Supporting Information for:

Construction from Destruction: Hydrogel Formation from Triggered Depolymerization-based Release of an Enzymatic Catalyst

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1. Materials:

Ethyl 4-amino benzoate, Sodium periodate, Potassium iodide, Diisobutylaluminium hydride (DIBALH), t-Butyl acrylate, Palladium acetate, Tetrabutylammonium chloride, Palladium on carbon (10 wt. %), Phenylchloroformate, Dibutyltin dilaurate (DBTL), 2-Nitrobenzyl alcohol, Polydiallyldimethylammonium chloride, 20 wt. % in water (PDADMAC), Rhodamine 6g and Peroxidase from horseradish (Type VI-A, salt free, lyophilized powder, >= 250 units/mg solid) were purchased from Sigma Aldrich and were used as such unless mentioned otherwise. Sodium chloride, Acetic acid, Potassium carbonate and Sodium bicarbonate were purchased from Fischer Scientific and used as such unless mentioned otherwise.

2. Instruments:

Nuclear Magnetic Resonance (NMR): ^{1}H NMR spectra were recorded using 400 MHz Bruker NMR spectrometer with residual proton for solvent as a standard. ^{13}C NMR spectra were recorded in 100 MHz Bruker NMR spectrometer using carbon signal of the deuterated solvent as a standard.

Dynamic Light Scattering (DLS): DLS was performed using Malvern nanozetasizer with a 637 nm laser source with noninvasive backscattering detected at 173°. Standard operating procedure was set up with the following parameters: sample equilibration for 1 min at 25 °C and then three measurements were taken while each measurement recorded 16 runs.

Transmission Electron Microscopy (TEM): The sample for DLS was drop casted on carbon coated copper grid and the sample was left for drying overnight. Subsequently, the imaging was done using JEOL-2000FX transmission electron microscope.

Cryogenic Transmission Electron Microscopy (cryo-TEM): Cryo-tem sample preparation was done using a fei vitrobot mkii plunge freezer at 4°C. The samples were cryo-transferred to a fei tecnai-t12 (120kV) and observed at-175°C under low-dose conditions.

Energy Dispersive X-ray Spectroscopy: Eds was performed using a JEOL jem-2200fs eftem equipped with an oxford x-max 80mm^2 eds detector. Samples were prepared as for TEM above but only allowed to dry for 5 minutes prior to observation. The samples appeared sufficiently stable at room temperature to obtain eds spectra for the confirmation of iron content without obvious beam damage.

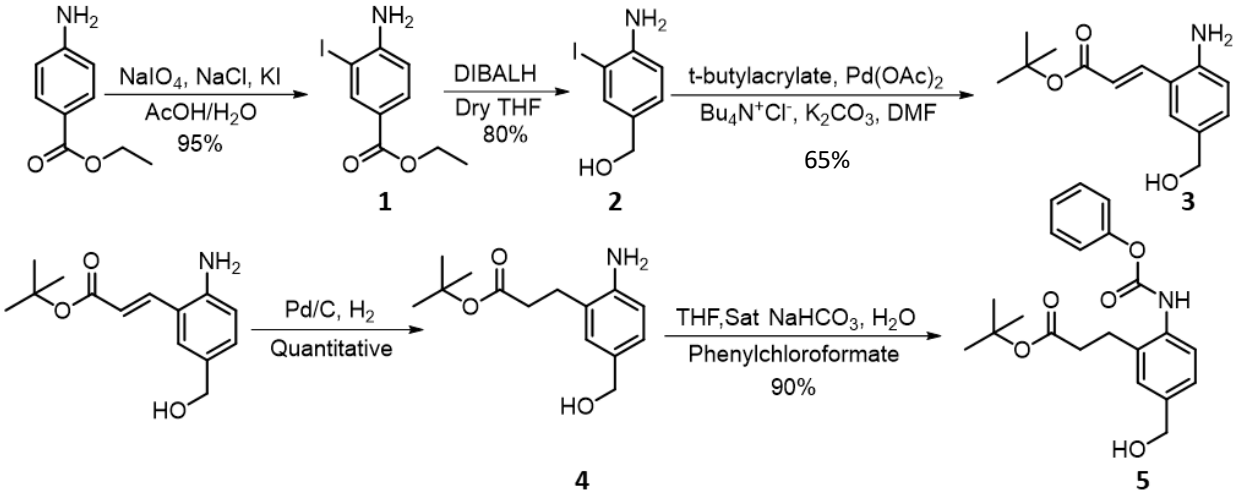
Atomic Force Microscopy (AFM): Samples were analyzed using a Veeco Bioscope AFM. AppNano ANSCM-PA probes were used for all measurements, which consist of an ~125 mm long Si cantilever that has been coated with PtIr resulting in tips with ~ 30nm radius and a resonant frequency of ca. 300 kHz. Measurements were performed with scans sizes of between 1.35 and 20 µm, at speeds between 1.0 and 1.5 Hz, and with either 256 x 256 or 512 x 512 pixel x lines of resolution. Analysis of topography images was performed in Gwyddion. Sample was cast on glass slide and dried overnight before analyzing.

UV-Vis Spectroscopy: UV-Vis measurement of the samples was done using PerkinElmer Lambda 35 spectrometer.

Fluorescence Spectroscopy: Fluorescence measurement of the samples was done using PerkinElmer LS 55 spectrometer. All the samples were excited at a wavelength of 510 nm.

Rheology: An AR2000ex rheometer (TA Instruments) equipped with a stainless steel 20 mm crosshatch parallel plate was used to perform rheological measurements.

3. Monomer and polymer synthesis:



Synthesis of 1: This compound was synthesized according to a previously reported procedure.[1]

Ethyl-4-aminobenzoate (10 g, 60.54 mmol), sodium periodate (12.94 g, 60.54 mmol), sodium chloride (7.08 g, 121.08 mmol) and potassium iodide (10.05 g, 60.54 mmol) were added in 500 mL round bottom flask and purged with argon for 10 minutes. Mixture of acetic acid (120 mL, 60.54 mmol) and water (13 mL) was added to the reaction mixture to dissolve all the starting materials and the solution was left for stirring overnight and monitored using TLC for completion. The reaction mixture was filtered and the filtrate was diluted with ethyl acetate. The solution was washed twice each time with brine solution, saturated solution of sodium thiosulfate and saturated solution of sodium bicarbonate respectively. Organic layer was collected and evaporated to obtain the crude product which was purified using column chromatography with pure product eluting at 30 % ethyl acetate in hexane mixture. (Yield 95 %).

^{1}H NMR (400 MHz, $CDCl_3$) δ= 8.38 (1H, s), 7.82-7.81 (1H, d), 6.71-6.79 (1H, d), 4.50 (2H, s), 4.34-4.29 (2H, q), 1.38-1.34 (3H, t).

Synthesis of 2: This compound was synthesized according to a previously reported procedure.[1]

Compound 1 (2.15 g, 7.41 mmol) was dissolved in 10 mL dry THF, cooled to 0 ^{o}C and purged with argon gas. DIBAL-H (3.96 mL, 22.23 mmol) was mixed with 5 mL THF and was added to the solution of compound 1 dropwise. The reaction was continued for an hour and was monitored for completion using TLC. To quench excess DIBAL-H, the reaction mixture was diluted with 20 mL methanol slowly followed by the addition of 20 mL THF and celite which led to the formation of suspension which was stirred for an hour. The solid was filtered and washed multiple times with THF to wash out the crude product as filtrate. The crude product was concentrated and purified using column chromatography with pure product eluting out at 30 % ethyl acetate in hexane mixture. (Yield 80 %).

^{1}H NMR (400 MHz, $CDCl_3$) δ= 7.66-7.65 (1H, d), 7.15-7.13 (1H, d), 6.74-6.72 (1H, d), 4.52(2H, d), 4.10 (2H, s).

Synthesis of 3: This compound was synthesized according to a previously reported procedure.[1]

Compound 2 (1.3 g, 5.22 mmol) was dissolved in 15 mL dry DMF and purged with argon. Subsequently Bu_4NBr (2.1 g, 6.53 mmol), $Pd(OAc)_2$ (59 mg, 0.26 mmol), *t*-butyl acrylate (1.13 mL, 7.83 mmol) and K_2CO_3 (3.6 g, 26.1 mmol) were added to the reaction mixture. The reaction was continued for 3 h at 65 ^{o}C and was monitored to completion using TLC. The reaction mixture was cooled, diluted with ethyl acetate and washed twice with brine and water each. The organic layer was concentrated to obtain crude product which was purified using column chromatography and the pure product eluted out at 35 % ethyl acetate in hexane mixture. (Yield 65 %).

^{1}H NMR (400 MHz, $CDCl_3$) δ= 7.70-7.67 (1H, d), 7.36 (1H, s), 7.17-7.15 (1H, d), 6.68-6.67 (1H, d), 6.31-6.28 (1H, d), 4.56-4.54 (2H, d), 3.95 (2H, s), 1.52 (9H, s).

Synthesis of 4: Compound 3 (1.6 g, 6.42 mmol) was dissolved in 200 mL methanol in a hydrogenation flask. Palladium on carbon (0.16 g, 0.65 mmoles) was added to the solution of compound 3. The reaction mixture was introduced to the Parr hydrogenation set up where the flask was filled with hydrogen at a pressure of 40 psi. The reaction was continued for an hour after which the flask containing reaction mixture was removed and reaction was monitored using TLC for completion. The reaction mixture was filtered and the solvent from the resulting filtered solution was evaporated to yield yellowish oil like crude product. The crude product was purified using column chromatography with pure product eluting at 30 % ethyl acetate in hexane mixture. The pure product was characterized using ^{1}H NMR, ^{13}C NMR and ESI-MS. (Yield=1.5 g, quantitative).

^{1}H NMR (400 MHz, $CDCl_3$) δ= 7.05-7.03 (2H, m), 6.67-6.65 (1H, d), 4.54 (2H, s), 3.80 (3H, s), 2.81-2.77 (2H, t), 2.58-2.53 (2H, t), 1.44 (9H, s).

^{13}C NMR (100 MHz, $CDCl_3$) δ= 172.92, 144.02, 131.24, 128.90, 126.72, 125.24, 115.84, 80.68, 65.28, 34.86, 28.10, 26.24.

ESI-MS: $[M + Na]^{+}$= 274.13

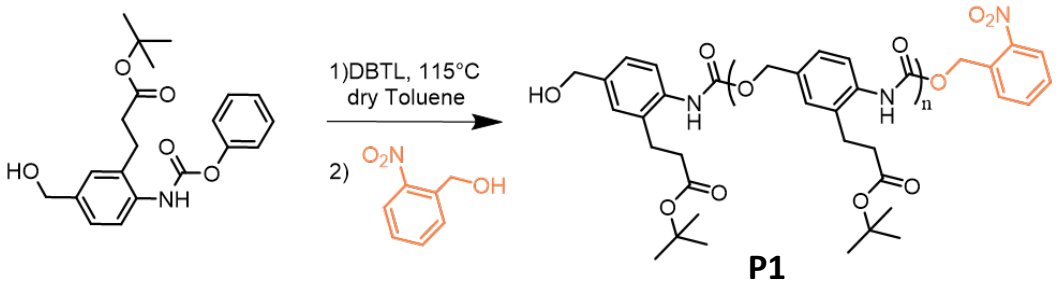
Synthesis of 5: Compound 4 (1.5 g, 5.95 mmol) was suspended in a 20 mL solution of THF: sat. $NaHCO_3$: H_2O (ratio of 2:2:1) in a round bottomed flask. Phenylchloroformate (0.85 mL, 6.55 mmol) was added dropwise to the suspension of 4 and the reaction mixture was stirred for 30 minutes while monitoring it using TLC for completion. The reaction mixture was diluted with ethyl acetate and was washed twice using saturated NH_4Cl. Solvent was evaporated to obtain crude product which was purified using column chromatography. Pure product was eluted at 30 % ethyl acetate in hexane mixture and was characterized using ^{1}H NMR, ^{13}C NMR and ESI-MS. (Yield 90 %).

^{1}H NMR (400 MHz, $CDCl_3$) δ= 8.34 (1H, s), 7.80 (1H, s), 7.41-7.37 (2H, m), 7.24-7.20 (5H, m), 4.66-4.65 (2H, d), 2.94-2.91 (2H, t), 2.68-2.65 (2H, t), 1.42 (9H, s).

^{13}C NMR (100 MHz, $CDCl_3$) δ= 173.65, 152.75, 150.95, 134.90, 129.31, 128.55, 125.97, 125.44, 121.76, 81.53, 64.99, 36.32, 28.06, 25.47.

ESI-MS: $[M + Na]^+$= 394.15

Synthesis of polymer P1:

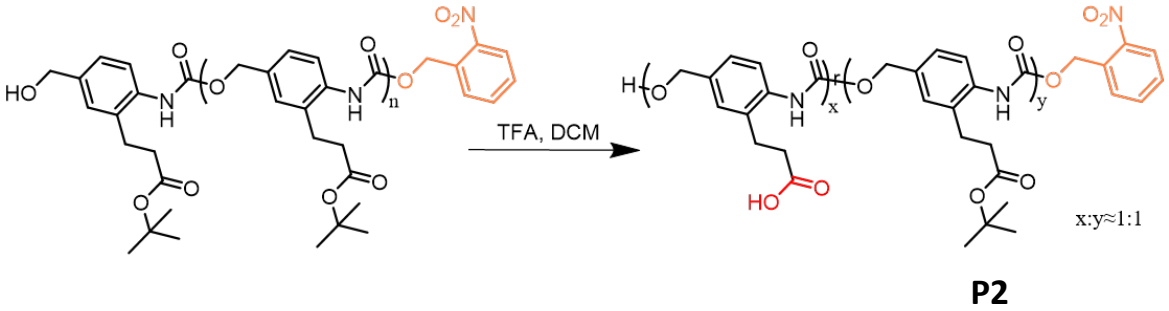


Monomer/Compound 5 (50 mg, 0.13 mmol) was dissolved in anhydrous toluene (0.15 mL) and to the mixture was added DBTL (8 µL, 0.013 mmol). The mixture was purged with argon for 5 minutes and subsequently transferred to an oil bath at 115 °C under argon atmosphere. The reaction mixture was stirred for 20 minutes after which 2- nitrobenzylalcohol (20 mg, 0.13 mmol) in a solution of anhydrous toluene (0.1 mL) was added dropwise. The reaction was stirred for 2 hours after which it was cooled to room temperature and purified by precipitation using cold diethyl ether. Brownish polymer powder was dried using high vacuum and characterized using ^{1}H NMR and ^{13}C NMR. (Yield=30 mg). Note: *The average repeat units in the polymer varied between 8-10 in different trials.*

^{1}H NMR (400 MHz, $CDCl_3$) δ= 8.14-8.12, 7.84, 7.77, 7.30-7.28, 7.21, 5.65, 5.15, 4.63, 2.85-2.82, 2.61-2.58, 1.38.

^{13}C NMR (100 MHz, $CDCl_3$) δ= 173.32, 154.31, 135.74, 129.79, 127.27, 81.33, 70.58, 66.50, 36.09, 28.04, 25.47.

Synthesis of polymer P2:

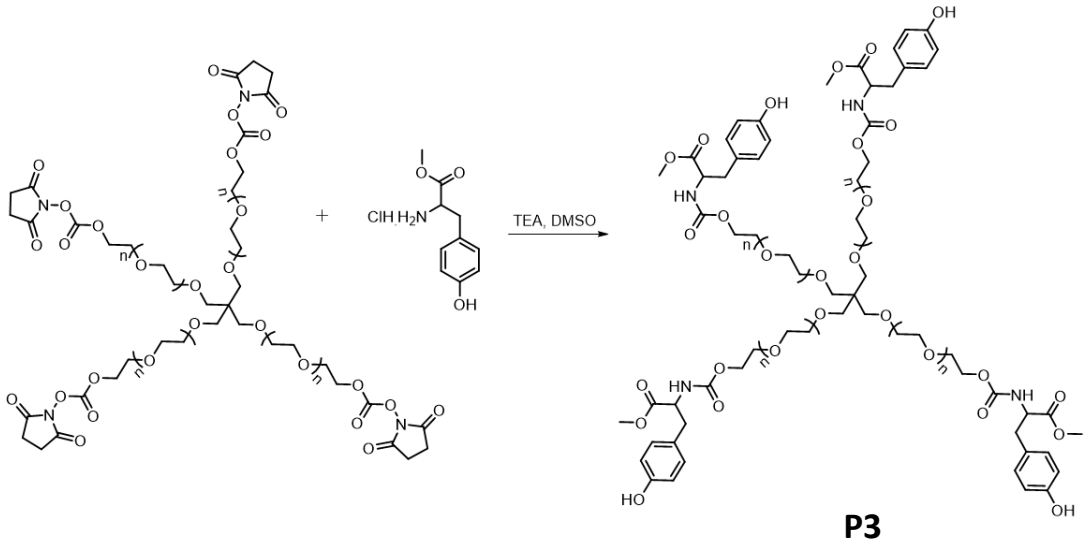


Polymer P1 (30 mg) was dissolved in a mixture of trifluoroacetic acid (0.1 mL) and dichloromethane (0.4 mL) and was stirred for three minutes at room temperature. It was diluted using cold diethyl ether to

obtain cloudy solution after which solvent and trifluoroacetic acid was evaporated using rotary evaporator. The solid was washed further with excess diethyl ether several times to obtain light brown polymer powder. The polymer was dried using high vacuum and characterized using ^{1}H NMR and ^{13}C NMR. (Yield=5 mg). *Note: This precursor polymer is very sensitive to trifluoroacetic acid and continuing the reaction even for a few extra minutes lead to polymer degradation.*

^{1}H NMR (400 MHz, MeOD) δ= 8.16-8.13, 7.77, 7.46, 7.28, 5.59, 5.16, 4.57, 2.91, 1.40.

^{13}C NMR (100 MHz, MeOD) δ= 173.04, 155.60, 135.35, 129.05, 126.18, 125.17, 115.11, 80.51, 66.02, 35.29, 30.12, 26.94, 25.97



P3

Synthesis of P3:

4arm-poly(ethylene glycol)-succinimidyl carbonate (average MW 10000) (1 g, 0.09 mmol) was dissolved in 10 mL DMSO in a round bottomed flask. In a vial, L-tyrosine methyl ester hydrochloride (208 mg, 0.9 mmol) was dissolved in 3 mL DMSO and triethyl amine (124 µL, 0.9 mmol) was added which led to a dark brown solution. The mixture was added dropwise to the solution of 4arm-poly(ethylene glycol)-succinimidyl carbonate and the reaction was continued for 3 hours. Upon completion of reaction, the reaction mixture was dialyzed using dialysis membrane of 6000-8000 MW cut off against methanol: chloroform (70: 30) mixture for 24 hours (solvent was changed for 5 times during dialysis). The product was concentrated and characterized using ^{1}H NMR and ^{13}C NMR.

^{1}H NMR (400 MHz, $CDCl_3$) δ= 7.10 (1H, s), 6.95-6.93 (8H, d), 6.79-6.77 (8H, d), 5.23-5.21 (4H, d), 4.59-4.54 (4H, m), 4.28-4.09 (8H, m), 3.83-3.80 (8H, t), 3.74 (12H, t), 3.64-3.41 (1132H, m), 3.11-3.06 (4H, dd), 2.98-2.93 (4H, dd).

^{13}C NMR (100 MHz, $CDCl_3$) δ= 172.10, 155.87, 130.24, 115.70, 70.96, 70.56, 70.35, 70.03, 69.50, 64.39, 54.82, 52.29, 45.53, 37.18

4. **Formulation of empty polymeric vesicles**:
   P2 (1 mg) was suspended in 2 mL deionized water and to the suspension, a catalytic amount of triethyl amine was added to obtain a clear solution. In a different vial, a stock solution of Polydiallyldimethylammonium chloride, 20 wt. % in water (PDADMAC) was prepared by dissolving 20 µL of 20 wt. % PDADMAC in 5 mL water. 100 µL PDADMAC from the stock solution was added dropwise to the P2 solution under stirring conditions. The mixture was left for stirring for 2 hours after which the vesicles were characterized using DLS and TEM. All the steps were performed at 25 °C
5. **Formulation of polymeric vesicles with Horseradish Peroxidase (HRP) encapsulation:**
   P2 (1 mg) was suspended in 2 mL deionized water and to the suspension, a catalytic amount of triethyl amine was added to obtain a clear solution. 1 mg (HRP) was added to the polymer solution to obtain a clear solution. pH of the solution was adjusted to 9 by carefully adding small amount of HCl. 100 µL PDADMAC from the stock solution was added dropwise to the (P2 + HRP) solution under stirring conditions. The mixture was left for stirring for 6 hours after which the free enzyme was washed out using centrifuge filter (100 kD MW cut off) and volume of nanoparticle solution was maintained by adding deionized water. The samples were centrifuged for 2 minutes at 5000 rpm multiple times until no absorbance peak corresponding to HRP's characteristic Soret band was observed in the UV-Vis spectrum of the filtrate.
   Vesicle solution with encapsulated HRP was diluted 10 times and its absorbance was measured using UV-Vis spectrum (by using empty nanoparticles as a blank) which indicated the characteristics Soret band of HRP. All the steps were performed at 25 °C
6. **Gelation experiments:**
   5 wt. % concentration of substrate was prepared in 1 mL water. To the substrate solution, 1 mL of nanoparticle solution with loaded enzyme was added and mixed gently. The mixture was irradiated with UV light for required durations in a UV chamber (350 nm, 2.2 mW/cm^2). After irradiation, sample was taken out and stoichiometric amount of H_2O_2 relative to P3 was added. In the control experiment, similar procedure was followed except the UV irradiation step. All the steps were performed at 25 °C

7. **Rheology experiments:**
   Hydrogels were loaded on the rheometer using a spatula. The upper geometry was lowered to a gap of ~1000 microns, based on the height of the gel, and a solvent trap filled with water was utilized to limit the solvent evaporation. All measurements were then performed at 25 °C using a normal force control of 1.50 ± 0.1 N (the normal force includes forces resulting from the solvent trap and the geometry compressing the sample). Each sample was pre-sheared using a stress of 0.06366 Pa for 30 s and then equilibrated for 5 min before performing a frequency sweep experiment. Frequency sweep experiments were performed from 100–0.1 rad/s using a 10% strain.

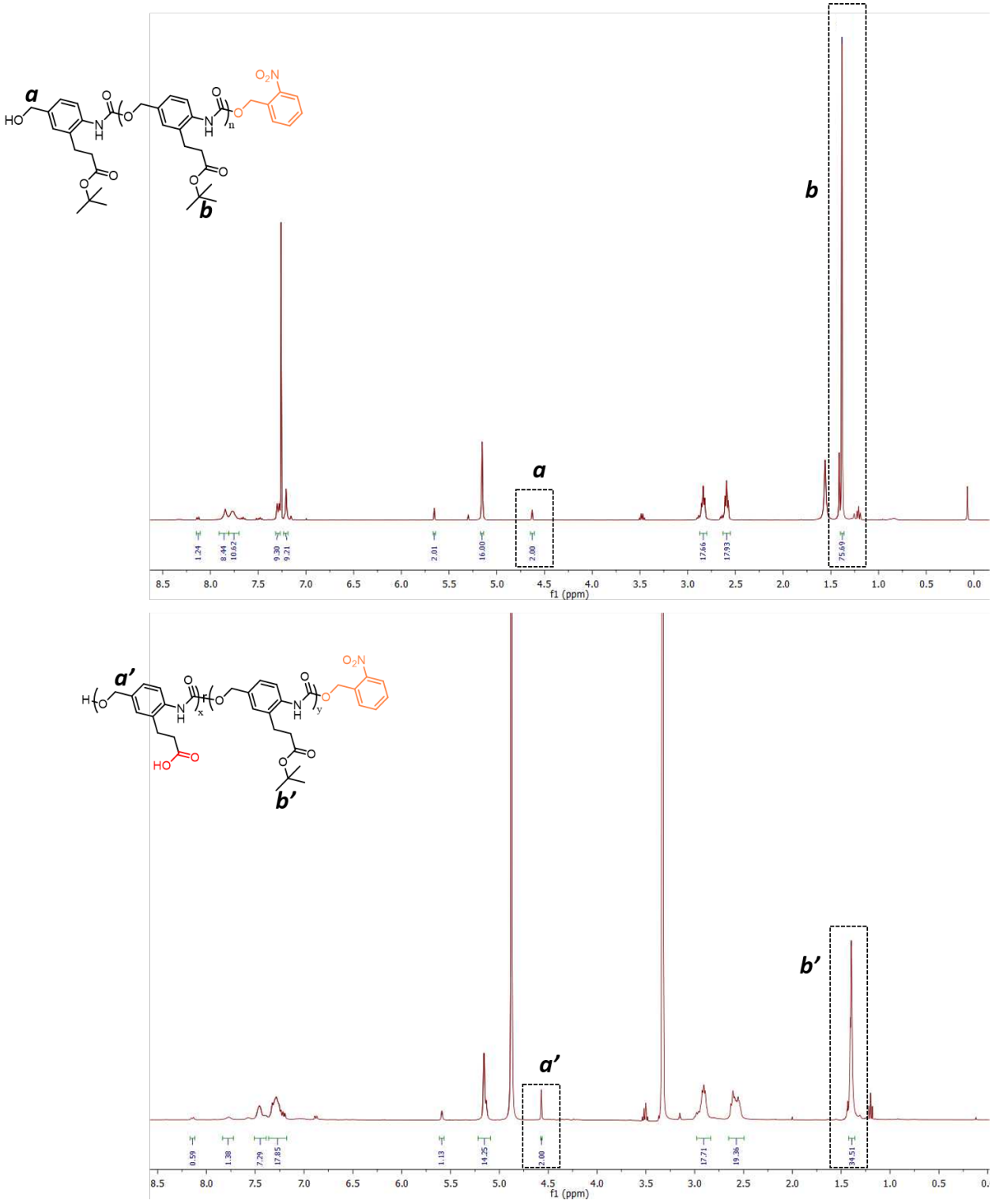


Figure S1: *^{1}H NMR spectra of P1 (top spectrum) and P2 (bottom spectrum). Note the relative integration values of terminal methylene protons (a or a') and protons corresponding to t-butyl groups (b or b'). The relative ratio of b': a' after TFA deprotection reduced by around 50 % implying 50 % t-butyl groups were deprotected.*

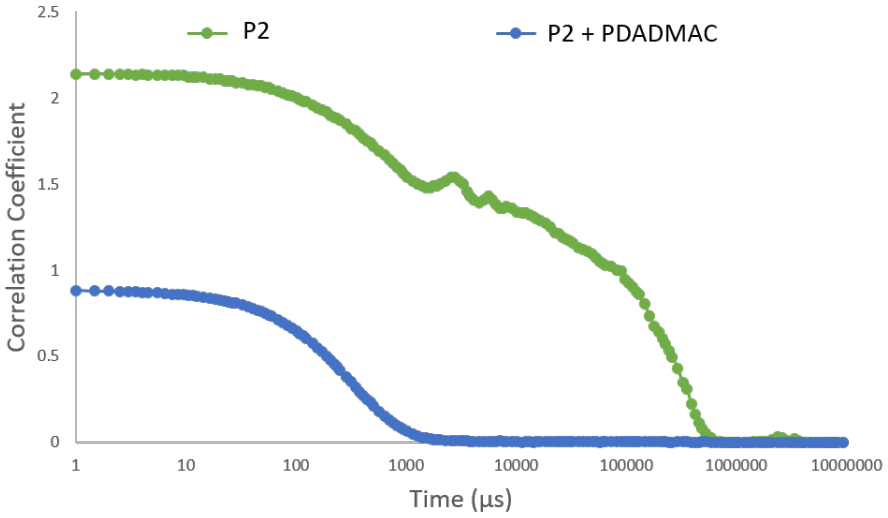


Figure S2: *Correlogram of P2 and (P2 + PDADMAC) nanoparticles*

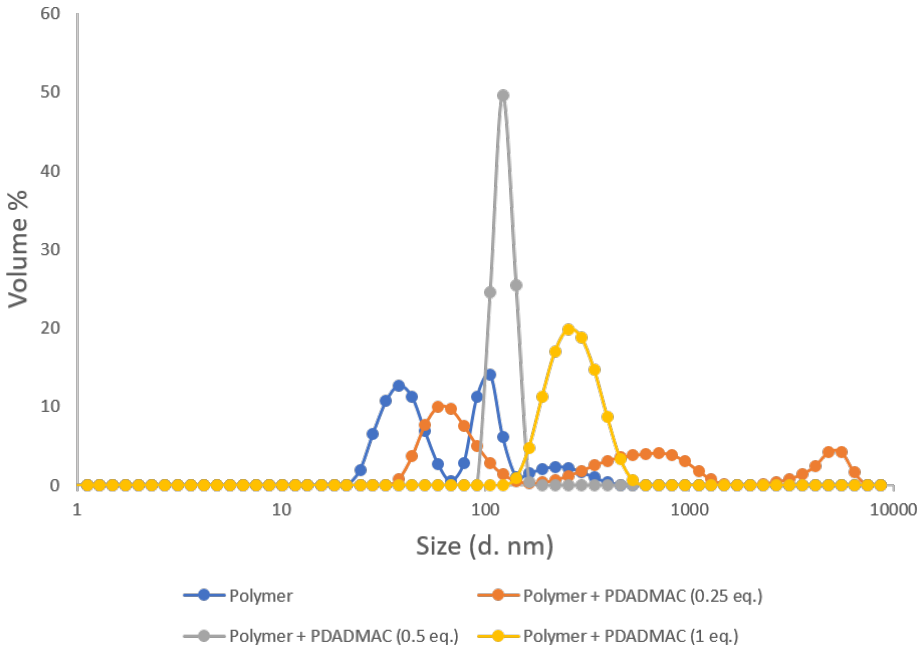


Figure S3: *DSL profile of (P2 + PDADMAC) nanoparticles with different equivalent of positive charge on PDADMAC, relative to P2. The PDI values for each of the samples were: 0.587 for P2; 0.752 for P2 + PDADMAC (0.25 eq.); 1 for P2 + PDADMAC (0.5 eq.) and 0.206 for P2 + PDADMAC (1 eq.).*

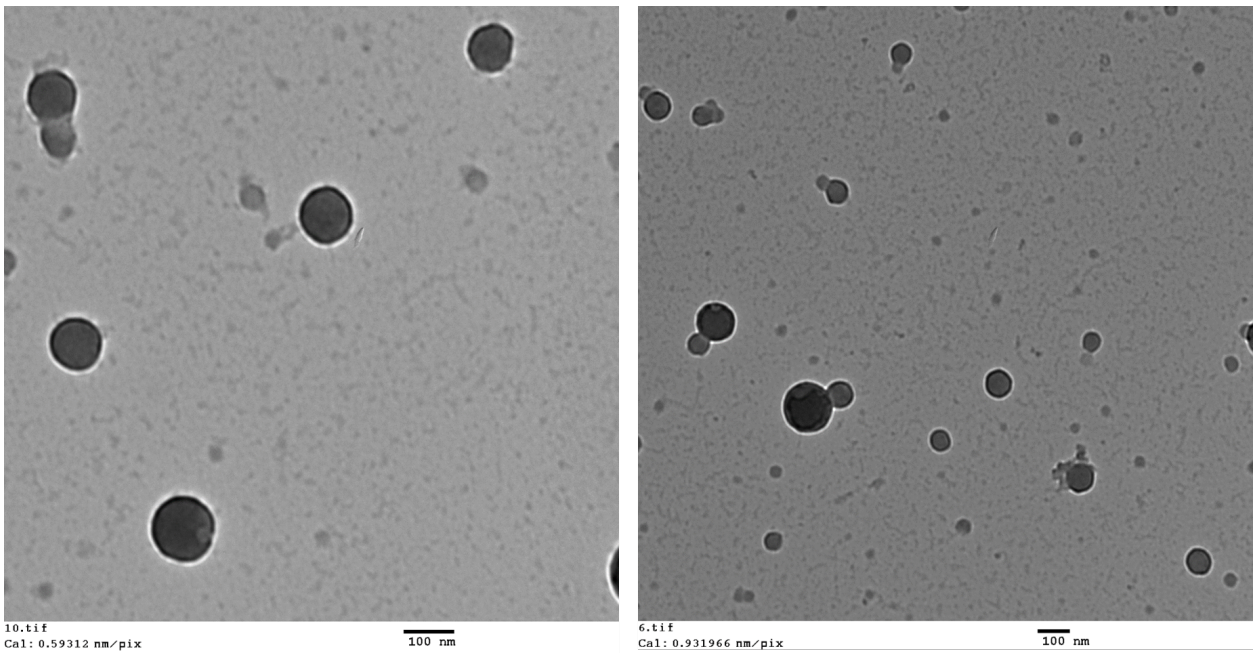


Figure S4: *Dry state TEM images of P2 + PDADMAC nanoparticles*

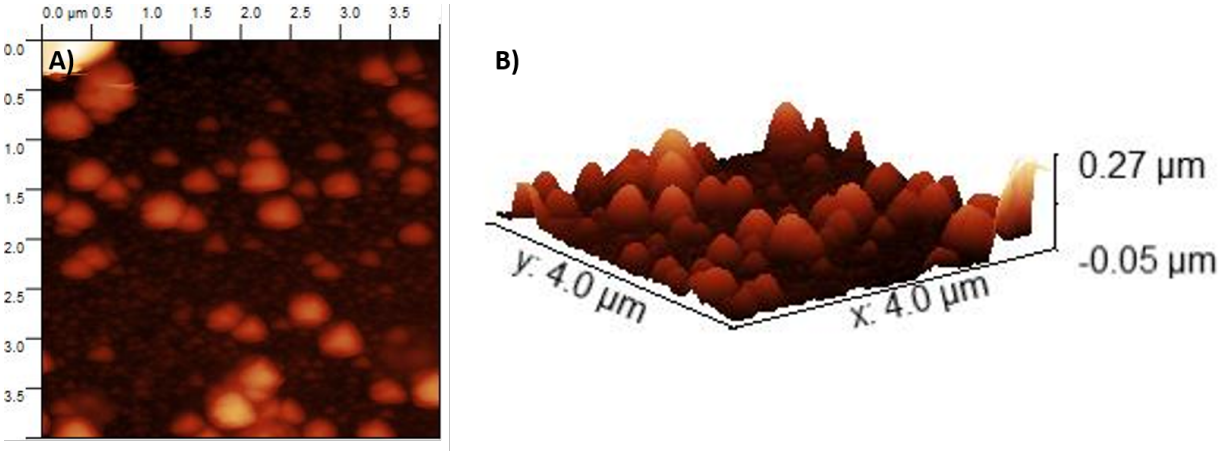


Figure S5: *AFM images A) height profile; B) 3-D height profile of P2 + PDADMAC nanoparticles*

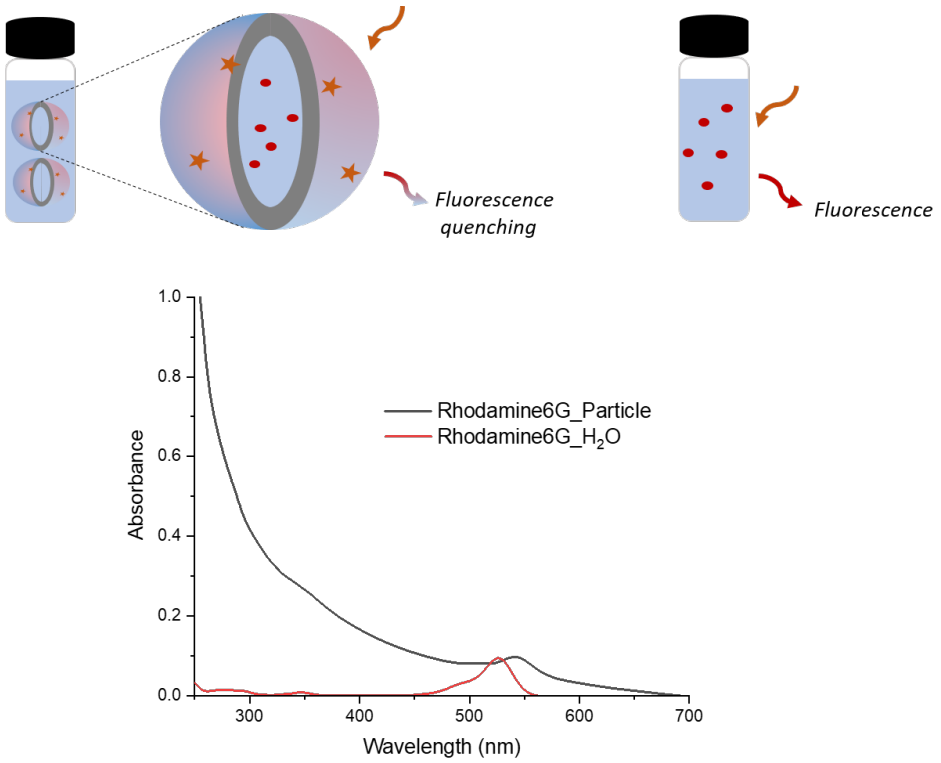


Figure S6: *Absorption matched emission spectra of rhodamine 6G in vesicles*

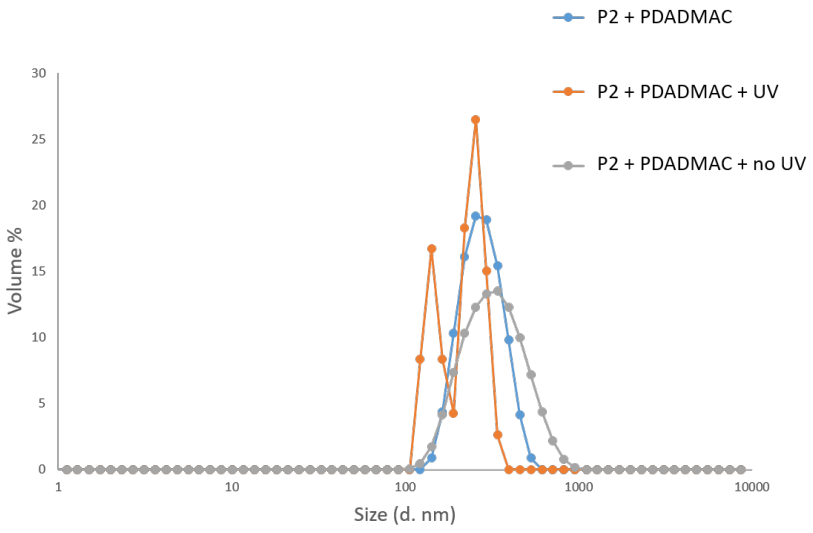


Figure S7: *DLS plot of the particles after UV illumination along with the control*

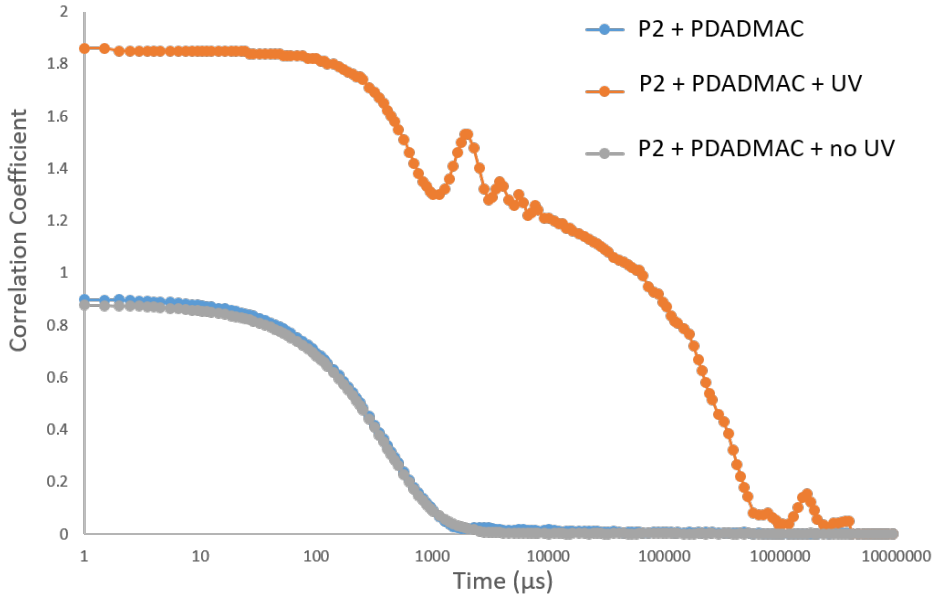


Figure S8: *Correlogram of the nanoparticles after UV illumination along with the control*

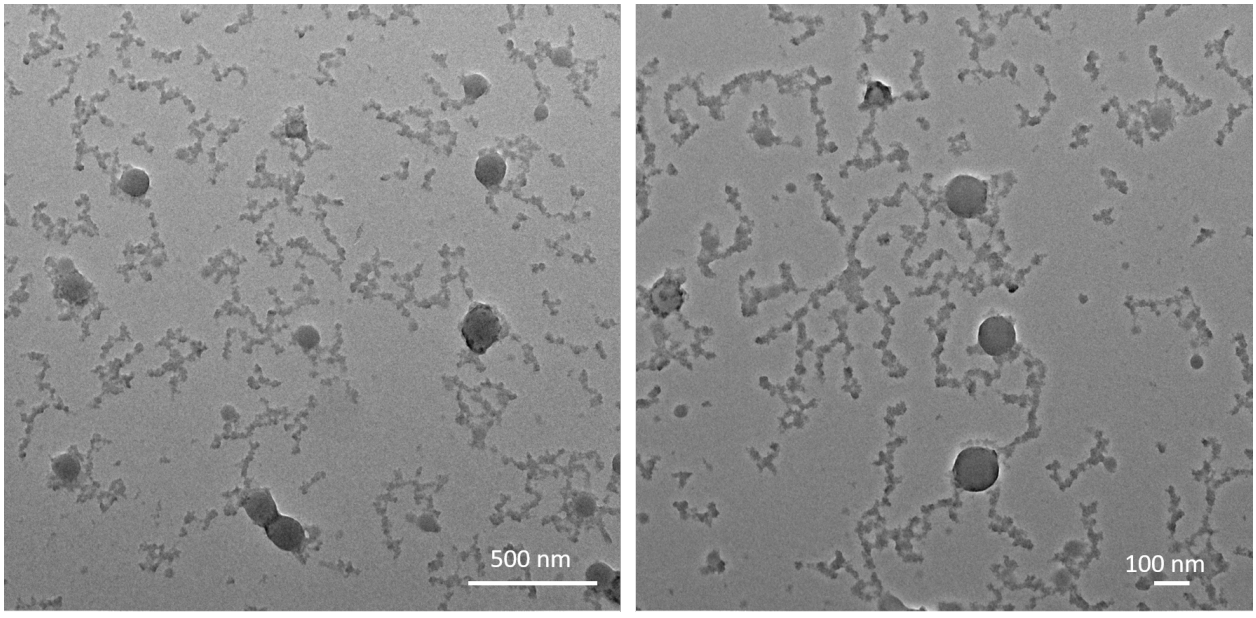


Figure S9: *TEM images of the nanoparticles after UV illumination*

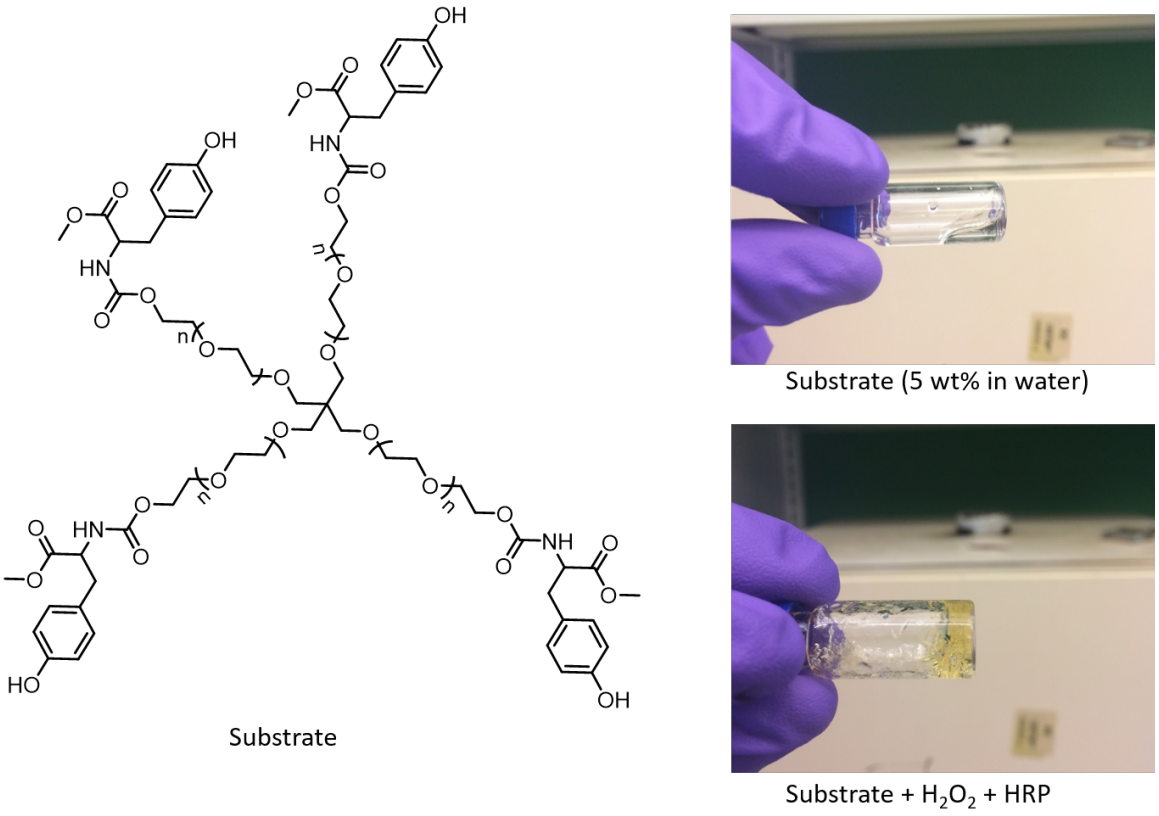


Figure S10*: HRP mediated gelation due to the cross-linking of tyrosine methyl ester*

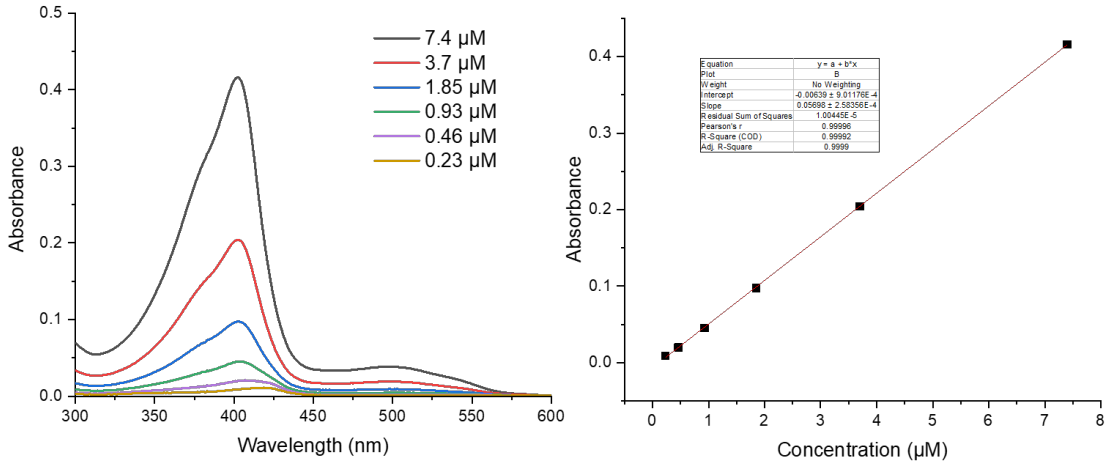


Figure S11*: Calibration curve for HRP quantification in the vesicles using the Soret band of porphyrin ring present in HRP at 403 nm.*

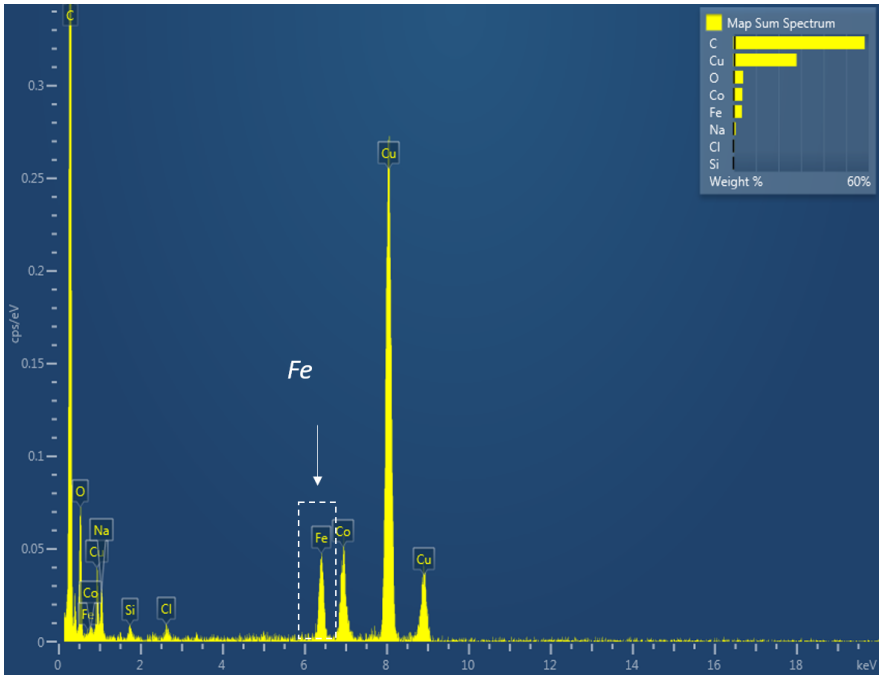


Figure S12: *EDS mapping confirming the presence of Fe in the vesicles encapsulated with HRP (Fe atoms are present in the porphyrin ring of HRP)*

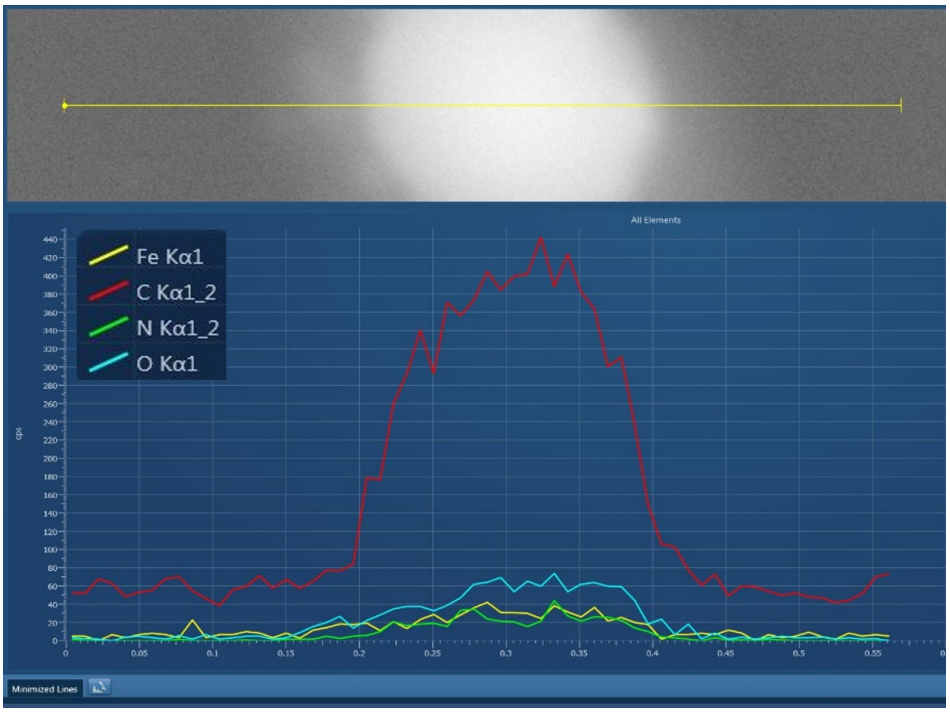


Figure S13: *EDS mapping of different atoms in the vesicle along the yellow line.*

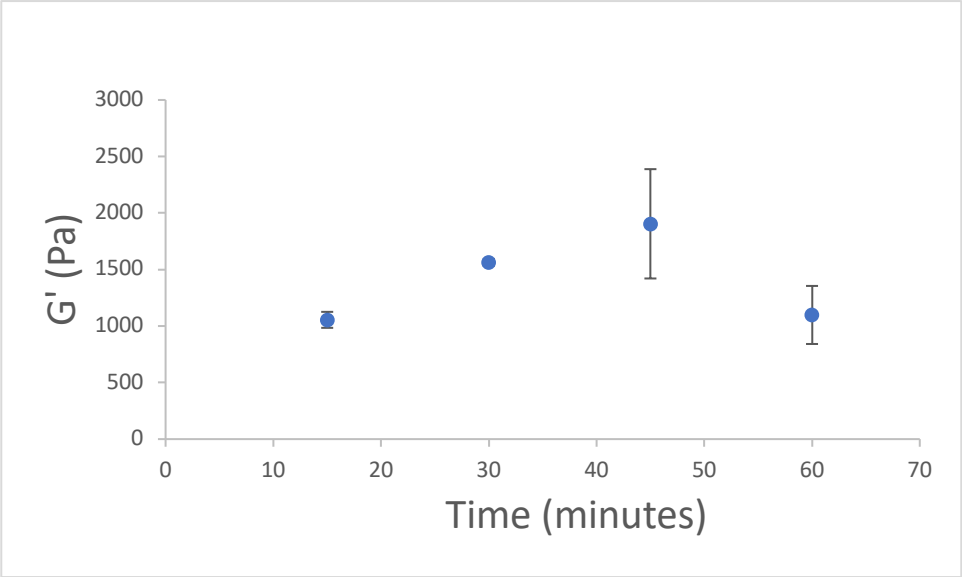


Figure S14: *G' value of gels with different UV exposure times. The experiment was done in duplicate.*

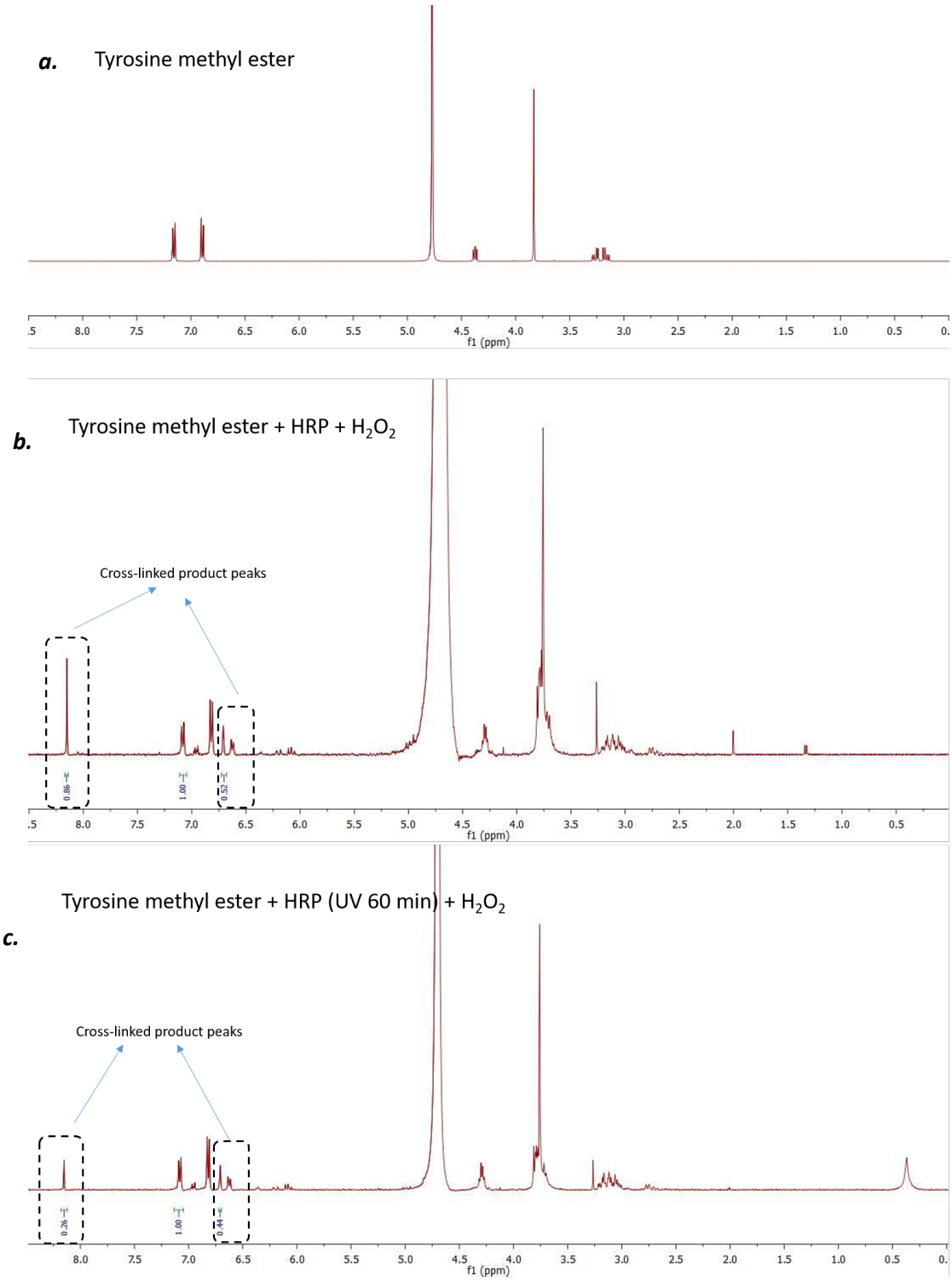


Figure S15: *1H NMR spectrum of tyrosine methyl ester (a); tyrosine methyl ester+ HRP + H_2O_2 (b); tyrosine methyl ester + HRP (treated with UV for 60 min) + H_2O_2. The relative intensity of cross-linked products after enzymatic reaction with respect to uncross-linked tyrosine methyl ester protons is less in 'c' compared to 'b'.*

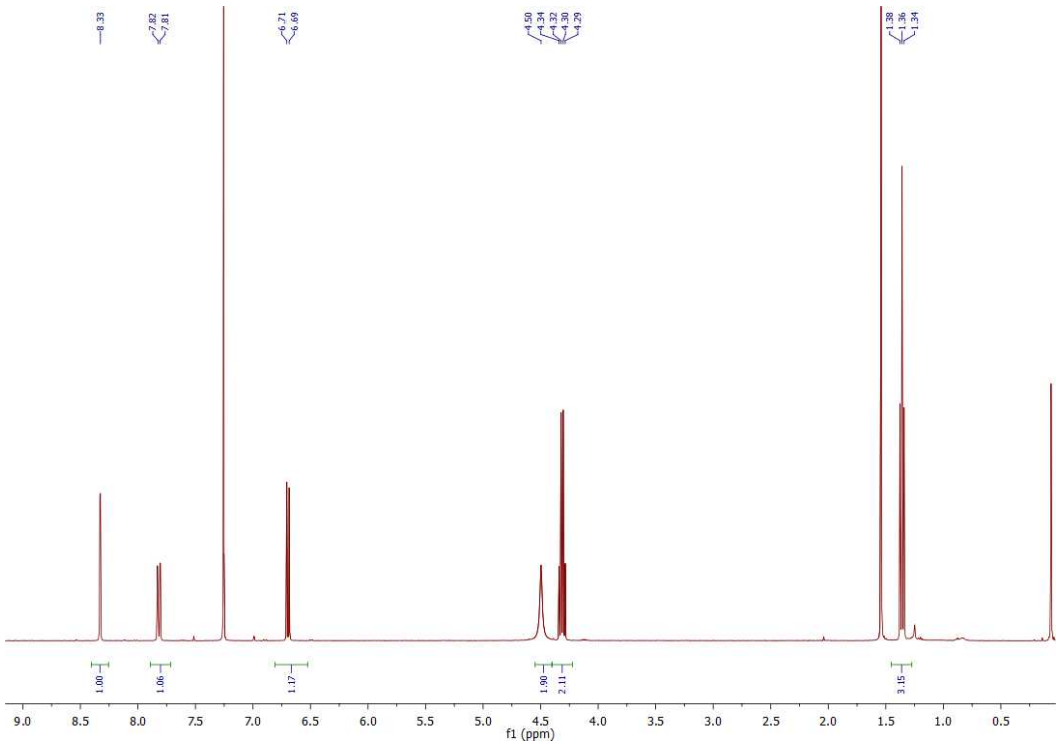


Figure S16: *^{1}H NMR spectrum of 1.*

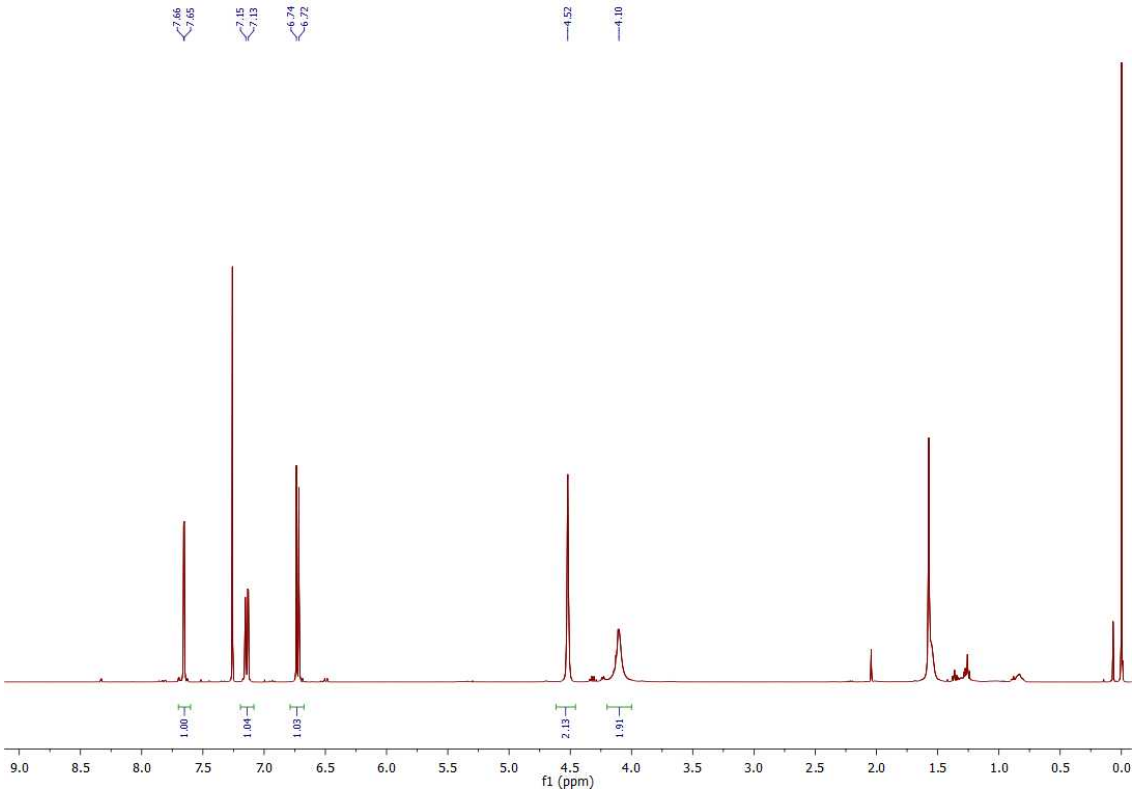


Figure S17: *^{1}H NMR spectrum of 2.*

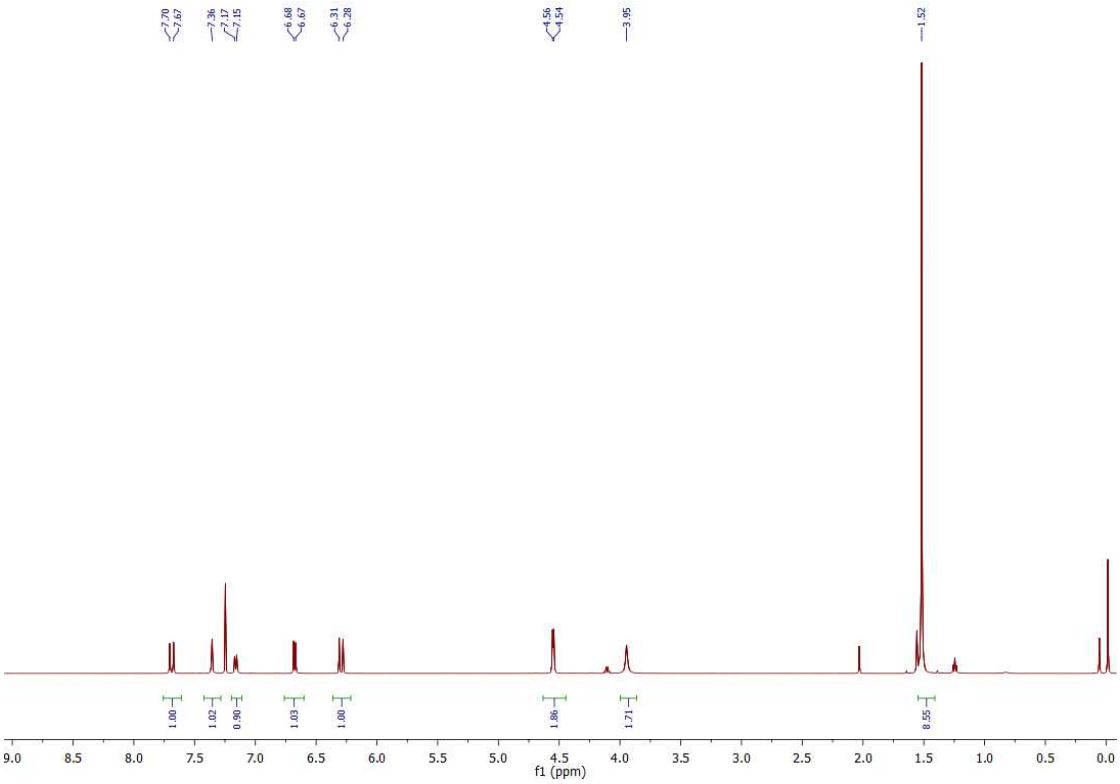
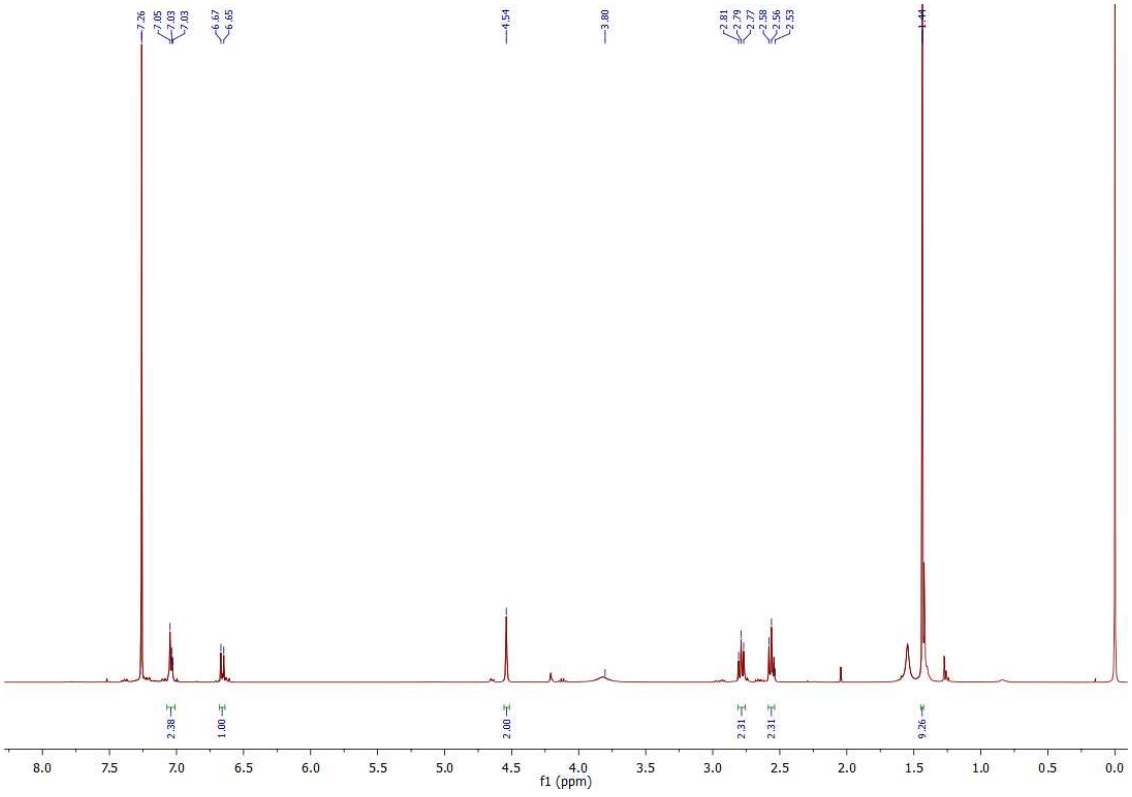


Figure S18*: [1]H NMR spectrum of 3*



. **Figure S19***: [1]H NMR spectrum of 4.*

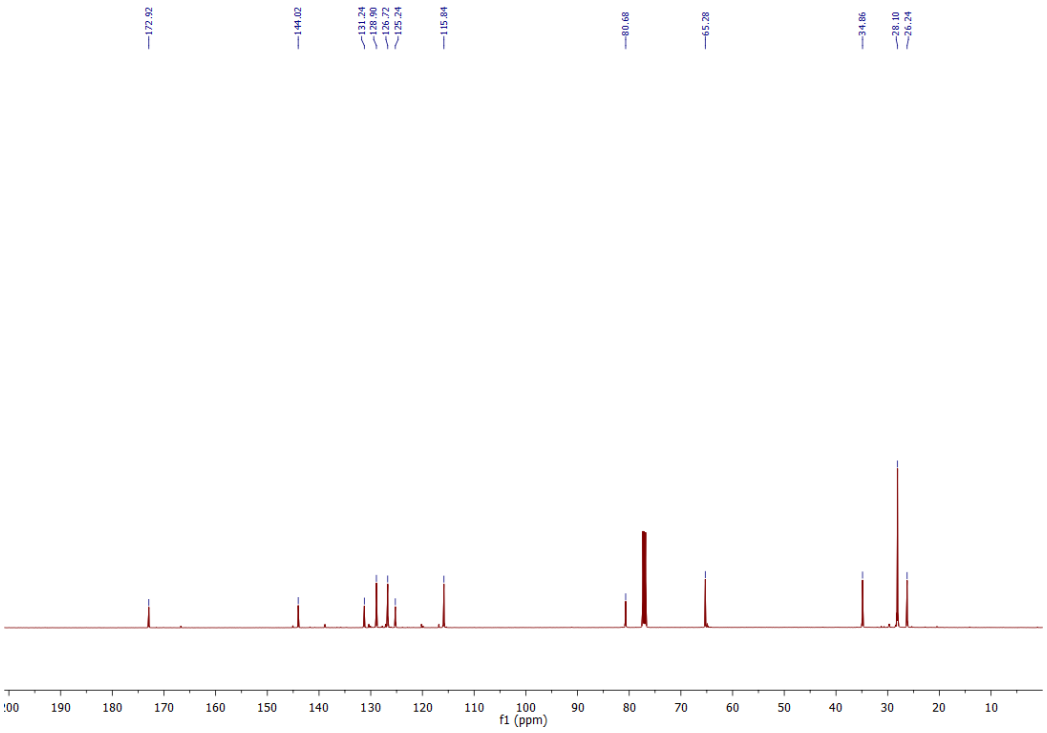


Figure S20: *^{13}C NMR spectrum of 4*

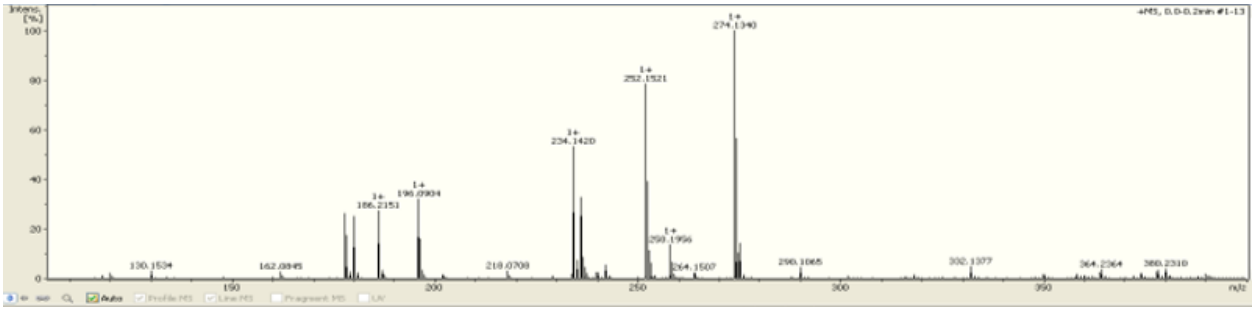


Figure S21: *ESI-MS spectrum of 4*

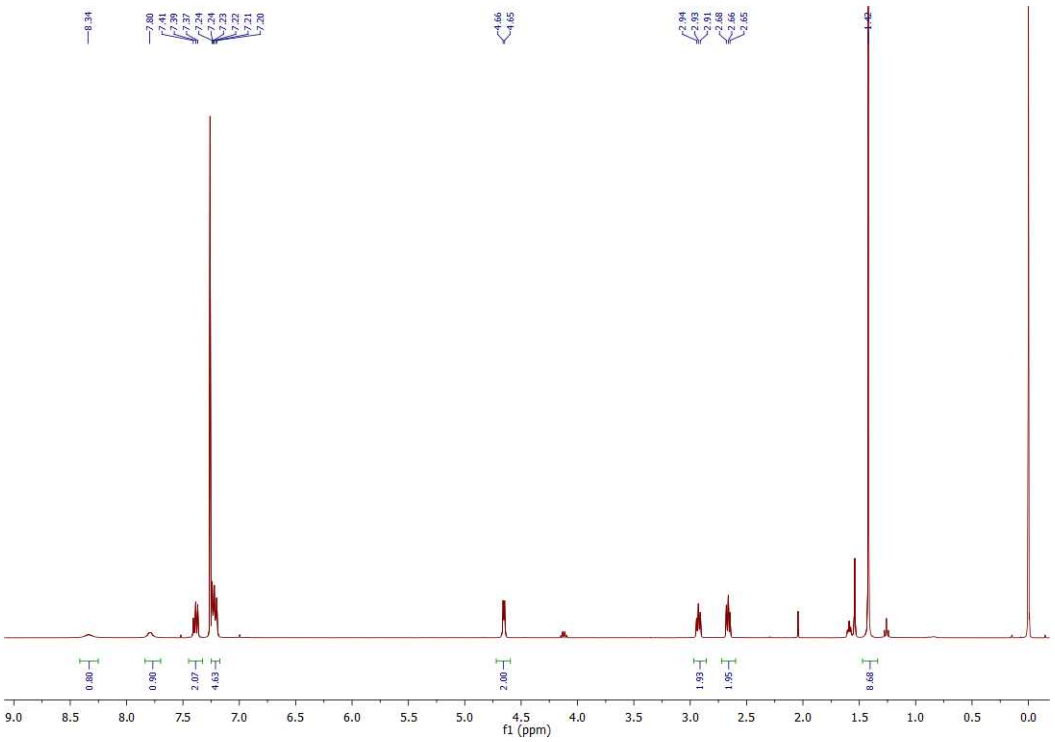


Figure S22*: [1]H NMR spectrum of 5.*

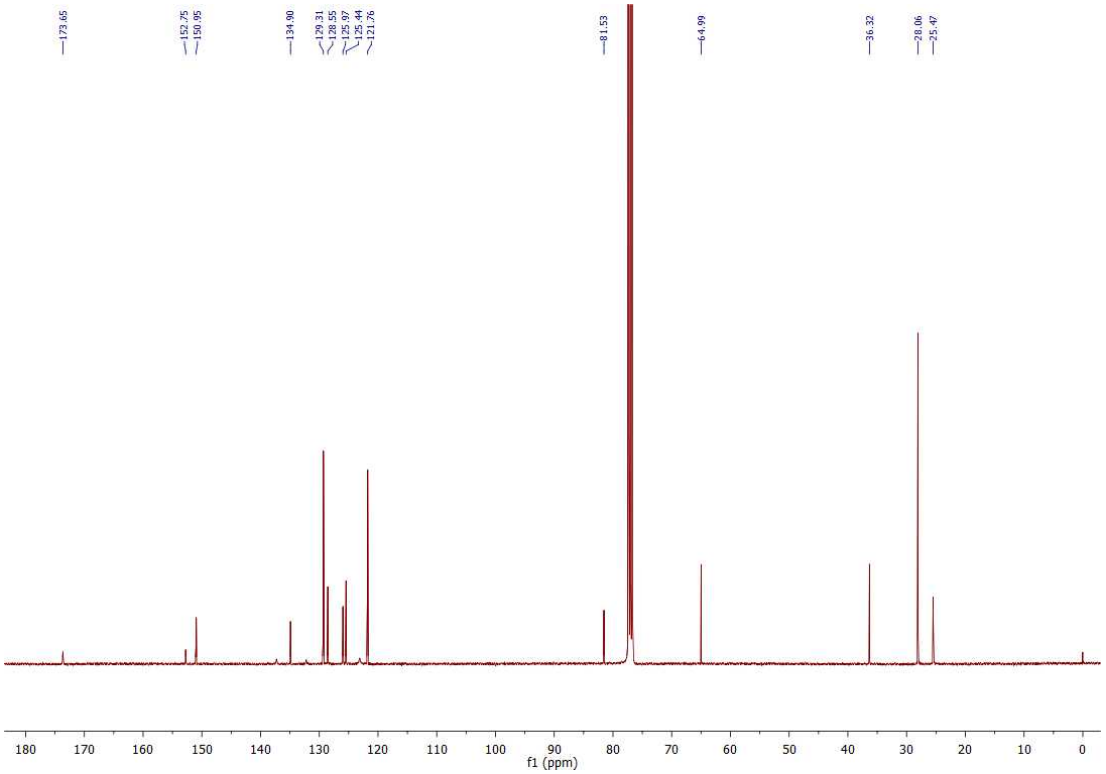


Figure S23*: [13]C NMR spectrum of 5.*

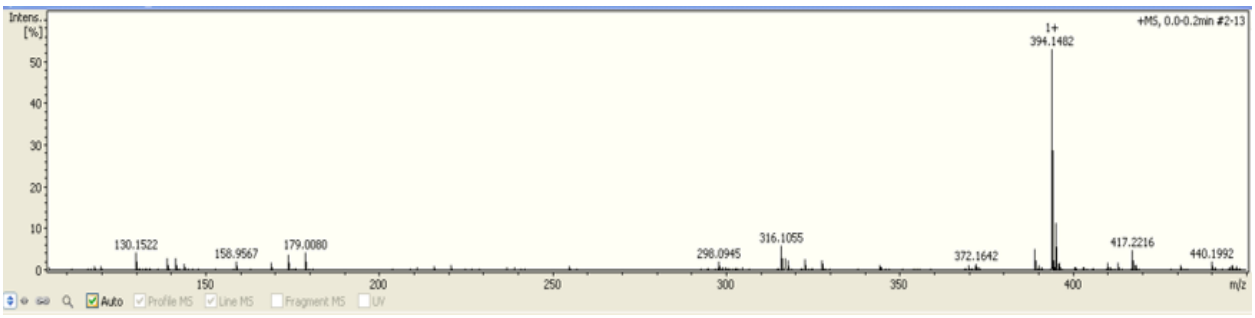


Figure S24: *ESI-MS spectrum of 5*

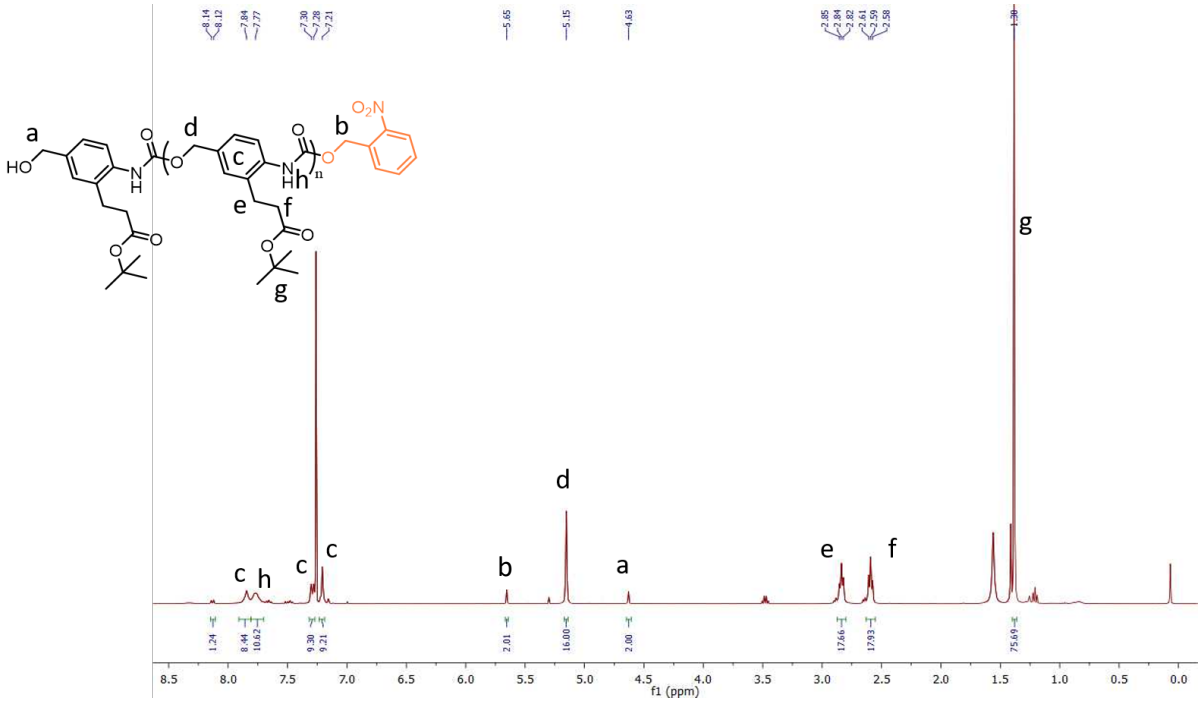


Figure S25: *^{1}H NMR spectrum of P1.*

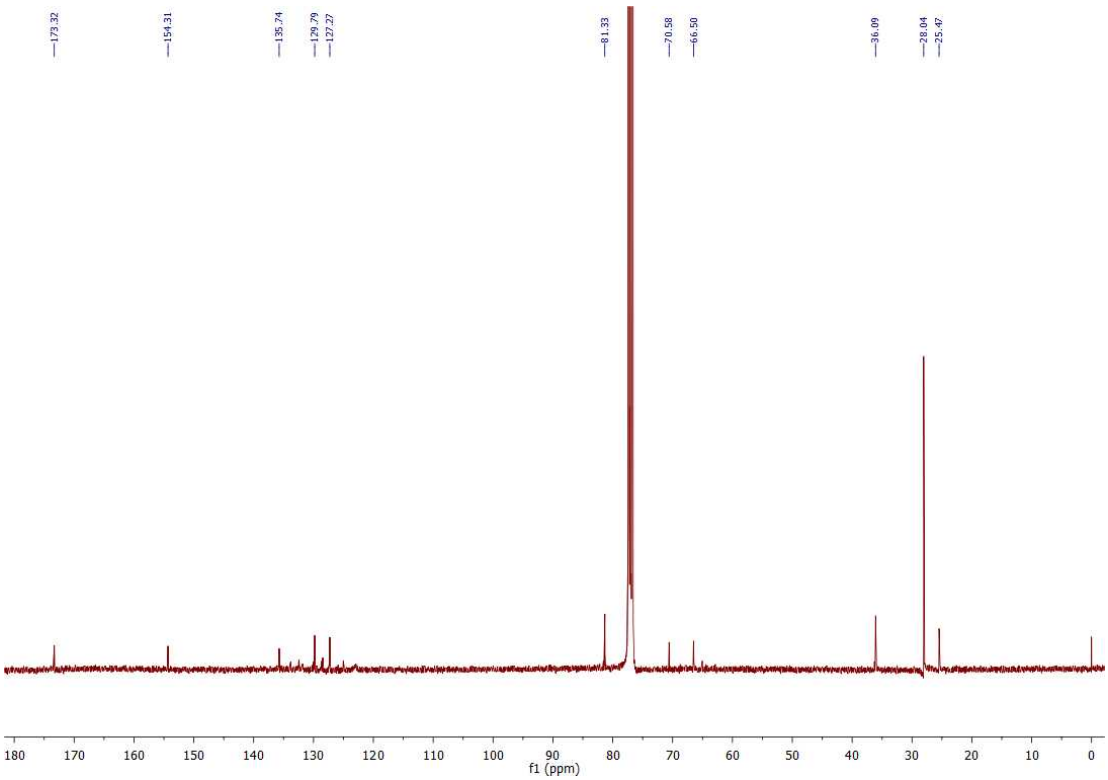


Figure S26: *^{13}C NMR spectrum of P1.*

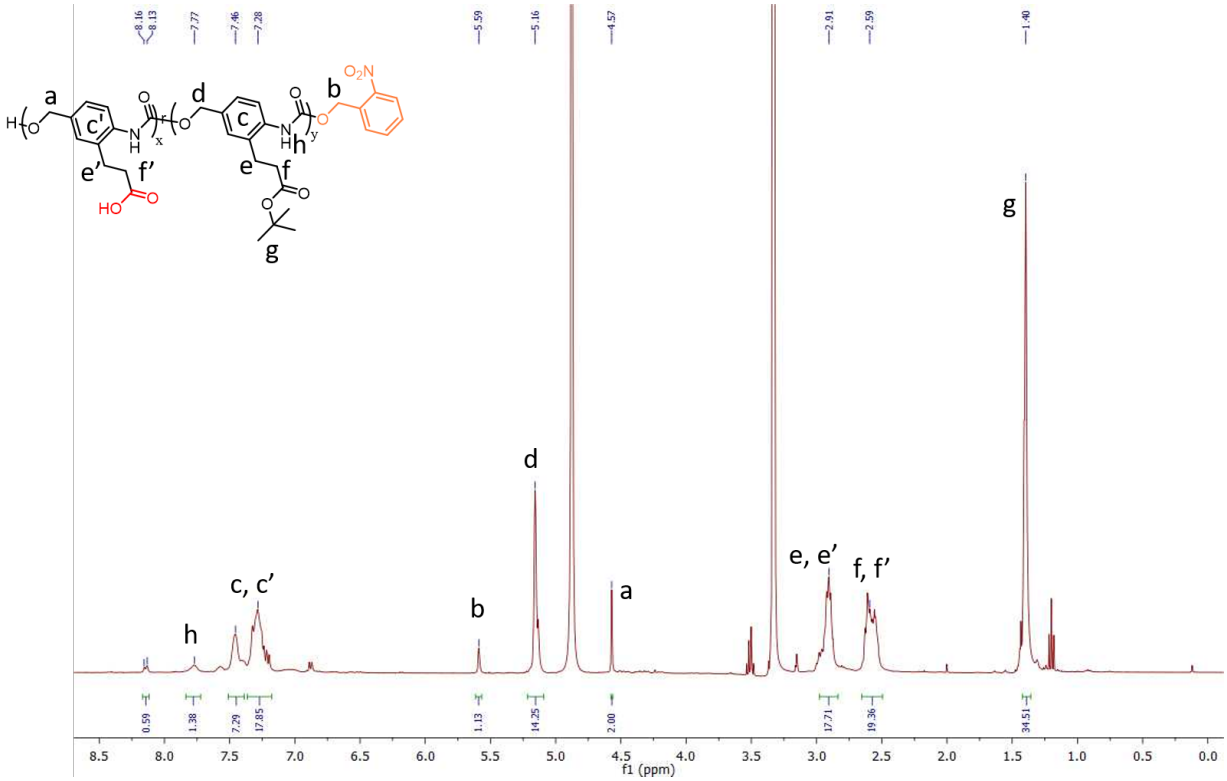


Figure S27: *^{1}H NMR spectrum of P2.*

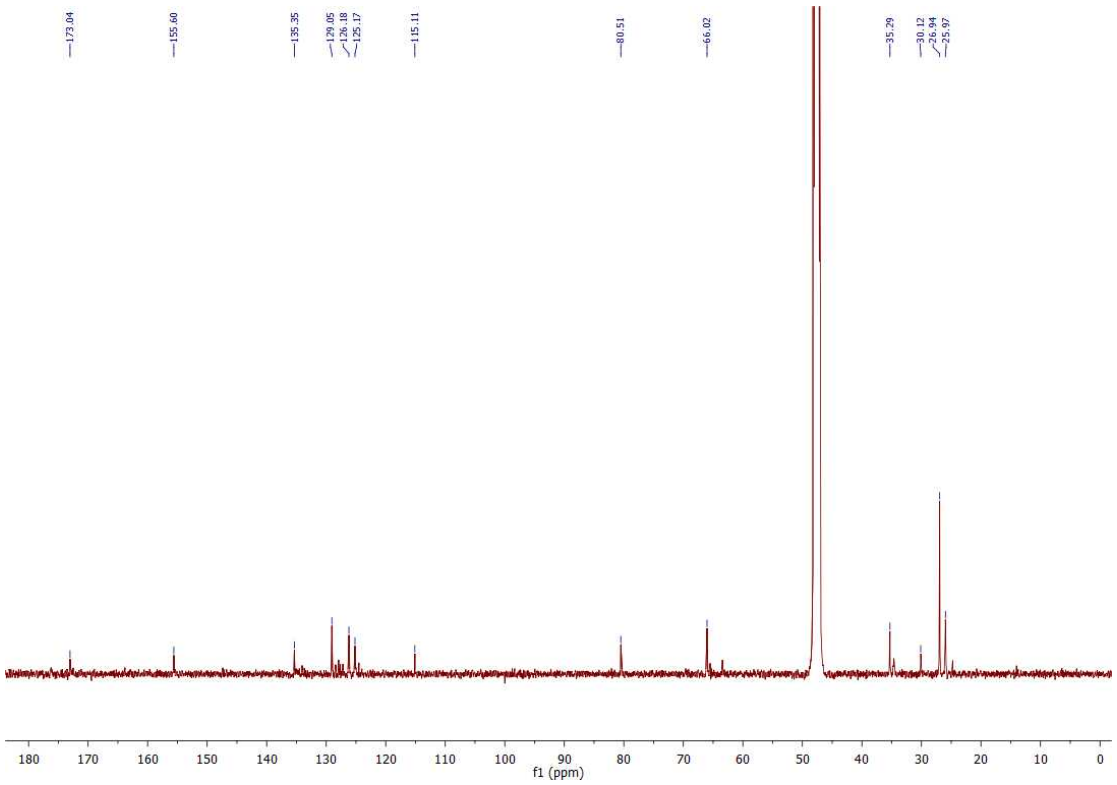


Figure S28: *^{13}C NMR spectrum of P2.*

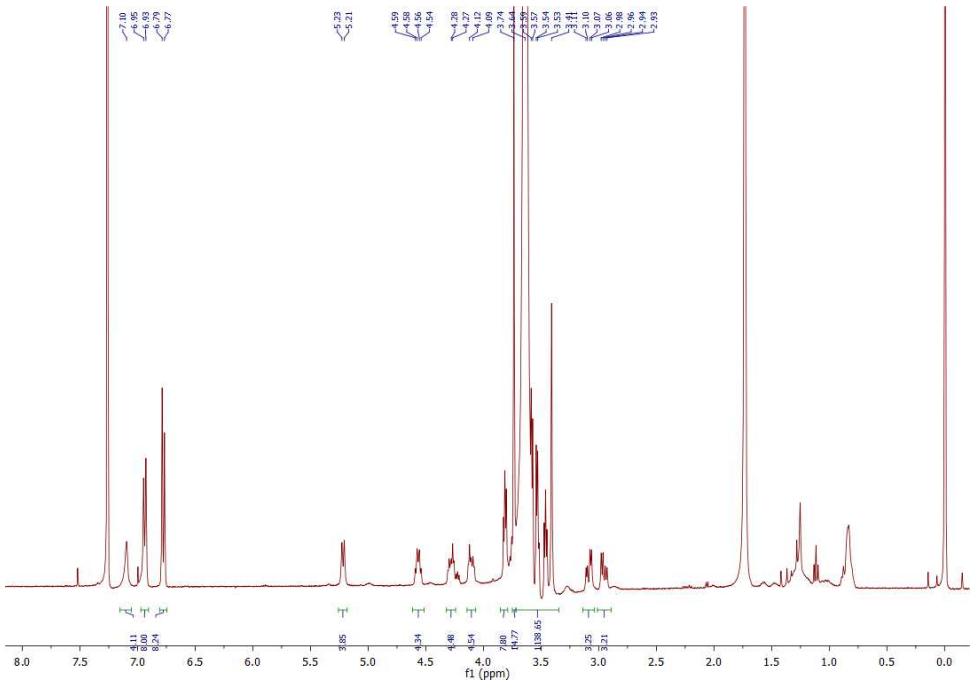


Figure S29: *^{1}H NMR spectrum of P3.*

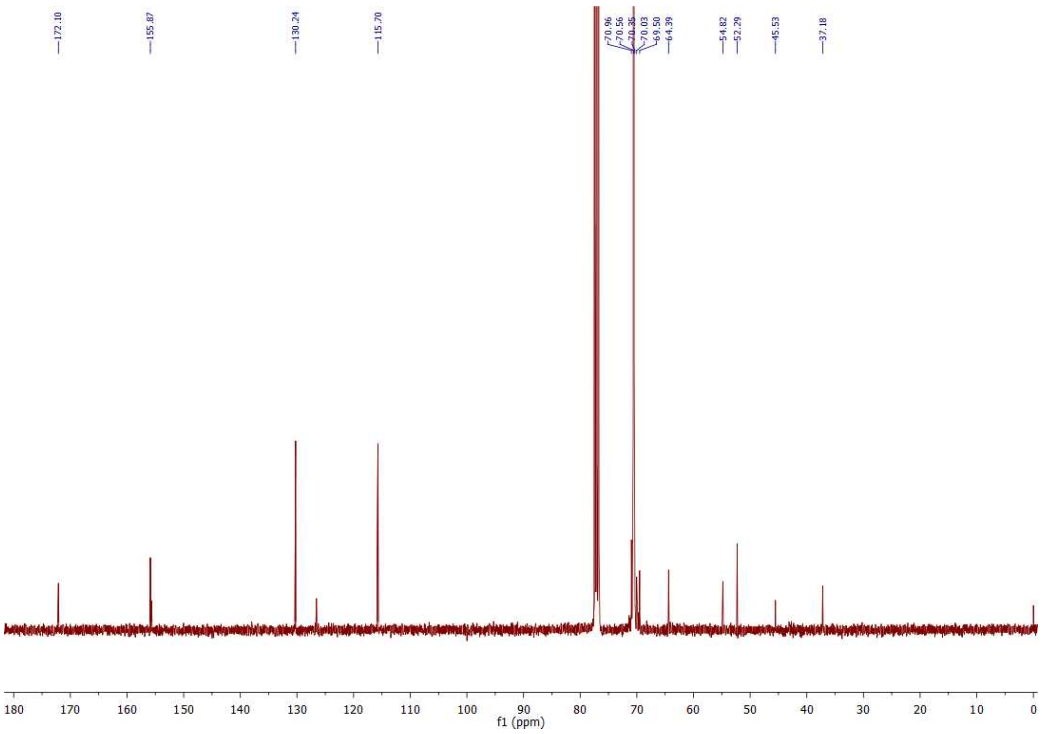


Figure S30*: ^{13}C NMR spectrum of P3.*

References:

[1] Sagi, A.; Weinstain, R.; Karton, N.; Shabat, D. Self-Immolative Polymers. *J. Am. Chem. Soc.* **2008**, *130* (16), 5434–5435.