Electronic Supplementary Information (ESI)

AIE Conjugated polyelectrolytes based on tetraphenylethene for efficient fluorescence imaging and lifetime imaging of living cells

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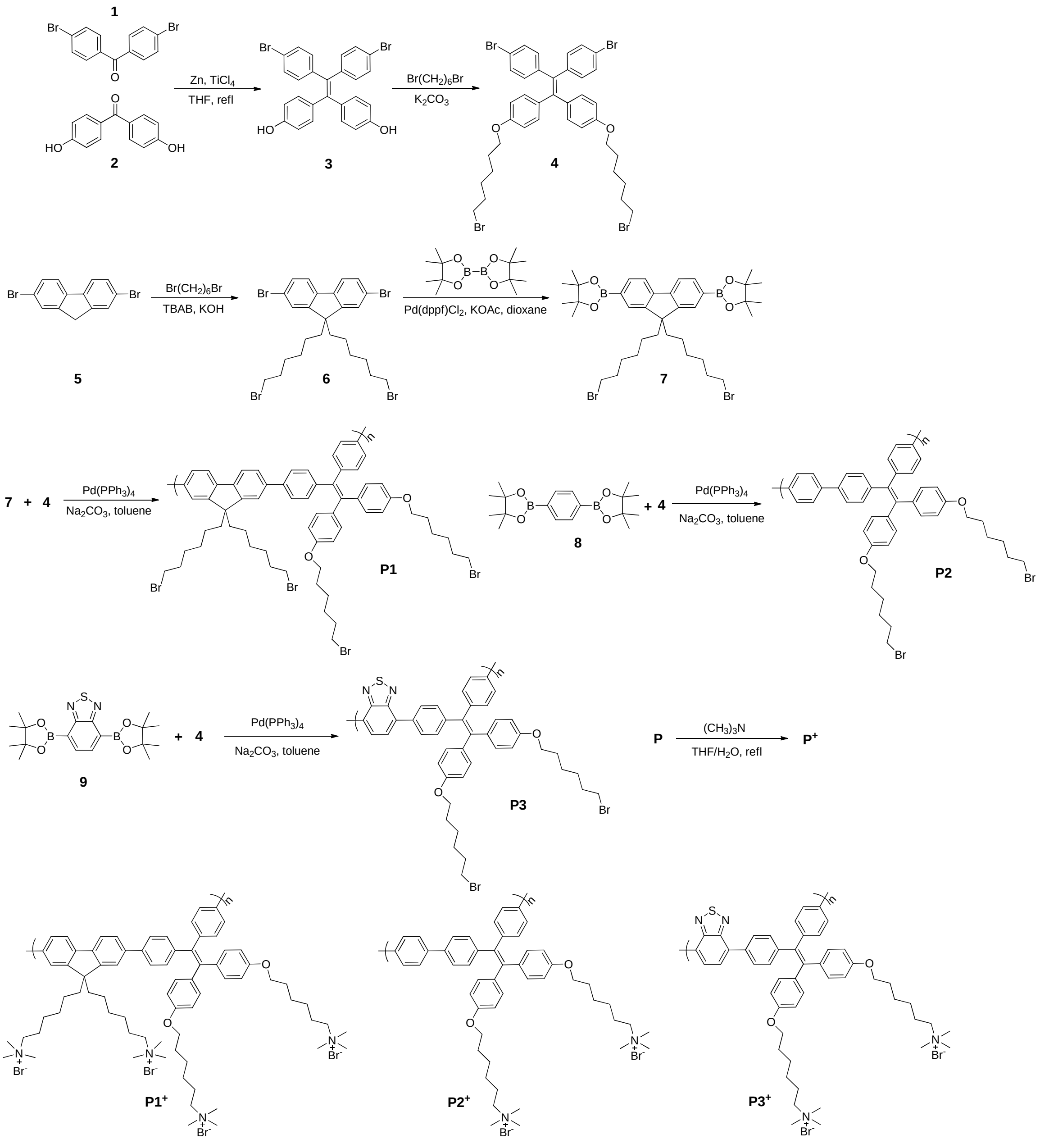
Experimental section

Materials and Instruments

THF was distilled from sodium benzophenone ketyl under dry nitrogen immediately prior to use. Chemicals and reagents were purchased from Aldrich and J&K Scientific Ltd., and used as received without further purification. HeLa cells were obtained from the American Type Culture Collection. ^{1}H and ^{13}C NMR spectra of small molecules were measured on a Bruker AV 500 spectrometer in deuterated solvents (chloroform, DMSO or dichloromethane) using tetramethylsilane (TMS; $\delta = 0$) as the internal reference. ^{1}H spectra of polymers P1-P3 were measured on an Agilent MR 400 spectrometer in deuterated chloroform using TMS as the internal reference. ^{13}C NMR of polymer P1-P3 spectra were measured on an Agilent 500 spectrometer in deuterated chloroform using TMS as the internal reference. ^{1}H NMR spectra of P1$^+$-P3$^+$ were measured on a Varian 600 spectrometer in deuterated DMSO using TMS as the internal reference. ^{13}C NMR spectra of polyelectrolytes P1$^+$-P3$^+$ were measured on an Agilent 500 spectrometer in deuterated DMSO using TMS as the internal reference. UV-vis absorption spectra were measured on a Shimadzu UV-2600 spectrophotometer. Photoluminescence was recorded on a Perkin-Elmer LS 55 spectrofluorometer. Fluorescent decay data were acquired using a Hamamatsu time-resolved spectrometer C11367-11 Quantaurus-Tau. Mass spectra were recorded on a Thermo Scientific™ Dionex UltiMate 3000 LC/Exactive™ Plus Orbitrap MS. The number average (M_n) and weight average (M_w) molecular weights and polydispersity indices (PDI or M_w/M_n) of the polymers were estimated by a Waters Associates gel permeation chromatography (GPC 2414) system equipped with RI and UV detectors. Aggregated particle morphologies of all the polymers were measured by a Hitachi HT-7700 transmission electron microscope (TEM) operated at 80 kV. The preparation of TEM samples was as follows: distribution of a polymer in solvent mixture was sonicated using a microtip probe sonicator (XL2000, Misonix Incorporated, NY) at 12 W output for 60 s; a drop of the mixture solution was dropped on a 300-mesh Formvar-coated copper grid and dried under vacuum at room temperature. Z-average particle sizes and polydispersities (PDIs) of particles were measured by DLS (Zetasizer Nanoseries) at 25 °C under a scattering angle of 90^o. Particle sizes were given as the average of three measurements. The ground-state geometries were optimized using the density function theory (DFT) method with B3LYP hybrid functional at the basis set level of 6-31G(d, p). All the calculations were performed using the Gaussian 09 package. Fluorescence lifetime imaging Microscopy (FLIM) images were acquired on a TCSPC-based system (Becker & Hickl, SPC150, Olympus IX71/FV300 confocal microscope using the frequency doubled output (380 nm) of a mode-locked and cavity dumped Titanium: sapphire laser, as described in detail elsewhere[1]) and Confocal Laser Scanning Microscopy (CLSM) images were obtained on a confocal microscope (Leica SP5, Germany) using the LAS AF imaging software.

Synthesis



Scheme S1. Molecular structures and synthetic routes to conjugated polyelectrolytes ($P1^+$-$P3^+$) based on TPE.

4,4'-(2,2-Bis(4-bromophenyl)ethene-1,1-diyl)diphenol (3): To a mixture of 4,4'-dibromobenzophenone (3.4 g, 10 mmol), 4,4'-dihydroxybenzophenone (2.14 g, 10 mmol), zinc dust (15.69g, 0.24 mol) in 80 mL dry THF was added dropwise titanium (IV) chloride (4.55g, 24 mmol) under N_2 at –78 ^{o}C. After stirring for 20 min, the reaction mixture was warmed to room temperature and then heated to reflux for 3.5 h. The mixture was cooled to room temperature and poured into water, and extracted with dichloromethane by three times. The combined organic layers were washed with saturated brine solution and water, and dried over anhydrous $MgSO_4$. After filtration and solvent evaporation, the residue was purified by silica-gel column chromatography using hexane/dichloromethane (2:1) as eluent. White solid of **3** was obtained in 59% yield. ^{1}H NMR (500 MHz, DMSO), δ (TMS, ppm): 9.41 (s, 2H), 7.32 (d, J = 8.5 Hz, 4H), 6.86 (d, J = 8.4 Hz, 4H), 6.75 (d, J = 8.1 Hz, 4H), 6.54 (d, J = 8.4 Hz, 4H). ^{13}C NMR (126 MHz, DMSO), δ (TMS, ppm): 156.74, 143.48, 142.57, 135.57, 133.97, 133.41, 132.54, 131.30, 119.78, 115.23. HRMS ($C_{26}H_{19}Br_2O_2$): *m/z* 522.9729 ([M+H]$^+$, calcd 522.9731).

4,4'-(2,2-Bis(4-((6-bromohexyl)oxy)phenyl)ethene-1,1-diyl)bis(bromobenzene) (4): To a mixture of **3** (2.09 g, 4 mmol), 1,6-dibromohexane (4.88 g, 20 mmol), K_2CO_3 (2.76 g, 20 mmol) and KI (33.2 mg, 0.2 mmol) in 30 mL acetone. The reaction mixture was heated to reflux for 12 h. The mixture was cooled to room temperature and poured into water, and extracted with dichloromethane by three times. The combined organic layers were washed with saturated brine solution and water, and dried over anhydrous $MgSO_4$. After filtration and solvent evaporation, the residue was purified by silica-gel column chromatography using hexane/dichloromethane (2:1) as eluent. White solid of **4** was obtained in 44% yield. ^{1}H NMR (500 MHz, $CDCl_3$), δ (TMS, ppm): 7.22 (d, *J* = 8.4 Hz, 4H), 6.87 (q, 8H), 6.63 (d, *J* = 8.7 Hz, 4H), 3.90 (t, *J* = 6.4 Hz, 4H), 3.42 (t, *J* = 6.8 Hz, 4H), 1.93–1.85 (m, 4H), 1.80–1.73 (m, 4H), 1.53–1.45 (m, 8H). ^{13}C NMR (126 MHz, $CDCl_3$), δ (TMS, ppm): 157.89, 135.46, 133.00, 132.49, 130.96, 113.70, 67.54, 33.79, 32.65, 29.08, 27.92, 25.29. HRMS ($C_{38}H_{40}Br_4O_2$): *m/z* 847.9705 (M^+, calcd 847.9721).

2,7-Dibromo-9,9'-bis(bromohexyl)fluorene (6): 2,7-Dibromofluorene (1.62 g, 5 mmol) was added to a mixture of aqueous NaOH (50 ml, 50%), tetrabutylammonium bromide (0.32 g, 1 mmol), and 1,6-dibromohexane (12.2 g, 50 mmol) at 75 ^{o}C. After 15 min, the mixture was cooled to room temperature. After extraction with dichloromethane by three times, the combined organic layers were washed successively with water, aqueous HCl (1M), water, and brine and then dried over anhydrous $MgSO_4$. After removal of the solvent and excess 1,6-dibromohexane, the residue was purified by silica-gel column chromatography using hexane/dichloromethane (2:1) as eluent. White solid of **6** was obtained in 76% yield. ^{1}H NMR (500 MHz, $CDCl_3$), δ (TMS, ppm): 7.53 (s, 1H), 7.52 (s, 1H), 7.47 (d, *J* = 1.7 Hz, 1H), 7.46 (d, *J* = 1.8 Hz, 1H), 7.43 (d, *J* = 1.6 Hz, 2H), 3.29 (t, *J* = 6.8 Hz, 4H), 1.98–1.88 (m, 4H), 1.72–1.62 (m, 4H), 1.20 (m, 4H), 1.08 (m,4H), 0.63–0.54 (m, 4H). ^{13}C NMR (126 MHz, $CDCl_3$), δ (TMS, ppm): 152.15, 139.05, 130.32, 126.07, 121.56, 121.22, 55.54, 40.02, 33.86, 32.59, 28.93, 27.74, 23.44. HRMS ($C_{25}H_{30}Br_4$): *m/z* 649.9031 (M^+, calcd 649.9040).

2,7-Bis[9,9(-bis(600-bromohexyl)-fluorenyl]-4,4,5,5-tetramethyl-[1.3.2]dioxaborolane (7): 2,7-Dibromo-9,9'-bis(bromohexyl)fluorene (6.5g, 10 mmol), bis-(pinacolato)diborane (6.0 g, 24 mmol), KOAc (7.0 g, 70 mmol), and dioxane (100 mL) were mixed together in a 250 mL flask. After degassing, [Pd(dppf)Cl_2] (0.5 g, dppf = 1,1'-bis(diphenylphosphanyl)ferrocene) was added. The reaction mixture was kept at 85 °C overnight, and then cooled to room temperature. The organic solvent was distilled out, and the residual solid was dissolved in dichloromethane and washed with water. After drying with anhydrous $MgSO_4$, the solvent was distilled out. The crude product was purified by flash chromatography using hexanes and dichloromethane (1:1) as the eluent to give **7** as white crystals (4.2 g, 89%). ^{1}H NMR (500 MHz, CD_2Cl_2), δ (TMS, ppm): 7.76 (d, 2H), 7.75–7.72 (m, 4H), 3.27 (t, J = 6.9 Hz, 4H), 2.08–1.99 (m, 4H), 1.67–1.58 (m, 4H), 1.36 (s, 24H), 1.18–1.11 (m, 4H), 1.11–1.03 (m, 4H), 0.53 (m, 4H). ^{13}C NMR (126 MHz, CD_2Cl_2), δ (TMS, ppm): 150.11, 143.80, 133.57, 128.77, 119.40, 83.77, 54.98, 40.01, 34.11, 32.71, 28.98, 27.76, 24.76, 23.51. HRMS ($C_{37}H_{55}B_2Br_2O_4$): *m/z* 745.2621 ($[M+H]^+$, calcd 745.2633).

P1: To a mixture of **7** (186 mg, 0.25 mmol), **4** (212 mg, 0.25 mmol), $Pd(PPh_3)_4$ (12 mg, 0.01 mmol) and K_2CO_3 (276 mg, 2 mmol) in a 10 mL round-bottomed flask. A mixture of toluene (1 mL) and water (1 mL) was added to the flask, and the reaction vessel was degassed. The mixture was vigorously stirred at 85 °C for 24 h and then cooled to room temperature. After extraction with dichloromethane, the combined organic layers were washed successively with water and then dried over anhydrous $MgSO_4$. The polymer was filtered and precipitated into methanol, and then dried under vacuum for 24 h to afford the neutral polymers P1 as a green solid in 68% yield. ^{1}H NMR (400 MHz, $CDCl_3$), δ (TMS, ppm): 7.77–7.54 (m, 6H), 7.47(d, *J* =7.9 Hz, 4H), 7.17 (d, *J* = 8.0Hz, 4H), 7.03(d, *J* = 8.2 Hz, 4H), 6.68 (d, *J* = 8.3 Hz, 4H), 3.91 (br, 4H), 3.41 (t, *J* = 6.5Hz, 4H), 3.25 (t, *J* = 6.7 Hz, 4H), 2.12–2.03 (m, 4H), 1.98–1.84 (m, 4H), 1.83–1.70 (m, 4H), 1.68–1.58 (m, 4H), 1.47(br, 8H), 1.24–1.14 (m, 4H), 1.13–0.98 (m, 4H), 0.69 (br, 4H). ^{13}C NMR (126MHz, $CDCl_3$), δ (TMS, ppm): 157.64, 151.22, 143.44, 140.53, 139.99, 139.55, 138.88, 138.75, 138.49, 136.40, 132.70, 131.98, 128.79, 127.15, 126.24, 125.89, 120.85, 119.98, 114.71, 113.64, 67.57, 55.14, 40.32, 33.93, 33.78, 32.65, 29.14, 29.05, 27.94, 27.75, 25.32, 23.57.

P2: The procedure was analogous to that described for P1. Green solid, yield 81%. ^{1}H NMR (400 MHz, $CDCl_3$), δ (TMS, ppm): 7.64 (d, *J* = 12.1 Hz, 4H), 7.41 (d, *J* = 7.9 Hz, 4H), 7.13 (d, *J* = 8.0 Hz, 4H), 6.99 (d, *J* = 8.2 Hz, 4H), 6.65 (d, *J* = 8.4 Hz, 4H), 3.89 (t, *J* = 5.5 Hz, 4H), 3.40 (t, *J* = 6.7 Hz, 4H), 1.88 (m, 4H), 1.76 (m, 4H), 1.48 (br, 8H). ^{13}C NMR (101MHz, $CDCl_3$), δ (TMS, ppm): 157.63, 143.46, 140.64, 139.34, 138.52, 138.17, 137.69, 136.27, 132.65, 131.94, 127.02, 126.04, 113.59, 67.52, 33.79, 32.65, 29.11, 27.93, 25.30.

P3: The procedure was analogous to that described for P1. Red solid, yield 79 %. ^{1}H NMR (400 MHz, $CDCl_3$), δ (TMS, ppm): 7.87–7.74 (m, 6H), 7.17–6.92 (m, 8H), 6.70–6.61 (m, 4H), 3.93–3.86 (m, 4H), 3.45–3.38 (m, 4H), 1.92–1.83 (m, 4H), 1.80–1.79 (m, 4H), 1.47 (br, 8H). ^{13}C NMR (126MHz, $CDCl_3$), δ (TMS, ppm): 157.76, 139.17, 137.52, 133.19, 132.72, 132.60, 131.78, 131.64, 130.91, 128.60, 128.47, 127.87, 113.68, 67.53, 33.76, 32.63, 29.10, 27.91, 25.29.

General Procedure for the Cationic Conjugated Polymers: Condensed trimethylamine (2 mL) was added drop-wise to a solution of the neutral polymer (50 mg) in THF (10 mL) at 0 °C. The mixture was allowed to warm to room temperature. The precipitate was redissolved by the addition of water (10 mL). After the mixture was cooled to 0 °C, additional trimethylamine (2 mL) was added, and the mixture was stirred at room temperature for 24 h. After removal of the solvent, acetone was added to precipitate the CCPs as powders in 81%, 82% and 85% yields for $P1^+$, $P2^+$, and $P3^+$ respectively.

P1$^+$: ^{1}H NMR (600 MHz, DMSO), δ (TMS, ppm): 7.83–7.58(m, 6H), 7.57–7.48 (m, 4H), 7.10 (d, J = 6.1 Hz, 4H), 6.95 (d, J = 6.7 Hz, 4H), 6.76–6.62(m, 4H), 3.89 (br, 4H), 3.28–3.22 (m, 4H), 3.03–3.01 (d, 22H), 2.89 (s, 18H), 2.06 (br, 4H), 1.70–0.99 (m, 20H), 0.84–0.57(m, 12H). ^{13}C NMR (125MHz, DMSO), δ (TMS, ppm): 157.70, 151.62, 143.50, 140.08, 139.06, 138.95, 138.57, 138.17, 138.02, 136.14, 132.54, 131.95, 131.82, 129.39, 127.25, 126.41, 126.29, 120.91, 120.74, 117.53, 114.19, 114.11, 65.58, 65.39, 52.51, 52.30, 29.62, 29.46, 29.17, 29.04, 28.91, 25.91, 25.68, 25.49, 22.40, 22.27.

P2$^+$: ^{1}H NMR (600 MHz, DMSO), δ (TMS, ppm): 7.70–6.65(m, 4H), 7.51(d, J = 6.6Hz, 4H), 7.10 (br, 4H), 6.93 (d, J = 7.7 Hz, 4H), 6.70 (d, J = 8.3, 4H), 3.89 (br, 4H), 3.28 (br, 4H), 3.04–3.03 (d, 18H), 1.72–1.65 (m, 8H), 1.46–1.40 (br, 4H), 1.35–1.30(br, 4H). ^{13}C NMR (101MHz, DMSO), δ (TMS, ppm): 157.74, 143.59, 138.61, 137.30, 136.08, 132.57, 131.99, 127.21, 125.22, 114.19, 67.56, 65.63, 52.58, 28.91, 25.93, 25.53, 22.42, 17.61.

P3$^+$: ^{1}H NMR (600 MHz, DMSO), δ (TMS, ppm): 7.96–7.81(m, 6H), 7.34–6.89(m, 8H), 6.76–6.68(m, 4H), 3.93–3.87(m, 4H), 3.33–3.25 (m, 4H), 3.06-3.04(d, 18H), 1.74–1.64 (m, 8H), 1.47–1.40 (m, 4H), 1.38–1.30 (br, 4H). ^{13}C NMR (126 MHz, DMSO), δ (TMS, ppm): 157.84, 155.61, 153.74, 144.43, 143.62, 141.51, 136.01, 133.43, 132.60, 132.53, 131.90, 131.57, 131.38, 129.02, 128.60, 128.27, 119.94, 114.26, 67.61, 65.64, 52.58, 28.91, 25.93, 25.54, 22.42.

Cell Culture: The HeLa cells were cultured in MEM containing 10% FBS, 100 U mL^{-1} penicillin, and 100 μg mL^{-1} streptomycin in a 5% CO_2 humidity incubator at 37 °C.

Cell Imaging: HeLa cells were grown in a 35 mm Petri dish with a coverslip at 37 °C. The living cells were stained with the three polyelectrolytes (20 μg mL^{-1}) for 30 min. The cells were washed for three times with phosphate-buffered saline (PBS) buffer and imaged under CLSM. P1$^+$ and P2$^+$ were excited at 405 nm and the fluorescence was collected at 450–550 nm. P3$^+$ was excited at 405 nm and the fluorescence was collected at 550–650 nm. The cells were further imaged by FLIM. For all polyelectrolytes, the excitation wavelength is 400nm. The emission were collected through a 500±15 nm band pass filter for P1$^+$ and P2$^+$, and through a 600±15 nm band pass filter for P3$^+$.

Cell Viability Evaluated by MTT Assay: Viability of the cells was assayed by using cell proliferation Kit I with the absorbance of 595 nm being detected using a Perkin-Elmer Victor plate reader. Five thousand cells were seeded per well in a 96-well plate. After overnight culture, various concentrations of CPEs were added into the 96-well plate. After 2 h treatment, 10 μL of 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide (MTT) solution (5 mg/mL in phosphate buffer solution) was added into the each well. After 4 h incubation at 37 °C, 100 μL of solubilization solution containing 10% SDS and 0.01 M HCl was added to dissolve the purple crystals. After 12 h incubation, the optical density readings at 595 nm were taken using a plate reader. Each of the experiments was performed at least 3 times.

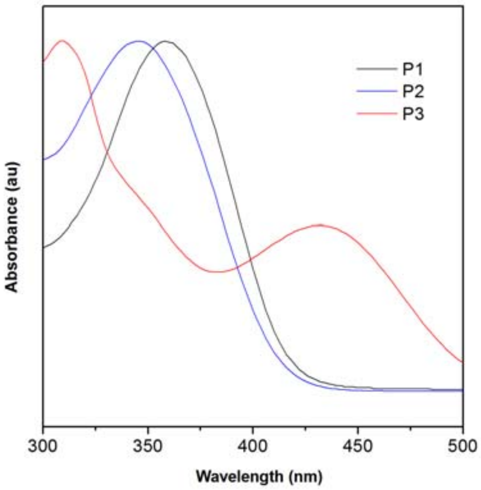


Fig. S1. Absorption spectra of polymers in chloroform solution.

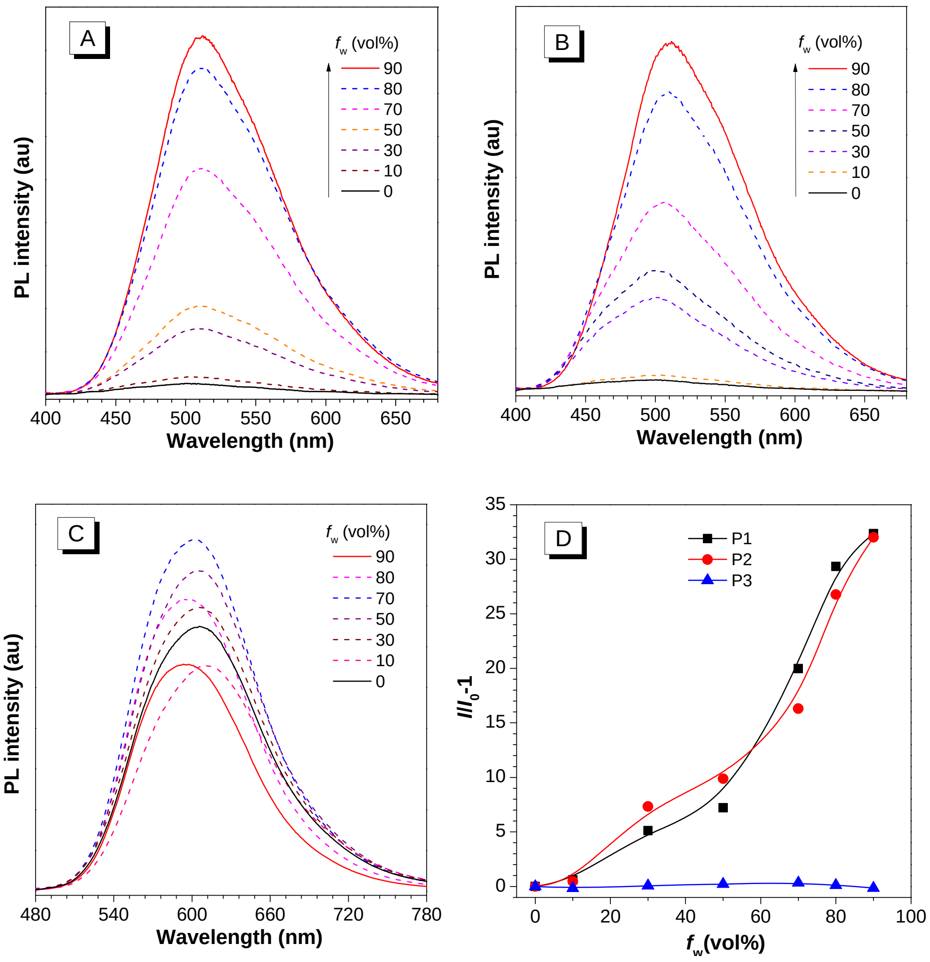


Fig. S2 Fluorescence emission spectra of neutral polymers in THF solution and THF-water mixture (A. P1, B. P2, C. P3). Plot of relative PL intensity ($I/I_0 - 1$) versus the water fraction in the mixture (D). Solution concentration: 10 mM; excitation wavelength: 350 nm (P1, P2) and 410 nm (P3).

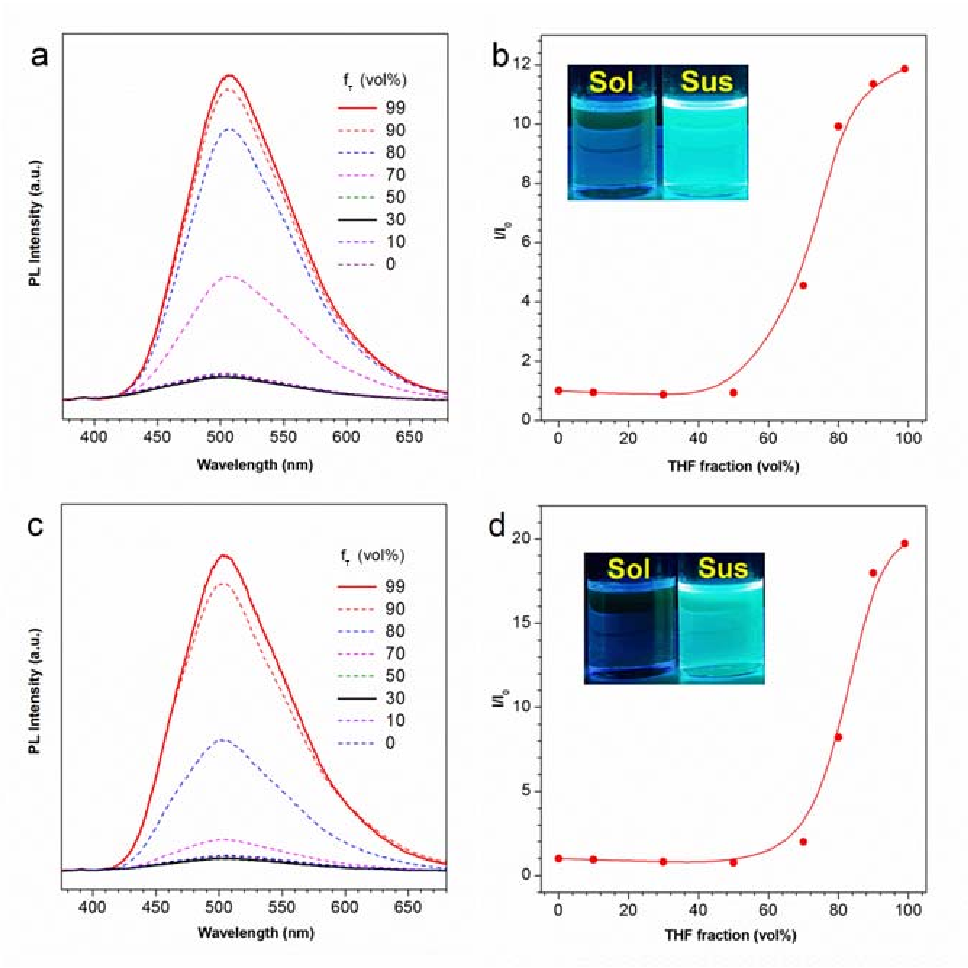


Fig. S3 Fluorescence emission spectra of CPEs in DMSO–THF mixtures (a. P1$^+$, c. P2$^+$). Plot of relative PL intensity (I/I_0) versus the composition of DMSO–THF mixtures of CPEs (b. P1$^+$, d. P2$^+$), Inset: photographs of polyelectrolytes in DMSO solution (Sol) and DMSO–THF mixture (1:9 v/v) (Sus) taken under illumination of a UV lamp. Concentration: 10 μM; excitation wavelength: 350 nm.

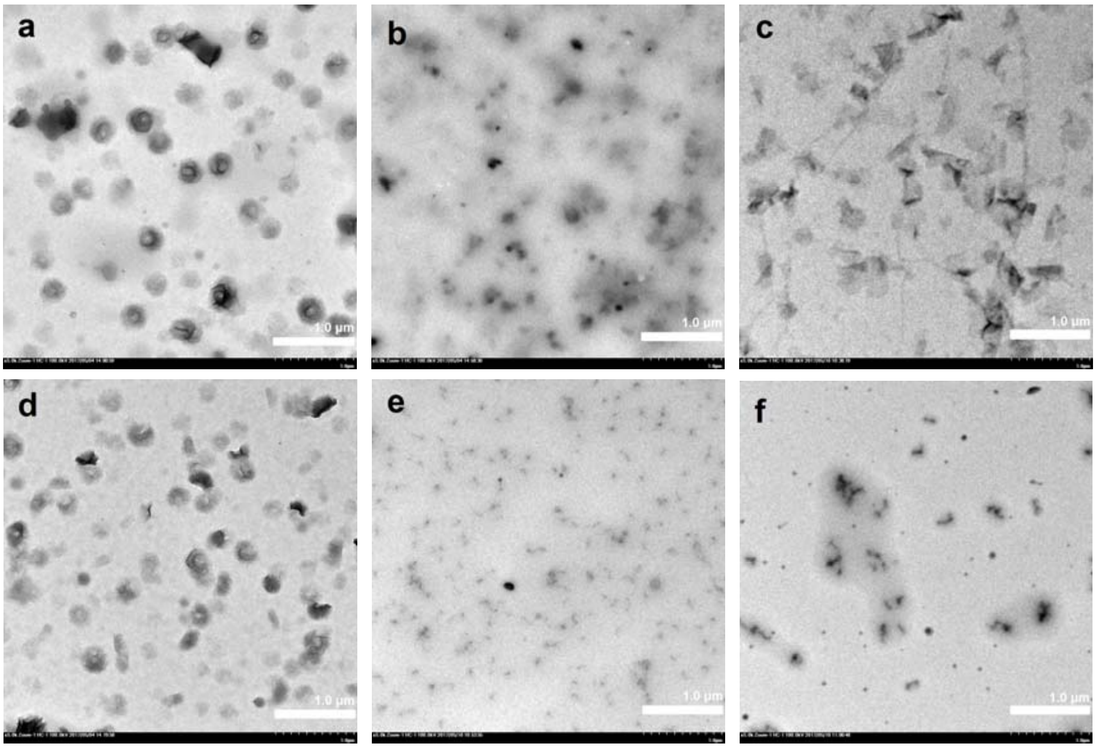


Fig. S4 TEM images of all the polymers (a. P1, b. P2, c. P3, d. P1$^+$, e. P2$^+$, f. P3$^+$). P1-P3 were measured in THF–H_2O mixture (1:9 v/v), P1$^+$-P3$^+$ were measured in DMSO–THF mixture (1:99 v/v).

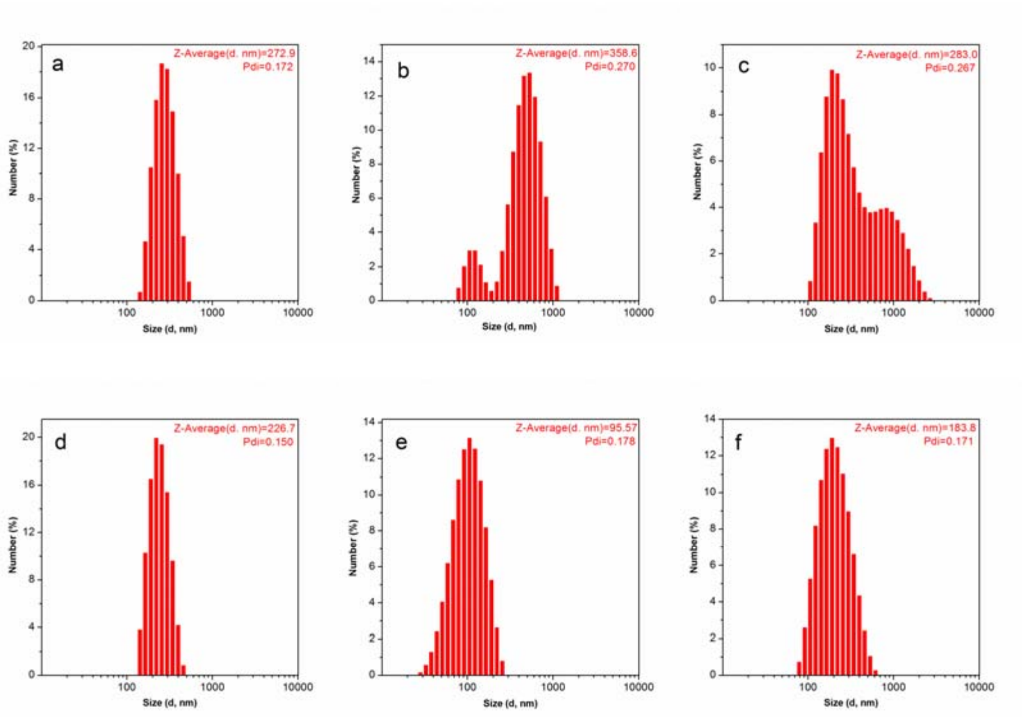


Fig. S5 Particle size distributions of all the polymers (a. P1, b. P2, c. P3, d. P1$^+$, e. P2$^+$, f.

$P3^+$). P1-P3 were measured in THF–H_2O mixture (1:9 v/v), $P1^+$-$P3^+$ were measured in DMSO–THF mixture (1:99 v/v).

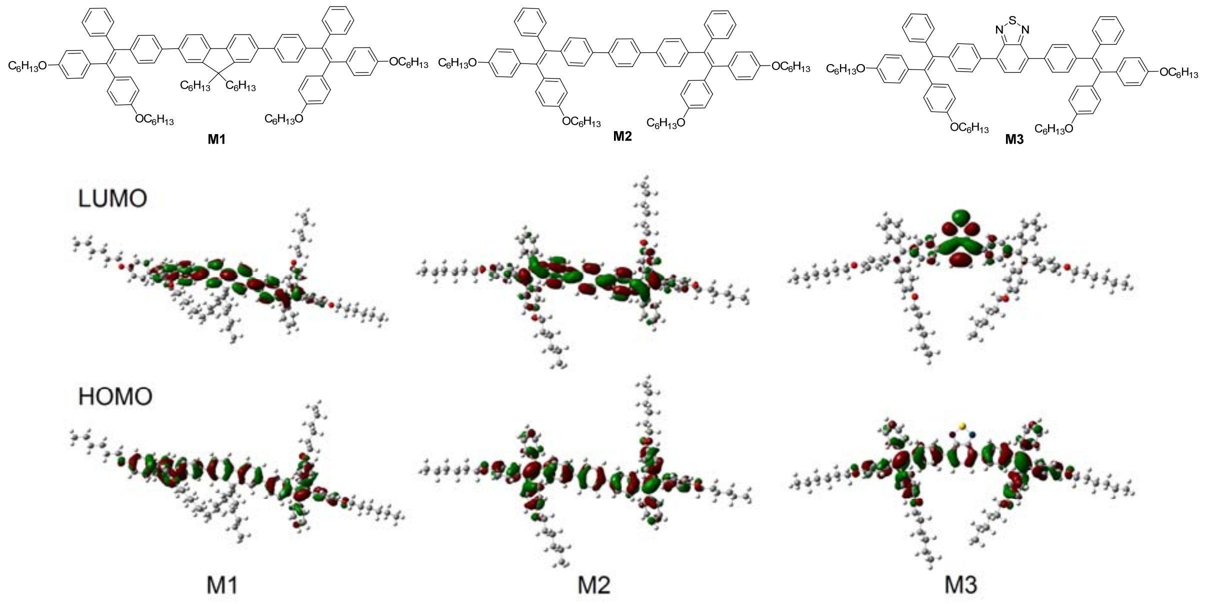


Fig. S6 Optimized conformations and calculated molecular orbital amplitude plots of HOMOs and LUMOs for the conjugated fragments of the polymers.

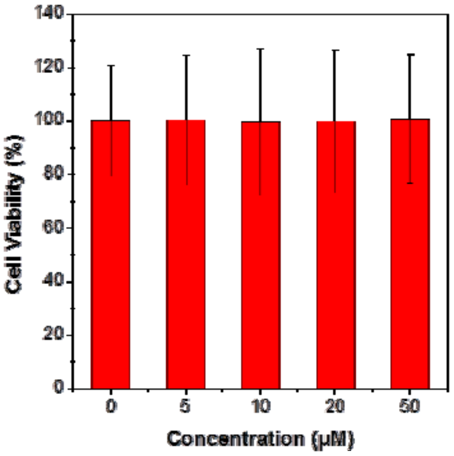


Fig. S7 Cytotoxicity of $P1^+$ on HeLa cells determined by MTT assay.

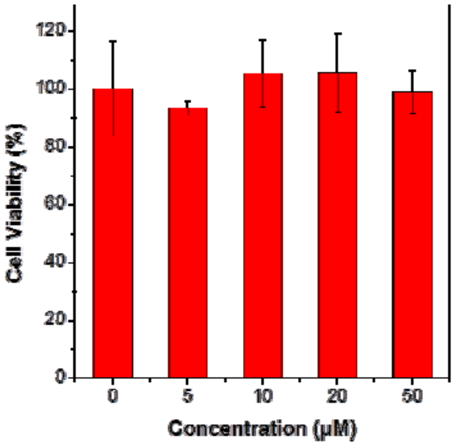


Fig. S8 Cytotoxicity of P2$^+$ on HeLa cells determined by MTT assay.

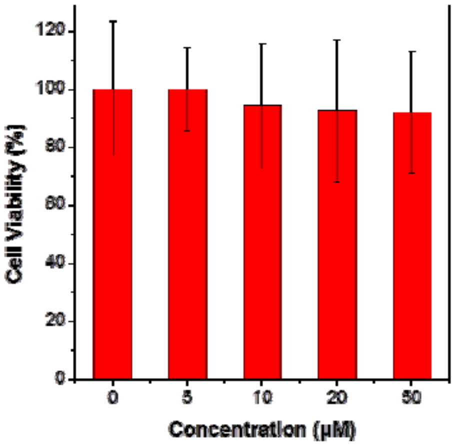


Fig. S9 Cytotoxicity of P3$^+$ on HeLa cells determined by MTT assay.

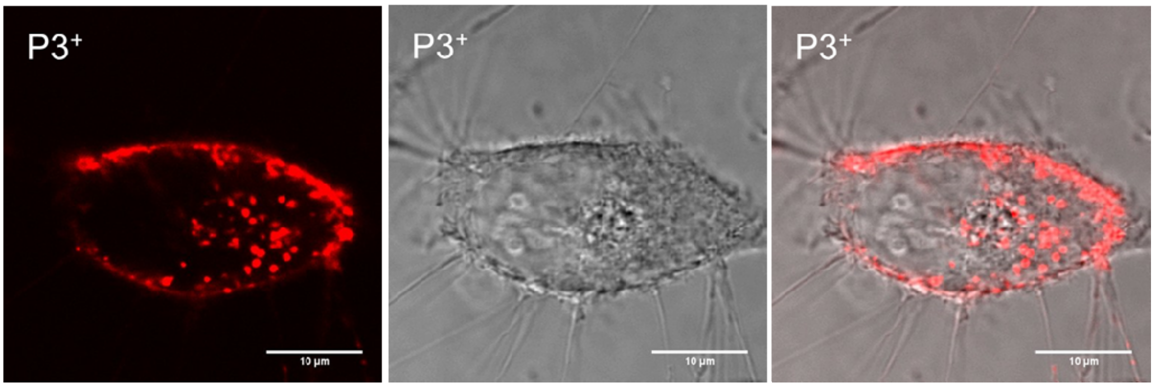
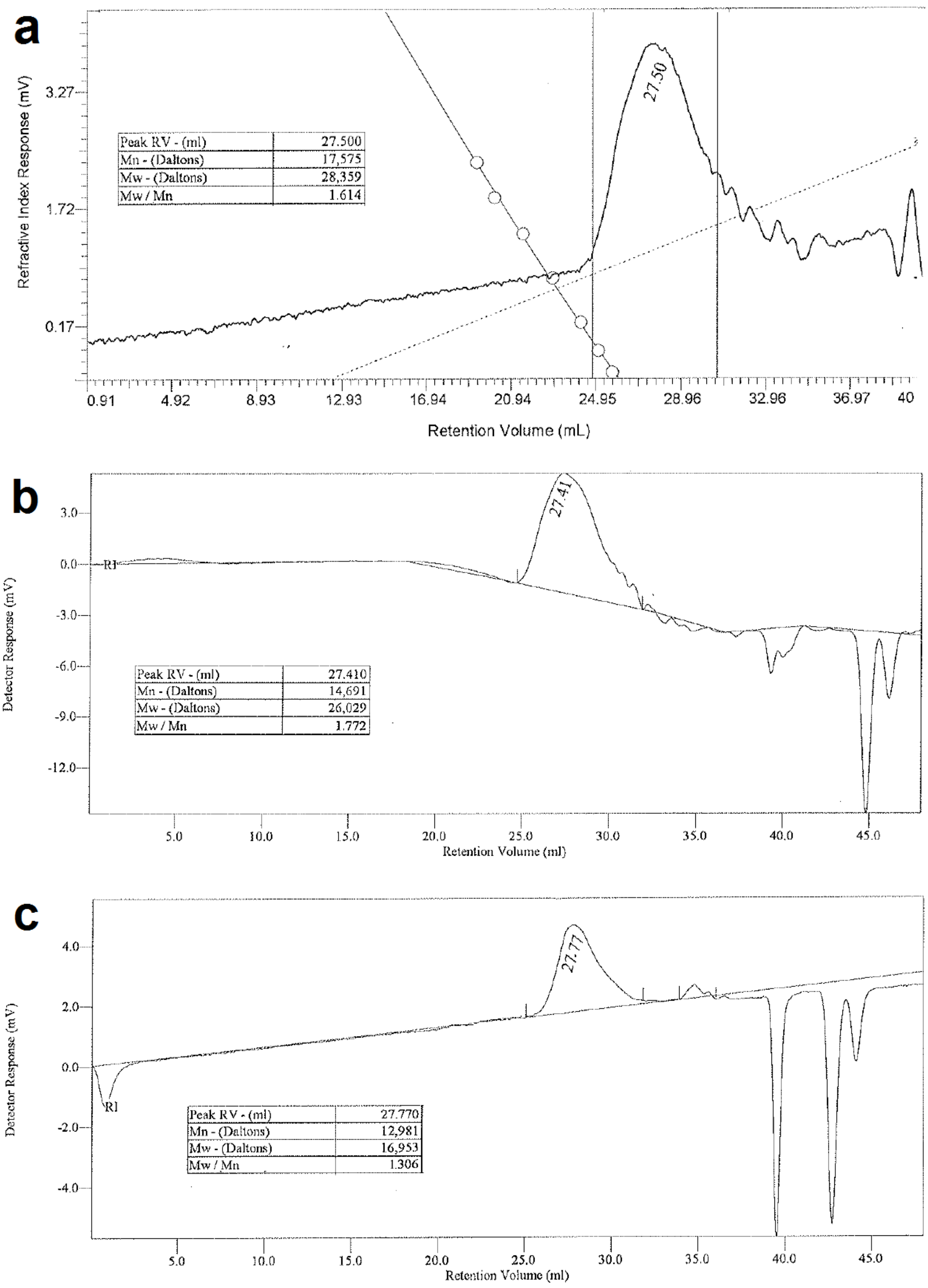


Fig. S10 Fluorescent images of HeLa cells stained with P3$^+$ (5 μg/mL) for 0.5 h at 37 °C (Left images are fluorescence images, middle images are bright field images, and right images are the overlay of fluorescence images and bright field images.



a

Peak RV - (ml)	27.500
Mn - (Daltons)	17,575
Mw - (Daltons)	28,359
Mw / Mn	1.614

b

Peak RV - (ml)	27.410
Mn - (Daltons)	14,691
Mw - (Daltons)	26,029
Mw / Mn	1.772

c

Peak RV - (ml)	27.770
Mn - (Daltons)	12,981
Mw - (Daltons)	16,953
Mw / Mn	1.306

Fig. S11 GPC figures of all neutral polymers (a. P1, b. P2, c. P3).

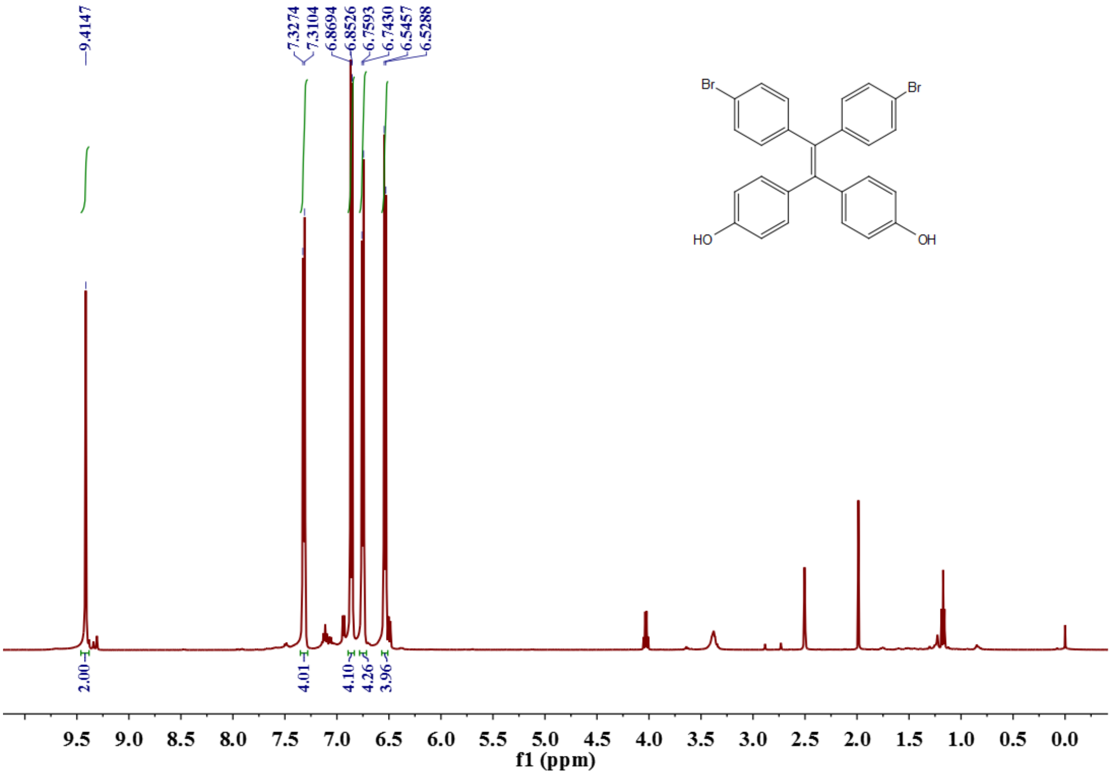


Fig. S12 ^{1}H NMR spectrum of compound **3.**

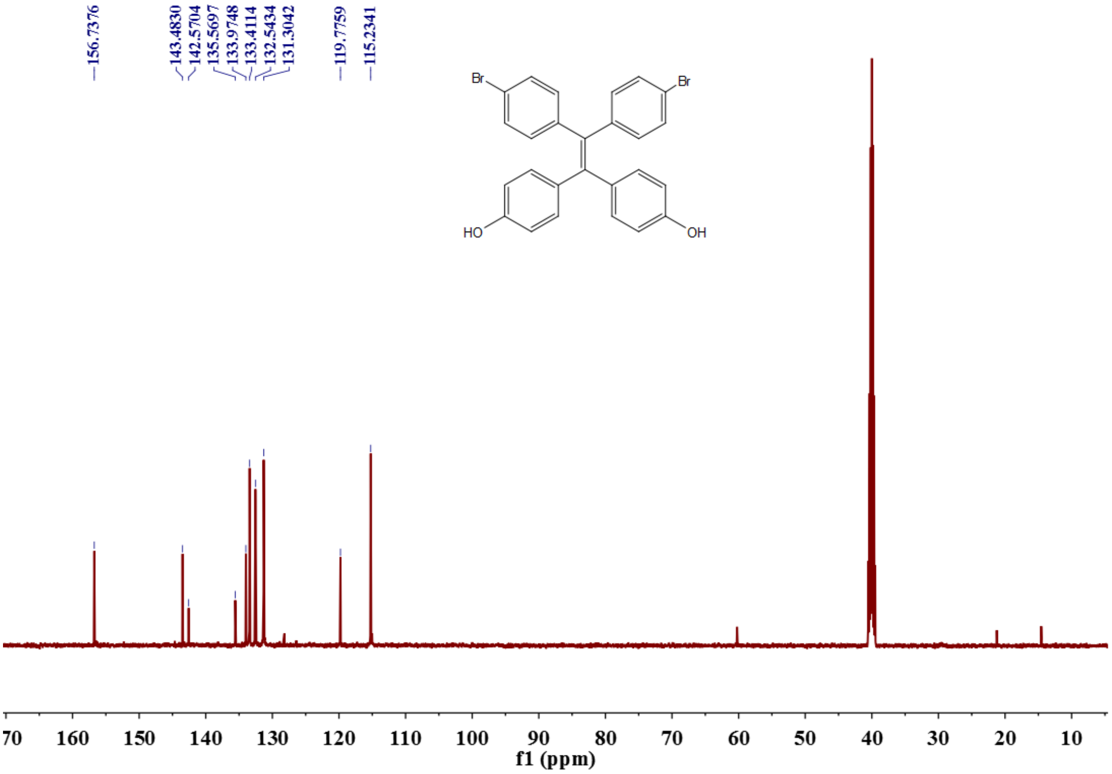


Fig. S13 ^{13}C NMR spectrum of compound **3.**

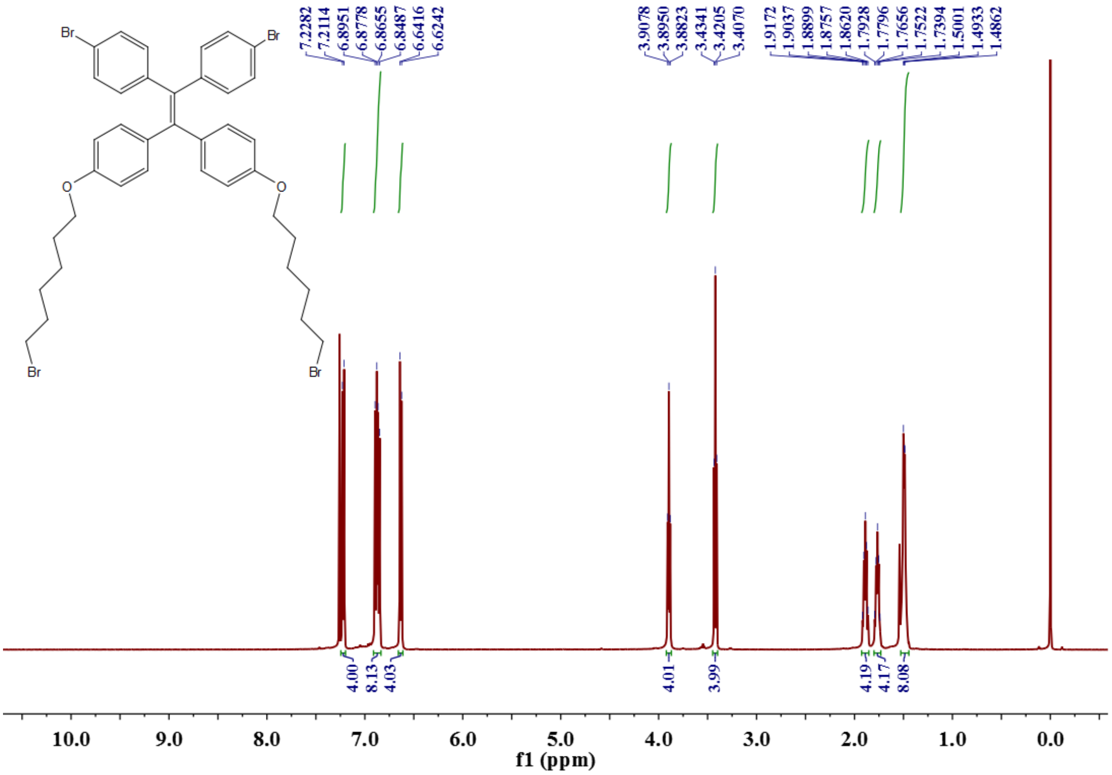


Fig. S14 ^{1}H NMR spectrum of compound **4.**

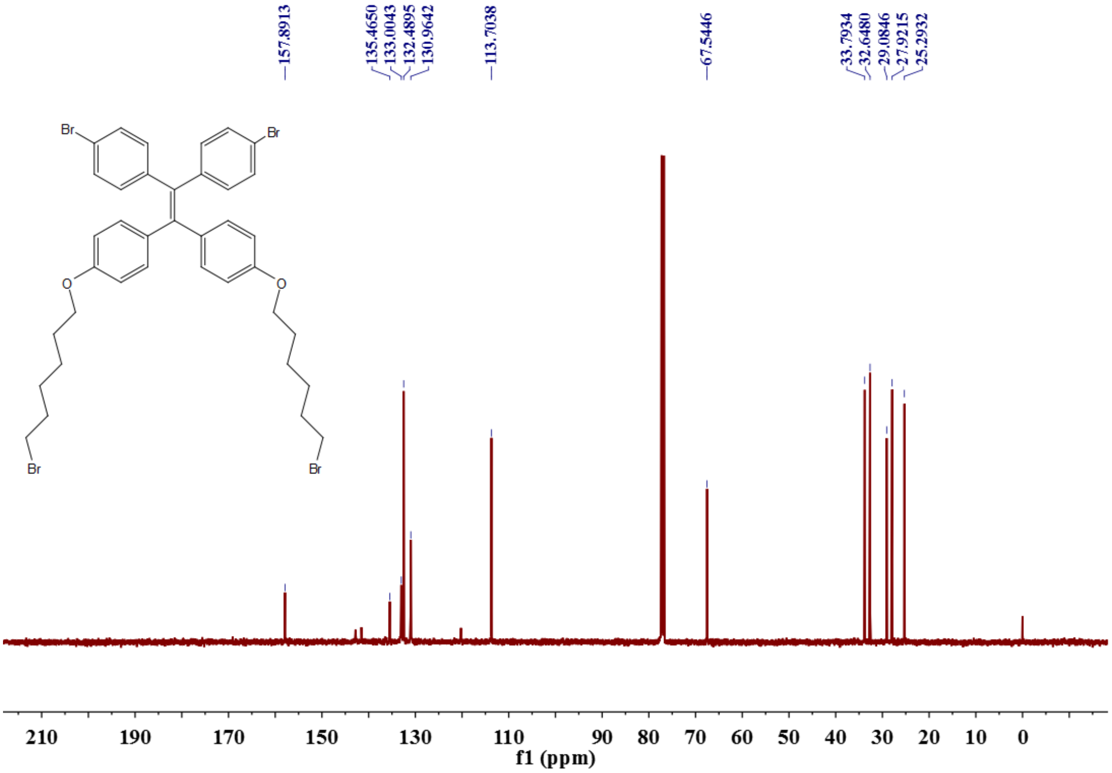


Fig. S15 ^{13}C NMR spectrum of compound **4.**

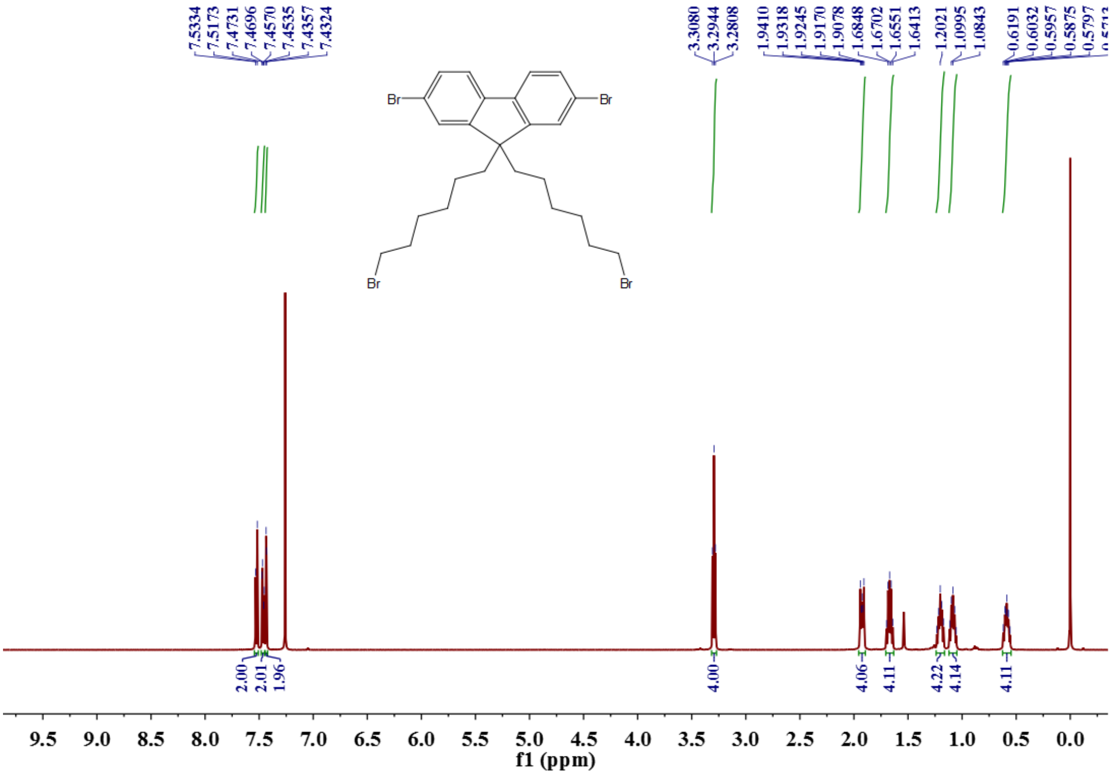


Fig. 16 ^{1}H NMR spectrum of compound **6.**

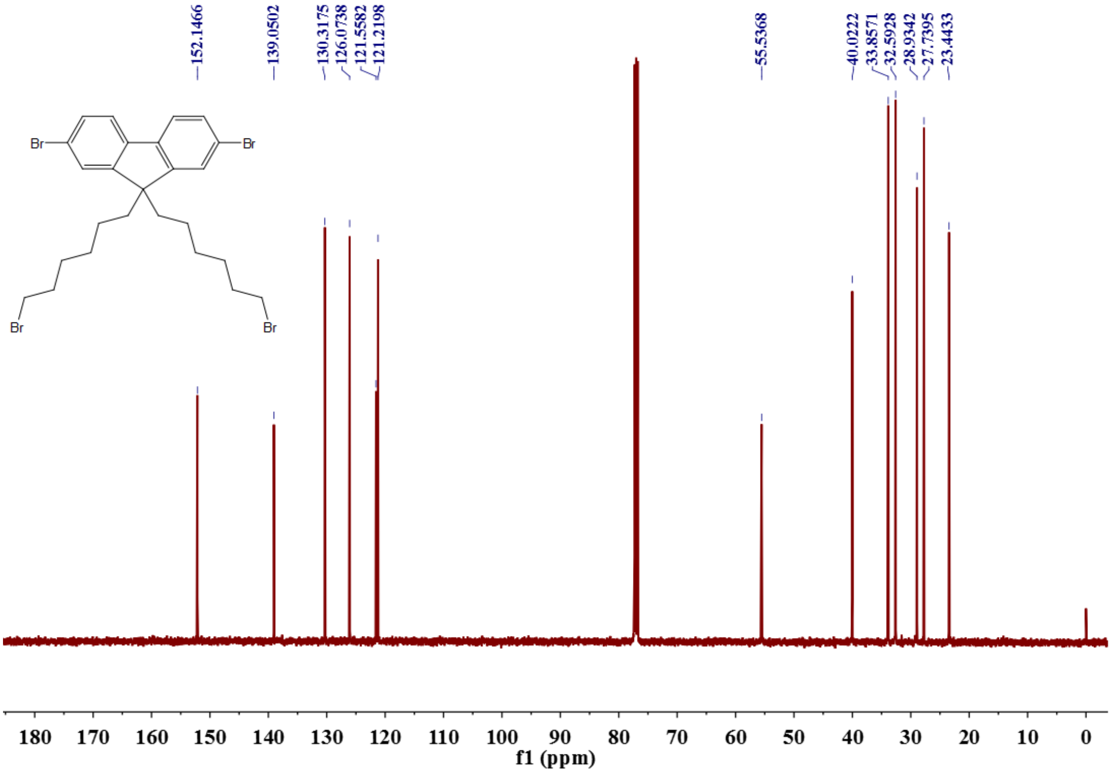


Fig. S17 ^{13}C NMR spectrum of compound **6.**

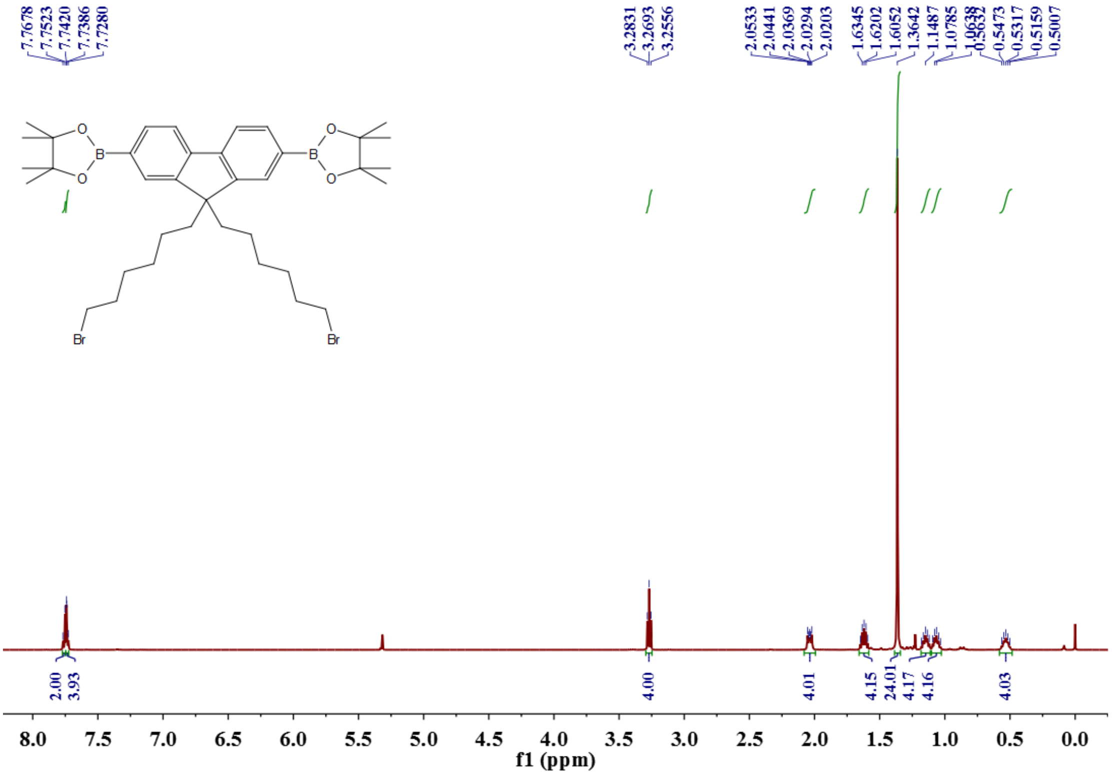


Fig. S18 ^{1}H NMR spectrum of compound **7.**

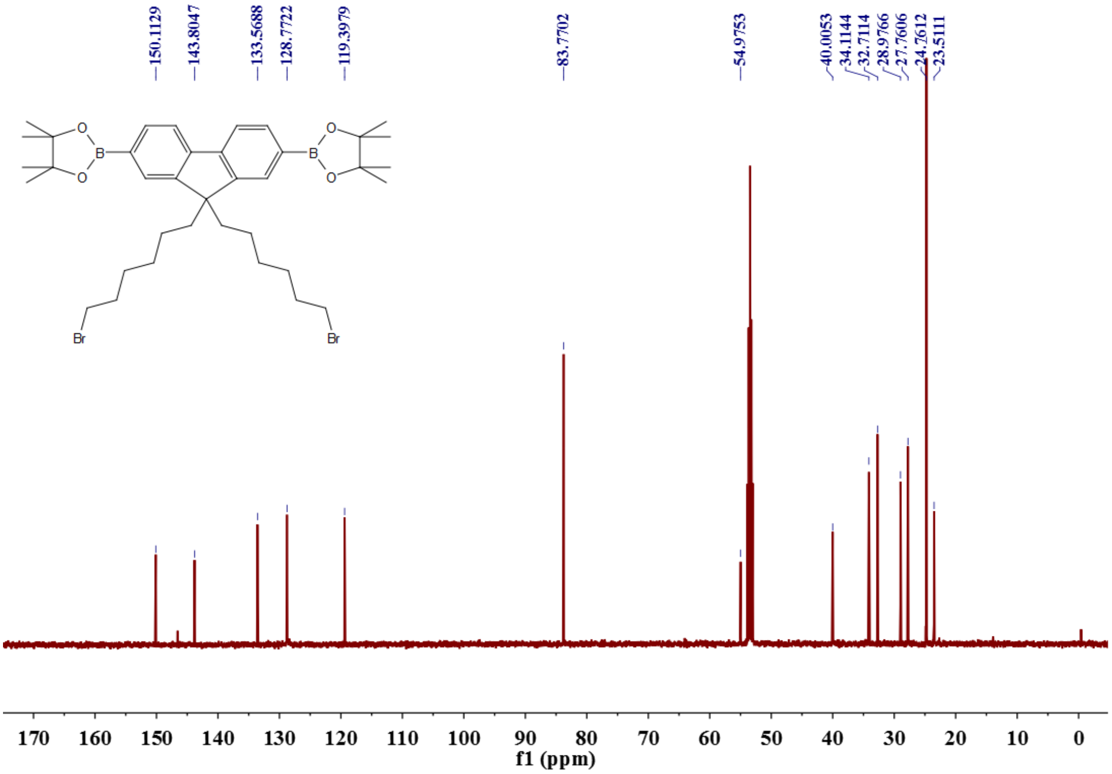


Fig. S19 ^{13}C NMR spectrum of compound **7.**

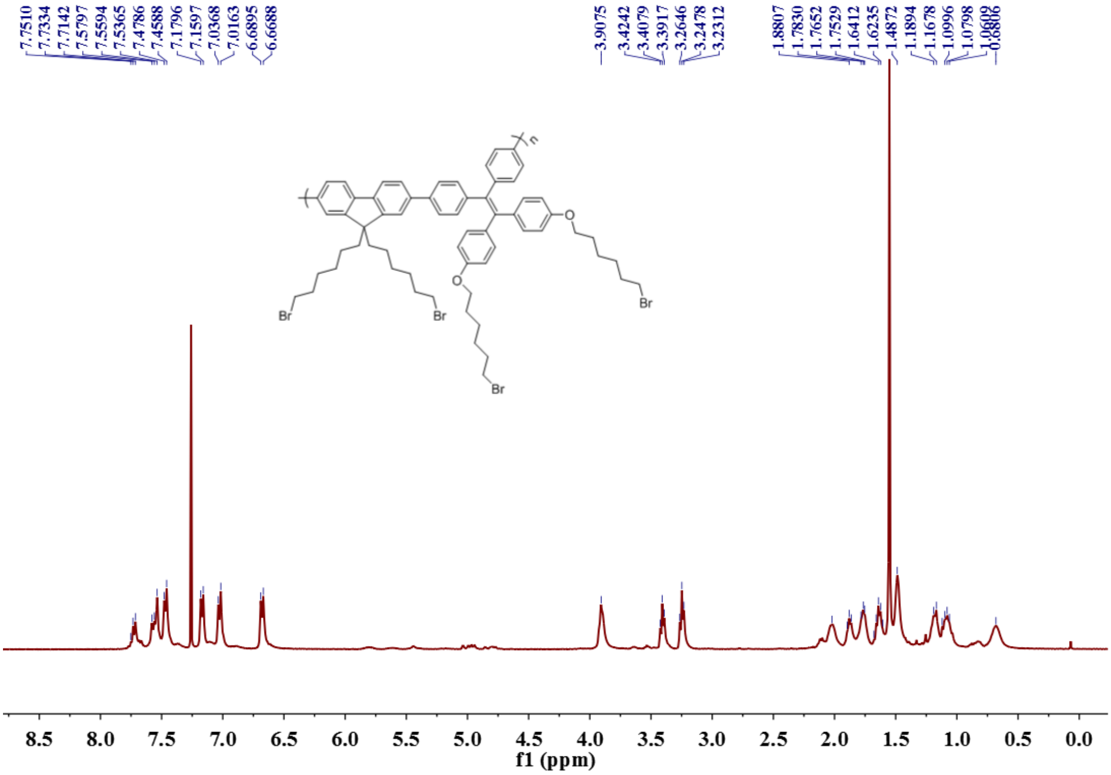


Fig. S20 ^{1}H NMR spectrum of copolymer **P1.**

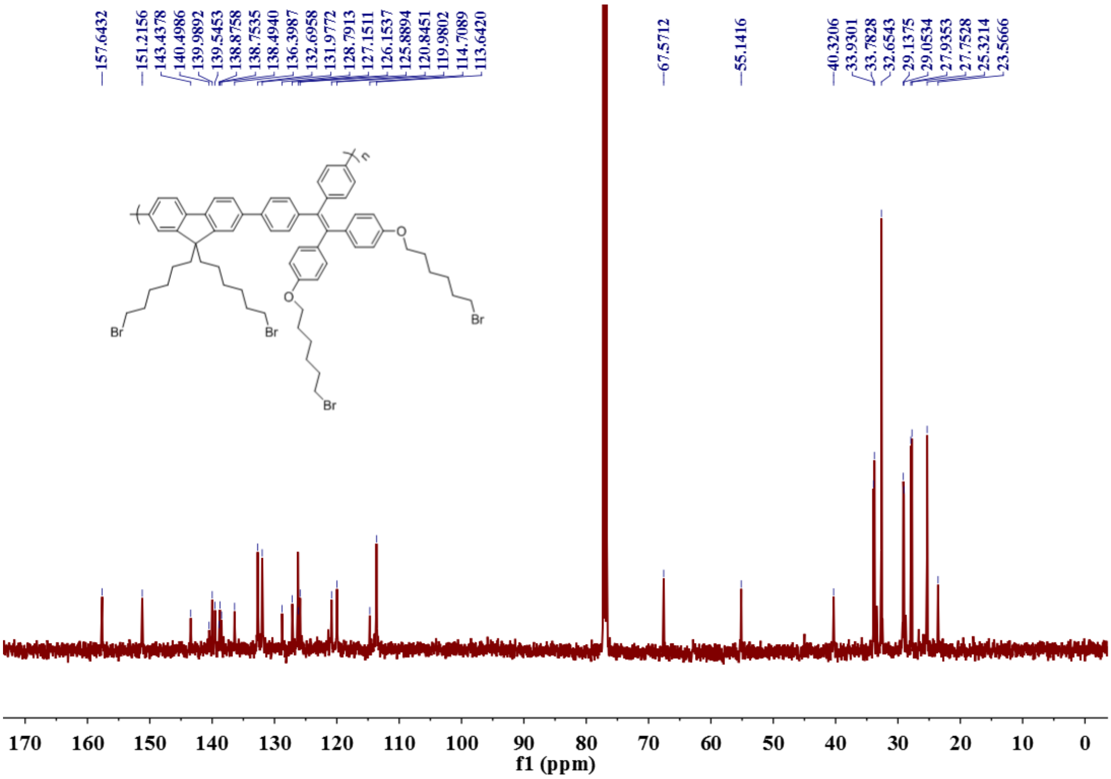


Fig. S21 ^{13}C NMR spectrum of copolymer **P1.**

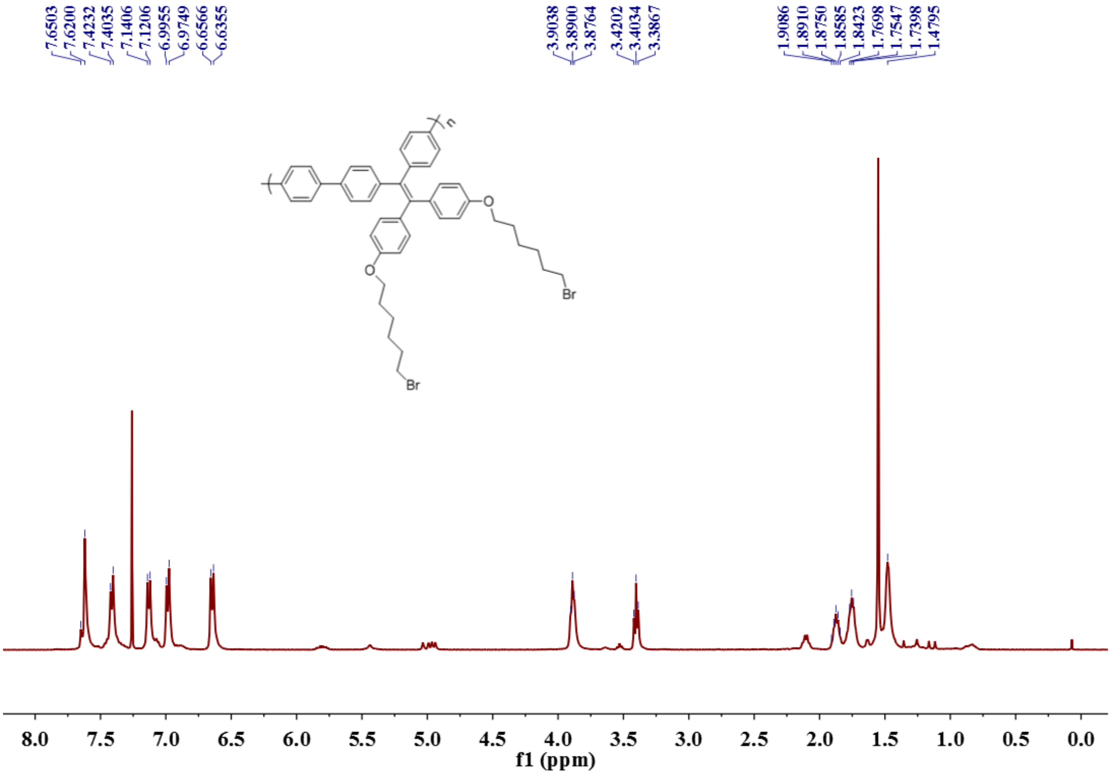


Fig. S22 ^{1}H NMR spectrum of copolymer **P2.**

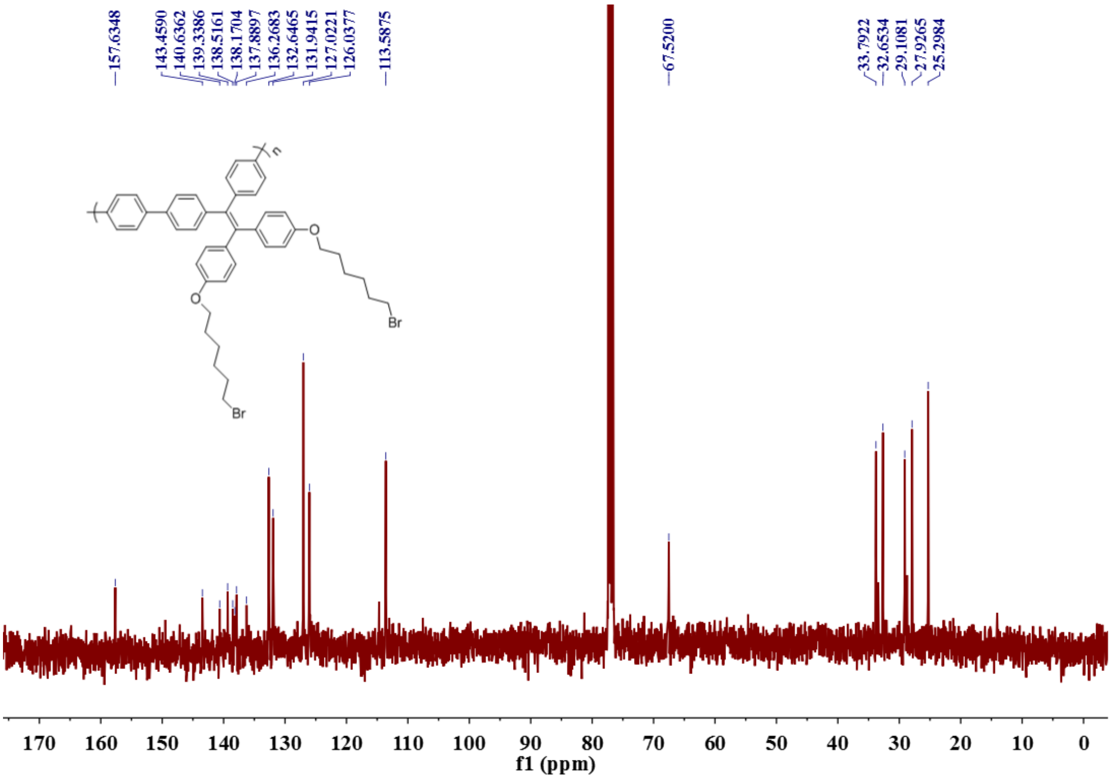


Fig. S23 ^{13}C NMR spectrum of copolymer **P2.**

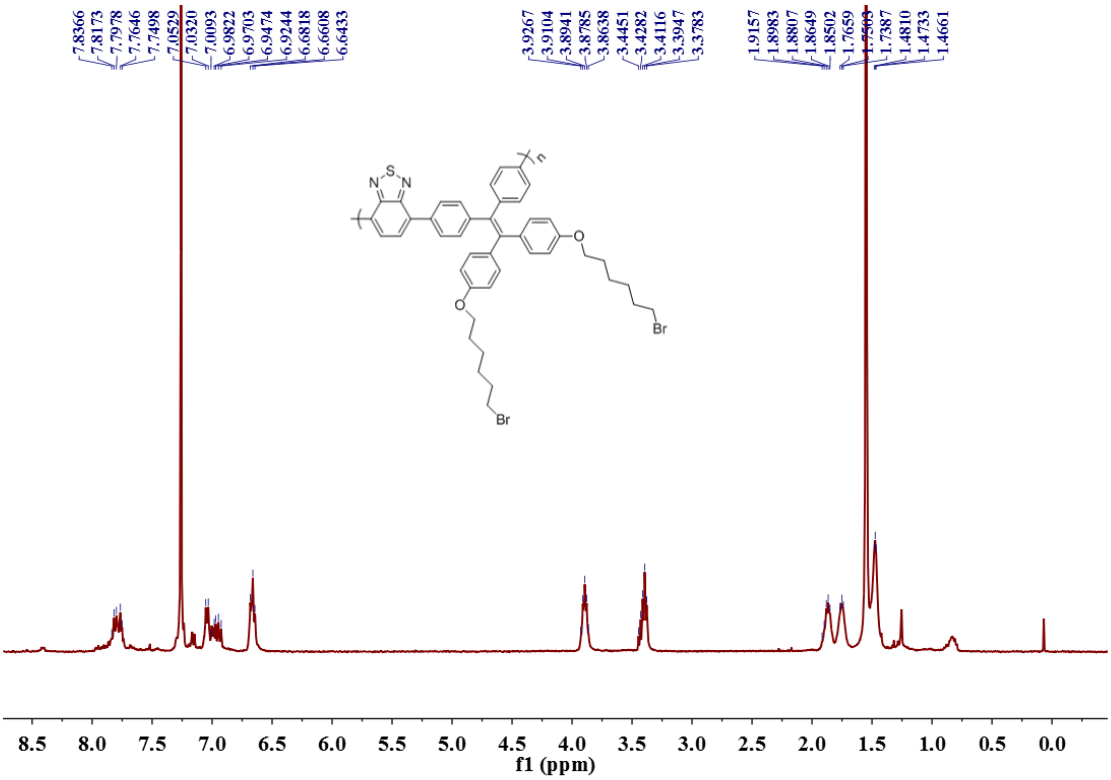


Fig. S24 ^{1}H NMR spectrum of copolymer **P3.**

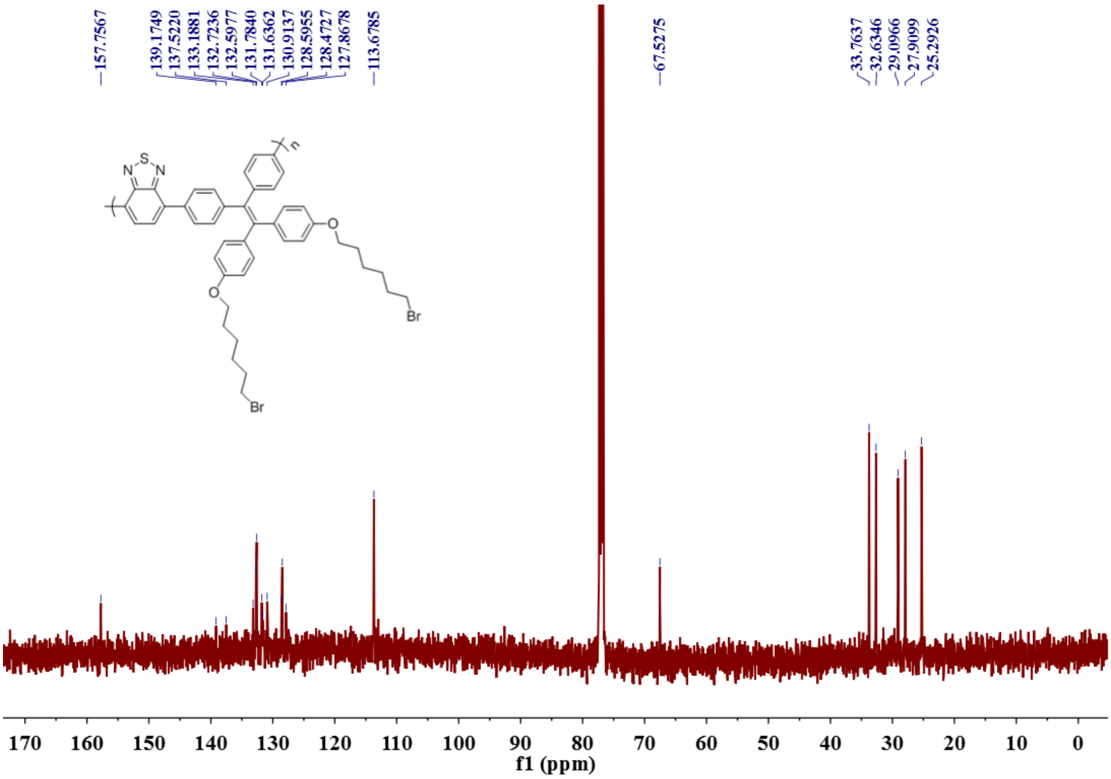


Fig. S25 ^{13}C NMR spectrum of copolymer **P3.**

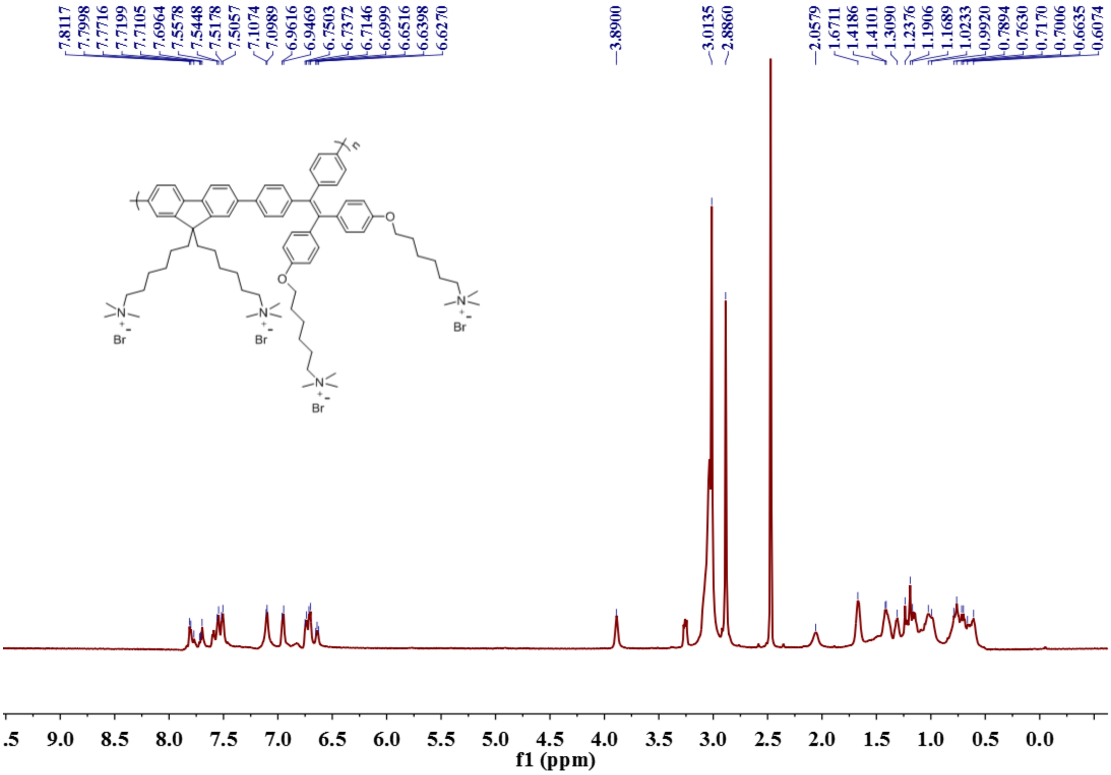


Fig. S26 ^{1}H NMR spectrum of copolymer **P1$^+$.**

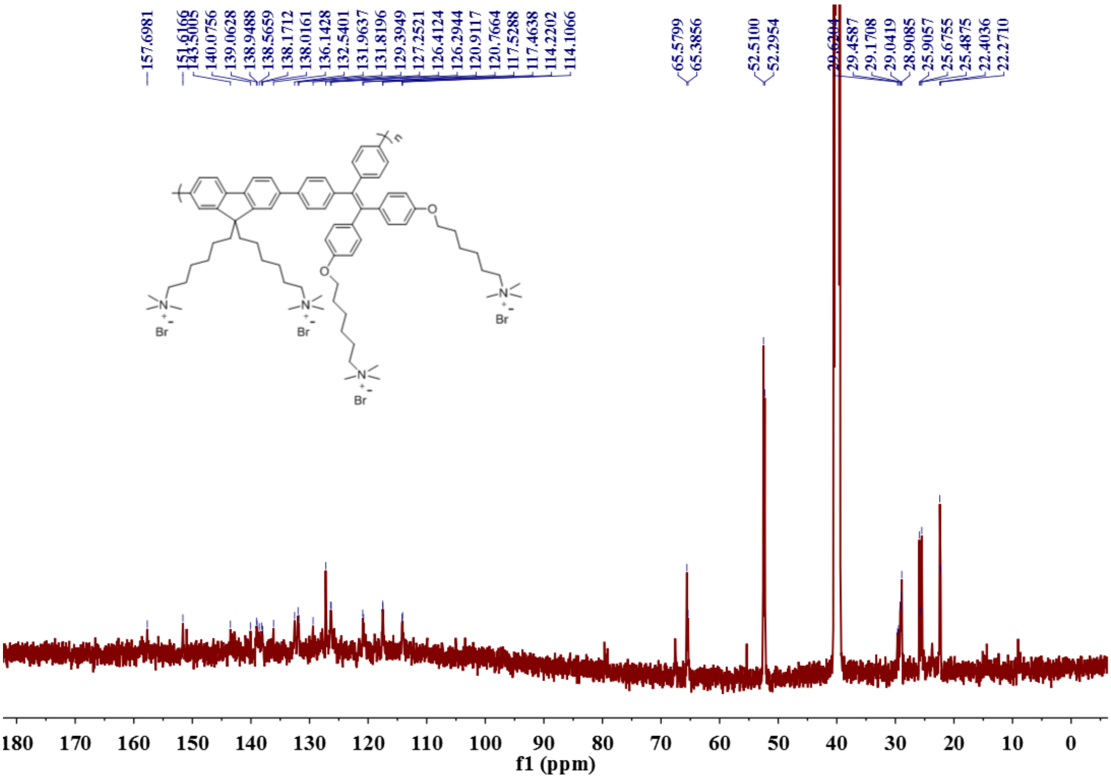


Fig. S27 ^{13}C NMR spectrum of copolymer **P1$^+$.**

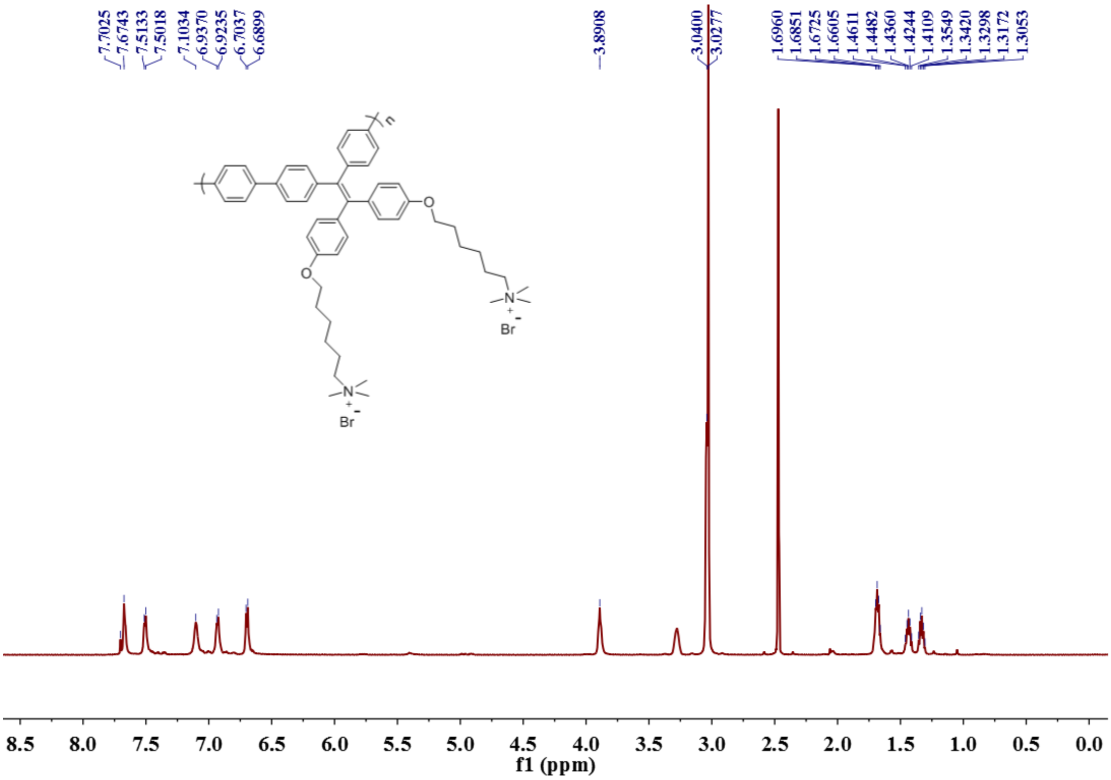


Fig. S28 ^{1}H NMR spectrum of copolymer **P2^{+}.**

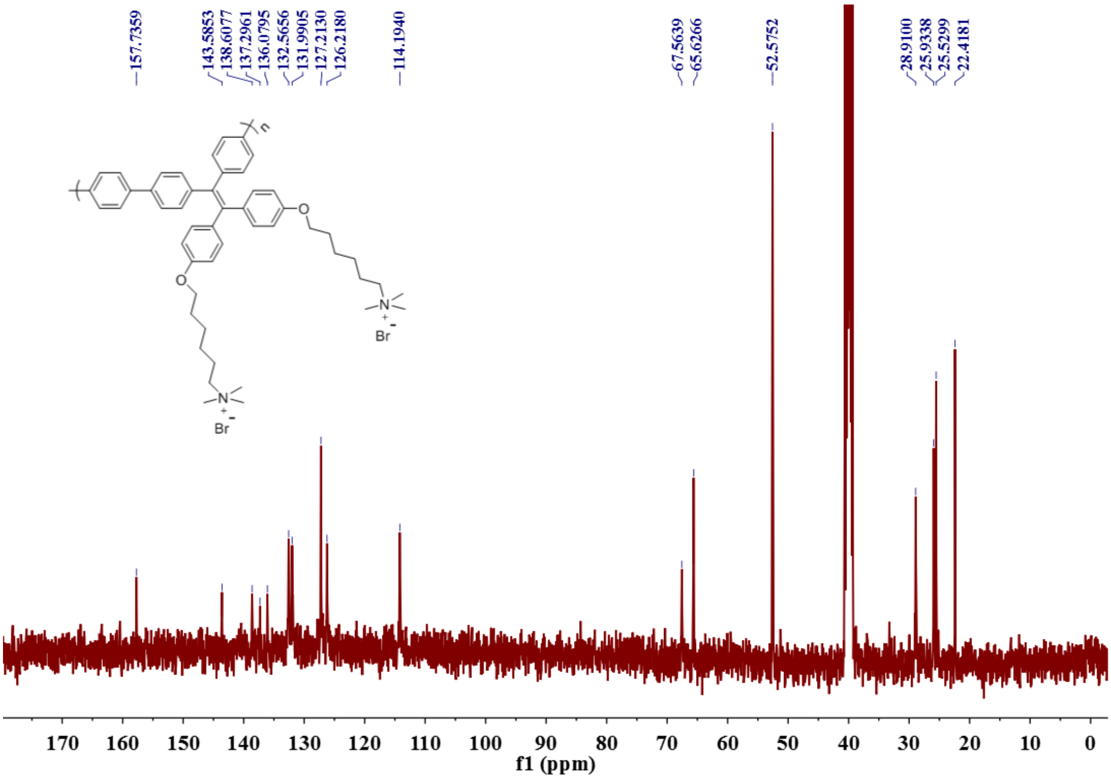


Fig. S29 ^{13}C NMR spectrum of copolymer **P2^{+}.**

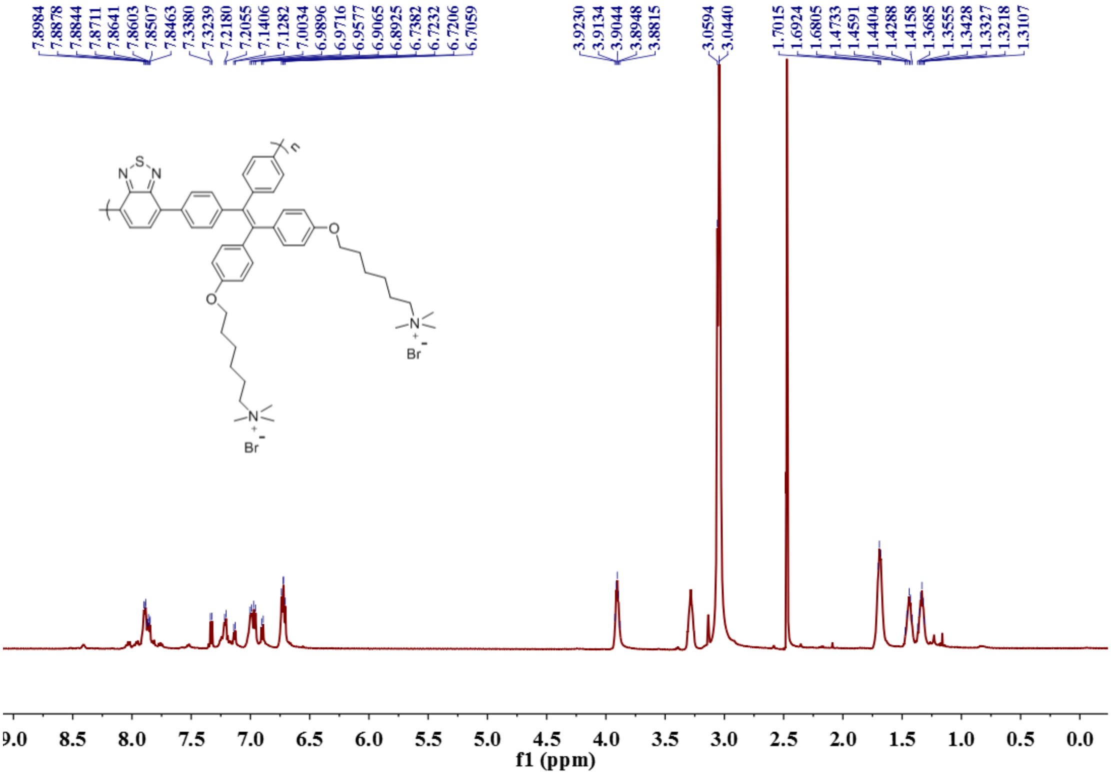


Fig. S30 ^{1}H NMR spectrum of copolymer **P3$^+$.**

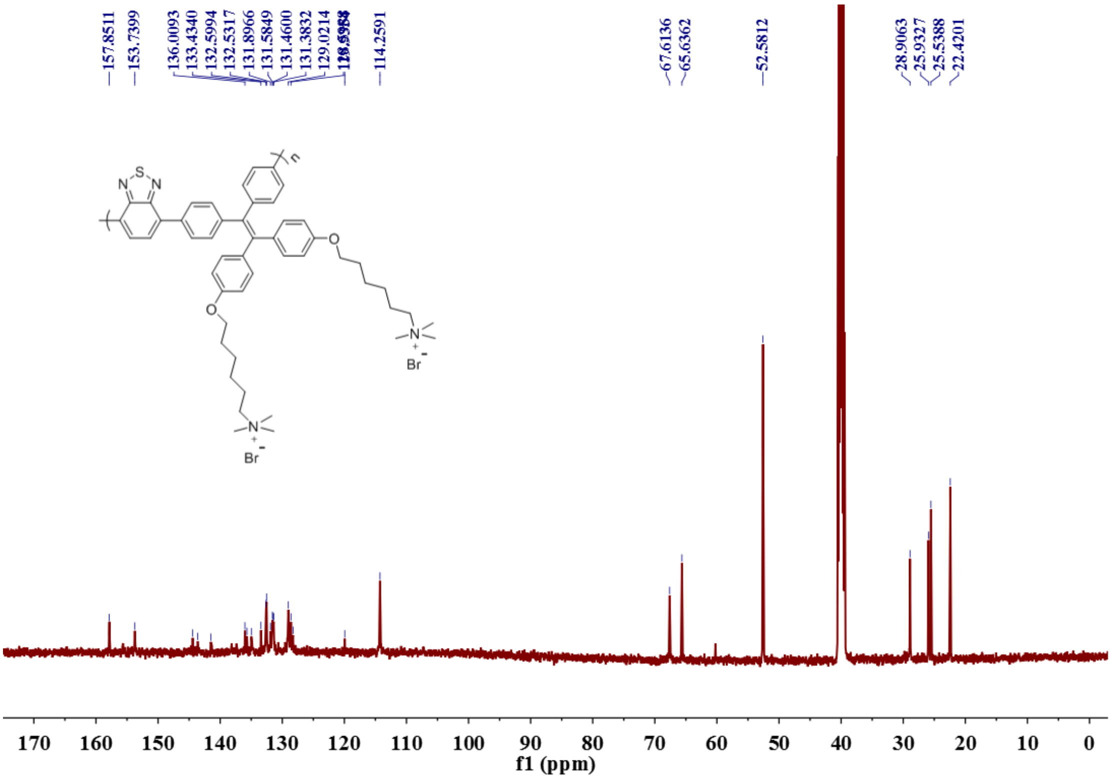


Fig. S31 ^{13}C NMR spectrum of copolymer **P3$^+$.**

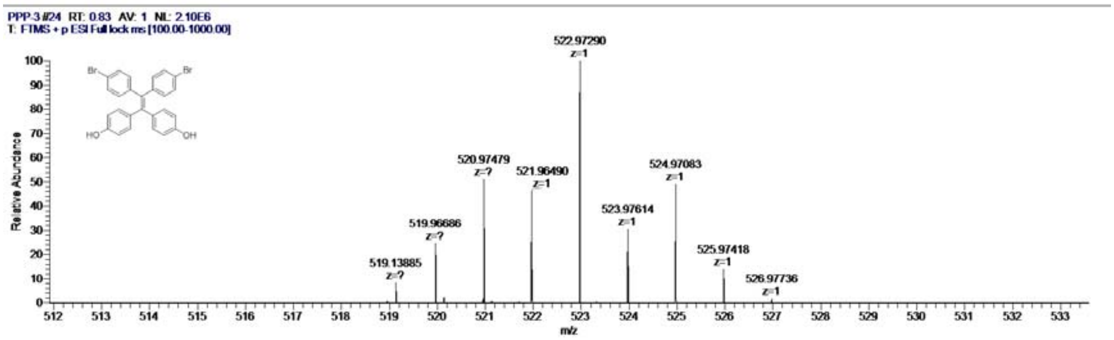


Fig. S32 Mass spectrum of compound **3.**

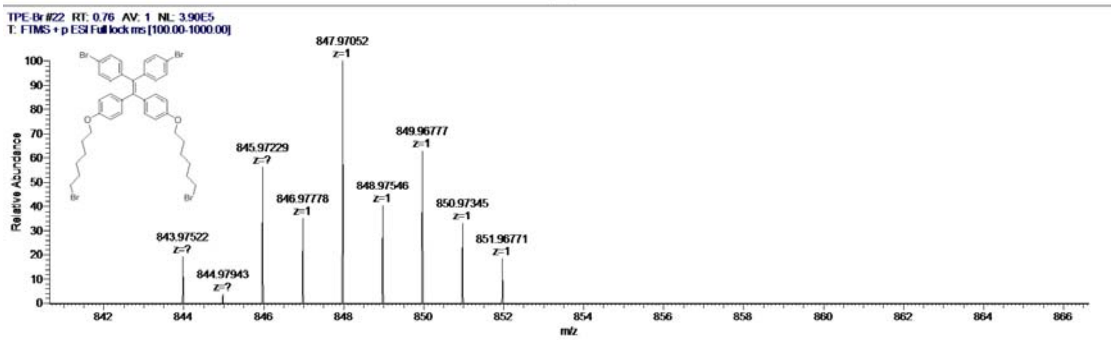


Fig. S33 Mass spectrum of compound **4.**

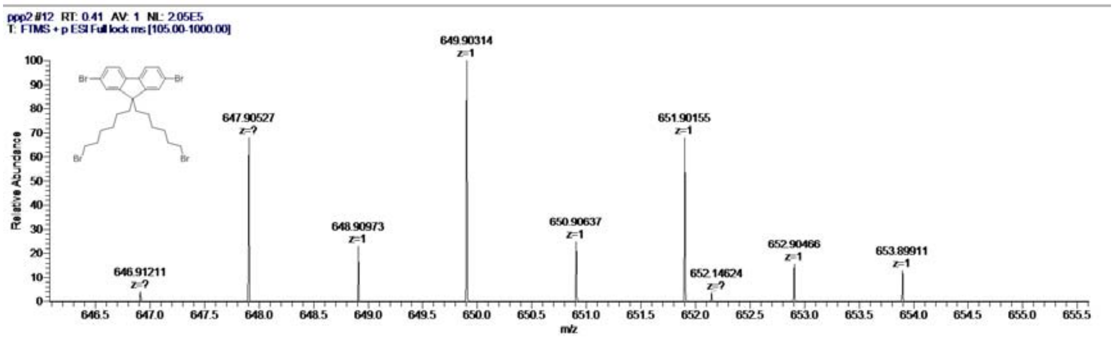


Fig. S34 Mass spectrum of compound **6.**

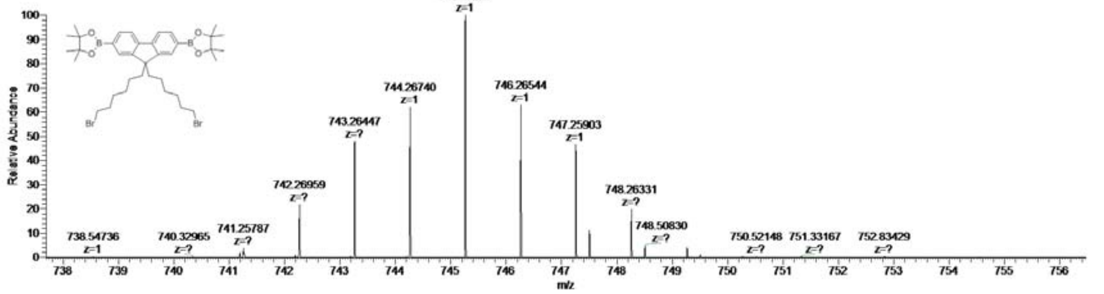


Fig. S35 Mass spectrum of compound **7.**

Reference

1 J. L. Hickey, J. L. James, C. A. Henderson, K. A. Price, A. I. Mot, G. Buncic, P. J. Crouch, J. M. White, A. R. White, T. A. Smith and P. S. Donnelly, *Inorg. Chem.*, 2015, **54**, 9556.