

## Supporting information

### A design strategy of D-A conjugated polymer for NIR-II fluorescence imaging

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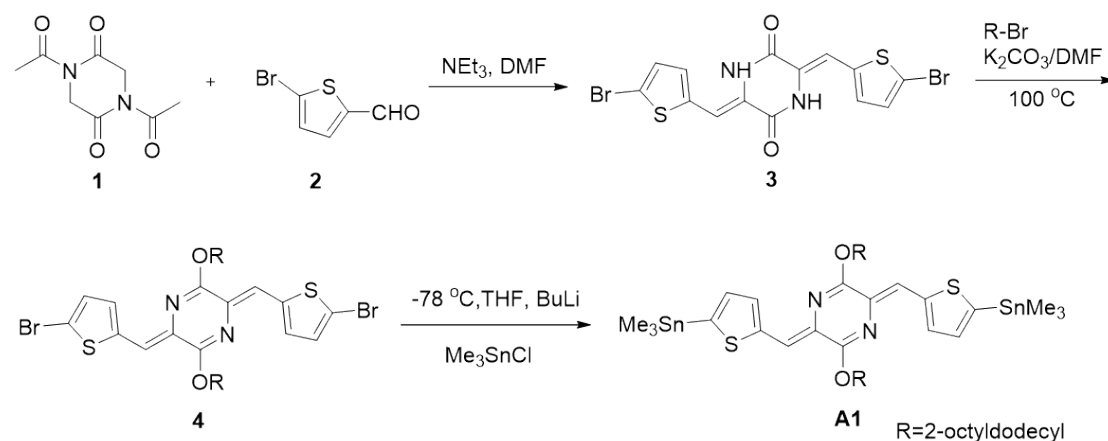
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## Synthesis



Scheme S1. The synthesis route of A1.

### 3,6-Bis((5-bromothiophen-2-yl)methylene)piperazine-2,5-dione (3)

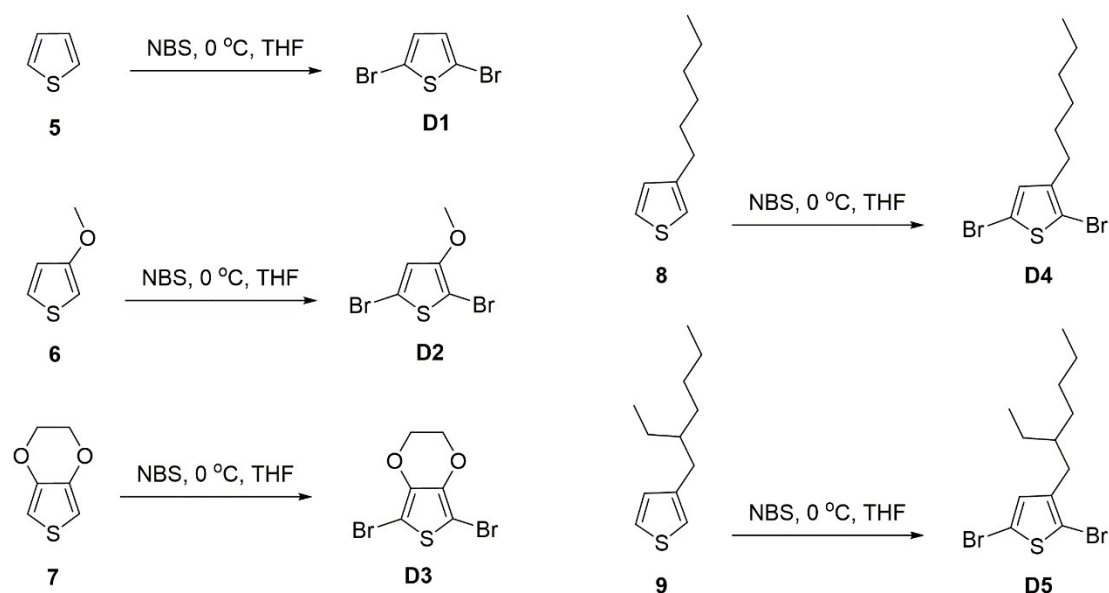
Into a mixture of 1 (1.19 g, 6.0 mmol) and 2 (2.87 g, 15.0 mmol) in DMF (30 mL) was syringe injected triethylamine (2.43 g, 24.0 mmol) at  $120\text{ }^\circ\text{C}$  under  $\text{N}_2$  sphere. The original colorless solution turned red immediately upon addition. Yellow precipitate was formed during the overnight reaction. After cooling to room temperature, the precipitate was collected by filtration and rinsed with acetone. The residue was recrystallized twice from ethanol to yield the desired compounds as yellow solid (1.48 g, 53.8%).  $^1\text{H NMR}$  (400 MHz, *d*-DMSO):  $\delta$  (ppm) 9.85 (s, 2H), 7.35 (s, 2H), 7.28 (s, 2H), 6.88 (s, 2H).

### 2,5-Bis((5-bromothiophen-2-yl)methylene)-3,6-bis(2-octyldodecyl)-2,5-dihydropyrazine (4)

A mixture of 3 (1.47 g, 3.19 mmol),  $\text{K}_2\text{CO}_3$  (2.20 g, 15.95 mmol) and 9-(bromomethyl)nonadecane (4.67 g, 12.90 mmol) in DMF (40 mL) was stirred at  $100\text{ }^\circ\text{C}$  for 2 hours under  $\text{N}_2$  sphere. After cooling to room temperature, the reaction mixture was filtered, and the filtrate was concentrated by rotary evaporation under reduced pressure. The residue was purified by column chromatography on silica gel (eluent: petroleum ether/ $\text{CHCl}_3$  gradient from 9:1 to 4:1) to give an orange solid (1.65 g, 50.0 %).  $^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  (ppm) 7.03 (d, 2H), 7.02 (s, 2H), 7.00 (d, 2H), 4.37 (d, 4H), 1.95 (m, 2H), 1.50-1.38 (m, 16H), 1.32-1.23 (m, 48H), 0.90-0.85 (m, 12H).  $^{13}\text{C NMR}$  ( $\text{CDCl}_3$ , 100 MHz):  $\delta$  157.90, 140.67, 129.92, 129.11, 128.49, 118.70, 116.45, 71.16, 37.23, 31.94, 31.65, 30.11, 29.75, 29.71, 29.69, 29.65, 29.40, 26.83, 22.71, 14.15.

### 2,5-Bis((5-(trimethylstannyl)thiophen-2-yl)methylene)-3,6-bis(2-octyldodecyl)-2,5-dihydropyrazine (A1)

Compound 4 (1.0 g, 0.97 mmol) was solubilized by 70 mL of dry THF under nitrogen atmosphere. The mixture was cooled down to -78 °C, and *n*-BuLi (1.37 mL of a 1.6 M solution in hexane, 2.20 mmol) was added dropwise. After being stirred at -78 °C for 1 h, trimethyltin chloride (2.5 mL of a 1.0 M solution in hexane, 2.5 mmol) was added, then the mixture turned clear. The reaction mixture was stirred overnight at room temperature, then poured into 100 mL of cool water, and was extracted with diethyl ether. The organic layers were combined, washed with brine, dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and concentrated under vacuum. The residue was washed twice by methyl alcohol to yield the desired compounds as yellow solid (0.88 g, 75.6%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ (ppm) 7.38 (d, 2H), 7.17 (s, 2H), 7.16 (d, 2H), 4.41 (d, 4H), 1.92 (m, 2H), 1.41-1.25 (m, 48H), 0.88-0.84 (m, 12H), 0.37 (t, 18H).



Scheme S2. The synthesis route of donor .

### 2,5-Dibromothiophene (D1)

5 (5.0 g, 59.43 mmol) and N-bromosuccinimide (NBS) (22.2 g, 124.79 mmol) were added into THF under stirring at 0 °C. The reaction mixture was stirred at a room temperature for 8 h, and then the reaction mixture was washed with brine and dried over anhydrous sodium sulfate. The solvent was removed under a reduced pressure. The residue was purified by column chromatography on silica gel (eluent: petroleum ether) to give a colourless oil (11.80 g, 82.0 %). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ (ppm) 6.84 (s, 2H).

#### **2,5-dibromo-3-methoxythiophene (D2)**

6 (0.5 g, 4.38 mmol) and NBS (1.59 g, 8.43 mmol) were added into THF under stirring at 0 °C. The reaction mixture was stirred at a room temperature for 8 h, and then the reaction mixture was washed with brine and dried over anhydrous sodium sulfate. The solvent was removed under a reduced pressure. The residue was purified by column chromatography on silica gel (eluent: petroleum ether) to give a light yellow solid (1.06 g, 89.3 %). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ (ppm) 6.79 (s, 1H), 3.86 (s, 3H).

#### **5,7-Dibromo-2,3-dihydrothieno[3,4-b][1,4]dioxine (D3)**

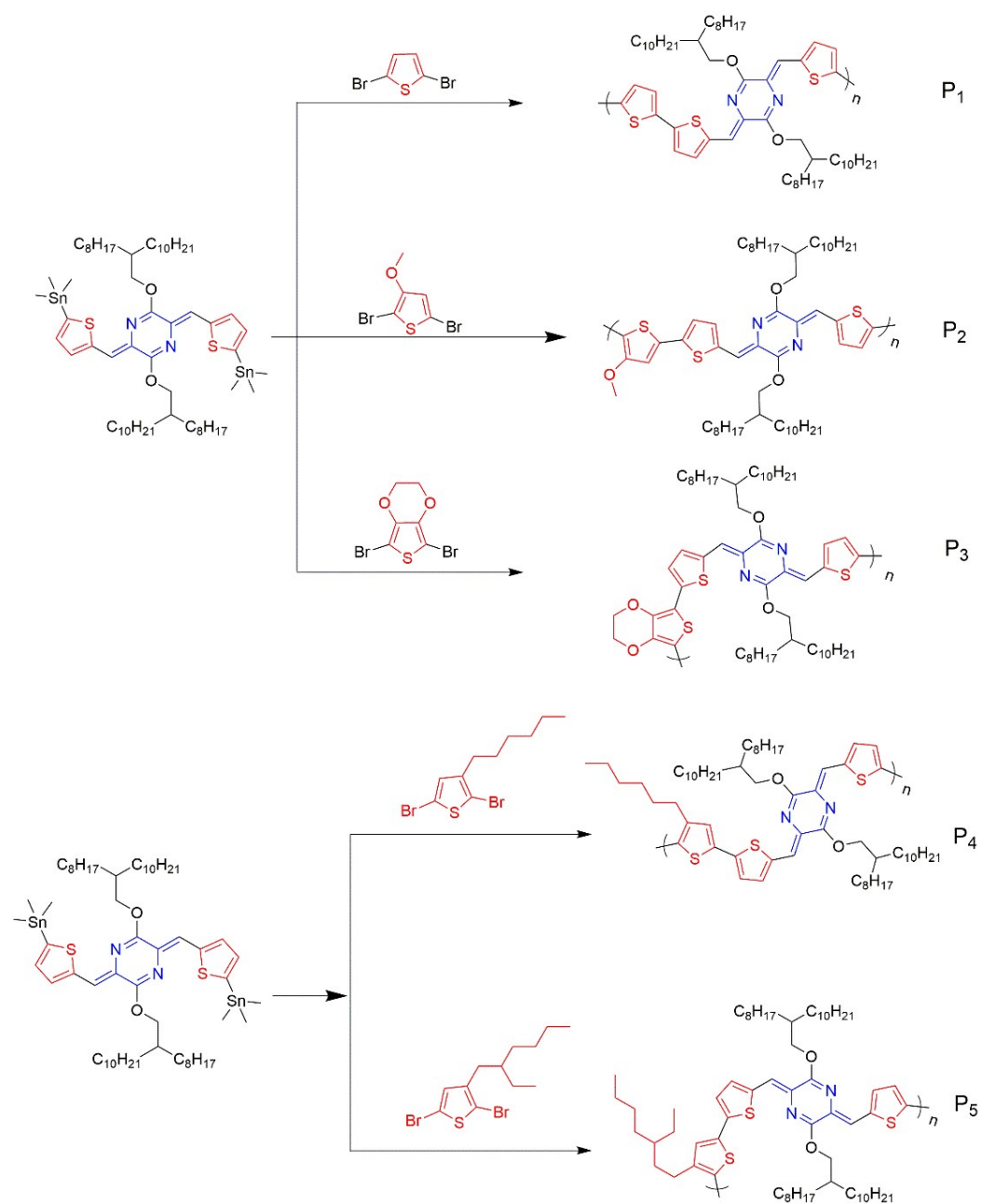
7 (2.0 g, 14.07 mmol) and NBS (5.13 g, 28.84 mmol) were added into THF under stirring at 0 °C. The reaction mixture was stirred at a room temperature for 8 h, and then the reaction mixture was washed with brine and dried over anhydrous sodium sulfate. The solvent was removed under a reduced pressure. The residue was purified by column chromatography on silica gel (eluent: petroleum ether) to give white power (4.01 g, 95.0 %). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ (ppm) 4.27 (s, 4H).

#### **2,5-Dibromo-3-hexylthiophene (D4)**

8 (3.0 g, 17.83 mmol) and NBS (6.50 g, 36.54 mmol) were added into THF under stirring at 0 °C. The reaction mixture was stirred at a room temperature for 8 h, and then the reaction mixture was washed with brine and dried over anhydrous sodium sulfate. The solvent was removed under a reduced pressure. The residue was purified by column chromatography on silica gel (eluent: petroleum ether) to give a colourless oil (5.23 g, 90.0 %). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ (ppm) 6.77 (s, 1H), 2.50 (t, 2H), 1.56-1.49 (m, 2H), 1.36-1.25 (m, 6H), 0.89 (t, J = 6.7 Hz, 3H).

#### **2,5-dibromo-3-(2-ethylhexyl)thiophene (D5)**

9 (2.0 g, 10.19 mmol) and NBS (3.72 g, 20.88 mmol) were added into THF under stirring at 0 °C. The reaction mixture was stirred at a room temperature for 8 h, and then the reaction mixture was washed with brine and dried over anhydrous sodium sulfate. The solvent was removed under a reduced pressure. The residue was purified by column chromatography on silica gel (eluent: petroleum ether) to give a light yellow oil (3.30 g, 91.4 %). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ (ppm) 6.74 (s, 1H), 2.44 (d, 2H), 1.58-1.52 (m, 1H), 1.32-1.23 (m, 8H), 0.90-0.84 (m, 6H).



Scheme S3. The synthesis route of polymers.

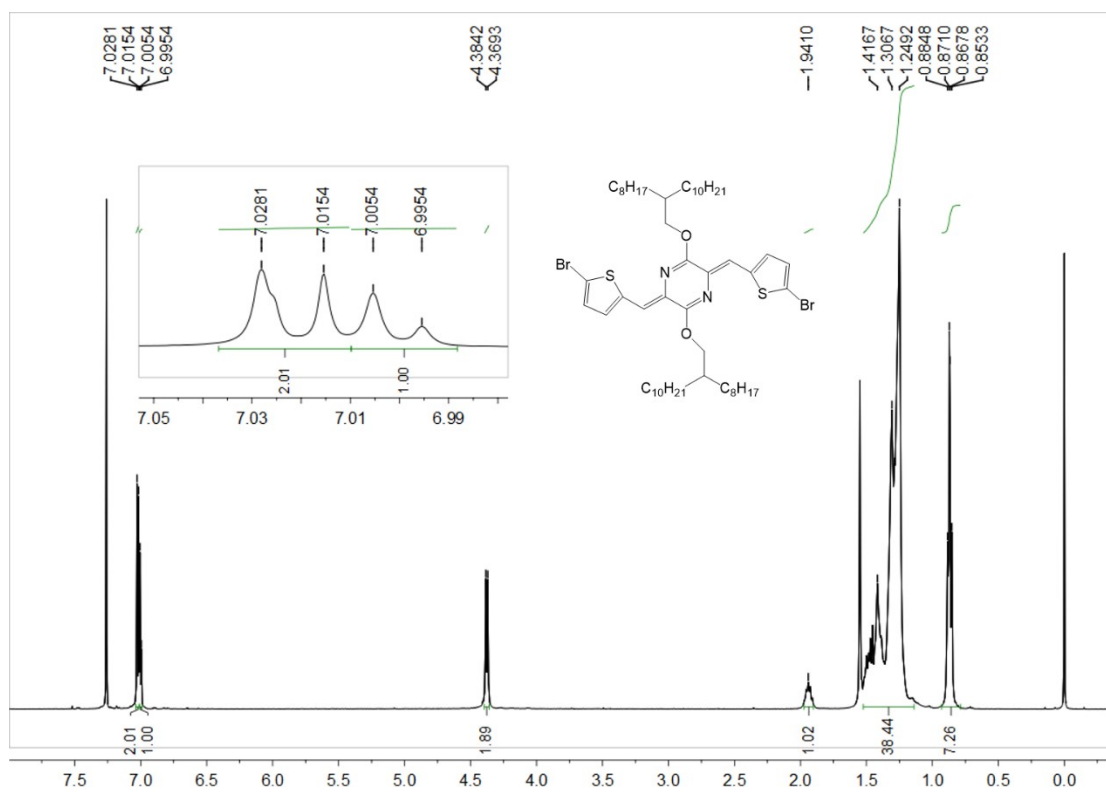


Figure S1.1 <sup>1</sup>H NMR Spectrum of the 4. The inset show a zoomed-in view of the spectrum in the 6.963-7.065 ppm region. (CDCl<sub>3</sub>, 298K)

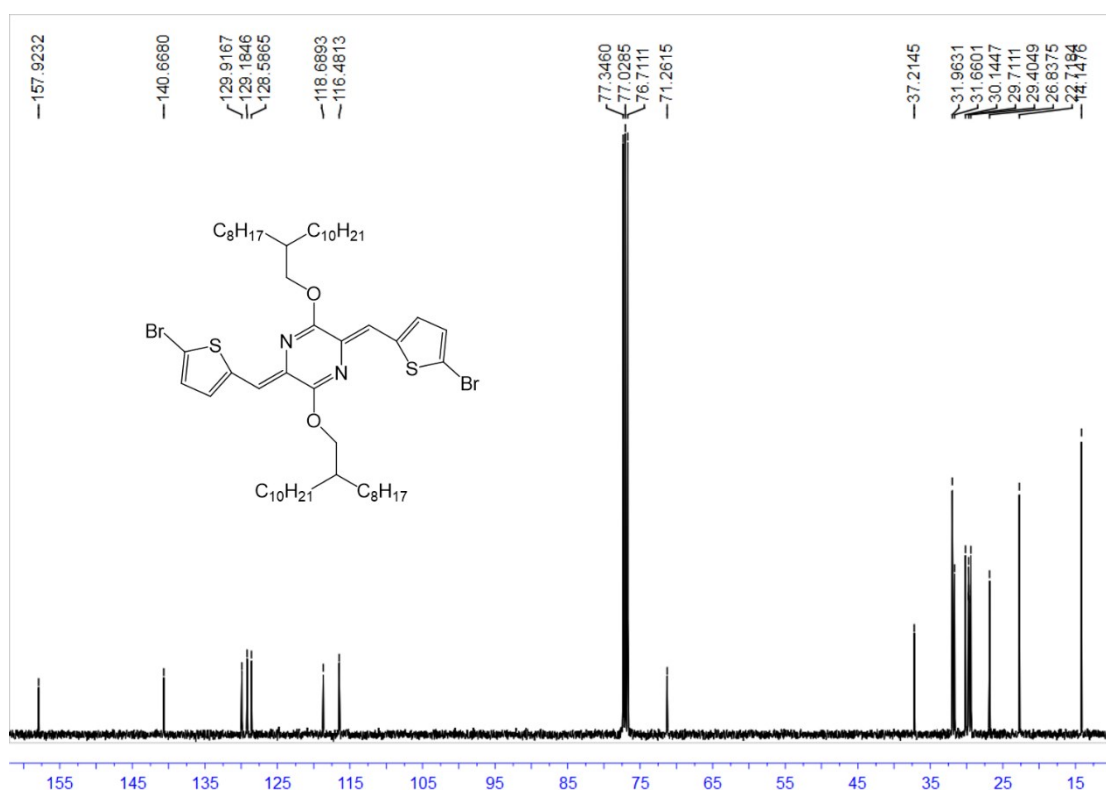


Figure S1.2 <sup>13</sup>C NMR Spectrum of the 4. (CDCl<sub>3</sub>, 298K)

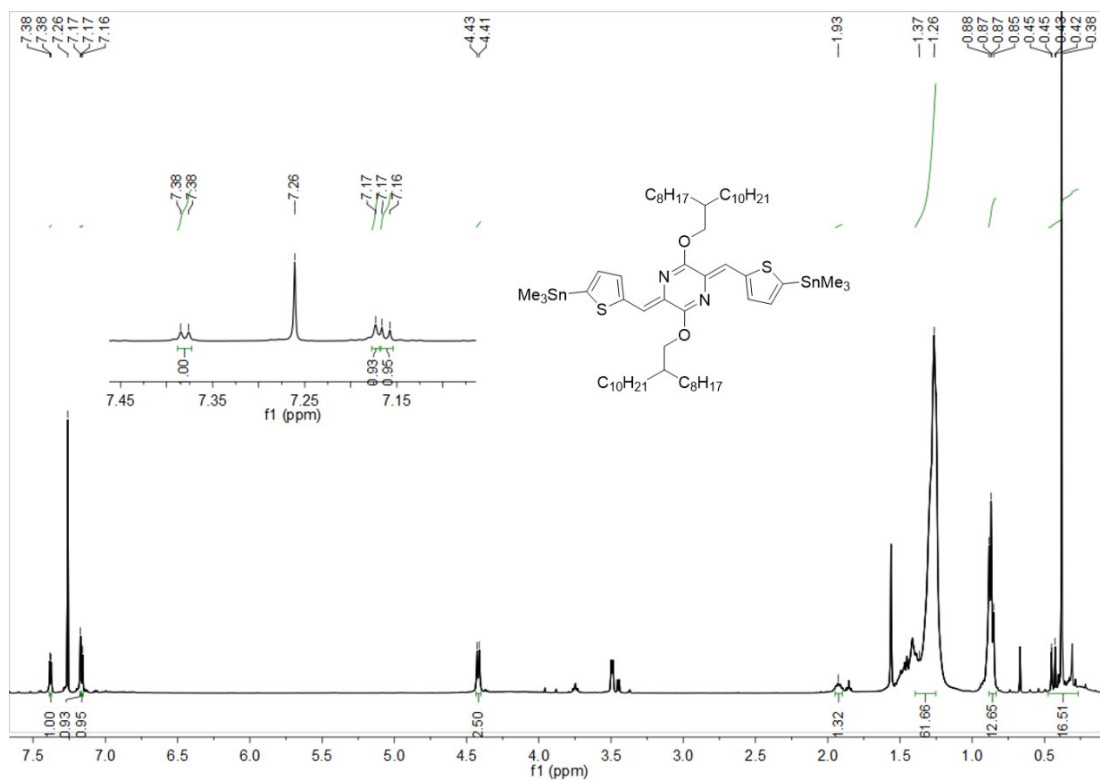


Figure S1.3  $^1\text{H}$  NMR Spectrum of the A1. The inset show a zoomed-in view of the spectrum in the 7.04-7.48 ppm region. ( $\text{CDCl}_3$ , 298K)

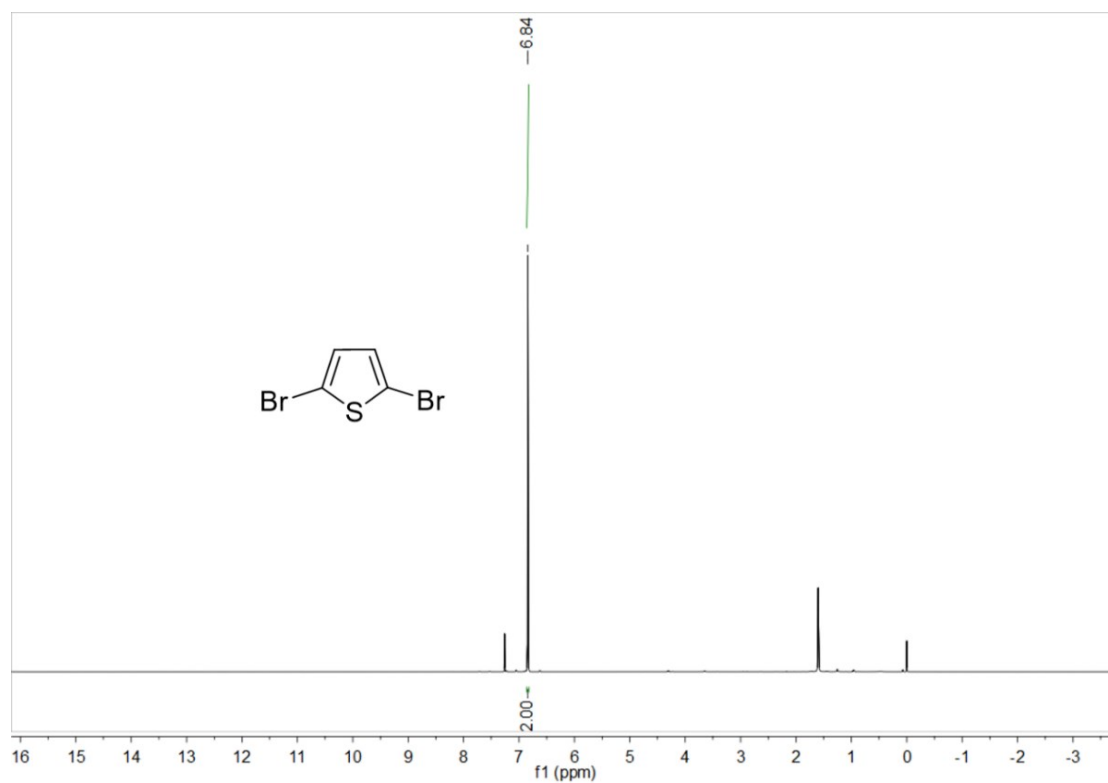


Figure S1.4  $^1\text{H}$  NMR Spectrum of the D1. ( $\text{CDCl}_3$ , 298K)

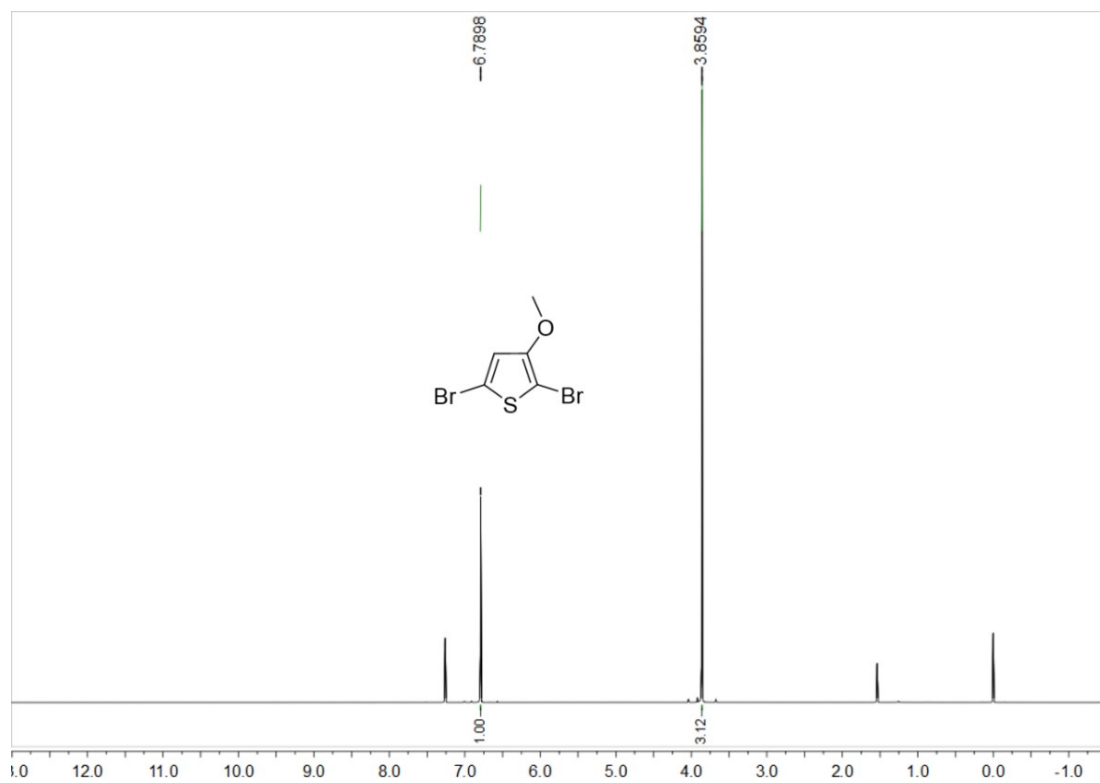


Figure S1.5 <sup>1</sup>H NMR Spectrum of the D2. (CDCl<sub>3</sub>, 298K)

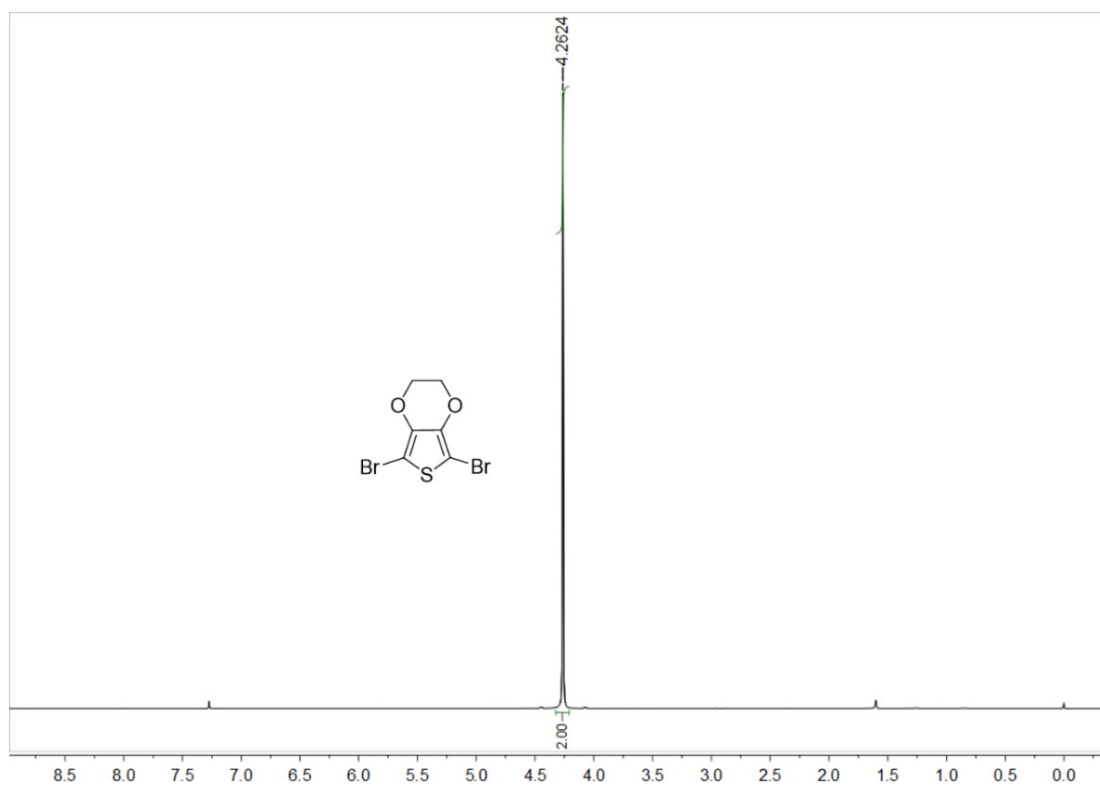


Figure S1.6 <sup>1</sup>H NMR Spectrum of the D3. (CDCl<sub>3</sub>, 298K)



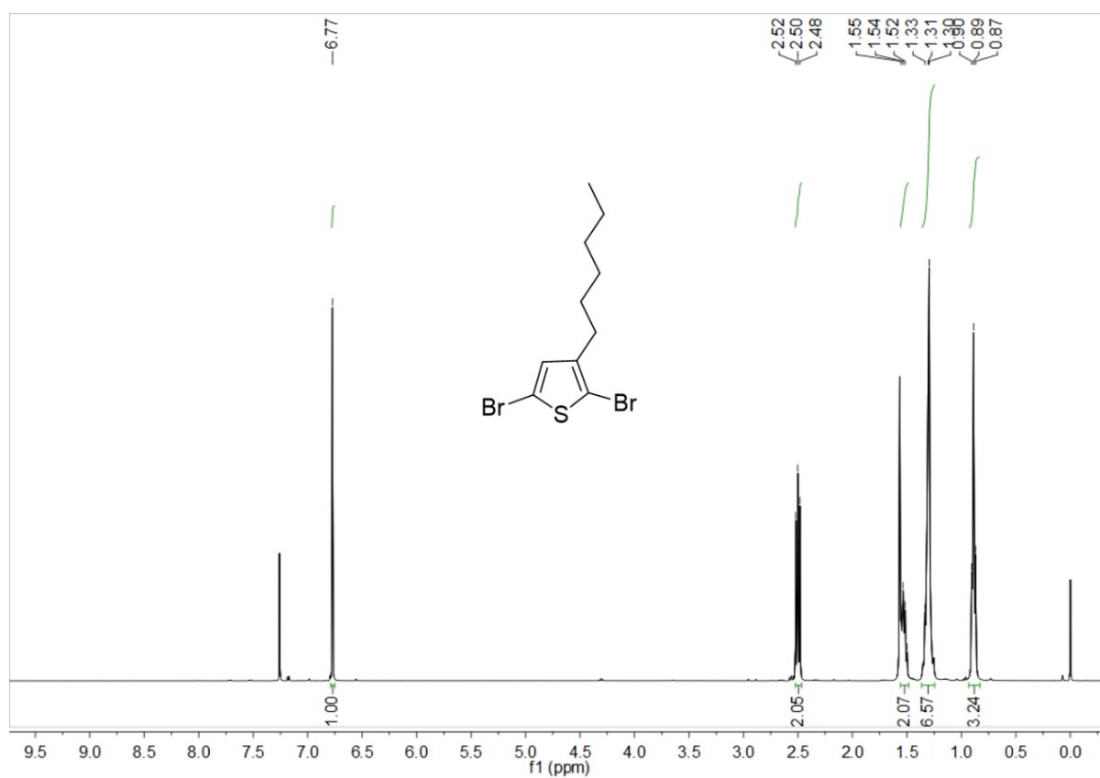


Figure S1.7  $^1\text{H}$  NMR Spectrum of the D4. ( $\text{CDCl}_3$ , 298K)

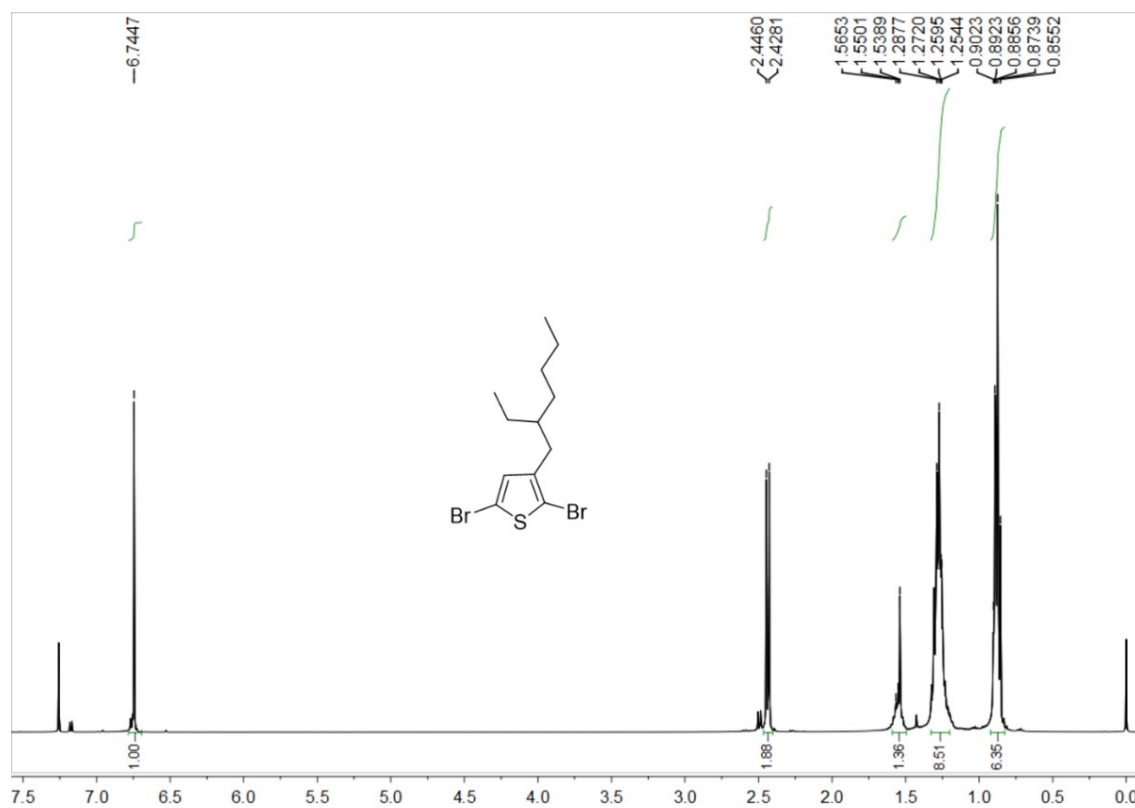


Figure S1.8  $^1\text{H}$  NMR Spectrum of the D5. ( $\text{CDCl}_3$ , 298K)

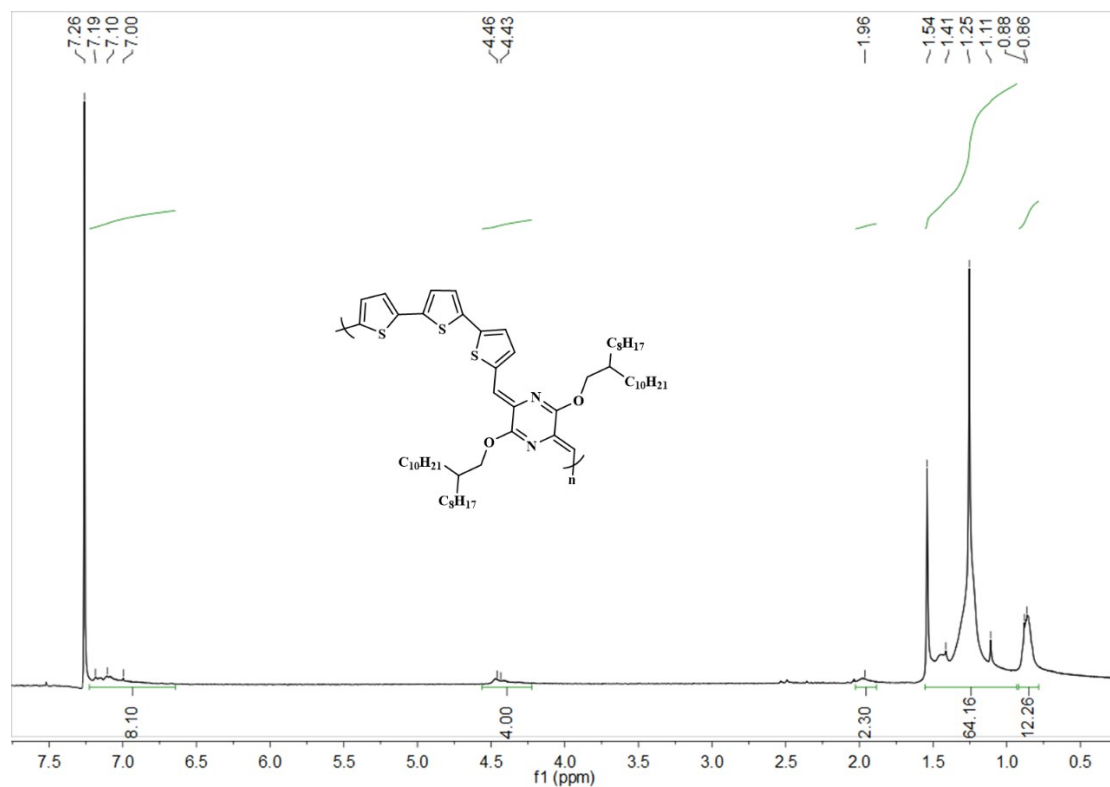


Figure S1.9 <sup>1</sup>H NMR Spectrum of the P1. (CDCl<sub>3</sub>, 298K)

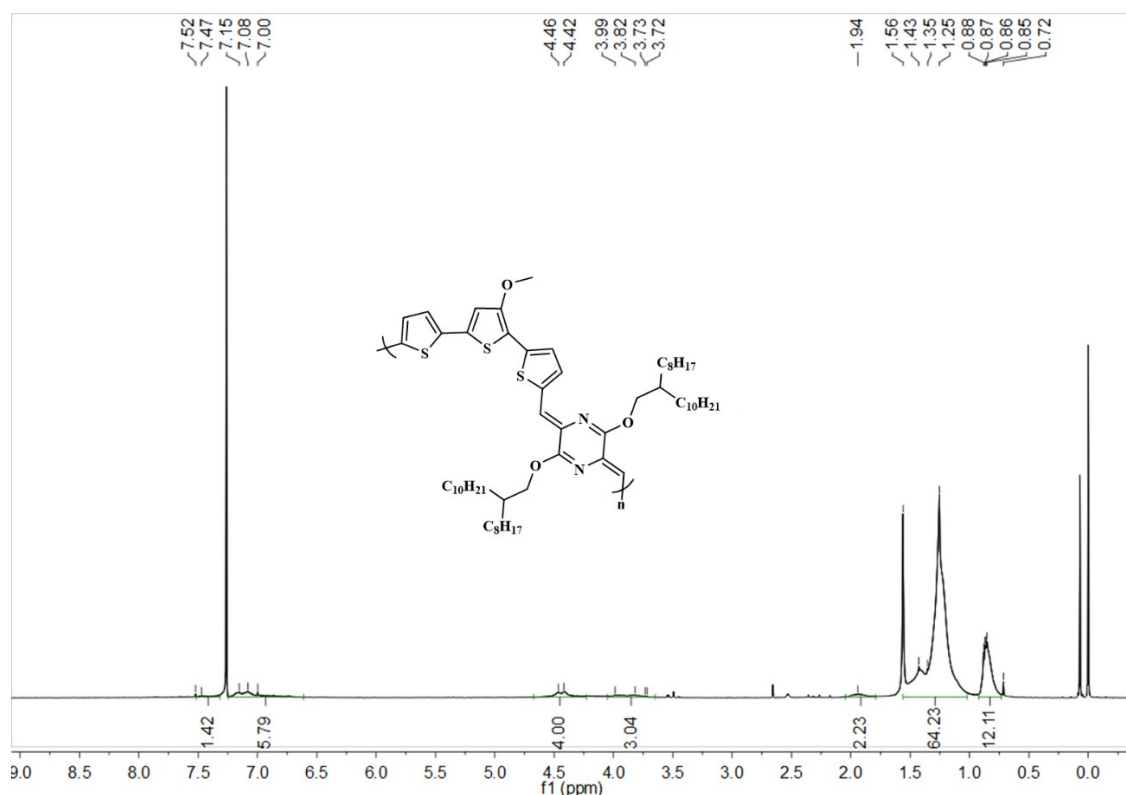


Figure S1.10 <sup>1</sup>H NMR Spectrum of the P2. (CDCl<sub>3</sub>, 298K)

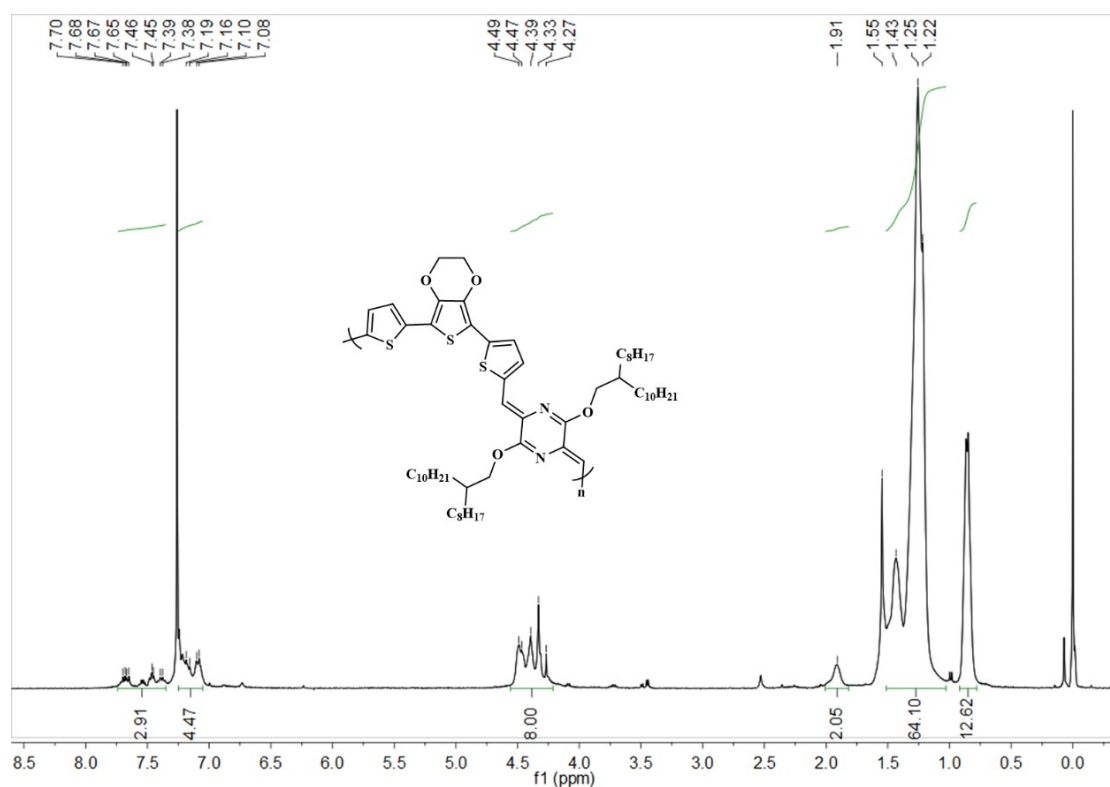


Figure S1.11  $^1\text{H}$  NMR Spectrum of the P3. ( $\text{CDCl}_3$ , 298K)

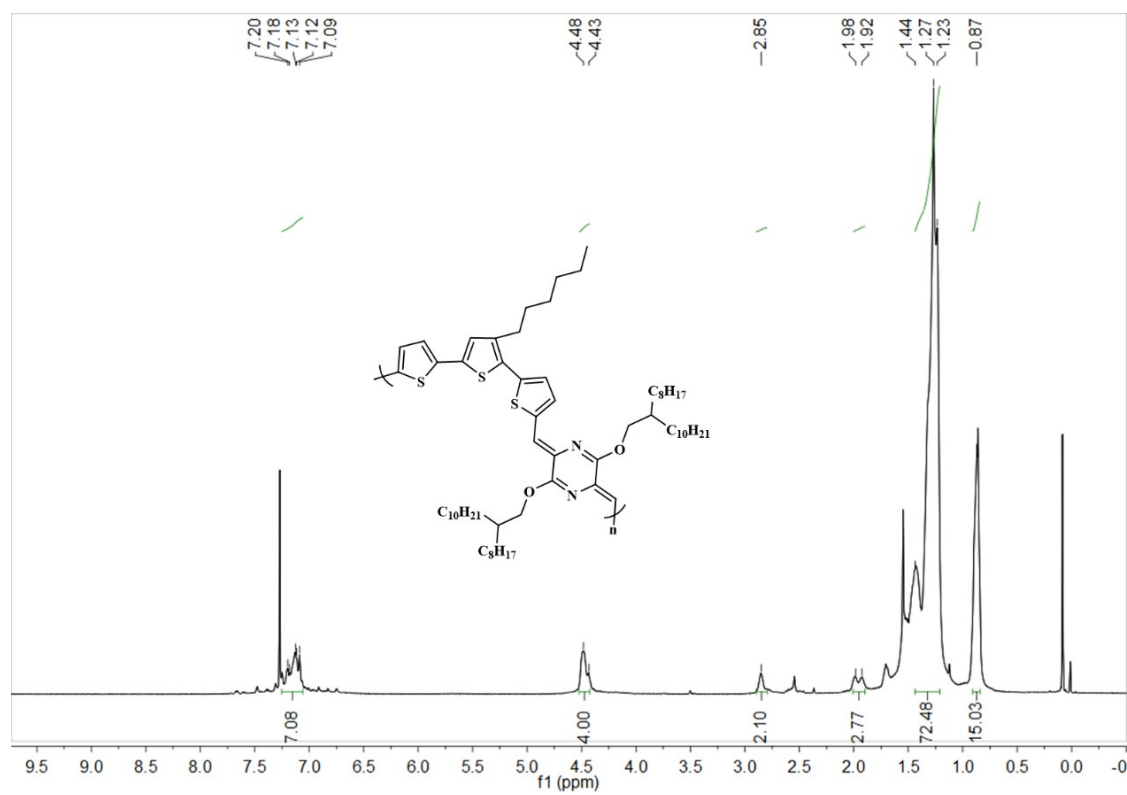


Figure S1.12  $^1\text{H}$  NMR Spectrum of the P4. ( $\text{CDCl}_3$ , 298K)

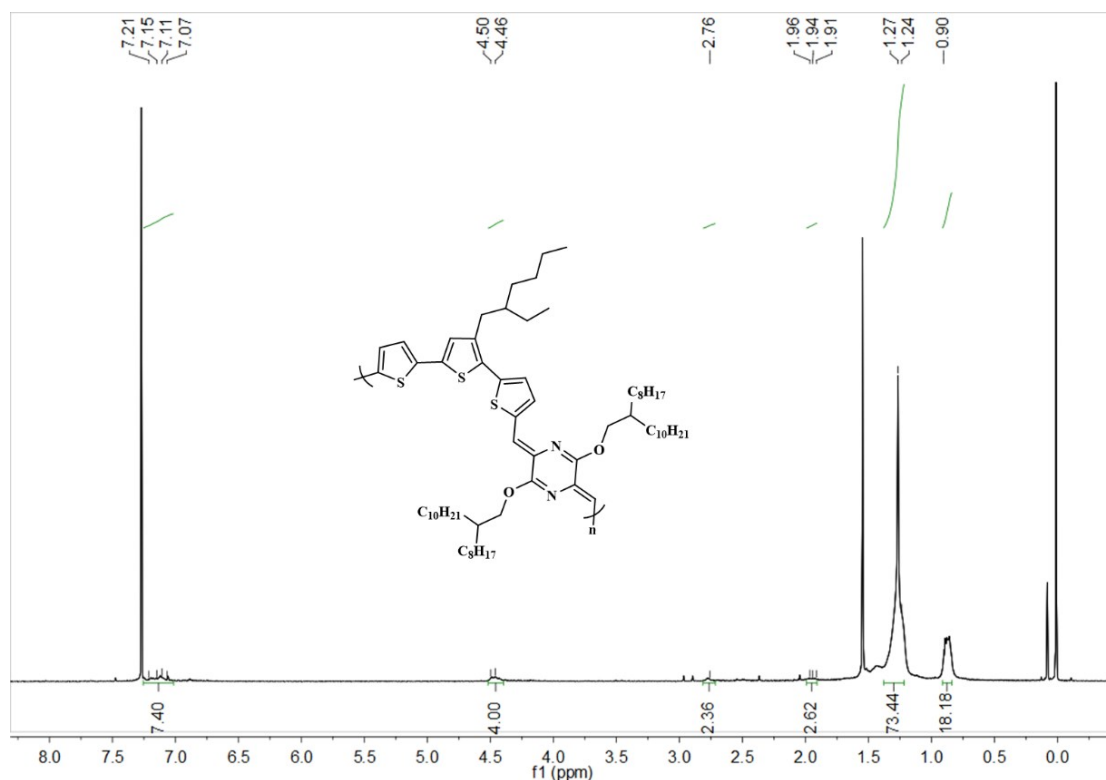


Figure S1.13  $^1\text{H}$  NMR Spectrum of the P5. ( $\text{CDCl}_3$ , 298K)

### Preparation of polymer nanoparticles (NPs) (S1)

First, 1 mg of polymer and 6 mg of DSPE-mPEG<sub>2000</sub> were dissolved in 4 mL THF and quickly added into 36 mL of distilled water under the action of strong ultrasound. After 5 min of ultrasound, THF and most of the water were removed by vacuum distillation to obtain 1 mL of NPs solution with a concentration of 1 mg mL<sup>-1</sup>.

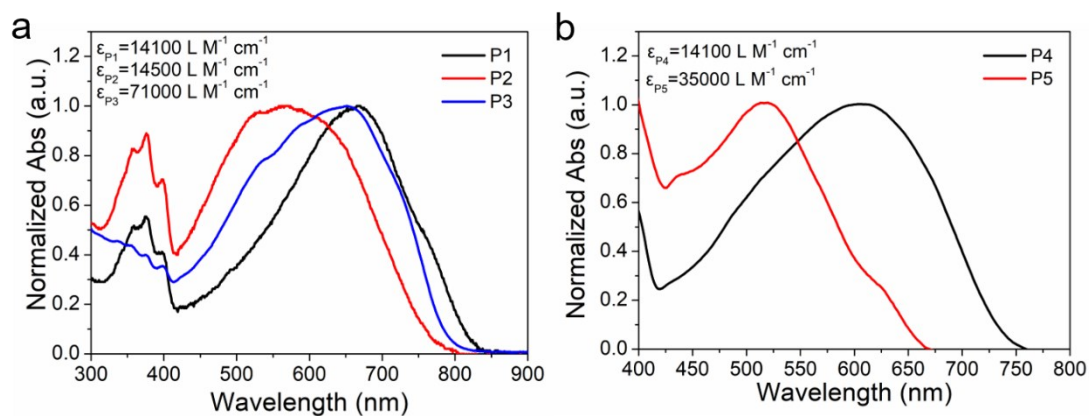


Figure S2 a) and b) the UV-Vis-NIR absorption spectrum of the P1 NPs, P2 NPs, P3 NPs, P4 NPs, and P5 NPs in chloroform solution, respectively.

Table S1. Gel Permeation Chromatography (GPC) Spectrum of polymers.

Dye	$M_w$ [g/mol]	$M_n$	PDI
P1	$7.457 \times 10^3$	$6.384 \times 10^3$	1.168
P2	$5.936 \times 10^3$	$5.973 \times 10^3$	0.994
P3	$7.572 \times 10^3$	$5.449 \times 10^3$	1.389
P4	$9.469 \times 10^3$	$5.787 \times 10^3$	1.636
P5	$3.455 \times 10^3$	$8.242 \times 10^2$	4.192

Molecular weight and polydispersity of polymer are listed in table. Number-average molecular weight:  $M_n$ ; Weight-average molecular weight:  $M_w$ ; Polydispersity index: PDI. Note: The polydispersity less than or near 1 in the above polymers may be due to error or other reasons.

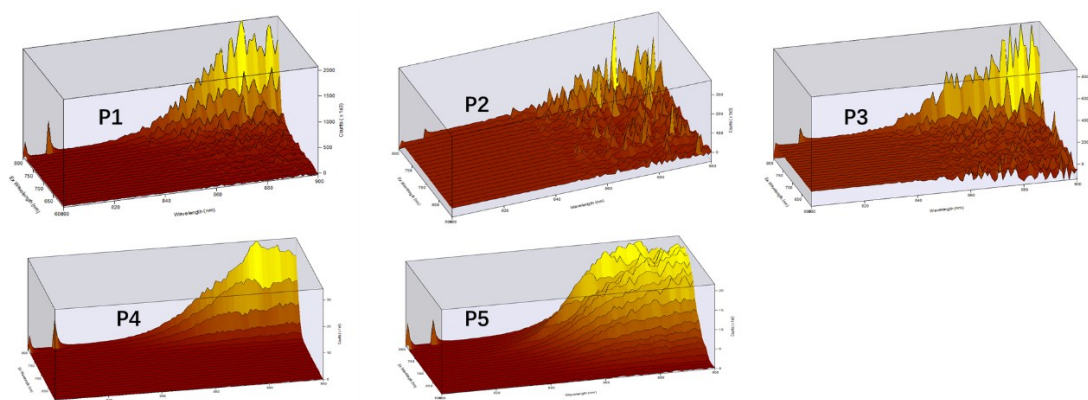
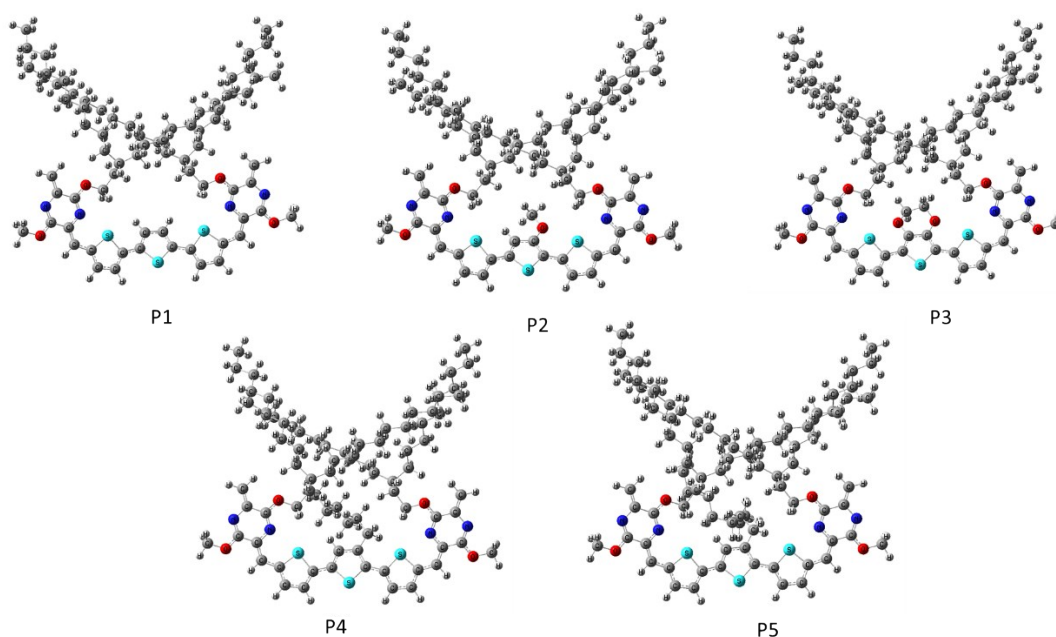
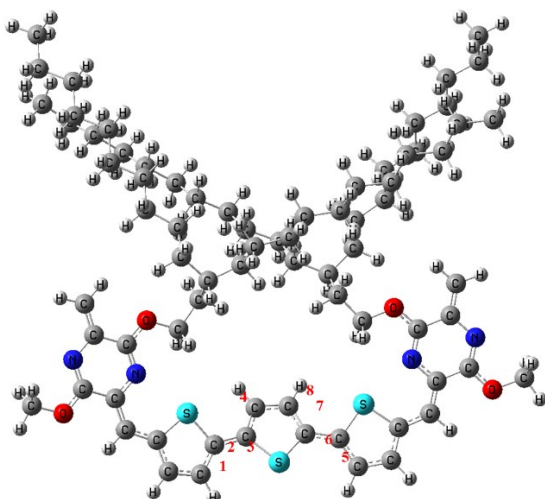


Figure S3 Photoluminescence excitation mapping of P1-P5.

### Theoretical Calculation (S2)



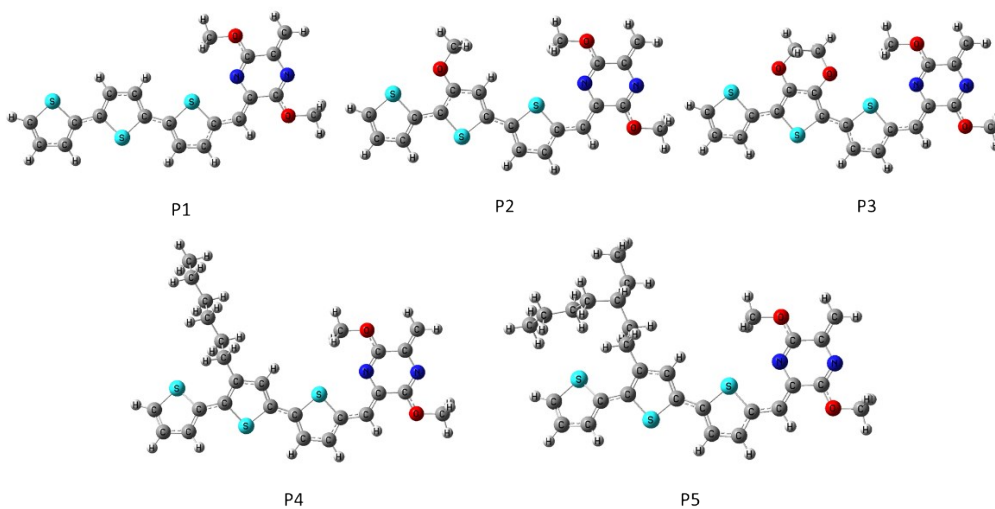
#### S2.1 P1~P5 simulation system.



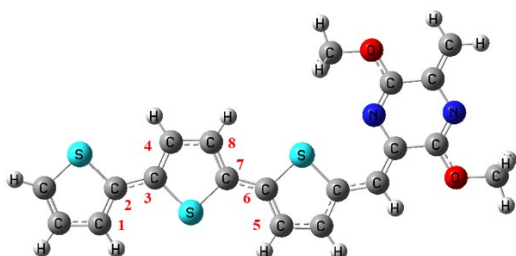
Structure	D <sub>(1-2-3-4)</sub> (°)	D <sub>(5-6-7-8)</sub> (°)	H-L Gap (eV)	$\lambda_{(Theo)}$ (nm)	$\lambda_{(expt)}$ (nm)
P1	16.18	20.15	2.31	613.14	637
P2	13.69	12.72	2.17	651.14	579
P3	11.69	14.35	2.21	638.6	594
P4	10.61	19.10	2.26	625.61	610
P5	12.22	16.57	2.26	627.49	512

Notes: D (1-2-3-4) is the size of the dihedral angle formed by atoms No. 1, 2, 3, and 4, i.e. the angle between the plane of atoms No. 1, 2, 3 and 2, 3 and 4. Level of Theory: B3LYP-D3/Def2-SV(P)

### S2.2 P1~P5 simulation system calculation.



### S2.3 P1~P5 simplify the system.



Structure	D <sub>(1-2-3-4)</sub> (°)	D <sub>(5-6-7-8)</sub> (°)	H-L Gap (eV)	$\lambda_{(Theo)}$ (nm)	$\lambda_{(expt)}$ (nm)
P1	7.39	0.84	2.55	537.92	637
P2	0.00	0.00	2.42	567.87	579
P3	0.30	0.22	2.46	557.31	594
P4	28.00	6.77	2.58	531.01	610
P5	28.91	9.57	2.59	529.26	512

Level of Theory: B3LYP-D3/Def2-TZVP

### S2.3 P1~P5 simplify the system calculation.

ligand	$\lambda$ (simulation, Theo) (nm)	$\lambda$ (simplification, Theo) (nm)	$\lambda$ (reference, Theo) (nm)	$\lambda$ (expt) (nm)
H	613.14	537.92	345.6	637
OCH <sub>3</sub>	651.14	567.87	364.26	579
(OCH <sub>2</sub> ) <sub>2</sub>	638.6	557.31	373.23	594
C <sub>6</sub> H <sub>13</sub>	625.61	531.01	356.4	610
C <sub>9</sub> H <sub>19</sub>	627.49	529.26	355.78	512

#### S2.4 Comparison of theoretical and experimental results.

Theoretical Calculation Additional Notes:

1. Simulation system. The molecular model is not simplified, but only 1.5 repeating units are selected. As can be seen from the structure, there is no molecular interaction between each repeating unit
2. The system is simplified. Compared with the simulated system, the two substituents of C<sub>10</sub>H<sub>21</sub> and C<sub>8</sub>H<sub>17</sub>, which are connected to the heterocyclic ring, are simplified
3. The reference system, which is a simple conjugate chain, only considers the influence of substituents on the absorption wavelength of the conjugate chain.

Because the experimental result is a periodic system, it is not easy to directly compare the absolute value of the absorbed light wavelength calculated in theory with the experimental result, but the relative value of the absorbed light wavelength in different structures should be able to compare with the experimental value. You can see that the three kinds of theoretical calculation results of absorption wavelength is relative order, C<sub>6</sub>H<sub>13</sub> and C<sub>9</sub>H<sub>19</sub> ligand replaced results are close to, the results of the two oxygen ligands are close to, illustrates a few kinds roughly theory model, the theoretical calculation result is stable, the result is reliable, similar substituent, replace the effect is similar, this is reasonable, but the theoretical results don't quite match the experimental ones, for reasons that are not clear.

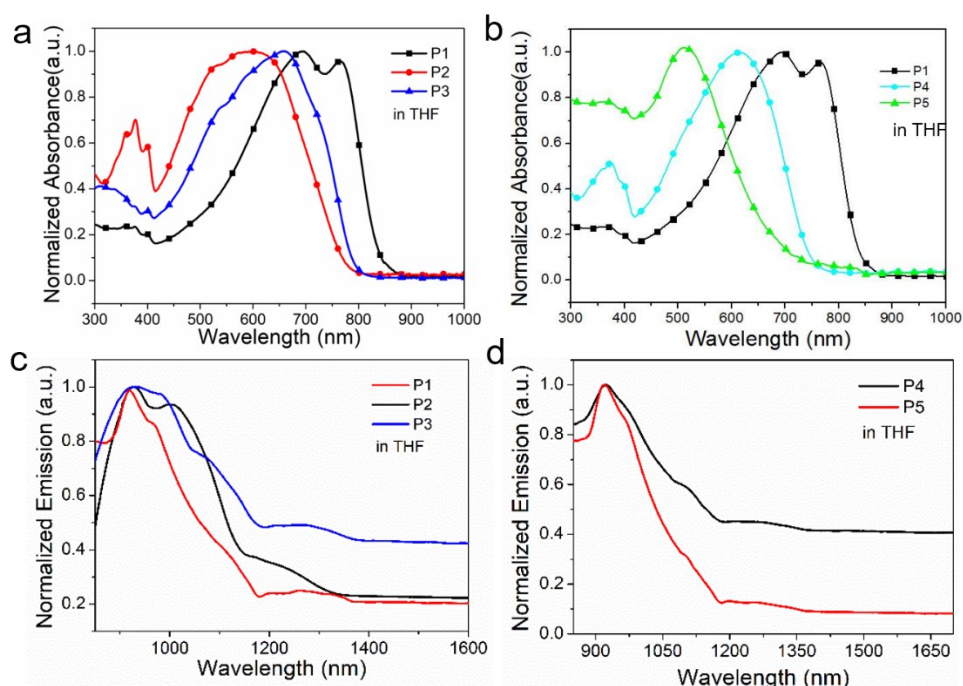


Figure S4 The absorption and Fluorescence emission spectrum in THF was obtained with an 808 nm excitation laser a) The UV-Vis-NIR absorption spectrum of the P1, P2, and P3; b) The UV-Vis-NIR absorption spectrum of the P4, and P5; c) The Fluorescence emission spectra of P1, P2, and P3; d) The Fluorescence emission spectra of P4, and P5.

## Reagents and Instruments (Table S2)

Table S2.1 Reagent

Name	Size	Source
Thiophene	99%	Aladdin
3-Methoxythiophene	98%	Aladdin
3,4-Ethylenedioxythiophene	99%	Aladdin
3-Hexylthiophene	98%	Aladdin
3-(2-Ethylhexyl)thiophene	97%	Aladdin
1,4-diacetylpiperazine-2,5-dione	96%	Macklin
5-Bromo-2-thiophenecarboxaldehyde	97%	Aladdin
Trimethyltin chloride	1.0M in hexane	J&K Chemical
n-Butyllithium	1.6M in hexane	Aladdin



Terakis(triphenylphosphine)palladium(0)	99%	Macklin
Calcium hydride	98.5%	Aladdin
N-Bromosuccinimide	98%	Aladdin
Dichloromethane	99.5%	Aladdin
Petroleum ether	AR	Aladdin
Methanol	99.5%	Aladdin
N,N-Dimethylformamide	99.5%	Aladdin
Tetrahydrofuran	99%	Aladdin
BCap 37 cells		Hangzhou Genuo Biomedical Technology Co., Ltd Co., Ltd
Fetal bovine serum		Hangzhou Genuo Biomedical Technology Co., Ltd Co., Ltd

**Table S2.2 Instrument**

Instrument	Type	Company
Vacuum pump	2XZ-2	Linhai Tan Vacuum Equipment Co., Ltd
Rotary evaporators	RV8	IKA group experimental equipment co., LTD
Aqua bi-distilling apparatus	SZ-93	Shanghai Yarong Biochemical Instrument Factory
Ultrasonic cleaner	QT08	Tianjin Ruipu Electronic Instrument Co., Ltd
Magnetic stirring apparatus	IKAC-MAG HS 7	Beijing Dalong Xingchuang Experimental Instrument Co. Ltd
Uv-Vis-NIR spectrophotometer	UH4150	Hitachi High-Technologies Corporation
Transmission electron microscopy	JSM-6309LV	JEOL company
Table centrifuge	L400	Hunan Xiangyi Laboratory Instrument Development Co. Ltd
Fluorescence spectrophotometer	FLS 1000	Edinburgh Instruments,EI
NIR-II living small animal imager	UniNano-NIR II	Huijia Biological Co., Ltd
Electronic scales	FA1004B	Shanghai Keping Instrument Co., Ltd

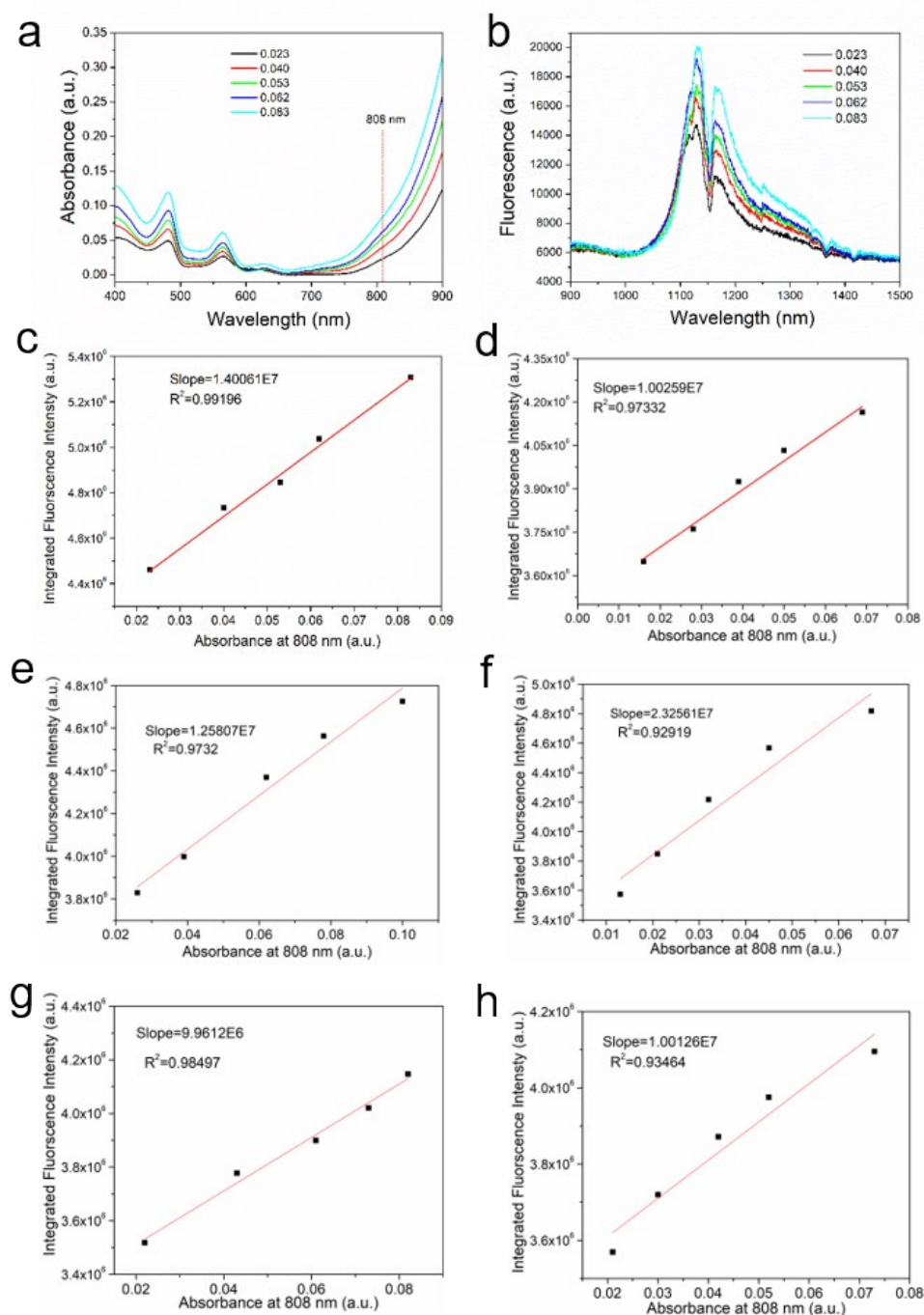


Figure S5 Fluorescence quantum yield measurement. Absorbance a) and fluorescence b) spectrum of IR-26 in DCE; c), d), e), f), g) and h) Linear fit of IR-26, P1 NPs, P2 NPs, P3 NPs, P4 NPs, and P5 NPs. The quantum yield was calculated in the following manner:

$$QY_{P1} = QY_{IR\ 26} \times (Slop_{P1} / Slop_{IR\ 26}) \times (n_{P1}^2 / n_{IR-26}^2)$$

The refractive indices (SNP) of water and dichloromethane solution was 1.333 and 1.424 in this case, respectively.