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ESI

AIE-Driven Fluorescent Polysaccharide Polymersome as Enzyme-responsive FRET Nanoprobe to Study the Real-time Delivery Aspects in Live-Cells

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Experimental Section

Materials: Benzophenone, 4-hydroxy benzophenone, Zinc powder, titanium tetrachloride, 3-Pentadecylphenol (PDP), Ethyl chloro acetate, triethylamine (TEA), dextran (mol. Wt 6000), dicyclohexyl carbodiimide (DCC), 4-dimethylamino pyridine (DMAP), doxorubicin hydrochloride (DOX), dextran (molecular weight = 6000 g/mol), nile red, rosebengal, dimethylsulfoxide (DMSO) and horse liver esterase enzyme, O-methyl-D-glucose were purchased from Aldrich chemicals. Dialysis tubing (MWCO 3500 Da) were purchased from spectrum chemicals. NaOH and all other necessary reagents and solvents were purchased locally and purified following the standard procedures. PDP acid (compound **4**) was made by following the procedure reported in our earlier reports.[1-2]

General Procedures: NMR spectra were recorded using a 400 MHz Jeol and 100-MHz Bruker NMR spectrometer using $CDCl_3$ or DMSOd6 containing a small amount of TMS as an internal standard. The electronic spectra were recorded using Perkin-Elmer Lambda 45 UV−visible, and a Fluorolog HORIBA JOBIN VYON fluorescence spectrophotometer. TCSPC lifetime measurements were also recorded using Fluorolog HORIBA JOBIN VYON fluorescence spectrophotometer using nano-LED with wavelength of 340 nm and lifetime were measured at emission maxima at 460nm. The size determination of the self assembled structures were carried out by dynamic light scattering (DLS), using a Nano ZS-90 apparatus utilizing a 633 nm red laser from Malvern instruments. The data shown here is mean of at least three independent measurements. Freshly cleaved mica surface was used for drop casting the samples for atomic force microscope (AFM) and recorded using Agilent instrument. The imaging was carried out in tapping mode using TAP-190AL-G50 probe from Budget sensors with a nominal spring constant of 48 N/m and resonance frequency of 190. TEM images were recorded using a Technai-300 instrument. Differential scanning calorimetry (DSC) measurements were performed on a TA Q20 differential scanning calorimeter (DSC) at heating and cooling rates of 5.0 °C/min. FE-SEM images were recorded using Zeiss Ultra Plus scanning electron microscope. The samples for FE-SEM analysis were prepared by drop casting on silicon wafers. For TEM Analysis, the samples were prepared by drop casting on copper grid. Confocal microscopic images were recorded using LSM10 Confocal Microscope.The mass analysis of the compoundswas carried out using high-resolution mass spectrometry (HRMS-ESI-Q-TOF LC-MS) and Applied Bio system 4800 PLUS matrix-assisted laser desorption/ionization (MALDI) TOF/TOF analyzer.

Synthesis of 4-(1,2,2-triphenylvinyl)phenol (TPE-OH) (1): In 250 mL round bottom flask, benzophenone (10.00 g, 55 mmol), 4-hydroxy benzophenone (10.50 g, 53 mmol), Zinc powder (9.25 g, 142 mmol) were dissolved in 100 mL of dry THF at 0 ºC under nitrogen purging. The reaction mixture was stirred for 0.5 h and then titanium tetrachloride (12.50 g, 66 mmol) was added drop wise at temperature below 10 ºC. The reaction mixture was brought to ambient temperature and heated to reflux for 12 h. The mixture was cooled to room, THF is evaporated under pressure and then 100 mL of dilute hydrochloric acid (0.1 N) was added. The product was extracted using ethyl acetate, dried over sodium sulphate and it was purified over 100-200 mesh silica gel column using 5-15 % ethyl acetate in petether solvent. Yield =5.6 g (32 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 4.76 (s, 1 H, Ar-OH) 6.54 - 6.60 (m, 2 H) 6.88 - 6.94 (m, 2 H) 7.01 - 7.08 (m, 6 H) 7.08 - 7.15 (m, 9 H).^{13}C NMR (100 MHz, $CDCl_3$)δ ppm: 76.69 (s, 1 C) 77.32 (s, 1 C) 114.56 (s, 1 C) 126.24 (s, 1 C) 126.35 (s, 1 C) 127.58 (s, 1 C) 127.69 (s, 1 C) 131.31 (s, 1 C) 131.34 (s, 1 C) 132.71 (s, 1 C) 136.33 (s, 1 C) 140.14 (s, 1 C) 140.39 (s, 1 C) 143.86 (s, 1 C) 143.96 (s, 1 C) 153.95 (s, 1 C). MALDI-TOF: (MW: 348.15) m/z: 371.09 ($M+Na^+$). HR-MS (ESI+): m/z [M + H+] Calculated for $C_{26}H_{20}O$ [M+] = 348.1600; Found =348.1516.

Synthesis of Ethyl 2-(4-(1,2,2-triphenylvinyl)phenoxy)acetate (TPE- Ester) (2): Compound **1** (5.00 g, 15 mmol) and K_2CO_3 (2.97 g, 22 mmol) were dissolved in 80.0 mL of acetonitrile and stirred at 80 ºC for 0.5 h. The reaction mixture was cooled to room temperature and ethyl chloro acetate (2.39 g, 15 mmol) was added drop wise. It was heated at 90 ºC for 12 h. The reaction mixture was cooled to room temperature, K_2 CO_3 was separated by filtration, acetonitrile was concentrated under reduced pressure. The product was extracted using ethyl acetate and dried over sodium sulphate. Crude product was purified by using 100-200 mesh silica gel column using 3-5 % ethyl acetate in pet ether solvent. Yield= 4.0 g (65 %).^{1}H NMR (400 MHz, $CDCl_3$) δ ppm:1.30 (t, 3 H) 4.28 (q, 2 H) 4.56 (s, 2 H) 6.67 (m, 2 H) 6.97 (m, 2 H) 7.01 - 7.08 (m, 6 H) 7.08 - 7.18 (m, 9 H).^{13}C NMR (100 MHz, $CDCl_3$)δ ppm:14.12 (s, 1 C) 61.27 (s, 1 C) 65.29 (s, 1 C) 76.69 (s, 1 C) 77.32 (s, 1 C) 113.74 (s, 1 C) 126.26 (s, 1 C) 126.36 (s, 1 C) 127.56 (s, 1 C) 127.68 (s, 1 C) 131.26 (s, 1 C) 131.32 (s, 1 C) 132.52 (s, 1 C) 137.11 (s, 1 C) 140.20 (s, 1 C) 140.36 (s, 1 C) 143.72 (s, 1 C) 143.78 (s, 1 C) 143.82 (s, 1 C) 156.25 (s, 1 C) 168.84 (s, 1 C).MALDI-TOF: (MW: 434.19) m/z : 473.09 (M + K^+). HR-MS (ESI+): m/z [M + H+] Calculated for $C_{30}H_{26}O_3$ [M+] = 435.1978; found =435.199.

Synthesis of 2-(4-(1,2,2-triphenylvinyl)phenoxy)acetic acid (TPE-Acid) (3): Compound **2** (4.00 g, 9.2 mmol), KOH (1.55 g, 28.0 mmol) were dissolved in 50 mL of dioxane and reaction mixture was reflux for 6 h. The reaction mixture was cooled to room temperature, dioxane was removed under reduced pressure. The the solid compound was dissolved in water, pH was maintained 6.0 using dilute hydrochloric acid (0.1 N) and product is extracted using ethyl acetate. Crude product is purified by passing over 100-200 mesh silica gel column using 20-40 % ethyl acetate: pet ether solvent. Yield = 3.6 g (98 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm:4.63 (s, 2 H) 6.64 - 6.70 (m, 2 H) 6.95 - 7.00 (m, 2 H) 7.01 - 7.07 (m, 6 H) 7.08 - 7.17 (m, 9 H).^{13}C NMR (100 MHz, $CDCl_3$)δ ppm: 64.35 (s, 1 C) 76.36 (s, 1 C) 76.68 (s, 1 C) 113.42 (s, 1 C) 125.99 (s, 1 C) 126.03 (s, 1 C) 126.09 (s, 1 C) 127.27 (s, 1 C) 127.30 (s, 1 C) 127.40 (s, 1 C) 130.95 (s, 1 C) 130.99 (s, 1 C) 132.34 (s, 1 C) 137.25 (s, 1 C) 139.74 (s, 1 C) 140.24 (s, 1 C) 143.34 (s, 1 C) 143.40 (s, 1 C) 143.43 (s, 1 C) 155.46 (s, 1 C) 173.79 (s, 1 C).MALDI-TOF: (MW: 406.16) m/z : 445.06 (M + K^+).HR-MS (ESI+): m/z [M + H+] Calculated for $C_{28}H_{22}O_3$ [M+] = 406.1600; found = 406.1572.

Synthesis of PDP and TPE substituted dextran derivative: In 100.0 mL round bottom flask, dextran (1.00 g, 6.10 mmol), PDP Acid (0.56 g, 1.55 mmol), and TPE-Acid (0.15 g, 0.37 mmol) were dissolved in 25.0 mL of dry DMSO and the reaction mixture was purged with nitrogen for 0.5 h. Meanwhile dimethyl amino pyridine (DMAP) (0.19 g, 1.55 mmol) and dicyclohexyl carbodiimide (DCC) (1.33 g, 6.45 mmol) were dissolved in dry DMSO separately and added to the reaction mixture and continued the reaction for 24 h at 37 ºC. After 24 h, the dicyclohexyl urea was filtrated and the DMSO was removed by vacuum distillation. The reaction mixture was precipitated in methanol and purified thrice by dissolving in small amount of DMSO and reprecipitating in methanol. Yield = 0.70 g (48 %). ^{1}H NMR (400 MHz, DMSOd6) δ ppm:δ 7.14-6.99 ppm (m, 10H, Ar−H), 6.98-6.95 ppm (m, 6H, Ar−H),6.88-6.86 ppm (t, 2H, Ar−H), 6.71-6.68 ppm (m, 5H, Ar−H) 4.63 ppm (s, dextran anomeric proton), 4.47, 4.82, 4.88 ppm(s, hydroxyl of dextran),3.14−3.69 ppm (dextran glucosidic protons), 2.49 ppm (2H, Ar−CH_2), 1.48 ppm (2H,Ar−CH_2−CH_2), 1.18−0.80 ppm(aliphatic protons).

Synthesis of TPE substituted dextran derivative: In 100.0 mL round bottom flask, dextran (1.0 g, 6.10 mmol), TPE-Acid (0.15 g, 0.37 mmol) were dissolved in 25.0 mL of dry DMSO and the reaction mixture was purged with nitrogen for 0.5 h. Meanwhile dimethyl amino pyridine (DMAP) (0.013 g, 0.011 mmol) and dicyclohexyl carbodiimide (DCC) (0.091 g,

0.44 mmol) were dissolved in dry DMSO separately and added to the reaction mixture and continued the reaction for 24 h at 37ºC. After completion of reaction, the formed dicyclohexyl urea was filtered out, DMSO was evaporated and product is obtained by precipitation in methanol. Yield = 0.70 g (48 %). ^{1}H NMR (400 MHz, DMSOd6) δ ppm:δ 7.14-6.99 ppm (m, 9H, Ar−H), 6.98-6.95 ppm (m, 6H, Ar−H),6.88-6.86 ppm (t, 2H, Ar−H), 6.71-6.68 ppm (m, 2H, Ar−H) 4.63 ppm (s, dextran anomeric proton), 4.47, 4.82, 4.88 ppm(s, hydroxyl of dextran),3.14−3.69 ppm (dextran glucosidic protons).

Synthesis of Methyl 4,6-O-Benzylidene-α-D-Glucopyranoside (compound- 5):[3] Methyl α-D-glucopyranoside (3 g, 15.46 mmol) was taken in a 100 mL round bottom flask, followed by the addition of 30 mL of acetonitrile. To above suspension was added, benzaldehyde dimethyl acetal (4.44 mL, 29.43 mmol) and 90 mg of CSA (10-camphorsulfonic acid). The system was then refluxed for 1 h under nitrogen. The reaction was allowed to cool at room temperature followed by the addition of 0.5 mL of triethylamine. The solvent was removed under vacuum followed by Ethyl acetate and water workup. The solid compound was dissolved into 30 mL of ethyl acetate and washed several times with water. The organic layer was then dried over anhydrous Na_2SO_4. The solution was then concentrated under vacuum to give thick syrup which was precipitated in DCM and Hexane (1:8). The solid was then filtered and dried under vacuum to afford product 2 in 89% yield. (^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.50 (m, 2 H, aromatic) 7.37 (m, 3H, aromatic) 5.54 (s, 1 H) 4.81 (d, 1 H) 4.32 (dd, 1H) 3.94 (t, 1 H) 3.82-3.79 (m, 1H) 3.78- 3.73 (m, 1 H) 3.65 (m, 1 H) 3.53 (t, 1H) 3.47 (s, 3 H, OMe) 2.78 (bs, 1H, -OH) 2.78 (bs, 1H, -OH). ^{13}C NMR (100 MHz, $CDCl_3$)δ ppm: 129.24 (aromatic, 1 C) 128.31 (aromatic, 1 C) 126.28 (aromatic, 1 C) 101.92 (1 C, CHPh) 99.75 (1 C, C1) 80.91 (1 C, C4) 72.85 (1 C, C2) 71.71(1 C, C3) 68.90 (1 C, C6) 62.34 (1 C, C1) 55.54(1C, C5). MALDI-TOF: (MW: 282.29) ,m/z $(M + H)^+$: 283.18

Synthesis of (4aR,6S,7R,8R,8aR)-7-hydroxy-6-methoxy-2-phenylhexahydropyrano[3,2-d][1,3]dioxin-8-yl 2-(3-pentadecylphenoxy)acetate (Compound-6): To a 100 mL 2 neck round bottom flask, compound **1** (0.5g, 1.77mmol), PDP- COOH (compound-4) (0.64g, 1.77mmol), EDC.HCl (0.35g, 3.5mmol), DMAP (44mg, 0.35 mmol) were taken and kept at high vacuum prior to reaction for 30 minutes. The system was removed off the vacuum and freshly dried DCM was added at 0ºC under nitrogen atmosphere. The stirring was continued for 30 minutes at 0ºC and then the reaction was continued for 12 hours at room temperature under nitrogen atmosphere. After 12 hours the reaction was stopped and works up was done

with 10% $NaHCO_3$ solution (1X 100 mL). The organic layer was extracted and then washed with water (2X 100mL). The organic layer was the dried over anhydrous Na_2SO_4. The solution was evaporated under reduced pressure to give the crude product. Column Chromatography in 230-400 mesh silica was done to extract compound 6 as a crystalline white solid (0.26g). (^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.45-7.43 (m, 2 H, aromatic) 7.38-7.35 (m, 3H, aromatic) 7.03 (t, 1 H) 6.78- 6.76 (m, 2 H) 6.67- 6.64 (dd, 1H) 5.48- 5.43 (s +t, 2 H) 4.83 (d, 1H) 4.68- 4.69 (s, 2 H) 4.33- 4.30 (dd, 1 H) 3.92- 3.88 (m, 1H) 3.79- 3.73 (t, 1 H,) 3.68 (m, 1H,) 3.64 (t, 1H), 3.49 (s, 3H, -OMe), 2.54 (t, 2H), 1.26 (broad multiplet, 24 H), 0.89 (t, 3H). ^{13}C NMR (100 MHz, $CDCl_3$)δ ppm: 169.46 (1 C), 158.10 (1 C), 145.06 (1 C), 137.16 (1 C), 129.43 (1 C), 128.57 (1 C), 126.49 (1 C), 122.19 (1 C), 115.66 (1 C), 111.44 (1 C), 101.85 (1 C), 100.32 (1 C), 73.46 (1 C), 78.73 (1 C), 71.85 (1 C), 69.16 (1 C), 65.59 (1 C), 63.01 (1 C), 55.92 (1 C), 36.19 (1 C), 32.22 (1 C), 31.57 (1 C), 21.99 (1 C), 23.00 (1 C), 14.43 (1 C), MALDI-TOF: (MW: 626.83) ,m/z $(M + K)^+$: 665.4315, m/z $(M + K)^+$: 649.5124.

Small amount of 2,3-substituted ester (compound-**7**) was also successfully isolated and its NMR spectrum is given in the latter pages.

Synthesis of (2R,3R,4S,5R,6S)-3,5-dihydroxy-2-(hydroxymethyl)-6-methoxytetrahydro-2H-pyran-4-yl 2-(3-pentadecylphenoxy)acetate (Model Compound)(Compound-8): To a 25 mL RB, compound 6 (100 mg, 0.159mmol) and Para-toluene sulphonic acid (PTSA, 10mg, 0.3, 0.004 mmol) were taken. To it was added 10 mL of (1:1) DCM/MeOH. The system was stirred at room temperature for 2 hours. After 2 hours, triethylamine was added to quench PTSA. The solvent was distilled under reduced pressure to afford yellowish white solid. The impure compound was then subjected for column chromatography in 100- 200 mesh silica using 1% MeOH in DCM as eluent. The product was isolated as crystalline white solid (47mg, 56%).(^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.13-7.09 (t, 1 H, aromatic) 6.76-6.74(d, 1H, aromatic) 6.7- 6.69 (m, 1 H) 6.66- 6.63 (m, 1 H) 5.13- 5.09 (t, 1 H) 4.71 (d, 1H) 4.66 (s, 2 H) 3.78-3.75 (m,2 H) 3.58- 3.57 (m, 2H) 3.53- 3.51 (m, 1 H,) 3.38 (s, 3H,) 2.51-2.47 (bs+t, 1H+2H), 2.10 (d,1H), 1.18 (24 H, b-multiplet), 0.83- 0.79 (t, 3H). ^{13}C NMR (100 MHz, $CDCl_3$)δ ppm: 170.51 (1 C), 157.71 (1 C), 145.05 (1 C), 129.33 (1 C), 122.15 (1 C), 115.09 (1 C), 111.32 (1 C), 99.38 (1 C), 71.28 (1 C), 70.78 (1 C), 68.80 (1 C), 65.39 (1 C), 62.01 (1 C), 55.51 (1 C), 35.97 (1 C), 31.95 (1 C), 29.72 (1 C), 22.71 (1 C), 14.14 (1 C) MALDI-TOF: (MW: 538.6) ,m/z $(M + K)^+$: 577.6

DOX encapsulation in dextran derivatives by dialysis method: 20.0 mg of fluorescent dextran polymer compound and 0.2 mg of doxorubicin hydrochloride molecule were dissolved in 2.0 mL of DMSO and stirred for 10 minutes. To the above solution 2.0 mL of milli-q water was added dropwise and the resulting solution was stirred for 12 h in the dark. The solution was then transfeered to dialysis bag (MWCO 3500Da) and dialysed against milli-q water for 48 h. The water from the reservoir was changed priodically to remove the unencapsulated molecules.After 48 h , the obtained solution is filtered through 0.45 u whatman filter paper, lyophilised and stored at $4^{o}C$. The amount of DOX loaded in the polymer was determined using lambert beers law using the molar extinction coefficient as 11500L mol^{-1} cm^{-1}.The drug loading content (DLC) and drug loading efficiency (DLE) were calculated following the equation in earlier report[4] and they were found to be 3.8 % and 60 % respectively.

Dye encapsulation in dextran derivatives by dialysis method: 20.0 mg of fluorescent dextran polymer compound and 0.2 mg of rose bengalor nile red dyemolecule were dissolved in 2.0 mL of DMSO and stirred for 10 minutes. To the above solution 2.0 mL of milli-q water was added dropwise and the resulting solution was stirred for 12 h in the dark. The solution was then transferred to dialysis bag (MWCO 3500) and dialysed against milli-q water for 48 h. The water from the reservoir was changed periodically to remove the unencapsulated molecules. After 48 h, the obtained solution is filtered through 0.45 u whattman filter paper, lyophilised and stored at $4^{o}C$. The amount of dye or drug loaded in the polymer was determined using Beer- Lambert's law. The drug loading content and drug loading efficiency were calculated following the equation in earlier report.[4] The dye loading contents (DLC) and dye loading efficiencies (DLE) for these dyes were found to be 1.5 % and 65 % for P_{TPE+RB}, and 1.8 % and 55 % for P_{TPE+NR}.

TPE acid encapsulation in dextran-PDP derivativesby dialysis method: In typical experiment, 20mg of dextran-PDP derivatives (without TPE conjugation) and 2 mg of TPE acid were dissolved in 2.0 mL milli-Q water and then 2.0 mL of DMSO was added slowly and the resulting mixture is stirred for 12 h in dark. The solution was then transferred to dialysis bag (MWCO 3500 da) and dialysed against milli-Q water for 48 hours. After dialysis, the solution was filtered through 0.45u whatman filter paper, lyophilised and stored in dark at $4^{0}C$. The lyophilised powder is used for further experiments.

***In Vitro* Drug Release Studies by dialysis method:** *In vitro* doxorubicin released from the fluorescent dextran vesicles was monitored using dialysis method described in our earlier report.[4] Typically 3.0 mg of DOX loaded dextran vesicles were taken in dialysis bag (MWCO 3500) in immersed in 10.0 mL of PBS at 37°C. To determine the dox concentration in the dialysate, 3.0 mL of dialysate was withdrawn periodically and absorbance was measrued to calculated the amount of dox released. The percent cumulative release was calculated following the equation. A similar protocol was followed to monitor the esterase enzyme action on the dox release.

Cumulative Release = amount of drug release at time t/ total amount of drug taken in dialysis tube *100

Photophysics and FRET Studies: All the absorption and emissionexperiments were carried out keeping the optical density of TPE fluorophore as 0.1. TPE chromophore was excited at 340 nm and DOX was excited at 480 nm. The other dyes like Nile red, and rose Bengal were excited at 560 , and 527 nm respectively. For Emission measurement the slit width was kept as 2 nm and it was held constant for all the measurements. For TCSPC lifetime studies nano-LED source with wavelength340 nm for exciting TPE chromophores was used, and their lifetime decay was obtained at emission maxima of 460 nm. These decays were fitted by using DAS6 software.

Cell Viability Assay and Cellular imaging: A cell viability assay was performed in MCF 7, WTMEF cells using the tetrazolium salt, 3-4,5 dimethylthiazol-2,5-diphenyl tetrazolium bromide (MTT) to compare the cytotoxicities of free drug and drug loaded vesicles.1000 cells per well were seeded in 96 well plate in 100 µL of complete DMEM and allowed to adhere for 16 h. Different concentrations of the free drug and drug encapsulated vesicles were then added to the cells and incubated for another 72 h. Thereafter the media was aspirated and 100 µL of freshly prepared MTT solution (0.5 mg/ mL in complete media) was added and incubated for another 4h at 37 °C. The formed formazan crystals were dissolved in 100 µL of DMSO and the absorbance was immediately measured using varioscan micro plate reader at 570 nm. The data represents the mean value from at least three independent measurements. The relative percentage values, with respect to control, were calculated and plotted against the concentration. To envision the intracellular fate of free drug and drug loaded vesicles,

cells were seeded at a density of 10^5 cells per well on 6 well-plates containing 2.0 mL of complete DMEM medium and incubated at 37 °C for 16 h. After 16 h, media was aspirated and cells were fed with 2.0 mL media having required concentration of the compounds. After incubating for 9 h, the media was removed and cells were washed using 1X PBS two times following the permeablisation using 4.0 % paraformaldehyde solution. After staining cells with necessary dyes, the cover slips were mounted on the surface of clean and flamed dried slides using 10% glycerol as mounting and were left to dry overnight at room temperature, away from light. The slides were then imaged using a confocal microscope employing 405 nm and 568 nm lasers as excitation sources. Image J software was used for processing the images. All the confocal images are recorded keeping all the parameters same for every measurement. DOX fluorescence intensity from the confocal images was calculated in Image J analysis software using the following equation:

Corrected Total Cell Florescence (CTCF) = Integrated Density – [Area of selected cell x Mean fluorescence of background reading]

Tissue Culture Conditions: Breast cancer cells (MCF 7) and wild type MEF (WTMEF) were cultured in complete DMEM media (with 5% fetal bovine serum (FBS) and 1% penicillin-streptomycin) at 37°C under 5% CO_2 humidified atmosphere. Cells were kept mycoplasma free by treating them with anti-mycoplasma agent sparfloxacin from Sigma. Cells were detached using trypsin and seeded in 60mm dishes or flasks as per requirement.

Phalloidin Staining of the Polymersomes: About 10^5 cells were seeded on 70% alcohol rinsed and flame dried coverslips and were placed carefully in a 6 well plate supplemented with DMEM media and 10 % FBS. The setup was incubated at 37 ^oC for 16h in a 5% CO2 atmosphere. A lyophilized powder of P_{TPE} containing 1μM of the flurophore and P_{RB} containing 5μM of flurophore was added independently in two different wells of the 6 well chambers and were further incubated for 4h at ambient conditions. The media was aspirated followed by the washing of cells with cold PBS solution (3 X 1mL). The coverslips having cells were chemically fixed using 4% paraformaldehyde solution in PBS for 10 minutes at 4^oC. Next, the cells were gently washed twice with 1mL cold PBS solution. Cells were then stained with Alexa Flouro-488 Phalloidin and kept at a dark place for 5 minutes at room temperature. The unbound dye was washed off of the coverslips by treating it twice with 1 mL cold PBS solution. The coverslips were gently mounted in an inverted position onto a

clean and flame dried glass slide using a drop of 10% glycerol solution as contact media. The coverslip was then sealed onto glass slide using transparent nail paint. CLSM imaging was carried using a confocal assisted microscope engaging 405 nm, 488nm and 568nm lasers respectively. A smart setup was created to avoid the spectral spill over. Images acquired were then processed using ImageJ software.

Lysosomal Tracking of the Polymersomes: A 4 well live cell chamber were cultured with approximately 25000 cells for about 16- 18 h. The cells were treated with desired amount of flurophore and were further incubated for necessary time. After the stipulated time period, the media was aspirated followed by a PBS wash. The cells were added with Lysotracker DND-26 in 1mL of DMEM media followed by imaging.

Live cell imaging Protocols: For a Time dependent live cell experiment, 25000 cells per well were seeded in a 4 well chamber for 16 hours in a CO2 incubator maintained at 37^0C. The chamber was then placed on a Stage top incubator of a Zeiss Multiphoton instrument (Verdi/Mira 900; Coherent), maintaining a CO_2 humidified atmosphere at 37^0C. The image focus (using DIC) at 40X oil immersion and other acquisition parameters were adjusted prior to compound addition. After the compound addition ($P_{TPE+DOX}$) to the first well, images were immediately acquired after 1 min, 5 min, 20 min, 45 min, and 60 min (lower time points) in the same ROI, focusing at the same cells (in one well only). Subsequently second, third and fourth wells were added with the compound and imaged at 2h, 3h and 4h(higher time points) respectively using a 405 nm and 488 nm laser respectively. For P_{TPE+RB} the compound was pre incubated for 24h, 12, 6h, 3h, 2h, 1h prior to imaging. For lower time point, compound addition was done on the stage top incubator and images were subsequently acquired using 405 and 561 nm laser respectively.

Enzyme Degradation Studies of Model Compound-8:[5] K_2HPO_4 (0.88 mg) was dissolved in 900 μL of D_2O (solution-1). Horse liver esterase enzyme (0.1 mg) of was then dissolved and warmed to 37 ^oC for 2 minutes. Compound-8 (10 mg) was dissolved in 100 μL of acetone- d^6 and was then added to 900 μL of enzyme containing solution-1. The system was allowed to equilibrate for 5 minutes at 37^oC and then NMR was recorded. The same sample was then incubated at 37^oC for 12 hours and was then subjected for NMR analysis. (Note:

Water suppression was not carried out as it would suppress the peaks of interest in its vicinity)

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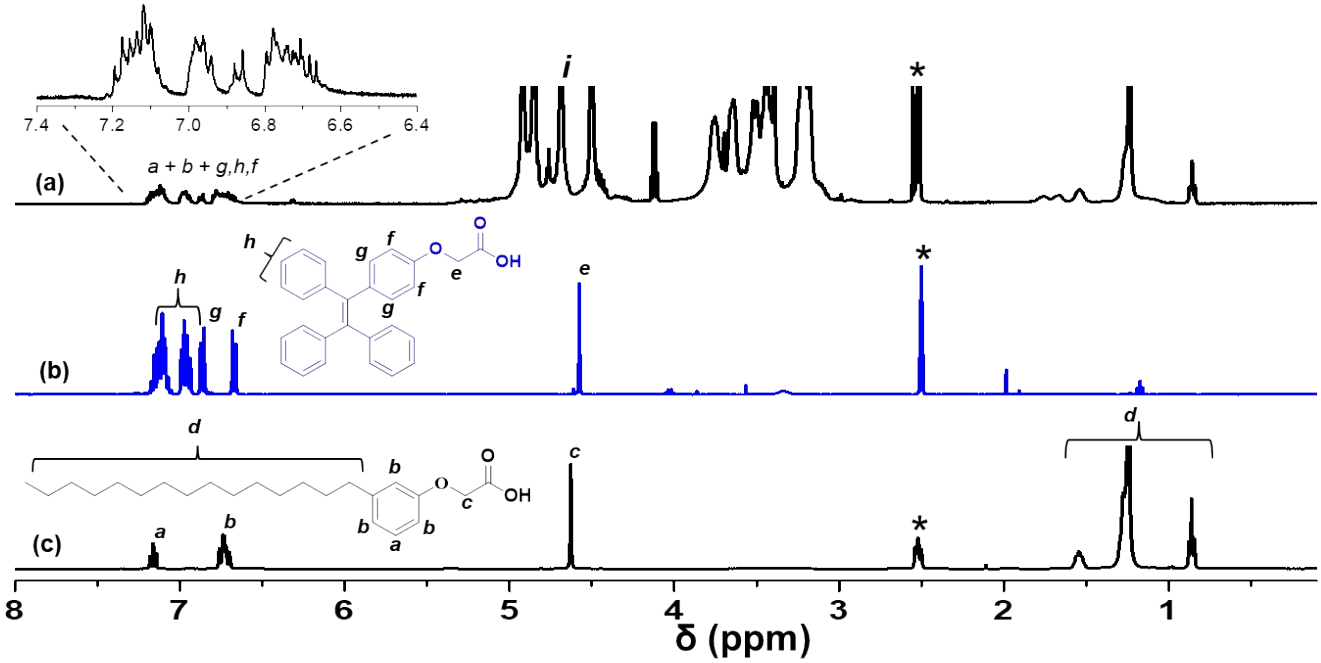


Figure S1: *(a) ^{1}H NMR spectrum of dextran TPE-conjugated derivative in DMSO-d_6 (b) ^{1}H NMR spectrum of TPE acid in DMSO-d_6, and (c) ^{1}H NMR spectrum of PDP acid in DMSO-d_6. The peaks in the NMR spectra are assigned alphabetically with respect to their chemical structure.*

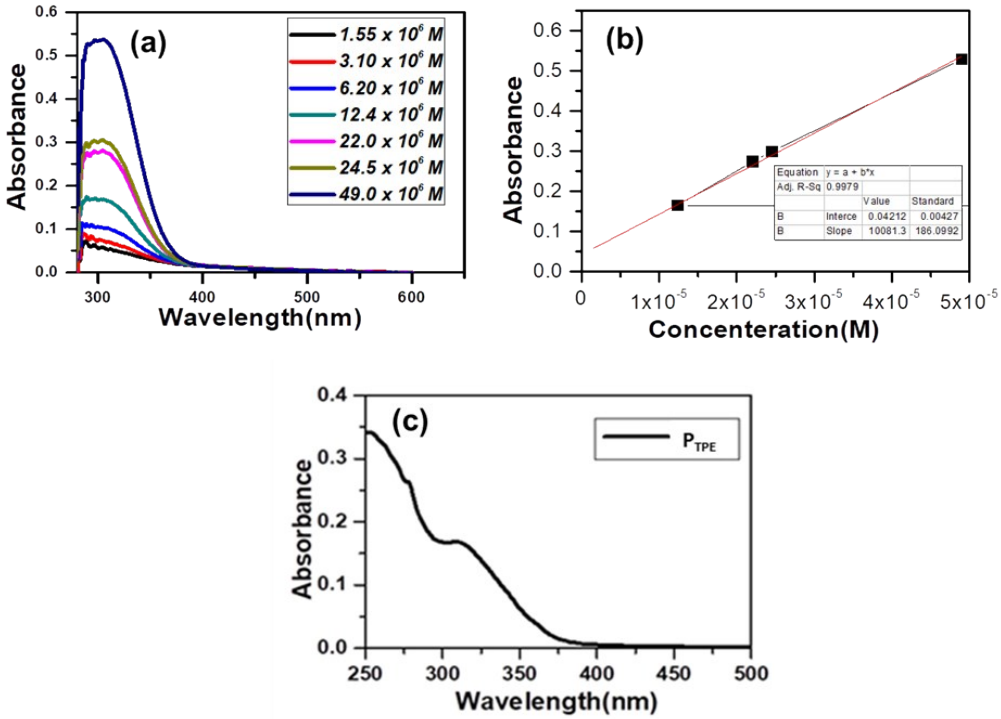


Figure S2: (a) *Absorption spectra of TPE-acid (3) at various concentrations in DMSO+ water (1:9 v/v). (b) Plot of molar extinction coefficient of TPE. (c)Absorption spectrum of P_{TPE} in water*

Note: *Calculation of percentage substitution of TPE via absorbance spectroscopy*
*TPE conjugated to dextran was synthesized and degree of substitution was maintained at 2% as quantified via NMR. UV spectroscopy was also used to quantify the degree of substitution of TPE tagged dextran derivative. For this a 10mg powdered sample of dextran derivative (in 1ml water + 1ml DMSO, concentration = 5mg/mL) was dialyzed against water for 2 days to afford Polymersomes tagged with TPE (**P_{TPE}**). The final volume after dialysis was 2.3mL. A 150uL of this dialyzed sample was then diluted to 2mL using MQ water for UV characterization. The peak at 310nm corresponds to λ_{max} of TPE (in figure S6b).*
Using Beer Lambert's Equation, concentration was calculated as follows:
A=Ecl, c= 0.16/10081 = 15.8μM
*Mass of TPE in 0.1mL of dialyzed sample = (M*molar mass of TPE* Volume)/1000 = 12.8 μg*
*Mass of TPE in 2.3mL of dialyzed sample = (12.8 μg *2.3mL)/ 0.15mL = 196.26 μg*
*% of TPE by weight (10mg of powdered sample) = 196.26 μg/10mg*100% = 1.96%*

The amount of TPE as calculated via UV absorption spectroscopy came out to be 1.96% which is almost the same as calculated via NMR (2%). Hence, the percentage substitution was quantified via both NMR and absorbance spectroscopy.

*** [**ε for Rose Bengal** = 90,400cm^{-1}/M from Ref: Biochemistry, **1967**, 6, 11, 3510-3518)]*

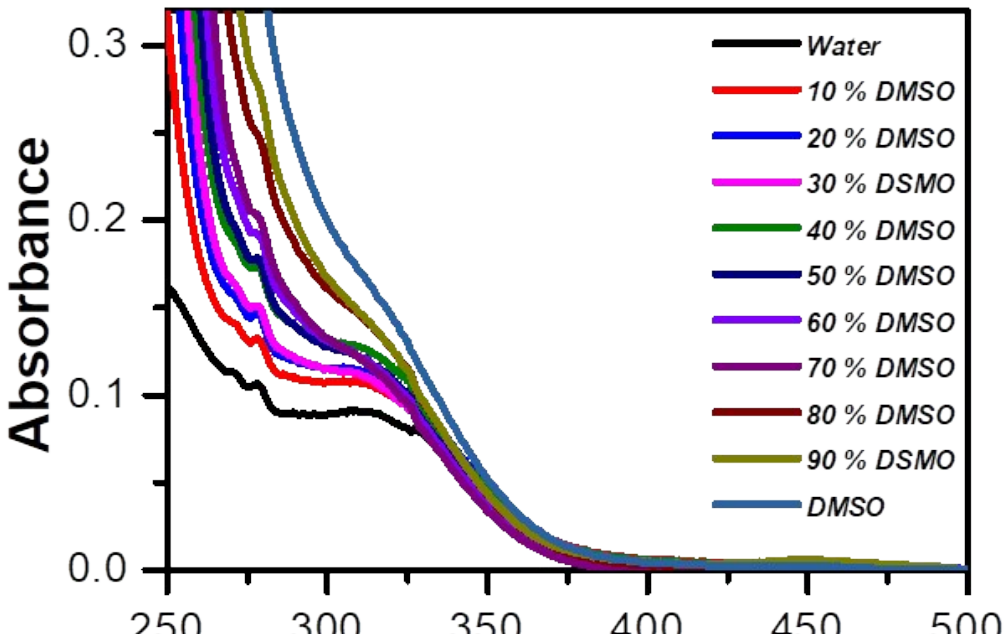


Figure S3: *Absorbance spectra of* P_{TPE} *in Water+DMSO solvent combinations.*

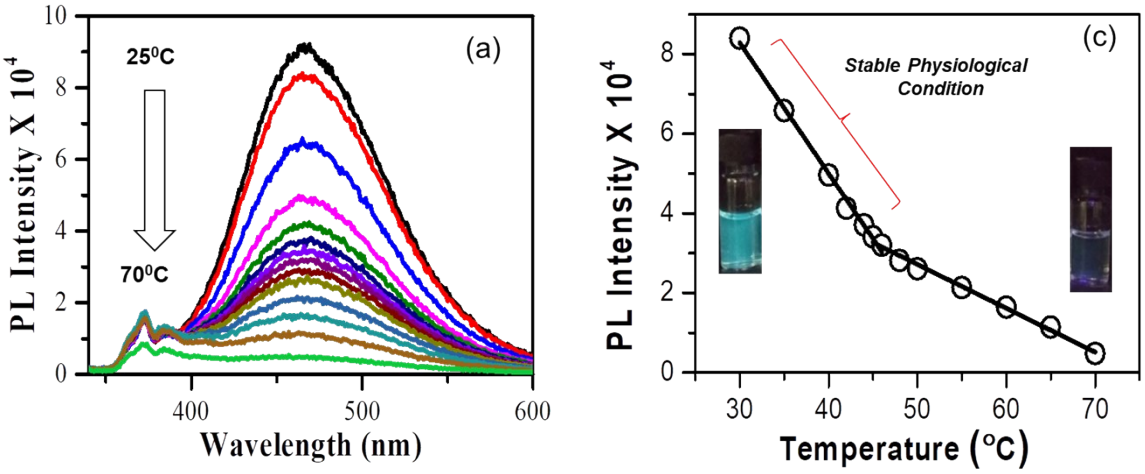


Figure S4: ***a)*** *Temperature dependent Fluorescence Spectra for DEX-TPE-PDP.* ***(b)*** *Plot showing the variation of Fluorescence intensity with varying temperature along with vials (inset)*

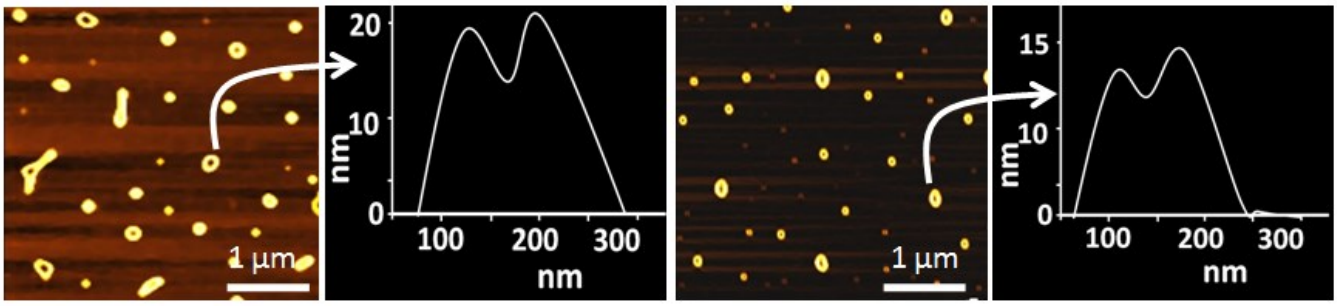


Figure S5: *AFM morphologies of Polymersomes* P_{TPE}

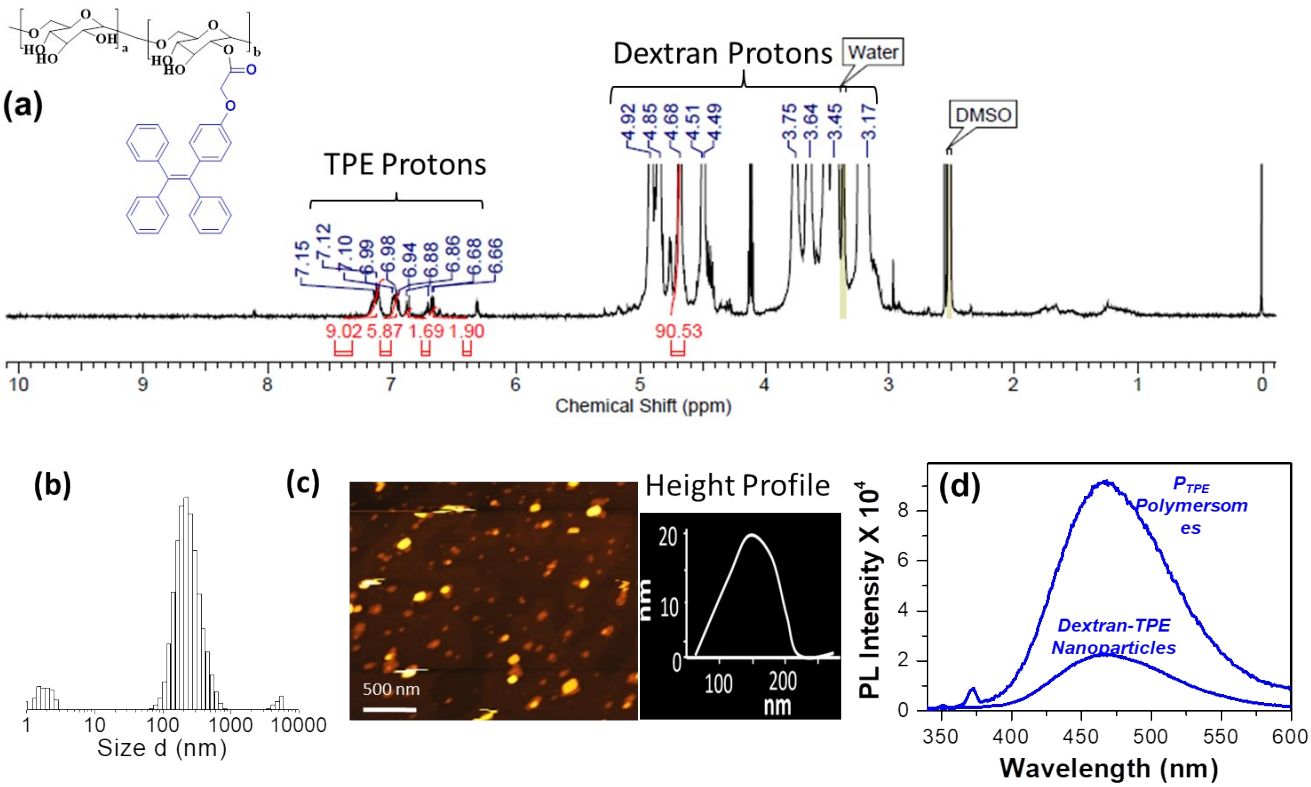


Figure S6: *(a) Structure and* ^{1}H *NMR spectrum of TPE substituted Dextran Derivative (without PDP) (b) DLS histogram, (c) AFM morphology, and (d) Fluorescence spectra of* P_{TPE} polymersomes *and TPE conjugated dextran nanoparticles in water. Excitation wavelength = 340 nm, slide width = 2nm*

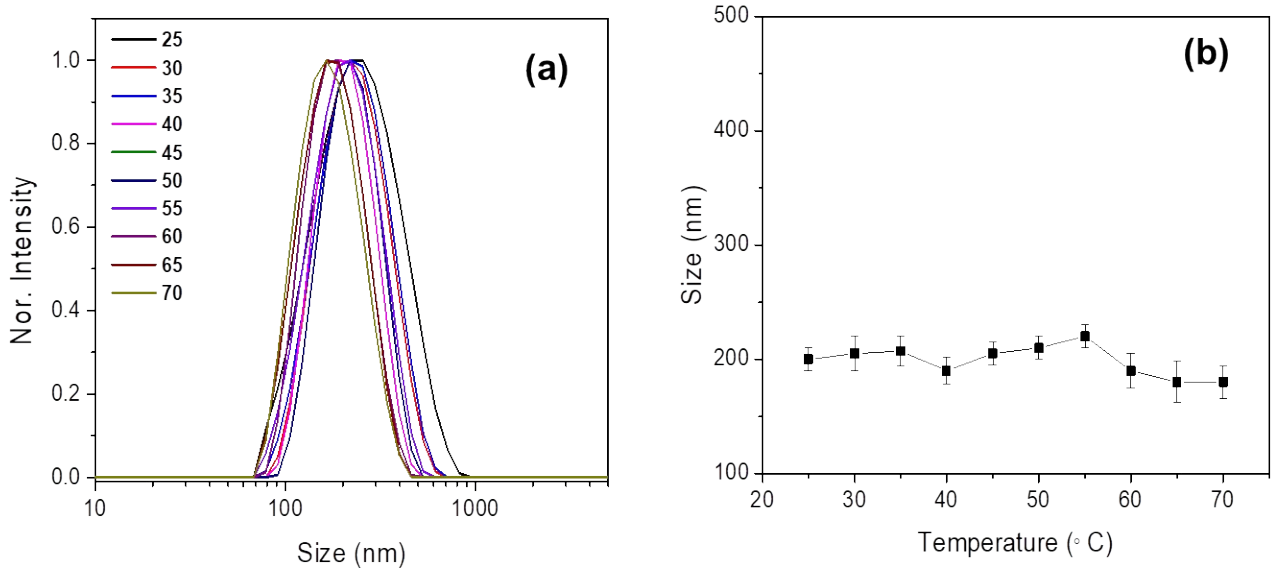


Figure S7: ***a)*** *Temperature dependent DLS measurement for DEX-TPE-PDP.* ***(b)*** *Temperature vs. Size plot for DEX-TPE-PDP (P_{TPE})*

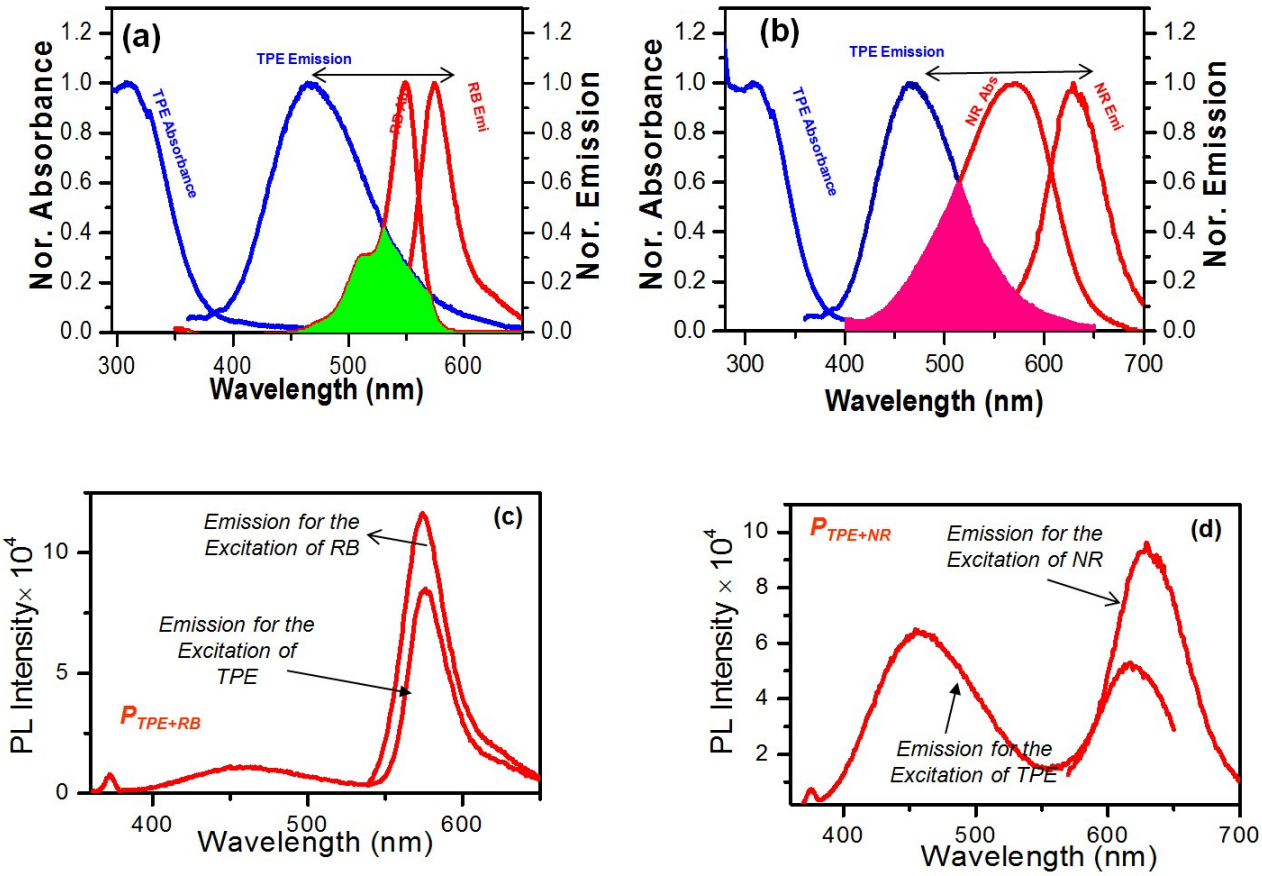
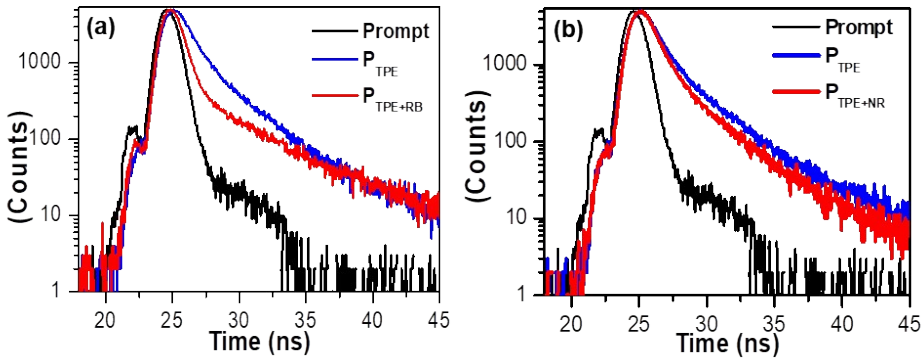


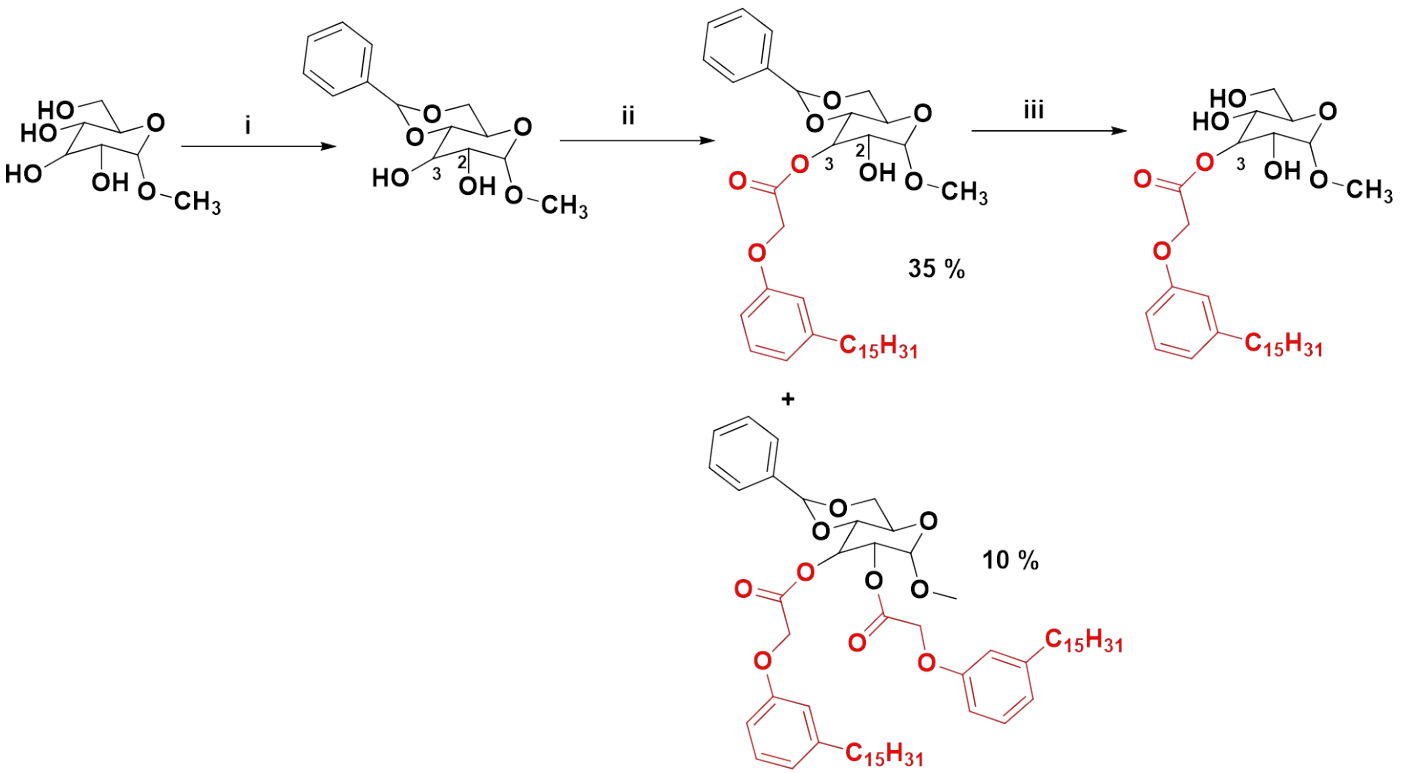
Figure S8: *(a) Plot showing spectral overlap between emission of P_{TPE} and absorbance of P_{RB}, and (b) Plot showing spectral overlap between emission of P_{TPE} and absorbance of $P_{NR.}$ It is evident from the spectrum that emission spectrum of donor (P_{TPE}) and emission spectrum of acceptor (both P_{RB} and P_{NR}) has a significant difference in their shifts and thus it facilitates the probing of FRET mechanism in cellular conditions using confocal microscopy. (c) Fluorescence intensity for P_{TPE+RB}* followed by excitation at TPE and RB**.** *(d) Fluorescence intensity for P_{TPE+NR}* followed by excitation at TPE and NR**.**



(f) FRET Parameters

Sample	P_{TPE+RB}	P_{TPE+NR}
J(λ) (M^{-1} cm^{-1} nm^4)	1.91×10^{13}	1.27×10^{15}
R_0 (Å)	15.67	26.99
τ_D (ns)	1.12	1.12
τ_{DA} (ns)	0.32	0.87
E_{FRET}	0.7142	0.223
R (Å)	13.41	32.30

Figure S9: *a) TCSPC analysis for* P_{TPE+RB} *b) TCSPC analysis for* P_{TPE+NR}



(i) *Benzaldehyde Dimethyl acetal, CSA, Dry ACN, 60°C, 2h* ***ii)*** *PDP Acid (compound-4), EDC.HCl, DMAP, Dry DCM, 25 °C,12h* ***iii)*** *PTSA, MeOH:DCM (1:1), 25 °C, 3h*

***Scheme S1**: Scheme for the synthesis of the model compound*

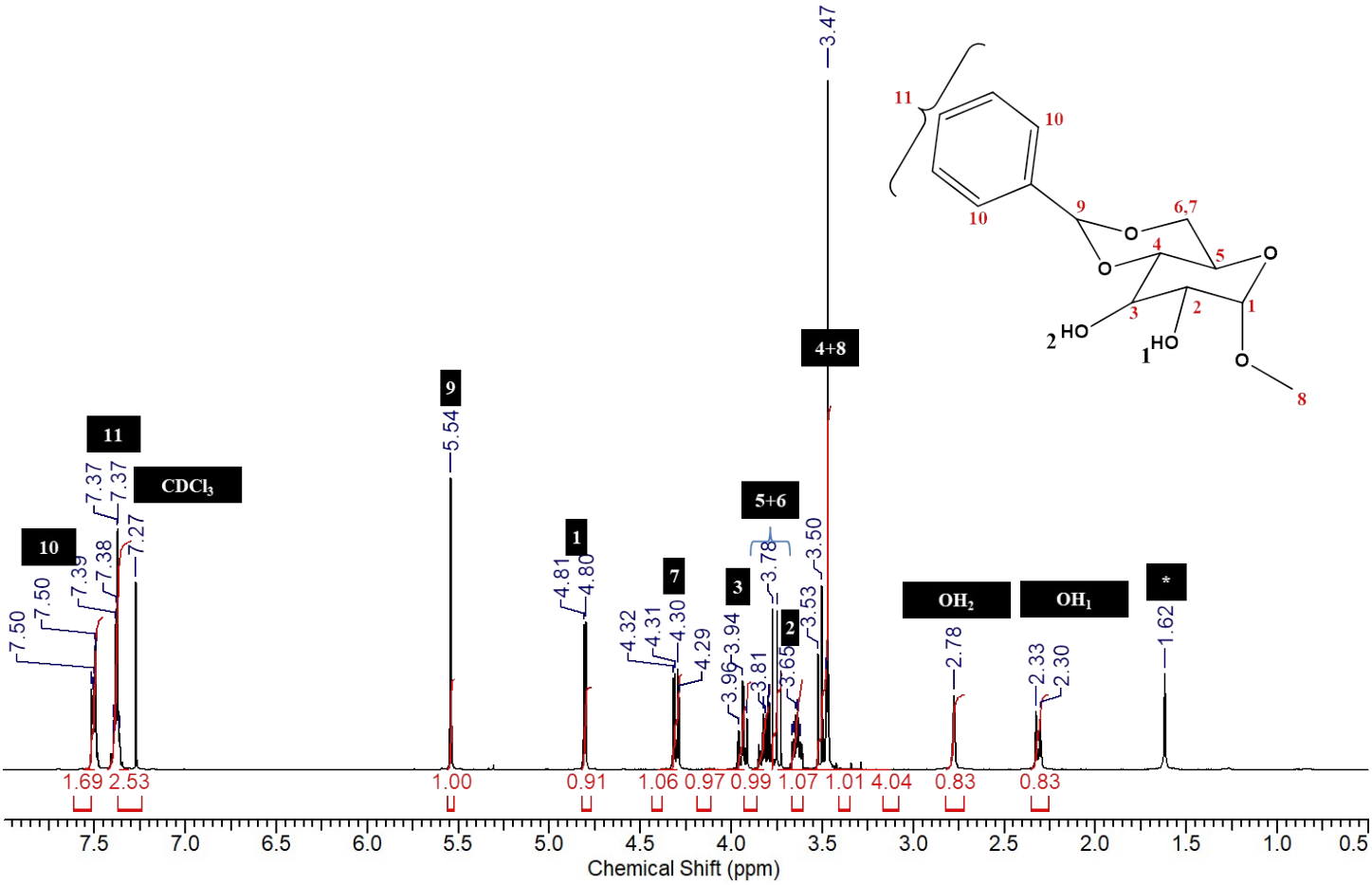


Figure S10: *^{1}H NMR for Compound-5*

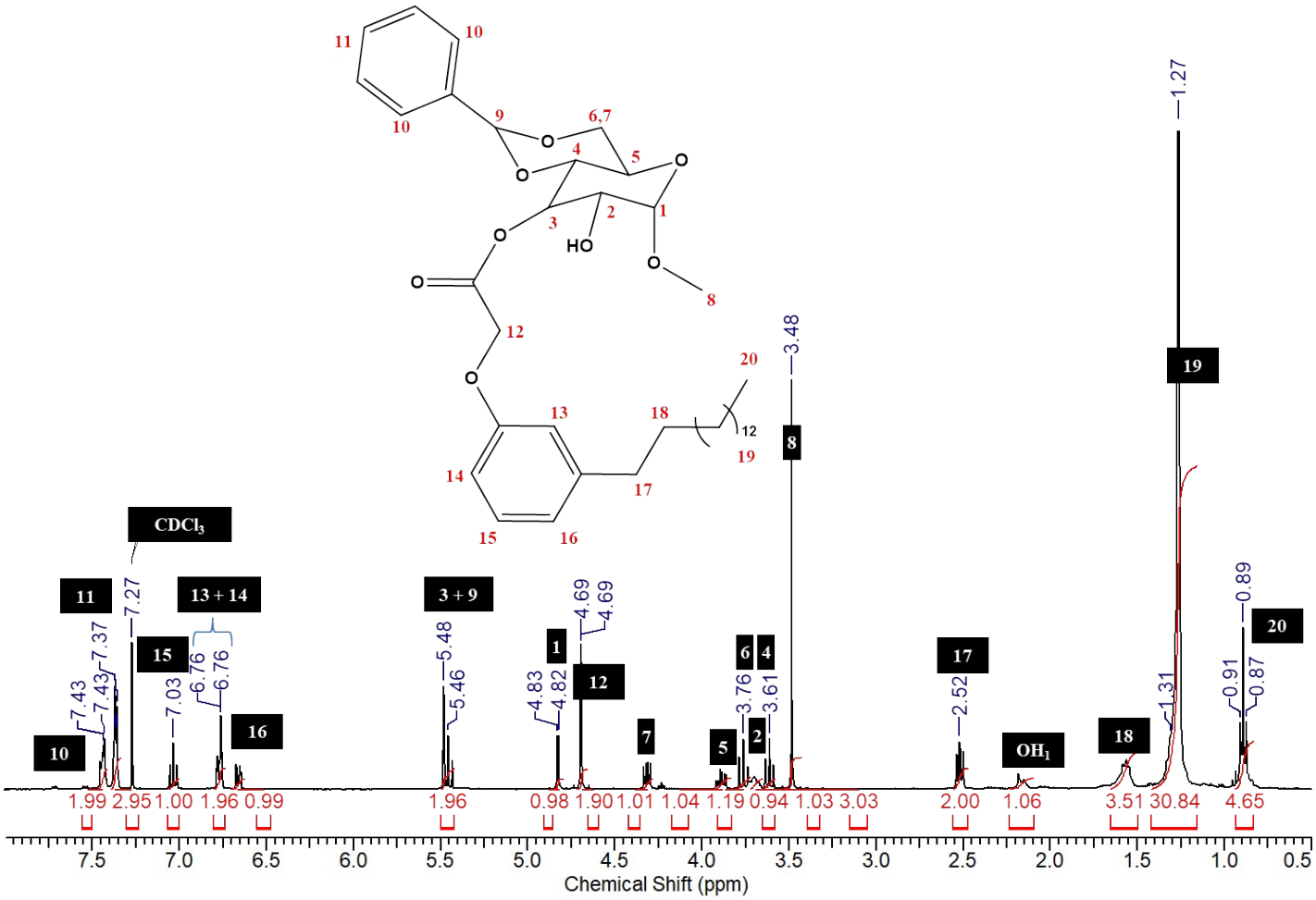


Figure S11: *^{1}H NMR for Compound-6*

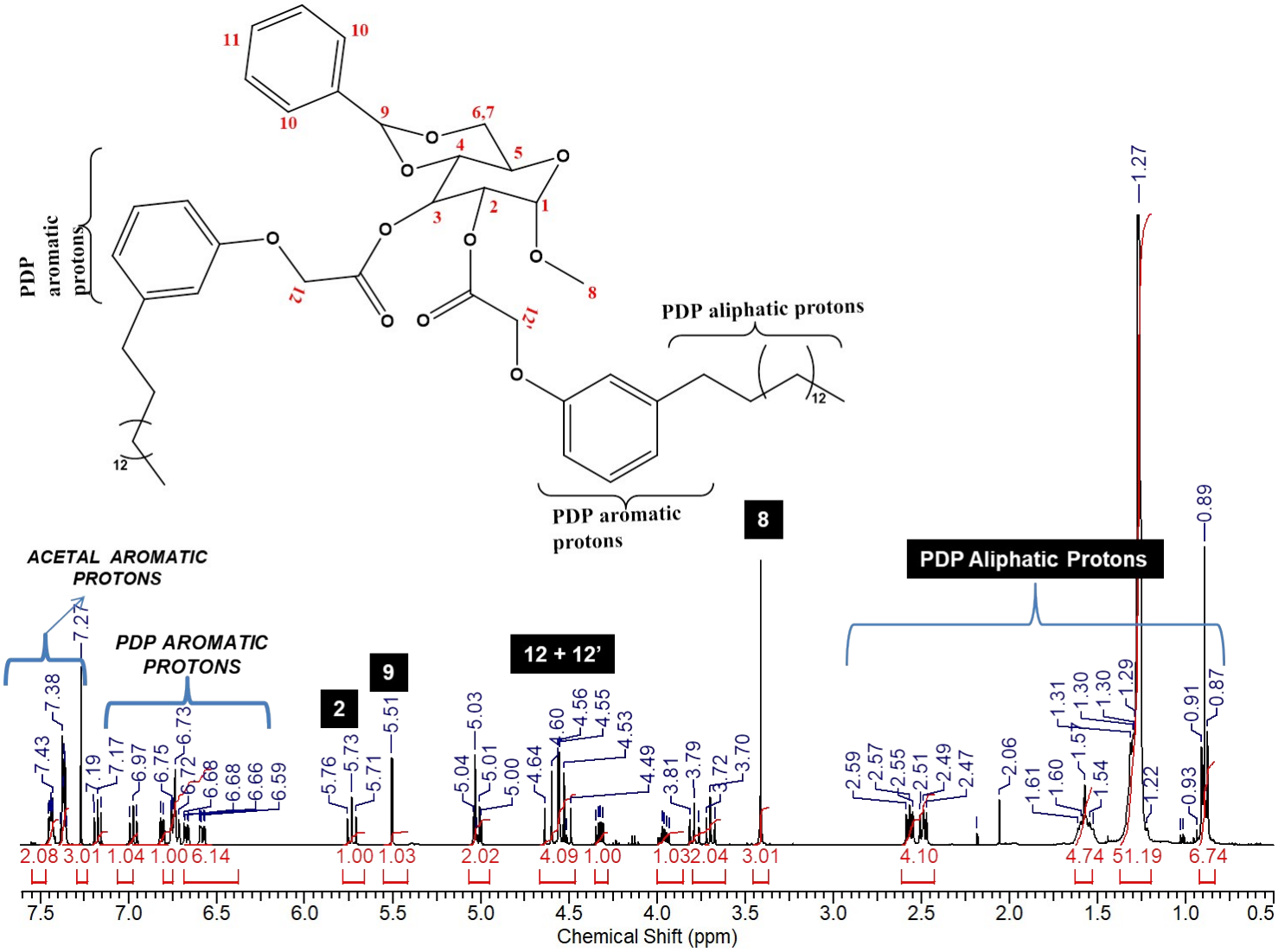


Figure S12: *^{1}H NMR for Compound-7*

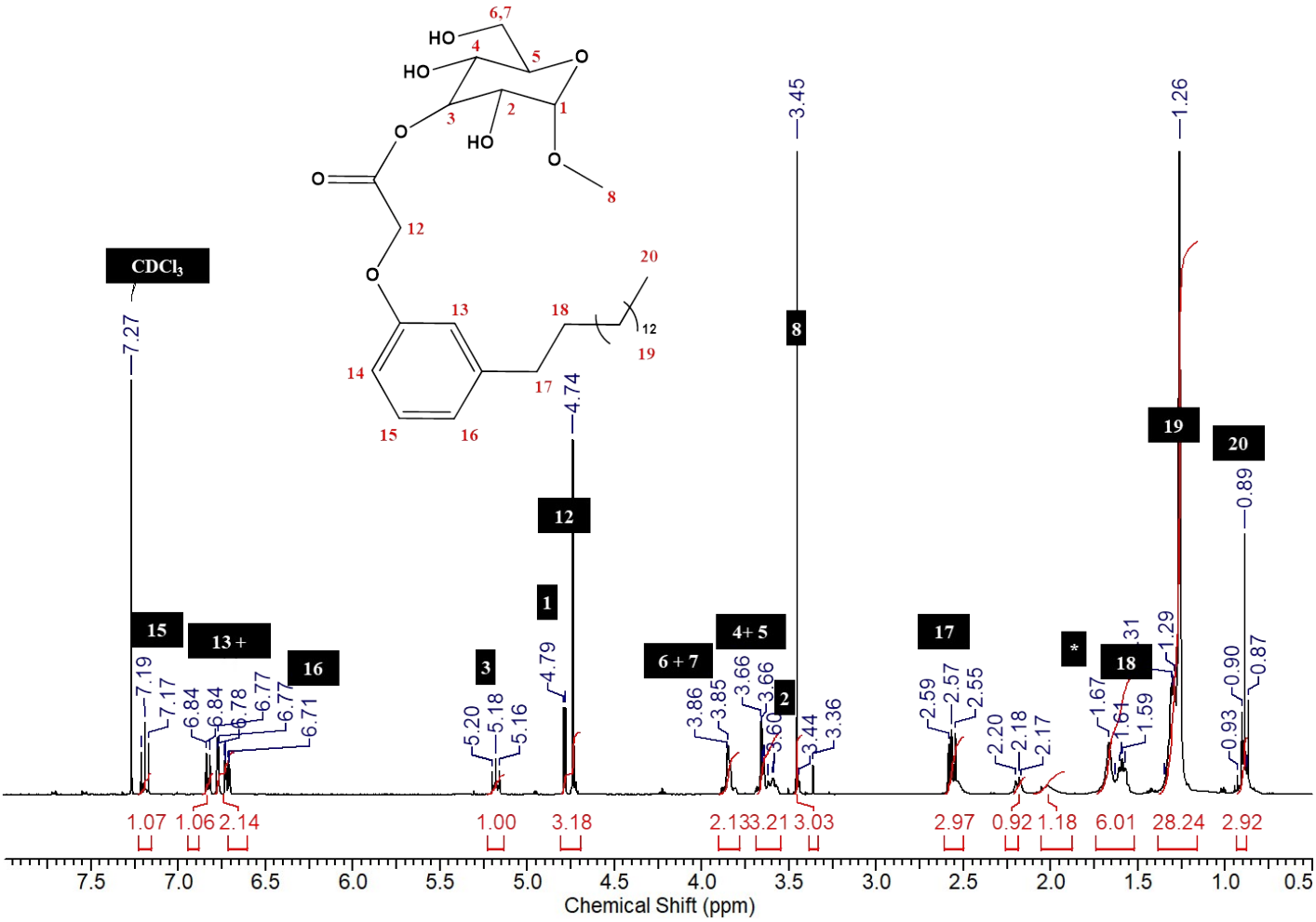


Figure S13: *^{1}H NMR for Compound-8*

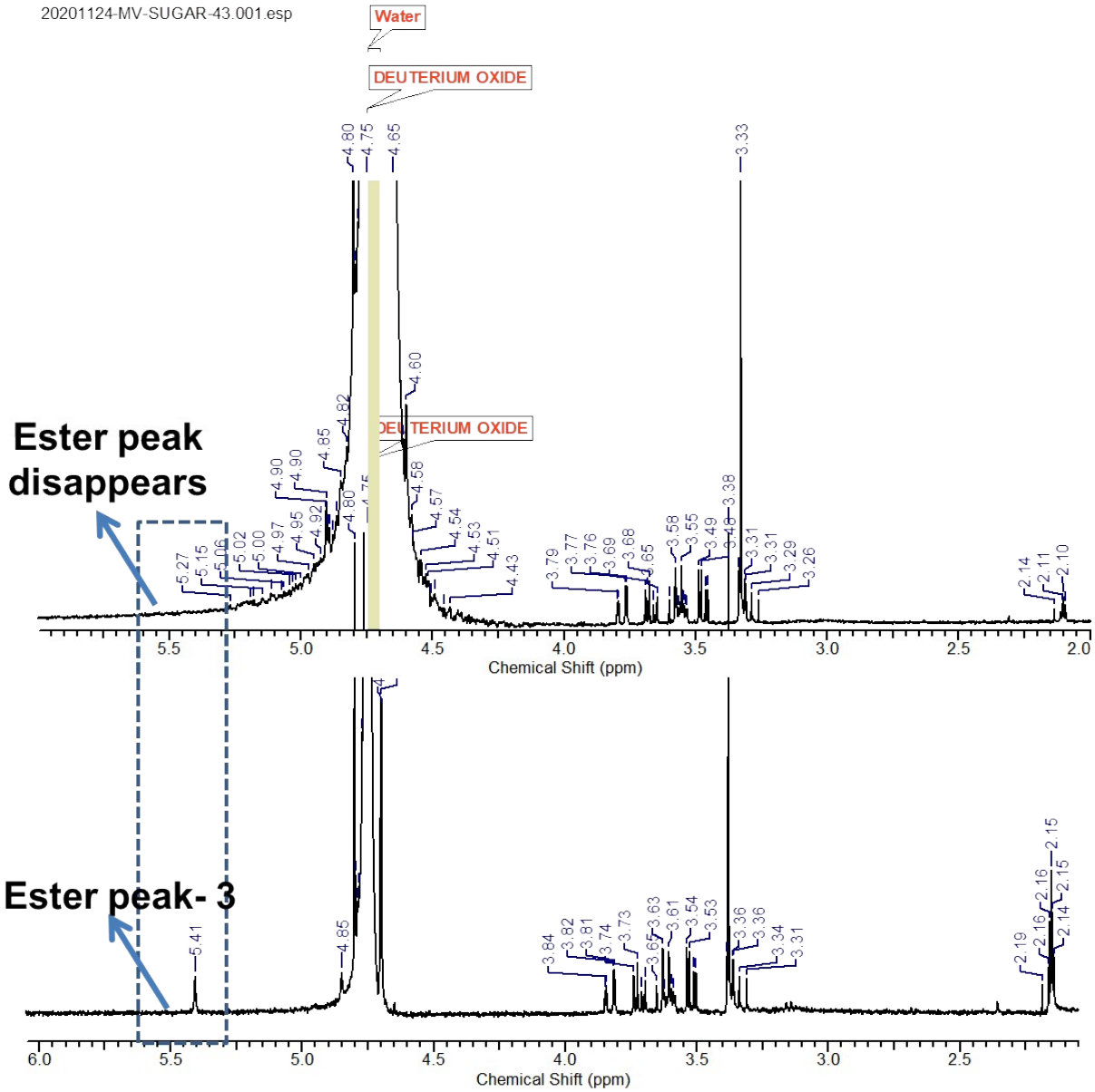


Figure S14: ^{1}H NMR spectra showing enzyme (Horse liver) mediated hydrolysis of sugar ester bond at 3 positions

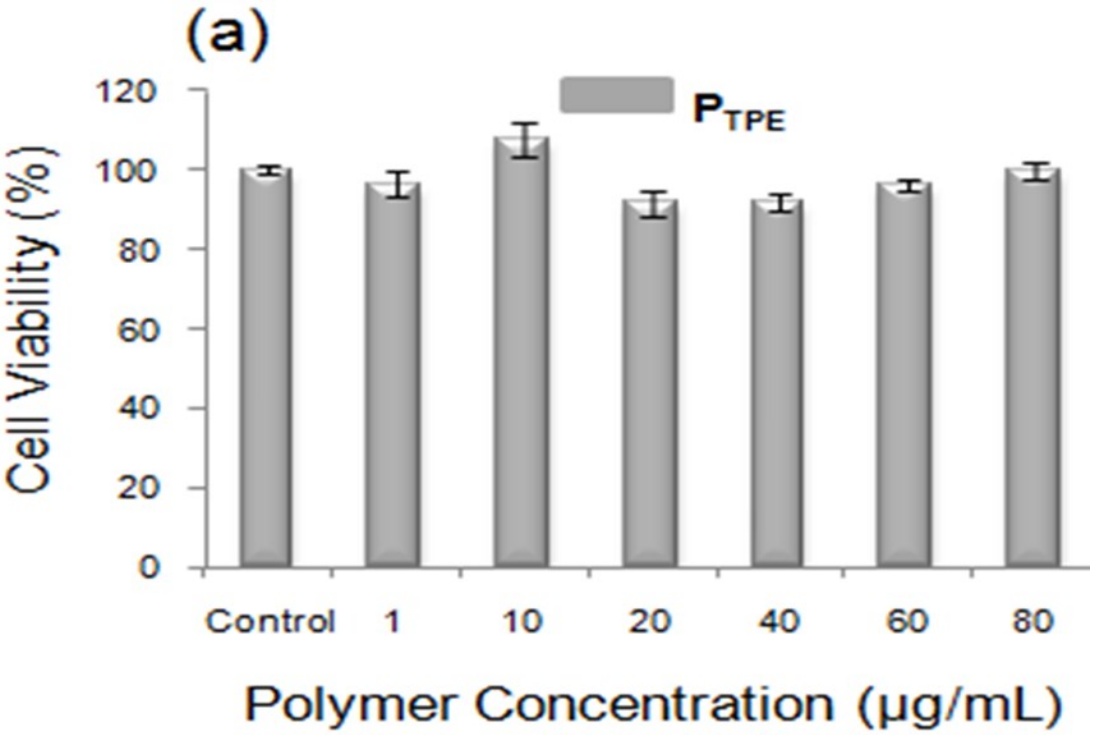


Figure S15: *In vitro cytotoxicity data of* P_{TPE} *in WT-MEF cell line.*

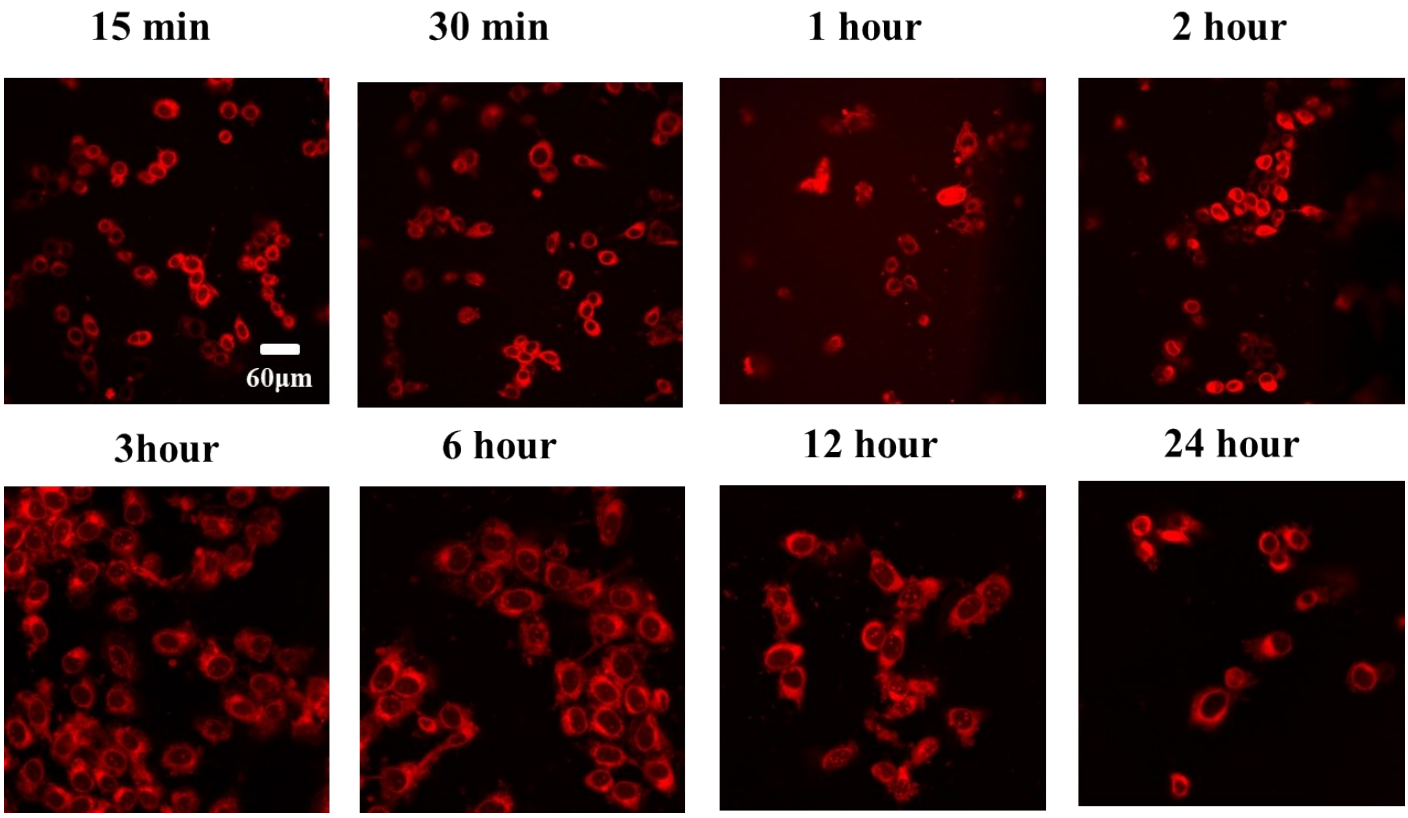


Figure S16: *Confocal images for self-emission from RB in P_{TPE+RB} when excited at RB excitation λ= 568nm laser, collection channel: 578-720nm; No of cells= 25000, seeded for 16 hours prior to compound addition; Concentration of RB in polymersome = 5µM. MCF cell line*

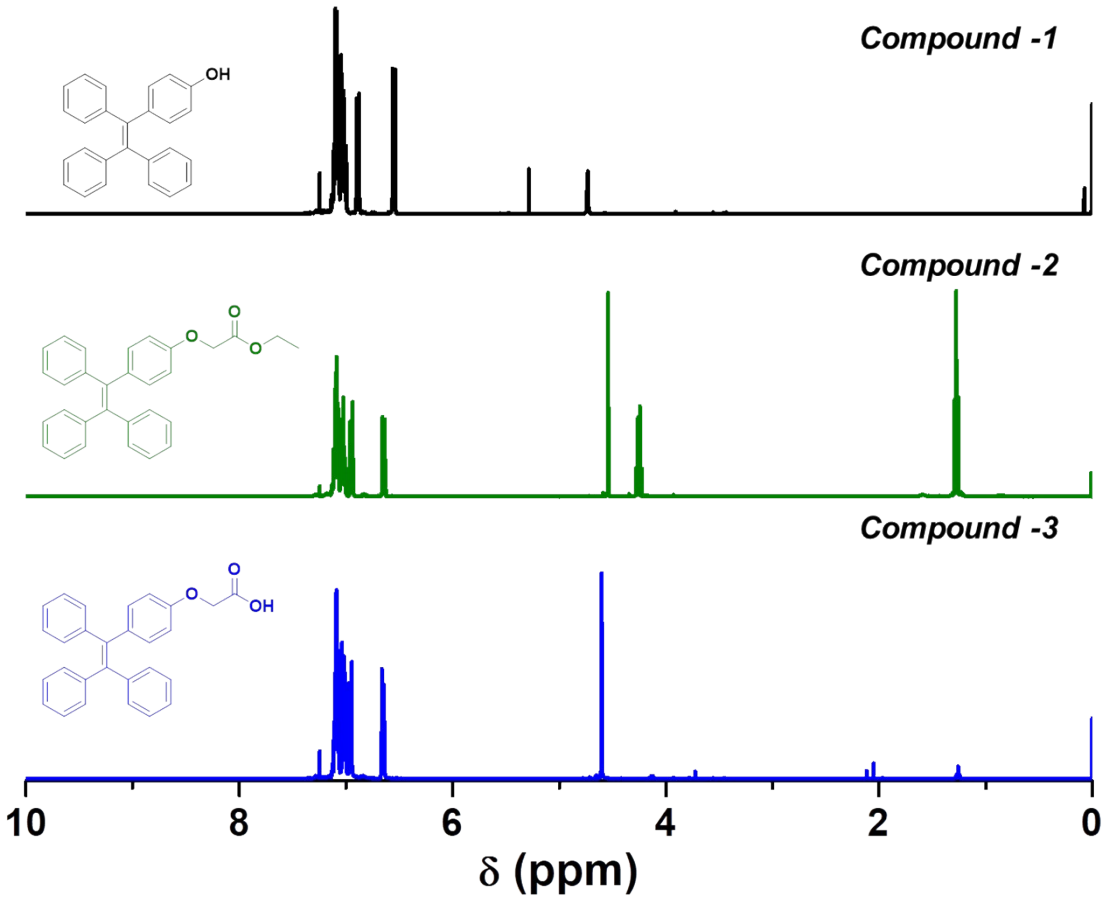


Figure S17: ^{1}H NMR for compounds (1), (2) and (3).

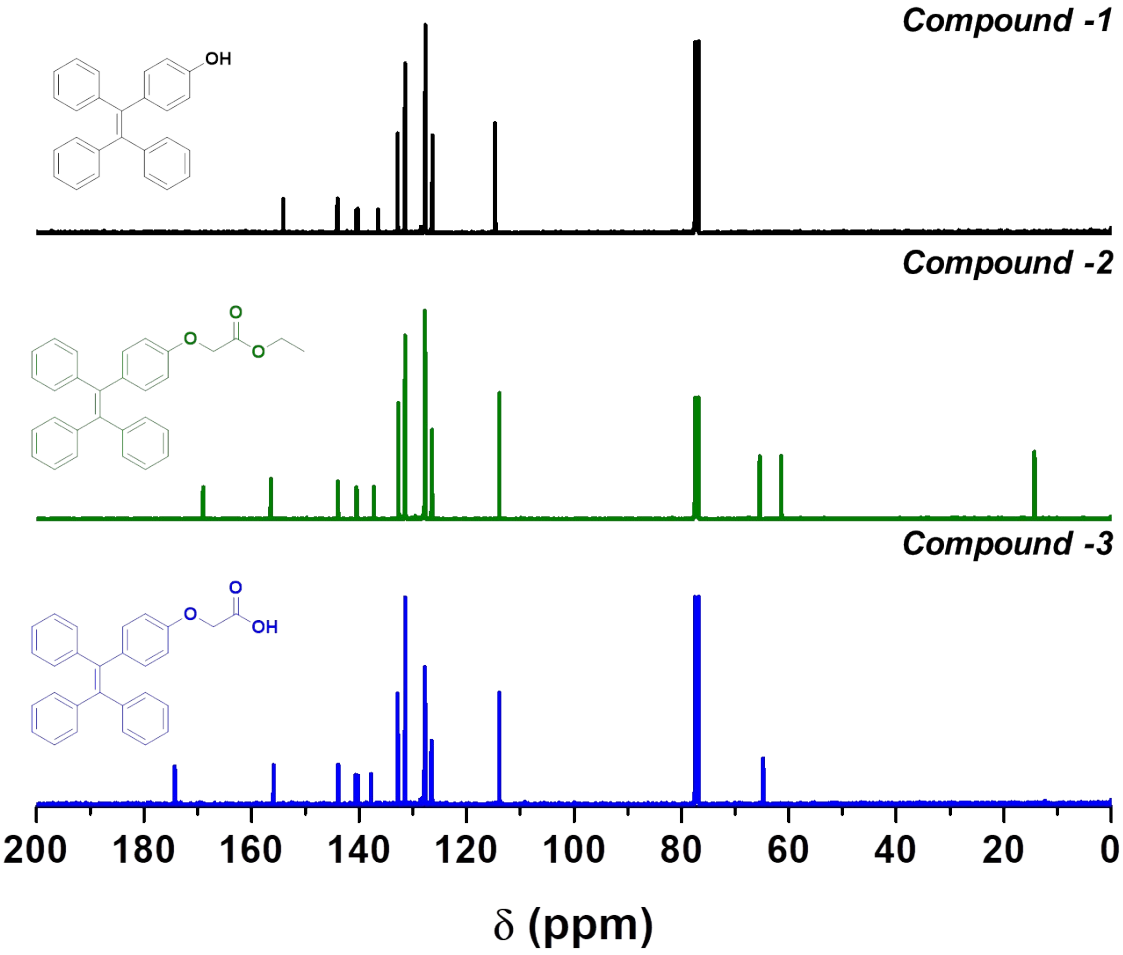


Figure S18: ^{13}C *NMR for compounds (1), (2) and (3).*

Compound -1

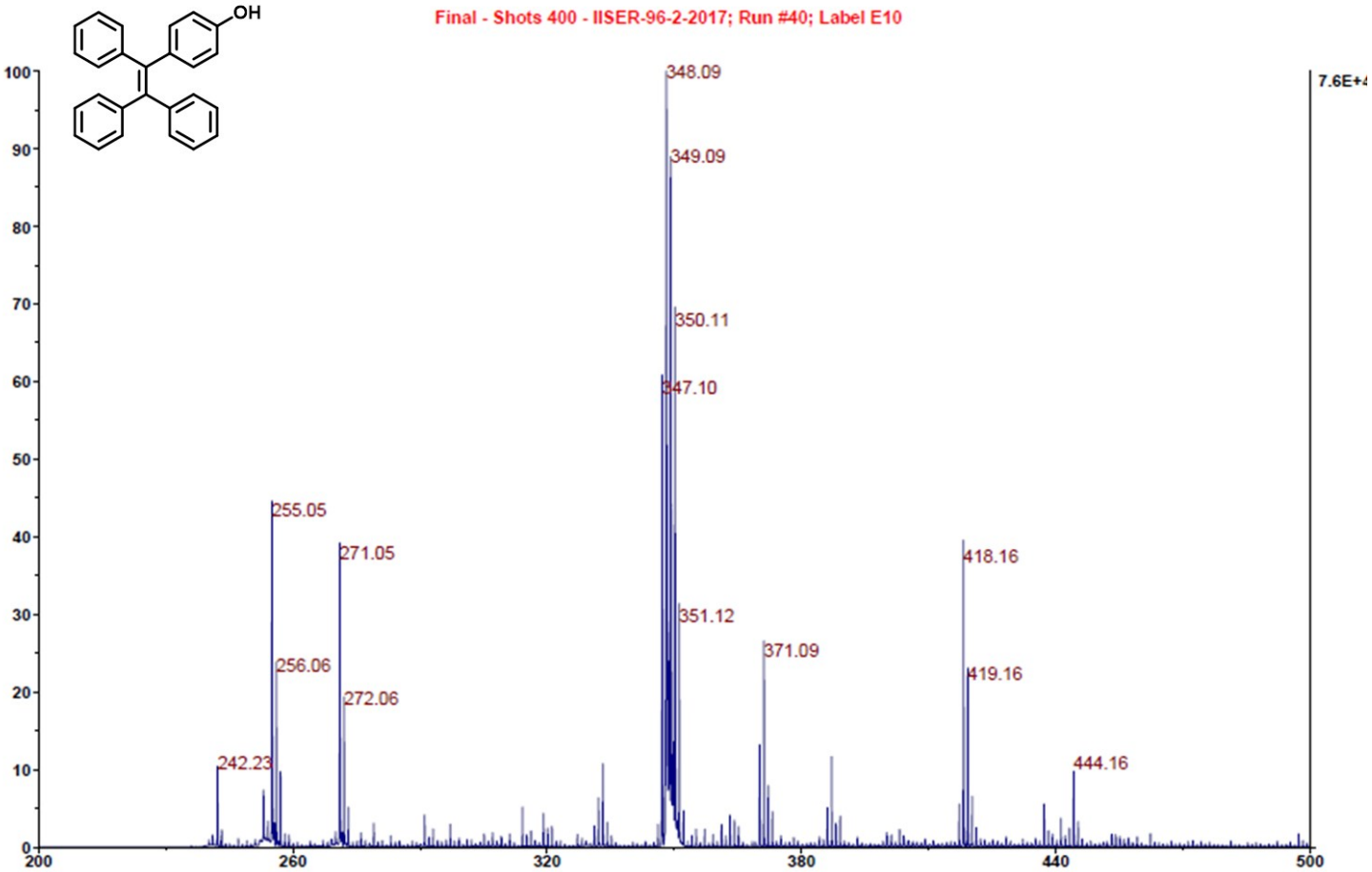


***Figure 19**: MALDI-TOF for Compound (1).*

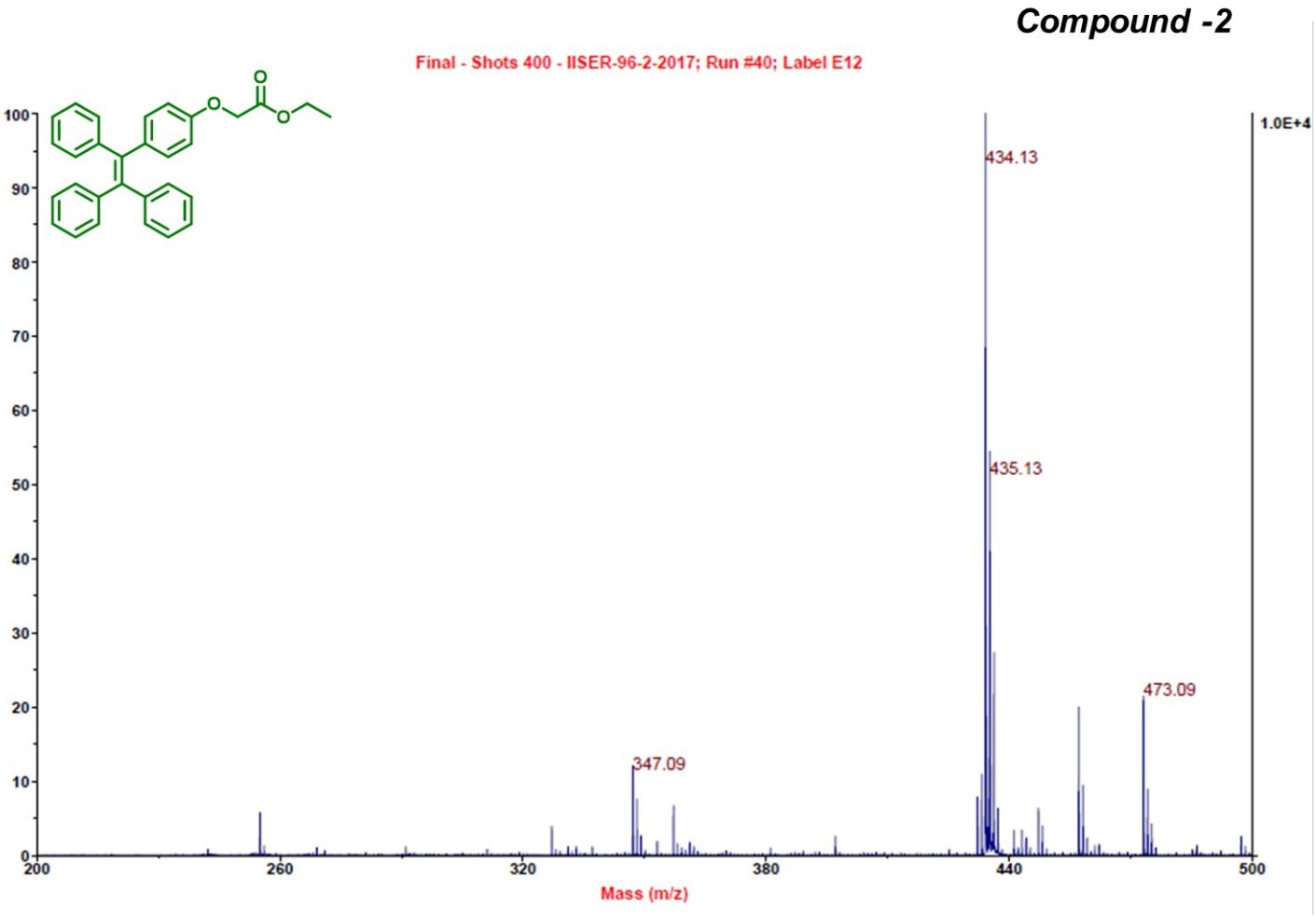


Figure S20: MALDI-TOF for Compound (2).

Compound -3

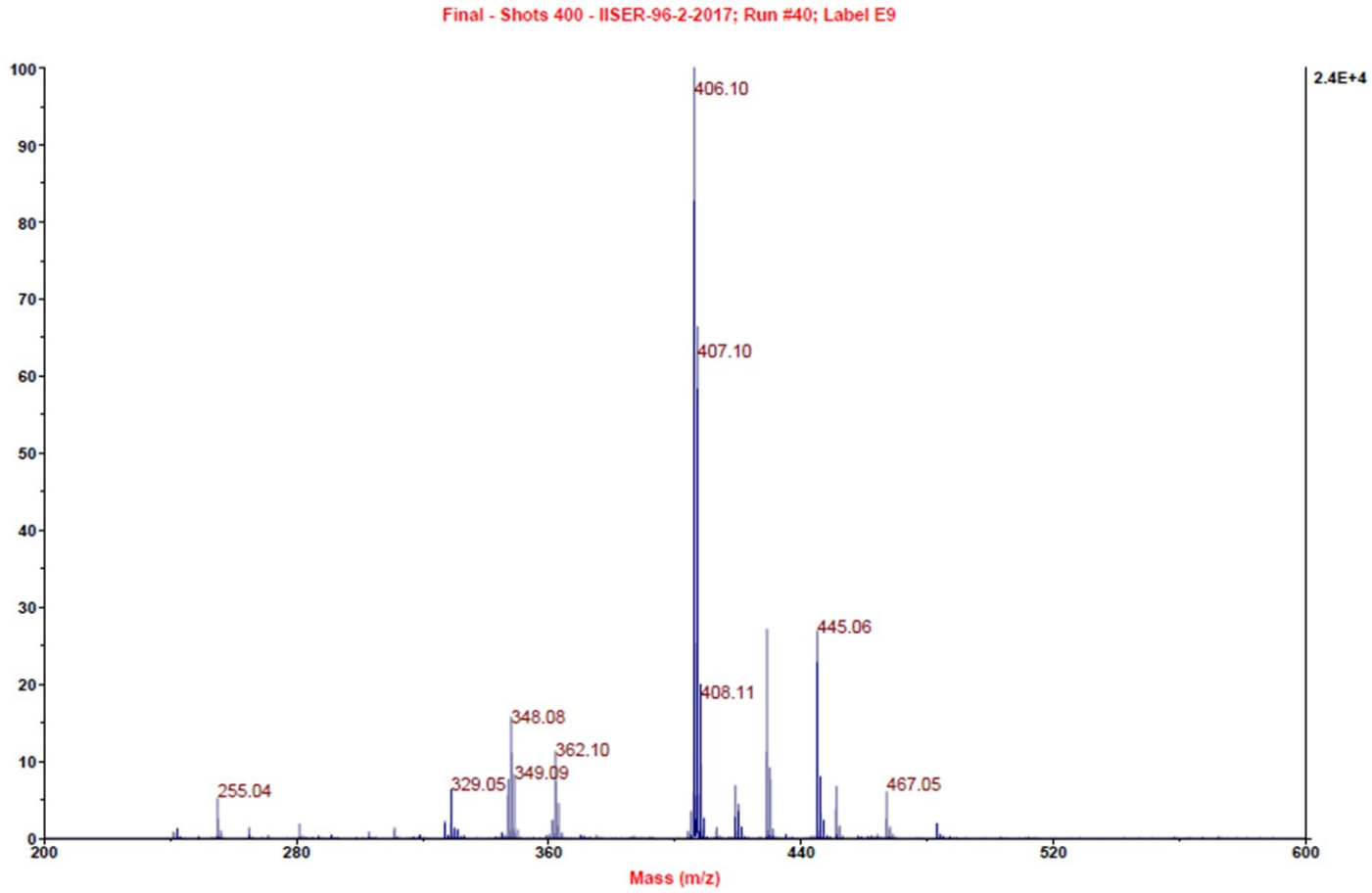


***Figure S21**: MALDI- TOF for Compound (3).*

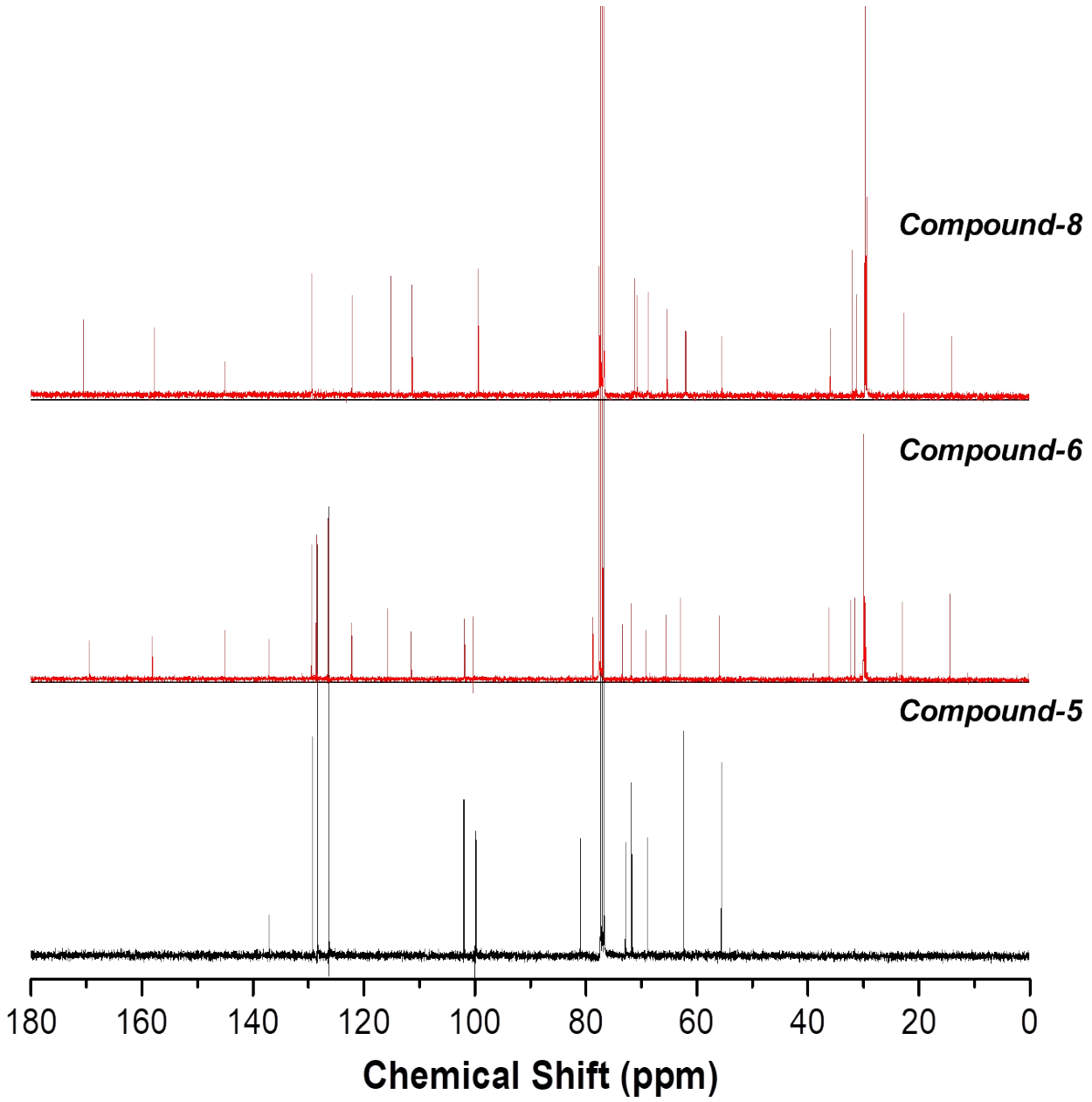


Figure S22: ^{13}C stack plot for compound 8, 6 and 5