Supporting Information

Diketopyrrolopyrrole-based multifunctional ratiometric fluorescent probe and γ-glutamyltranspeptidase-triggered activatable photosensitizer for tumor therapy

Zhicheng Yang, [‡a] Weibo Xu, [‡bc] Jian Wang,[a] Lingyan Liu,[d] Yanmeng Chu,[e] Yu Wang,[*bc] Yue Hu,[e] Tao Yi[*d] and Jianli Hua[*a]

[a]Key Laboratory for Advanced Materials, Joint International Research Laboratory for Precision Chemistry and Molecular Engineering, Institute of Fine Chemicals, School of Chemistry and Molecular Engineering, East China University of Science and Technology, 130 Meilong Road, Shanghai 200237, PR China.

[b]Department of Oncology, Shanghai Medical College, Fudan University, 130 Dong'an Road, Shanghai 200032, PR China.

[c]Department of Head and Neck Surgery, Fudan University Shanghai Cancer Center, 270 Dong'an Road, Shanghai 200032, PR China.

[d]Department of Chemistry, Fudan University, 2005 Songhu Road, Shanghai 200438, PR China.

[e]Michael Grätzel Center for Mesoscopic Solar Cells, Wuhan National Laboratory for Optoelectronics, Huazhong University of Science and Technology, 1056 Luoyu Road, Wuhan 430074, Hubei, PR China.

Table of Contents

Section S1. Experimental procedures

Materials

All reagents were bought from commercial sources (Energy Chemical, Sigma-Aldrich, TCI) and used without further processing. All solvents were purified and dried before using by standard methods. The solvents used in spectrum analysis were of HPLC grade. The solutions for analytical studies were prepared with deionized water treated using a Milli-Q System (Billerica, MA, USA). The mitochondria-targeting agent Mito-Tracker Green FM was provided by Beyotime biotechnology Corporation.

Instruments

^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AM-400 MHz NMR spectrometer. Chemical shifts were expressed in ppm (in chloroform-d ($CDCl_3$) and DMSO-d^6; TMS as an internal standard) and coupling constants (*J*) in Hz. Electrospray ionization and time-of-flight analyzer (ESI-TOF) mass spectra were determined using a Waters Micromass LCT mass spectrometer. Fluorescence spectra were recorded using a Horiba Fluoromax-4 spectrofluorometer. Absorption spectra were recorded on a P Varian Cary 500 UV-vis spectrophotometer. Fluorescence images were captured using an Olympus FV-1000 laser scanning confocal fluorescence microscope. Imaging of living mouse cell was observed using Bruker in vivo imaging system (In Vivo Xtreme) with a Xe lamp as an excitation.

Calculation of the limit of detection

The limit of detection of γ-GT was estimated by **3σ/s**, which **σ** and **s** represent for the standard deviation of blank measurements of **DPP-GGT** and the slope obtained from the linear plot of **DPP-GGT** fluorescence ratio from 10-50 U/L, respectively.

General procedure for in vitro monitoring γ-Glutamyltranspeptidase activity

Probes were dissolved in dimethyl sulfoxide (DMSO, AR) to obtain 1.0 mM stock solutions. All UV-vis absorption and fluorescence spectra measurements were carried out in DMSO/PBS buffer solution (2:8, v/v, pH = 7.4). In a 1.5 mL tube, PBS buffer (0.8 mL) and DMSO (190 μL) solution were mixed, and then the probe (10 μL) was added to obtain a final concentration of 10 μM. γ-Glutamyltranspeptidase was dissolved in a PBS buffer, and an appropriate volume was added to the sample solution. After rapid mixing of the solution, it was transferred to a 10 ×10 mm quartz cuvette and incubated at 37 °C for in vitro detection.

Cytotoxicity of DPP-py, DPP-dipy, DPP-dibpy and DPP-GGT

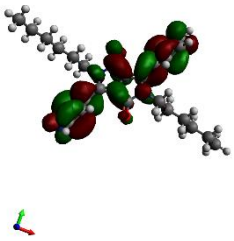
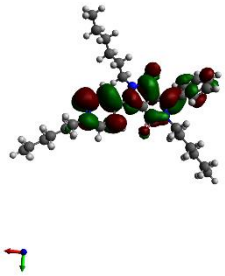
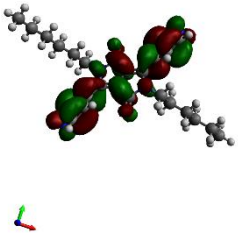
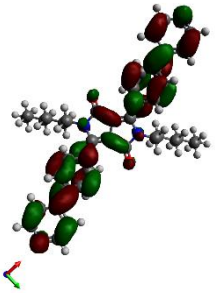
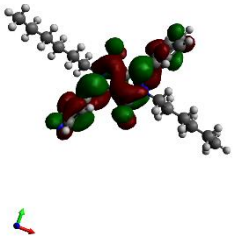
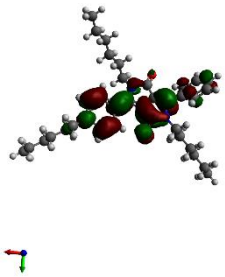
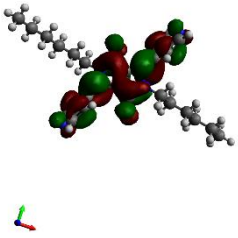
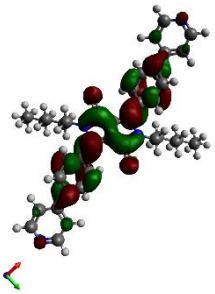
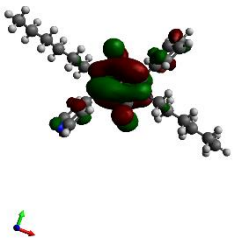
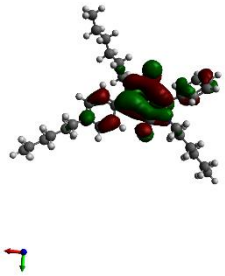
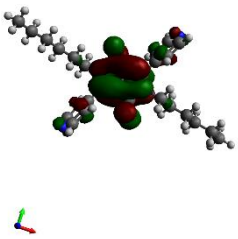
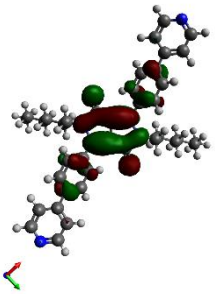
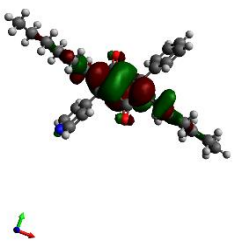
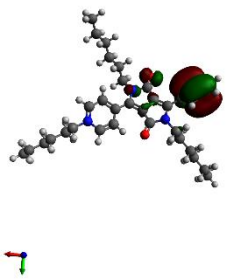
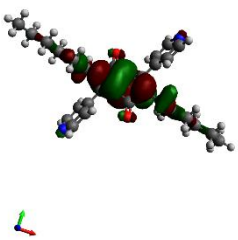
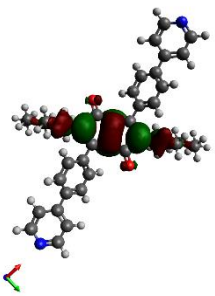
To evaluate the biocompatibility and security of **DPP-py**, **DPP-dipy**, **DPP-dibpy** and **DPP-GGT**, the cytotoxicity to cancer cells (HepG2 cells and MCF-7 cells) and normal cells (L02 cells) was evaluated by Cell Counting Kit 8 (CCK-8) assays. HepG2 cells, L02 cells, and MCF-7 cells were seeded in 96-well plates and cultured in standard 0.2ml DMEM medium containing 10% FBS（Invitrogen, Calsbad, CA, USA）and 1% antibiotics (penicillin, 10 000 U mL^{-1}, streptomycin 10 mg mL^{-1}) for 24 h (37 °C, 5% CO_2). The concentration of DPP-based

photosensitizers was 0-80 μM. After incubation for 24 h, absorbance was measured at 450 nm using multifunctional microplate reader (Synergy H1, BioTek Instruments, America). The relative cell survival rate (%) was calculated by the following formula: cell survival rate = ($OD_{treated}/OD_{control}$) × 100%.

Animals and tumor xenograft models

Female BALB/c nude mice (6–8 weeks old) were purchased from Shanghai SLAC Laboratory Animal Co.,LTD. All animal experiments were performed according to procedures approved by the Fudan University Committee on Animal Care and Use.

Table S1. Molecular orbital distributions and energy optimized in vacuum (isodensity=0.020 a.u.).

	DPP-py	**DPP-pys**	**DPP-dipy**	**DPP-dibpy**
LUMO+1	 -1.41 eV	 -4.67eV	 -1.70 eV	 -1.91 eV
LUMO	 -2.86eV	 -6.31eV	 -3.13 eV	 -2.94 eV
HOMO	 -5.70eV	 -8.38 eV	 -5.94 eV	 -5.68 eV
HOMO-1	 -6.99eV	 -9.59 eV	 -7.19 eV	 -7.07 eV
S_1	2.6404eV	1.7982 eV	2.6138 eV	2.5173 eV
T_1	0.6872 eV	0.4461 eV	0.6132 eV	0.6881eV
T_2	2.6017 eV	2.3060 eV	2.5448 eV	2.3653eV
S_1-T_2	0.0387 eV	1.3521 eV	0.069 eV	0.152eV

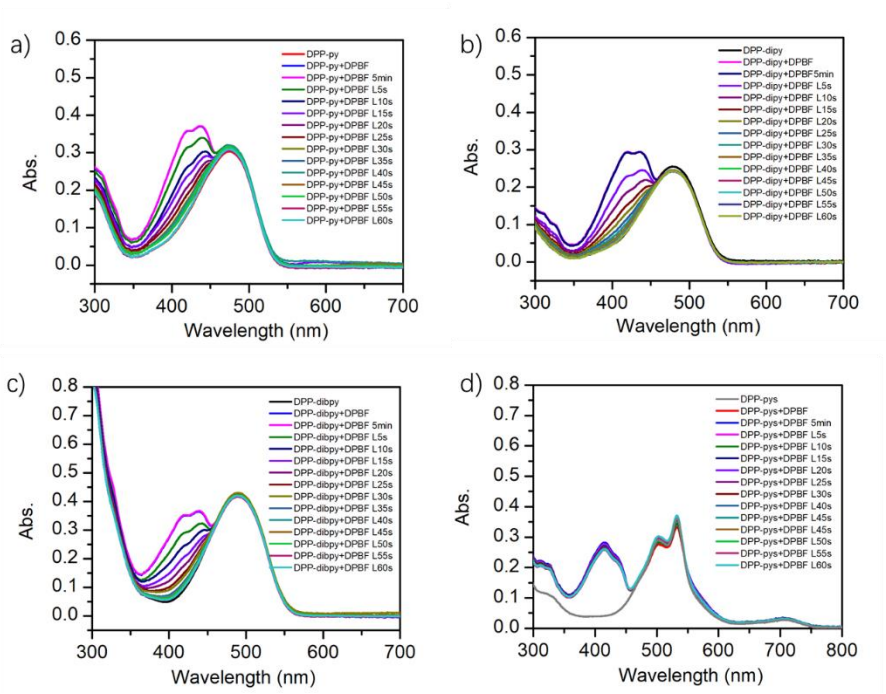


Fig. S1 The absorption spectra of **DPP-py** (a), **DPP-dipy** (b), **DPP-dibpy** (c) and **DPP-pys** (d) with DPBF under irradiation (530 nm, 20 mW/cm^2, 0-60s).

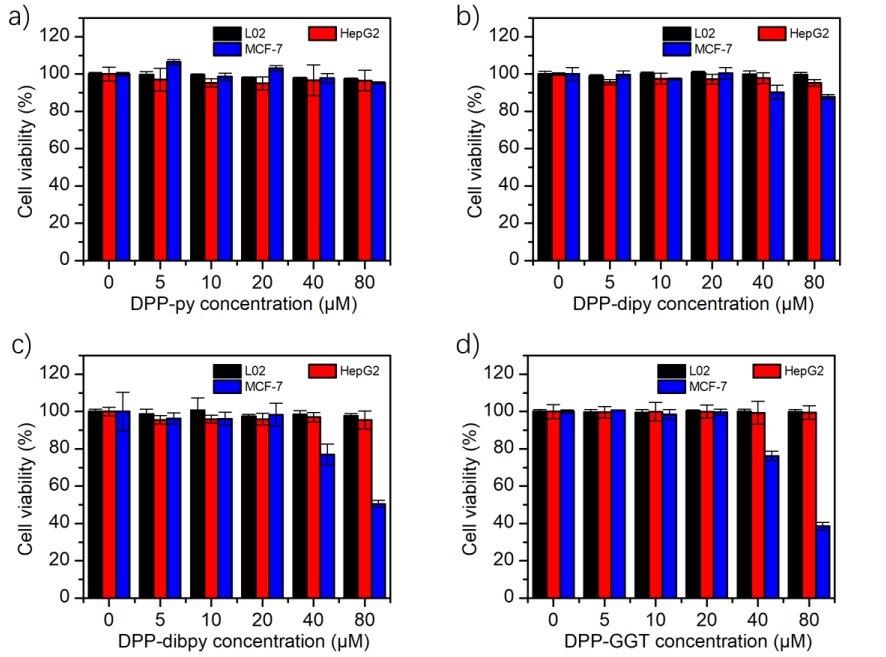


Fig. S2 The viability of **DPP-py** (a), **DPP-dipy** (b), **DPP-dibpy** (c) and **DPP-GGT** (d) treated L02, HepG2 and MCF-7 cells in the dark.

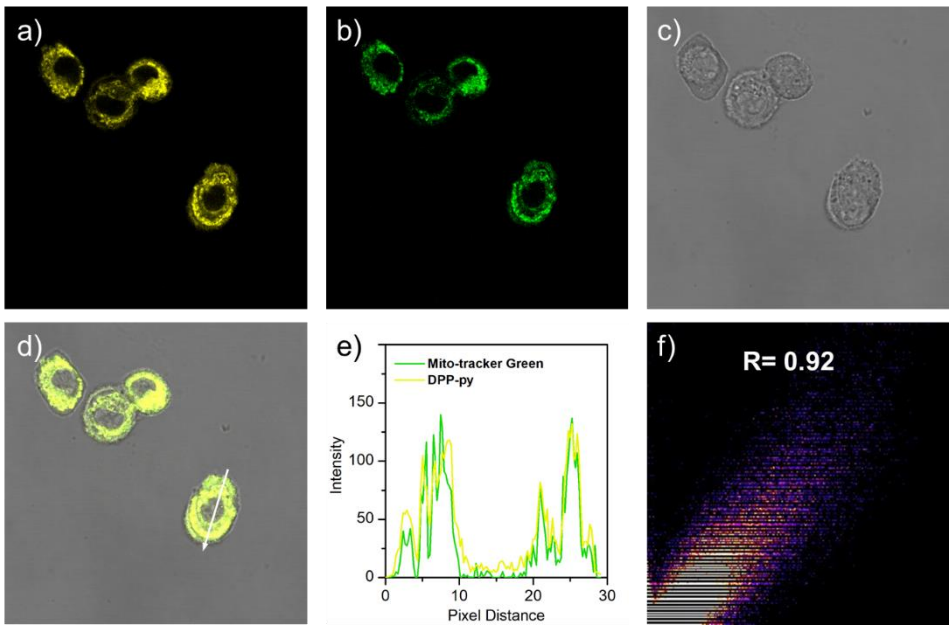


Fig. S3 Confocal fluorescence images of HepG2 cells incubated with a) 10 μM **DPP-GGT** (yellow channel) at 37 °C for 120 min, b) 200 nM Mito Tracker Green FM (green channel) at 37 °C for 30 min, c) Bright field. d) Merged image of parts a−c. e) Intensity profiles of ROI across HepG2 cells; yellow lines represent the intensity of **DPP-GGT**, green lines represent the intensity of Mito Tracker Green FM. f) Correlation plot of Mito Tracker Green FM and **DPP-GGT** intensities, R = 0.92. Yellow channel, 540−570 nm; green channel, 500−520 nm; λex = 490 nm.

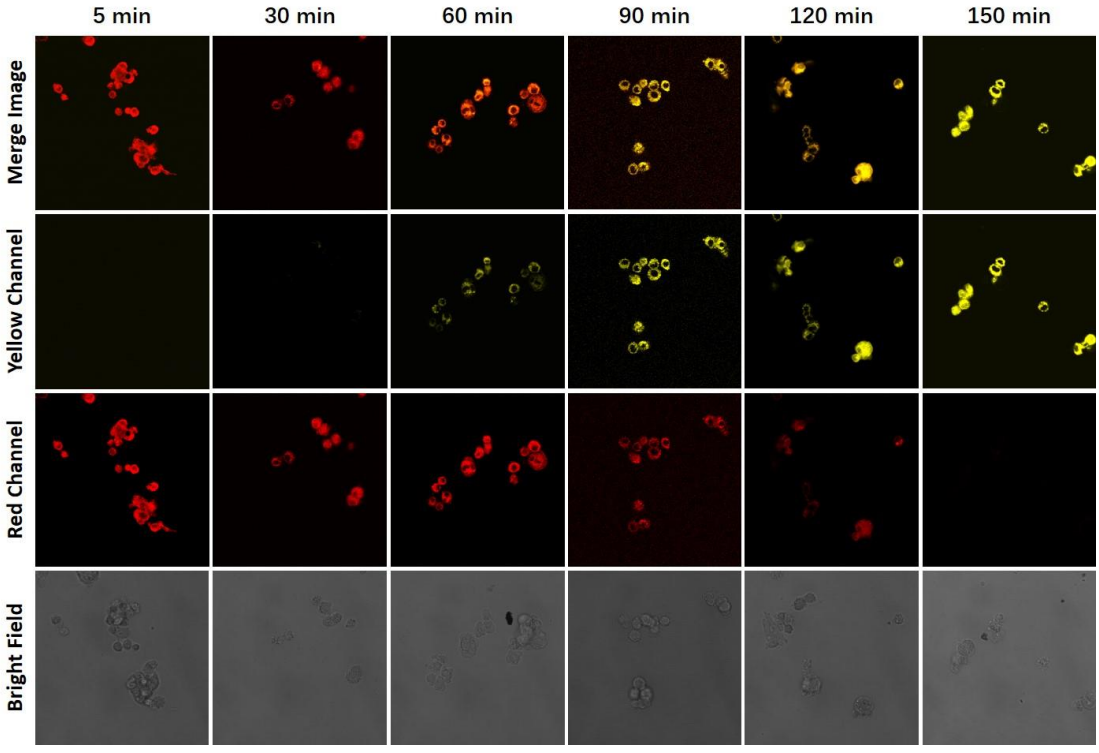


Fig. S4 Confocal fluorescence images of HepG2 incubated with **DPP-GGT** for 5-150min.

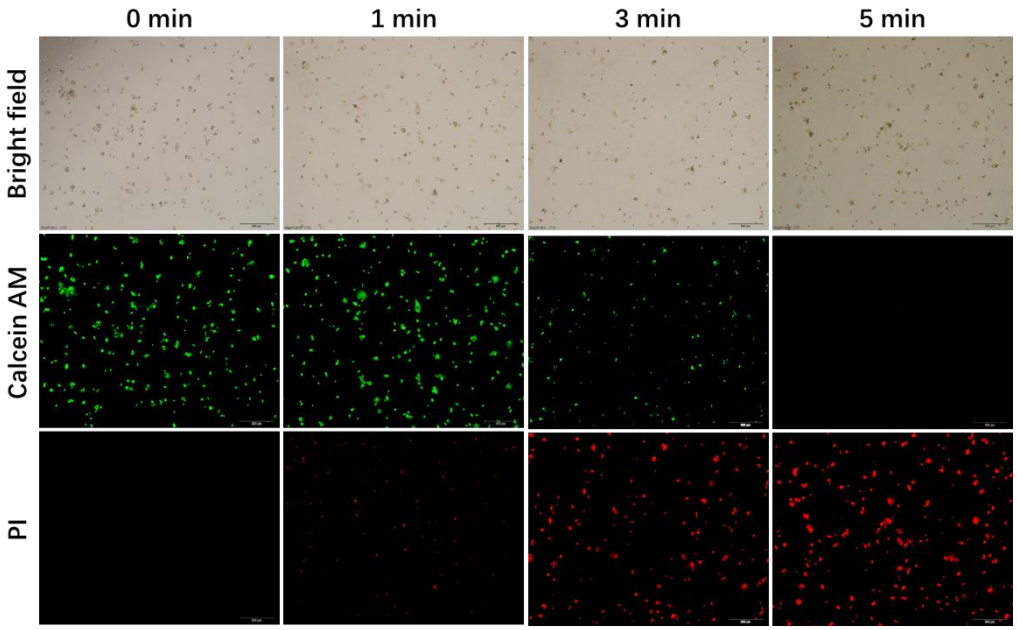
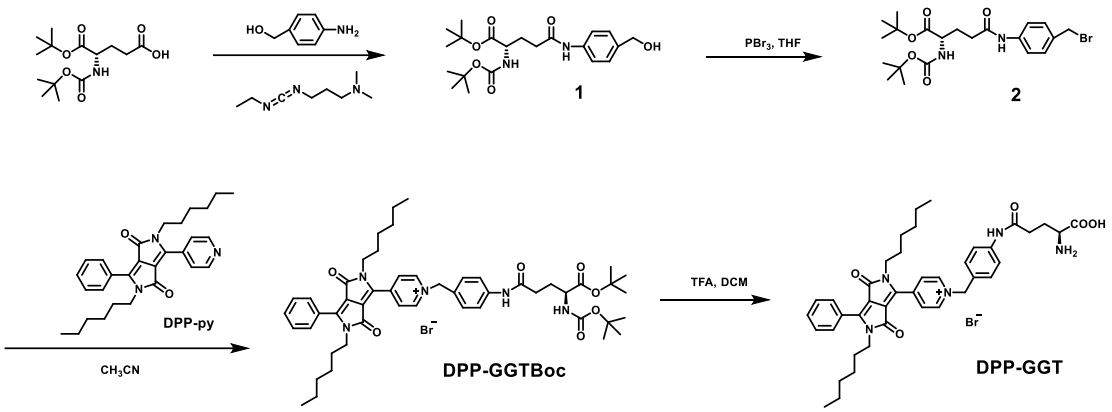


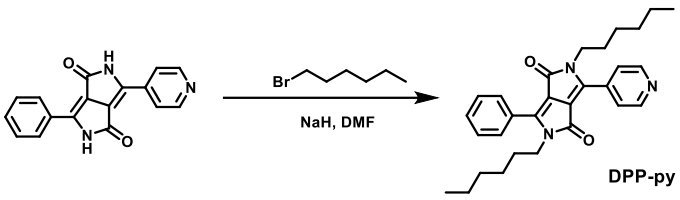
Fig. S5 Fluorescent image of Calcein-AM and propidium iodide staining HepG2 cells (incubating **DPP-GGT** for 150 min) after irradiation (530 nm, 20 mW/cm^{-2}).

Section S2. Synthesis and characterization



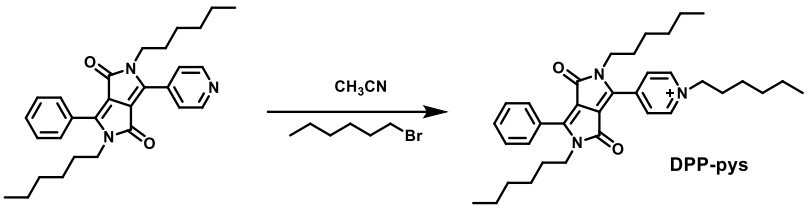
Scheme S1. Synthesis route of **DPP-GGT**

Synthesis of DPP-py



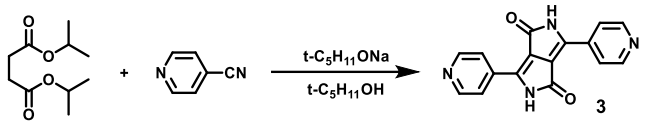
Compound 3-phenyl-6-(pyridin-4-yl)-2,5-dihydropyrrolo[3,4-c]pyrrole-1,4-dione (578.59 mg, 2.0 mmol) and sodium hydride (200 mg, 8.3 mmol) were added into a 50 mL two-necked flask, then 20 mL dry N,N-dimethylformamide was added, subsequently, the mixture was stirred at 0 °C for 1 hour under the protection of an argon atmosphere. Then the reaction solution was warmed to room temperature and 1-bromohexane (1.0 mL, 7.1 mmol) was added. The mixture was keep stirred about 8 hours at 100 °C. Solvent was removed under vacuum and the crude product was purified by column chromatography on silica gel using dichloromethane / petroleum (3:1 v/v) as eluent to provide pure product **DPP-py** (130.70 mg, 14.28% yield) as an orange solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm): δ 8.81 (d, *J* = 6.0 Hz, 2H), 7.83 – 7.78 (m, 2H), 7.68 (d, *J* = 6.1 Hz, 2H), 7.57 – 7.52 (m, 3H), 3.78 – 3.72 (m, 4H), 1.57 (q, *J* = 6.9 Hz, 4H), 1.26 – 1.17 (m, 12H), 0.83 (td, *J* = 6.7, 2.3 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 162.57, 162.10, 150.78, 150.62, 144.23, 135.62, 131.66, 129.00, 128.74, 127.81, 122.05, 111.59, 109.81, 109.55, 42.02, 41.91, 31.19, 31.16, 29.55, 29.33, 26.36, 26.34, 22.44, 13.93. HRMS (ESI-MS, m/z): Calcd for $[M + H]^+$, 458.2808; Found, 458.2791.

Synthesis of DPP-pys

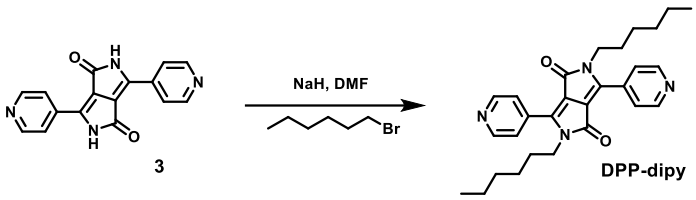


Compound **DPP-py** (50mg, 0.11 mmol) and 1-bromohexane (1.0 mL, 7.1 mmol) were dissolved in dry acetonitrile (15 mL), then the mixture was stirred about 24 hours at 100 °C under argon protection. After removal of the solvent under vacuum, the crude product was purified by column chromatography on silica gel using dichloromethane/ ethanol (20:1 v/v) as eluent to give **DPP-pys** (50 mg, 72.99%) as a purple solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.54 (d, *J* = 6.7 Hz, 2H), 8.49 (d, *J* = 6.7 Hz, 2H), 7.81 (d, *J* = 8.4 Hz, 2H), 7.56 (dd, *J* = 14.0, 7.3 Hz, 3H), 5.10 (t, *J* = 7.4 Hz, 2H), 3.91 – 3.84 (m, 2H), 3.81 – 3.74 (m, 2H), 2.13 – 2.01 (m, 3H), 1.74 (s, 6H), 1.57 (dq, *J* = 12.9, 6.7, 5.9 Hz, 5H), 1.42 (ddd, *J* = 16.7, 9.1, 5.5 Hz, 5H), 0.91 – 0.80 (m, 14H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 162.35, 161.23, 155.12, 145.26, 142.35, 138.18, 132.85, 129.16, 129.03, 126.77, 125.62, 116.27, 110.11, 61.83, 42.43, 42.36, 32.04, 31.24, 31.15, 31.06, 29.89, 29.09, 26.40, 26.25, 25.78, 22.45, 22.39, 22.36, 13.96, 13.93. HRMS (ESI-MS, m/z): Calcd for $[M]^+$, 542.3747; Found, 542.3759.

Synthesis of DPP-dipy

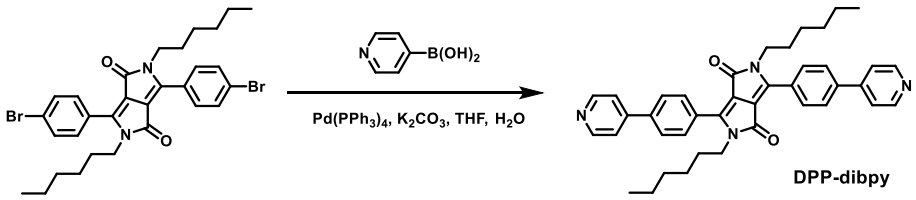


Compound 4-cyanopyridine (8.94 g, 86 mmol) were added in to a solution of sodium tert-pentoxide in 100mL of 2-methyl-2-butanol and heat up to 60 ℃. To a solution of diisopropyl succinate (8.70 g, 43 mmol) in 30 mL of 2-methyl-2-butanol were added dropwise into reacting solution. After addition, the mixture was stirred at 90 °C overnight under argon protection. After the reaction was complete, a mixture of methanol 200 mL and acetic acid 20 mL was added into solution. The resulting solution was purified by suction filtration and wash by a mixture of water/ methanol for three times to afford **compound 3** as a red solid (9.20g, 73% yield).



Compound 3 (580.56 mg, 2.0 mmol) and sodium hydride (200 mg, 8.3 mmol) were added into a 50 mL two-necked flask, then 20 mL dry DMF was added, subsequently, the mixture was stirred at 0 °C for 1 hour under the protection of an argon atmosphere. Then the reaction solution was warmed to room temperature and 1-bromohexane (2.0 mL, 14.2 mmol) was added. The mixture was keep stirred about 8 hours at 100 °C. After the reaction was complete, solvent was removed under vacuum and the residue was purified by column chromatography on silica gel using dichloromethane/ ethyl acetate (4:1, v/v) as eluent to provide the corresponding pure **DPP-dipy** (120 mg, 13.10%) as a red solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.85 (d, *J* = 5.6 Hz, 4H), 7.69 (d, *J* = 6.0 Hz, 4H), 3.78 – 3.73 (m, 4H), 1.28 – 1.17 (m, 16H), 0.86 – 0.82 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 161.87, 150.73, 146.51, 135.13, 121.99, 111.22, 41.98, 31.14, 29.46, 26.31, 22.41, 13.91. HRMS (ESI-MS, m/z): Calcd for $[M + H]^+$, 459.2760; Found, 459.2751.

Synthesis of DPP-dibpy



Compound 3,6-bis(4-bromophenyl)-2,5-dihexyl-2,5-dihydropyrrolo[3,4-c]pyrrole-1,4-dione (307.21 mg, 0.5 mmol), pyridine-4-boronic acid (153.66 mg, 1.25 mmol) and $Pd(PPh_3)_4$ (58 mg, 0.05 mmol) were dissolved in tetrahydrofuran (15 mL). To an aqueous solution of K_2CO_3 (5 mL, 2 M) were added into reaction, then the mixture was stirred at 80 °C for 12 h under argon protection. After the reaction was complete, solvent was removed under vacuum and extraction. The residue was purified by column chromatography on silica gel using dichloromethane/ ethyl acetate (5:1, v/v) as eluent to provide the **DPP-dibpy** (220 mg, 72.04 %) as an orange solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.75 – 8.70 (m, 4H), 7.98 (d, *J* = 8.5 Hz, 4H), 7.82 (d, *J* = 8.5 Hz, 4H), 7.59 – 7.54 (m, 4H), 3.85 – 3.78 (m, 4H), 1.69 – 1.62 (m, 4H), 1.28 – 1.21 (m, 12H), 0.86 – 0.81 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 162.65, 150.46,

147.82, 147.10, 140.70, 129.46, 128.74, 127.54, 121.55, 110.30, 42.08, 31.22, 29.49, 26.41, 22.47, 13.97. HRMS (ESI-MS, m/z): Calcd for $[M + H]^+$, 611.3386; Found, 611.3394.

Synthesis of the fluorescent probe DPP-GGT

Synthesis of compound 1

To a solution of 3.0 mmol N-Boc-Glu-OtBu and 3.0 mmol 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide (EDC) hydrochloride in 6 mL of CH_2Cl_2 was dropwise added a solution of 4aminobenzyl alcohol (2.97 mmol) in 4 mL of CH_2Cl_2 at 0 °C and the resulting solution was stirred for an additional 1.5 h. After the reaction was complete, all the solvent was removed under reduced pressure and the crude product was purified by column chromatography on silica gel (EtOAc-Hexane-MeOH as eluent, 1:2:0.5 v/v) to afford 945 mg of **compound 1** as a white solid (78% yield). ^{1}H NMR (400 MHz, CD_3OD): δ 7.52 (d, *J* = 8.4 Hz, 2H), 7.29 (d, *J* = 8.4 Hz, 2H), 4.55 (s, 2H), 4.06 – 3.97 (m, 1H), 2.46 (t, *J* = 7.5 Hz, 2H), 2.22 – 2.10 (m, 1H), 1.94 (dd, *J* = 14.8, 8.1 Hz, 1H), 1.47 (s, 9H), 1.43 (s, 9H).

Synthesis of compound 2

Compound 1 (200 mg, 0.490 mmol) from last step was dissolved in 5.0 mL anhydrous THF, and PBr_3 (46 μL) was added under ice bath. The reaction mixture was stirring at 0 °C for another 1 h. The mixture was added to a saturated $NaHCO_3$ solution (10 mL) and then diluted with 50 mL H2O, followed by extracting with ethyl acetate (50 mL) for three times. The organic phases were combined and dried by anhydrous Na_2SO_4. The solvent was then removed under vacuum and the crude product was purified by column chromatography on silica gel using petroleum/ethyl acetate (10:1 v/v) as eluent to afford the **compound 2** which was used directly for next step.

Synthesis of DPP-GGTBoc

Compound **DPP-py** (91.52 mg, 0.2 mmol) and **compound 2** (94.28 mg, 0.2 mmol) were dissolved in dry acetonitrile (15 mL), then the mixture was stirred at 80 °C overnight under argon protection. After removal of the solvent under vacuum, the crude product was purified by column chromatography on silica gel using dichloromethane/ethanol (20:1 v/v) to give product **DPP-GGTBoc** (55 mg, 29.60% yield) as a purple solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.12 (s, 1H), 9.33 (d, *J* = 6.6 Hz, 2H), 8.51 (d, *J* =

6.6 Hz, 2H), 7.89 (d, *J* = 6.4 Hz, 2H), 7.67 (t, *J* = 8.8 Hz, 5H), 7.56 (d, *J* = 8.5 Hz, 2H), 7.17 (d, *J* = 7.7 Hz, 1H), 5.85 (s, 2H), 3.83 (dt, *J* = 7.2, 4.2 Hz, 1H), 3.80 – 3.72 (m, 4H), 2.43 – 2.38 (m, 2H), 2.02 – 1.94 (m, 1H), 1.80 (dd, *J* = 8.8, 5.5 Hz, 1H), 1.39 (s, 9H), 1.37 (s, 9H), 1.17 (dt, *J* = 29.8, 11.3 Hz, 22H). ^{13}C NMR (100 MHz, DMSO): δ 211.01, 203.95, 191.24, 191.14, 190.66, 188.36, 185.14, 183.68, 180.10, 179.07, 172.10, 170.72, 164.51, 163.18, 147.33, 146.32, 145.83, 144.65, 143.76, 140.85, 140.35, 135.01, 129.66, 129.52, 128.71, 128.52, 128.16, 127.33, 126.76, 120.25, 119.38, 107.58, 98.06, 93.76, 93.04, 78.27, 77.51, 73.92, 62.38, 58.45, 52.19, 52.04, 49.77, 30.84, 30.63, 26.13, 22.05, 21.87, 13.82. HRMS (ESI-MS, m/z): Calcd for $[M]^+$, 848.4962; Found, 848.4963.

Synthesis of DPP-GGT

To a solution of **DPP-GGTBoc** (100mg, 0.10mmol) in 20 mL of CH_2Cl_2 were added 0.1 mL CF_3CO_2H in an ice bath. The resulting solution was cooled down to 0 °C and further stirred for 4 h. After the reaction was complete, all the volatiles were removed under reduced pressure and the crude solid was washed with petroleum to afford **DPP-GGT** (79 mg, 95% yield) as a purple solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.32 (s, 1H), 9.31 (d, *J* = 6.8 Hz, 2H), 8.51 (d, *J* = 6.9 Hz, 2H), 7.96 (t, *J* = 7.5 Hz, 2H), 7.90 – 7.87 (m, 3H), 7.69 – 7.64 (m, 5H), 7.56 (d, *J* = 8.4 Hz, 2H), 5.83 (s, 2H), 3.76 (q, *J* = 8.6, 8.0 Hz, 5H), 2.07 – 1.98 (m, 2H), 1.44 – 1.39 (m, 2H), 1.05 (t, *J* = 7.0 Hz, 12H), 0.76 (dt, *J* = 6.7, 3.3 Hz, 10H). ^{13}C NMR (100 MHz, DMSO): δ 170.79, 170.05, 145.13, 135.68, 135.53, 135.51, 134.10, 134.03, 133.93, 130.45, 130.36, 130.23, 130.12, 130.00, 129.87, 129.09, 128.81, 126.28, 119.52, 119.47, 119.02, 117.30, 116.42, 109.07, 99.48, 60.38, 51.54, 51.41, 31.45, 31.40, 30.52, 30.35, 28.99, 27.44, 25.58, 25.48, 21.79, 13.76. HRMS (ESI-MS, m/z): Calcd for $[M]^+$, 692.3812; Found, 692.3817.

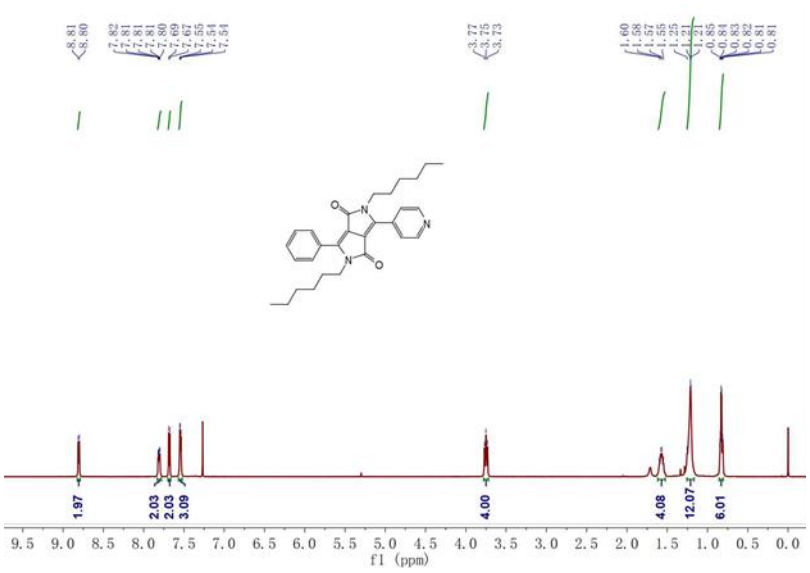


Fig. S6 ^{1}H NMR spectrum (400 MHz) of **DPP-py** in $CDCl_3$.

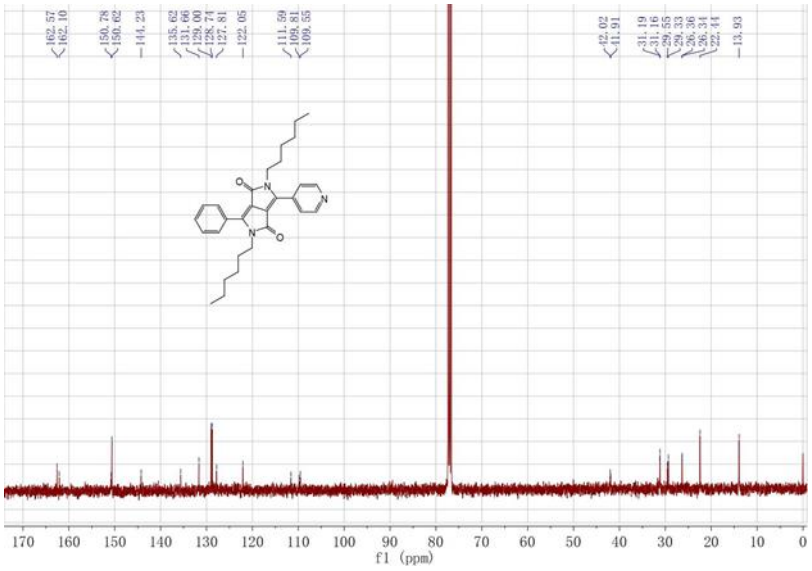


Fig. S7 ^{13}C NMR spectrum (100 MHz) of **DPP-py** in $CDCl_3$.

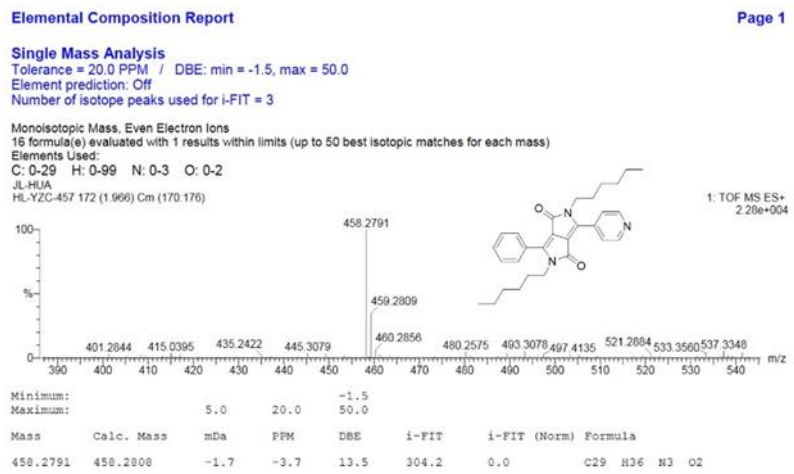


Fig. S8 HRMS of **DPP-py**.

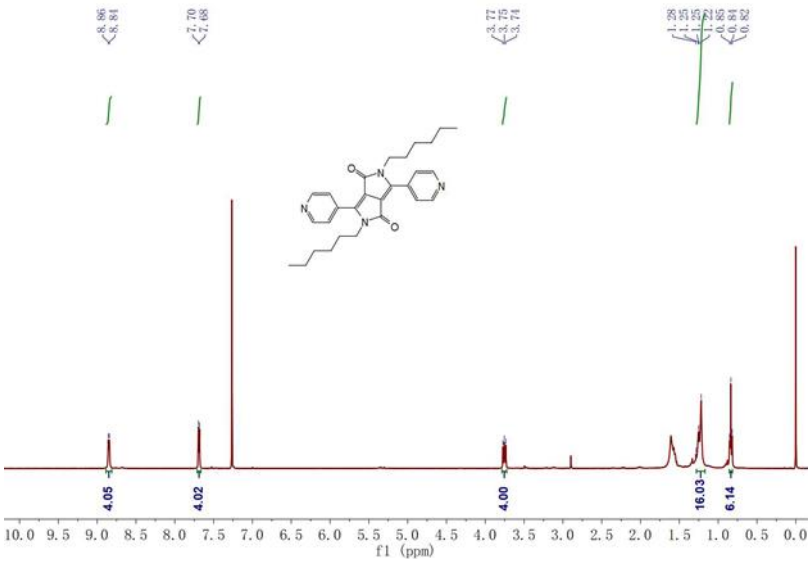


Fig. S9 ^{1}H NMR spectrum (400 MHz) of **DPP-dipy** in $CDCl_3$.

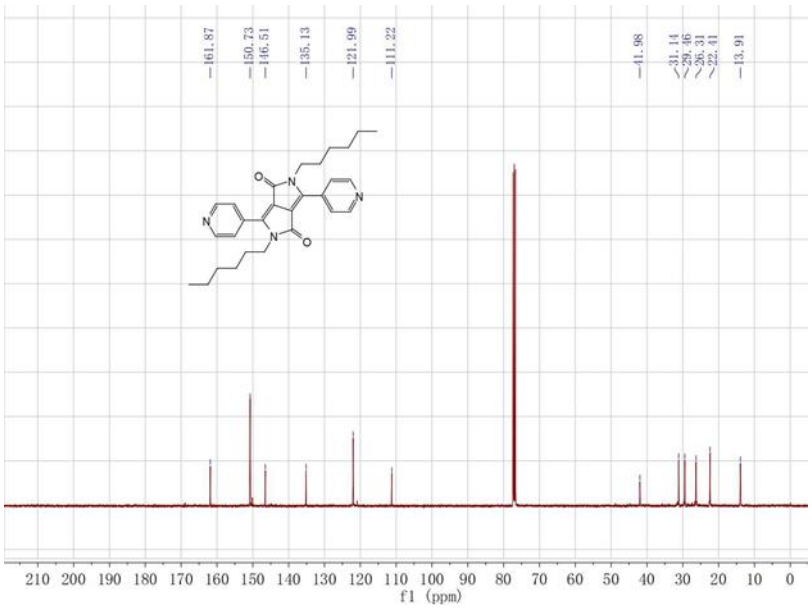


Fig. S10 ^{13}C NMR spectrum (100 MHz) of **DPP-dipy** in $CDCl_3$.

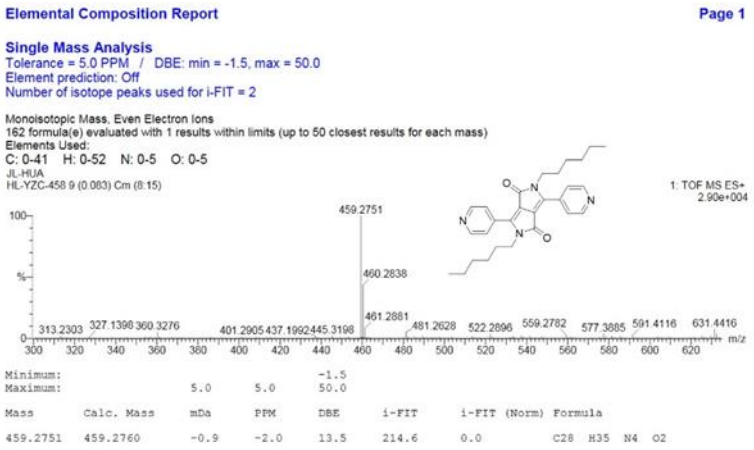


Fig. S11 HRMS of **DPP-dipy**.

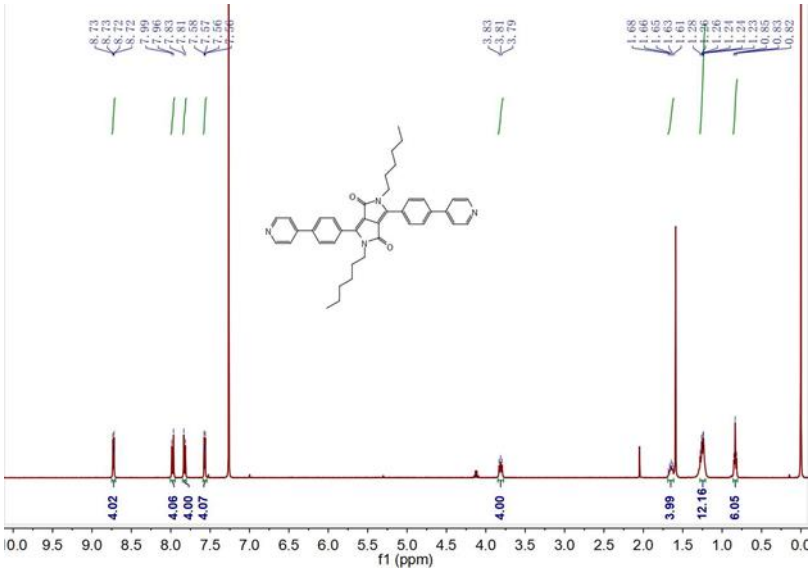


Fig. S12 ^{1}H NMR spectrum (400 MHz) of **DPP-dibpy** in $CDCl_3$.

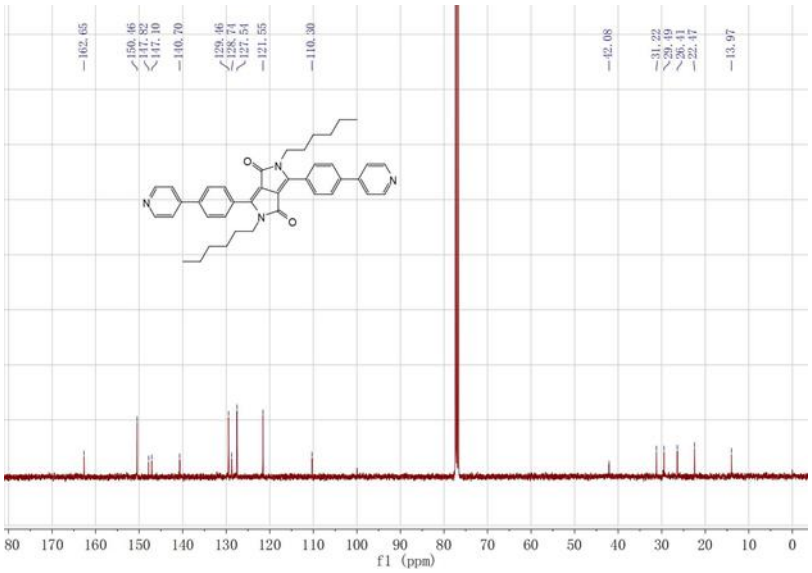


Fig. S13 ^{13}C NMR spectrum (100 MHz) of **DPP-dibpy** in $CDCl_3$.

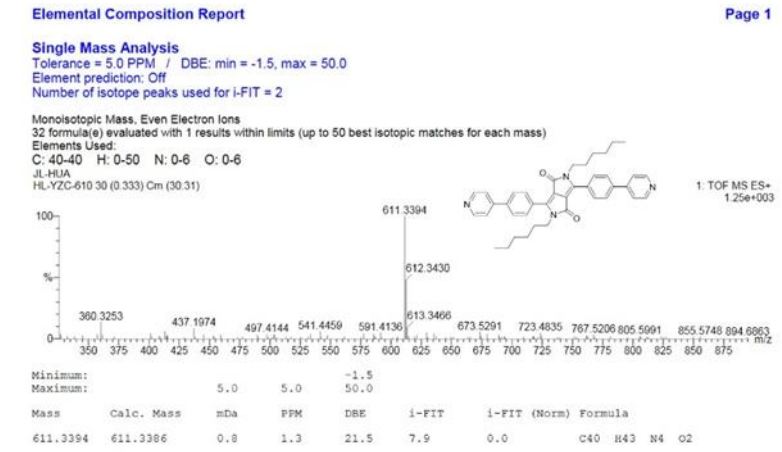


Fig. S14 HRMS of **DPP-dibpy**.

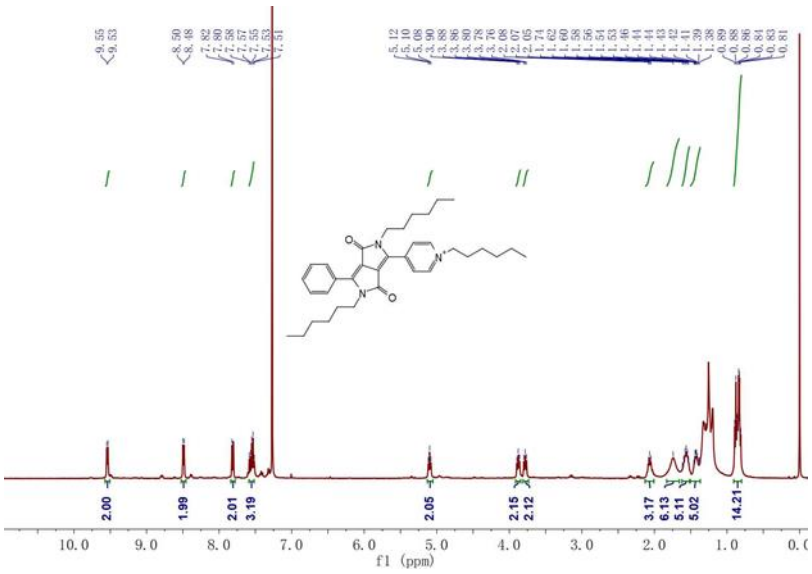


Fig. S15 ^{1}H NMR spectrum (400 MHz) of **DPP-pys** in $CDCl_3$.

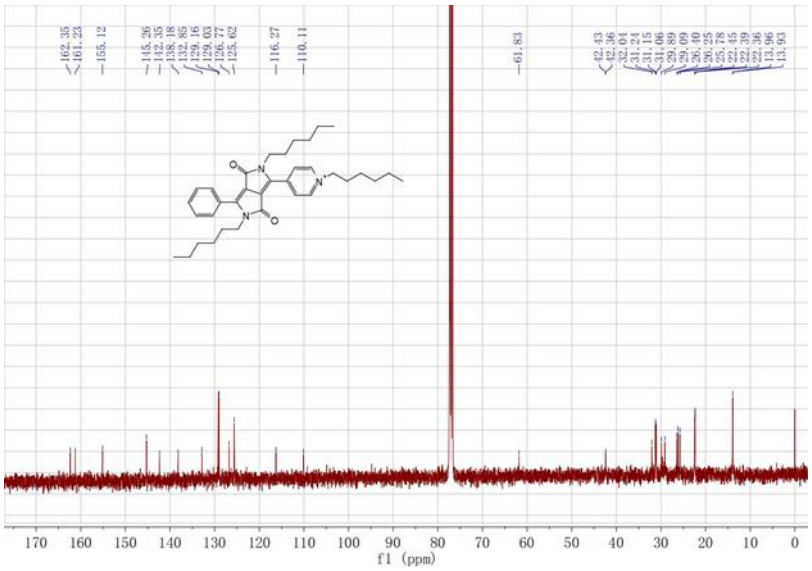


Fig. S16 ^{13}C NMR spectrum (100 MHz) of **DPP-pys** in $CDCl_3$.

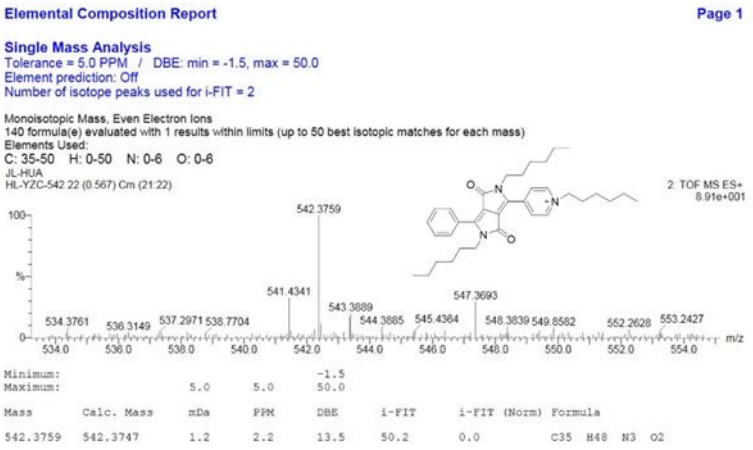


Fig. S17 HRMS of **DPP-pys**.

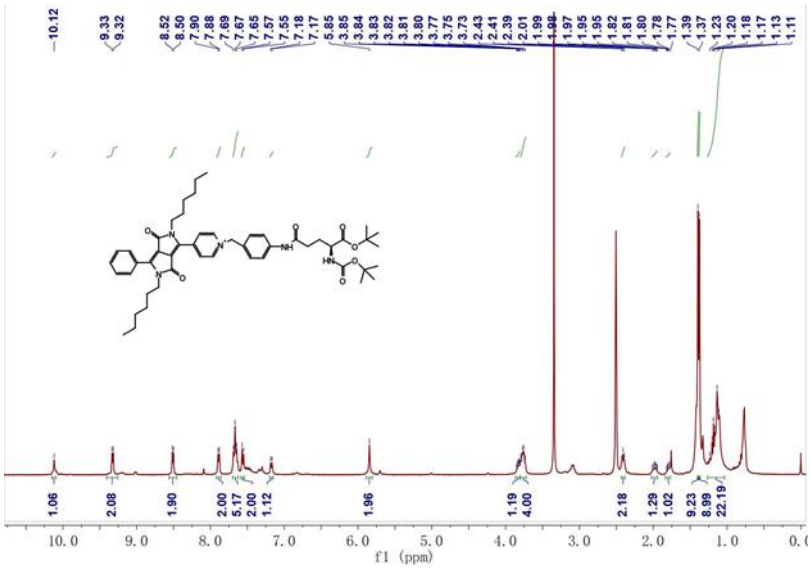


Fig. S18 ^{1}H NMR spectrum (400 MHz) of **DPP-BocGGT** in DMSO-d_6.

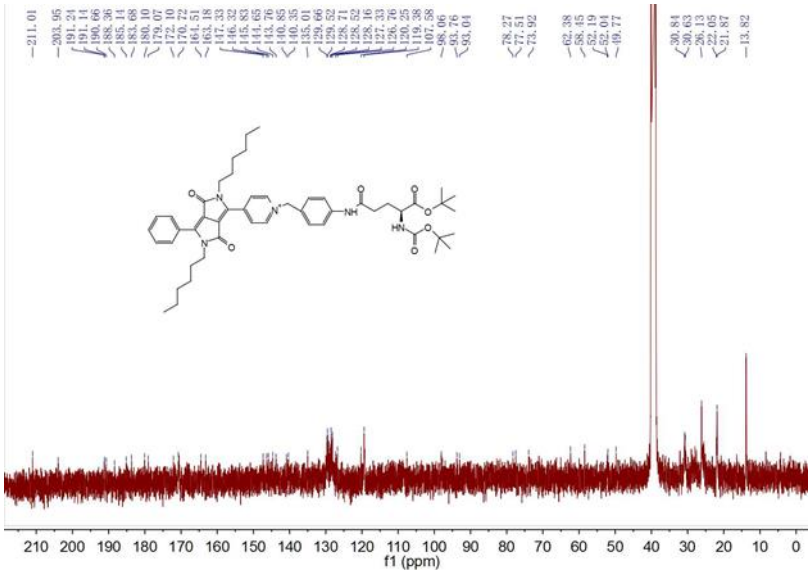


Fig. S19 ^{13}C NMR spectrum (100 MHz) of **DPP-BocGGT** in DMSO-d_6.

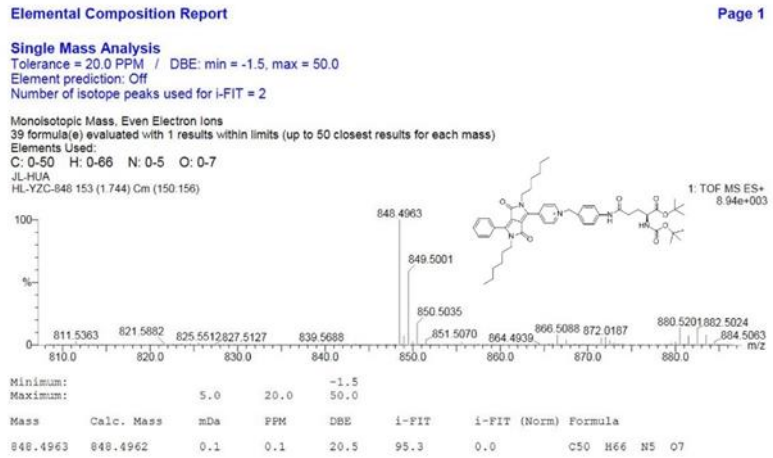


Fig. S20 HRMS of **DPP-BocGGT**.

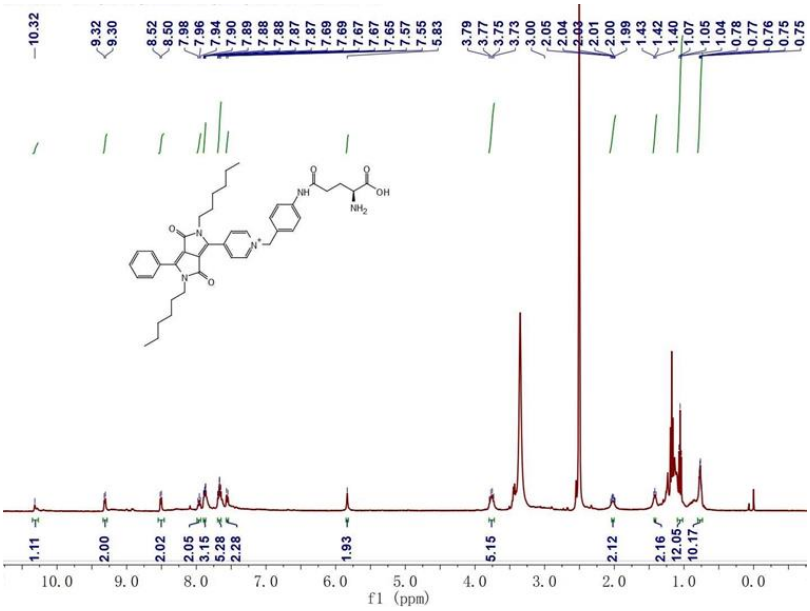


Fig. S21 ^{1}H NMR spectrum (400 MHz) of **DPP-GGT** in DMSO-d_6.

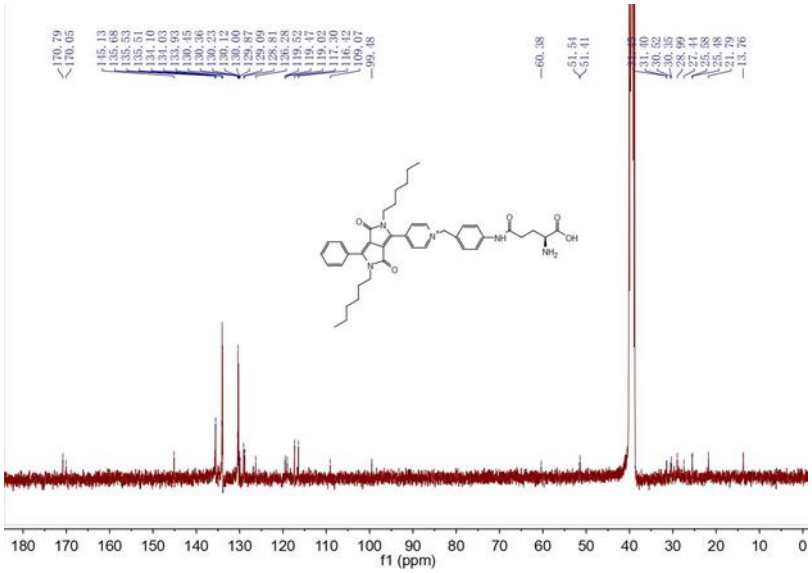


Fig. S22 ^{13}C NMR spectrum (100 MHz) of **DPP-GGT** in DMSO-d_6.

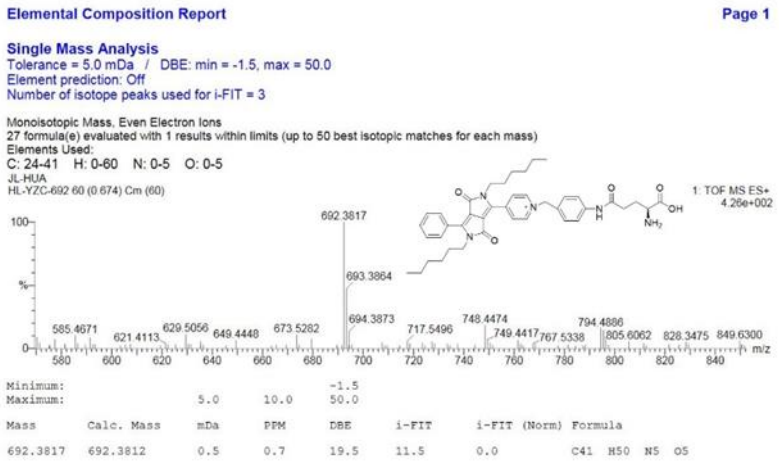


Fig. S23 HRMS of **DPP-GGT**.