Supporting Information

Thiophene-fused 1,10-phenanthroline toward a far-red emitting conjugated polymer and its polymer dots: synthesis, properties and subcellar imaging

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Materials and Methods.

General. Unless noted otherwise, all chemicals were purchased from Aldrich, Acros or Adama and used without further purification. The amphiphilic functional polymer poly(styrene-co-maleic anhydride) (PSMA, cumene-terminated, average M_W~1,700, styrene content = 68%) and streptavidin were purchased from Sigma-Aldrich (Shanghai, China). 1-Ethyl-3-(3-

(dimethylamino)propyl)carbodiimide hydrochloride (EDC) was purchased from Thermo Scientific. Dichloromethane (DCM) and tetrahydrofuran (THF) were dried by standard methods before using. All reactions were performed under an atmosphere of nitrogen and monitored by TLC. Column chromatography was carried out on silica gel (200–300 mesh). The catalyst precursor $Pd(PPh_3)_4$ was prepared according to the literature (*S1*), and stored in a Schlenk tube under nitrogen. 4,7-bis(5-bromothiophen-2-yl)benzo[*c*][1,2,5]thiadiazole (**M1**) , 2,9-dibromo-4,7-di-tert-butyldithieno[3,2-c:2',3'-i][1,10]phenanthroline (**M2**) and 2,2'-(9,9-dioctyl-9H-fluorene-2,7-diyl)bis(4,4,5,5-tetramethyl-1,3,2-dioxaborolane) (**M3**), 2,2'-(9,9-bis(6-bromohexyl) -9H-fluorene-2,7-diyl)bis(4,4,5,5-tetramethyl-1,3,2-dioxaborolane) (**M3'**) were prepared according to the literature procedures (*S2*, *S3*, *S4*).

Measurements. ^{1}H NMR (400 MHz) spectra and ^{13}C NMR (100 MHz) spectra were recorded on a 400 MHz Bruker AV 400 spectrometer. Tetramethylsilane was used as an internal standard in $CDCl_3$ and DMSO-d_6. UV-vis absorption spectra were obtained on a PerkinElmer Lambda 750 UV/VIS/NIR spectrometer. Fluorescence emission spectra were recorded on a Hitachi F-7000 fluorescence spectrophotometer. Fluorescence quantum yields were measured using a Hamamatsu photonic multichannel analyzer C10027 equipped with CCD and integrating sphere. In quantum yield calibration, we used the solvent as the reference. We also used dyes in different solvents for calibration and found the measured QY values were very close to the reported values for the dyes. Number-average (M_n) and weight-average (M_w) molar masses were determined by an Agilent Technologies 1200 series gel permeation chromatography (GPC) instrument running in chlorobenzene at 80 ^oC, using two PL mixed B columns in series, and calibrated against narrow polydispersity polystyrene standards. Thermal gravimetric analysis (TGA) and differential scanning calorimetry (DSC) measurements were performed on TA Q50 and TA Q20, respectively,

under a nitrogen atmosphere at a heating (and cooling) rate of 10 ^{o}C/min to record TGA and DSC curves. The particle size of Pdots in bulk solution was characterized by dynamic light scattering (Malvern Zetasizer NanoS). The surface topography of Pdots was imaged with a Bruker Mutimode 8 AFM in TAPPING mode.

Experimental Details.

Polymer **P1**. A mixture of **M1** (36.6 mg, 80.1 μmol), **M2** (5 mg, 8.9 μmol), **M3** (57.1 mg, 89 μmol), $NaHCO_3$ (175 mg, 2.08 mmol), THF (6 mL) and H_2O (3 mL) was carefully degassed before and after $Pd(PPh_3)_4$ (2 mg, 1.7 μmol) was added. The mixture was heated to reflux and stirred under nitrogen for 72 h. The reaction mixture was extracted with $CHCl_3$ (3 × 30mL), and combined organic layers were dried over anhydrous $MgSO_4$. After the removal of most of the solvent, the residue was precipitated into methanol. The resulted precipitate was collected by filtration and dried under vacuum to give **P1** as a purple-red solid (90 mg, 91%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.14 (s, 2H), 7.97–7.61 (m, 12H), 7.49 (s, 1H), 7.15 (s, 1H), 2.30 (d, *J* = 6.8 Hz, 1H), 2.04 (d, *J* = 7.2 Hz, 5H), 1.86 (s, 2H), 1.76 (d, *J* = 9.5 Hz, 1H), 1.59 (d, *J* = 16.5 Hz, 2H), 1.40 (s, 10H), 1.34–0.97 (m, 51H), 0.77 (t, *J* = 47.9 Hz, 16H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.53, 152.78, 152.57, 152.51, 152.43, 152.30, 152.05, 150.30, 146.82, 143.71, 140.93, 140.68, 138.32, 134.04, 130.90, 130.86, 129.07, 129.03, 127.47, 127.22, 125.44, 125.37, 125.24, 125.17, 124.92, 124.22, 120.79, 120.22, 119.28, 114.97, 83.94, 77.53, 77.21, 76.89, 69.03, 62.29, 55.49, 40.46, 34.25, 31.98, 30.16, 29.90, 29.41, 25.14, 22.78, 14.33, 14.27.

Polymer **P2**. A mixture of **M1** (36.6 mg, 80.1 μmol), **M2** (5 mg, 8.9 μmol), **M3'** (66.2 mg, 89 μmol), $NaHCO_3$ (175 mg, 2.08 mmol), THF (6 mL) and H_2O (3 mL) was carefully degassed before and after $Pd(PPh_3)_4$ (2 mg, 1.7 μmol) was added. The mixture was heated to reflux and stirred under nitrogen for 72 h. The reaction mixture was extracted with $CHCl_3$ (3 ×30mL), and combined

organic layers were dried over anhydrous $MgSO_4$. After the removal of most of solvent, the residue was precipitated into methanol. The resulted precipitate was collected by filtration and dried under vacuum to give **P2** as a purple-red solid (97 mg, 90%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.17 (d, *J* = 17.3 Hz, 2H), 7.78 (m, *J* = 55.6, 26.2, 18.0 Hz, 12H), 7.51 (s, 1H), 7.15 (s, 1H), 3.25 (t, *J* = 19.0 Hz, 4H), 2.23–1.49 (m, 22H), 1.33 (d, *J* = 60.6 Hz, 126H), 0.88 (t, *J* = 5.8 Hz, 20H).

Polymer **P3**. Trimethylamine (1 mL, 30% wt) was added dropwise to a solution of the neutral precursor **P2** (22.5 mg, 28 μmol) in THF (5 mL) at -78 ^{o}C under N_2.The mixture was allowed to warm up to room temperature and the precipitate was redissolved by addition of water (10 mL). After the mixture was cooled down to -78 ^{o}C, trimethylamine (1 mL, 30% wt) was added and the mixture was stirred for 24 h at room temperature. The purple-red product was collected after removing the solvent (20 mg, 83%). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.21 (s, 4H), 8.05–7.59 (m, 12H), 7.45 (s, 2H), 5.39 (s, 2H), 3.44 (s, 20H), 3.20 (d, *J* = 24.7 Hz, 10H), 2.98 (s, 12H), 2.20 (d, *J* = 21.1 Hz, 7H), 1.89–1.45 (m, 16H), 1.43–0.48 (m, 53H).

Preparation of P1 Pdots. Firstly, **P1** was dissolved in THF to make a stock solution with a concentration of 0.5 mg/mL. PSMA was also dissolved in THF and mixed with a diluted solution of **P1** to produce a solution mixture with a **P1** concentration of 33 μg/mL and a PSMA concentration of 7 μg/mL. The mixture was sonicated to form homogeneous solutions. A 3-mL quantity of the solution mixture was added quickly to 10 mL of MilliQ water in a bath sonicator. The THF was removed by nitrogen stripping, and the solution was concentrated by continuous nitrogen stripping to 10 mL on a 90 ^{o}C hotplate, followed by filtration through a 0.2 micron filter. **P3** Pdots were prepared similarly to **P1** Pdots.

Bioconjugation of P1 Pdots.

Bionconjugation of **P1** Pdots was performed in an EDC-catalyzed reaction between carboxyl

groups on Pdots surface and amine groups of biomolecules. In a typical reaction, 20 μL poly (ethylene glycol) (5% w/v PEG, M_W~3350) and 20 μL HEPES buffer (1 M, pH = 7.2) were respectively added to 1 mL **P1** Pdots solution (50 μg/mL in Milli-Q water). Then, 60 μL streptavidin solution (1 mg/mL in 20 mM HEPES buffer, Sigma-Aldrich) was added and mixed well on a vortex. Finally, 20 μL EDC solution (5 mg/mL in Milli-Q water) was freshly prepared and added to the solution. The resulting mixture was held on the rotary shaker at the speed of 15 r/min for 4 h at room temperature. Pdot-streptavidin bioconjugates were separated from free streptavidin molecules by using a centrifugal filter (MWCO=100,000) at the speed of 4000 r/min.

Subcellular Labeling.

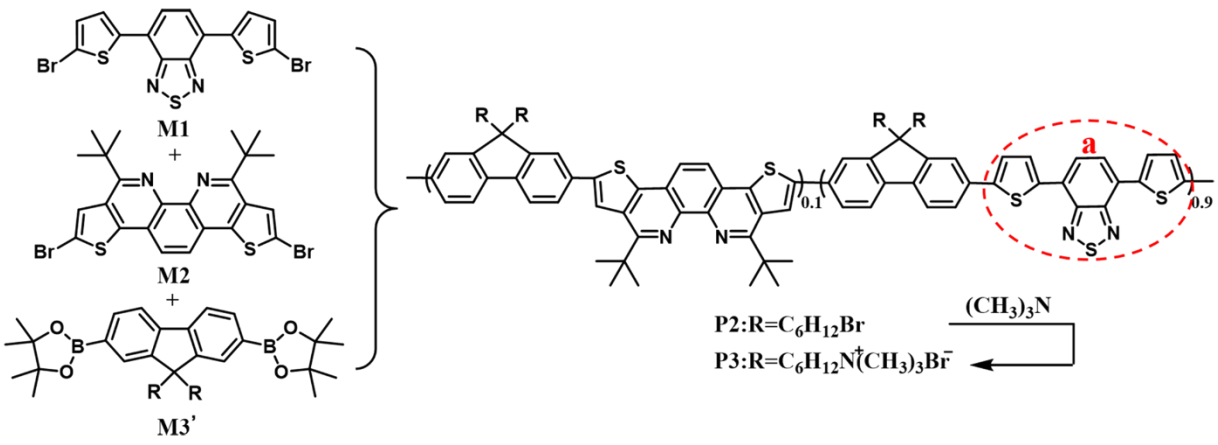
For microtubulin labeling, HeLa cells were plated on glass-bottomed dishes and cultured in Dulbecco's modified Eagle Medium (DMEM) with 10% Fetal Bovine Serum (FBS), 50 U/mL penicillin, and 50 μg/mL streptomycin overnight (5% CO_2, 37 ^oC). At the confluence of 80%, cells were pre-extracted with extracting buffer containing 0.1 M PIPES, 1 mM EGTA, 1 mM $MgCl_2$, and 0.2% TritonX-100 for 1min, followed by rinsing with PBS for three times. Then, cells were fixed with 4% v/v paraformaldehyde (PFA) and 0.1% glutaraldehyde (GA) in PBS for 15 min at room temperature. After fixation, cells were washed with PBS for three times and subsequently permeabilized with 0.5% Triton-X 100 for 5min. Then the cells were incubated with blocking buffer containing 5% BSA and 0.1% Triton-X 100 for 30 min. Next, Biotinylated anti-alpha tubulin antibodies (ab74696, Abcam) were diluted in blocking buffer and incubated for 1 hour at room temperature. The cells were washed three times with PBS, after which streptavidin-conjugated **P1** Pdots were diluted in the same blocking buffer and incubated with the cells for 1 hour at room temperature. Finally, the cells were washed well with PBS and mounted on a total internal reflection fluorescence (TIRF) microscope (Olympus IX71) equipped with a 405-nm laser

beam (Coherent, USA).

In Vitro Cytotoxicity Analysis of P1 Pdots.

The cytotoxicity of **P1** Pdots was assessed by MTT assay. HeLa cells were seeded into a 96-well plate (10,000 cells/well) and cultured for 24 h in 100 μL growth medium which contains **P1** Pdots at a series of concentrations (0, 10, 20, 40, 80, 100 μg/mL) and subsequently measured by a BioTek Cytation3 imaging reader (BioTek Instruments Inc., USA). Each data point represents an averaged value with corresponding standard deviation from 40 wells.

Supporting Figures.



Scheme S1. Synthetic route of the control polymer **P3**.

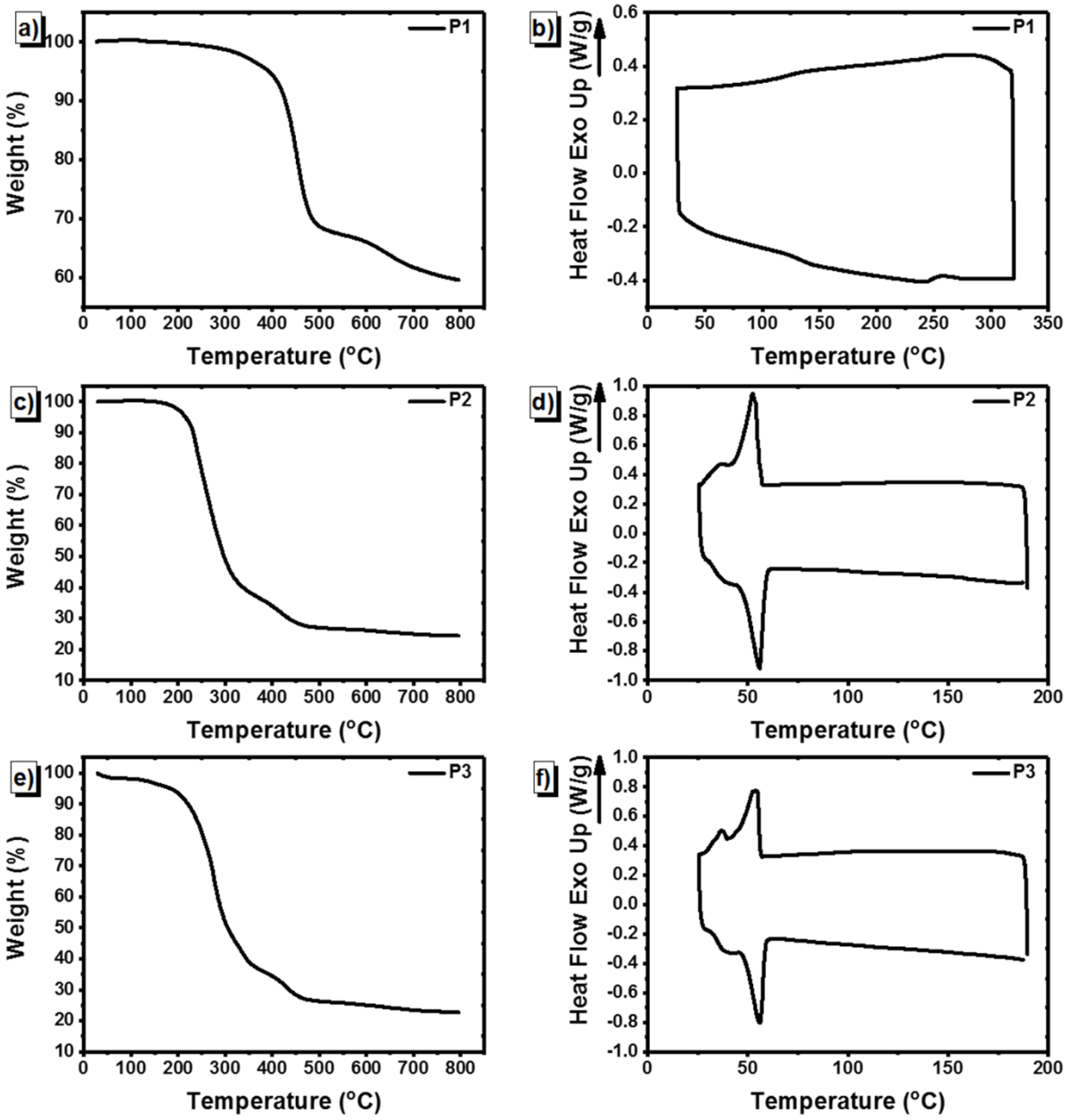


Figure S1. TGA traces of (a) **P1**, (c) **P2** and (e) **P3** and DSC second heating and cooling traces of (b) **P1**, (d) **P2** and (f) **P3**, (10 ^{o}C/min).

P2 and **P3** showed less than 10% decomposition up to 220 ^{o}C, and a residual weight of about 24% and 23%, respectively, at 800 ^{o}C under a nitrogen atmosphere at a heating rate of 10 ^{o}C/min. **P2** and **P3** exhibited a distinct endothermal peak at about 56 ^{o}C in the DSC trace upon heating and an exothermic peak at about 53 ^{o}C in the cooling trace at a rate of 10 ^{o}C/min. These peaks are probably ascribed to an undefined solid to solid transition (*S5*, *S6*).

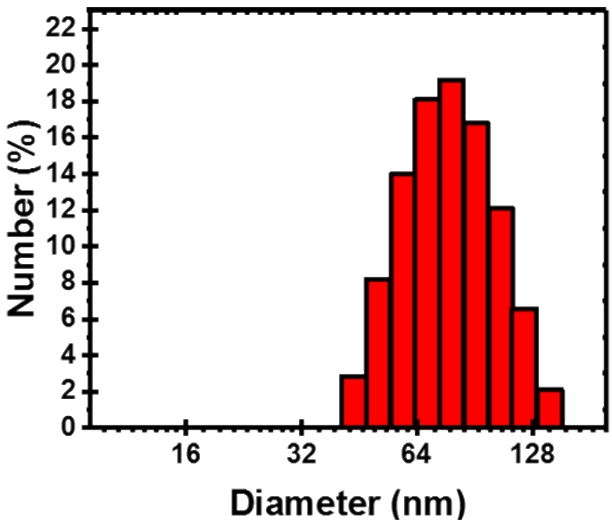


Figure S2. Hydrodynamic diameter of **P3** Pdots measured by dynamic light scattering.

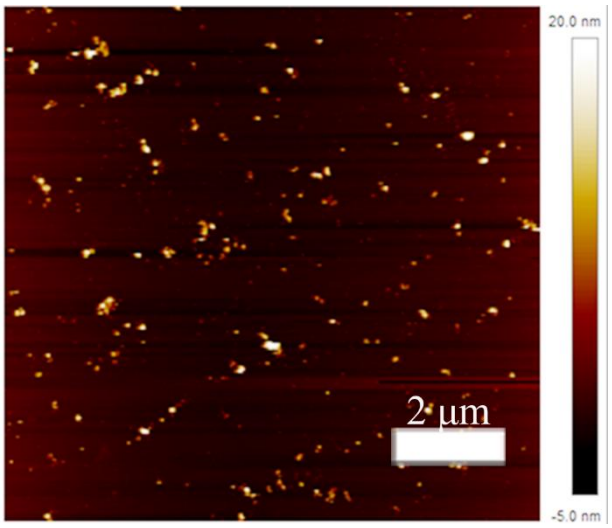


Figure S3. Typical AFM image of **P3** Pdots.

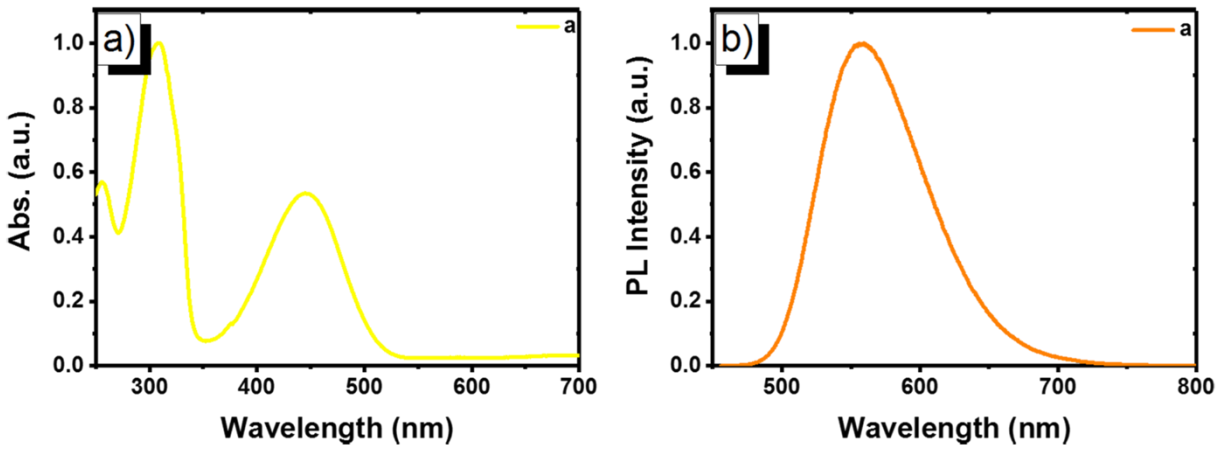


Figure S4. Normalized (a) UV-vis absorption and (b) PL emission spectra of the repeating unit **a** in chloroform.

Table S1. Photophysics of copolymer **P1** in chloroform solution and its Pdots in water.

Entry	M_n (KDa)	PDI	λ_{peak}^{abs} (nm)[a]	λ_{max}^{em} (nm)[b]	Stokes shifts (nm)	Φ(%)
P1	1.7	2.1	397, 552	653	101	72.6[c]
Pdots	—	—	369, 515	677	162	6.2[d]

[a]Absorption peak. [b]Fluorescence maximum. [c]Relative fluorescence quantum yield. Rhodamine B as the standard for **P1** (Φ= 0.69 in EtOH). [d]Absolute fluorescence quantum yield.

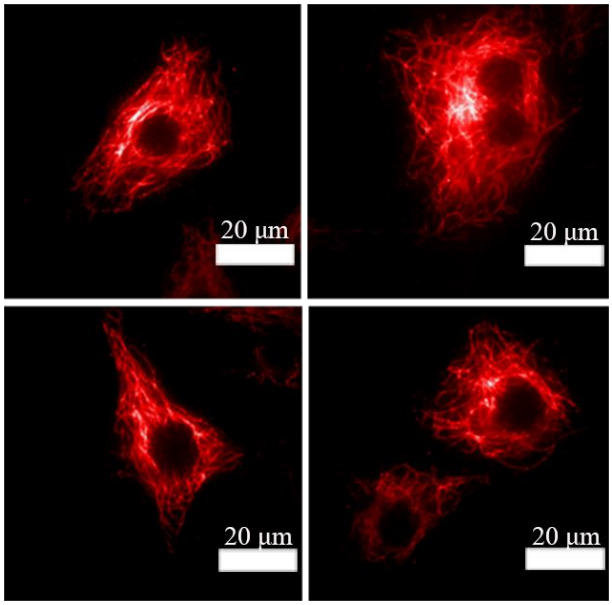


Figure S5. TIRFM (total internal reflection fluorescence microscope) microscopy images of microtubules in HeLa cells labeled with streptavidin conjugated **P1** Pdots in the presence of biotinylated anti-alpha tubulin antibody (excited by 405 nm laser).

NMR spectra

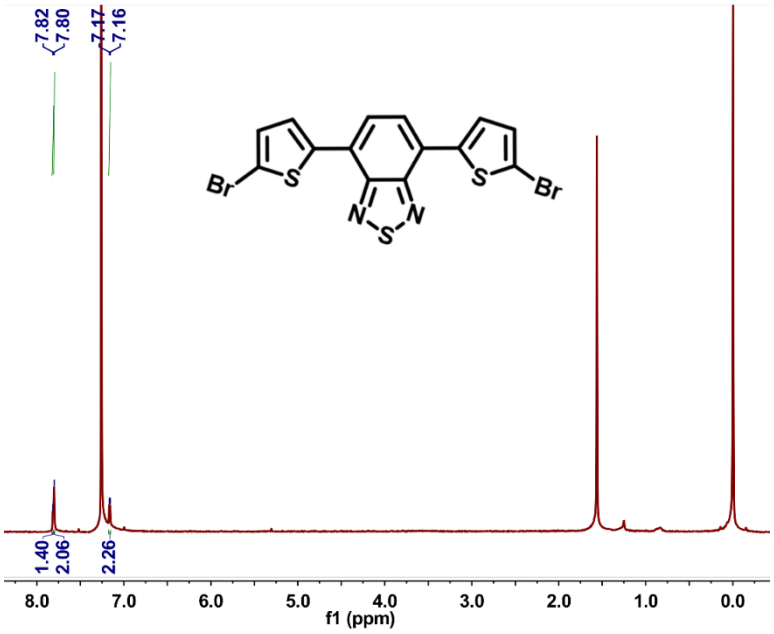


Figure S6. [1]H-NMR spectrum of compound **M1**.

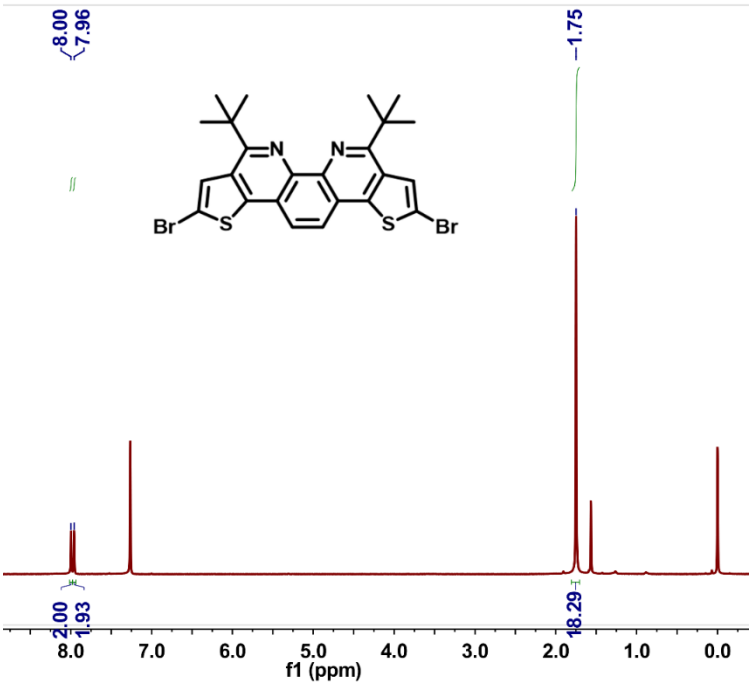


Figure S7. [1]H-NMR spectrum of compound **M2**.

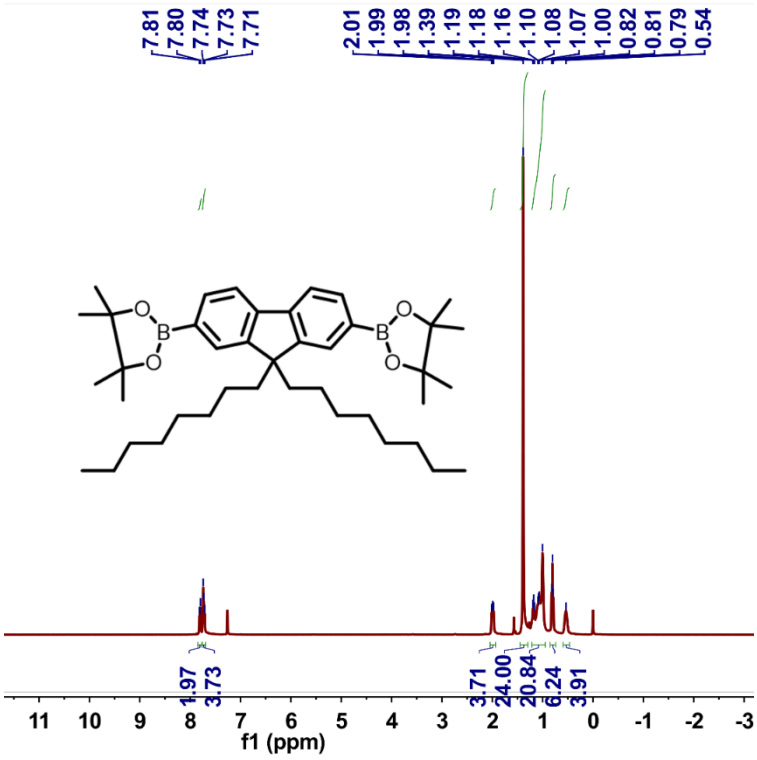


Figure S8. [1]H-NMR spectrum of compound **M3**.

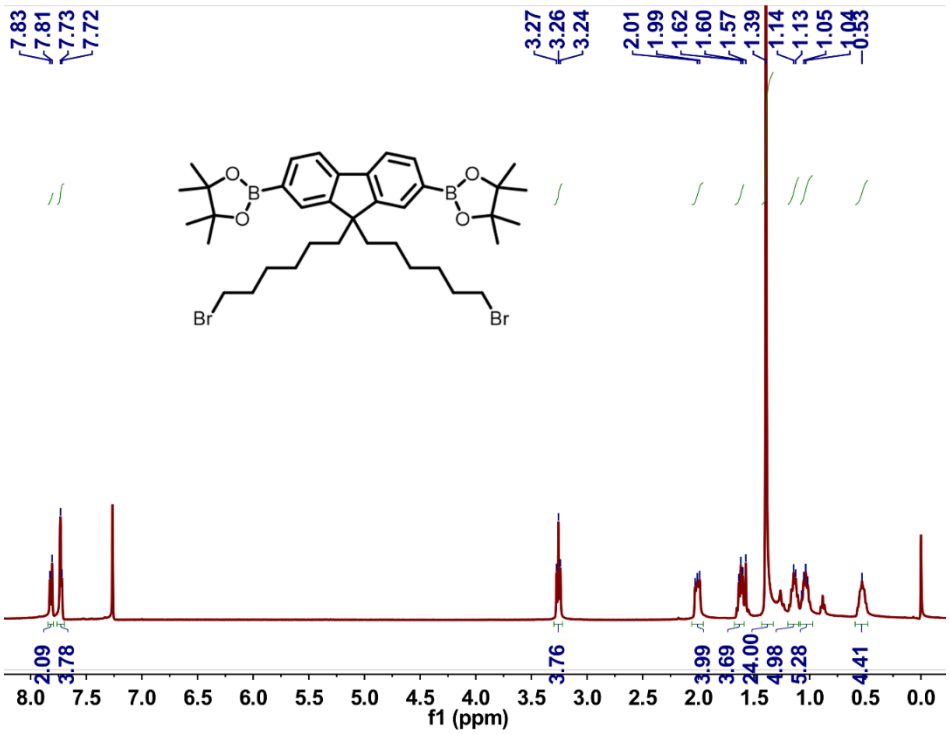


Figure S9. [1]H-NMR spectrum of compound **M3'**.

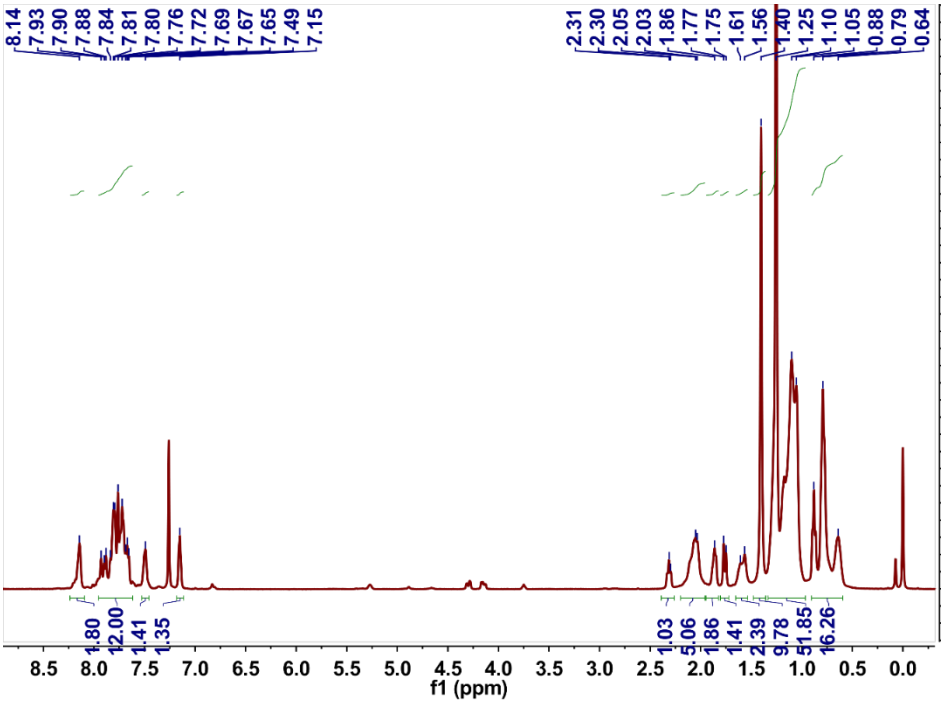


Figure S10. ^{1}H-NMR spectrum of polymer **P1**.

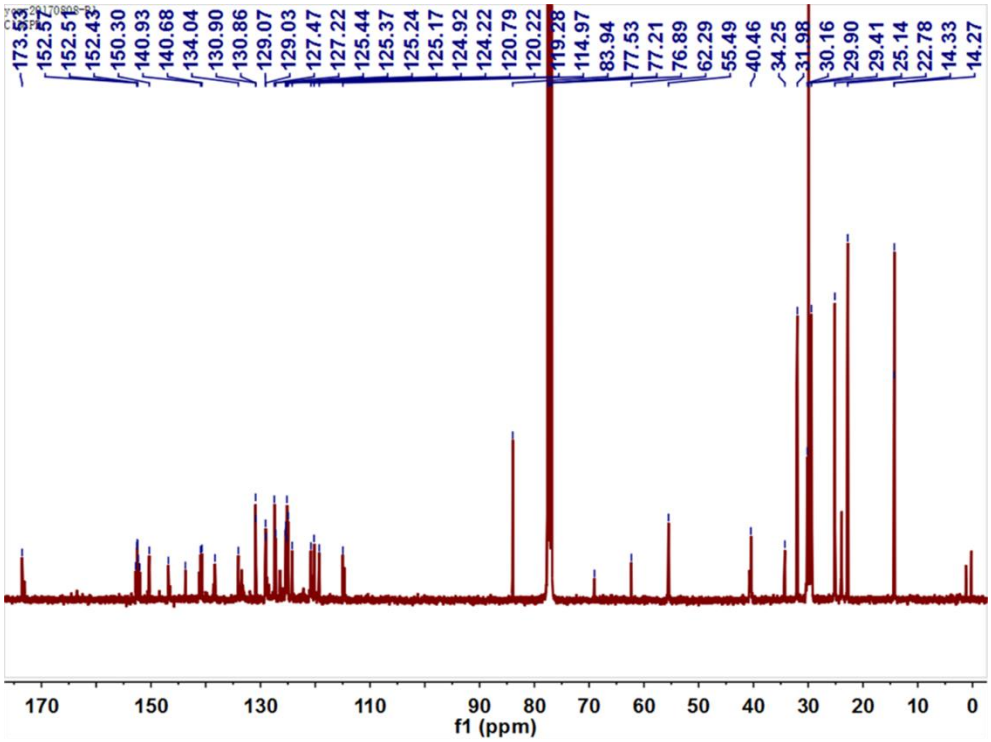


Figure S11. ^{13}C-NMR of polymer **P1**.

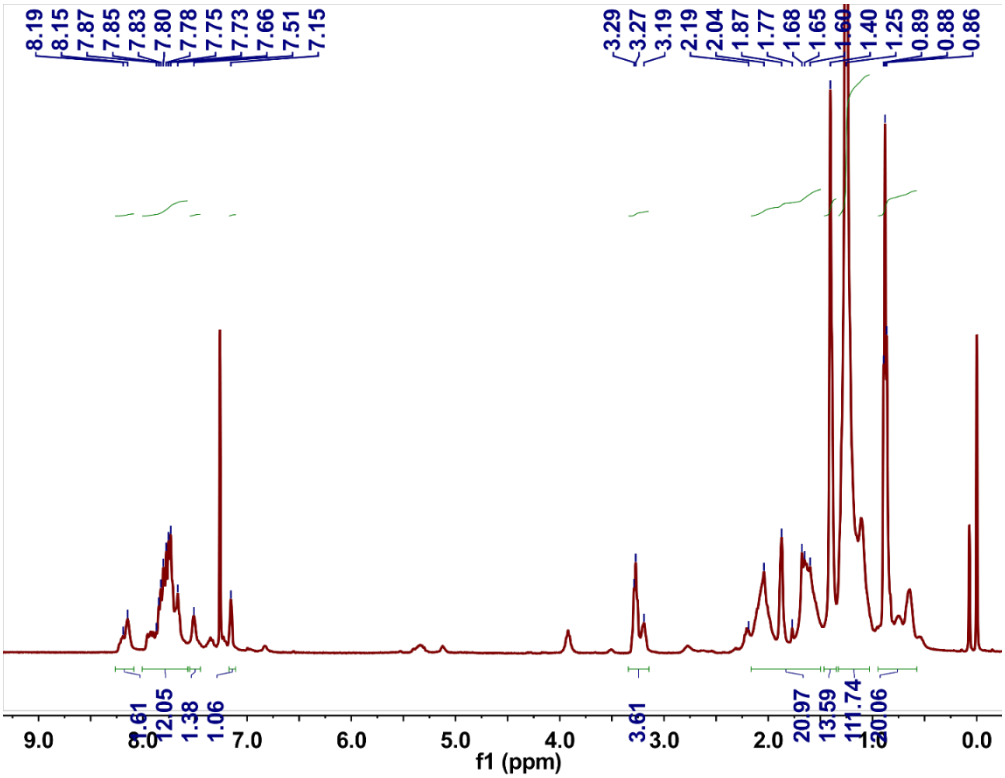


Figure S12. ^{1}H-NMR spectrum of polymer **P2**.

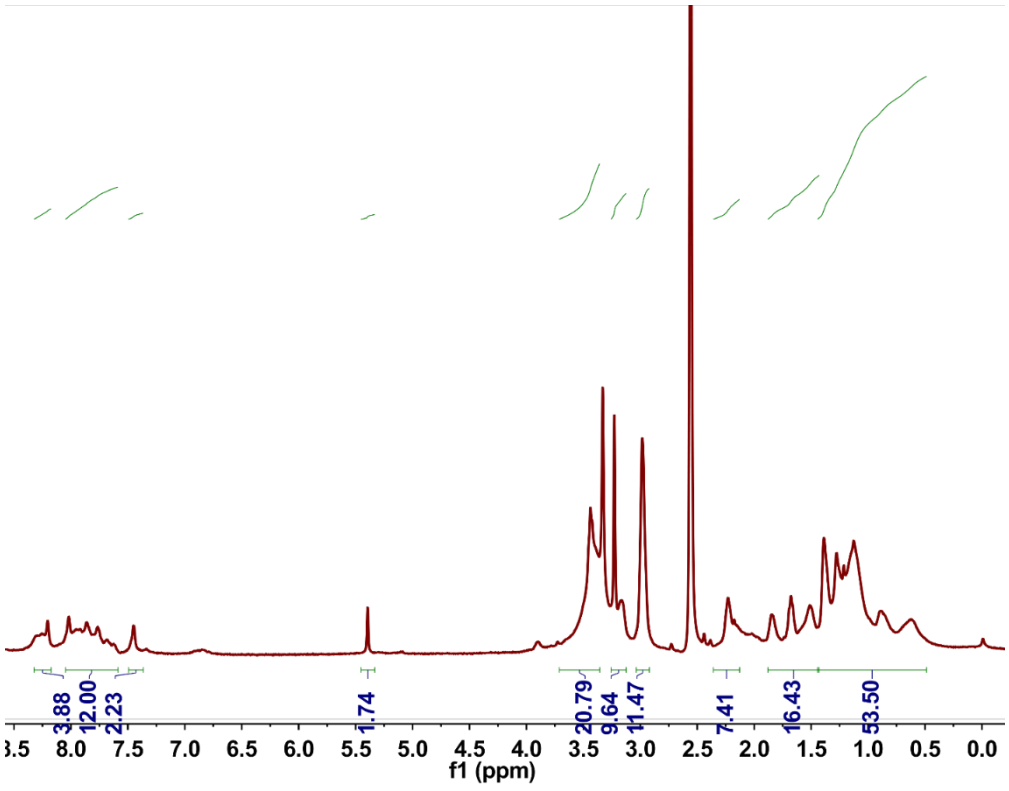


Figure S13. ^{1}H-NMR spectrum of polymer **P3**.

References.

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