Electronic Supplementary Material (ESI) for Polymer Chemistry.

Supplementary Information

Poly(oxanorbornene)s Bearing Triphenylphosphonium and PEGylated Zinc(II) Phthalocyanine with Boosted Photobiological Activity and Singlet Oxygen Generation

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1. Synthesis of Compounds

Materials and Characterization: IR spectra were recorded on a Thermo Scientific iS10 FT-IR (ATR sampling accessory) spectrophotometer and electronic spectra on a Shimadzu UV-2450 spectrophotometer. NMR spectra were recorded on a Bruker Avance III 500 MHz spectrometer using TMS as an internal reference. Mass spectra were measured on a Bruker Microflex LT MALDI TOF-MS. All reagents and solvents were of reagent grade quality and were obtained from commercial suppliers. Monomer M2, 4-((2,5,8,11-tetraoxatridecan-13-yl)oxy)phthalonitrile, 4-(4-hydroxyphenoxy)phthalonitrile, and tosylated oxanorbornene derivative were synthesized according to published procedures.[1-4]

Synthesis of 4-((2,5,8,11-tetraoxatridecan-13-yl)oxy)phthalonitrile: 4-nitrophthalonitrile (0.400 g, 2.31 mmol) and tetraethyleneglycol monomethyl ether (0.962 g, 4.62 mmol) were taken into a 50 mL flask and dissolved with 8 mL of DMF. The reaction mixture was stirred at room temperature for 24 hours after K_2CO_3 (1.280 g, 9.24 mmol) was added under nitrogen atmosphere. The resulting mixture was poured into ice-water, and extracted with ethyl acetate. It was dried with Na_2SO_4, filtered and the organic phase was evaporated. The final substance was dried in a vacuum oven at 40 °C. An oily light green substance was obtained (Yield: 563 mg, 73%). FT-IR υ_{max} (cm^{-1}): 3077.12, 2915.63, 2229.39, 1596.46, 1561.88, 1491.74, 1454.23, 1349.46, 1319.97, 1254.84, 1199.75, 1095.97, 1043.86, 966.07, 842.28, 729.36, 525.81. MALDI-TOF MS (matrix: dithranol): *m/z* calcd. 334.370; found: 373.483 $[M+K]^+$, 357.518 $[M+Na]^+$. ^{1}H-NMR (500 MHz, $CDCl_3$) (ppm): δ 7.71 (d, 1H), 7.31 (d, 1H), 7.24 (dd, 1H), 4.22 (t, 2H), 3.88 (t, 2H), 3.70 (t, 2 H), 3.65 (m, 8H), 3.54 (t, 2H), 3.37 (s, 3H). ^{13}C-NMR (126 MHz, $CDCl_3$) (ppm): δ 162.02, 135.15, 119.84, 119.57, 117.32, 115.66, 115.25, 107.35, 70.59, 70.49, 69.21, 68.68, 59.00.

Synthesis of M1: 4-(4-hydroxyphenoxy)phthalonitrile (19.31 mg, 0.081 mmol), 4-((2,5,8,11-tetraoxatridecan-13-yl)oxy)phthalonitrile (246 mg, 0.735 mmol), $Zn(CH_3COO)_2$ (37.49 mg, 0.204 mmol) and a few drops of DBU were stirred at the reflux temperature of 3 mL hexanol for 4 hours. The reaction mixture was poured into hexane and the oily substance was separated by centrifugation. The purification was conducted by silica gel chromatography (chloroform:methanol, 30:1 (v/v)). Symmetrical and unsymmetrical phthalocyanine mixture was obtained. The resulting residual (274 mg), tosylated oxanorbornene (175 mg, 0.464 mmol), KI (77 mg, 0.464 mmol) and K_2CO_3 (116 mg, 0.840 mmol) were stirred in 10 mL of DMF at room temperature for 7 days. The resulting mixture was poured into cold water and extracted with ethyl acetate. It was dried with Na_2SO_4, filtered and the organic phase was evaporated. The residue was dried in a vacuum oven at 30 °C. Column chromatography was made on the dried substance in a 25:1:1 (v/v/v) mixture of dichloromethane, ethanol, and acetone on silica gel. An oily green substance was obtained (Yield: 5 mg, 5% in two steps). FT-IR υ_{max} (cm^{-1}): 3067.12, 2871.78, 1770.49, 1698.36, 1606.56, 1487.78, 1450.28, 1394.42, 1337.05, 1282.19, 1218.86, 1088.05, 1060.78, 953.42, 852.36, 773.76, 745.06, 729.10, 655.71, 588.74. UV-vis (DMSO, λ_{max}) (nm): 682, 615, 356. MALDI-TOF MS (matrix: dithranol): *m/z* calcd. 1507.520; found: 1507.707 $[M]^+$, 1439.201 $[M\text{-furan}]^+$. ^{1}H-NMR (500 MHz, DMSO-d_6) (ppm): δ 9.16-8.52 (m, 8H), 7.78-7.65 (m, 4H), 7.46 (d, 2H), 7.19 (d, 2H), 6.56 (s, 2H), 5.19 (s, 2H), 4.66 (m, 5H), 4.09 (m, 5H), 3.81-3.34 (m, 38H), 2.21 (m, 9H), 2.98 (s, 2H), 2.63 (m, 2H), 2.36 (m, 2H), 2.01 (m, 2H).

General synthesis procedure for copolymers (P1-P3): M1 and M2 were dissolved in 1.5 mL of $CHCl_3$ in a nitrogen atmosphere and Grubbs' catalyst (3rd gen) in 0.5 mL of DCM was added in one shot to the stirring monomers' solution. The reaction was monitored by TLC and, after 3 hours, 0.5 mL of 30% ethyl vinyl ether was added and

stirred for 30 minutes. It was then precipitated and washed with diethyl ether. After centrifugation, it was dried in a vacuum oven.

P1: M1 (0.0304 g, 0.02 mmol), M2 (0.0129 g, 0.0235 mmol) and Grubbs' catalyst (3rd gen) (3.8 mg, 0.0043 mmol) were used. A blue colored substance P1 was obtained (theoretical molecular weight = 10,000 g mol^{-1}). Yield: 0.025 g (5%7). FT-IR υ_{max} (cm^{-1}): 3060.16, 2870.18, 1775.18, 1701.26, 1606.15, 1487.90, 1470.33, 1438.67, 1394.51, 1371.40, 1334.69, 1280.00, 1218.77, 1197.66, 1111.81, 1087.50, 1058.07, 1039.71, 996.62, 951.24, 828.65, 773.24, 747.75, 724.07, 689.31, 659.02. UV-vis (DMSO, λ_{max}) (nm): 679, 613, 356. ^{1}H-NMR (500 MHz, DMSO-d_6) (ppm): δ 9.21-7.08, 5.93, 5.73, 4.87, 4.42, 4.05, 3.77-3.41, 3.21, 2.01, 1.77, 1.61. ^{31}P-NMR (DMSO-d_6) (ppm): δ 24.11.

P2: M1 (0.031 g, 0.02 mmol), M2 (0.031 g, 0.0565 mmol) and Grubbs' catalyst (3rd gen) (5.4 mg, 0.0062 mmol) were used. A blue colored substance P2 was obtained (theoretical molecular weight = 10,000 g mol^{-1}). Yield: 0.040 g (64%). FT-IR υ_{max} (cm^{-1}): 3057.23, 2869.78, 1775.23, 1699.25, 1607.19, 1588.66, 1487.86, 1438.06, 1396.55, 1369.70, 1335.31, 1280.68, 1219.63, 1111.95, 1089.31, 1057.88, 1040.07, 996.56, 956.25, 848.55, 747.59, 736.11, 723.77, 689.98. UV-vis (DMSO, λ_{max}) (nm): 680, 612, 359. ^{1}H-NMR (500 MHz, DMSO-d_6) (ppm): δ 9.25-7.08, 5.92, 5.75, 4.86, 4.42, 4.03, 3.76-3.39, 3.20, 2.01, 1.80, 1.60. ^{31}P-NMR (DMSO-d_6) (ppm): δ 24.14.

P3: M1 (0.0334 g, 0.022 mmol), M2 (0.0778 g, 0.0155 mmol) and Grubbs' catalyst (3rd gen) (2.54 mg, 0.141 mmol) were used. A blue colored substance P3 was obtained (theoretical molecular weight = 10,000 g mol^{-1}). Yield: 0.080 g (71%). FT-IR υ_{max} (cm^{-1}): 3055.89, 2923.93, 2869.01, 1774.55, 1698.46, 1667.76, 1607.09, 1587.56, 1487.19, 1437.47, 1397.55, 1369.11, 1335.42, 1238.18, 1219.78, 1151.50, 1111.82, 1037.45, 996.16, 969.81, 848.76, 806.15, 747.34, 736.98, 723.05, 689.38, 660.34. UV-vis (DMSO, λ_{max}) (nm): 680, 612, 359. ^{1}H-NMR (500 MHz, DMSO-d_6) (ppm): δ 9.29-7.09, 5.92, 5.73, 4.84, 4.44, 4.03, 3.77-3.40, 3.20, 2.02, 1.81, 1.62. ^{31}P-NMR (DMSO-d_6) (ppm): δ 24.17.

Synthesis of homopolymer P4: M1 (0.041 g, 0.0271 mmol) was dissolved in 1.5 mL of $CHCl_3$ in a nitrogen atmosphere and Grubbs' catalyst (3rd gen) (3.61 mg, 0.00408 mmol) in 0.5 mL of DCM was added in one shot to the stirring monomer solution. The reaction was monitored by TLC and, after 3 hours, 0.5 mL of 30% ethyl vinyl ether was added and stirred for 30 minutes. It was then precipitated and washed with diethyl ether. After centrifugation, it was dried in a vacuum oven. A blue colored substance P4 was obtained (theoretical molecular weight = 10,000 g mol^{-1}). Yield: 0.020 g (48%). FT-IR υ_{max} (cm^{-1}): 3068.32, 2921.73, 2870.19, 1772.93, 1702.51, 1606.59, 1503.15, 1487.61, 1470.24, 1450.36, 1394.22, 1336.86, 1280.58, 1219.77, 1198.18, 1178.23, 1088.90, 1058.52, 1040.58, 952.46, 825.74, 774.12, 746.16, 729.99, 686.19, 656.84. UV-vis (DMF, λ_{max}) (nm): 677, 615, 350. ^{1}H-NMR (500 MHz, DMSO-d_6) (ppm): δ 9.18-7.07, 5.99, 5.84, 5.00, 4.54, 4.05, 3.78-3.42, 3.21, 2.06.

Singlet oxygen quantum yields: Singlet oxygen quantum yields were determined using the relative method. $^{1}O_2$ photogeneration rates in DMF were derived using 1,3-diphenylisobenzofuran (DPBF). The initial absorbance of DPBF was adjusted to about 1.0, and then the PS was added to reach absorbance about 0.2-0.3. The photooxidation of DPBF was monitored between 0 s to 40 s. $^{1}O_2$ photogeneration rates in water were derived using 9,10-anthracenediyl-*bis*(methylene)dimalonic acid (ADMA) as a fluorescent monitor (λ_{exc} = 370 nm) for photosensitized bleaching rates. The Φ_Δ for the samples was calculated according to the following equation:

$$\Phi_{\Delta}^{S} = \Phi_{\Delta}^{R} \cdot \frac{r_S}{r_R} \cdot \frac{\int_{\lambda 1}^{\lambda 2} I_0(\lambda) \cdot (1 - 10^{-A_R(\lambda)}) \cdot d\lambda}{\int_{\lambda 1}^{\lambda 2} I_0(\lambda) \cdot (1 - 10^{-A_S(\lambda)}) \cdot d\lambda} \quad (1)$$

where r is the slope of the monitor's bleaching over time (plotted as ln (Φ/Φ_0)), $\lambda_1 - \lambda_2$ is the irradiation wavelength interval, $I_0(\lambda)$ the incident spectral photon flow, $A(\lambda)$ the absorbance, and the subscripts *R* and *S* are the reference (unsubstituted ZnPc, Φ_Δ = 0.56 in DMF or methylene blue, Φ_Δ = 0.52 in H_2O) and sample, respectively. The incident intensity can be approximated by a constant value, drawn out of the integral and canceled. Freshly prepared dye solution in dark flasks was mixed with the Polymer-PS conjugate only immediately before taking the samples at "0 time." The samples were irradiated in 5 or 20 s steps with a LED light array 660 ± 24 nm. Samples were mixed vigorously between each irradiation step. Recorded graphics plotted against the irradiation time *t* are shown in Figures S23 and S24.

Bacterial strains and culture conditions: Bacterial strains *B. subtilis* DB104 and *E. coli* Nissle 1917 were grown on lysogeny broth (LB; Miller) agar and stored at 4 °C. One single isolated colony was taken from this plate, transferred to 3 mL LB broth, and incubated aerobically overnight at 37 °C in a shaker incubator at 180 rpm (rotations per minute). Bacteria were then suspended in 10 mL of fresh LB medium to an optical density OD_{600} ≈ 0.1 and grown to attenuation of OD_{600} ≈ 0.4. The bacterial suspensions were then centrifuged at 4000 rpm for 5 min and PBS was added to achieve the concentration of approximately 1 x 10^{-8} cells per mL. This suspension was used for the irradiation experiments.

Photoinactivation of bacteria: 1 mL bacterial suspension containing a certain amount of photosensitizer was incubated in the dark for 15 min at 37 °C in 24-well plate. Irradiation was performed with an LED lamp (660 ± 24 nm) ("GLU-150" from RoHs). Fluence rates were routinely measured using a power meter ("Solarmeter 9.6" from Solartech). After irradiation, the living bacterial cells were determined by serial dilutions of the bacterial suspension using LB agar plating method. After the incubation overnight at 37 °C, the number of CFU/mL was counted using an automated colony counter "ProtoCOL2" from Synbiosis. The results are the mean of three replicates ± standard deviation.

Live/Dead assay: Bacterial cells were treated in the same way as for evaluation with CFU counting. After irradiation, samples were incubated with the dyes contained in the "LIVE/DEAD®" assay ("*Bac*Light™ Bacterial Viability Kit", Invitrogen L13152) for 15 min according to the manufacturer's instructions and fluorescence images were acquired using a Zeiss Axio Observer 7 equipped with a Colibri 7 multi-color LED illumination for fluorescence and Zeiss AxioCam 506 mono CCD camera.

Biofilm growth: For biofilms, overnight cultures of the *E. coli* Nissle 1917 and *B. subtilis* DB104 were diluted 1:10 into LB broth and 1:100 into TSB, correspondingly, and 125 µL aliquots were transferred to the wells of a 96-well polystyrene microtiter plate (Nunc). After growth of the samples for 72 h at 37 °C, medium and unbound cells were discarded, the wells were rinsed with PBS, and adhered cells were stained by incubation with 150 µL of the corresponding amount of PS in the buffer solution for 30 min and irradiated for 30 min using the same regime as for planktonic cultures.

Viability assay; XTT staining: Viability of the cells was determined using an XTT assay based on the reduction of 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino)carbonyl]-2H-tetrazolium hydroxide (XTT) in the

metabolically active microbial cells to a water-soluble formazan. XTT solution: (1 mg/mL) was prepared using PBS and filtered with a filter of 0.22 µm pore size. Menadione solution (0.4 mM) was prepared, filtered with a filter of 0.22 µm pore size, and mixed with XTT solution at a volume ratio of 1:2 right before each assay. After irradiation, the solution was removed and the biofilm was carefully washed with PBS. XTT-menadione solution was diluted with PBS in a 1:2 ratio and 200 µL of it was added to each well and incubated for 3 h. An aliquot of 100 µL was then taken and transferred to a fresh 96-well plate. The plate was then assayed at 492 nm with a microplate reader. The results were expressed as the relative viability (% control).

Zeta potential analysis: Stock solution of copolymers were prepared in dimethyl sulfoxide (DMSO) at a concentration of 1 mg/mL. 100 µL of these stock solutions were added to 900 µL of PBS giving a final concentration of 0.1 mg/mL and zeta potentials were measured.

2. Characterization of Compounds

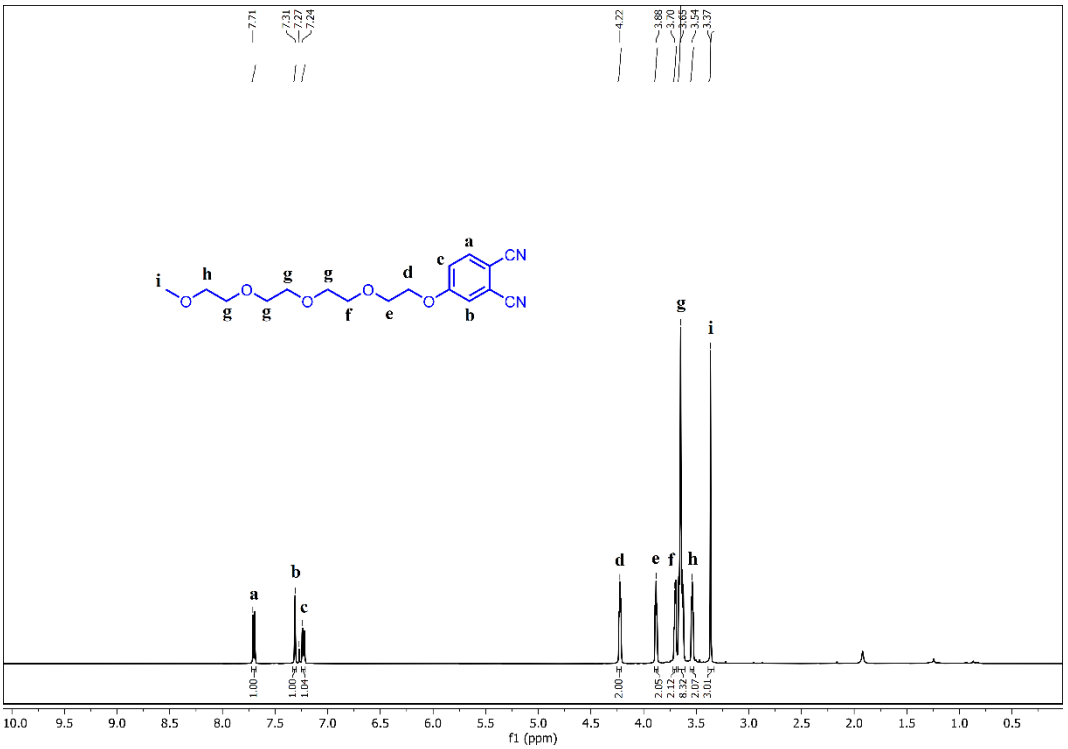

Fig. S1 ^{1}H-NMR spectrum of 4-((2,5,8,11-tetraoxatridecan-13-yl)oxy)phthalonitrile in $CDCl_3$.

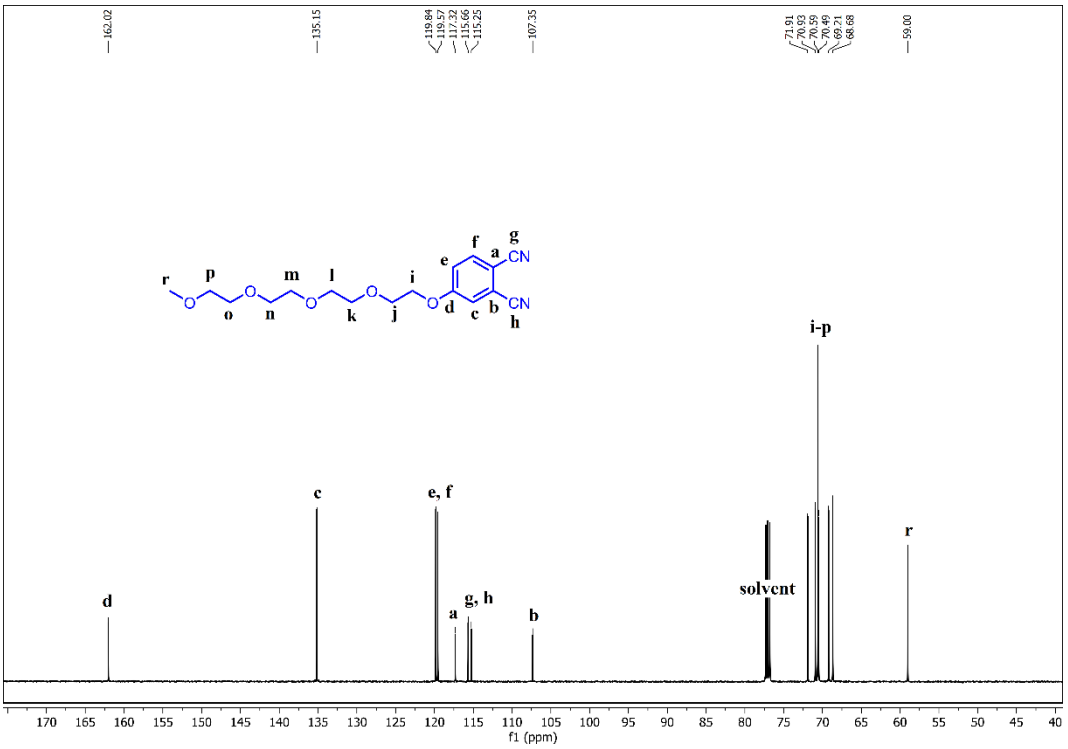

Fig. S2 ^{13}C-NMR spectrum of 4-((2,5,8,11-tetraoxatridecan-13-yl)oxy)phthalonitrile in $CDCl_3$.

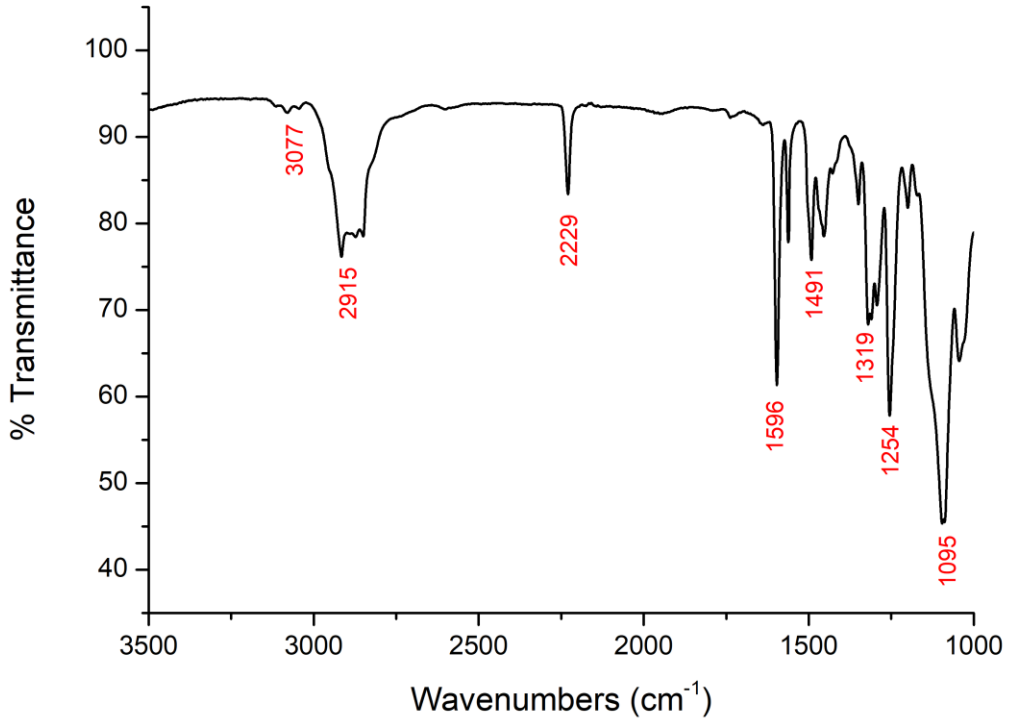

Fig. S3 FT-IR spectrum of 4-((2,5,8,11-tetraoxatridecan-13-yl)oxy)phthalonitrile.

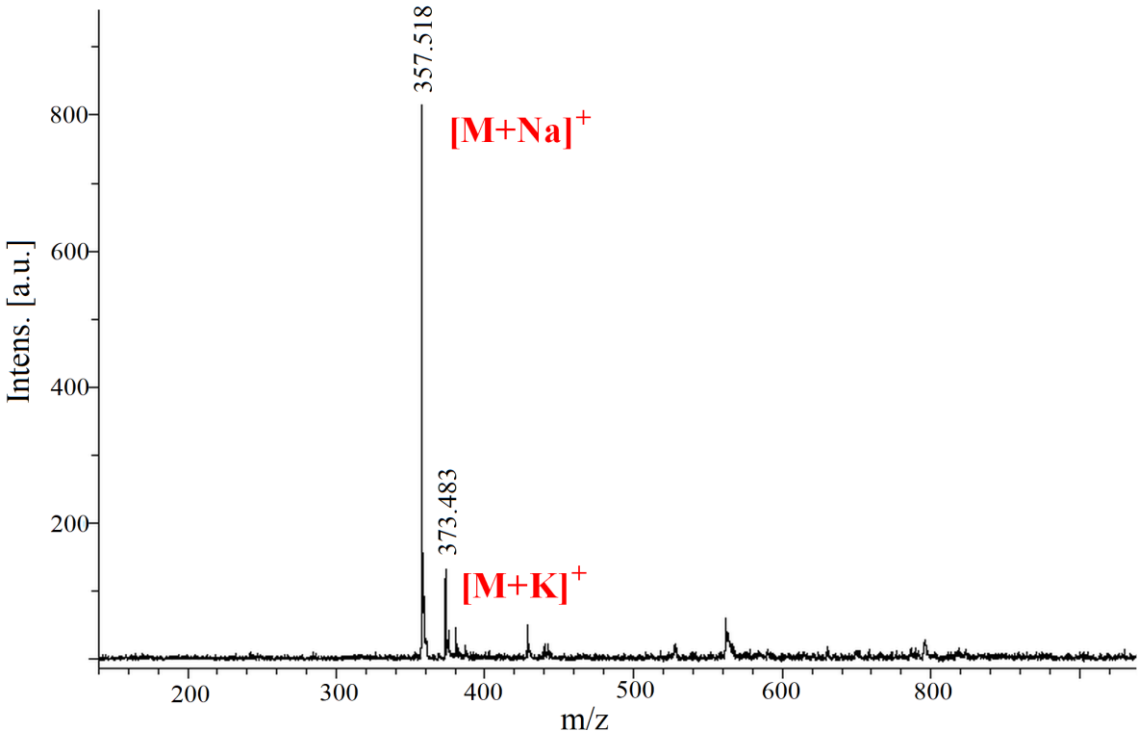

Fig. S4 MALDI-TOF MS spectrum of 4-((2,5,8,11-tetraoxatridecan-13-yl)oxy)phthalonitrile.

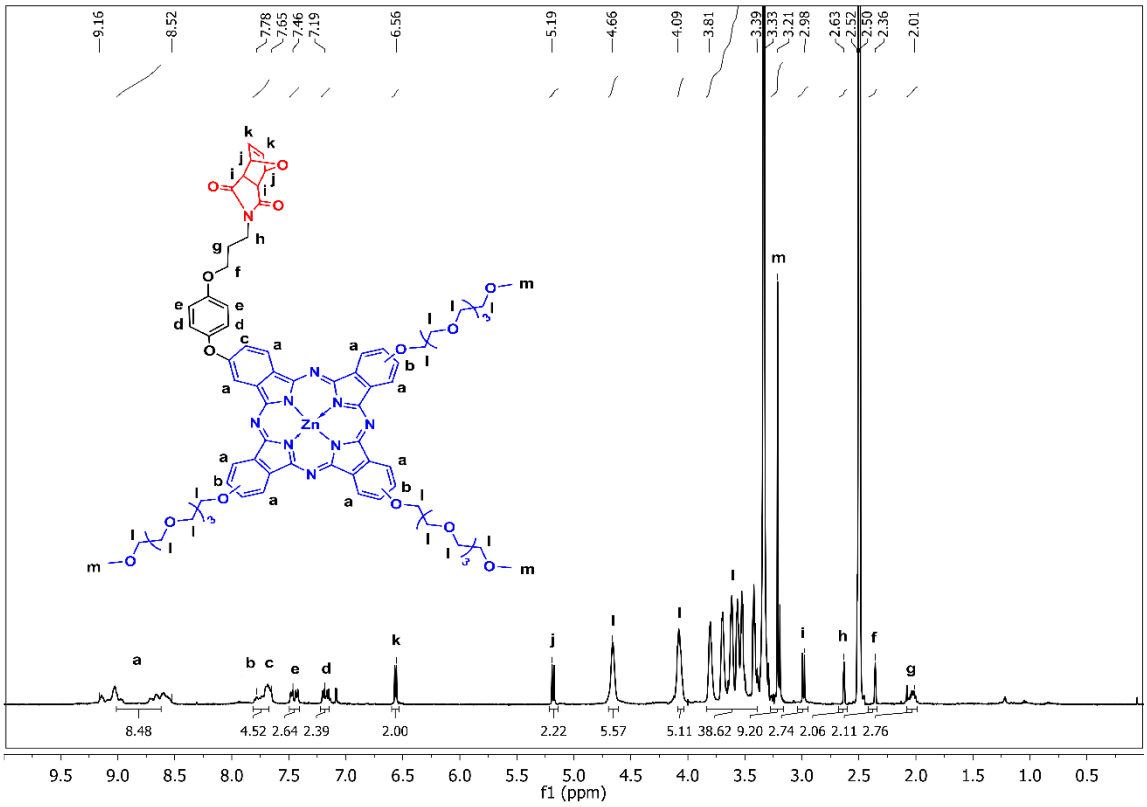

Fig. S5 ^{1}H-NMR spectrum of monomer **M1** in DMSO-d_6.

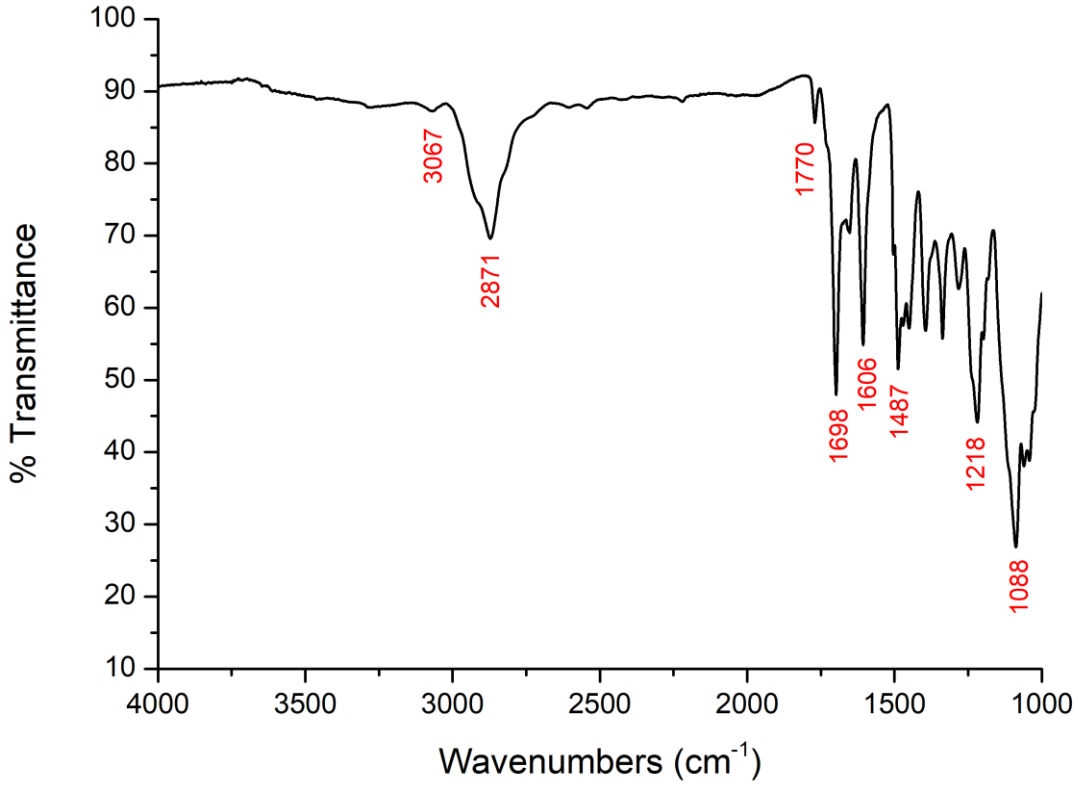

Fig. S6 FT-IR spectrum of monomer **M1**.

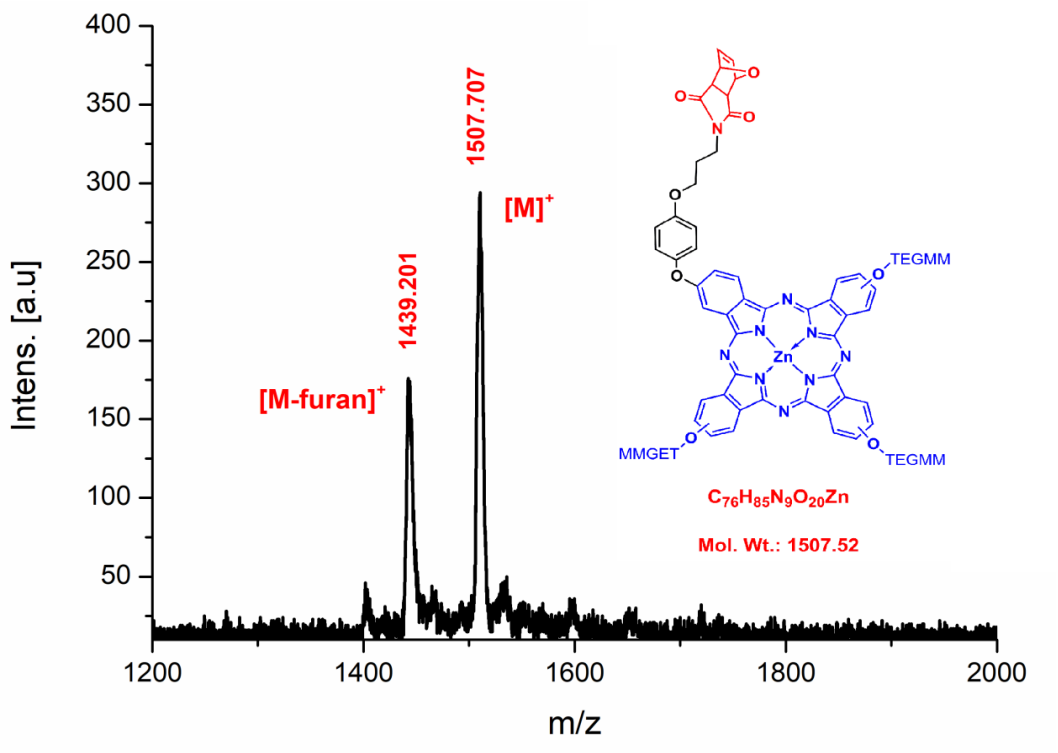

Fig. S7 MALDI-TOF MS spectrum of monomer **M1**.

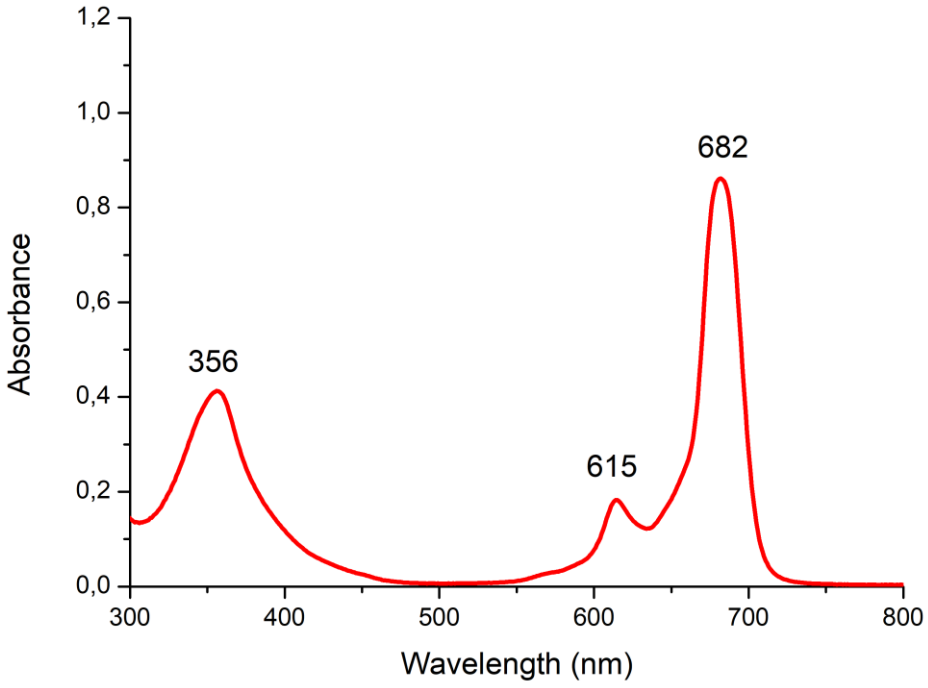

Fig. S8 UV-vis spectrum of monomer **M1** in THF.

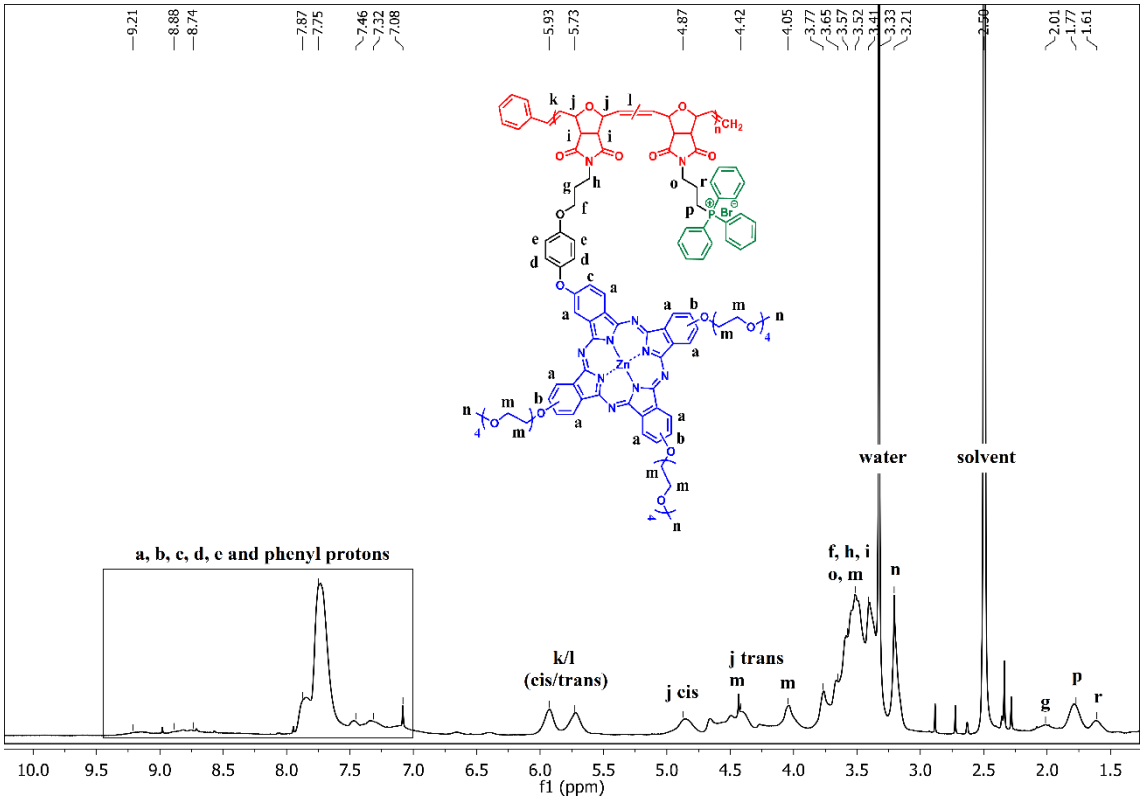

Fig. S9 ^{1}H-NMR spectrum of copolymer **P1** in DMSO-d_6.

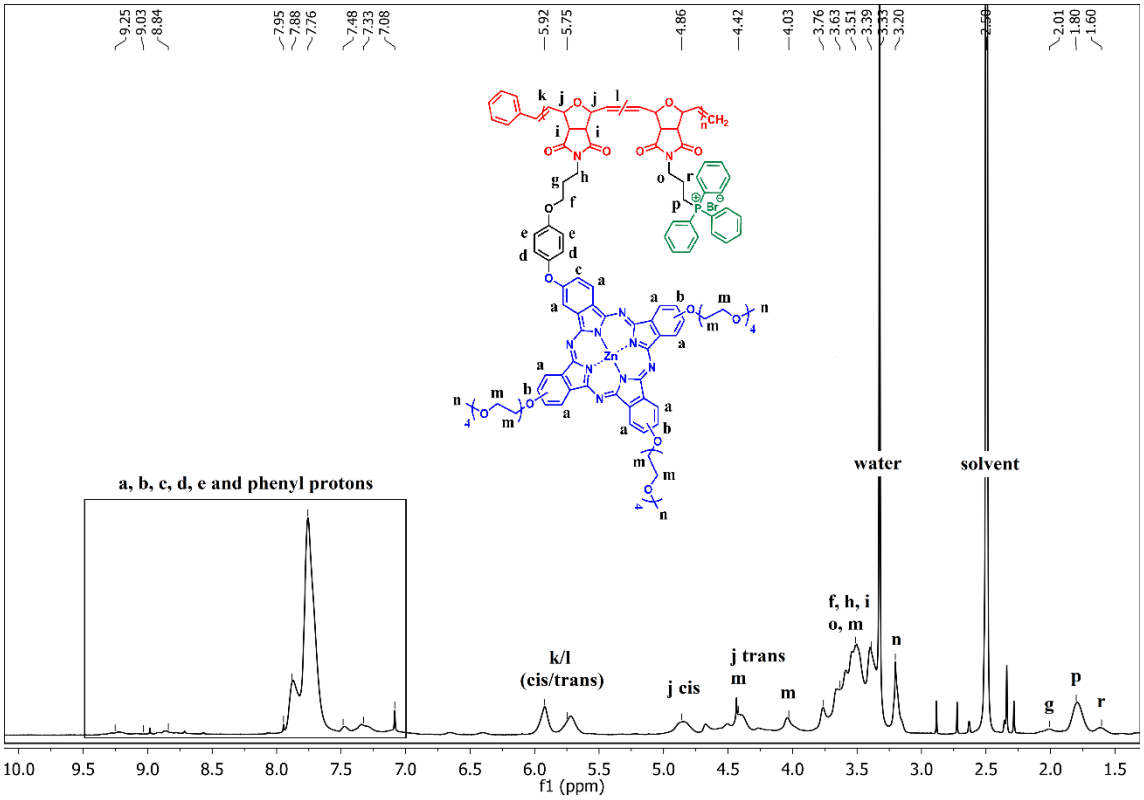

Fig. S10 ^{1}H-NMR spectrum of copolymer **P2** in DMSO-d_6.

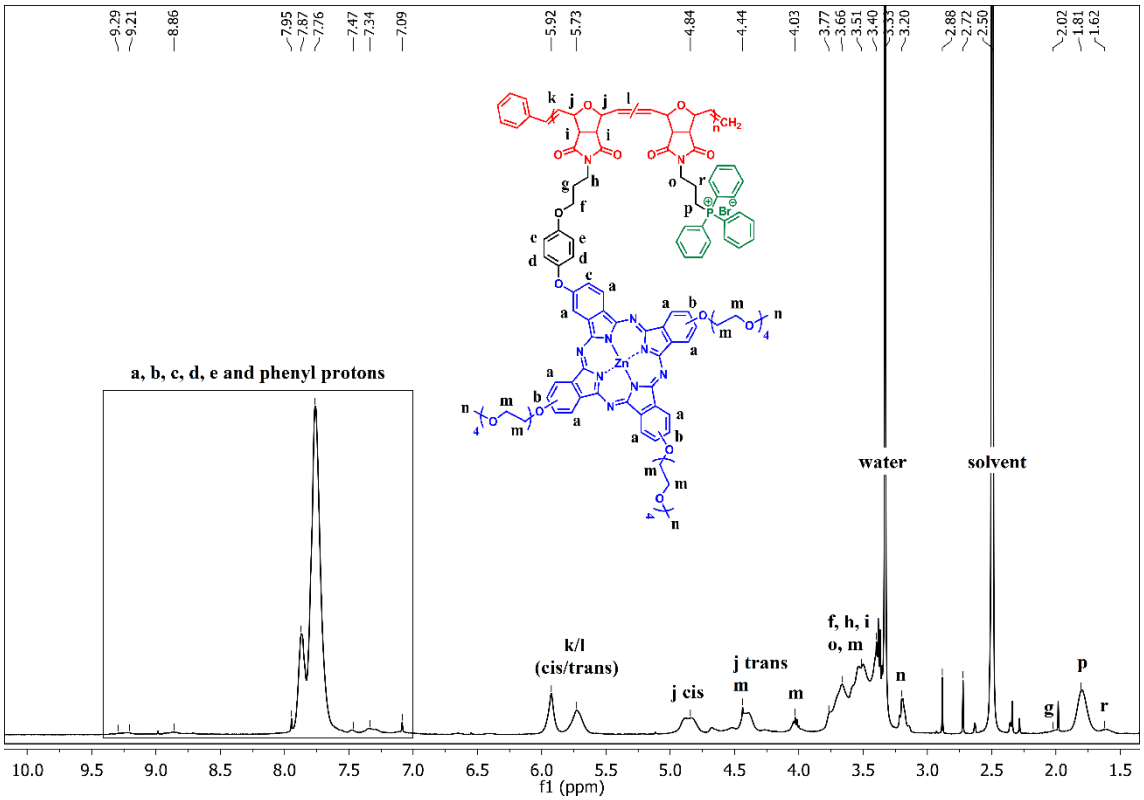

Fig. S11 ^{1}H-NMR spectrum of copolymer **P3** in DMSO-d_6.

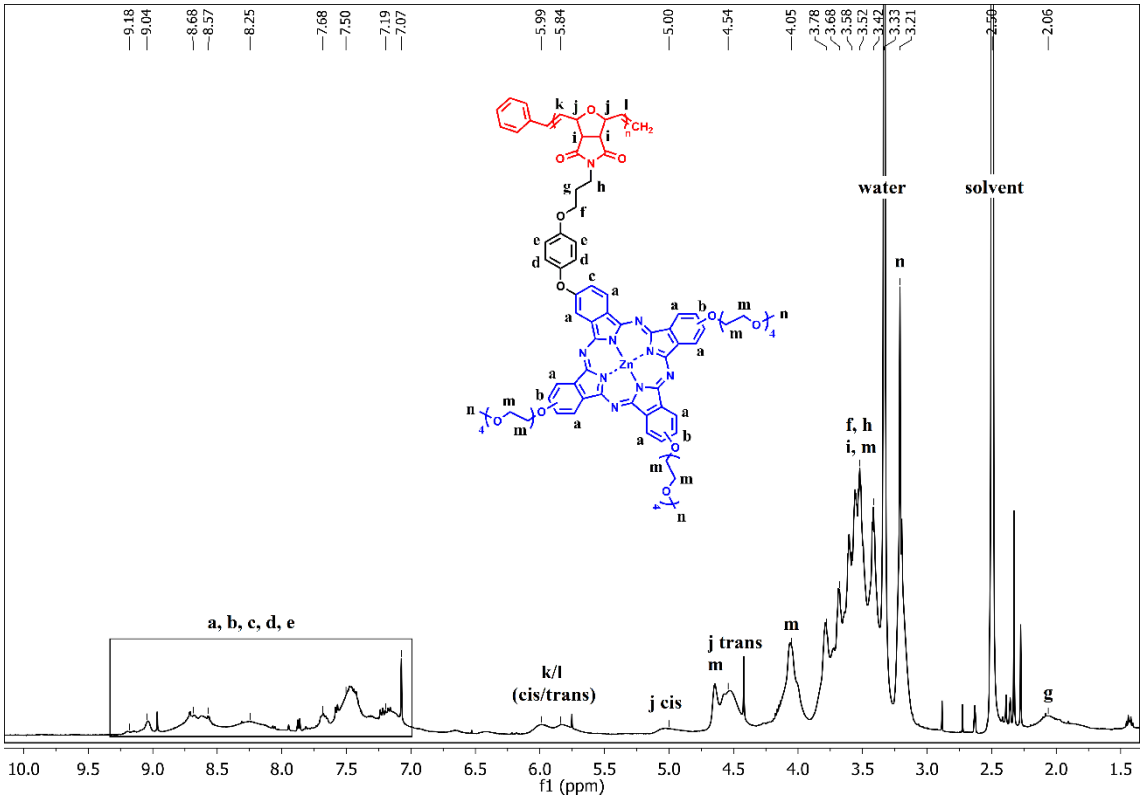

Fig. S12 ^{1}H-NMR spectrum of homopolymer **P4** in DMSO-d_6.

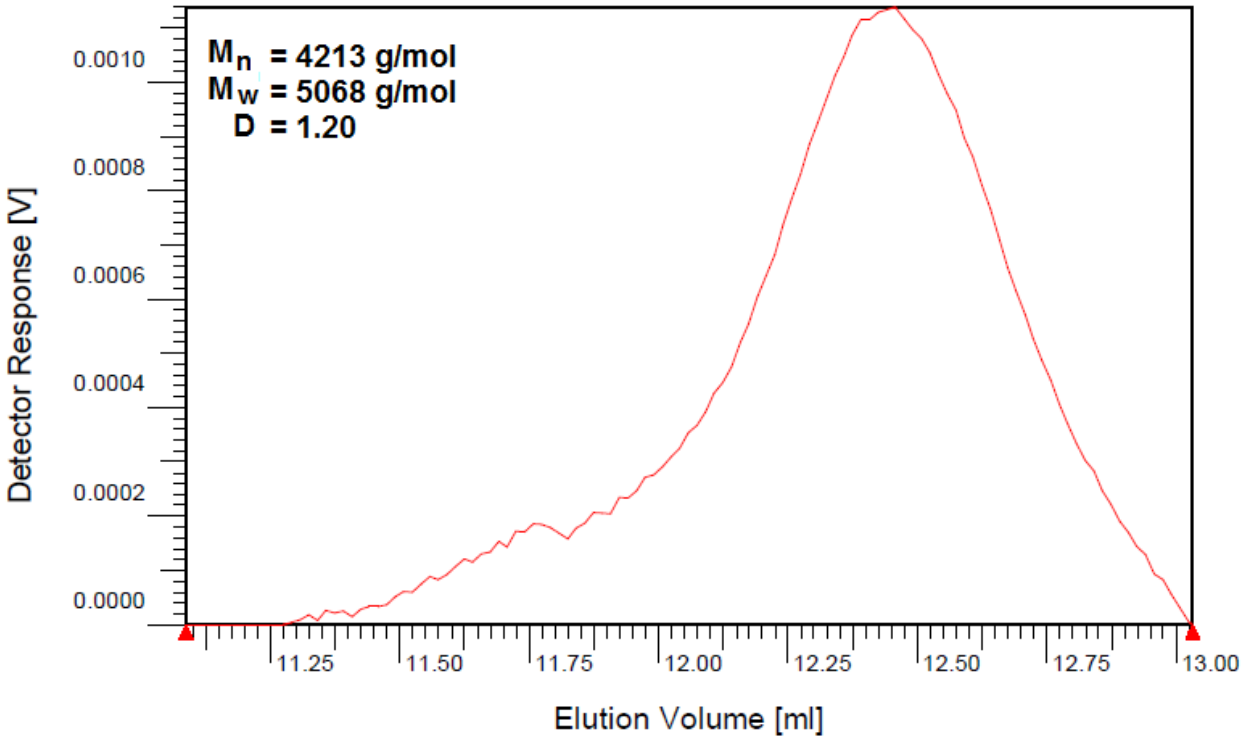

Fig. S13 GPC elugram of the copolymer **P1** in DMAC (RI detector, PMMA standard).

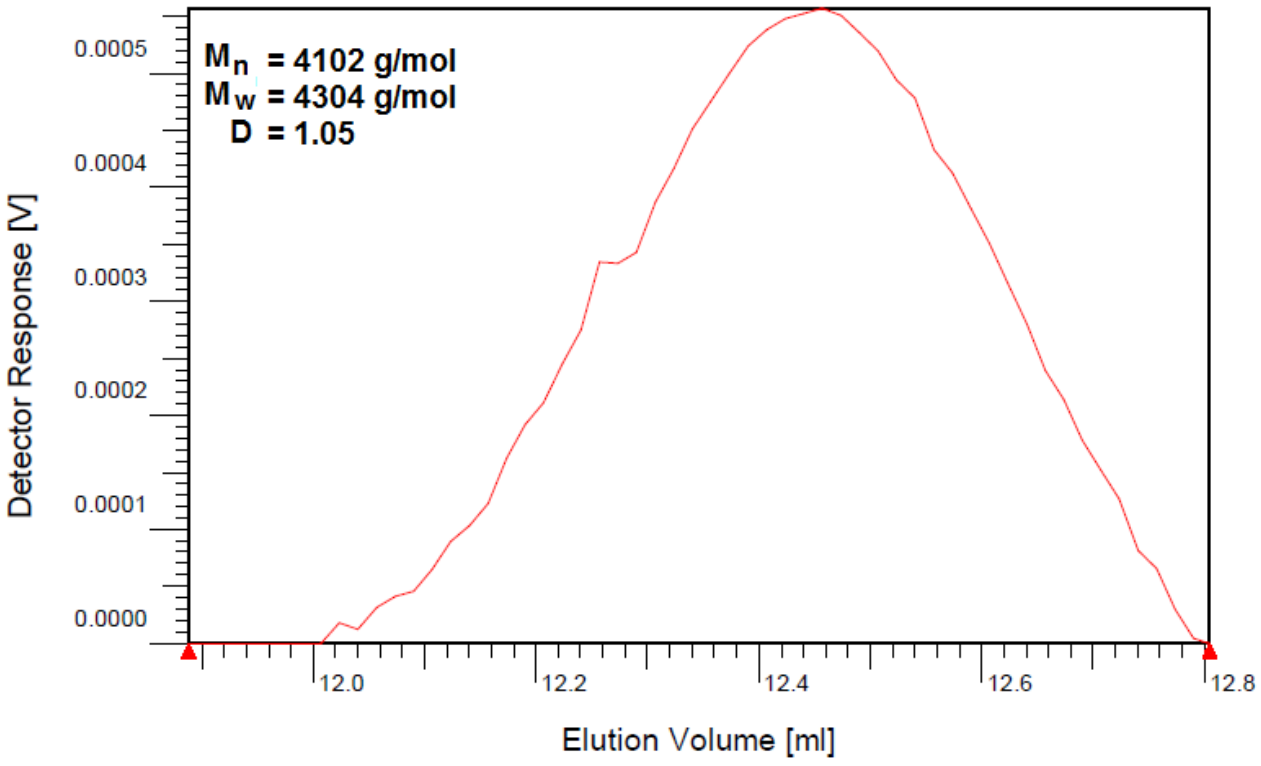

Fig. S14 GPC elugram of the copolymer **P2** in DMAC (RI detector, PMMA standard).

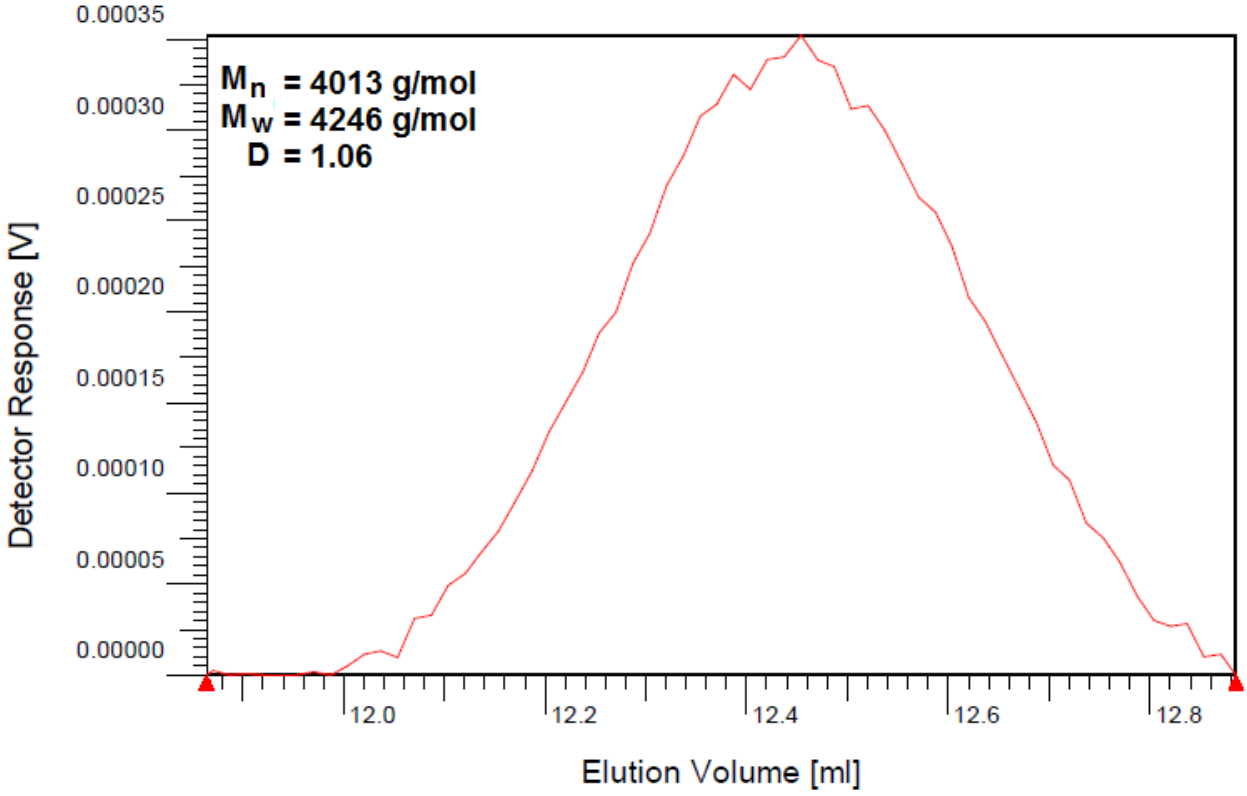

Fig. S15 GPC elugram of the copolymer **P3** in DMAC (RI detector, PMMA standard).

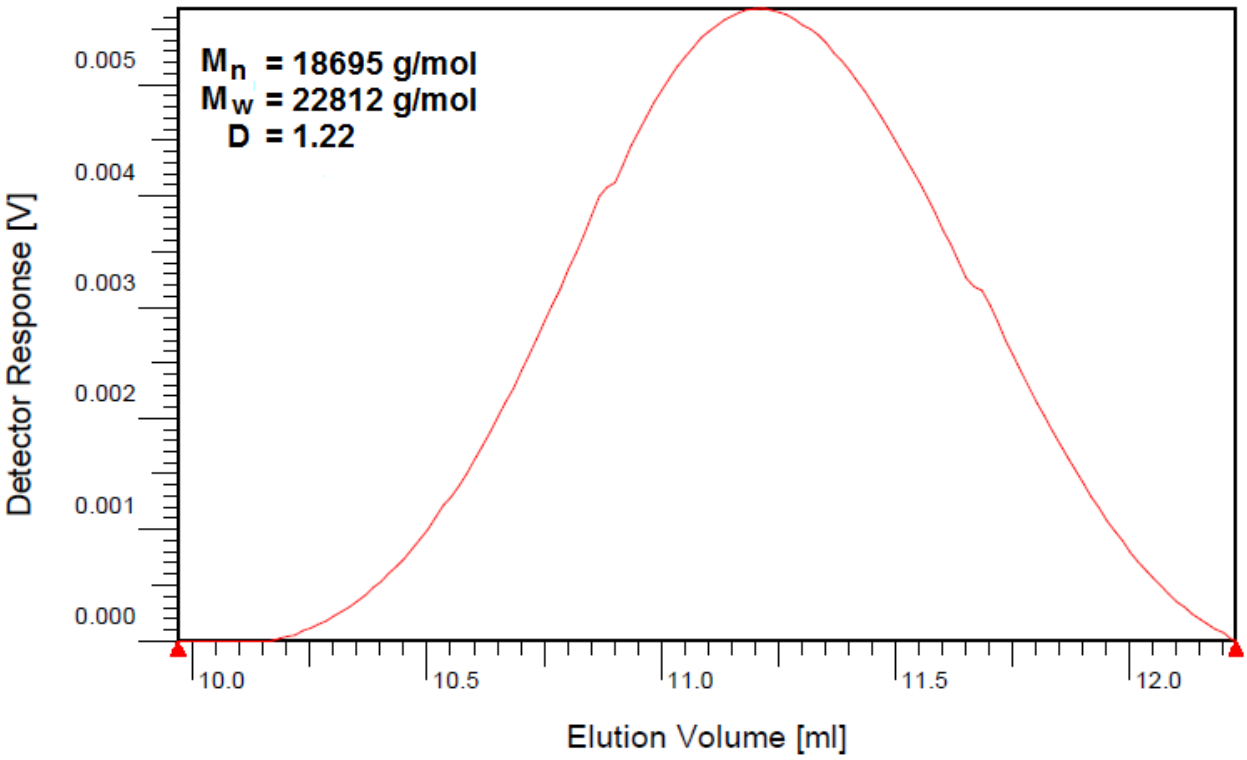

Fig. S16 GPC elugram of the homopolymer **P4** in DMAC (RI detector, PMMA standard).

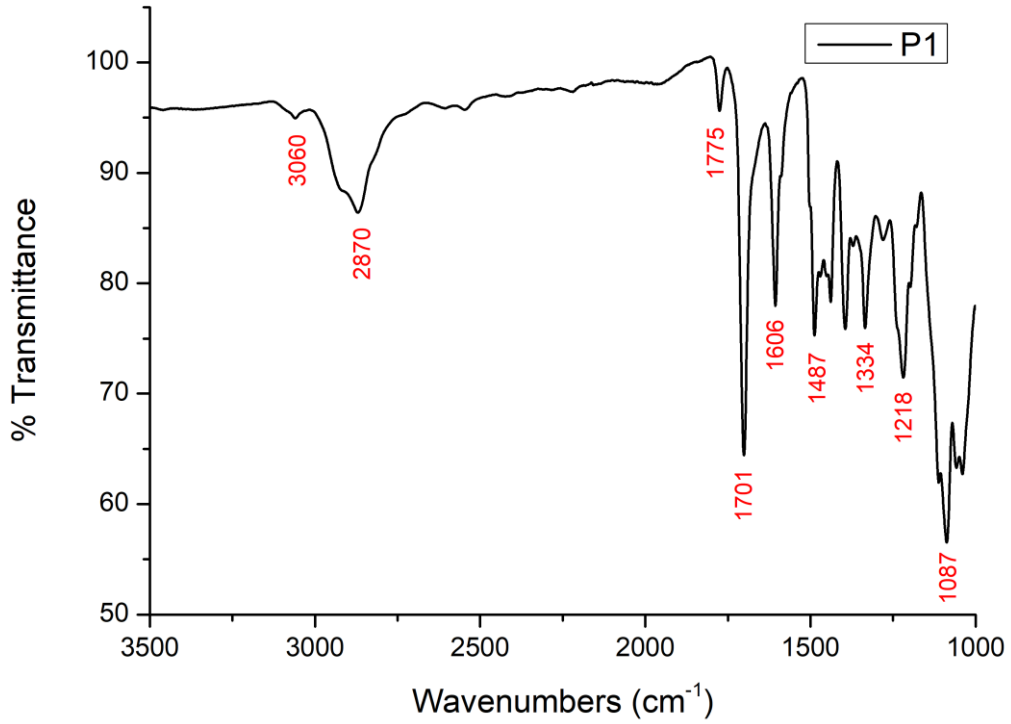

Fig. S17 FT-IR spectrum of **P1**.

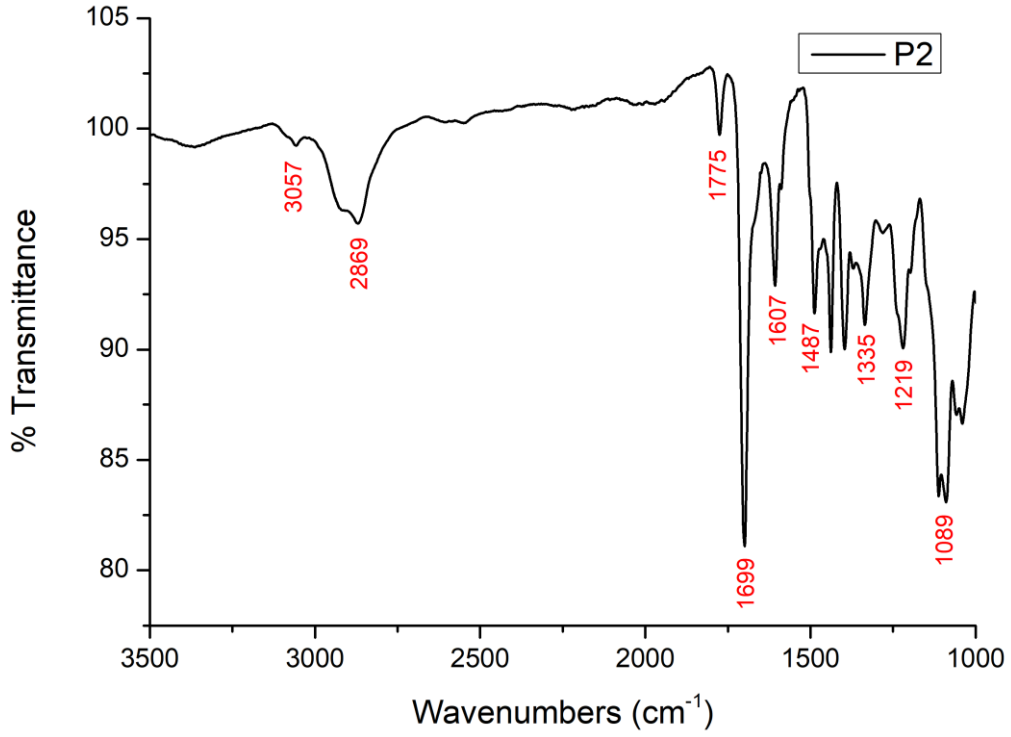

Fig. S18 FT-IR spectrum of **P2**.

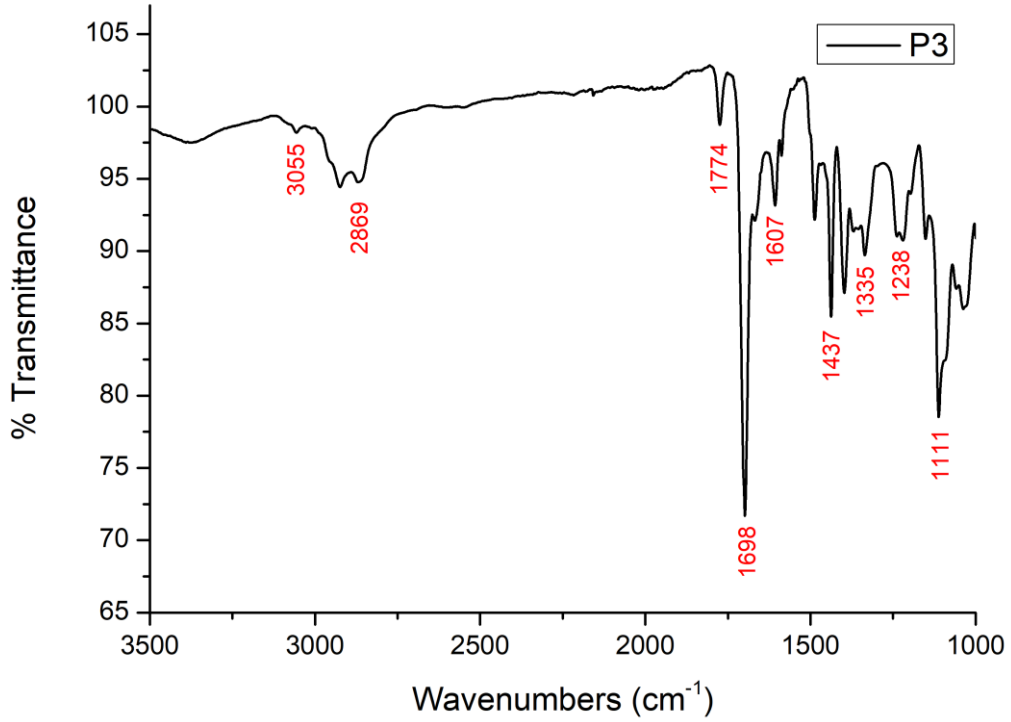

Fig. S19 FT-IR spectrum of **P3**.

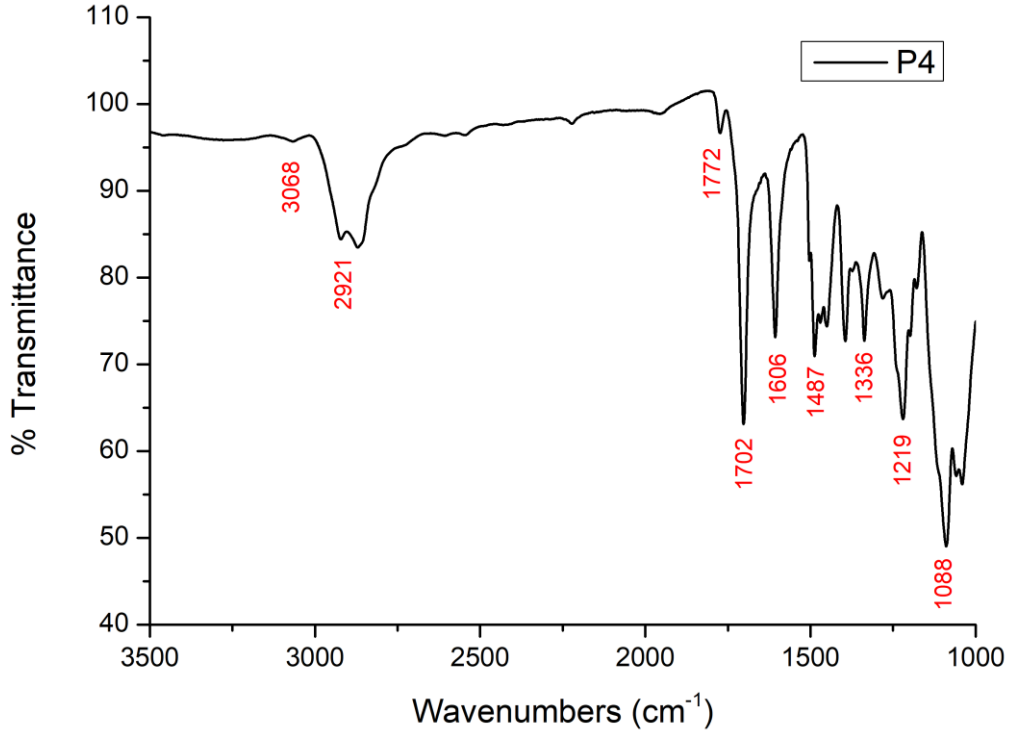

Fig. S20 FT-IR spectrum of **P4**.

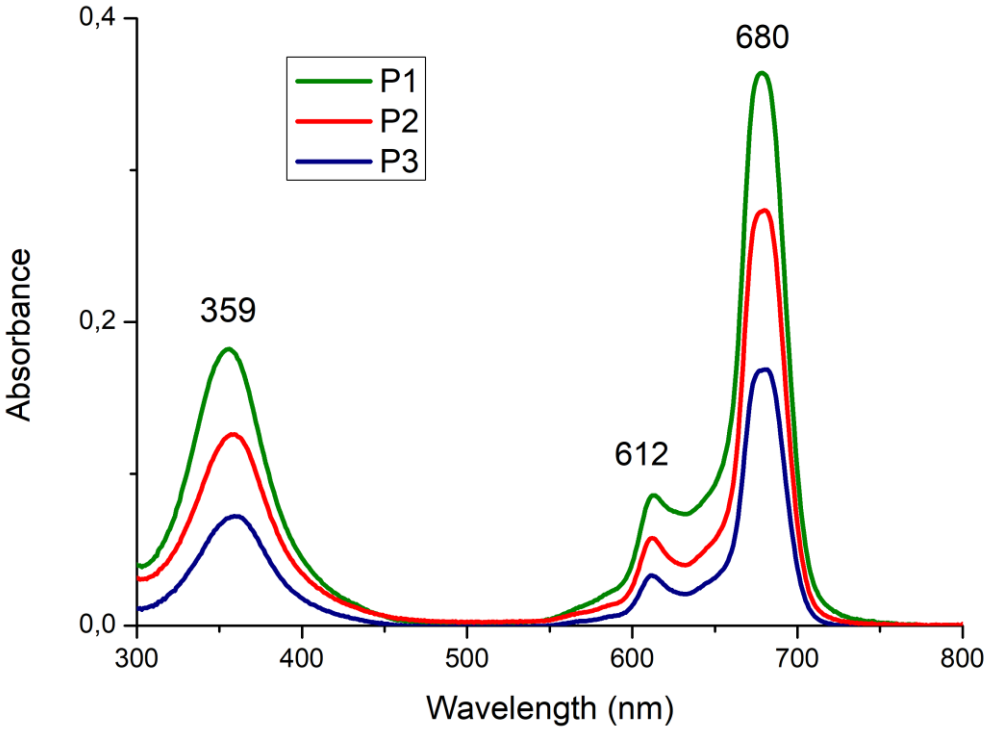

Fig. S21 UV-vis spectra of copolymers (**P1**-**P3**) in DMF (concentration: 7 µg/mL).

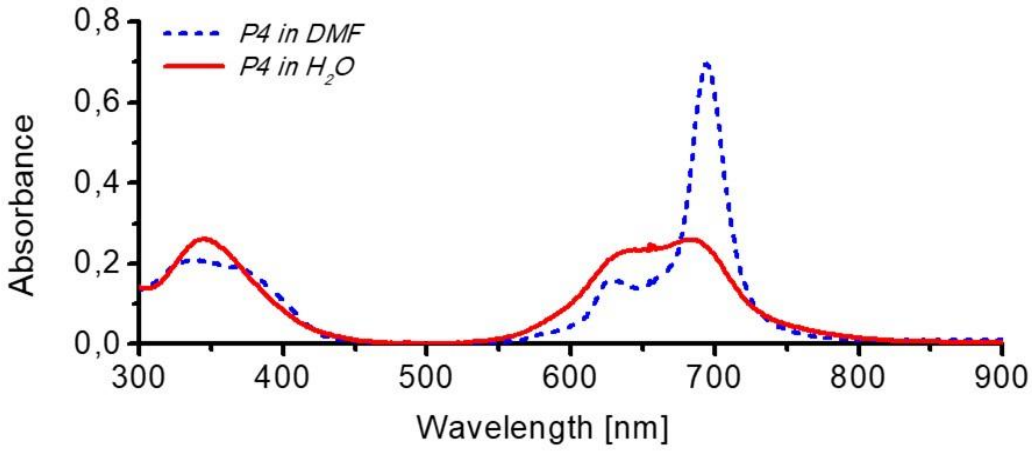

Fig. S22 UV-vis spectrum of homopolymer (**P4**) in DMF and H_2O (concentration: 10 µg/mL).

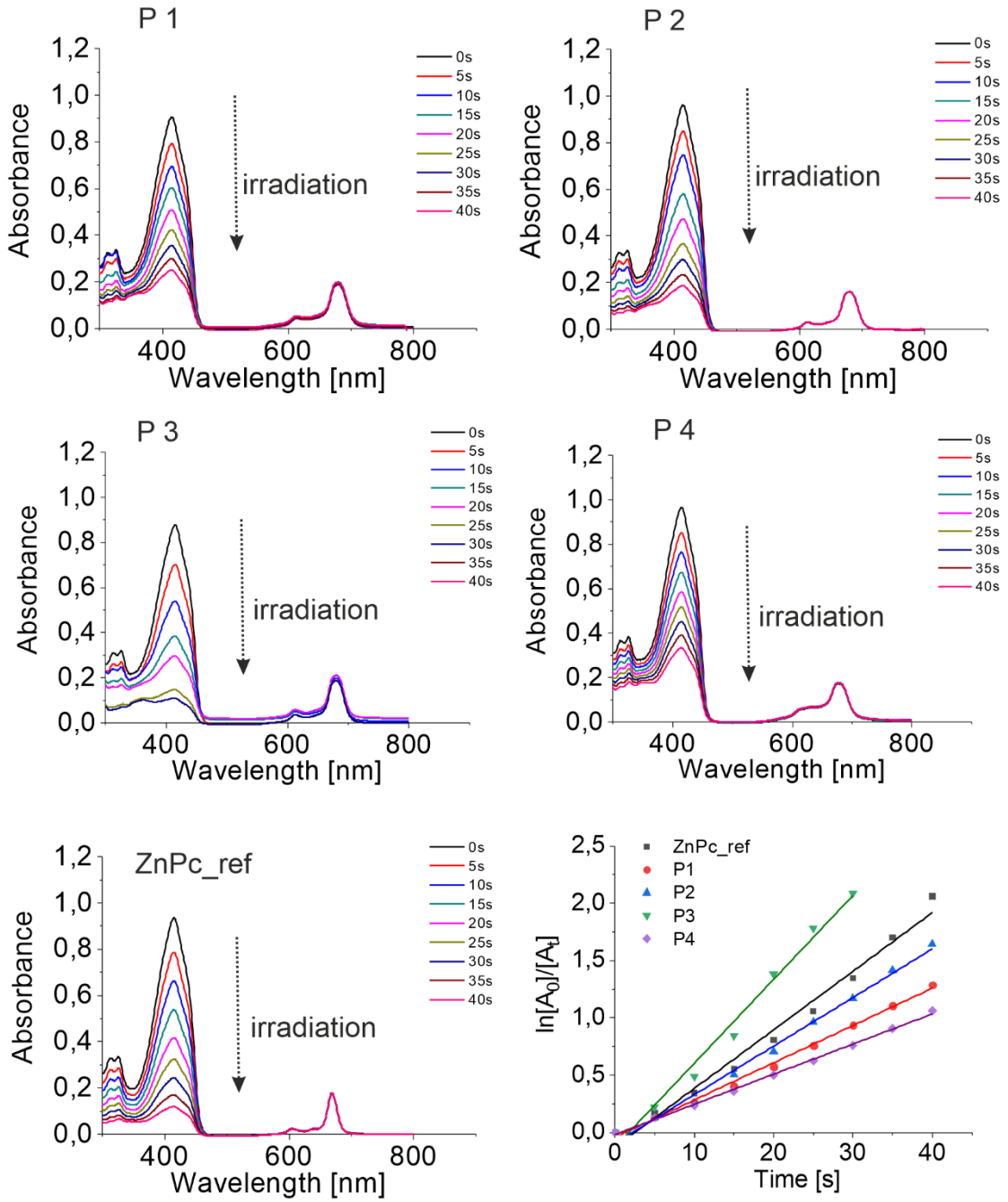

Fig. S23 Time-dependent decomposition of DPBF photosensitized by **P1-P4** and unsubstituted ZnPc as a reference in DMF upon irradiation with light of 660 ± 20 nm and corresponding first-order plots.

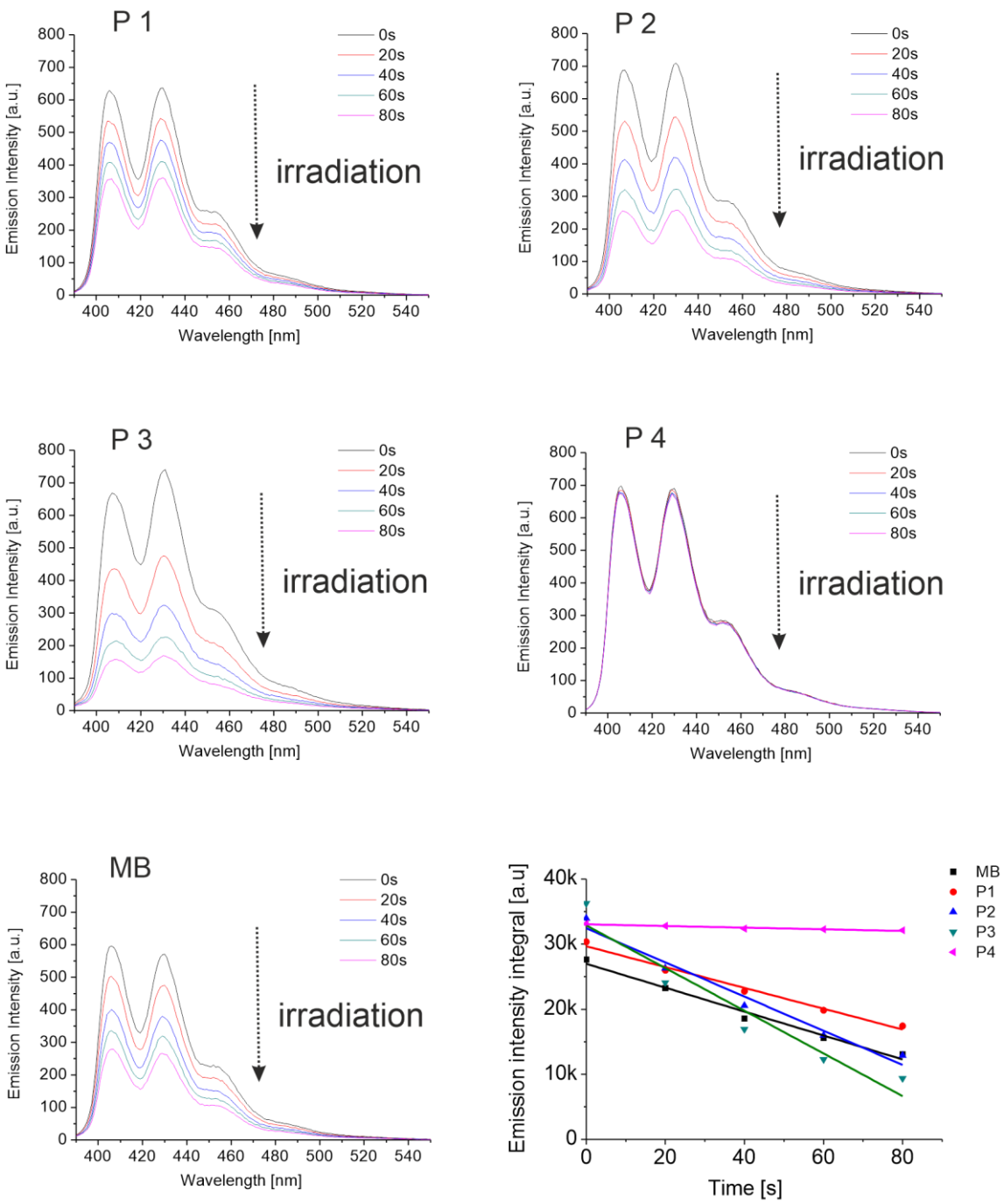

Fig. S24 Time-dependent decomposition of ADMA photosensitized by **P1-P4** and MB as a reference in H_2O upon irradiation with light of 660 ± 20 nm and corresponding first-order plots.

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