

ADVANCED MATERIALS

Supporting Information

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Semiconducting Polymer Nanoparticles for Centimeters-Deep
Photoacoustic Imaging in the Second Near-Infrared Window

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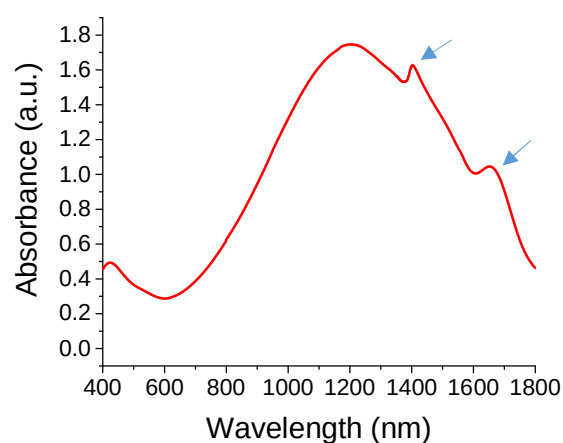


Figure S1. UV/VIS/NIR absorption spectrum of TSPN in D₂O. The small peaks at 1400 nm and 1650 are generated from H₂O residues in D₂O.

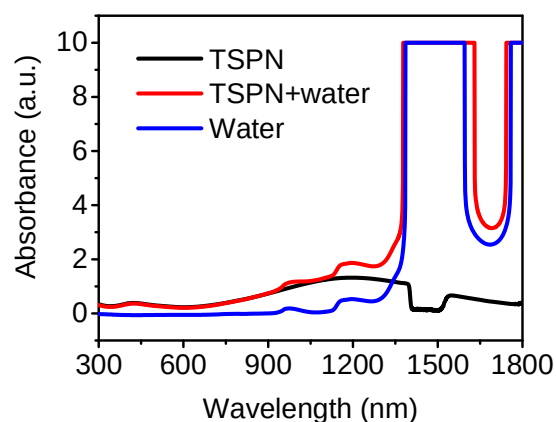


Figure S2. Raw absorption spectra of (1) aqueous solution of TSPN using water as a reference (black) (2) aqueous solution of TSPN using an empty cuvette as a reference (red) (3) water using an empty cuvette as a reference (blue).

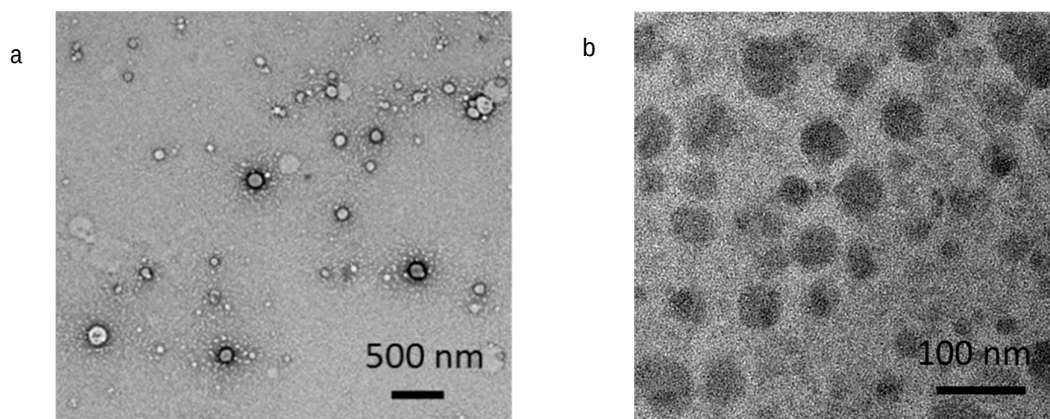


Figure S3. TEM images of TSPNs (a) with negative staining (b) without negative staining.

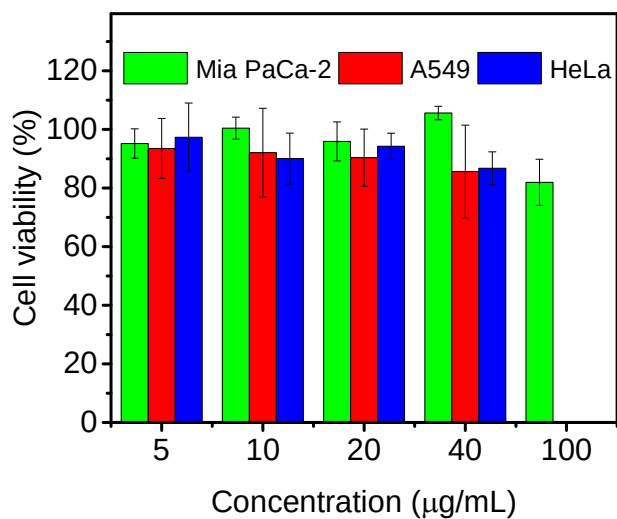


Figure S4. Cytotoxicity studies of TSPN. In vitro viability of Mia PaCa-2, A549 and HeLa cells were treated with TSPN solutions at concentrations of 5, 10, 20, 40 µg/mL for 24 h, respectively. The percentage cell viability of treated cells is calculated relative to that of cells treated with the same volume of PBS (viability was arbitrarily defined as 100%). Error bars represent standard deviations of four separate measurements.

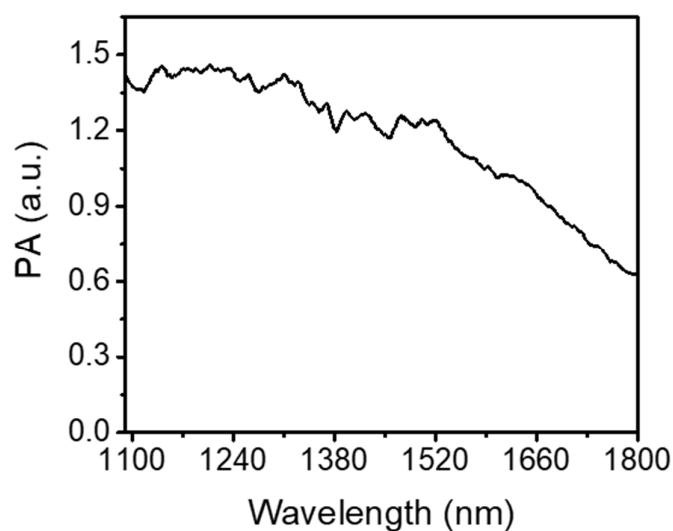


Figure S5. PA spectrum of TSPN in D₂O with a concentration of $\sim 2.5 \mu\text{g}\cdot\text{mL}^{-1}$. Laser energy, 45 μJ .

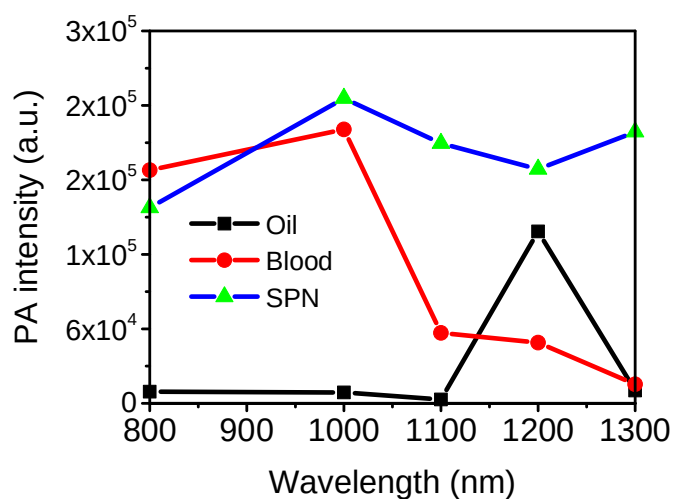


Figure S6. PA intensities extracted from PA images (**Figure 2b**) of blood, $40 \mu\text{g}\cdot\text{mL}^{-1}$ TSPN, and lipid (represented by olive oil) contained in transparent plastic tubes with a laser wavelength at 1000, 1100, 1200 and 1300 nm, respectively. The averaged laser energy density was approximately $10 \text{ mJ}\cdot\text{cm}^{-2}$ at 800 nm, $9 \text{ mJ}\cdot\text{cm}^{-2}$ at 1000 nm, $18 \text{ mJ}\cdot\text{cm}^{-2}$ at 1100 nm, $5 \text{ mJ}\cdot\text{cm}^{-2}$ at 1200 nm and $4 \text{ mJ}\cdot\text{cm}^{-2}$ at 1300 nm. Each image was normalized with the corresponding laser energy density.

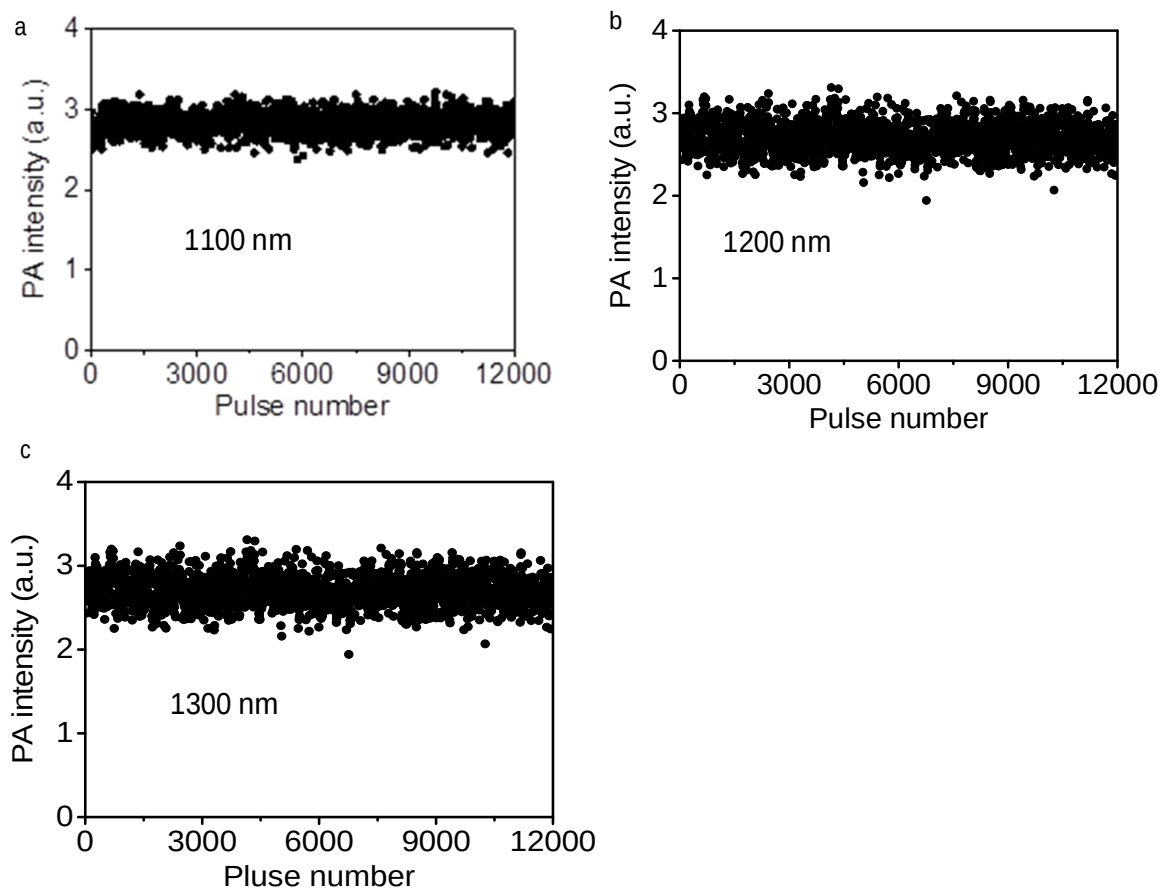


Figure S7. PA amplitude of $40 \mu\text{g}\cdot\text{mL}^{-1}$ SPN over 12000 pulses with a laser wavelength at (a) 1100 nm (b) 1200 nm (c) 1300 nm. The total time is 55 minutes for repeat sweeping wavelength from 1100-1350 nm for 2000 times. The laser light energy at the focus point is $\sim 45 \mu\text{J}$.

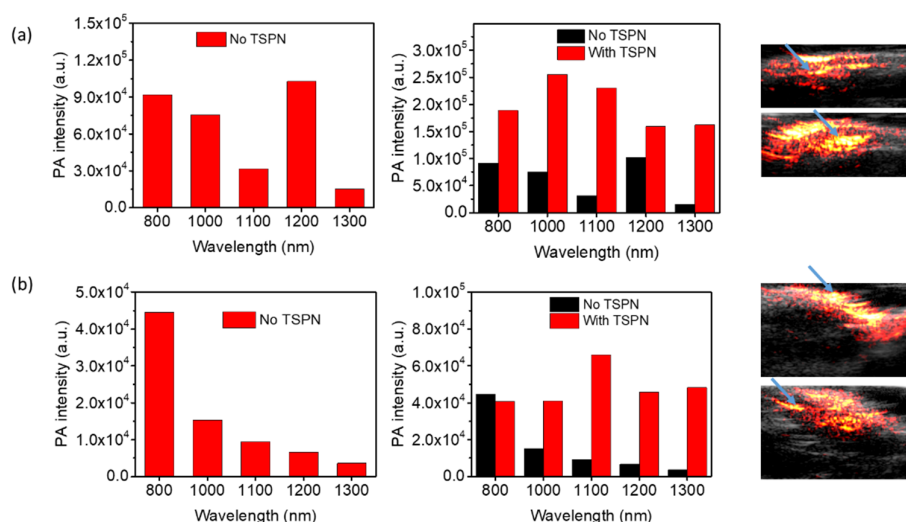


Figure S8. The PA intensities extracted from in **Figure 4** (a) rat skin without injection of TSPN (upper) and with injection of 50 μL , $40\mu\text{g}\cdot\text{mL}^{-1}$ matrigel inclusions of TSPN (dashed circles) (lower), and in (b) mouse tumor (dashed circles) without injection of TSPN (upper) and with injection of 50 μL , $40\mu\text{g}\cdot\text{mL}^{-1}$ aqueous solution of TSPN (lower). The region of interest is indicated by an arrow.

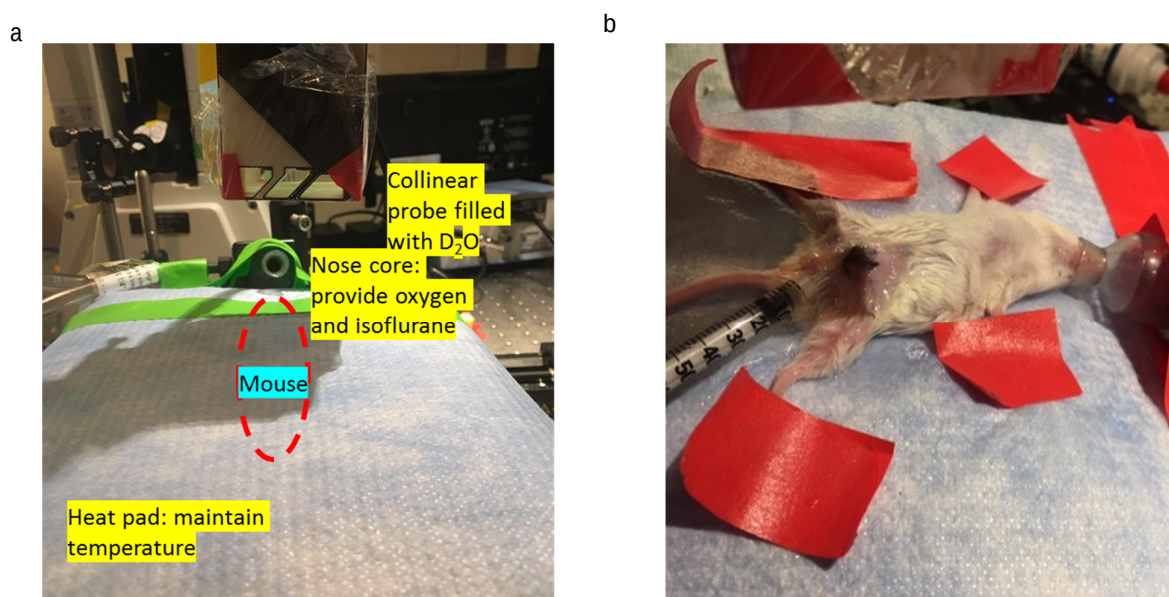


Figure S9. (a) Photograph of *in vivo* PA tomography imaging setup. (b) Photograph of the intratumoral injection.

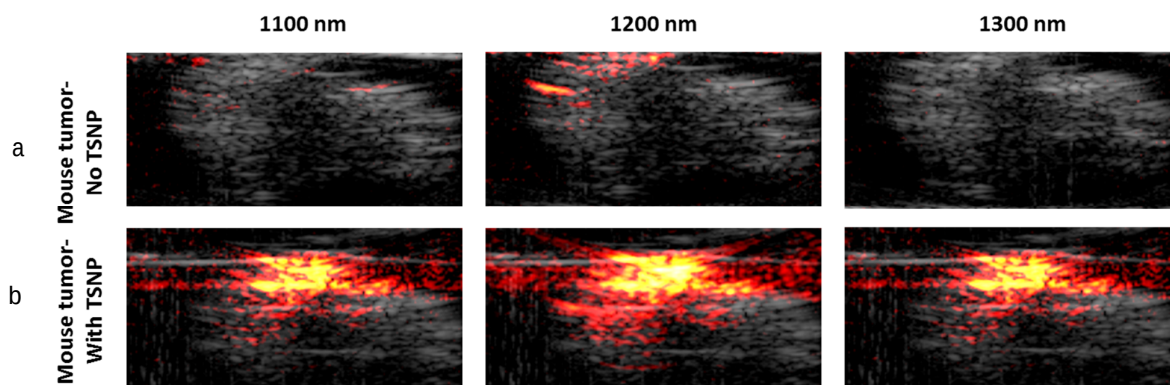
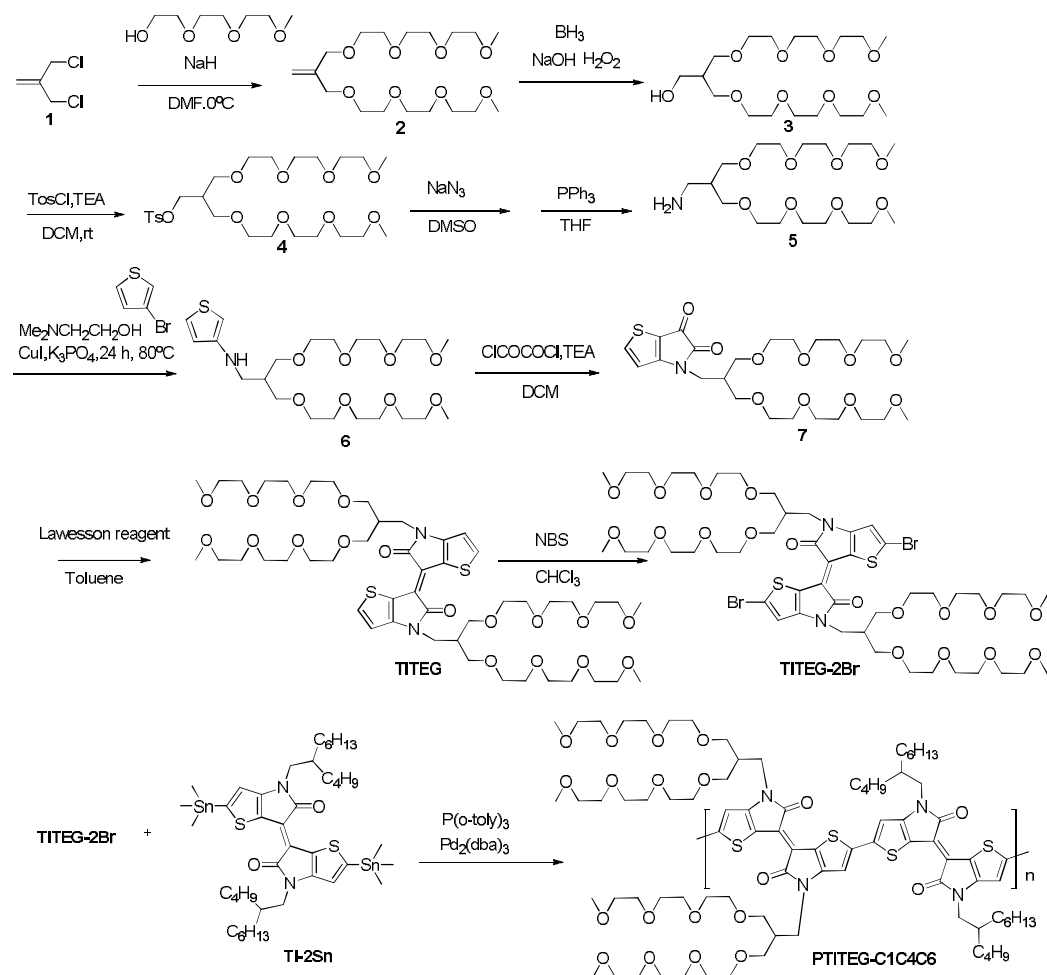


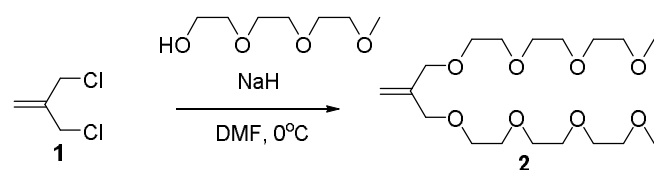
Figure S10. Ex vivo images of the mouse prostate tumor (a) without injection of TSPN and (b) with injection of $40 \mu\text{g}\cdot\text{mL}^{-1}$ TSPN. Laser energy density, $10 \text{ mJ}\cdot\text{cm}^{-2}$.

Materials and characterizations

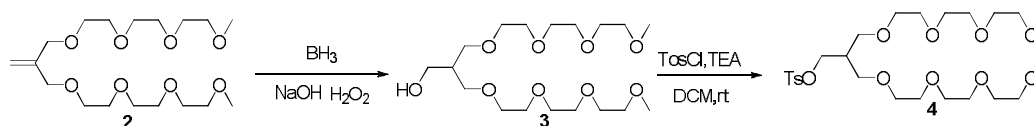
All reagents were purchased from Sigma Aldrich and used without further purification unless otherwise noted. Anhydrous *N,N*-dimethylformamide is prepared from MB-SPS solvent purifying system.¹H and ¹³C NMR spectra were recorded on a Bruker ARX 400 at 293 K with deuterized chloroform as solvent.



Scheme S1. Synthetic routes for PTITEG-C1C4C6

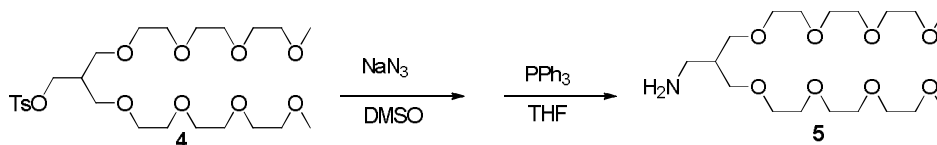


2-(2-(2-methoxyethoxy)ethoxy)ethan-1-ol (19.7 g, 120.0 mmol) was added dropwise to a mixture of NaH (60% in mineral oil) (4.97 g, 124.0 mmol), methallyl dichloride (5.00 g, 40.0 mmol) and THF (70.0 mL) at room temperature. Then the mixture was stirred at 65 °C for 18 h. Upon completion, the reaction mixture was quenched with water and extracted with dichloromethane. The organic phase was dried over anhydrous MgSO₄ and concentrated under vacuum and subjected to flash column chromatography(DCM/Acetone=20/1), yielding a colorless liquid . (14.0 g, 92 %) ^[45]. ¹H NMR (400 MHz, CDCl₃), δ (ppm): 5.07 (s, 2H), 3.91 (s, 4H), 3.55-3.42 (m, 24H), 3.26 (s, 6H). ¹³C NMR (100 MHz, CDCl₃), δ (ppm): 142.4, 113.7, 71.7, 71.6, 70.4, 70.4, 70.3, 69.3, 58.8.

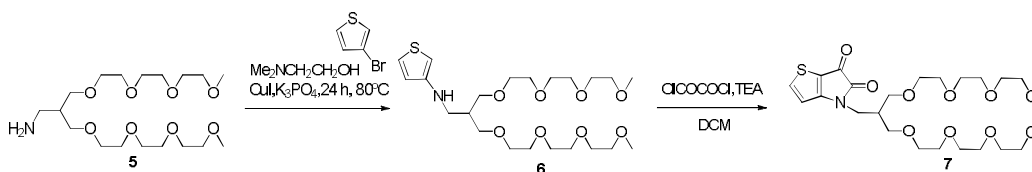


A 1 M BH₃ solution in THF (26.3 mL) was slowly added to a solution of **2** (10.00 g, 26.3 mmol) in 60.0 mL of THF. Then the reaction mixture was stirred at 0 °C for 2 h. After completion, the reaction was quenched with a 3M NaOH aqueous solution and stirred for an additional 15 mins. This was followed by the addition of 30% H₂O₂ (20 mL) and then the mixture was stirred at room temperature for 30 min. The reaction mixture was saturated with K₂CO₃ and extracted with methylene chloride. After the organic layer was dried over anhydrous MgSO₄, it was concentrated under vacuum. . Filtration of the oil through a short silica gel pad resulted in a crude colorless liquid which was used without further purification. TosCl (8.7 g, 46 mmol) was added to a DCM solution of crude **3** (100.0 mL). Then TEA (5.0 g, 49.5 mmol) was also added slowly. The mixture was stirred for 20 h. Then the solution was washed with water and brine. The organic phase was dried over MgSO₄ and the crude oil was submitted to flash column chromatography(DCM/Acetone=10/1) to get the product as a colorless oil(25g, 95 %). ¹H NMR (400 MHz, CDCl₃), δ (ppm): 7.75 (d, 2H, *J* = 8.4 Hz), 7.34

(d, 2H, $J = 8.4$ Hz), 4.08 (d, 2H, $J = 5.6$ Hz), 3.62-3.34 (m, 34H), 2.12-2.25 (m, 1H). ^{13}C NMR (100 MHz, CDCl_3), δ (ppm): 144.6, 132.9, 129.7, 127.8, 71.8, 70.5, 70.4, 70.2, 68.5, 68.2, 58.9, 39.4, 21.5.



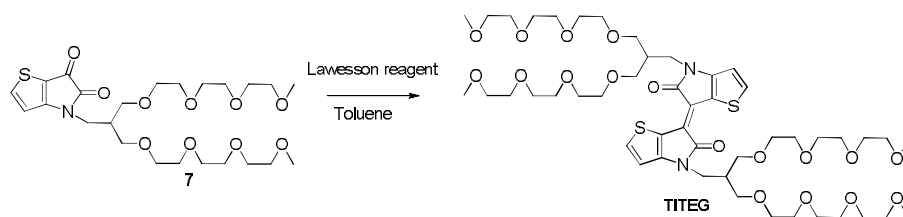
NaN_3 (3.5 g, 54 mmol) was added into a DMSO solution of Ts-TEG (25 g, 45.2 mmol) (75 mL). Then the solution was stirred at 50 °C for 15 h. The reaction was quenched with 200 mL of water and extracted with DCM. Then the solvent was removed and the resulting product was directly used for next step. Then, 100 mL THF and PPh_3 (14.2 g, 54 mmol) was added in sequence. The solution was stirred for 2 h before the addition of 20 mL water. After the reaction finished, solvent was removed first and then extraction was performed with DCM and water. The organic phase was filtered through a silica gel pad. The solvent was removed to get crude product (12.6 g, 70%). ^1H NMR (400 MHz, CDCl_3), δ (ppm): 3.79-3.36 (m, 36H), 1.97-1.95 (m, 1H).



A schlenk tube was charged with tribasic potassium phosphate (4.45 g, 21 mmol, 2.0 equiv.), copper (I) iodide (0.2 g, 1.1 mmol, 0.1 equiv.) and a stir bar, and then vacuumed and purged with N_2 for three times. 3-bromothiophene (1.71 g, 10.48 mmol, 1.0 equiv.), TEG-amine 5 (5 g, 12.58 mmol, 1.2 equiv.), N,N -dimethylaminoethanol (11 mL, 1M based on 3-bromothiophene) was added to the tube. This mixture was heated to 80 °C for 48 h, after which it was poured into a saturated NH_4Cl solution, extracted three times with DCM, dried

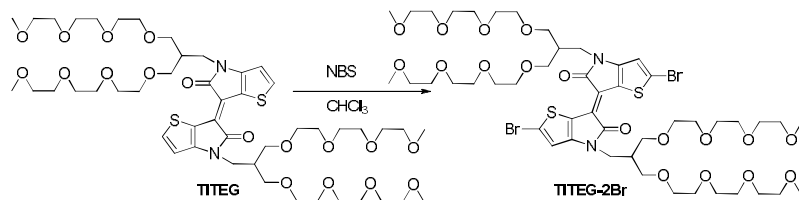
over MgSO_4 , filtered, and concentrated via rotary evaporation. The residue was purified by flash silica gel chromatography (10% EtOAc/DCM), 2.2g (44%) yellow oil was as the crude material **6**.

To a solution of oxalyl chloride (0.79 g, 6.2 mmol) diluted in anhydrous dichloromethane (8 mL) and cooled at 0 °C under a nitrogen atmosphere, a solution of Th-TEG **6** (2.2 g, 4.6 mmol) diluted in anhydrous dichloromethane (4 mL) was added dropwise over 30 min. After 30 min, triethylamine (2.1 g, 20.1 mmol) was added dropwise over 30 min. The resulting mixture was allowed to warm to room temperature. After stirring for 15 h at room temperature, the mixture was quenched with water and then extract with dichloromethane. The organic phase was evaporated under reduced pressure. The residue was purified by silica gel chromatography, eluting with (CH_2Cl_2 /Acetone 10/1) to provide 0.6 g (24%) **7** as a red oil. ^1H NMR (400 MHz, CDCl_3), δ (ppm): 8.04 (d, 1H, $J = 4.8$ Hz), 7.11 (d, 1H, $J = 4.8$ Hz), 3.77-3.35 (m, 36H), 2.30-2.27 (m, 1H). ^{13}C NMR (100 MHz, CDCl_3), δ (ppm): 172.7, 166.0, 162.0, 144.4, 114.3, 110.5, 71.7, 70.4, 70.3, 70.2, 69.5, 58.7, 41.1, 39.3.

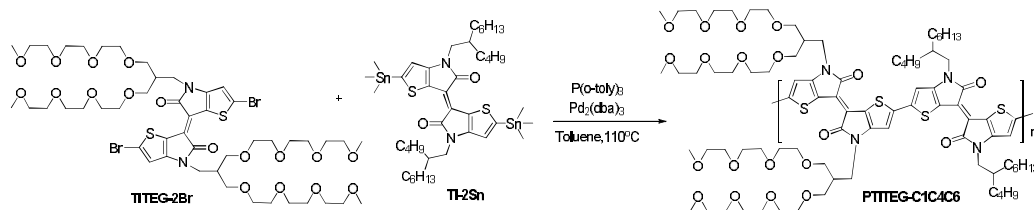


Compound **7** (0.6g, 1.1mmol) and Lawesson reagent (0.44 g, 1.1mmol) was added to a schlenk tube, then 10mL of toluene was added and the mixture was heated to 60 °C for 2 h. When the reaction finished, the solvent was evaporated and then the mixture was submitted to column chromatography (DCM:Acetone=10:1) to get a red oil (0.12 g, 20 %). ^1H NMR (400 MHz, CDCl_3), δ (ppm): 7.47 (d, 2H, $J = 5.2$ Hz), 6.96 (d, 2H, $J = 5.2$ Hz), 3.84 (d, 4H, $J = 6.4$ Hz), 3.59-3.44 (m, 56H), 3.30 (s, 12H), 2.36-2.32 (m, 2H). ^{13}C NMR (100 MHz, CDCl_3), δ

(ppm): 171.2, 151.7, 134.3, 120.7, 113.8, 112.0, 71.8, 70.5, 70.4, 70.4, 70.2, 69.6, 58.9, 40.7, 39.5.



TITEG (0.65 g, 0.63 mmol) was added to a flask, then 10 mL of DCM and NBS (0.25 g, 1.38 mmol) was added slowly, stirring at 25 °C for 4 h. After the reaction was done, the solution was submitted to column chromatography (DCM:Acetone=10:1) to get the desired product as a purple oil (0.2 g, 27%). ^1H NMR (400 MHz, CDCl_3), δ (ppm): 7.03 (s, 2H), 3.79 (d, 4H, $J = 6.0$ Hz), 3.62-3.42 (m, 56H), 3.32 (s, 12H), 2.32-2.28 (m, 2H). ^{13}C NMR (100 MHz, CDCl_3), δ (ppm): 170.3, 150.4, 122.9, 119.4, 115.7, 114.3, 71.8, 70.5, 70.4, 70.3, 69.6, 58.9, 40.8, 39.6. MS (MALDI): Calcd for $\text{C}_{48}\text{H}_{76}\text{Br}_2\text{N}_2\text{O}_{18}\text{S}_2$ 1192.3 m/z. Found: 1215.4 m/z $[\text{M} + \text{Na}]^+$



TITEG-2Br (42 mg, 0.035 mmol), **TI-2Sn** (33 mg, 0.035 mmol), and $\text{P}(o\text{-tolyl})_3$ (2.5 mg, 0.008 mmol) was added to a schlenk tube, then vacuumed and purged with N_2 three times. 1 mL of toluene (bubbled with N_2 for 15 mins before using) was added with a syringe. After that, $\text{Pd}_2(\text{dba})_3$ (1 mg, 0.001 mmol) was added under N_2 . The reaction mixture was heated to 110 °C and reacted for 48 h. A black colored solution was formed which was washed with water and precipitated with hexane, and then underwent soxhlet purification with methanol, hexane and chloroform. The chloroform fraction was collected and reprecipitated in hexane. 32 mg (54 %) of black solid was recovered.

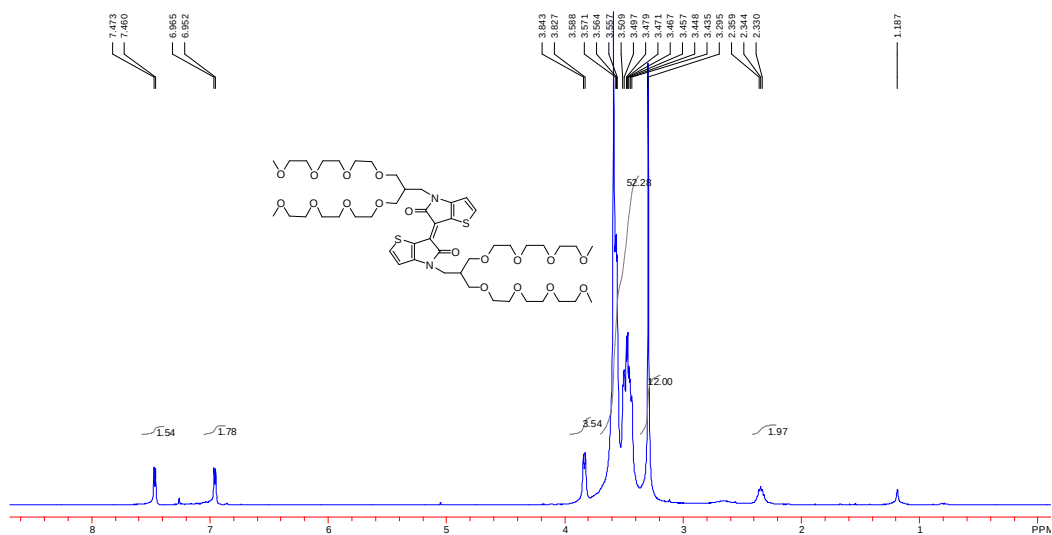


Figure S11. ¹H NMR spectrum of compound **TITEG**.

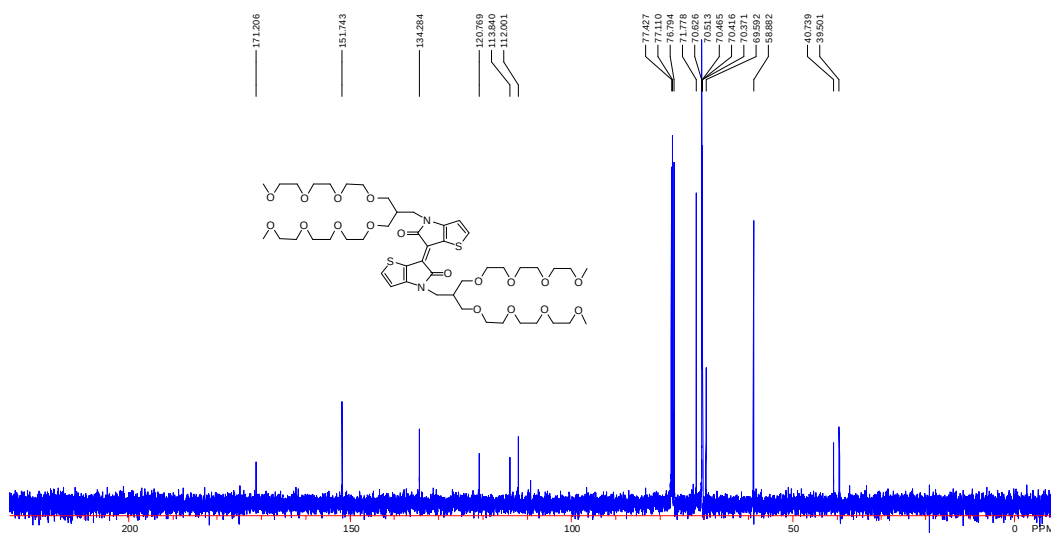


Figure S12. ¹³C NMR spectrum of compound **TITEG**.

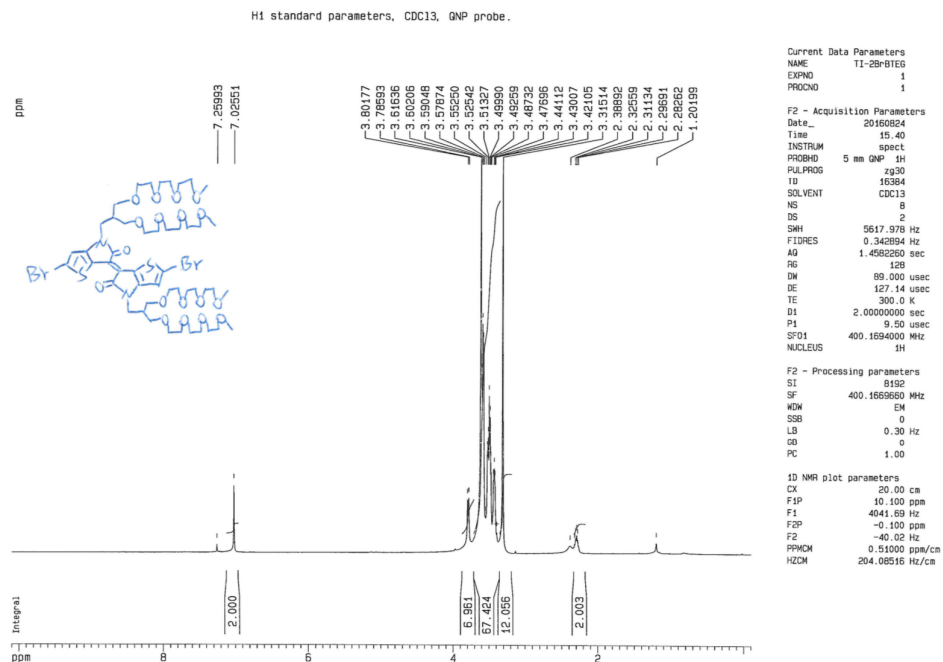


Figure S13. ^1H NMR spectrum of compound **TITEG-2Br**.

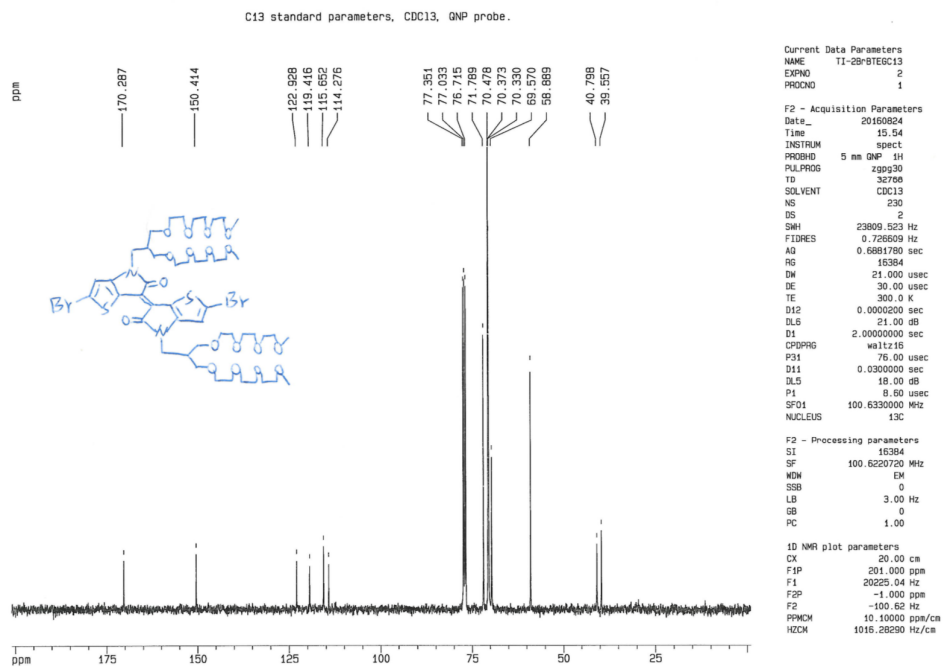
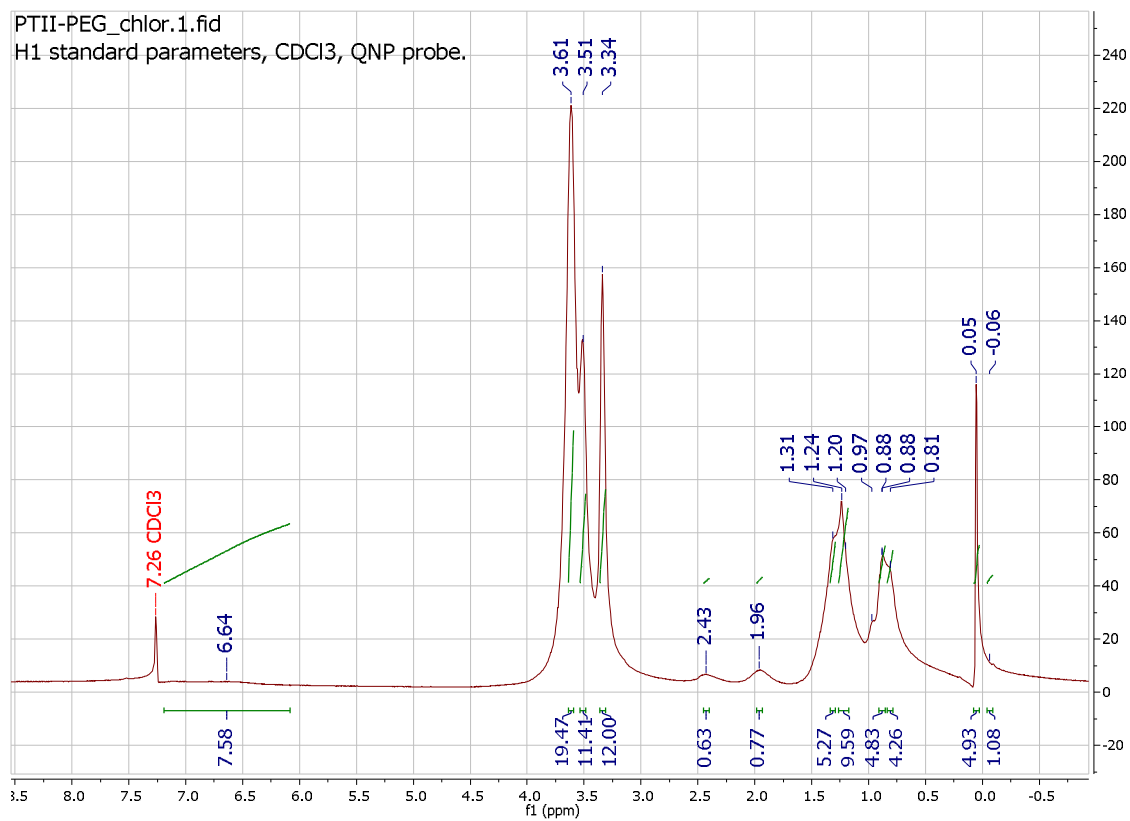


Figure S15. TGA of polymer PTITEG-C1C4C6**Figure S16.** ¹H NMR spectrum of polymer PTITEG-C1C4C6