Supporting Information

Self-assembled Amphiphilic NIR-II Emissive Nano-micelles for Imaging-Guided Photothermal Therapy of Colorectal cancer

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1. Experimental materials and methods Procedures

1.1 General information

All air and moisture-sensitive reactions were carried out in flame-dried glassware under a nitrogen atmosphere. Reactive liquid compounds were measured and transferred by gas-tight syringes and were added in the reaction flask through rubber septa. Tetrahydrofuran (THF) was freshly distilled from sodium benzophenoneketyl. Toluene was distilled from CaH_2. Unless otherwise noted, all reagents were obtained commercially and used without further purification.

1.2 NMR spectrum

^{1}H and ^{13}C NMR spectra were collected on 500 MHz NMR spectrometers (Bruker AVANCE) using CDCl3. Chemical shifts are reported in parts per million (ppm). Chemical shifts for protons are reported in parts per million downfield and are referenced to residual protium in the NMR solvent ($CDCl_3$ = δ 7.26). Chemical shifts for carbon are reported in parts per million downfield and are referenced to the carbon resonances of the solvent (CDCl3 = δ 77.0). Data are represented as follows: chemical shift, multiplicity (br = broad, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet), coupling constants in Hertz (Hz), integration.

1.3 Mass spectroscopy

Mass spectra were in general recorded on a QSTAR Elite (ABI).

1.4 Chromatography

Column chromatography was performed with silica gel (200-300 mesh ASTM).

1.5 UV- VIS-NIR spectroscopy

Ultraviolet-visible-near Infrared (UV-VIS-NIR) absorption spectra were recorded on Shimadzu UV-3600Plus.

1.6 Size exclusion chromatography

Size exclusion chromatography (SEC) was performed on Malvern Viscotek 270max, 10 μm PLgel 600 × 7.5 mm column, THF used as the mobile phase at a flow rate of 1.0 mL/min at 40℃, linear polystyrene calibration, equipped with refractive index (RI) and ultraviolet (UV) detector.

1.7 Quantum yield test

The fluorescence quantum yield (QY) of the IR-BTGP was measured with the previous equation [1,2]. The fluorescence spectra in the region of 900-1800 nm were measured by an array detector and a spectrometer under an 808 nm diode laser excitation (RMPC lasers, 160 mW). The fluorescence quantum yield was determined against the reference fluorophore IR-FEP with a known quantum yield of 2.0% (Φ_{st}) in an aqueous solution [1,2]. All samples were measured at 25℃, and reference fluorophore IR-FEP was used with an optical density (OD) of 0.1 at 808 nm. Their NIR-II fluorescence emission intensities were measured under 808 nm excitation.

With the parameters of optical density (OD) at 808 nm, spectrally integrated fluorescence intensity (F) and refractive index of the solvent η, the QY of the sample can be calculated according to the following equation:

$$QY_{\text{sample}} = QY_{\text{ref}} \times \frac{slope_{\text{sample}}}{slope_{\text{ref}}} \times \frac{\eta^2_{\text{sample}}}{\eta^2_{ref}}$$

Both η_{sample} and η_{ref} are the refractive index of water.

1.8 Photothermal conversion efficiency

The photothermal conversion efficiency was calculated using the following equation: where h is the heat transfer coefficient, S is the surface of the container, T_{max} is the steady-state maximum temperature, T_{surr} is the ambient room temperature, T is the

instantaneous temperature during cooling, t represents the time it takes by T cooled to room temperature, C represents approximate to the specific heat capacity of water, m is the mass of the solution (g), Q_0 represents the energy input by the same solvent without in the same quartz cuvette after the same laser irradiation, A_λ is the absorbance of IR-BTGP at 808 nm, and I is the laser power density[1,2]. According to the above equation, the η value of IR-BTGP was determined to be about 30.2%.

$$\eta = \frac{\mathrm{hs}(T_{\max} - T_{\mathrm{surr}}) - Q_0}{I(1-10^{-A_\lambda})}$$

2. Synthetic procedures and characterization

2.1 Synthetic routes for NIR-II fluorophores

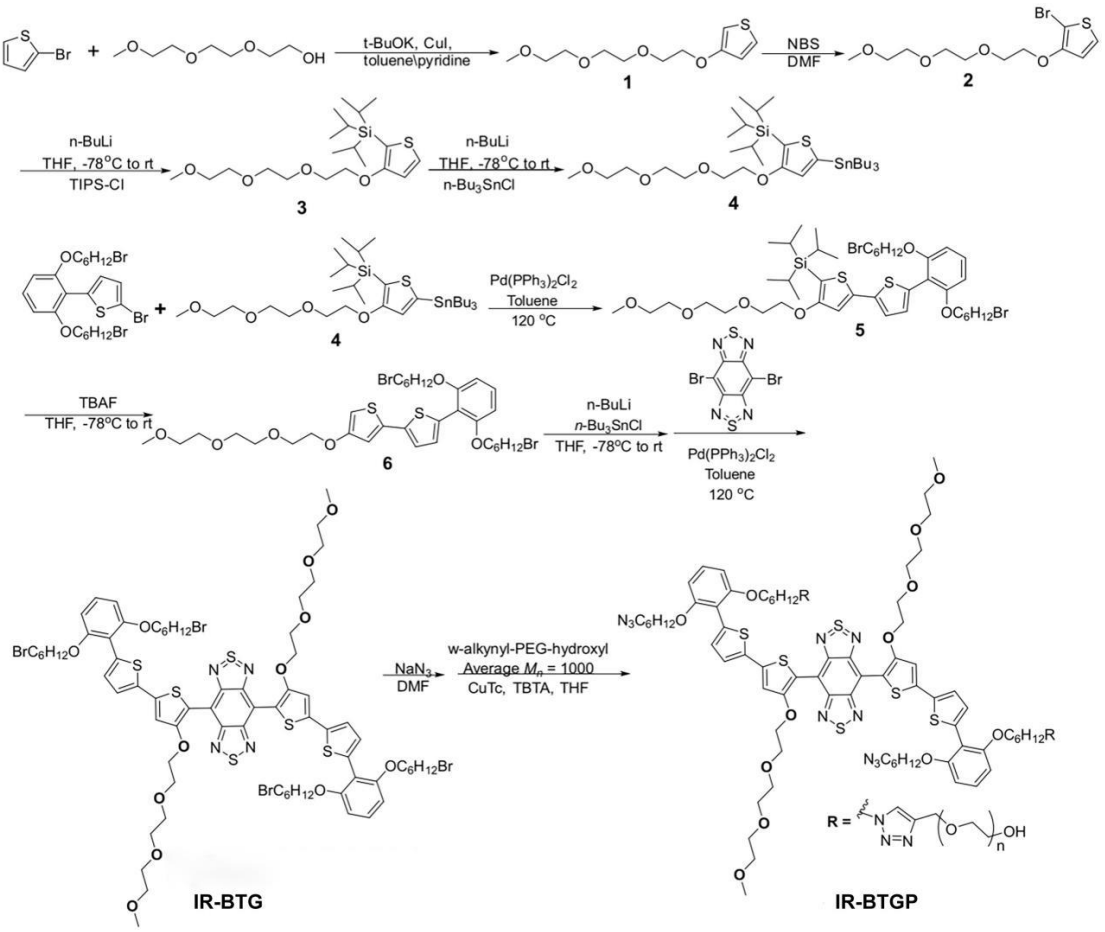

Fig. S1 Synthetic routes of IR-BTG and IR-BTGP

2.2 Synthetic procedures of compound 1

3-(2-(2-(2-methoxyethoxy)ethoxy)ethoxy)thiophene (1) A dry 250 mL two-neck round bottom flask with condenser and magnetic stir bar was charged with 10.5 g potasstium tertbutylate (94 mmol) and 2.34 g copper iodide (12 mmol). Next, 10 mL pyridine and 100 mL toluene mixture solution were added and stirred until dissolved, and 15 mL triethylene glycol monomethyl ether was added. The reaction mixture was then allowed to stir at room temperature for 30 min. 5.8 mL of 3-bromothiophene (62 mmol) was then added in one portion and the reaction mixture was heated to 110 ^{o}C for 24 h. After cooling to room temperature, the reaction mixture was centrifuged and concentrated under a vacuum. 50 mL dichloromethane was added and washed with 5 M HCl(aq). The aqueous layers were extracted with DCM, and the combined organic portions were dried with MgSO4 and concentrated under reduced pressure. The crude material was purified via column chromatography, eluting with 1:1 ethyl ether in hexanes (v/v). The desired product was isolated as a yellow tinted oil (10.35 g, 68% yield). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.18 (dd, J = 5.3, 3.1 Hz, 1H), 6.79 (dd, J = 5.3, 1.5 Hz, 1H), 6.28 (dd, J = 3.1, 1.5 Hz, 1H), 4.17 – 4.10 (m, 2H), 3.89 – 3.83 (m, 2H), 3.77 – 3.72 (m, 2H), 3.72 – 3.64 (m, 4H), 3.57 (dd, J = 5.7, 3.6 Hz, 2H), 3.40 (s, 3H).

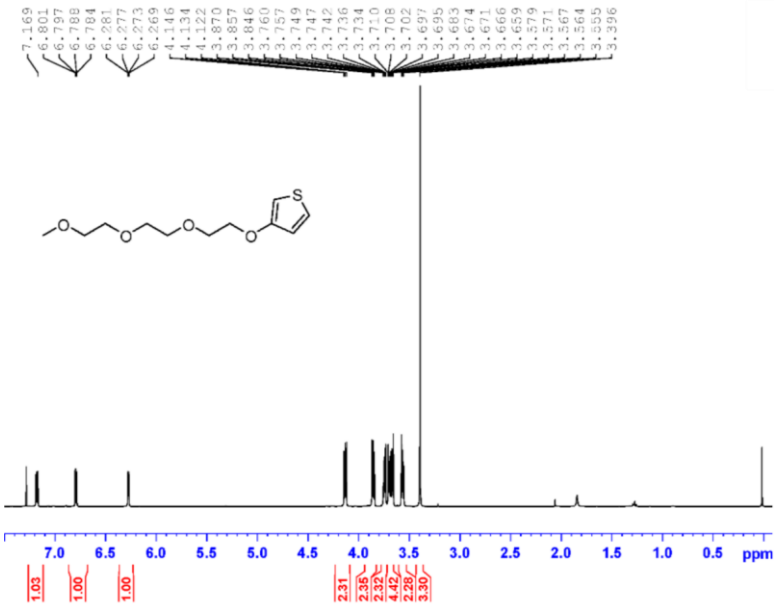

Fig. S2 ^{1}H NMR of compound 1.

2.3 Synthetic procedures and characterization of compound 2

bromo-3-(2-(2-(2-methoxyethoxy)ethoxy)ethoxy)thiophene (2) Compound 1 2.46 g (10 mmol) was dissolved in 15 mL DMF, and 1.78 mg NBS (10 mmol) was added in portions at 0 oC. After 18 h at rt, it was added just as much water as to dissolve all solids. Then it was extracted twice with ethyl acetate, the combined organic phase was washed with Na2SO3(aq) three times and dried with MgSO4 and evaporated in vacuo without further purification and afford 2 as a yellow tinted oil (3.0 g, 92% yield). ^{1}H NMR (400 MHz, CDCl3) δ 7.18 (d, J = 6.0 Hz, 1H), 6.77 (d, J = 5.9 Hz, 1H), 4.28 – 4.13 (m, 2H), 3.86 – 3.79 (m, 2H), 3.76 – 3.70 (m, 2H), 3.70 – 3.63 (m, 4H), 3.55 (dd, J = 5.6, 3.6 Hz, 2H), 3.38 (s, 3H).

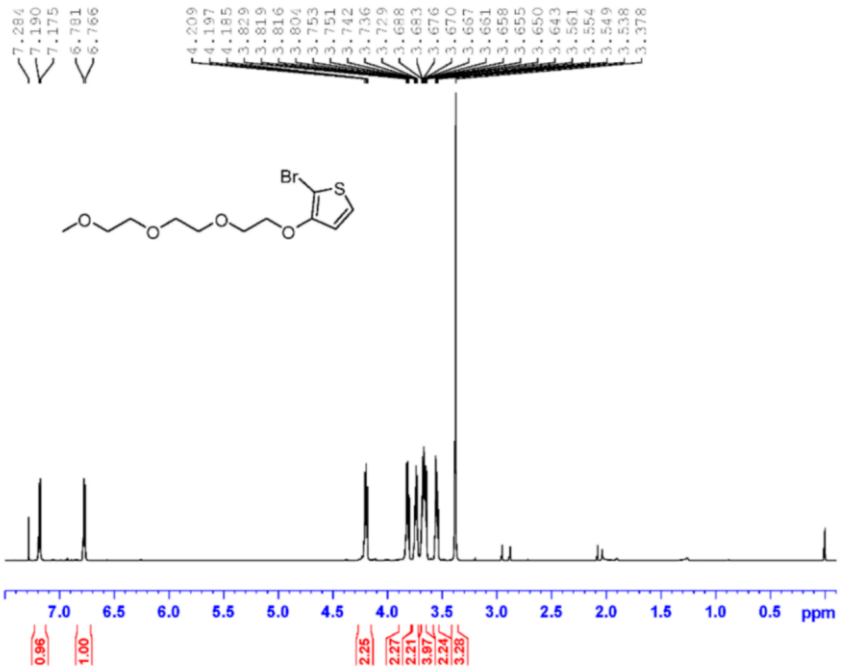

Fig. S3 ^{1}H NMR of compound 2.

2.4 Synthetic procedures and characterization of compound 3

triisopropyl(3-(2-(2-(2-methoxyethoxy)ethoxy)ethoxy)thiophen-2-yl)silane (3) To a solution of Compound 2 3.25 g(10 mmol) in 20 mL THF at -78 ^{o}C under protection gas atmosphere, n-BuLi(1.6 M in Hexane, 6.25 mL, 20 mmol)was added dropwise. After the

mixture was stirred at this temperature for another 2.0 h, triisopropylsilyl chloride (1.9 g, 12 mmol) was added to the solution. Then slowed warmed to room temperature and stirred for 12 h. After that the mixture was poured into water and extracted twice with ethyl acetate, the combined organic phase was dried with $MgSO_4$ and evaporated in vacuo. The crude material was purified via column chromatography, eluting with 2:1 ethyl ether in hexanes (v/v). The desired product was isolated as a yellow tinted oil (3.53 g, 88% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.46 (d, J = 5.0 Hz, 1H), 6.95 (d, J = 5.0 Hz, 1H), 4.16 – 4.10 (m, 2H), 3.79 (t, J = 5.2 Hz, 2H), 3.71 – 3.66 (m, 6H), 3.59 – 3.55 (m, 2H), 3.40 (s, 3H), 1.49 – 1.35 (m, 2H), 1.10 (d, J = 7.5 Hz, 19H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 163.62, 129.85, 116.51, 108.99, 71.95, 70.72, 70.67, 70.06, 69.96, 59.07, 18.78, 12.03. HRMS(ESI) calcd for $C_{20}H_{39}O_4SSi^+$, ([M+H$^+$]) 402.2294, Found 403.2333.

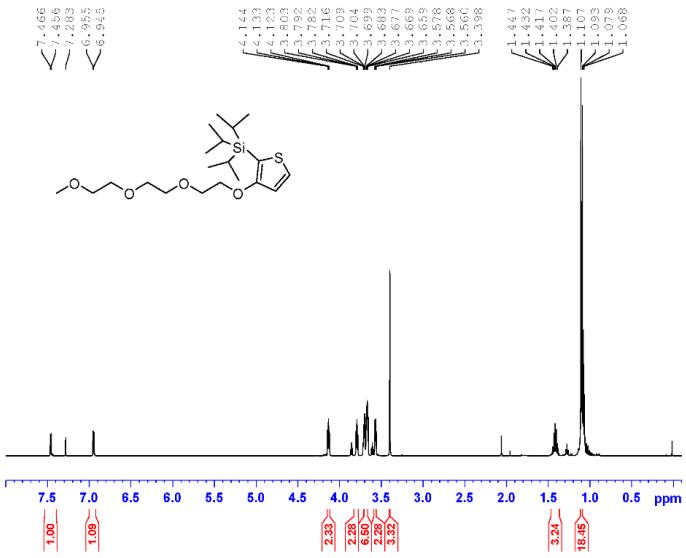

Fig. S4 ^{1}H NMR of compound 3.

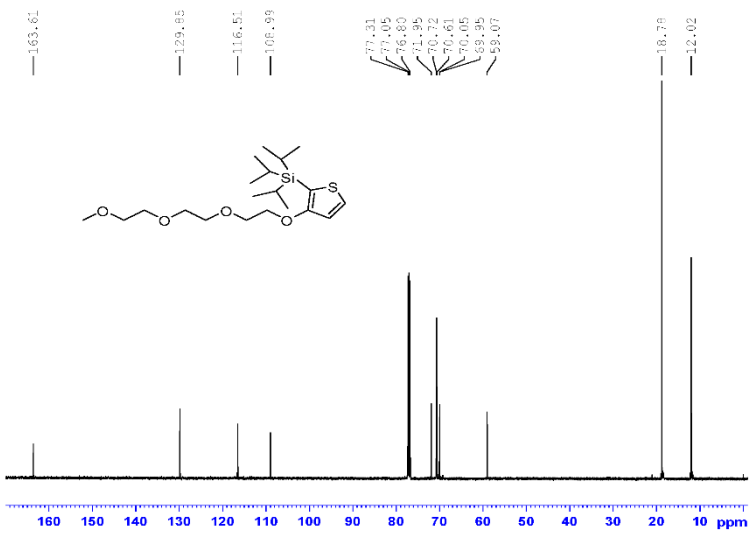

Fig. S5 ^{13}C NMR of compound 3.

2.5 Synthetic procedures of compound 4

Triisopropyl(3-(2-(2-(2-methoxyethoxy)ethoxy)ethoxy)-5-(tributylstannyl)thiophen-2-yl)silane (4) To a solution of Compound 3 402 mg(1.0 mmol) in 5 mL THF at -78 oC under protection gas atmosphere, n-BuLi(1.6 M in Hexane, 0.625 mL, 1.2 mmol)was added dropwise. After the mixture was stirred at this temperature for another 2.0 h, trinbutyltinchloride (390 mg, 1.2 mmol) was added to the solution. Then slowed warmed to room temperature and stirred for 8 h. After that the mixture was poured into water and extracted twice with ethyl acetate, the combined organic phase was dried with MgSO4 and evaporated in vacuo without further purification.

2.6 Synthetic procedures and characterization of compound 5

(5'-(2,6-Bis((6-bromohexyl)oxy)phenyl)-4-(2-(2-(2-methoxyethoxy)ethoxy)ethoxy)-[2,2'-bithiophen]-5-yl)triisopropylsilane (5) To a solution of compound 2-(2,6-bis((6-bromohexyl)oxy)phenyl)-5-bromothiophene 1.19 g (2.0 mmol), compound 4 1.66 g (2.4 mmol) in 10 mL toluene under protection gas atmosphere. Pd(PPh3)4 142 mg (0.122 mol) was added. The mixture was stirred at 110 oC for 18 h. After cooling to room temperature, the mixture was poured in to water and extracted twice with Ethyl

acetate, dried with MgSO4 and evaporated in vacuo. The crude product was subjected to column chromatography on silica gel with PE/EA 2:1 to afford 5 as a light yellow oil (1.18 g, 54 %). 1H NMR (400 MHz, CDCl3) δ 7.44 (d, J = 3.9 Hz, 1H), 7.19 (dd, J = 6.1, 4.5 Hz, 2H), 7.05 (s, 1H), 6.64 (d, J = 8.4 Hz, 2H), 4.04 (t, J = 6.3 Hz, 4H), 3.82 (t, J = 5.1 Hz, 2H), 3.76 – 3.63 (m, 8H), 3.58 (dd, J = 5.7, 3.6 Hz, 2H), 3.46 – 3.34 (m, 7H), 1.92 – 1.80 (m, 8H), 1.68 (s, 3H), 1.55 – 1.47 (m, 8H), 1.14 (d, J = 7.4 Hz, 18H). 13C NMR (101 MHz, CDCl3) δ 163.19, 157.12, 141.65, 136.97, 133.40, 129.67, 128.50, 122.15, 112.88, 112.48, 108.23, 105.46, 71.97, 70.75, 70.73, 70.62, 70.04, 68.94, 59.04, 33.80, 32.54, 29.03, 27.84, 25.42, 18.83, 12.11. HRMS(ESI) calcd for C42H67O6Br81BrS2Si, ([M+H+]) 919.24891, Found 919.24902.

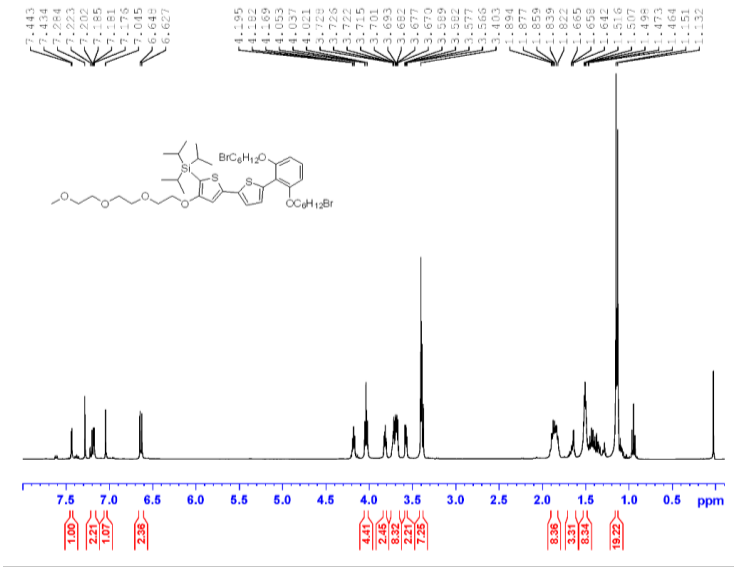

Fig. S6 [1]H NMR of compound **5.**

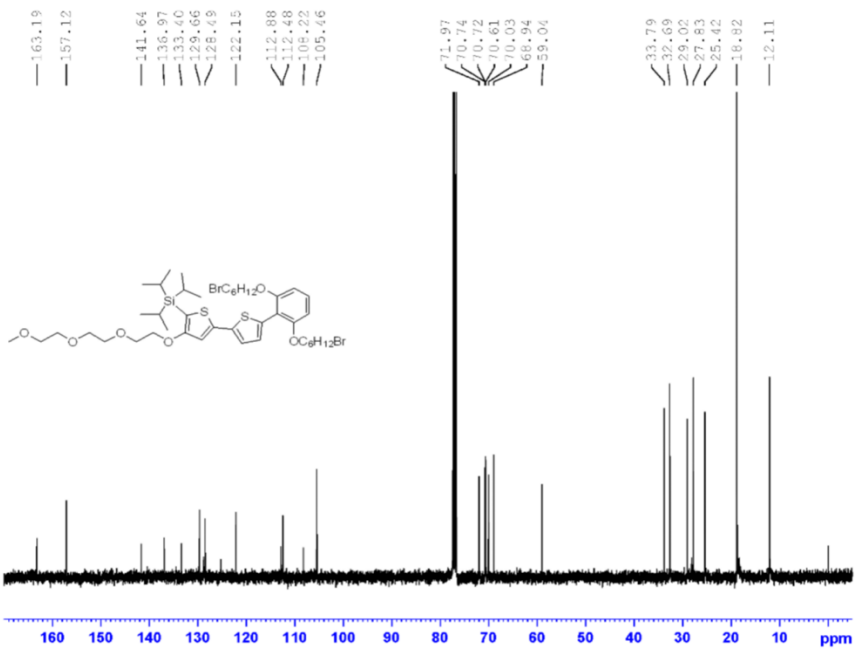

Fig. S7 ^{13}C NMR of compound **5.**

2.7 Synthetic procedures and characterization of compound 6

5'-(2,6-Bis((6-bromohexyl)oxy)phenyl)-4-(2-(2-(2-methoxyethoxy)ethoxy)ethoxy)-2, 2'-bithiophene (6) To a solution of compound 5 918 mg(1 mmol) in 4 mL THF at -78 oC under protection gas atmosphere, Tetrabutylammonium fluoride (1.0 M in THF, 4 mL, 4 mmol) was added. After the mixture was stirred at this temperature for another 1.0 h. Then slowed warmed to room temperature and stirred for 4 h. After that the mixture was poured into water and extracted twice with ethyl acetate, the combined organic phase was dried with MgSO4 and evaporated in vacuo. The crude material was purified via flash column chromatography, eluting with hexanes to ethyl acetate. The desired product was isolated as a yellow tinted oil (722 mg, 95 %). ^{1}H NMR (400 MHz, CDCl3) δ 7.43 (d, J = 3.9 Hz, 1H), 7.30 (d, J = 3.9 Hz, 1H), 7.19 (t, J = 8.3 Hz, 1H), 7.05 (d, J = 5.5 Hz, 1H), 6.91 (d, J = 5.5 Hz, 1H), 6.63 (d, J = 8.4 Hz, 2H), 4.02 (t, J = 6.3 Hz, 4H), 3.93 – 3.84 (m, 2H), 3.75 (dd, J = 5.8, 3.5 Hz, 2H), 3.71 – 3.60 (m, 4H), 3.54 (dd, J = 5.7, 3.6 Hz, 2H), 3.44 – 3.34 (m, 7H), 1.90 – 1.78 (m, 8H), 1.56 – 1.44 (m, 8H). ^{13}C NMR (101 MHz,

CDCl3) δ 157.15, 151.93, 134.56, 132.35, 129.03, 128.23, 122.23, 121.03, 117.11, 113.30, 105.56, 71.93, 71.17, 70.91, 70.68, 70.57, 70.06, 68.94, 59.01, 33.88, 32.70, 29.04, 27.86, 25.41.HRMS(ESI) calcd for C33H47O6Br81BrS2, ([M+H+]) 763.1155, Found 763.1154.

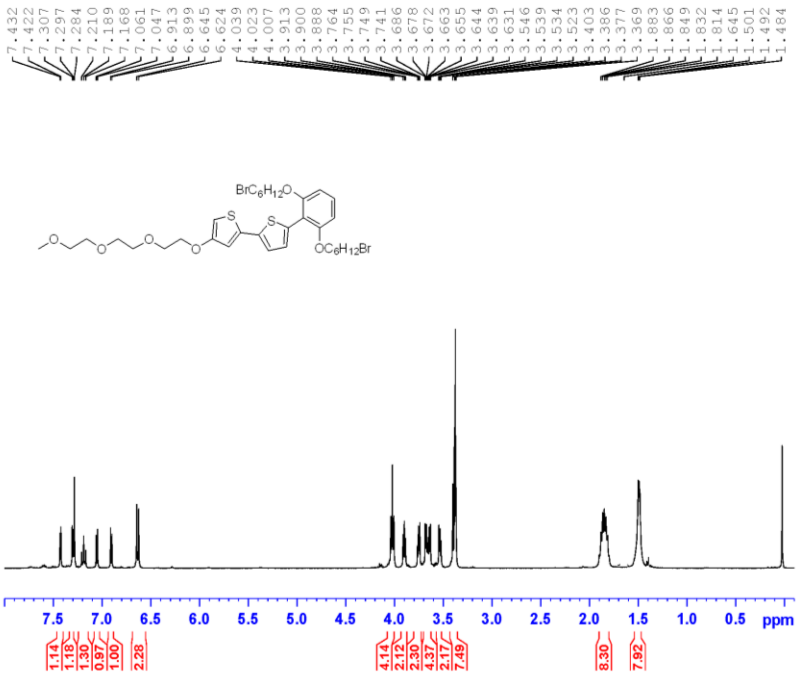

Fig. S8 [1]H NMR of compound **6.**

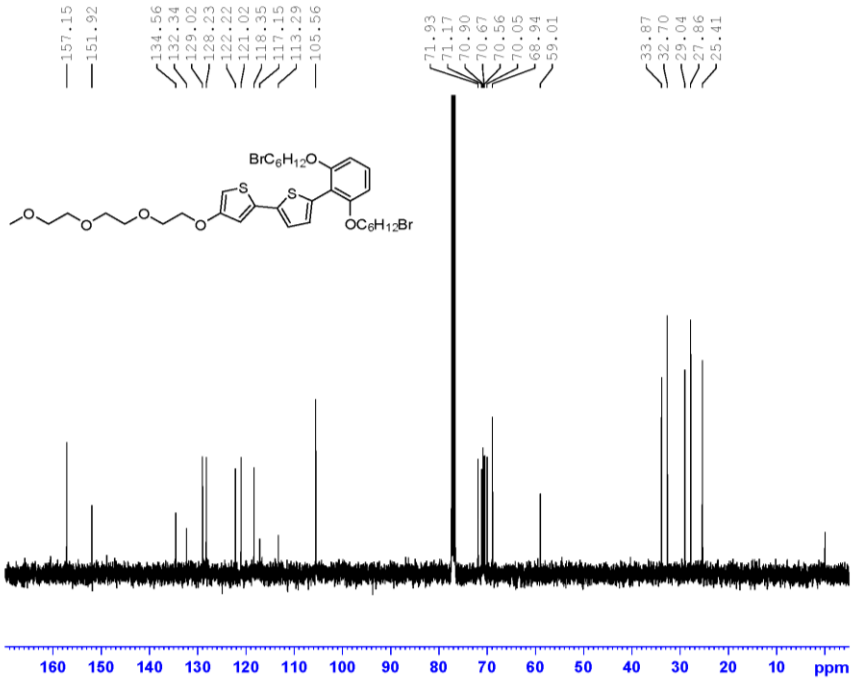

Fig. S9 ^{13}C NMR of compound **6.**

2.8 Synthetic procedures and characterization of compound IR-BTG

4,8-(Di-2,6-bis((6-bromohexyl)oxy)phenyl)-4-(2-(2-(2-methoxyethoxy)ethoxy)ethoxy)-2,2'-bithiophene)-1H,5H-benzo[1,2-c:4,5-c']bis([1,2,5]thiadiazole) (IR-BTG). To a solution of compound 6 1.52 g (2 mmol) in 15 mL THF at -78 ^{o}C under protection gas atmosphere, n-BuLi (1.6 M in Hexane, 1.5 mL, 2.4 mmol) was added dropwise. After the mixturewas stirred at this temperature for another 2.0 h, trinbutyltinchloride (0.812 mg, 2.5 mmol) was added to the solution. Then slowed warmed to room temperature and stirred for 8 h. After that the mixture was poured into water and extracted twice with ethyl acetate, the combined organic phase was dried with $MgSO_4$ and evaporated in vacuo without further purification. To a solution of 4,8-dibromo-1H,5H-benzo[1,2-c:4,5-c']bis([1,2,5]thiadiazole) (a) 234 mg(0.67 mmol), the crude product (2 mmol) in 15 mL toluene under protection gas atmosphere then $Pd(PPh_3)_2Cl_2$ 150 mg was added. The mixture was stirred at 110 ^{o}C for 12 h. After cooling to room temperature, the mixture was poured in to water and extracted twice with

ethyl acetate, dried with $MgSO_4$ and evaporated in vacuo. The crude product was subjected to column chromatography on silica gel with PE/EA 3:1 to afford IR-BTG as a dark green solid (505 mg, 43 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.56 (dd, J = 8.6, 4.4 Hz, 2H), 7.33 (dd, J = 3.7, 1.4 Hz, 2H), 7.23 (dd, J = 15.5, 4.8 Hz, 4H), 6.70 – 6.59 (m, 4H), 4.41 (d, J = 4.2 Hz, 4H), 4.07 (t, J = 6.2 Hz, 8H), 3.72 (t, J = 5.0 Hz, 4H), 3.63 – 3.47 (m, 16H), 3.42 (t, J = 6.7 Hz, 8H), 3.36 (s, 6H), 1.97 – 1.83 (m, 16H), 1.60 – 1.45 (m, 16H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 157.02, 156.28, 152.68, 140.16, 138.60, 136.36, 134.70, 129.95, 128.69, 123.09, 114.85, 113.50, 112.45, 105.29, 71.89, 71.12, 70.60, 70.04, 68.97, 59.03, 34.00, 32.72, 29.09, 27.89, 25.94.^{13}C NMR (125 MHz, $CDCl_3$) δ 159.25, 152.93, 150.90, 149.60, 140.11, 138.00, 135.70, 129.38, 124.74, 123.08, 122.44, 120.18, 118.16, 109.81, 102.14, 73.68, 70.37, 69.96, 69.54, 69.25, 57.85, 33.30, 31.58, 30.68, 28.80, 26.58. HRMS(ESI) calcd for $C_{72}H_{91}Br_4N_4O_{12}S_6^+$, ([M+H$^+$]) 1715.1572, Found 1715.1568. Optical parameters in DMSO: λ_{ex} = 765 nm, λ_{em} = 1090 nm, absorption coefficient K= 11.2 L/g.cm, quantum yield is 0.94% (with 808 nm excitation)

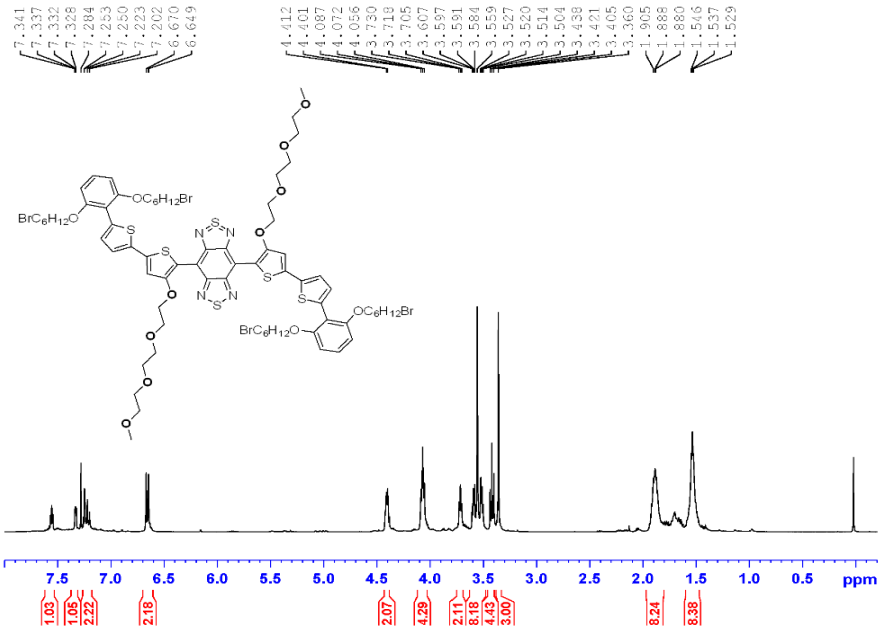

Fig. S10 ^{1}H NMR of compound IR-BTG.

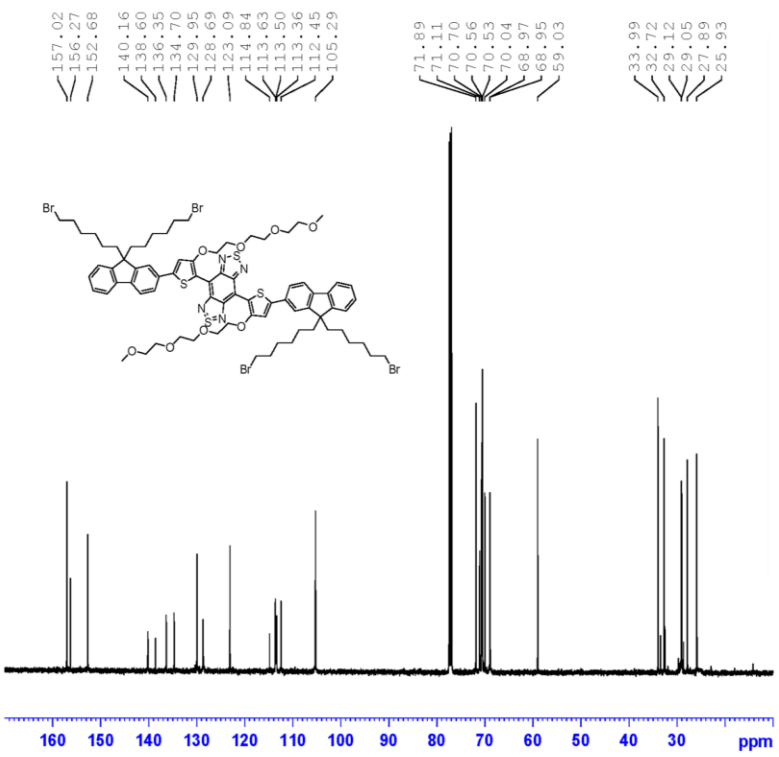

Fig. S11 ^{13}C NMR of compound IR-BTG.

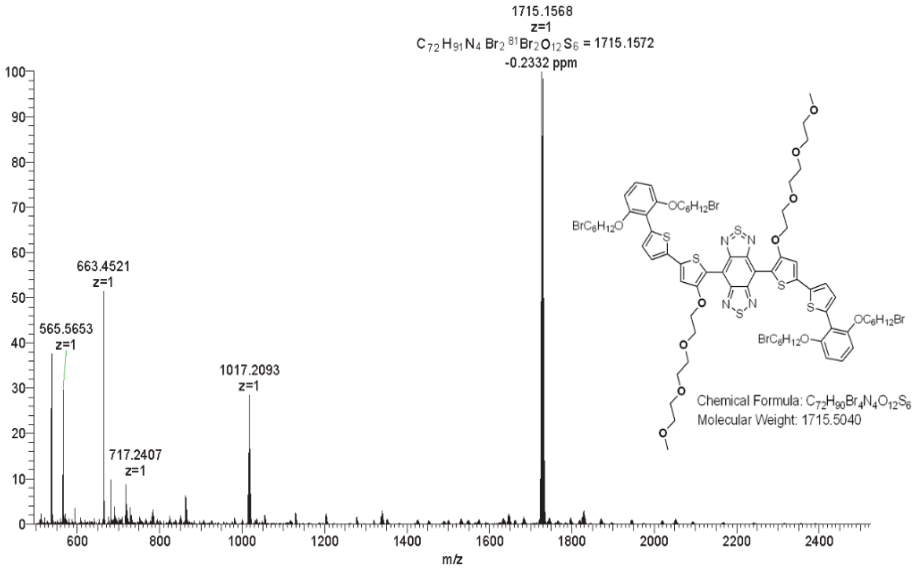

Fig. S12 HRMS of compound IR-BTG.

2.9 Synthetic procedures and characterization of IR-BTGP

Compound IR-BTG 100 mg (0.058 mmol) was dissolved in 10 mL DMF and sodium azide 47 mg (0.72 mmol) and heated for 3 h at 70 ^{o}C, then added just as much water as

possible to dissolve all solids. Then it was extracted twice with ethyl acetate, the combined organic phase was dried with $MgSO_4$ and evaporated in vacuo. The crude product was subjected to flash column chromatography on silica gel to afford dark green solid 97 mg. The dark green solid was dissolved in 5 mL THF and CuTc 10 mg, w-alkynyl-PEG-hydroxyl (Mn = 2000) 230 mg, and TBTA (5 mg) was added. The system was stirred at rt for 1.0 h. Then filtered with diatomite, and the solution was evaporated in vacuo. When all the organic solvent was removed, the crude product was purified by thin layer chromatography eluting with DCM/MeOH 10:1. IR-BTGP (240 mg) was afforded as a green oil.

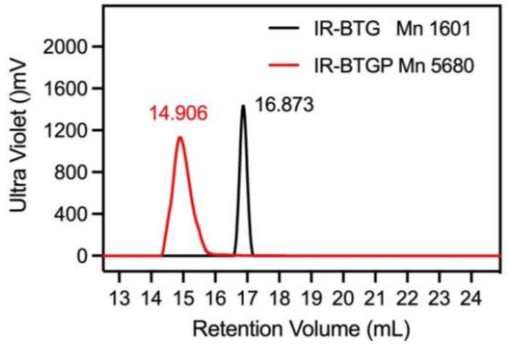

Fig. S13 Size exclusion chromatography (SEC) of IR-BTG and IR-BTGP.

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