **P4b** in $CDCl_3$. "*" denotes residual solvent peaks.

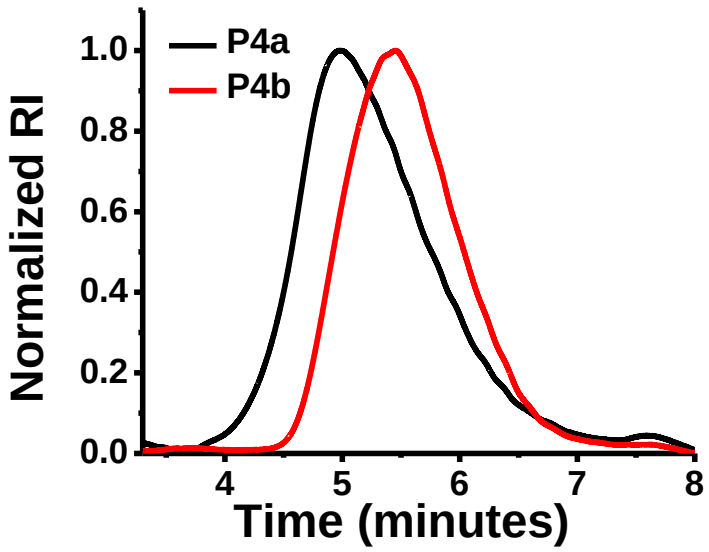


Figure S9: SEC traces of **P4a** and **P4b** with DMF as eluent.

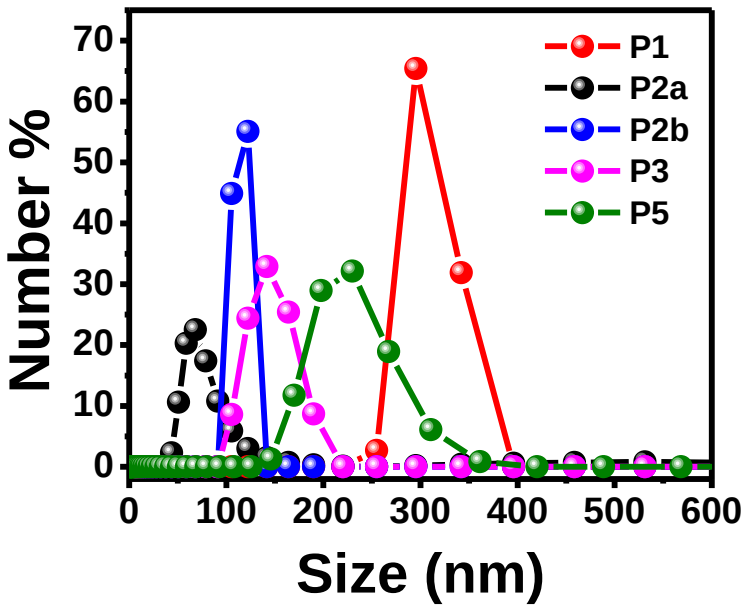


Figure S10: DLS plot of polyesters in water (*C* = 0.05 mg/mL)**.**

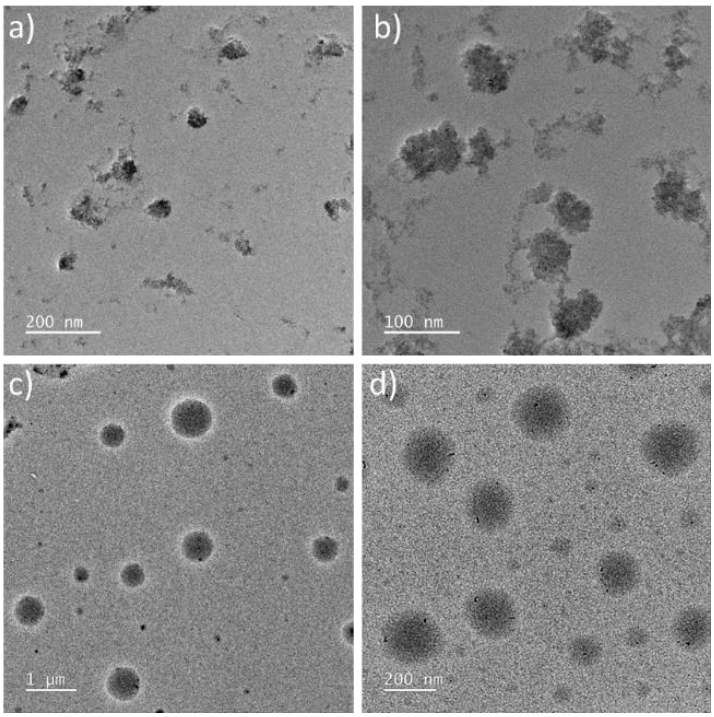


Figure S11: TEM images of **P3** (a,b) and **P5** (c,d) in water (*C* = 0.05 mg/mL).

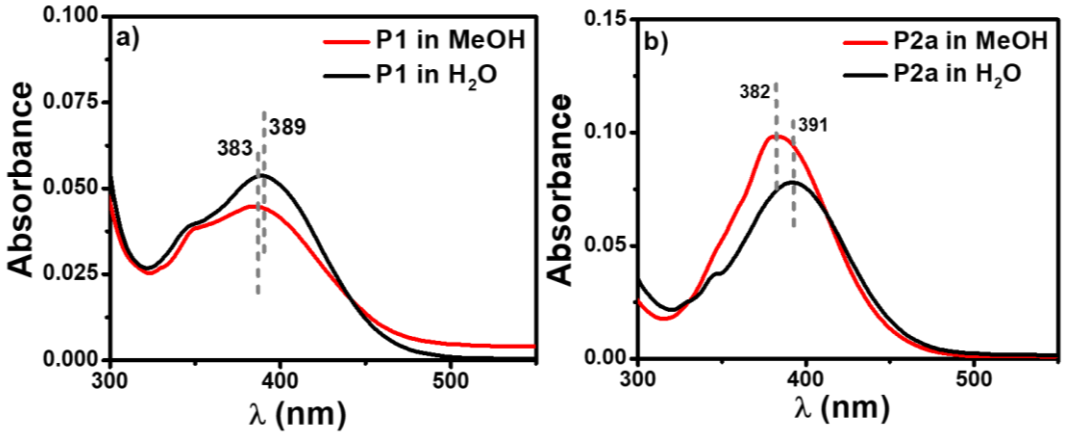


Figure S12: Compared UV/vis spectra of (a) **P1** and (b) **P2a** in MeOH and water (*C* = 0.1 mg/mL).

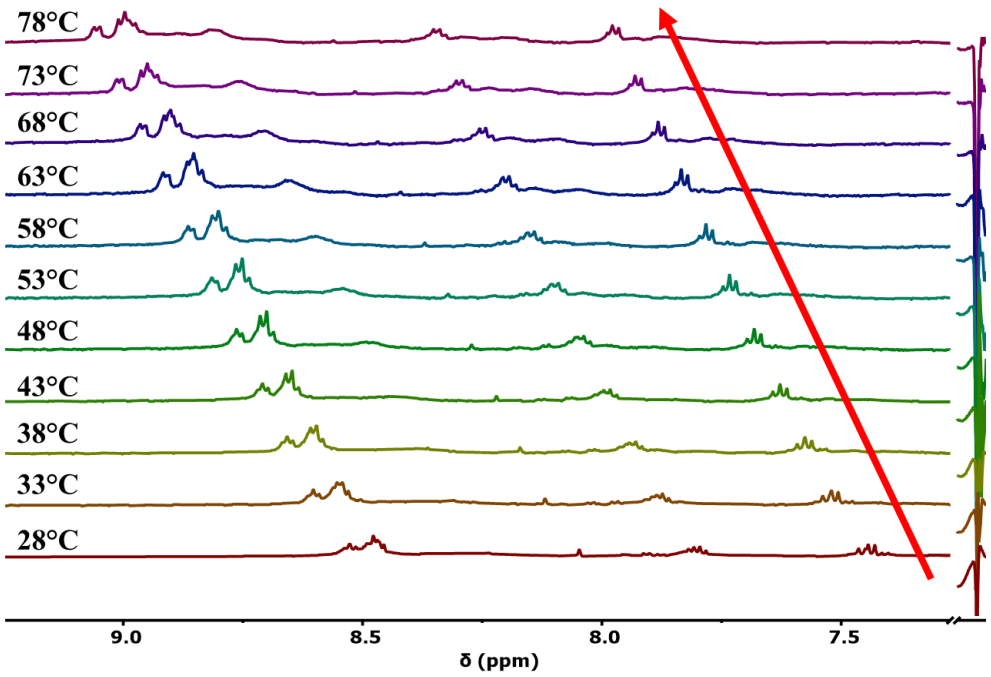


Figure S13: Variable-Temperature ^{1}H NMR stack plot of **P2a** in D_2O (in water suppression mode) showing deshielding effect with increasing temperatures.

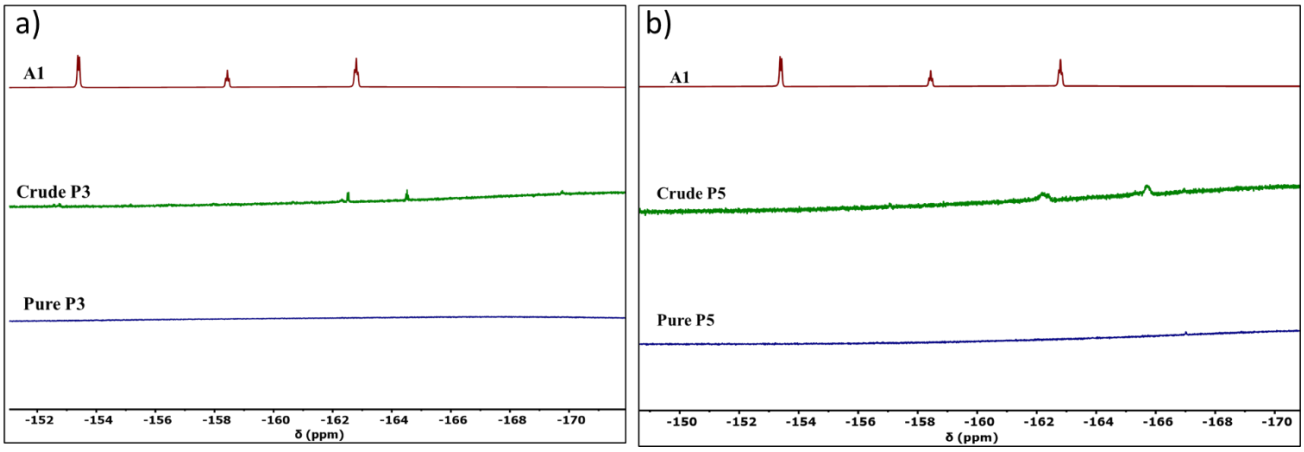


Figure S14: Stacked ^{19}F NMR spectra of (a) **P3** and (b) **P5** in $CDCl_3$.

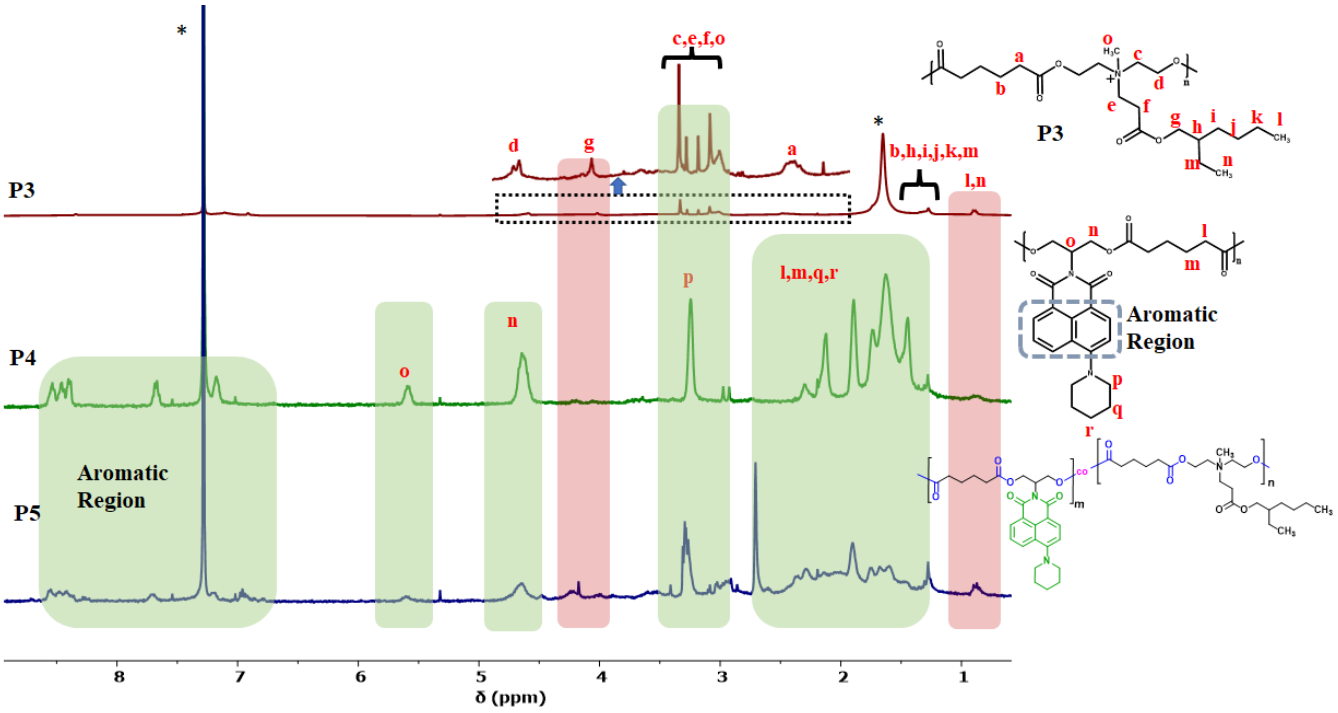


Figure S15: Stacked ^{1}H NMR of **P3, P4** and **P5** in $CDCl_3$.

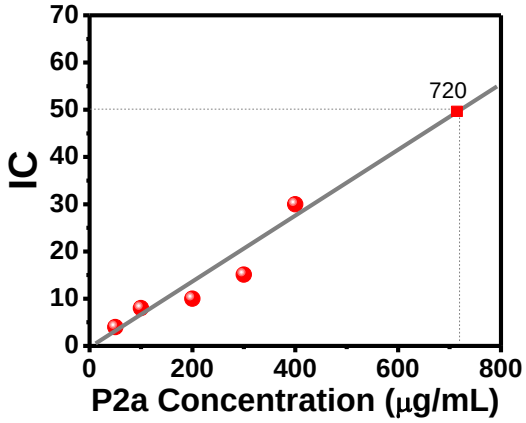


Figure S16: Extrapolation curve of IC values against HeLa cell line derived from Figure 7(b) at different concentrations of **P2a** for the estimation of IC_{50}.

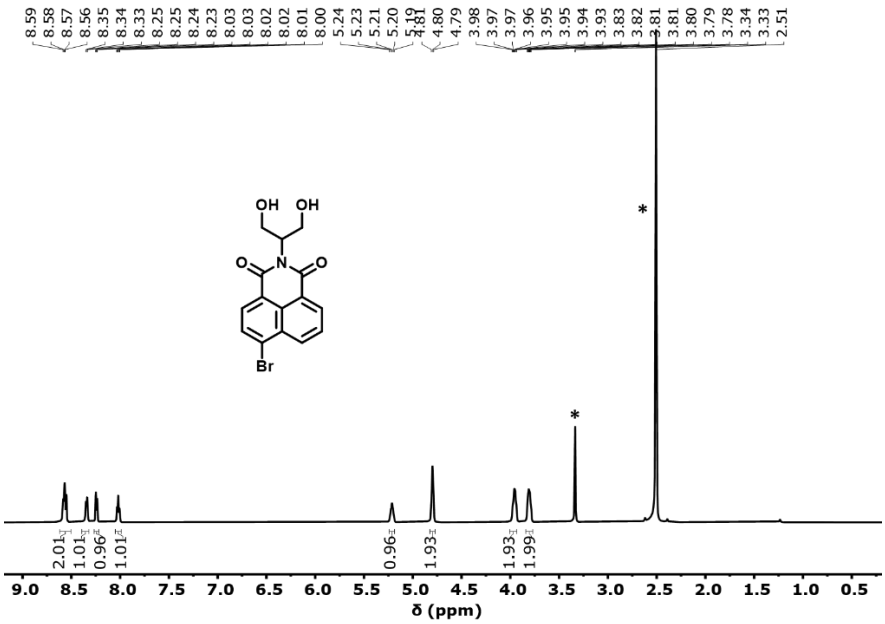


Figure S17: [1]H NMR spectrum of **I1** in DMSO-d_6. (*) denotes residual solvent peaks.

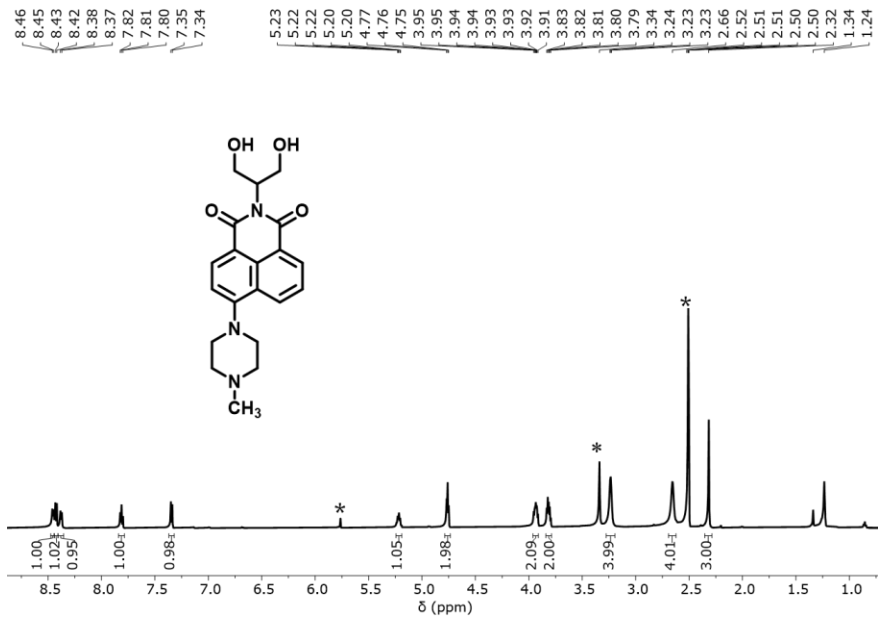


Figure S18: [1]H NMR spectrum of **I2** in DMSO-d_6. (*) denotes residual solvent peaks.

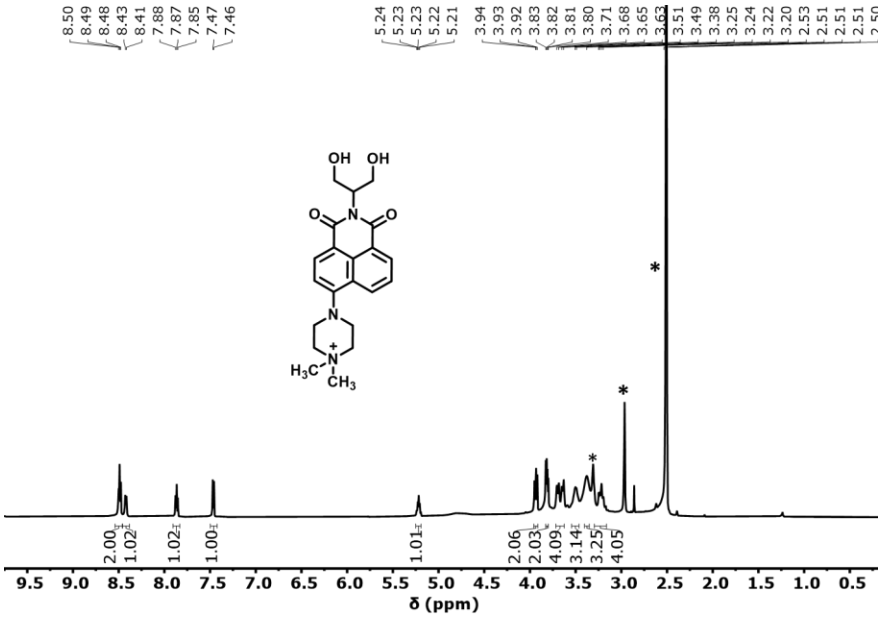


Figure S19: ^{1}H NMR spectrum of **M1** in DMSO-d_6. (*) denotes residual solvent peaks.

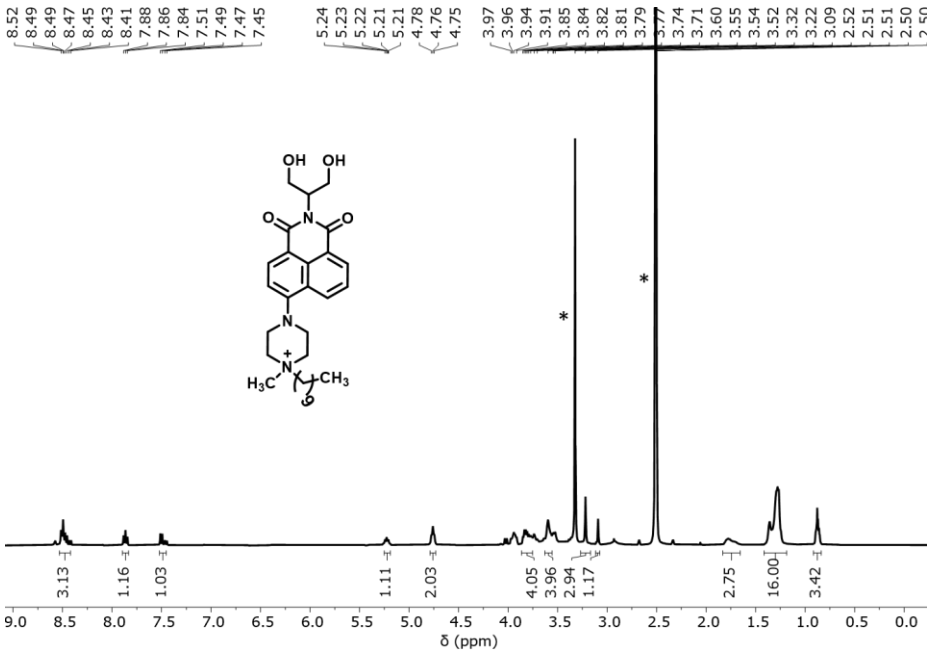


Figure S20: ^{1}H NMR spectrum of **M2** in DMSO-d_6. (*) denotes residual solvent peaks.

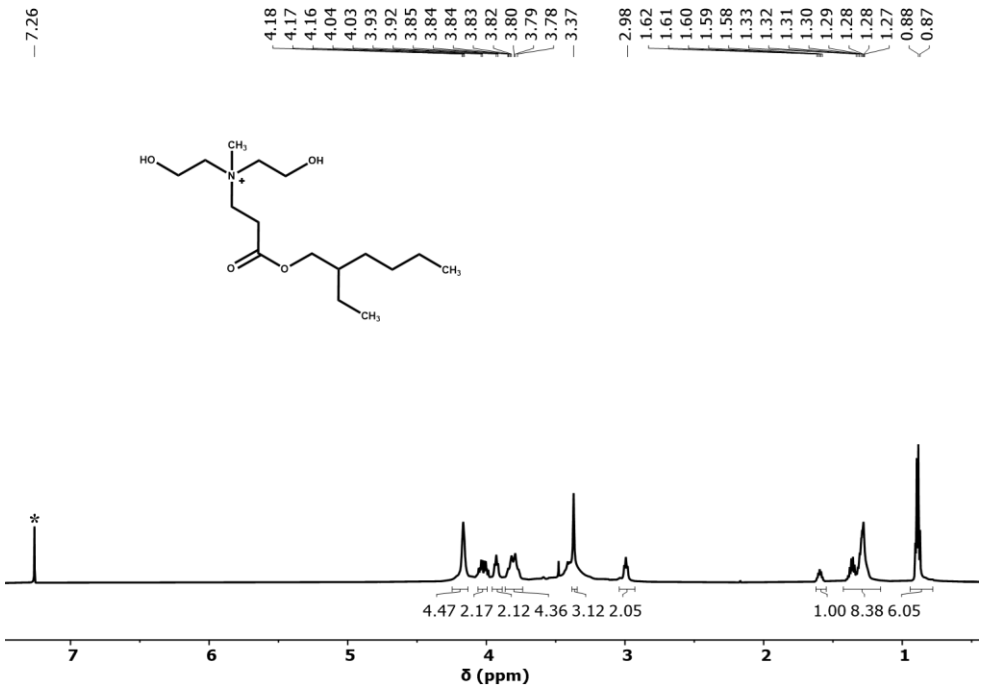


Figure S21: [1]H NMR spectrum of **M3** in $CDCl_3$. (*) denotes residual solvent peaks.

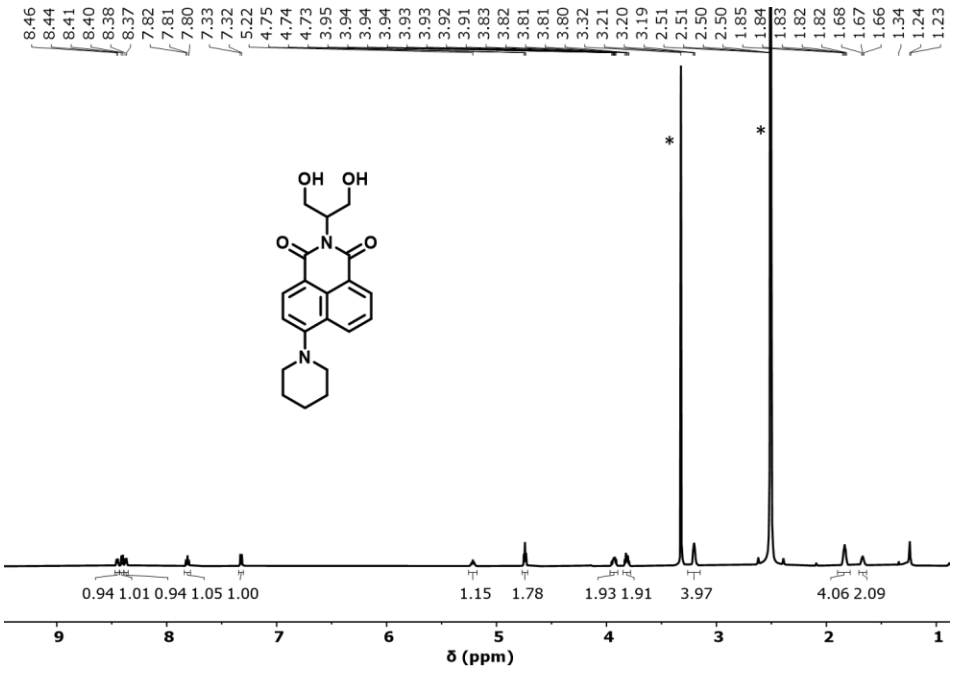


Figure S22: [1]H NMR spectrum of **M4** in DMSO-d_6. (*) denotes residual solvent peaks.

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1. S. Biswas and A. Das, *Chem. Eur. J*, 2023, **29**, e202203849.
2. Y. Fujikawa, K. Terakado, T. Nampo, M. Mori and H. Inoue, *Talanta*, 2019, **204**, 633–640.
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6. P. Pazhanisamy, M. Ariff, and Q. Anwaruddin, *J. Appl. Polym. Sci.*, 2005, **98**, 1100–1105.