Supplementary information

ROS-Responsive and active targeted drug delivery based on conjugated polymers nanoparticles for synergistic chemo-/photodynamic therapy

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Experimental section

Materials and measurements. All the compounds mentioned in the experiments were purchased from Shanghai Aladdin, Sigma-Aldrich, TCI (Shanghai) and J&K Scientific, and used without any purification. Nuclear magnetic resonance (NMR) spectra were recorded on Bruker Ascend 300 MHz and 400 MHz spectrometers. Mass spectra were measured by a Bruker maXis II mass spectrometer (ESI+). The spectral properties were taken on SHIMADZU UV-2600 spectrophotometer and Hitachi F-7000 spectrophotometer equipped with a Xe lamp. Confocal laser scanning microscopy images were recorded on Olympus Fluo-view 1200. In MTT analysis, the absorbance was evaluated by Spectramax M5. Flow cytometry analysis was taken on a BD Accuri C6 flow cytometer.

Synthesis of compound 1. 4-(hydroxymethyl) phenylboronic acid pinacol ester (1.17 g, 5 mmol), *p*-nitrophenyl chloroformate (1.12 g, 5.5 mmol), triethylamine (1.4 mL, 1 mmol) and THF (10 mL) were stirred at room temperature for 12 h. Then the mixture was extracted with ethyl acetate, dried over anhydrous magnesium sulfate, and concentrated to obtain a white solid (1.26 g, 63%) after column chromatography. The eluent was petroleum ether/ethyl acetate (v/v=3: 1). ^{1}H NMR (300 MHz, $CDCl_3$), δ (ppm): 8.26 (d, J = 9.0 Hz, 2 H), 7.84 (d, J = 6.0 Hz, 2 H), 7.42 (d, J = 9.0 Hz, 2 H), 7.36 (d, J = 9.0 Hz, 2 H), 5.31 (s, 2 H), 1.35 (s, 12 H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 155.6, 152.4, 140.3, 137.1, 135.2, 132.9, 127.6, 125.3, 121.8, 84.0, 77.2, 77.0, 76.8, 70.8, 24.9.

Synthesis of compound 2. First *p*-methoxyphenol (2.25 g, 18 mmol) and K_2CO_3

(4.88 g, 36 mmol) were completely dissolved in DMF (40 mL), and then the mixture was heated to 100 °C for 30 min followed by adding ethyl 7-bromoenanthate (2.58 mL). After react at 100 °C for another 1 h the mixture was cooled to room temperature, extracted with ethyl acetate and saturated brine, dried and concentrated, and then separated by column chromatography to obtain a colorless oily liquid (2.3 g, 53%). The eluent was petroleum ether/ethyl acetate (v/v=10: 1). ^{1}H NMR (300 MHz, $CDCl_3$), δ (ppm): 6.82 (s, 4 H), 4.11 (dd, 2 H), 3.89 (t, J = 6.0 Hz, 2 H), 3.75 (s, 3 H), 2.3 (t, J = 9.0 Hz, 2 H), 1.17-1.18 (m, 2 H), 1.60-1.68 (m, 2 H), 1.35-1.5 (m, 4 H), 1.25 (t, J = 9.0 Hz, 3 H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.8, 153.7, 153.3, 115.5, 114.7, 77.3, 77.0, 76.7, 68.5, 60.2, 55.8, 34.3, 34.2, 29.2, 28.9, 25.8, 24.9, 14.3. HRMS (ESI): m/z: calcd. for $C_{16}H_{24}O_4Na^+$ 303.1575 $[M+Na]^+$, found 303.1565 $[M+Na]^+$.

Synthesis of monomer 1. Firstly, compound 1 (1.68 g, 6 mmol), I_2 (1.02 g, 4 mmol), KIO_3 (0.51 g, 2.4 mmol) were dissolved in glacial acetic acid (30 mL), and then concentrated sulfuric acid (0.7 mL) and distilled water (3.5 mL) were added. The mixture was heated to 120 °C for 3 h. The mixture was cooled to room temperature before excess I_2 was removed by 10% Na_2SO_3 solution, followed by pouring the mixture into ice water (100 mL). The precipitation was collected by filtration, wash with distilled water several times and dry to obtain a white solid (2.25 g, 75%). Next, methanol (5 mL) and concentrated sulfuric acid (0.6 mL) were added to a 25 mL double-necked flask. After bubbling N_2 for 20 min, the above crude product (0.5 g, 1 mmol) was added. The mixture was heated to 75 °C for 2 h. After cooled to room temperature the mixture was extracted with ethyl acetate, drying over anhydrous

magnesium sulfate and concentrated, then separated by column chromatography to obtain a pale yellow oil (0.45 g, 87%). The eluent was petroleum ether/ethyl acetate (v/v=10: 1). ^{1}H NMR (300 MHz, $CDCl_3$), δ (ppm): 7.17 (t, J = 3.0 Hz, 2 H), 3.93 (t, J = 6.0 Hz, 2 H), 3.80 (d, J = 6.0 Hz, 3 H), 3.65 (d, J = 6.0 Hz, 3 H), 1.75-1.85 (m, 2 H), 1.63-1.73 (m, 2 H), 1.49-1.58 (m, 2 H), 1.37-1.45 (m , 2 H). ^{13}C NMR (101 MHz, CDCl3) δ 174.20, 153.29, 152.90, 122.95, 121.49, 86.36, 85.43, 77.36, 77.04, 76.73, 70.18, 57.19, 51.51, 34.00, 28.95, 28.78, 25.75, 24.85. HRMS (ESI): m/z: calcd. for $C_{15}H_{20}I_2O_4Na^+$ 540.9351 $[M+Na]^+$, found 540.9350 $[M+Na]^+$.

Calculation of drug loading efficiency and drug loading. The drug encapsulation efficiency (EE) and drug loading (DL) were calculated using the equations following:

$$EE(\%) = \frac{\text{the weight of drugs in CPNs}}{\text{total weight of drugs for preparation}} \times 100\%$$

$$DL(\%) = \frac{\text{the weight of drugs in CPNs}}{\text{the weight of CPNs}} \times 100\%$$

Supporting figures

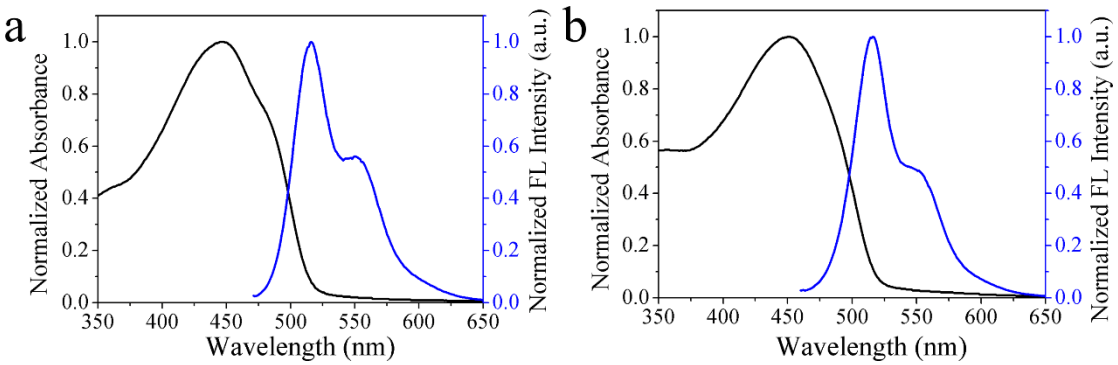

Fig. S1 Photophysical properties of (a) PFV and (b) iRGD@CPNs in aqueous solution.

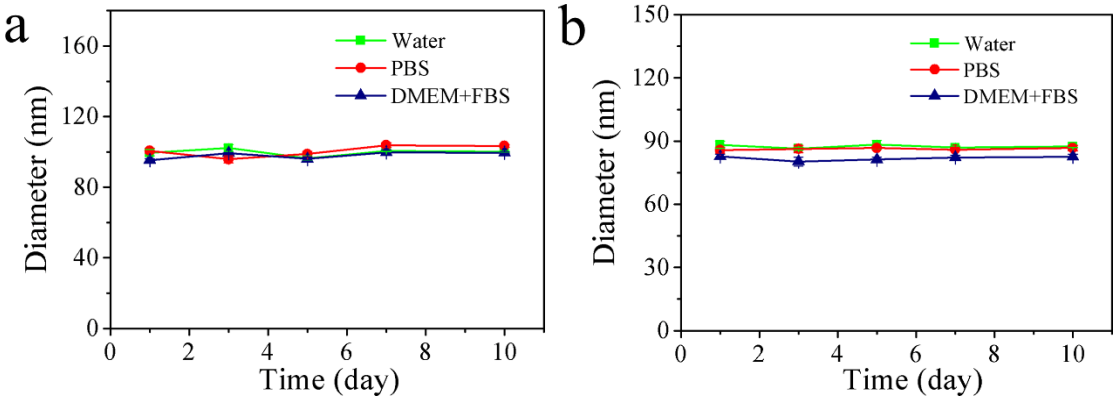

Fig. S2 Particle size stability of (a) iRGD-BDOX@CPNs and (b) and iRGD@CPNs in different solutions within ten days.

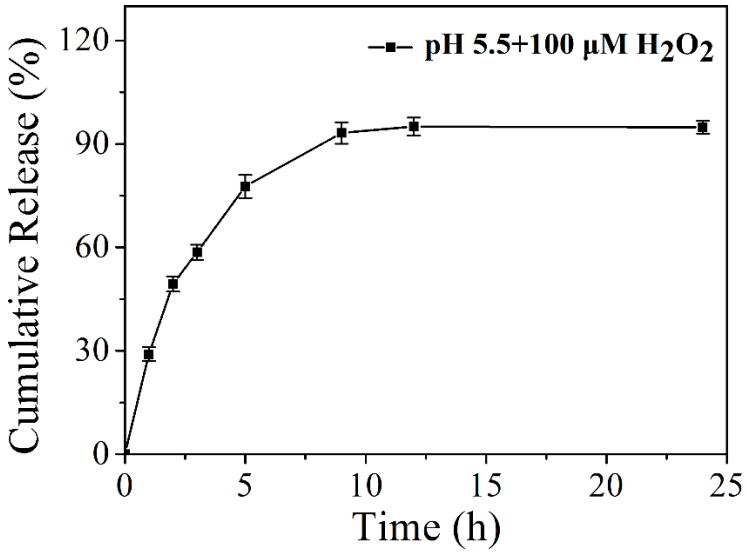

Fig. S3 DOX release profile of iRGD-BDOX@CPNs at pH 5.5.

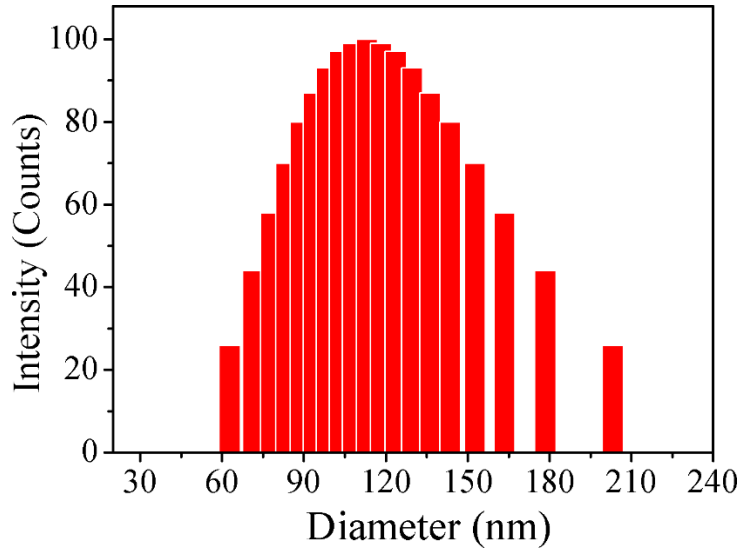

Fig. S4 Size distribution of iRGD-BDOX@CPNs after drug release determined by DLS.

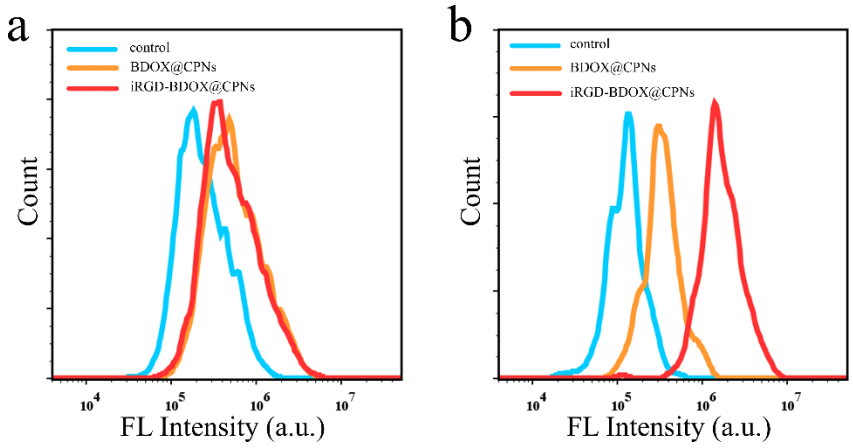

Fig. S5 Flow cytometric profiles of (a) MCF-7 cells and (b) PC-3 cells treated with BDOX@CPNs or iRGD-BDOX@CPNs for 2 h. [PFV] = 5 μM.

Fig. S6 Confocal laser scanning microscopy (CLSM) imaging of PC-3 cells incubation with free DOX for 2 h. [DOX] = 2.5 μM. The fluorescence signal was collected at 500-560 nm (λ_{ex}: 488 nm).

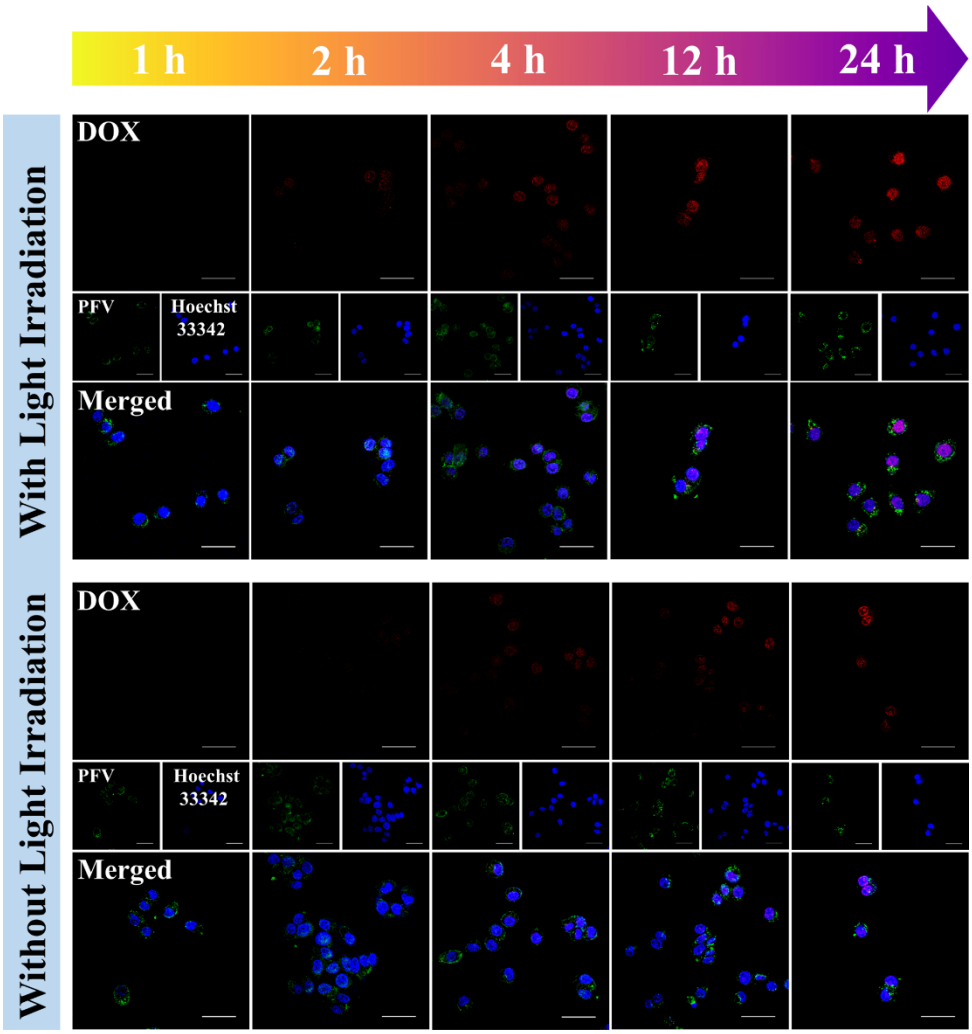

Fig. S7 Drug release monitoring of iRGD-BDOX@CPNs in MCF-7 cells without or with light irradiation (90 mW cm^{-2}) for 15 min. The fluorescence signal was collected at 425-475 nm (λ_{ex}: 405 nm) for Hoechst 33342, 500-560 nm (λ_{ex}: 488 nm) for PFV and 570-610 nm (λ_{ex}: 488 nm) for DOX, respectively. Scale bar is 40 μm.

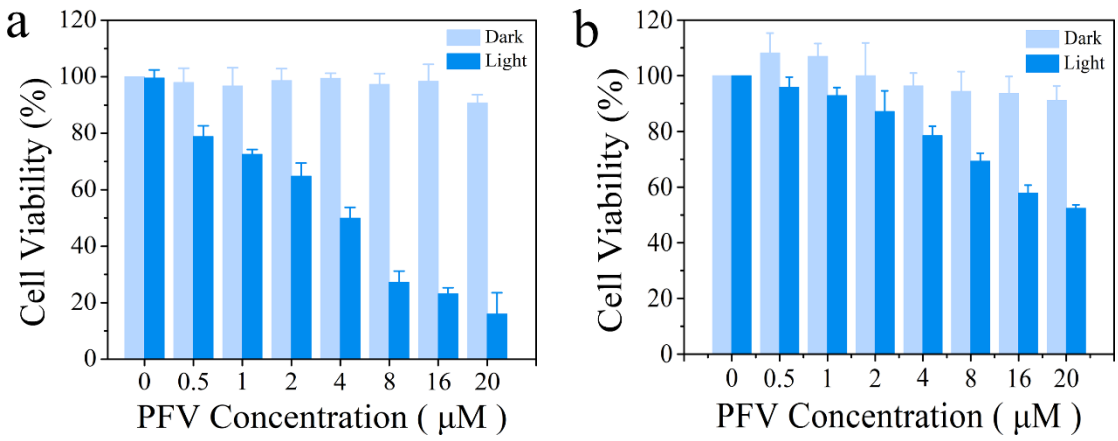

Fig. S8 Cytotoxicity of iRGD@CPNs to (a) PC-3 and (b) MCF-7 cells without or with light irradiation (25 mW cm^{-2}) for 30 min.

Compound characterization

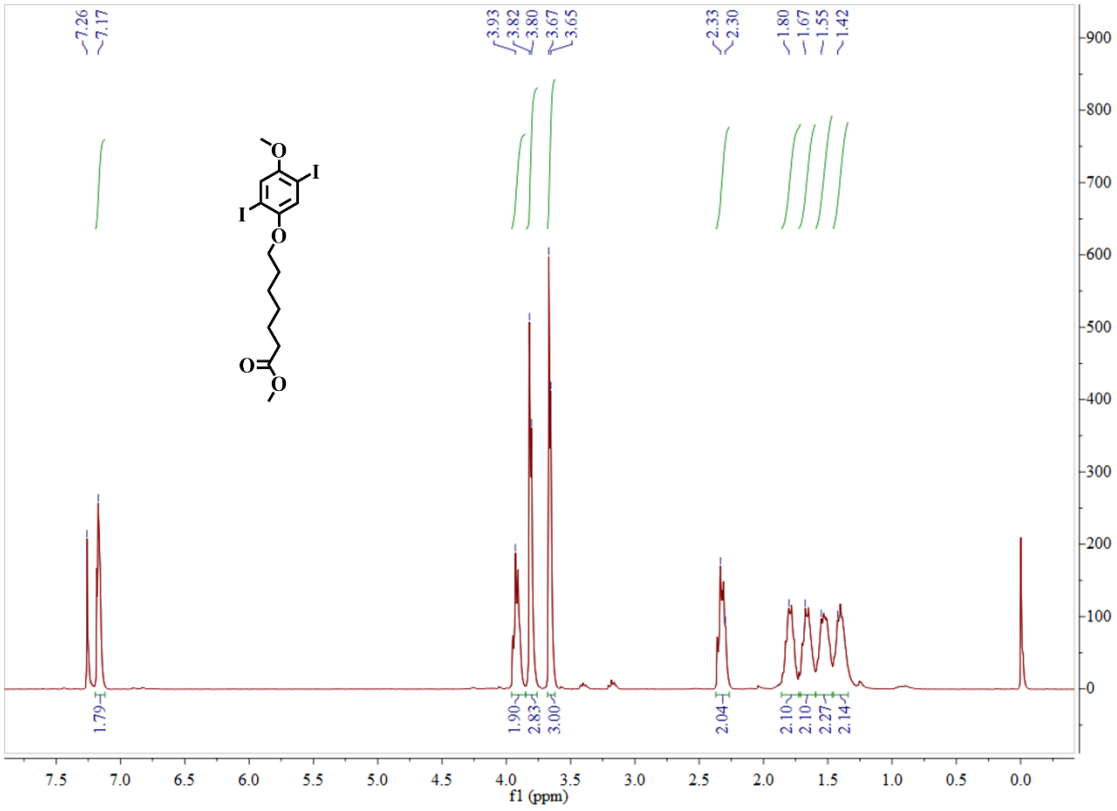

Fig. S9 ^{1}H NMR spectrum of Monomer 1 (300 MHz, $CDCl_3$).

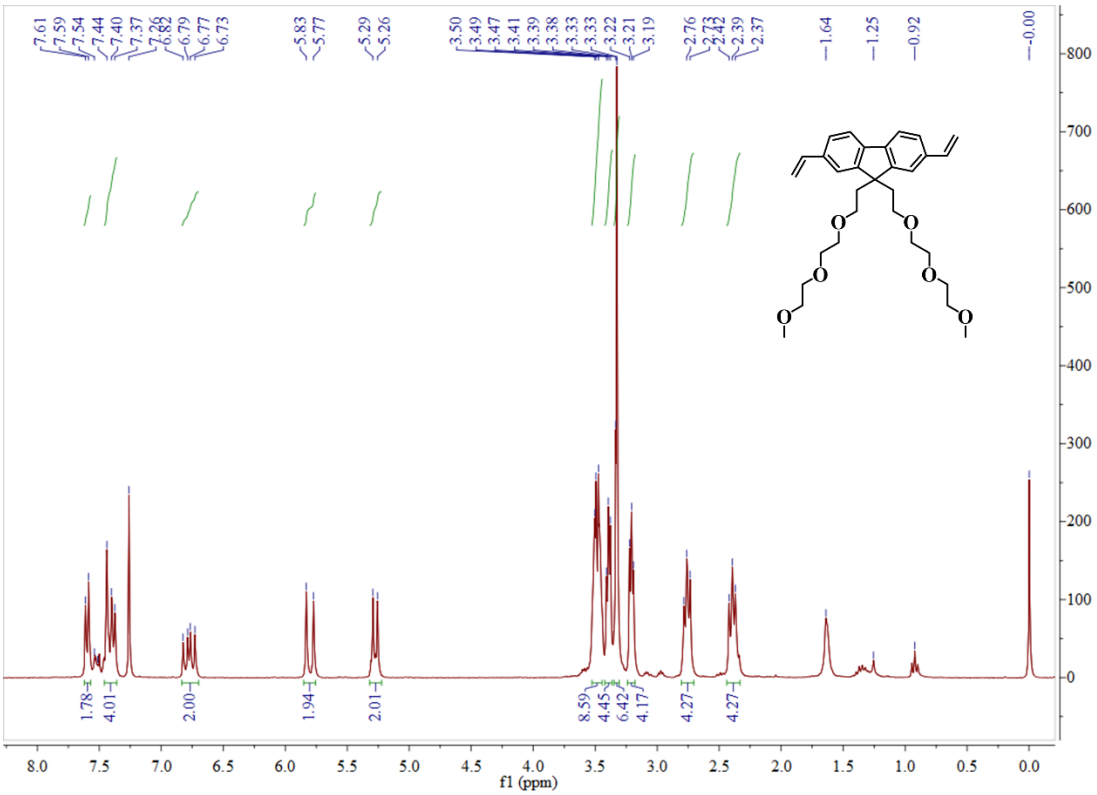

Fig. S10 ^{1}H NMR spectrum of Monomer 2 (300 MHz, $CDCl_3$).

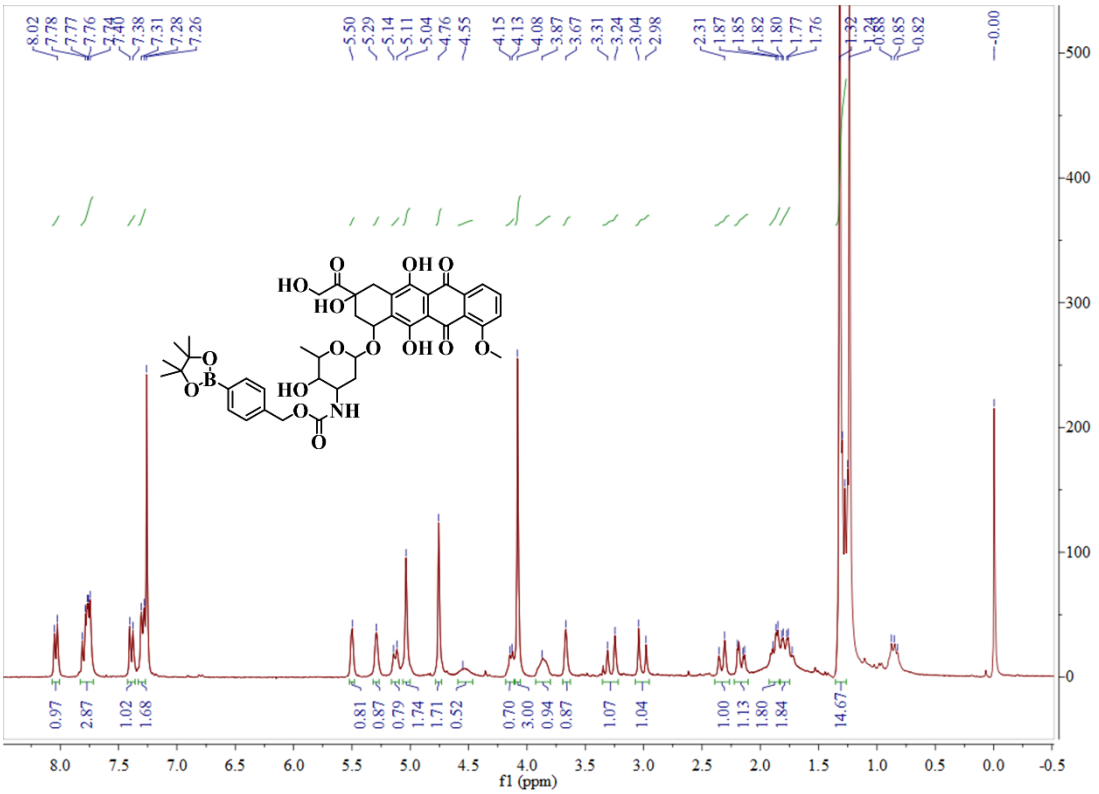

Fig. S11 ^{1}H NMR spectrum of BDOX (400 MHz, $CDCl_3$).

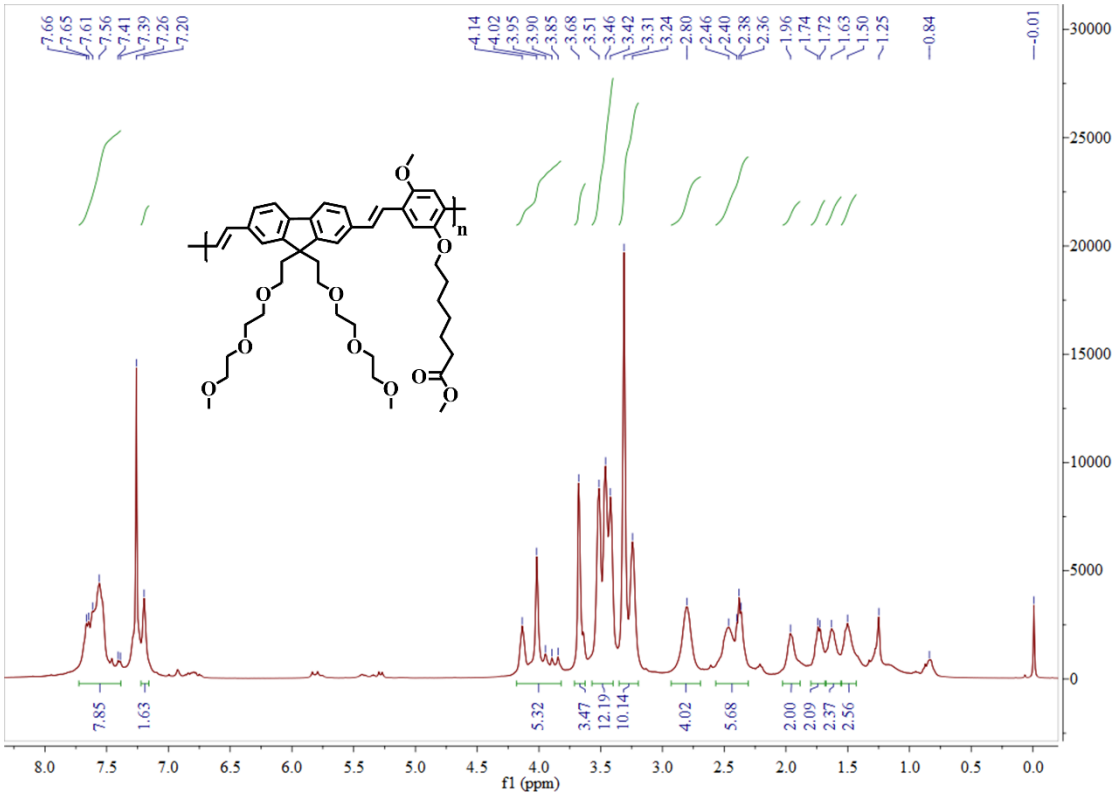

Fig. S12 ^{1}H NMR spectrum of PFV (400 MHz, $CDCl_3$).