

Thieno[3,2-*b*]thiophene-DPP-based Near Infrared Nanotheranostic Agent for Dual Imaging-Guided Photothermal/Photodynamic Synergistic Therapy

Xue Yang,^a Qing Yu,^a Nan Yang,^a Lei Xue,^a Jiawei Shao,^a Buhong Li,^{b*} Jinjun Shao,^{a*}
Xiaochen Dong^{a*}

^aKey Laboratory of Flexible Electronics (KLOFE) & Institute of Advanced Materials (IAM),
Nanjing Tech University (NanjingTech), Nanjing 211800, China.

**E-mail: iamjjshao@njtech.edu.cn, iamxcdong@njtech.edu.cn*

^bMOE Key Laboratory of OptoElectronic Science and Technology for Medicine, Fujian
Provincial Key Laboratory for Photonics Technology, Fujian Normal University, Fuzhou
350007, China.

**Email: bhli@fjnu.edu.cn*

Table of Contents

1. Materials and apparatus.
2. Cell migration study.
3. *Ex Vivo* Histology Examination
4. Figures and spectra.

1. Materials and apparatus

Phenothiazine (**1**), 1,4-dibromobenzene (**2**), bis(triphenylphosphine)palladium dichloride, sodium *tert*-butoxide, tri-*tert*-butylphosphine tetrafluoroborate, triethyl phosphonoacetate, 1,8-diazabicyclo[5.4.0]undec-7-ene, methyl trioctyl ammonium chloride, bis(pinacolate)diboron, tetrakis(triphenylphosphine)palladium, *N*-Bromosuccinimide and *N,N*-dimethylformamide were purchased from Energy Chemical (Shanghai, China). 3,6-bis(5-bromothiopheno[3,2-*b*]thiophen-2-yl)-2,5-bis(2-decyltetradecyl)-2,5-dihydropyrrolo[3,4-*c*]pyrrole-1,4-dione (**7**) was purchased from Solarmer Materials Inc. (Beijing, China). 1,3-diphenylisobenzofuran (DPBF) was purchased from Aladdin (Shanghai, China). Methylene blue trihydrate (MB) was purchased from Tianjin Chemical Reagent Research Institute Co. Ltd. (Tianjin, China). Potassium carbonate, phosphorus oxychloride, sodium sulfate, potassium hydroxide, sodium bicarbonate, chloroform, tetrahydrofuran, dichloromethane, petroleum ether and ethyl acetate were obtained from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). All the chemical agents are analytical pure and are used as received.

¹H NMR and ¹³C NMR were obtained by a Bruker Ultra Shield Plus AV400 or DR500 spectrometer (CDCl₃, δ = 7.26 ppm for ¹H NMR, and δ = 77.0 ppm for ¹³C NMR). UV-vis-NIR absorption and fluorescence spectra were recorded on UV-3600 spectrophotometer (Shimadzu, Japan) and an F-4600 Spectro-fluorophotometer (HITACHI, Japan). MALDI-TOF (Matrix-assisted laser desorption ionization time-of-flight mass spectrometry) Mass spectra were recorded on a Bruker Autoflex speed MALDI-TOF Mass spectrometer. The NPs morphology was characterized by transmission electron microscopy (JEOL JEM-2100), and the size distribution was investigated by dynamic light scattering (DLS, Nanoplus-3*). The temperature of the solution samples for photothermal conversion measurement was recorded by an IR thermal camera (E50, Arlington, VA).

2. Cell migration study

HeLa cells were seeded into two 6-well plates at a density of 5000 cells per well and incubated in the culture media (2 mL) at 37 °C under a 5% CO₂ atmosphere for 24 h. After removing

media, the cells were washed thrice with PBS solution, then a pipette-tips was used to draw a line to separate the cells into two parts. The cells were further incubated with **PDBr** NPs (200 μ L) at different concentration per well for 24 hours. Then FBS (200 μ L) was added, and the photographs were recorded under microscope after 12 h.

3. *Ex Vivo* Histology Examination.

After 20 days of treatment, all mice are sacrificed and tumor, heart, liver, spleen, lung, and kidney are collected for histology analysis. After dehydration and staining with haematoxylin and eosin (H&E), the images are viewed by microscope.

4. Figures and spectra.

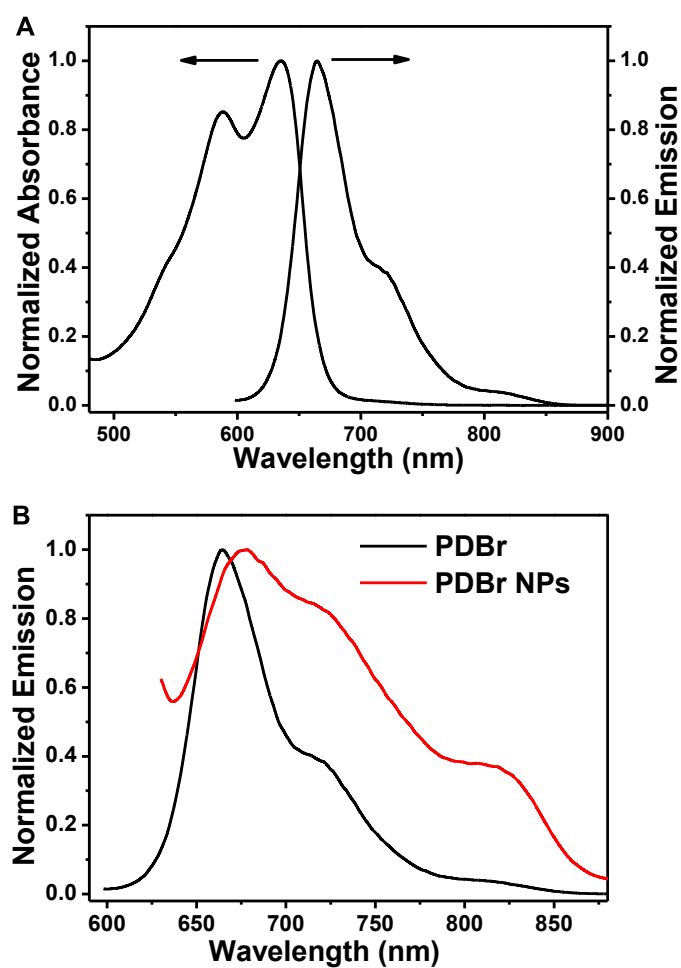


Fig. S1 (A) The normalized UV and fluorescence spectra of **PDBr** in DCM. (B) The normalized fluorescence spectra of PDBr in DCM and PDBr NPs in water.

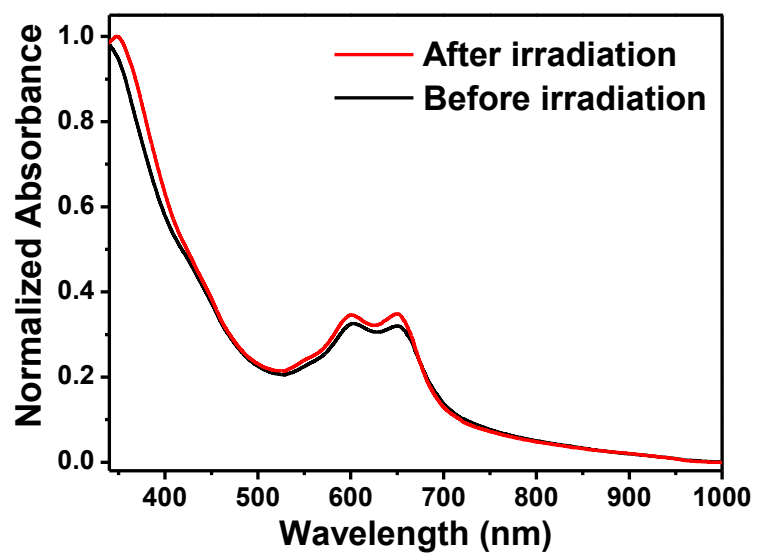


Fig. S2 Absorption spectra of **PDBr** NPs before (red) and after (black) 660-nm laser irradiation in water (1.0 W/cm^2 , 22 min).

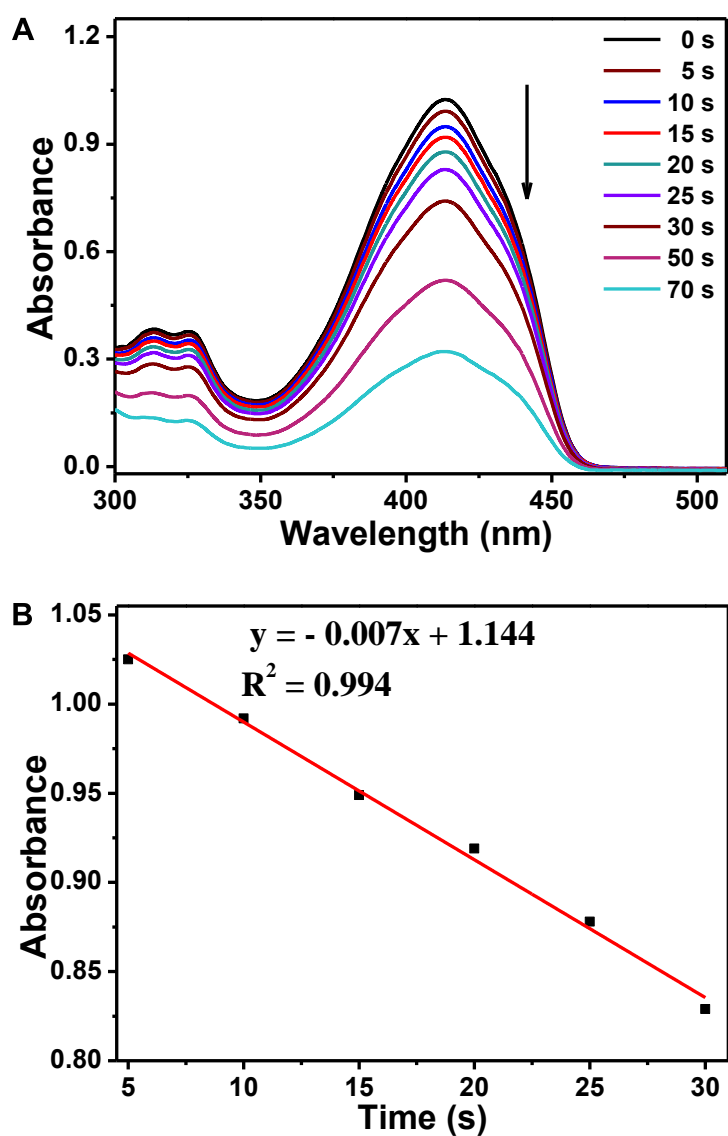


Fig. S3 (A) Absorption spectra of DPBF (ROS reporter) in the presence of MB and 660 nm laser irradiation (0.6 W/cm^2) for different illumination time. (B) Linear fitting of the degradation of DPBF.

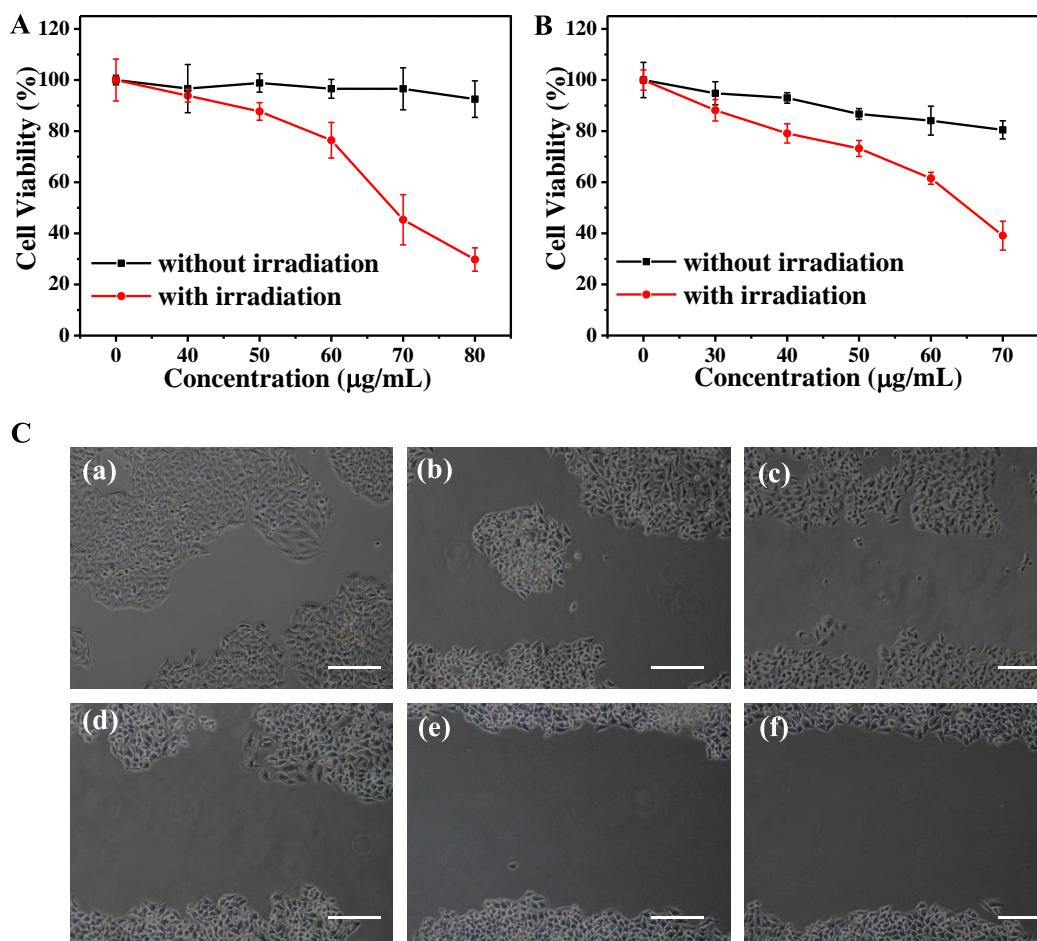


Fig. S4 Cell viability of (A) HCT-116, (B) A2780 cells incubated with **PDBr** NPs at various concentrations, respectively. (C) The photographs of HeLa cells migration at various concentrations of **PDBr** NPs (from a to f, 0, 10, 20, 30, 40, 50 µg/mL). Scale bar: 50 µm.

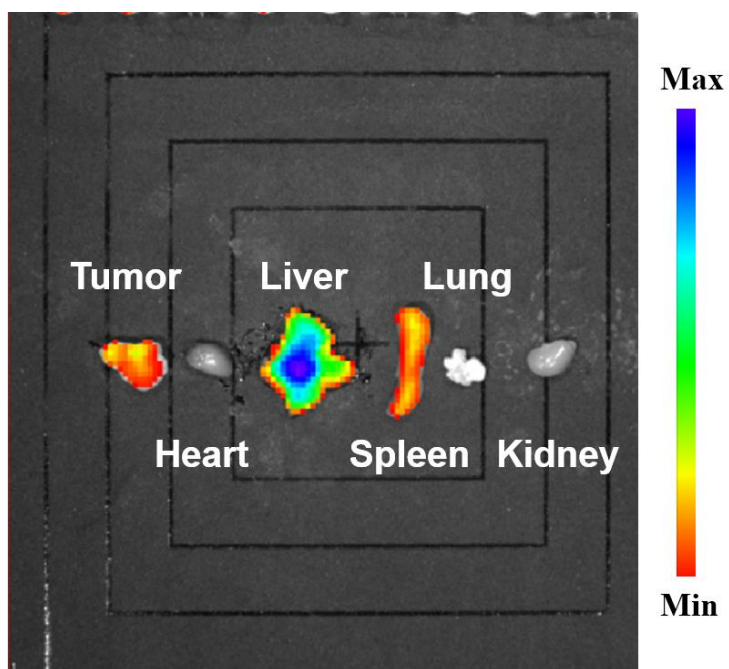


Fig. S5 *Ex vivo* fluorescence imaging of major organs of mice 24 h after systemic administration of **PDBr** NPs.

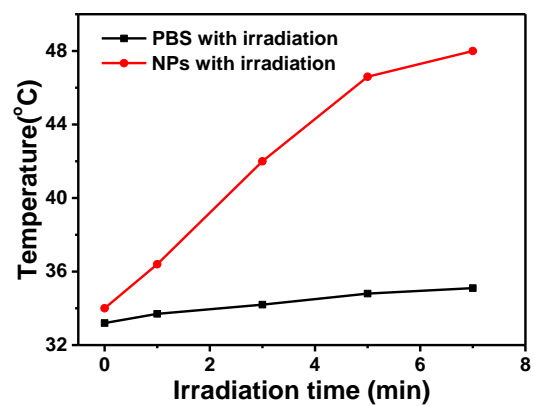


Fig. S6. Temperature increase within 7 min for mice with PBS and NPs injections, respectively.

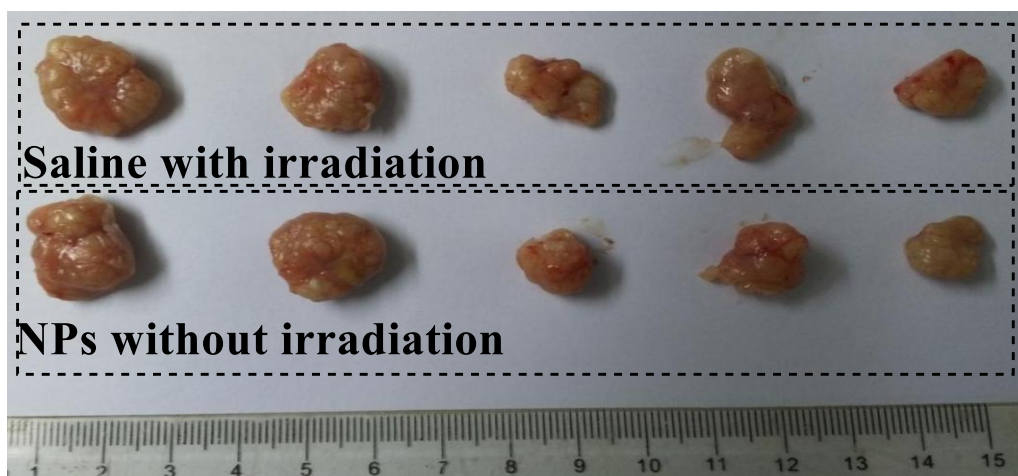


Fig. S7. Photographs of tumor-bearing mice after different treatment.

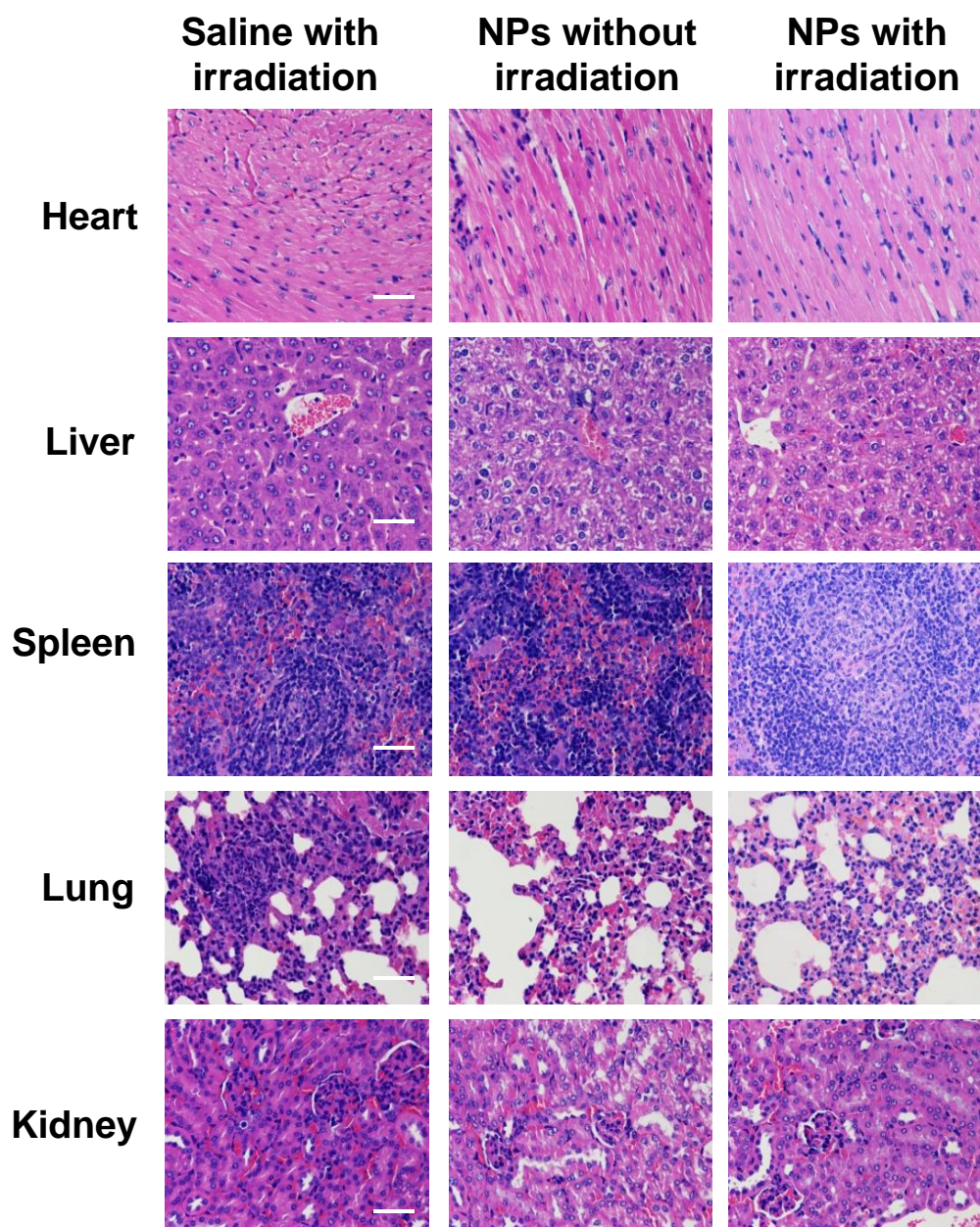


Fig. S8 H&E stained images of major organs (heart, liver, spleen, lung, and kidney) after different treatment. Scale bars: 100 μ m

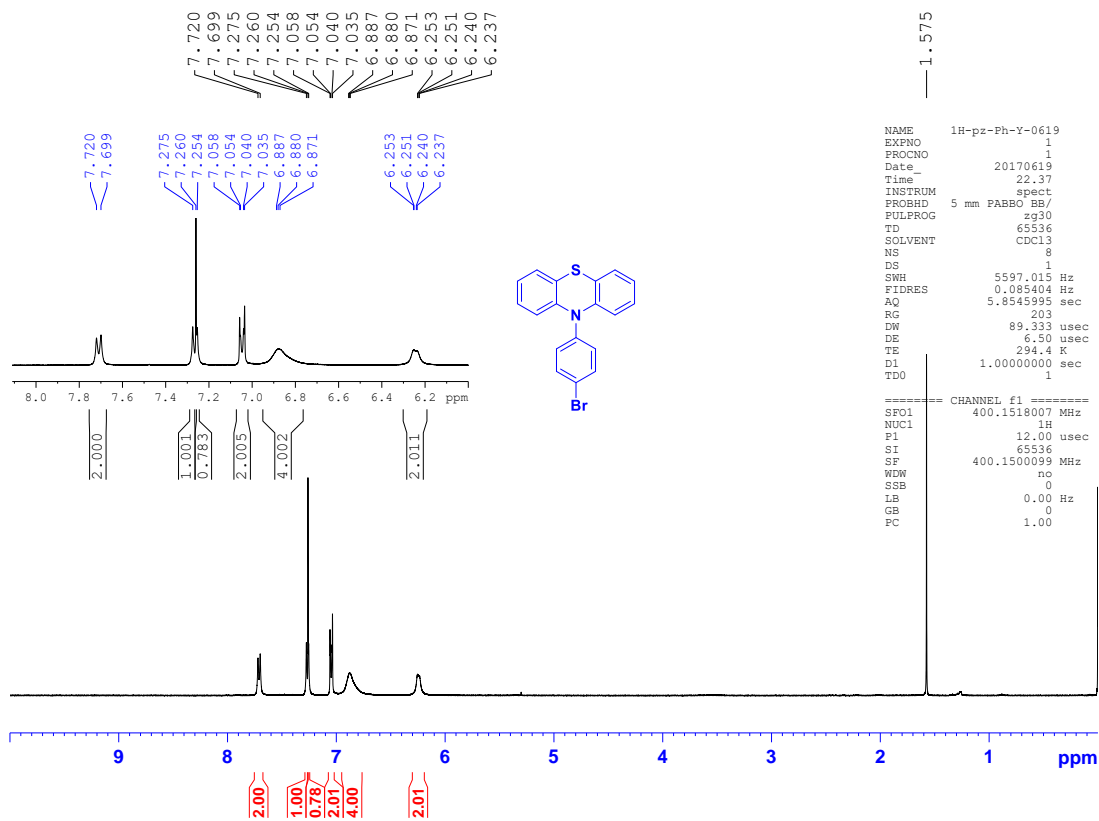


Fig. S9 ¹H NMR of 3

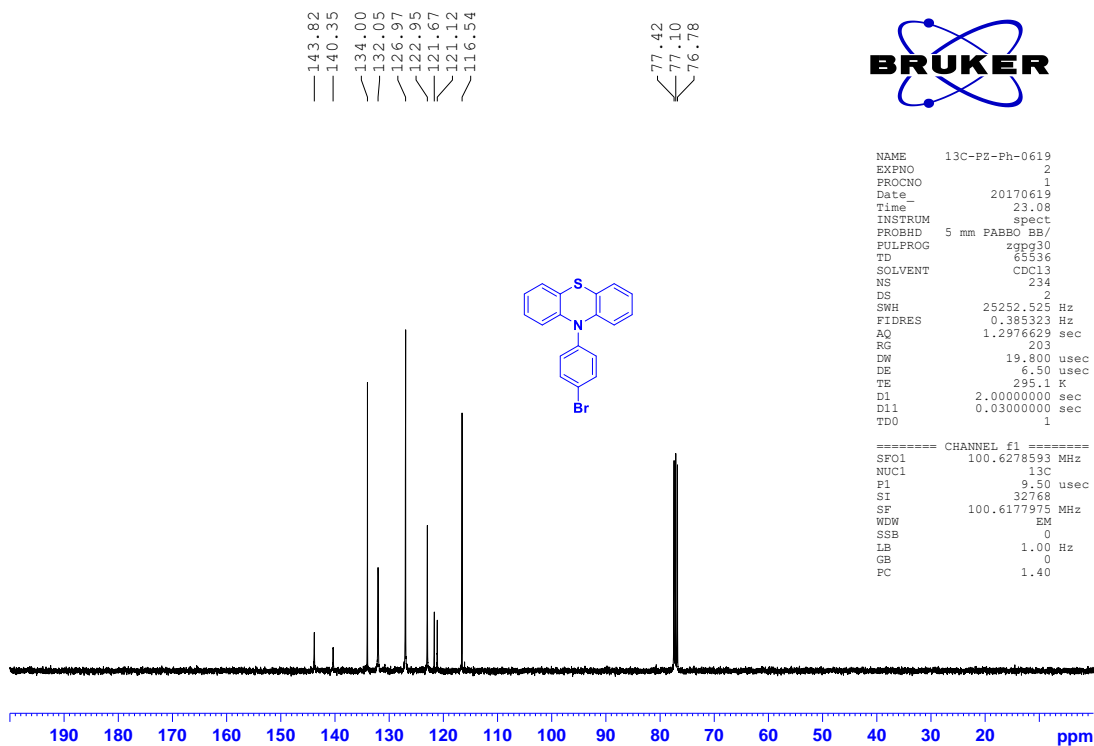


Fig. S10 ¹³C NMR of 3

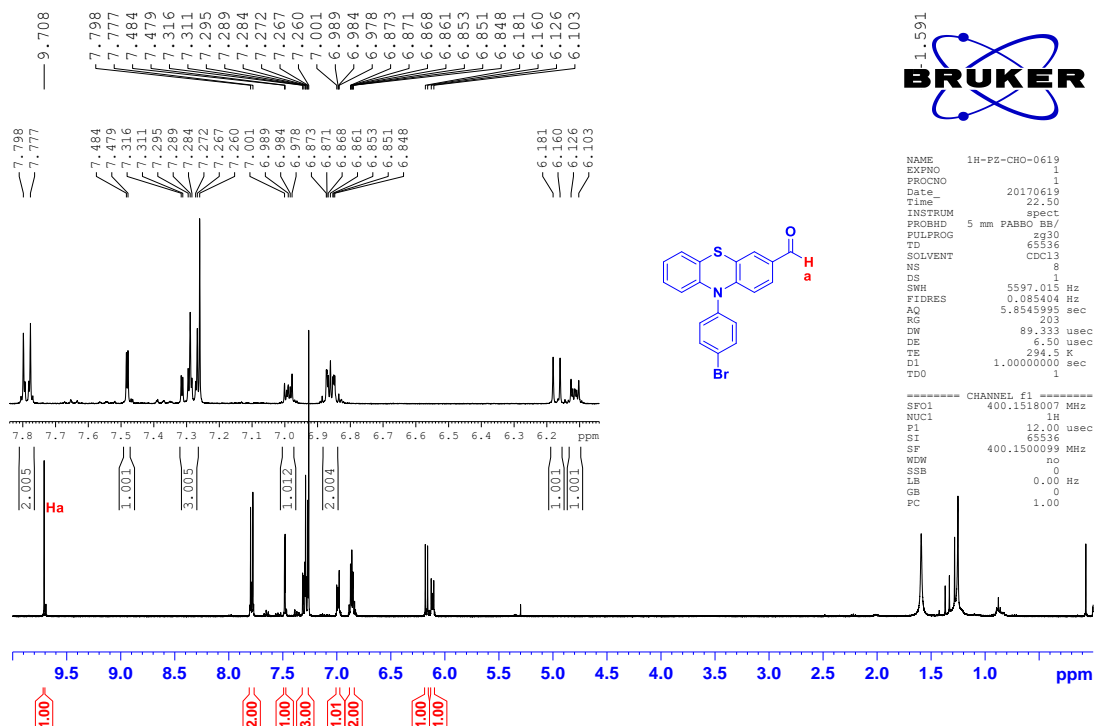


Fig. S11 ¹H NMR of 4

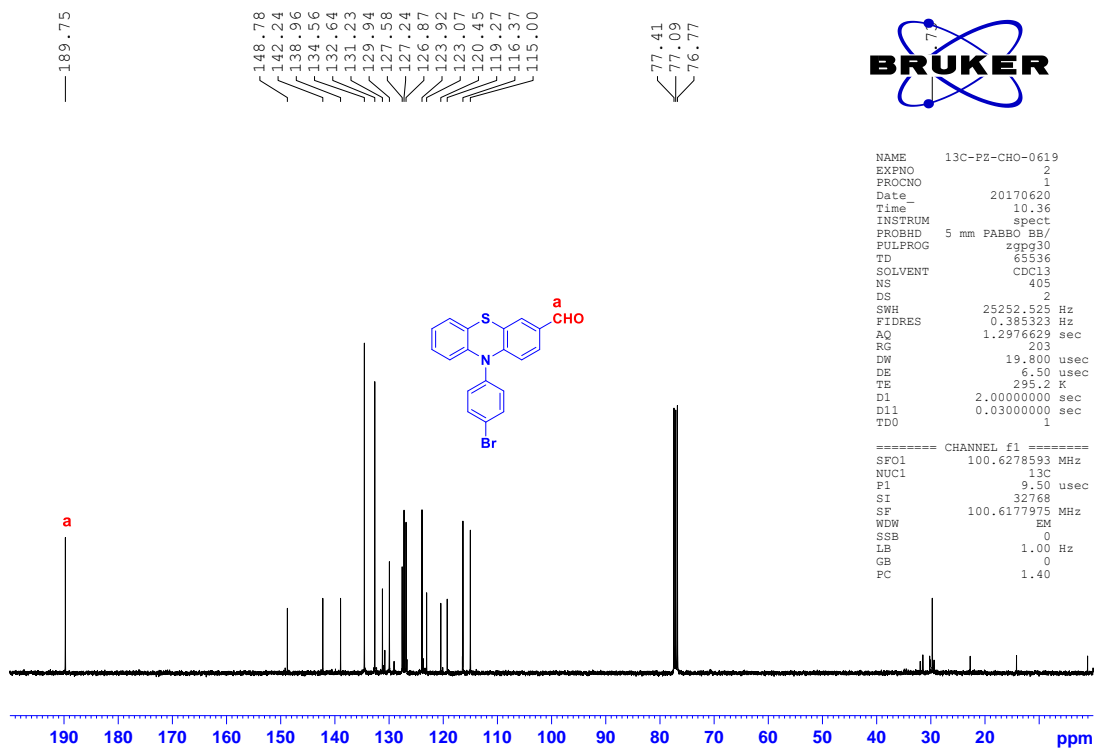


Fig. S12 ¹³C NMR of 4

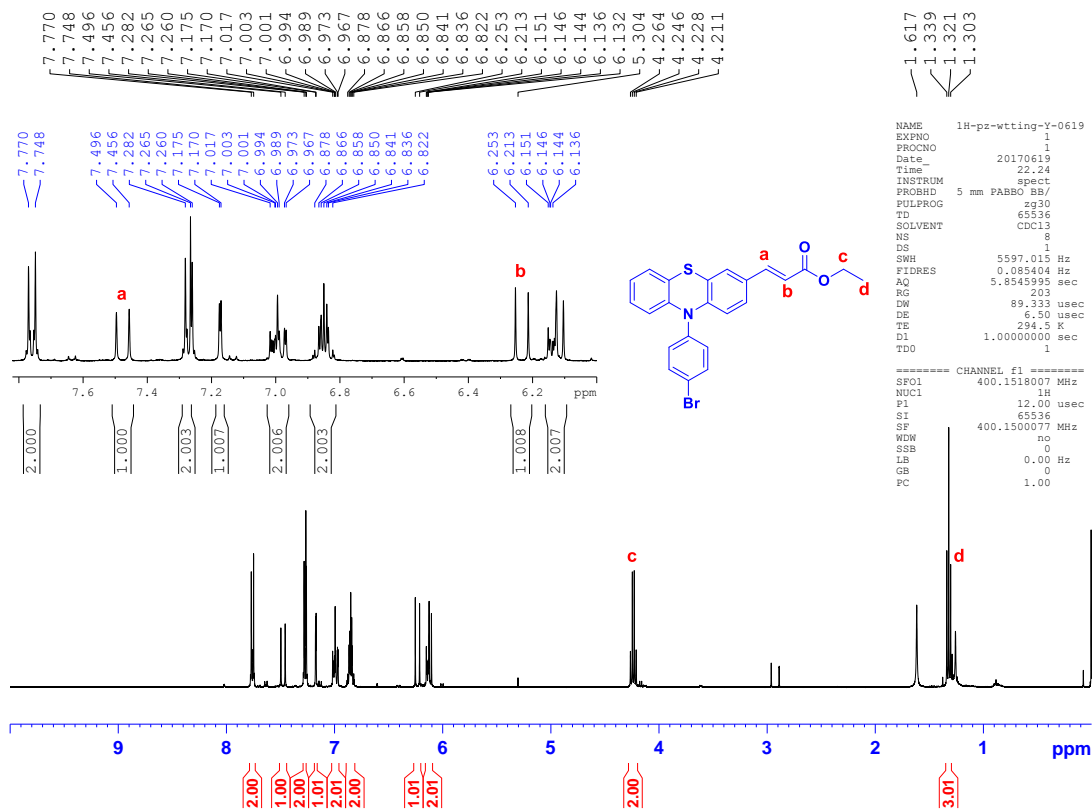


Fig. S13 ¹H NMR of 5

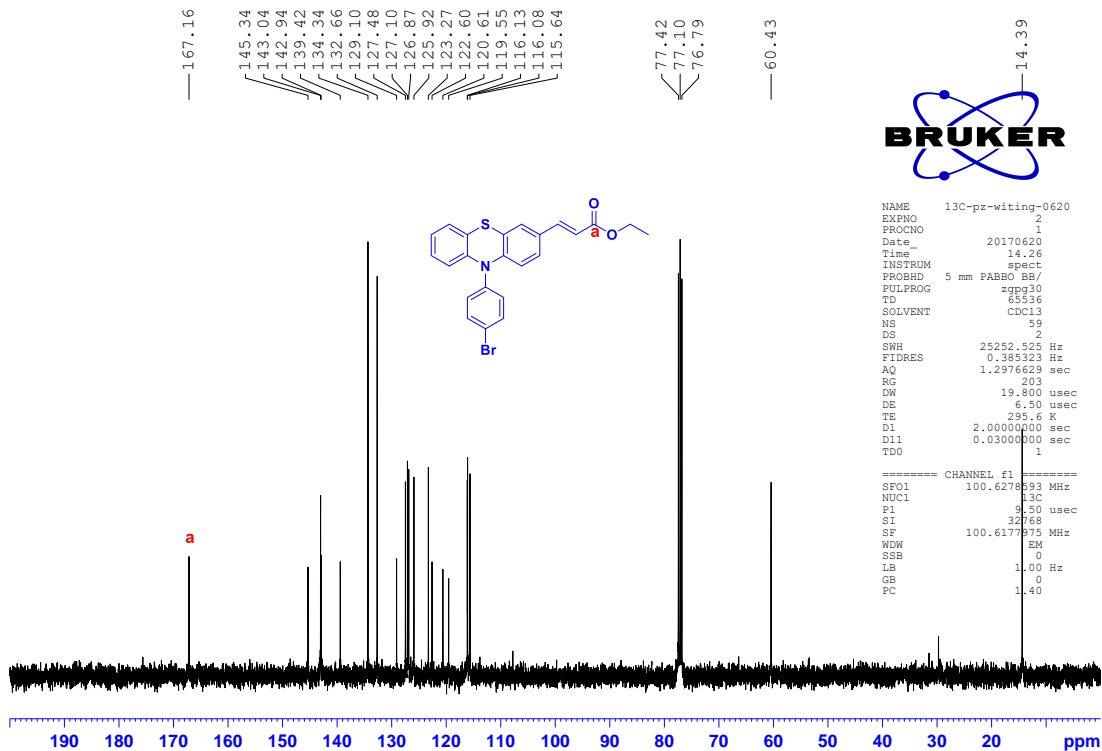


Fig. S14 ¹³C NMR of 5

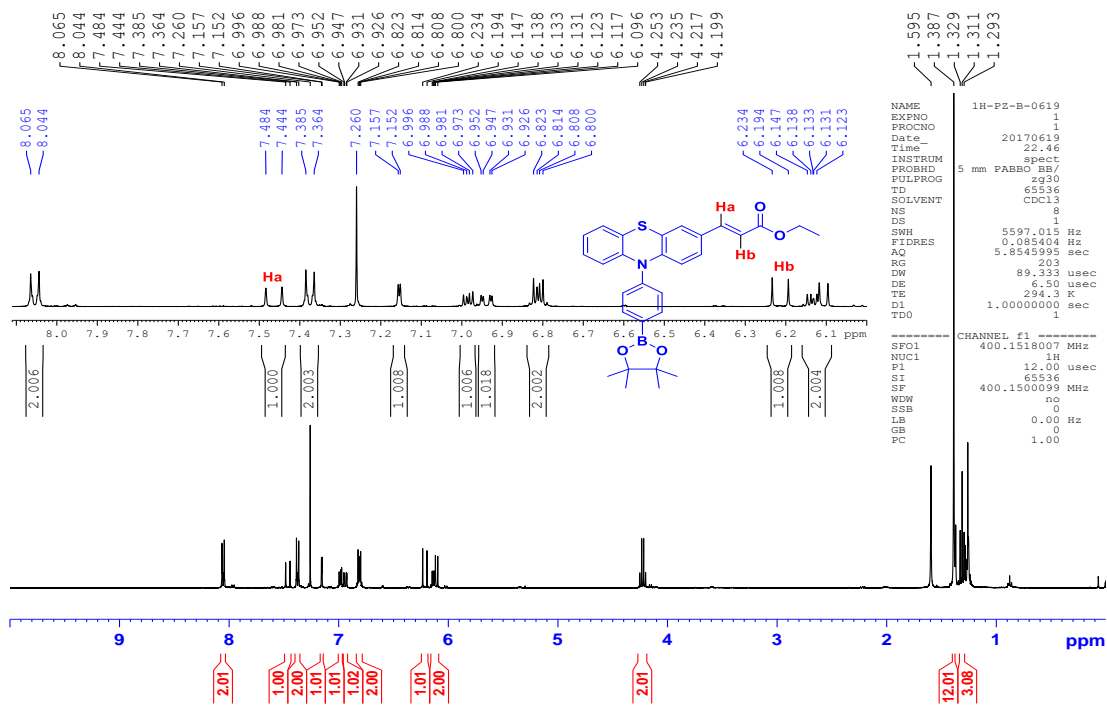


Fig. S15 ¹H NMR of 6

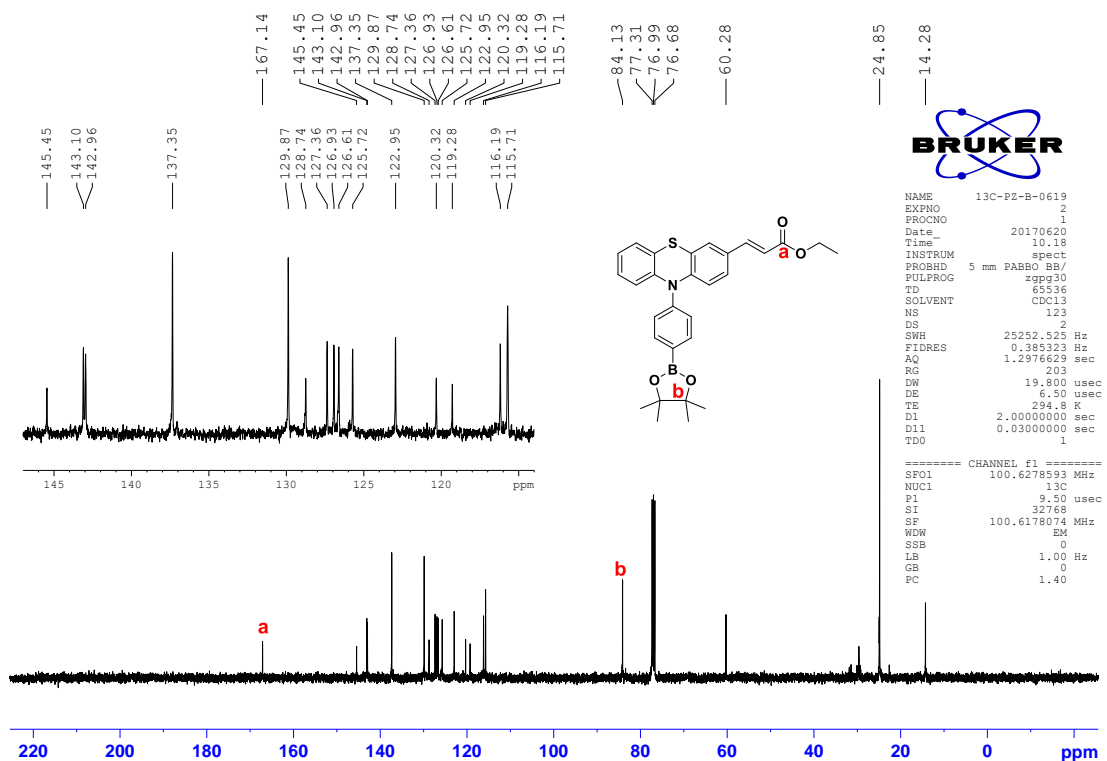


Fig. S16 ¹³C NMR of 6

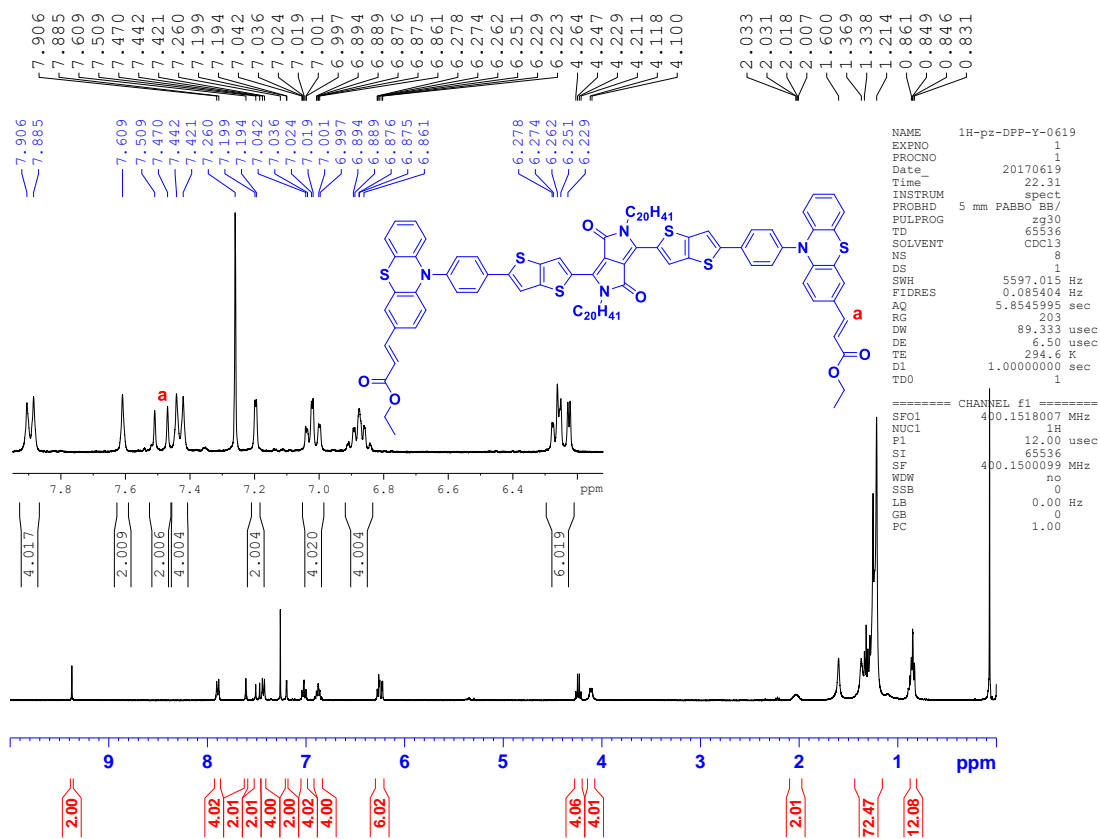


Fig. S17 ¹H NMR of PD

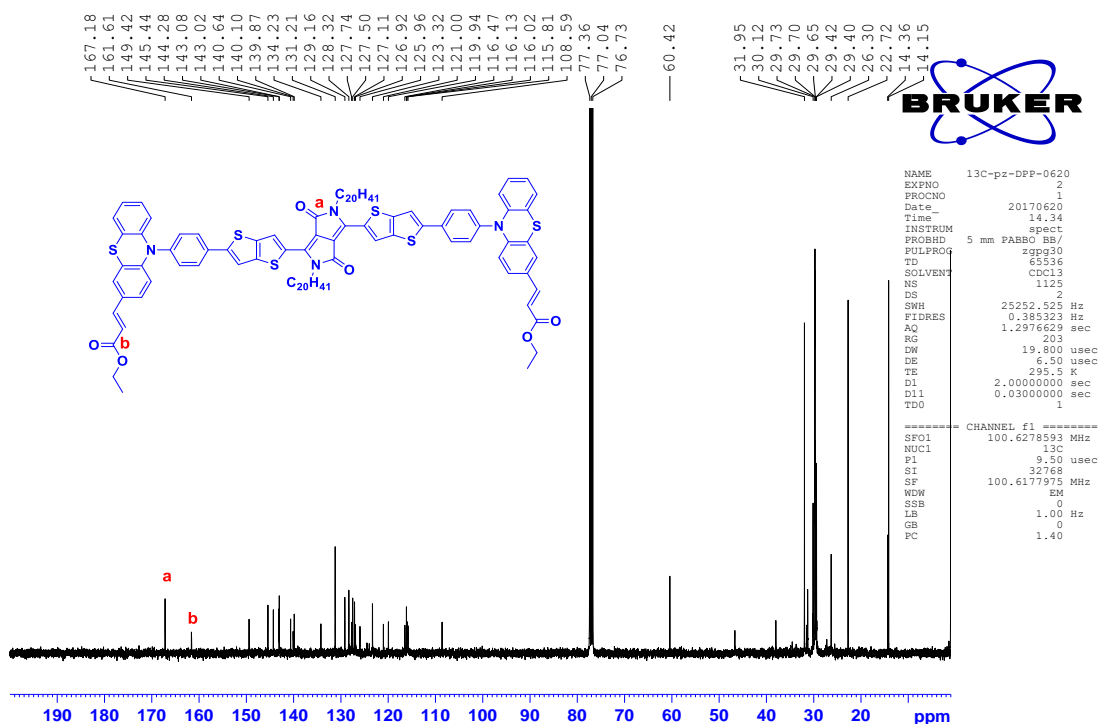
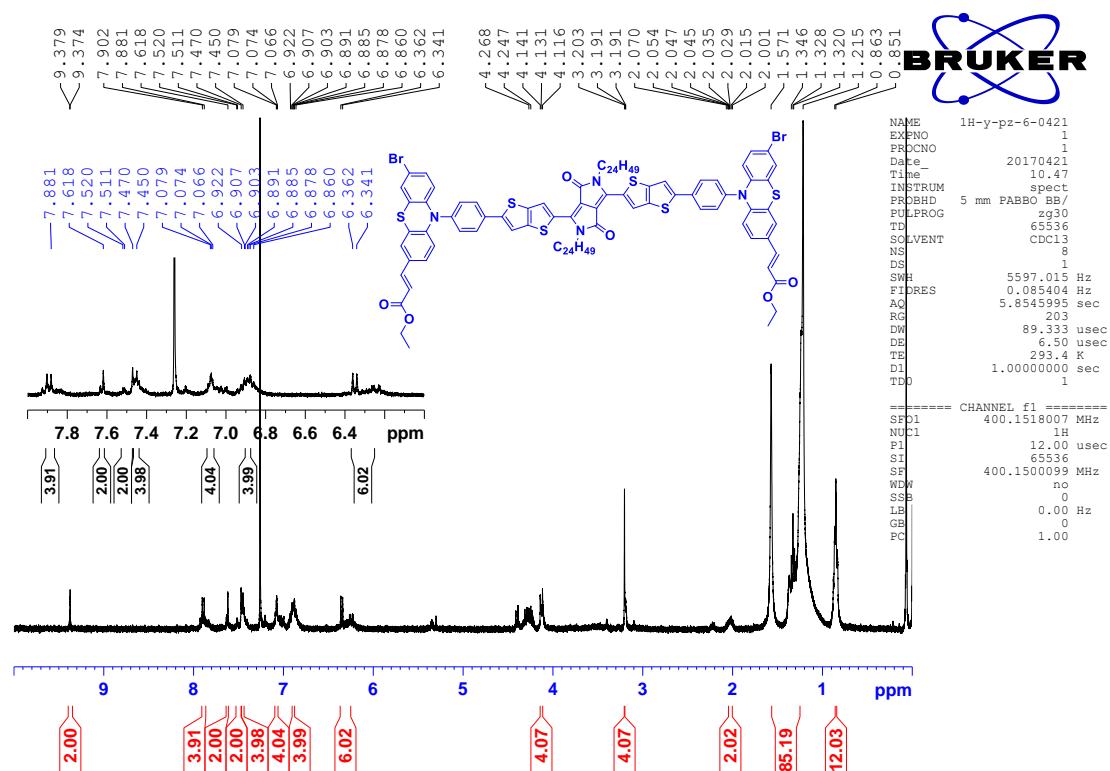


Fig. S18 ¹³C NMR of PD

Fig. S19 ^1H NMR of PDBr