# Supporting Information

# Water-Soluble thienoviologen derivatives for bacteria imaging and antimicrobial photodynamic therapy

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## 1. Materials and instrumentation

**General.** All reactions were performed using standard Schlenk and glovebox (Vigor) techniques under argon atmosphere. $Et_2O$, THF, DMF and toluene were distilled from sodium/benzophenone prior to use. All the chemicals used in the experiments were purchased from Energy Chemical Inc, Sigma, Thermo Fisher Scientific Inc. and Sangon Biotech. 1-bromo-6-(trimethylammonium)hexyl bromide was prepared according to literature procedures. If no other special indication, other reagents and solvents were used as commercially available without further purification. Column chromatographic purification of products was accomplished using 200-300 mesh silica gel. NMR spectra were measured on a Bruker Avance-400 spectrometer in the solvents indicated; chemical shifts are reported in units (ppm) by assigning DMSO-$d_6$ resonance in the $^1H$ spectrum as 2.50 ppm, $CDCl_3$ resonance in the $^1H$ spectrum as 7.26 ppm, DMSO-$d_6$ resonance in the $^{13}C$ spectrum as 39.52 ppm, $CDCl_3$ resonance in the $^{13}C$ spectrum as 77.16 ppm. Coupling constants are reported in Hz with multiplicities denoted as s (singlet), d (doublet), t (triplet), q (quartet) and m (multiplet). UV-vis measurements were performed using DH-2000-BAL Scan spectrophotometer. The absolute fluorescence quantum yields were measured on the Hamamatsu C9920 Quantum Efficiency Measurement. Fluorescence measurements were conducted on FLS920 system (Edinburgh Instruments) and Hitachi F-7000. Bacterial killing assay processed under visible light via a Mejiro Genossen MVL-210. HT7700 transmission electron microscopy was used to record the TEM images. SEM images were examined by Quanta 250 feg scanning electron microscope. Delsa Nano C was used to measure the zeta potentials. CLSM characterization was conducted with a confocal laser scanning biological microscope (Leica TCS SP8 STED 3X). Photographs were taken using a Nikon D5100 digital camera.

## 2. Experimental conditions

### Bacterial growth conditions

Bacterial samples were transferred from the frozen state onto agar slants incubated at 37℃ overnight and then held at 4℃ for up to 2 weeks. A single colony from the slants was incubated in 50 mL of LB for shaking overnight at 37℃. After growth, the bacterial culture was centrifuged at 7000 rpm for 2 min at 4℃ and the pellet was suspended in PBS. This washing procedure was repeated trice. The final concentration of bacteria was around $1\times10^9$ CFU/mL.

### *In vitro* antibacterial experiments

The antibacterial activities of compounds were determined by incubation with bacterial cells suspensions for 10 min in the dark at room temperature. Then the mixture solutions were exposed to 70 mW/cm$^2$ white light for 60 min, or incubated in the dark for 60 min. And then all of the bacterial suspensions were serially diluted $1\times10^4$ fold with PBS. A 100 μL portion of the dilution with bacteria was spread on the solid LB agar plate, and the colonies formed after 12 h incubation at 37℃ were counted. The inhibition ratio was determined by dividing the number of colony-forming unite (CFU). The inhibition ratio (IR) was calculated according to the following equation:

$$\mathrm{IR} = \frac{\mathrm{C_0} - \mathrm{C}}{\mathrm{C_0}} \times 100\%$$

Where C is the CFU of t he experimental group treated with compounds, and $C_0$ is the CFU of the control group without any treatment.

### Confocal laser scanning microscopy (CLSM) characterization

*E. coli* was routinely grown at 37℃ in LB medium overnight, and then bacteria was collected and suspended in PBS with an optical density (OD) 1.0 at 600 nm. 10 μL of 1 mM **2TPyC$_6$** stock solution was added to the bacteria suspension (the final concentration was 10 μM) and co-cultured at 37℃ for 10 min at 220 rpm. The bacteria without addition of **2TPyC$_6$** was used as control group. After centrifugation at 4000 rpm, the collected cells were washed and suspended in PBS with 10 μg/mL DAPI (4',6-diamidino-

2-phenylindole) and stained for 5 min at room temperature. Then the cells were washed twice and prepared on glass for confocal imaging. The wavelength of stimulating laser of **2TPyC$_6$** and DAPI is 500 nm and 405 nm, respectively. The emission of **2TPyC$_6$** was collected from 518 nm to 676 nm and the emission of DAPI was collected from 413 nm to 521 nm.

**Zeta potential measurements**

Bacteria (*E. coli* and *S. aureus*) in PBS (10 mM, pH=7.4) was incubated separately with **nTPy-R**s for 15 min at 37℃. The bacteria were obtained by centrifuging (1000 g for 5 min, 4℃), and the precipitated pellets were suspended in ultrapure water and the suspensions were kept on ice for zeta potential measurements. As negative controls, untreated bacteria (without any kind of materials) were also disposed under exactly the same process.

**Reactive oxygen species (ROS) measurements**

2, 7-dichlorofluorescein diacetate (DCFH-DA) was used to probe the generation of ROS. Under alkaline conditions, DCFH-DA was converted into 2,7-dichlorofluorescin (DCFH), which was followed by transforming into highly fluorescent 2,7-dichloro fluorescein (DCF, excitation 488nm, emission at 525 nm, quantum yield: 90%) in the presence of ROS. Compounds were added into the solutions of activated DCFH (40 μM), respectively (The final concentration is 1 μM). The solutions were irradiated under white light (5 mW/cm$^2$) for 5 min, and emission intensity of DCF solution at 525nm was recorded every minute with the excitation wavelength of 488 nm.

**TEM measurements**

TEM was applied to study the specific differences of **nTPy-R**s in killing bacteria. After the treatment described in antibacterial experiments, bacteria were immediately fixed with glutaraldehyde (0.5%) in PBS at room temperature for 30 min. The bacteria were centrifuged (10000 g for 5 min) and the supernatant was removed, and then the pellets were suspended in sterile water. 2-3 μL of bacterial suspension was dropped onto clean copper grid followed by drying naturally in the air. Once the specimens became dry, 0.1% glutaraldehyde was added to fix it for 1 h and then 0.5% glutaraldehyde for another 2

h. Next, the specimens were washed twice with sterile water and then were dehydrated by adding ethanol in a graded series (70% for 6 min, 90% for 6 min, and 100% for 6 min), and dried after that. Finally, the specimens were put into the experiment of TEM.

**SEM measurements**

To gain more insight on the toxicity of **nTPy-R**s towards bacteria in the presence and absence of light source, SEM characterization was included in this study. 1 mL of bacterial suspension (~$10^9$ colony forming units (CFU)/mL) was mixed with different **nTPy-R**s followed by incubation under visible light irradiation or in the dark for 1 h. The mixture of bacteria cells and **nTPy-R**s was centrifuged at 8000 g for 5 minutes. The cell pellets were resuspended with 0.5% glutaraldehyde and incubated at 4℃ overnight, followed by washing with 0.01 M PBS buffer for two times and sterile water for 2 times. Then, the fixed cells were dehydrated by sequential treatment with increasing concentrations of ethanol (70% for 6 min, 90% for 6 min, and 100% for 6 min) and then dried. At last, the totally dried specimens were sputter-coated with platinum before examination in SEM.

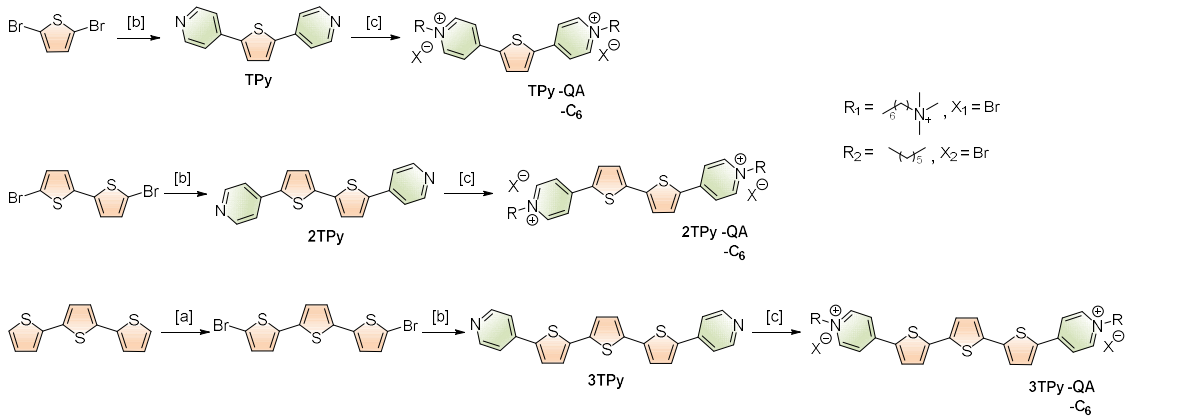
**Cell viability assay**

L-929 mouse fibroblast were cultured in Dulbecco's modified eagle medium supplemented with 10% FBS at 37℃ in a humified atmosphere containing 5% $CO_2$. L-929 mouse fibroblast were seeded in 96-well U-bottom plates at a density of $6\times10^3$ cells/well until adherent, and then were incubated with the same concentration of agents and as the antibacterial experiments at 37℃ for 24 h. Subsequently, MTT (1 mg/mL in medium, 100 μL/well) was added to the wells after the supernatant was removed followed by incubation at 37℃ for 4 h. The supernatant was removed and 100 μL DMSO per well was added to dissolve the produced formazan. After shaking the plates for 10 min, absorbance values of the wells were read with a microplate reader at 520 nm. The cell viability rate (VR) was calculated according to the following equation:

$$\mathrm{VR} = \frac{\mathrm{A}}{\mathrm{A_0}} \times 100\%$$

where A is the absorbance of the experimental groups, and the $A_0$ is the absorbance of the control group.

## 3. Synthetic procedures

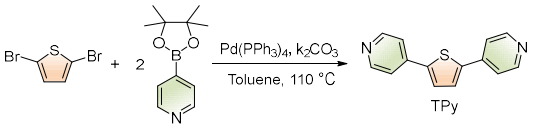



Conditions:[a] NBS (0°C to rt, DMF). [b] 4-Pyridylboronic acid pinacol ester,$Pd(PPh_3)_4$, $K_2CO_3$, (toluene, 110°C). [c] 1-bromohexane / (6-bromohexyl)thrimethylammonium bromide (Chloroform, 60°C).

**Scheme S1.** Synthesis of thienoviologen derivatives.

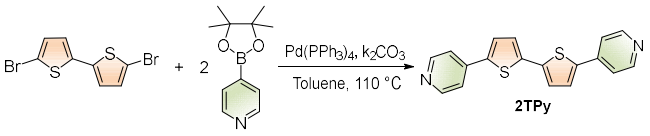
### Synthesis of thienoviologen derivatives.

Each step of synthesis was performed more than 3 times, and the target products were thoroughly characterized by multinuclear NMR ($^1$H and $^{13}$C NMR), UV-vis spectroscopy and HRMS.

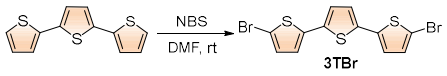



**TPy:** 4-Pyridylboronic acid pinacol ester (6.36 g, 31 mmol) tetrakis(triphenylphosphine) palladium(0) (286 mg, 0.248 mmol) and sodium carbonate (7.95 g, 75 mmol) were added into a 250 mL Schlenk flask. Under argon atmosphere, toluene (50 mL), 2,5-dibromothiophene (1.40 mL, $d$ = 2.15 g/mL, 3 g, 12.4 mmol) and 15 mL $H_2O$ were added, and the suspension was stirred for 48 h at 90°C. After cooling down to room temperature, the toluene was removed under reduced pressure. The product was extracted with chloroform three times. Concentrated HCl (ca. 2 mL) was then added to the organic phase, resulting in precipitation of the product from solution. The precipitate was collected by filtration and then dissolved in $H_2O$. Finally, aqueous NaOH (15 M) was added dropwise to the $H_2O$ layer until the pH was ca. 8-9, resulting the precipitation of pure TPy (1.83 g, 7.68 mmol, 61.9%) as a yellow solid.[1] $^1$H NMR (400 MHz,

DMSO-$d_6$): $\delta$ 8.63 (dd, $J$ = 6.4 Hz, 4H, Py*H*), 7.93 (s, *2*H, T*H*), 7.72 (dd, $J$ = 6.4 Hz, 4H, Py*H*). $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 150.56, 141.74, 139.88, 128.11, 119.51. HRMS (ESI) m/z: calcd. for [$C_{14}H_{10}N_2S$ + $H^+$]$^+$ 239.0637; found 239.0632; UV/vis (in DMF): $\lambda_{max}$ (ε) = 334 nm (4.046 × $10^4$ $M^{-1}$ $cm^{-1}$); Fluorescence emission (in DMF) ($\lambda_{ex}$ = 339 nm): $\lambda_{emis}$ = 386 nm; Mp (°C): 153.8-160.5.

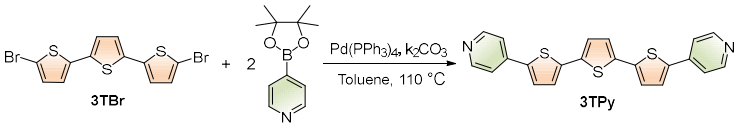



**2TPy:** 4-Pyridylboronic acid pinacol ester (3.29 g, 16.04 mmol), 5,5'-Dibromo-2,2'-bithiophene(2 g, 6.17 mmol), tetrakis(triphenylphosphine) palladium(0) (357 mg, 0.31 mmol) and sodium carbonate (7.95 g, 75 mmol) were added into a 250 mL Schlenk flask. Under argon atmosphere, toluene (50 mL) and 15 mL $H_2O$ were added, and the suspension was stirred for 48 h at 90°C. After cooling down to room temperature, the toluene was removed under reduced pressure. The product was extracted with chloroform three times. Concentrated HCl (ca. 2 mL) was then added to the organic phase, resulting in precipitation of the product from solution. The precipitate was collected by filtration and then dissolved in $H_2O$. Finally, aqueous NaOH (15 M) was added dropwise to the $H_2O$ layer until the pH was ca. 8-9, resulting the precipitation of pure 2TPy (1.27 g, 3.95 mmol, 63.9%) as a yellow solid. $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 8.60 (d, $J$ = 5.6 Hz, 4H, Py*H*), 7.86 (d, $J$ = 4 Hz, 2H, T*H*), 7.68 (d, $J$ = 5.6 Hz, 4H, Py*H*), 7.55 (d, $J$ = 4 Hz, 2H, T*H*). $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 150.49, 139.87, 139.65, 137.44, 127.95, 126.38, 119.30. HRMS (ESI) m/z: calcd. for [$C_{18}H_{12}N_2S_2$ + $H^+$] 321.0515; found 321.0512; UV/vis (in DMF): $\lambda_{max}$ (ε) = 385 nm (5.066 × $10^4$ $M^{-1}$ $cm^{-1}$); Fluorescence emission (in DMF) ($\lambda_{ex}$ = 396 nm): $\lambda_{emis}$ = 454 nm; Mp (°C): 233.0-235.3.




**3TBr:** 2,2':5',2''-terthiophene (3 g, 12.08 mmol) and N-bromosuccinimide (5.37 g, 30.20 mmol) were added into a 250 mL and 100 mL Schlenk flask respectively. 50 mL DMF was added to dissolve N-bromosuccinimide under argon atmosphere. Then the solution was added into 2,2':5',2''-terthiophene dropwise in dark, and the suspension was stirred for 48 h at room temperature under argon. 50 mL $H_2O$ was added to quenching the reaction and the precipitate was isolated via filtration and washed with *abs*

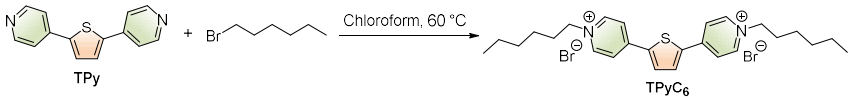
EtOH to afford crude product. The further purification used column chromatography (silica gel, chloroform: methyl alcohol=1:1) to give 3TBr (4.36 g, 10.73 mmol, 89%) as a light yellow solid. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 7.00 (s, 2H, T*H*), 6.98 (d, $J$ = 4 Hz, 2H, T*H*), 6.91 (d, $J$ = 4 Hz, 2H, T*H*). Mp (°C): 129.9-140.9.




**3TPy:** 4-Pyridylboronic acid pinacol ester (2.52 g, 12.31 mmol), 5,5''-dibromo-2,2':5',2''-terthiophene(2 g, 4.92 mmol), tetrakis (triphenylphosphine) palladium(0) (114 mg, 0.10 mmol) and sodium carbonate (7.95 g, 75 mmol) were added into a 250 mL Schlenk flask. Under argon atmosphere, toluene (50 mL) and 15 mL $H_2O$ were added, and the suspension was stirred for 48 h at 90℃. After cooling down to room temperature, the toluene was removed under reduced pressure. The product was extracted with chloroform three times. Concentrated HCl (ca. 2m L) was then added to the organic phase, resulting in precipitation of the product from solution. The precipitate was collected by filtration and then dissolved in $H_2O$. Finally, aqueous NaOH (15 M) was added dropwise to the $H_2O$ layer until the pH was ca. 8-9, resulting the precipitation of pure 3TPy (1.15 g, 2.85 mmol, 58%) as a yellow solid. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 8.61 (d, $J$ = 5.6 Hz, 4H, Py*H*), 7.47 (d, $J$ = 6 Hz, 4H, Py*H*), 7.46 (d, $J$ = 4 Hz, 2H, T*H*), 7.22 (d, $J$ = 4 Hz, 2H, T*H*), 7.19 (s, 2H, T*H*). $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 150.55, 141.02, 140.02, 138.72, 136.42, 126.44, 125.31, 125.12, 119.62. HRMS (ESI) m/z: $[C_{22}H_{14}N_2S_3 + H^+]^+$ calcd. for 403.0392; found 403.0386; UV/vis (in $H_2O$): $\lambda_{max}$ ($\varepsilon$) = 416 nm ($5.372 \times 10^4$ $M^{-1}$ $cm^{-1}$); Fluorescence emission (in DMF) ($\lambda_{ex}$ = 419 nm): $\lambda_{emis}$ = 476 nm; Mp (°C): 267.1-269.4.

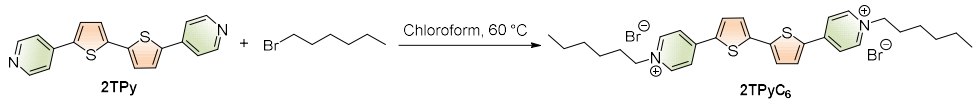
**nTPyC6: nTPy** (1 mmol) and 1-bromohexane (2.2 mmol) were added to dry N,N-dimethylformamide (30 mL) in a round-bottom flask. The reaction mixture was heated under reflux for 72 h, during which precipitate was formed. The precipitate that formed was isolated via vacuum filtration and washed with dry dichloromethane to afford **nTPyC$_6$**.

**TPyC$_6$:**



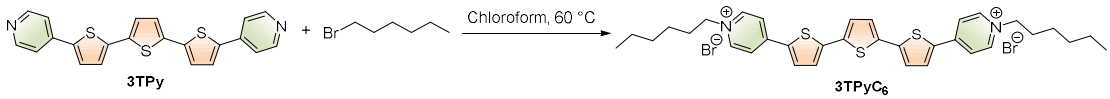

A brown solid. Yield: 88%. $^{1}$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 9.13 (d, $J$ = 6.8 Hz 4H, Py*H*), 8.49 (d, $J$ = 6.8 Hz, 4H, Py*H*), 8.46 (s, 2H, T*H*), 4.57 (t, $J$ = 14.8 Hz, 4H, NC$H_2$), 1.90-1.87 (m, 4H, C$H_2$), 1.26 (s, 12H, C$H_2$), 0.84-0.82 (m, 6H, C$H_2$); $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 146.56, 145.18, 142.51, 133.68, 123.19, 60.06, 34.27, 30.58, 25.06, 21.86, 13.83. HRMS (ESI) m/z: [2TPyC$_6$-2Br$^-$]$^{2+}$ calcd. for 204.1294; found 204.1286; UV/vis (in DMF): $\lambda_{max}$ ($\varepsilon$) = 375 nm (4.574 × 10$^4$ M$^{-1}$ cm$^{-1}$); Fluorescence emission (in DMF) ($\lambda_{ex}$ = 450 nm): $\lambda_{emis}$ = 503 nm; Mp (°C): 180.1-182.6; *LogP* = -1.51; $\Phi$ = 52.17%.

**2TPyC$_6$:**




A yellow solid. Yield: 86%. $^{1}$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 9.03 (d, $J$ = 6.4 Hz 4H, Py*H*), 8.41 (d, $J$ = 6.4 Hz, 4H, Py*H*), 8.35 (d, $J$ = 4.4 Hz 2H, T*H*), 7.88 (d, $J$ = 4 Hz, 2H, T*H*), 4.55 (t, $J$ = 14.8 Hz, 4H, NC$H_2$), 1.918-1.87 (m, 4H, C$H_2$), 1.30 (s, 12H, C$H_2$), 0.89-0.85 (m, 6H, C$H_2$); $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 146.99, 144.78, 141.68, 137.25, 133.73, 128.69, 122.39, 59.79, 30.62, 30.57, 25.10, 21.90, 13.87. HRMS (ESI) m/z: [2TPyC$_6$-2Br$^-$]$^{2+}$ calcd. for 245.1233; found 245.1221; UV/vis (in DMF): $\lambda_{max}$ ($\varepsilon$) = 435 nm (4.916 × 10$^4$ M$^{-1}$ cm$^{-1}$); Fluorescence emission (in DMF) ($\lambda_{ex}$ = 467 nm): $\lambda_{emis}$ = 505 nm; Mp (°C): 251.9-253.0; *LogP* = -0.82; $\Phi$ = 58.37%.

**3TPyC$_6$:**

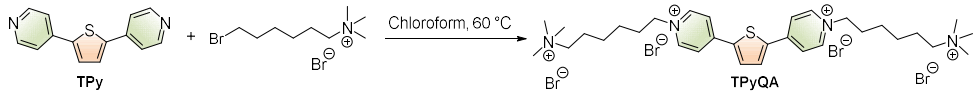



A brown solid. Yield: 83%. $^{1}$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 8.95 (d, $J$ = 6.8 Hz 4H, Py*H*), 8.33 (d, $J$ = 6.4 Hz, 4H, Py*H*), 8.27(d, $J$ = 4 Hz 2H, T*H*), 7.71 (d, $J$ = 4 Hz, 2H, T*H*) 7.64 (s, 2H, T*H*), 4.48 (t, $J$ = 14.8 Hz, 4H, NC$H_2$), 1.87-1.84 (m, 4H, C$H_2$), 1.25 (s, 12H, C$H_2$), 0.84-0.81 (m, 6H, C$H_2$); $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 146.99, 144.49, 142.81, 135.80, 135.74, 133.67, 127.87, 127.28, 121.95, 59.52; 34.17, 30.46, 24.95, 21.75, 13.71. HRMS (ESI) m/z: [3TPyC$_6$-2Br$^-$]$^{2+}$ calcd. for 286.1171; found 286.1159;

UV/vis (in DMF): $\lambda_{max}$ (ε) = 467 nm (4.531 × $10^1$ $M^{-1}$ $cm^{-1}$); Fluorescence emission (in DMF) ($\lambda_{ex}$ = 467 nm): $\lambda_{emis}$ = 579 nm; Mp (°C): 262.8-264.7.

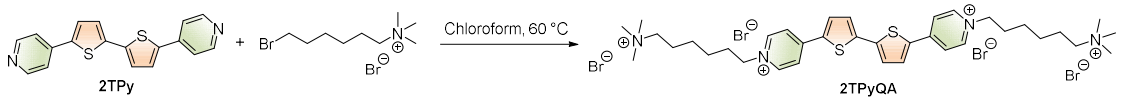
**nTPyQA: nTPy** (1 mmol) and (6-bromohexyl)trimethylammonium bromide (2.3 mmol) were added to dry N,N-dimethylformamide (30 mL) in a round-bottom flask. The reaction mixture was heated under reflux for 72 h, during which precipitate was formed. The precipitate that formed was isolated via vacuum filtration and wash with *abs* EtOH to afford **nTPyQA**.

**TPyQA:**




A carroty solid. Yield: 91%. $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 9.20 (d, $J$ = 6.8 Hz 4H, Py*H*), 8.54 (d, $J$ = 6.4 Hz, 4H, Py*H*), 8.51 (s, 2H, T*H*), 4.63 (t, $J$ = 14.8 Hz, 4H, NC$H_2$), 3.06 (s, 18H, NC$H_3$), 1.99-1.95 (m, 5H, C$H_2$), 1.73-1.66 (m, 5H, C$H_2$), 1.35-1.32 (m, 10H, C$H_2$); $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 146.58, 145.23, 142.52, 133.72, 123.19, 65.07, 59.86, 52.18, 30.28, 25.14, 24.90, 21.82. HRMS (ESI) m/z: [TPyQA-4$Br^-$]$^{4+}$ calcd. for 131.0973; found 131.0962; UV/vis (in DMF): $\lambda_{max}$ (ε) = 376 nm (5.196 × $10^4$ $M^{-1}$ $cm^{-1}$); Fluorescence emission (in DMF) ($\lambda_{ex}$ = 450 nm): $\lambda_{emis}$ = 503 nm; Mp (°C): 202.6-210.2; *LogP* = -1.78; $\Phi$ = 44.63%.

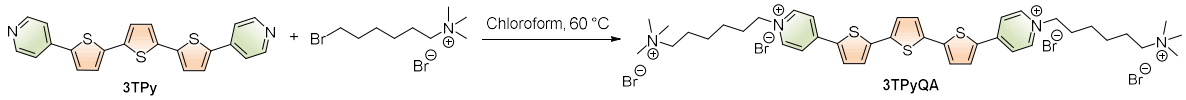
**2TPyQA:**




An origin solid. Yield: 89%. $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 9.07 (d, $J$ = 6.8 Hz 4H, Py*H*), 8.44 (d, $J$ = 6.8 Hz, 4H, Py*H*), 8.37 (d, $J$ = 4.4 Hz 2H, T*H*), 7.89 (d, $J$ = 4 Hz, 2H, T*H*), 4.57 (t, $J$ = 14.8 Hz, 4H, NC$H_2$), 3.30-3.27 (m, 4H, C$H_2$), 3.05 (s, 18H, NC$H_3$), 1.99-1.93 (m, 4H, C$H_2$), 1.69-1.65 (m, 4H, C$H_2$), 1.34 (m, 8H, C$H_2$); $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 147.00, 144.82, 141.69, 137.23, 133.77, 128.70, 122.38, 65.04, 59.52, 52.16, 30.26, 25.14, 24.89, 21.82. HRMS (ESI) m/z: [2TPyQA-4$Br^-$]$^{4+}$ calcd. for

151.5942; found 151.5958; UV/vis (in DMF): $\lambda_{max}$ (ε) = 435 nm (5.880 × $10^4$ $M^{-1}$ $cm^{-1}$); Fluorescence emission (in DMF) ($\lambda_{ex}$ = 467 nm): $\lambda_{emis}$ = 505 nm; Mp (℃): 235.2-238.8; *LogP* = -2.17; $\Phi$ = 52.9%.

**3TPQA:**




A wine-colored solid. Yield: 87%. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 9.05 (d, *J* = 6.8 Hz 4H, Py*H*), 8.40 (dd, *J* = 6.4 Hz, 4H, Py*H*), 8.34 (d, *J* = 4 Hz, 2H, T*H*), 7.77 (d, *J* = 4 Hz, 2H, T*H*) 7.69 (s, 2H, T*H*), 4.57 (t, *J* = 14.8 Hz, 4H, NC$H_2$), 3.31 (m, 4H, C$H_2$), 3.06 (s, 18H, N$CH_3$), 1.96-1.93 (m, 4H, C$H_2$), 1.70-1.66 (m, 4H, C$H_2$), 1.35-1.44 (m, 8H, C$H_2$); $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 147.15, 144.67, 142.64, 135.94, 135.86, 133.85, 128.03, 127.45, 122.06, 65.05, 59.41, 52.17, 30.25, 25.15, 24.89, 21.83. HRMS (ESI) m/z: $[3TPyQA\text{-}4Br^-]^{4+}$ calcd. for 172.0911; found 172.0914; UV/vis (in DMF): $\lambda_{max}$ (ε) = 472 nm (5.772 × $10^4$ $M^{-1}$ $cm^{-1}$); Fluorescence emission (in DMF) ($\lambda_{ex}$ = 467 nm): $\lambda_{emis}$ = 576 nm; Mp (℃): 215.7-218.6.

## 4. Photoluminescence (PL) spectra

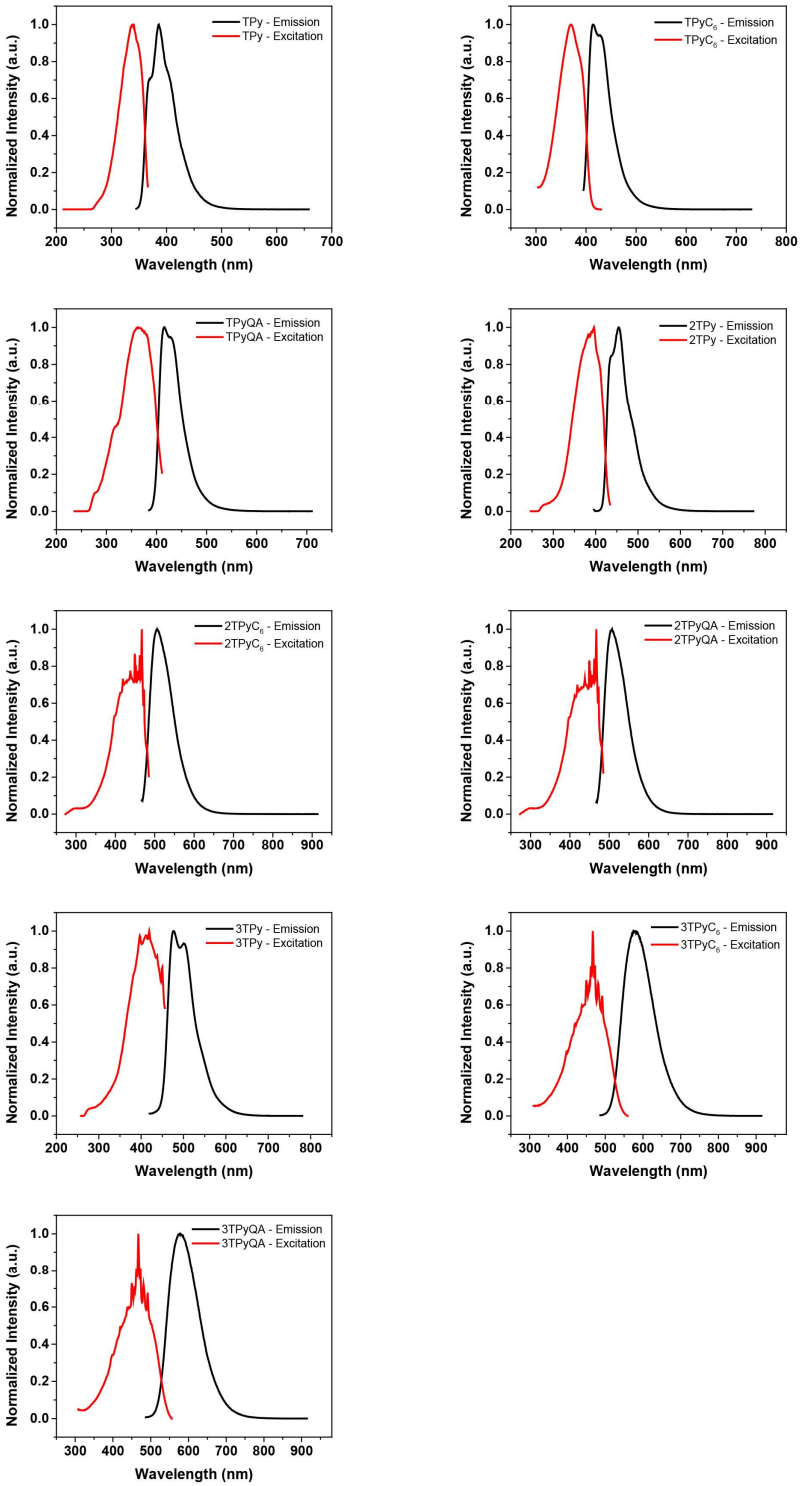



**Fig. S1.** Photoluminescence (PL) spectra of thienoviologens.

## 5. UV−Vis spectra

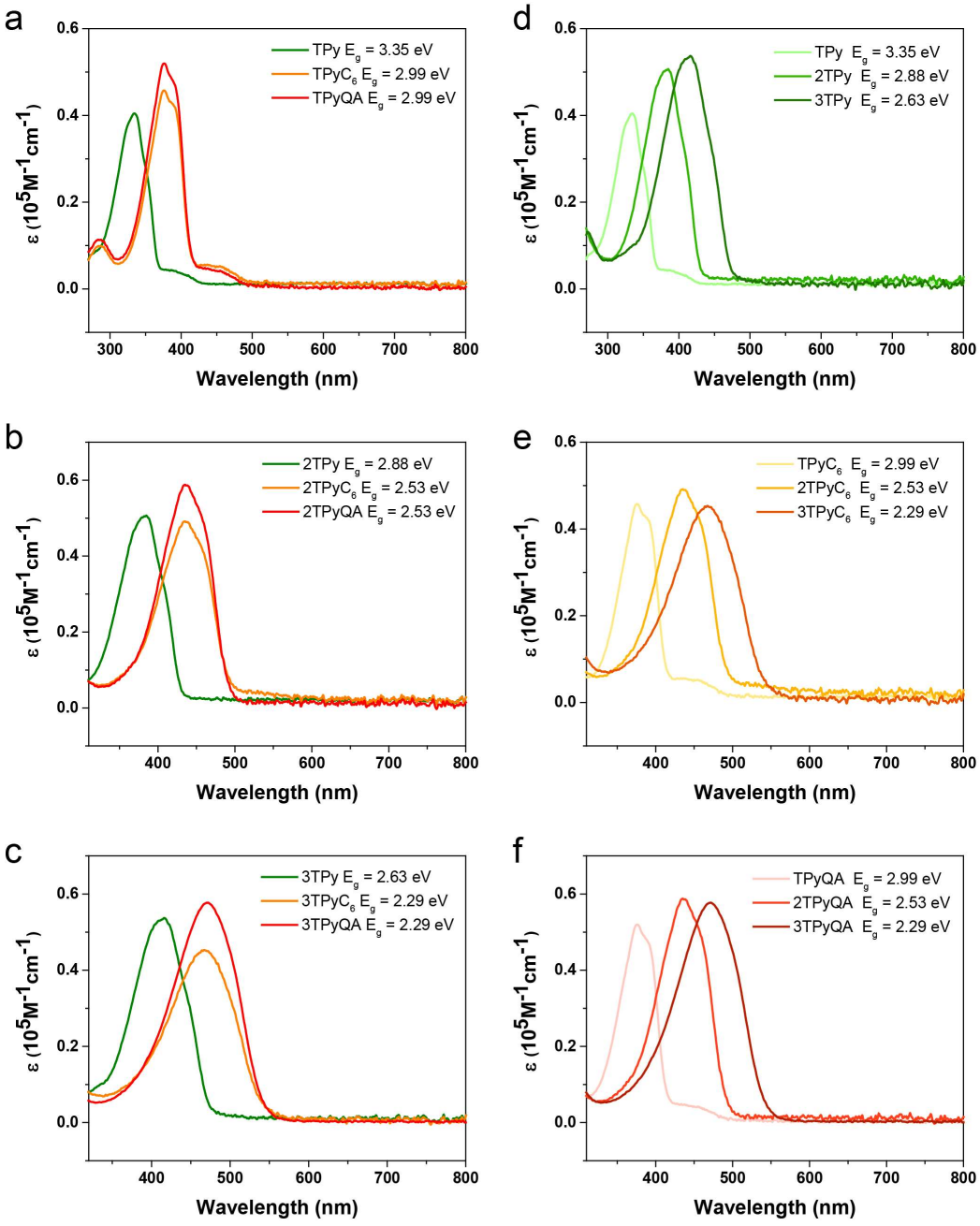



**Fig. S2.** UV−Vis spectra of cationic thienoviologens.

## 6. Cyclic voltammogram

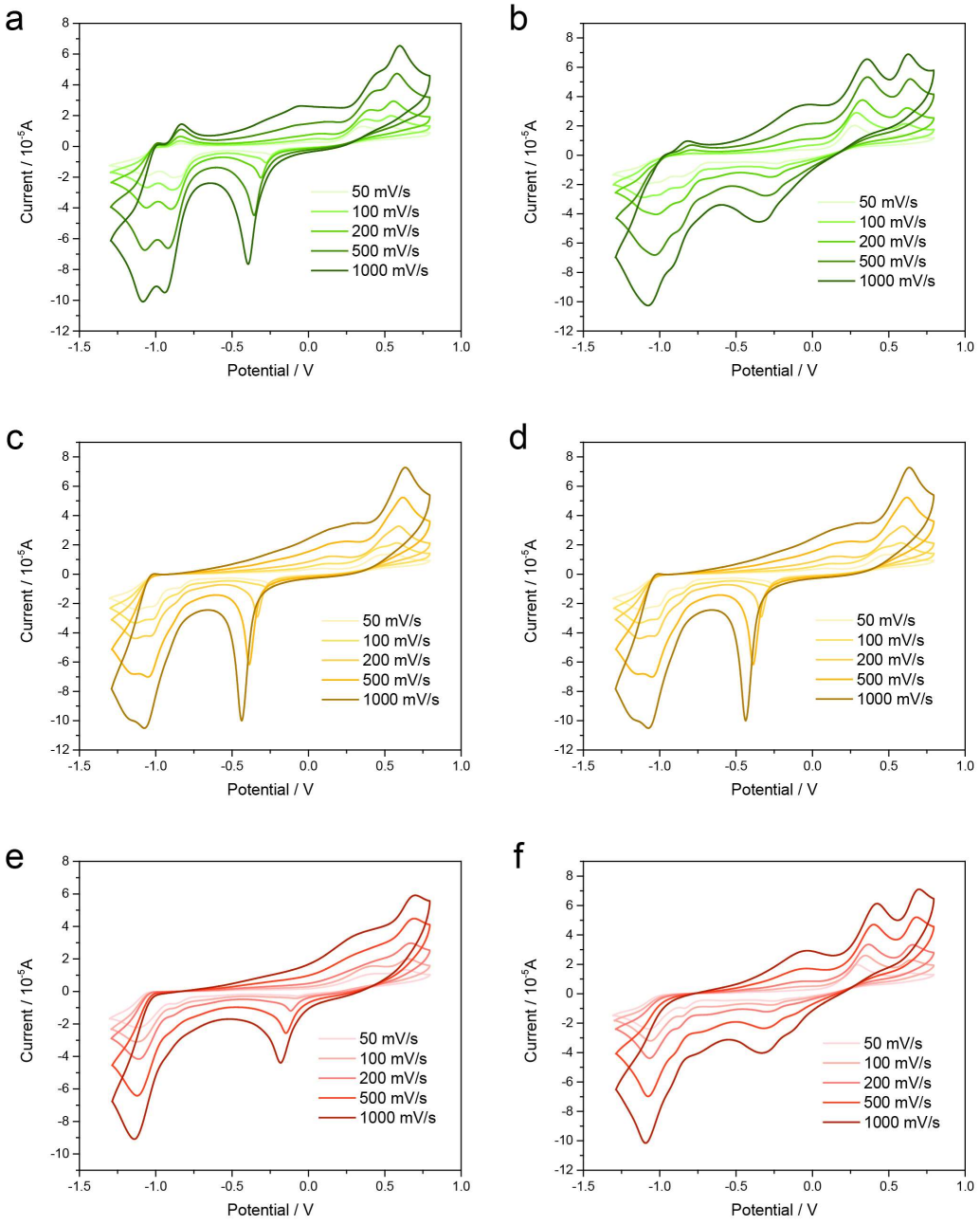



**Fig. S3.** Cyclic voltammogram of (a) **TPyC$_6$**, (b) **TPyQA**, (c) **2TPyC$_6$**, (d) **2TPyQA**, (e) **3TPyC$_6$** and (f) **3TPyQA** ($10^{-3}$ M in DMF solution, 0.1 M, *vs*. Fc/Fc$^+$).

## 7. Summary of various PDT agents

**Table S1**. The comparison of **2TPyC$_6$** with other PDT agents.

| PDT agents | Irradiation Source | Light Dosage, Irradiation Time | Kill Rate | Concentration | Ref., Feature |
|---|---|---|---|---|---|
| **2TPyC$_6$** | White light | 70 mW/cm$^2$<br>60 min | 100% | 20 ng/mL (*E. coli*)<br>4.5 ng/mL (*S. aureus*) | This work |
| **OTE** | White light | 90 mW/cm$^2$<br>60 min | 50% | 17 ng/mL (*E. coli*)<br>7 ng/mL (*S. aureus*) | 2 |
| **TBD-anchor** | White light | 25 mW/cm$^2$<br>10 min | 96%<br>99.5% | 5 μM (*E. coli*)<br>2 μM （*S. aureus*） | 3 |
| **UCNPs/PSeV** | 980 nm | 150 mW/cm$^2$<br>4 min | 98.3% | 2.5 μM (MRSA) | 4 |
| **SeMV$^{2+}$(6b)** | White light | 70 mW/cm$^2$<br>60 min | 87.8%<br>75.5% | 10 μM (*E. coli*)<br>40 μM (*S. aureus*) | 5 |
| **TIdBO** | White light | 30 mW/cm$^2$<br>10 min | 100% | 5 μM (*S. aureus*) | 6 |
| **PFH/F-I** | 532 nm | 10 min | 98.3% | 500 μg/mL (*P. aeruginosa*) | 7 |
| **Se4** | 1064 nm | 1 W/cm$^2$<br>5 min | 100% | 50 μg/mL (*S. aureus*) | 8 |
| **PPIX-PEI NPs** | 635 nm | 100 mW/cm$^2$<br>10 min | 100% | 850 μg/mL (*E. coli*)<br>212.5 μg/mL (*P. aeruginosa*)<br>425 μg/mL (*S. aureus*)<br>26.6 μg/mL (*P. epidermidis*) | 9 |
| **DNase-AuNCs** | 808 nm | 2 W/cm$^2$<br>10 min | 100% | 100 μg/mL (*E. coli* and *S. aureus*) | 10 |
| **PS-M-R** | LED | 30 min | 100% | 20 μM (*E. coli*) | 11 |
| **Cholo-PEG-PplX** | White light | 5 mW/cm$^2$<br>30 min | 99.9% | 4 μM (*E. coli*) | 12 |
| **ZIF8-SQ** | Red light | 30 min | 86% | 100 nM (MRSA) | 13 |
| **$C_{70}$[>M($C_3N_6^+C_3$)$_2$]** | White light | 100 J/cm$^2$ | 99.9%<br>99% | 40 μM (*E. coli*)<br>1 μM (*S. aureus*) | 14 |
| **$C_{60}$[>M($C_3N_6^+C_3$)$_2$]** | White light | 100 J/cm$^2$ | 99.9%<br>99.9% | 80 μM (*E. coli*)<br>1 μM (*S. aureus*) | 14 |
| **EPS-RB NPs** | White light | 5 mW/cm$^2$<br>10 min | 100% | 8 μM (*E. coli*)<br>500 nM (*S. aureus*) | 15 |
| **TCPP-TG NPs** | Visible light | 8 mW/cm$^2$<br>10 min | 100% | 4 μM (*E. coli*)<br>8 μM (*S. aureus*) | 16 |

| | | | | | |
|---|---|---|---|---|---|
| **TPOR/(CB[7])$_4$** | White light | 1 J/cm$^2$ | 97% | 0.72 μM (*E. coli*) | [17] |
| **1-PtNPs** | Visible light | 50 mW/cm$^2$<br>120 min | 100% | 20 μg/mL | [18] |
| **2a** | White light | 10 J/cm$^2$ | 100% | 0.13 μg/mL (*E. coli*)<br>0.13 μg/mL (*P. aeruginosa*)<br>1.4 μg/mL (*C. albicans*)<br>> 100 μM (*S. aureus*) | [19] |
| **MNPSiNH-TCPP** | Visible light | 90 mW/cm$^2$<br>30 min | 99.9% | 3 μM (*E. coli*, *S. aureus*, *C. albicans*) | [20] |
| **PPO** | Visible light | 12 mW/cm$^2$<br>10 min | 99.4%<br>99.9% | 4 μM (*E. coli*)<br>100 nM (*S. aureus*) | [21] |
| **ZnTPyP@NO** | Simulated sunlight | 30 min (*S. aureus*)<br>120 min (*E. coli*) | 100% | 100 μg/mL | [22] |
| **PTTP-Glu-Ac NPs** | Visible light | 22 mW/cm$^2$<br>10 min | 100% | 18 μg/mL | [23] |
| **OC-UCNP-ZnPc** | 980 nm | 400 mW/cm$^2$<br>15 min | 100% | 250 μg/mL (MSSA) | [24] |
| **$Bi_2Se_3$ NPs/PEI** | 808 nm | 1 W/cm$^2$<br>10 min | 99%<br>97% | 80 μg/mL (*S. aureus*)<br>80 μg/mL (*E. coli*) | [25] |
| **UCNP@$SiO_2$-RB/HMME** | 980 nm | 1 W/cm$^2$, 20 min (*S. aureus*)<br>2 W/cm$^2$, min (*E. coli*) | 100% | 125 μg/mL | [26] |

## 8. Supporting figures

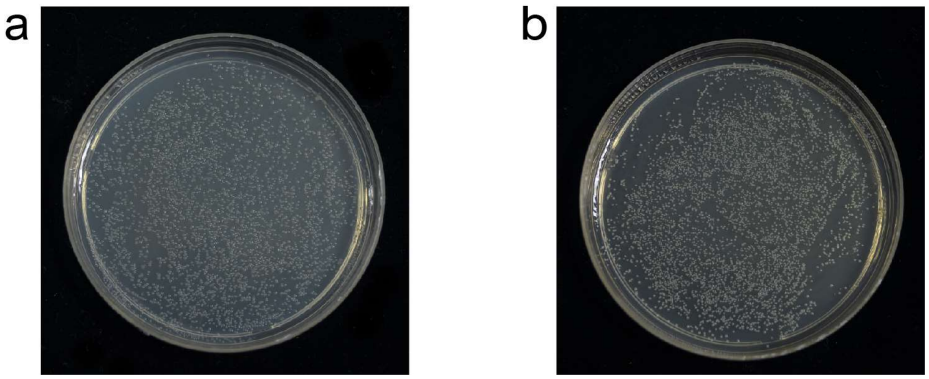



**Fig. S4.** Colony-forming units (CFU) for (a) *E. coli* and (b) *S. aureus* without treatment under irradiation for 60 min.

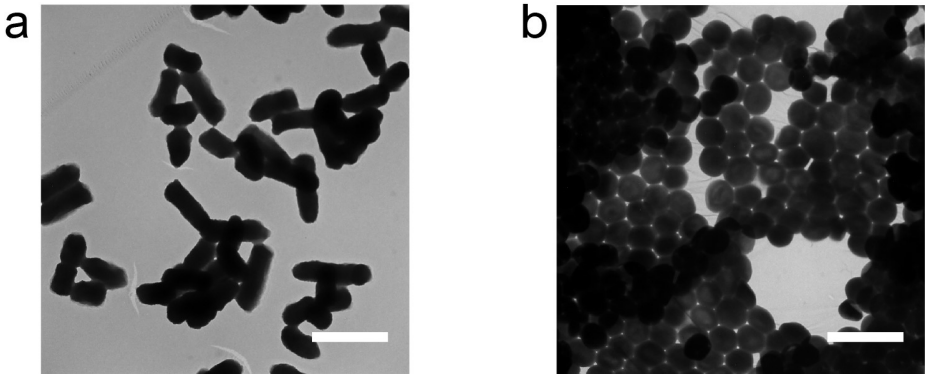



**Fig. S5.** TEM images of (a) *E. coli* and (b) *S. aureus* in PBS without irradiation. The scale bar is 2 μm.

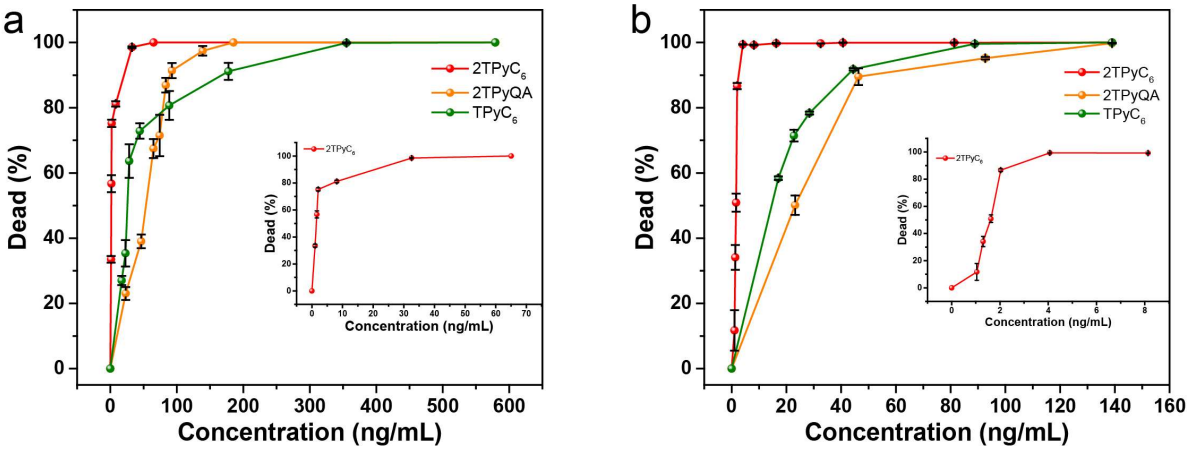



**Fig. S6.** (a) *E. coli* and (b) *S. aureus* viability against **2TPyC$_6$**, **2TPyQA** and **TPyC$_6$** upon exposure to visible light at various concentrations for 60 min.

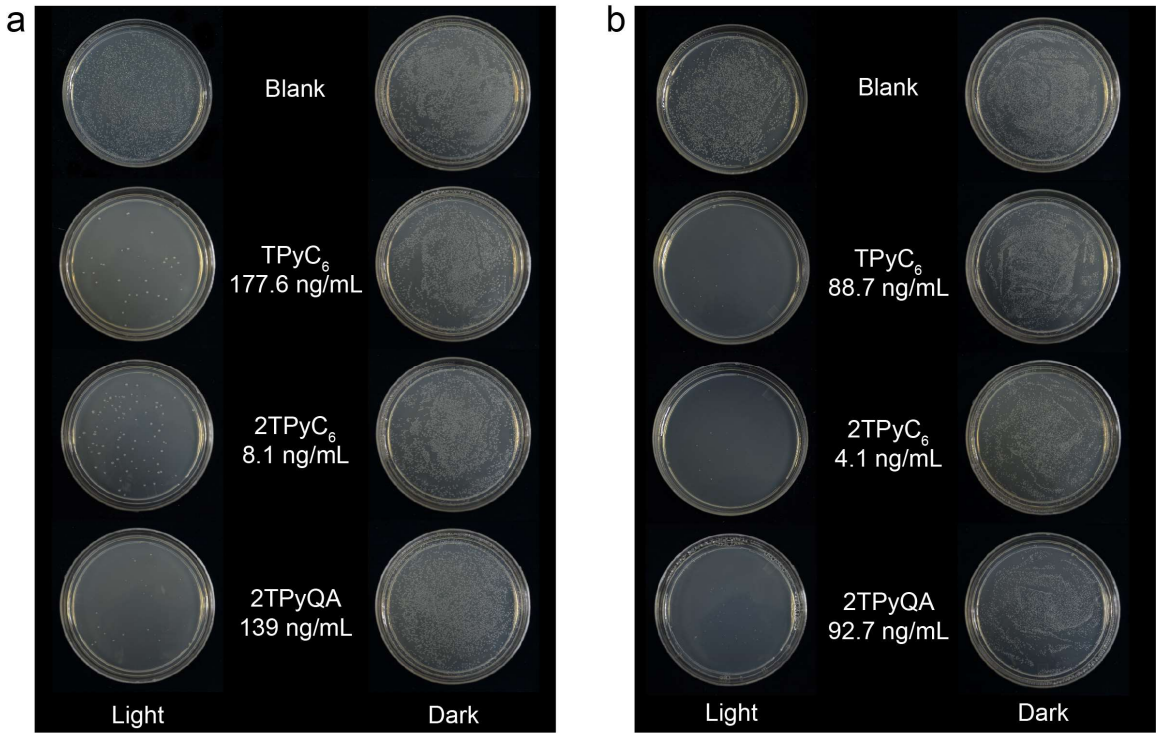



**Fig. S7**. Colony-forming units (CFU) incubated with pure medium components of **TPyC6**, **2TPyC6** and **2TPyQA** irradiation for 60 min and without irradiation for (a) *E. coli* at the concentration of 177.6 ng/mL, 8.1 ng/mL, 139.0 ng/mL, respectively and (b) *S. aureus* at the concentration of 88.7 ng/mL, 4.1 ng/mL, 92.7 ng/mL, respectively.

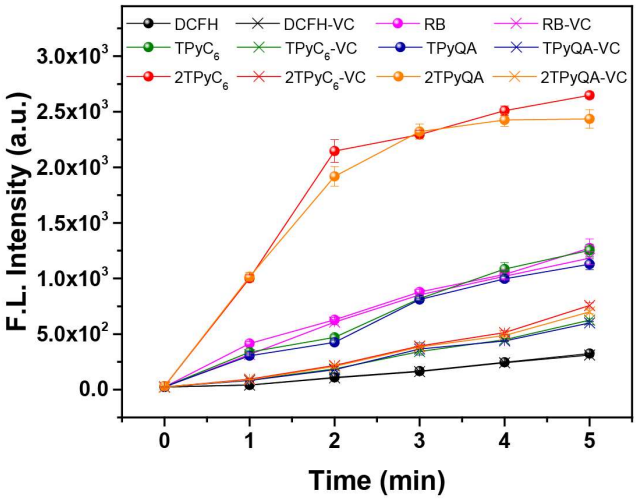



**Fig. S8**. **RB, TPyC6, TPyQA, 2TPyC6 and 2TPyQA** sensitized ROS with and without VC upon white light irradiation (0−5 min) with an excitation of 488 nm. The error bars represent the standard deviations of three parallel measurements.

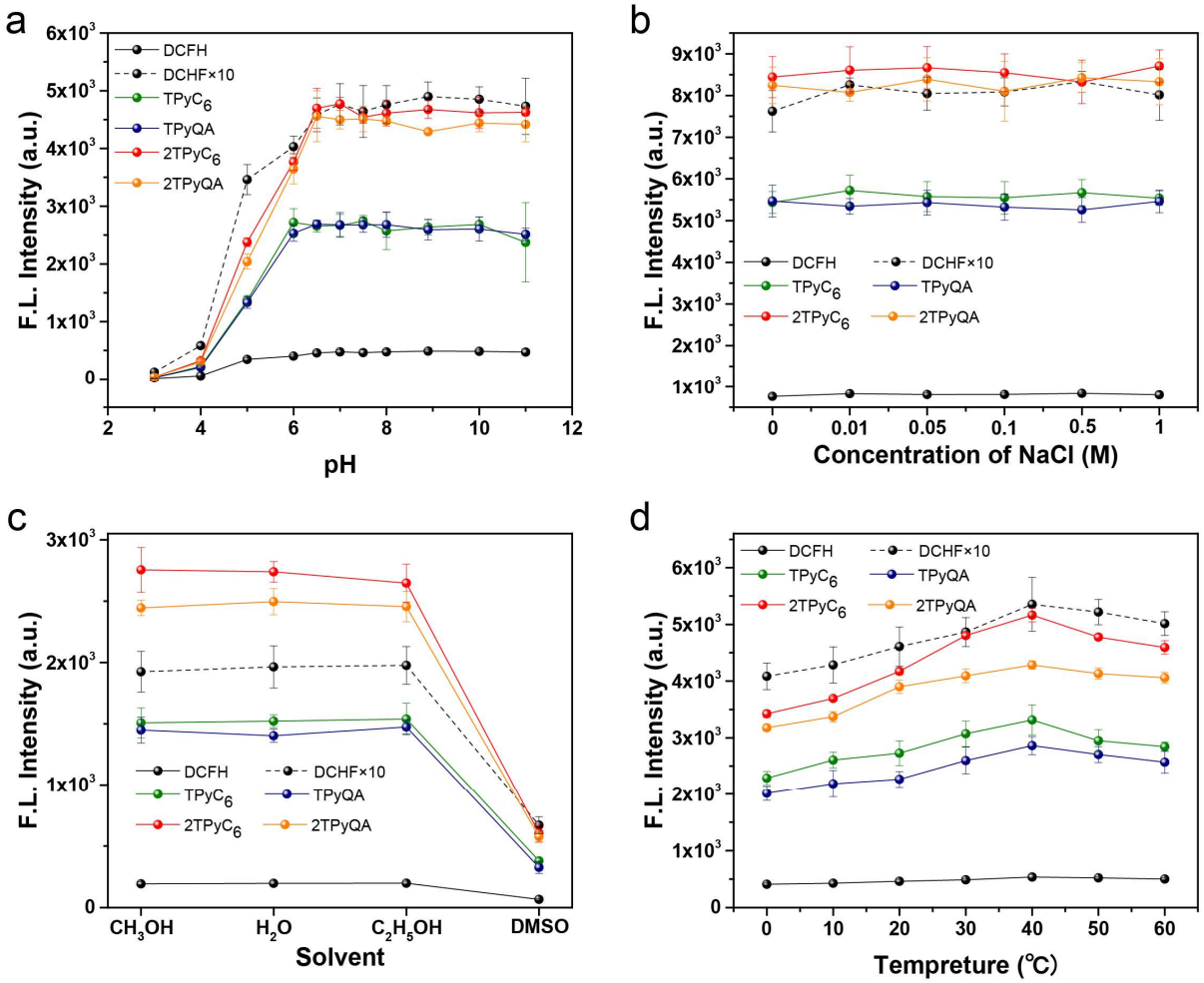



**Fig. S9**. **TPyC$_6$, TPyQA, 2TPyC$_6$ and 2TPyQA** sensitized ROS (a) in different pH; (b) in the presence of different concentrations of NaCl; (c) in different solvent; (d) at different temperature after 5 min white light irradiation with an excitation of 488 nm. The error bars represent the standard deviations of three parallel measurements.

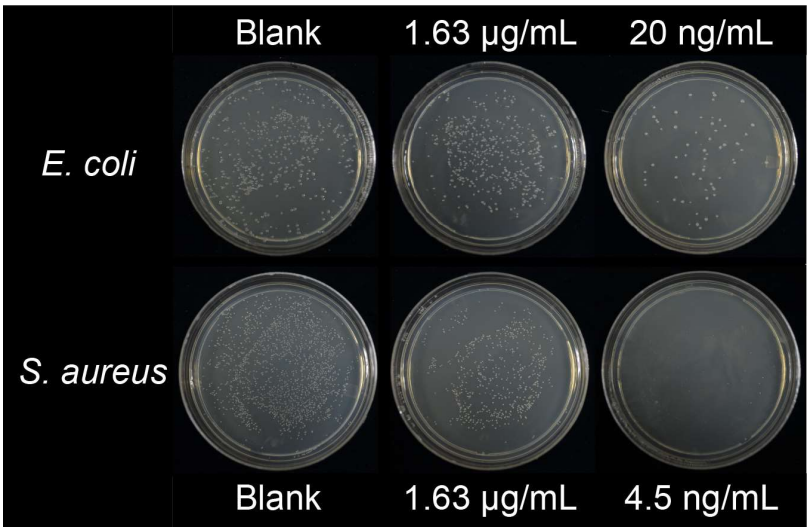



**Fig. S10**. Colony-forming units (CFU) incubated with pure medium components of **2TPyC$_6$** irradiation for 30 min for *E. coli* at the concentration of 1.63 μg/mL and 20 ng/mL, and *S. aureus* at the concentration of 1.63 μg/mL and 4.5 ng/mL.

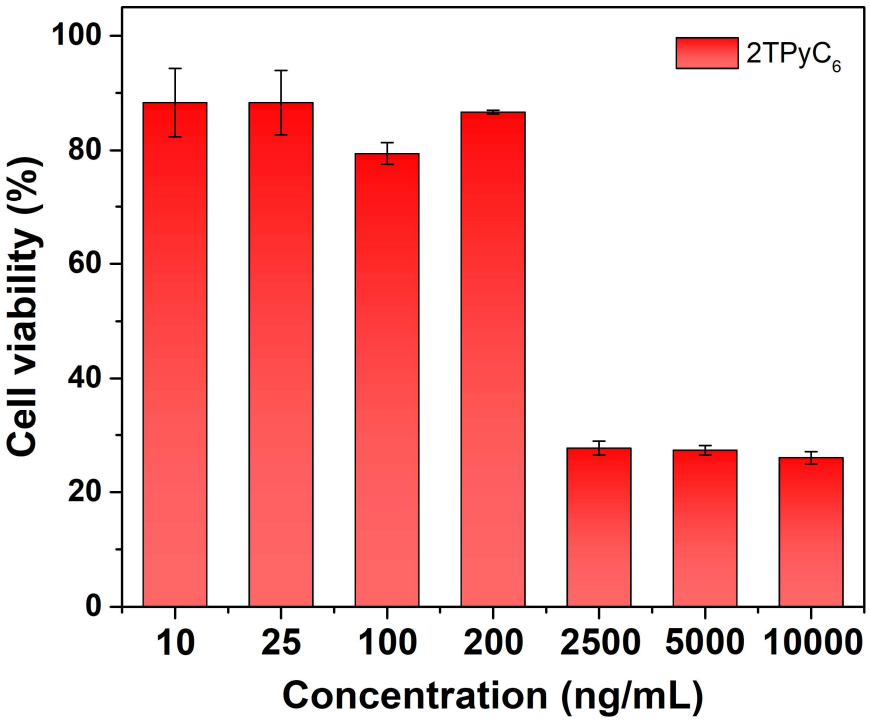



**Fig. 11.** Cell viability of **2TPyC$_6$** against L-929 cells at different concentrations upon white light irradiation for 60 min (70 mW/cm$^2$).

**Table S2.** Minimum inhibitory concentration (MIC) of *E. coli* of different **nTPy-R**s (μg/mL) in dark

| **TPy** | **TPyBnBr** | **TPyC$_6$** | **TPyMeOTf** | **TPyQA** |
|---|---|---|---|---|
| >1000 | 500 | 3.9 | 125 | 250 |
| **2TPy** | **2TPyBnBr** | **2TPyC$_6$** | **2TPyMeOTf** | **2TPyQA** |
| >1000 | 500 | 0.49 | 15.63 | 15.63 |

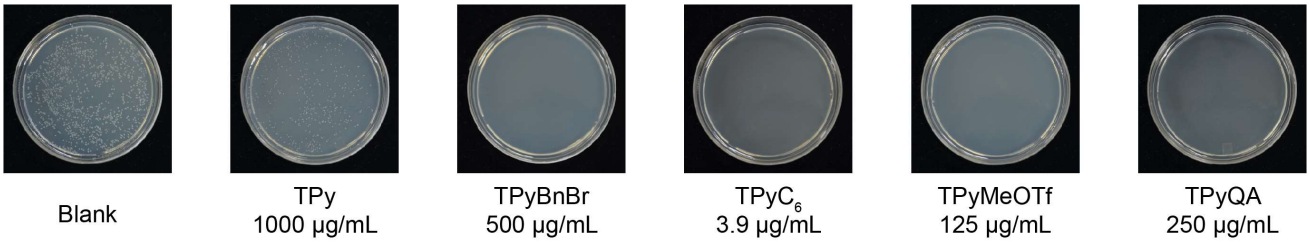



**Table S3.** Minimum inhibitory concentration (MIC) of *S. aureus* of different **nTPy-R**s (μg/mL) in dark

| **TPy** | **TPyBnBr** | **TPyC$_6$** | **TPyMeOTf** | **TPyQA** |
|---|---|---|---|---|
| >1000 | 500 | 3.9 | 125 | 250 |
| **2TPy** | **2TPyBnBr** | **2TPyC$_6$** | **2TPyMeOTf** | **2TPyQA** |
| >1000 | 500 | 0.98 | 15.63 | 31.25 |

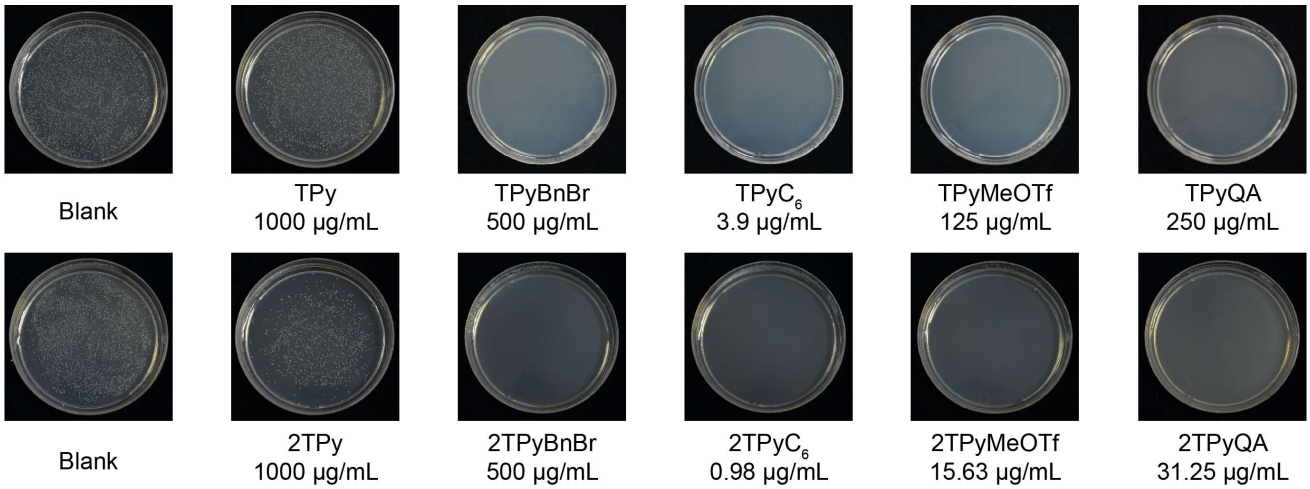


**Table S4.** Zeta potential of *E. coli* and *S. aureus* with different **nTPy-R**s (mV)

| ***E. coli*** | **TPyC$_6$** | **TPyQA** | **2TPyC$_6$** | **2TPyQA** |
|---|---|---|---|---|
| -17.5 | -7.05 | -4.3 | -7.77 | -2.28 |
| ***S. aureus*** | **TPyC$_6$** | **TPyQA** | **2TPyC$_6$** | **2TPyQA** |
| -26.5 | -10.5 | -4.62 | -3.32 | -5.68 |

**Table S5.** Electrochemical potentials and energy levels of the **nTPy-R**s

| **nTPy-R**s | $E_{red}$ **(V)**[a] | $E_g$ **(eV)**[b] | **LUMO**[c] | **HOMO**[d] |
|---|---|---|---|---|
| **TPyC$_6$** | -0.776 | 2.99 | -4.024 | -1.034 |
| **2TPyC$_6$** | -0.815 | 2.53 | -3.985 | -1.452 |
| **3TPyC$_6$** | -0.940 | 2.29 | -3.860 | -1.57 |
| **TPyQA** | -0.844 | 2.99 | -3.956 | -0.966 |
| **2TPyQA** | -0.862 | 2.53 | -3.938 | -1.408 |
| **3TPyQA** | -0.884 | 2.29 | -3.916 | -1.626 |

[a] Reduction potential measured by cyclic voltammetry with ferrocene as the standard (as oxidation potential of ferrocene set as zero).

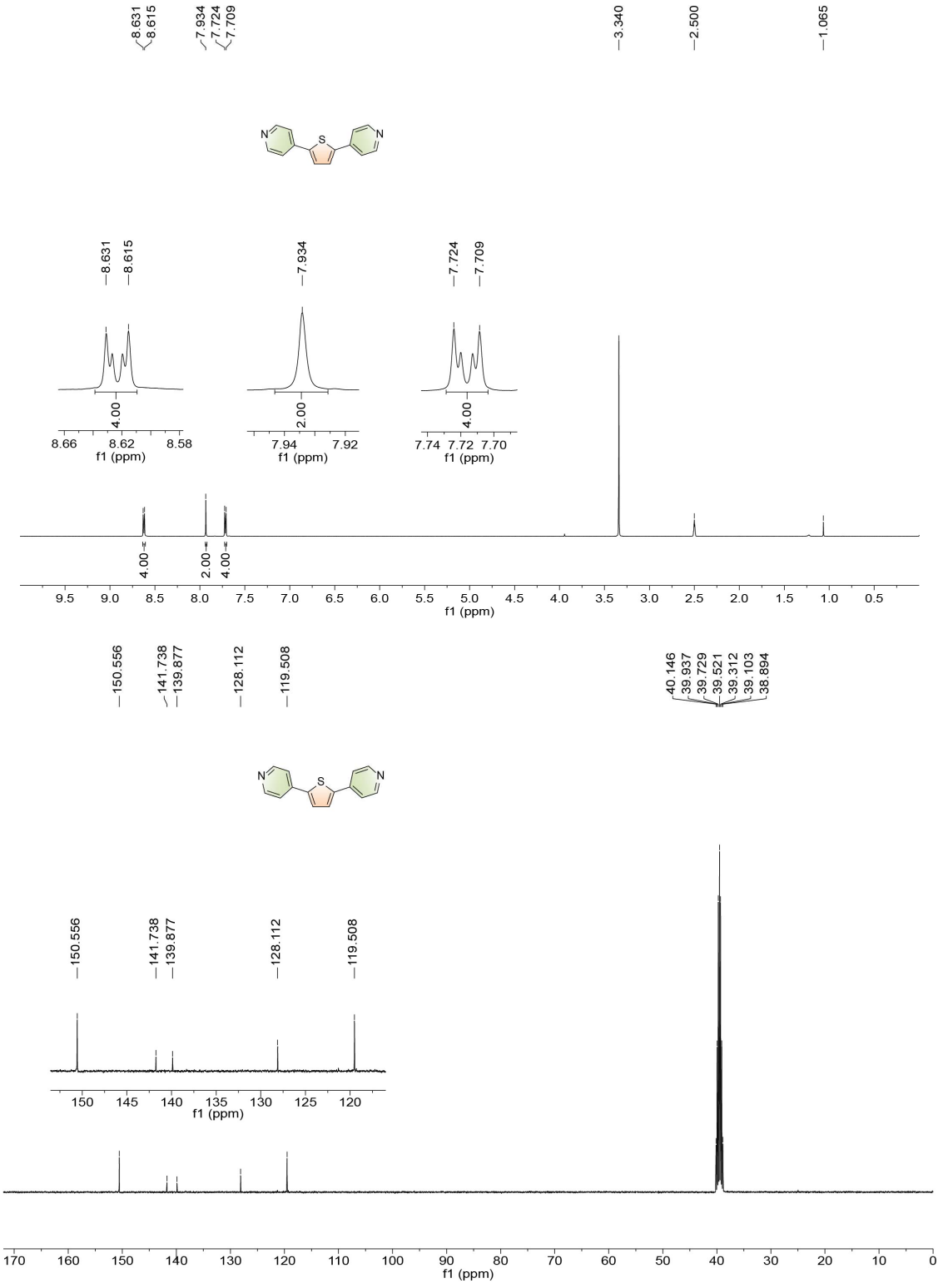
[b] Band gap estimated from the UV-vis absorption spectrum.

[c] Calculated from the reduction potentials.

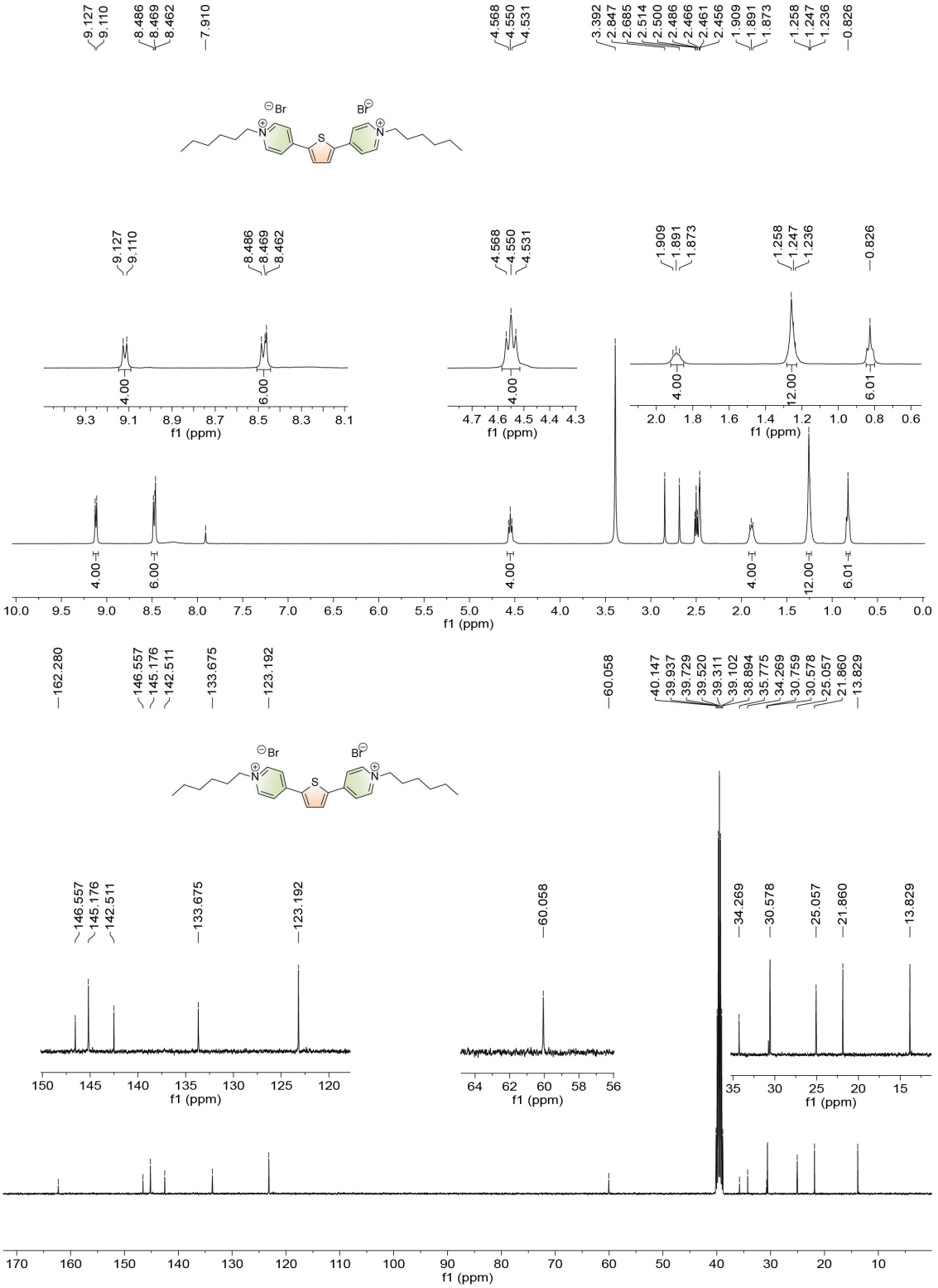
[d] Deduced from the LUMO and $E_g$.

## 9. $^{1}$H, $^{13}$C NMR spectra

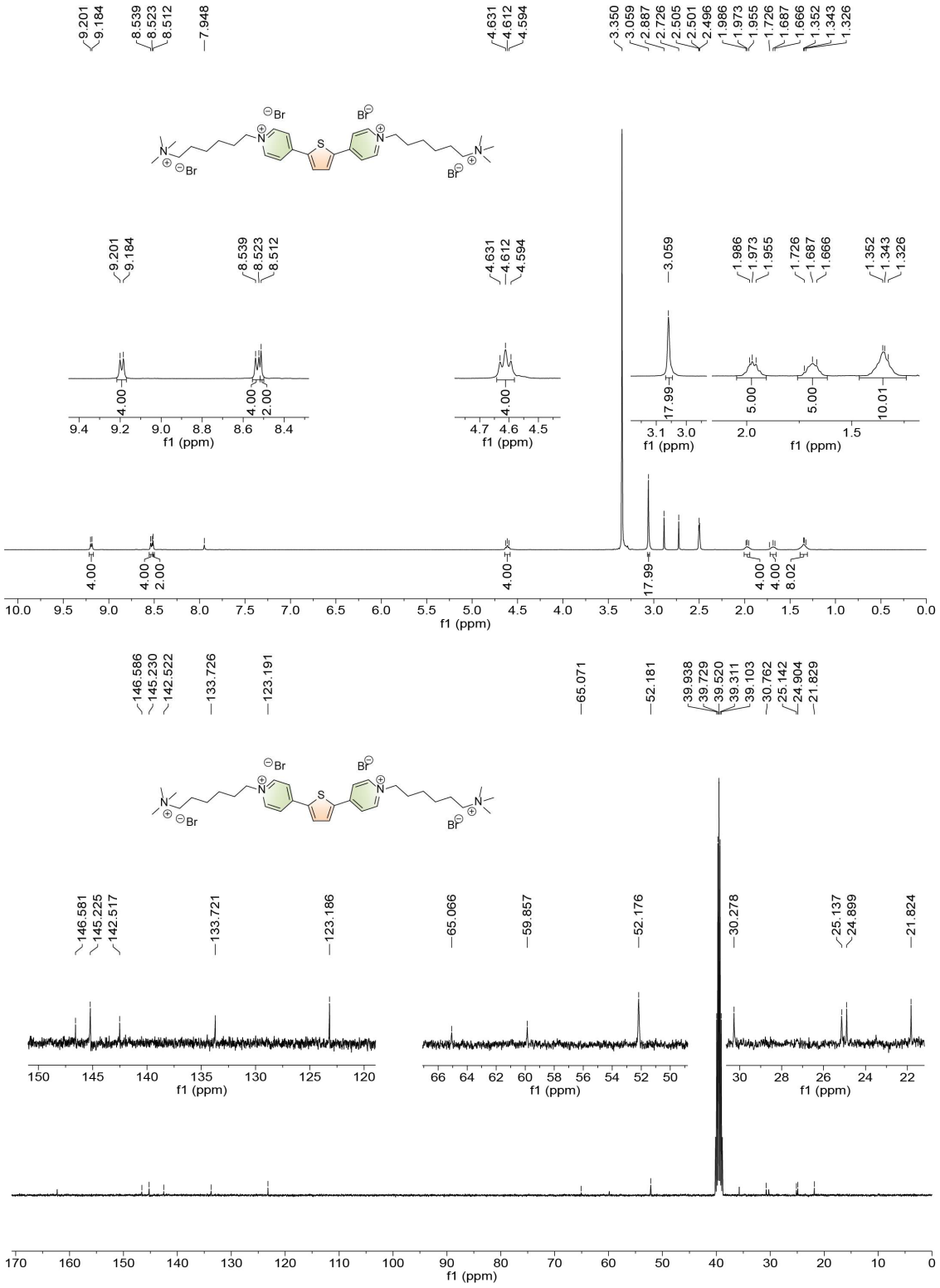
$^{1}$H NMR (DMSO-$d_6$, 400 MHz) and $^{13}$C NMR (DMSO-$d_6$, 100 MHz) spectra of **TPy**.



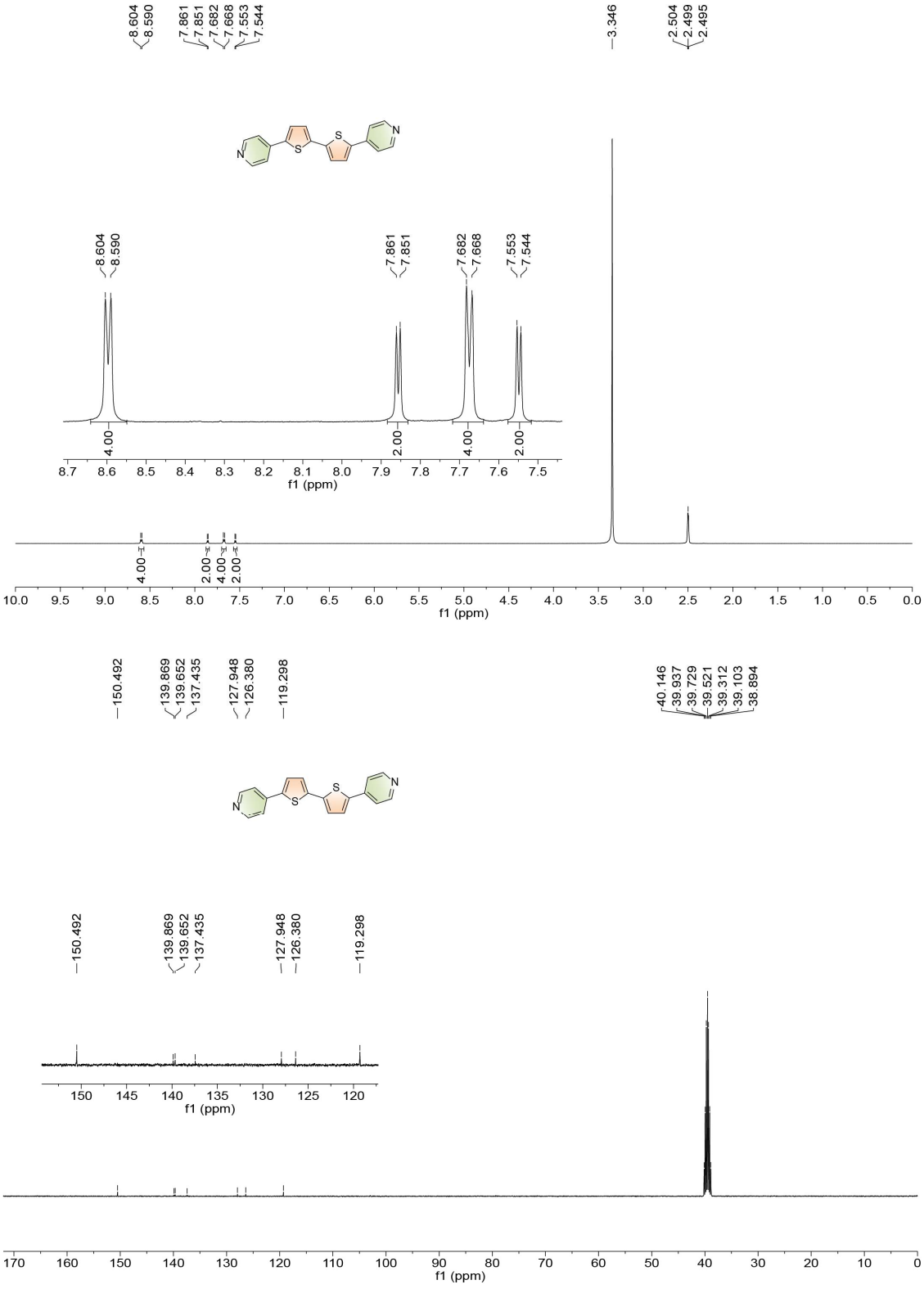
$^{1}$H NMR (DMSO-$d_6$, 400 MHz) and $^{13}$C NMR (DMSO-$d_6$, 100 MHz) spectra of **TPyC$_6$**.



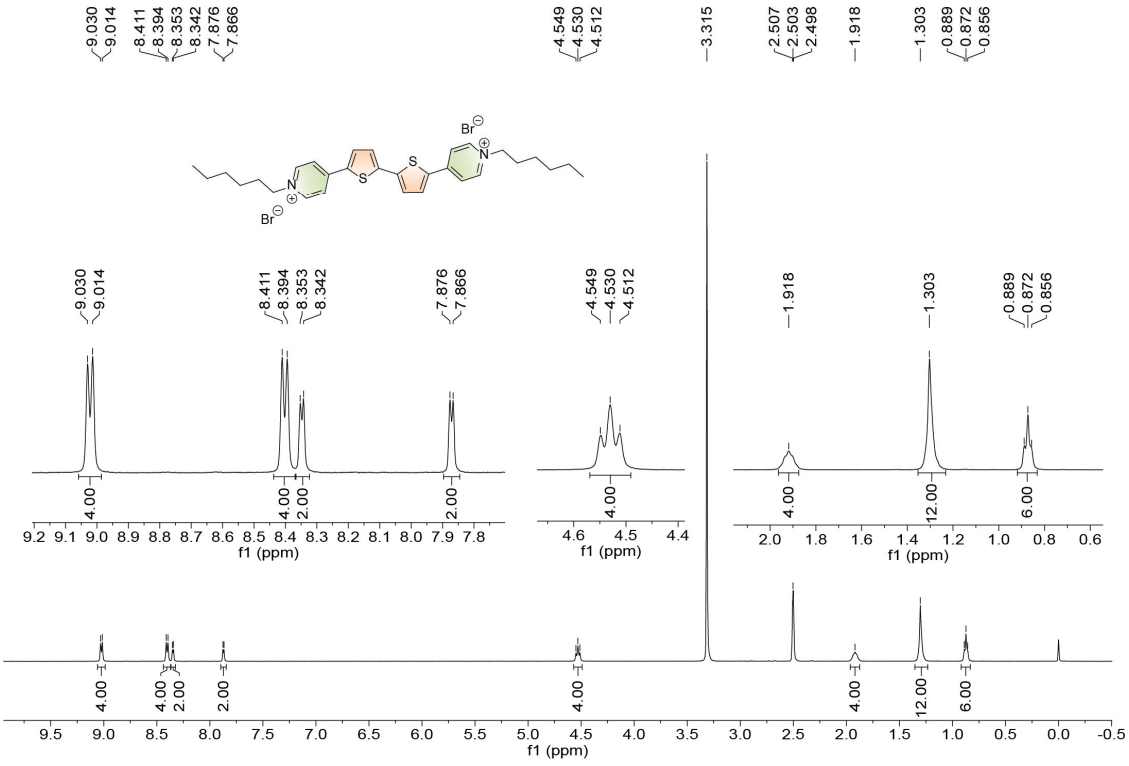
$^{1}$H NMR (DMSO-$d_6$, 400 MHz) and $^{13}$C NMR (DMSO-$d_6$, 100 MHz) spectra of **TPyQA**.

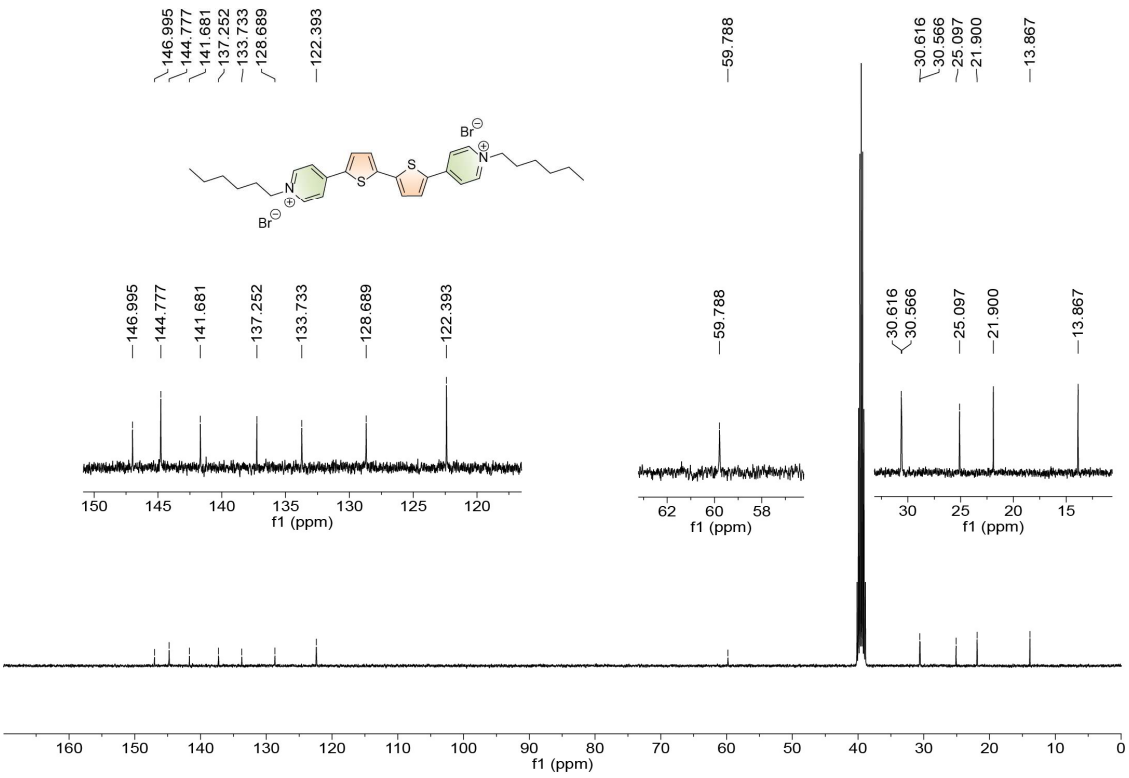


$^{1}$H NMR (DMSO-$d_6$, 400 MHz) and $^{13}$C NMR (DMSO-$d_6$, 100 MHz) spectra of **2TPy**.

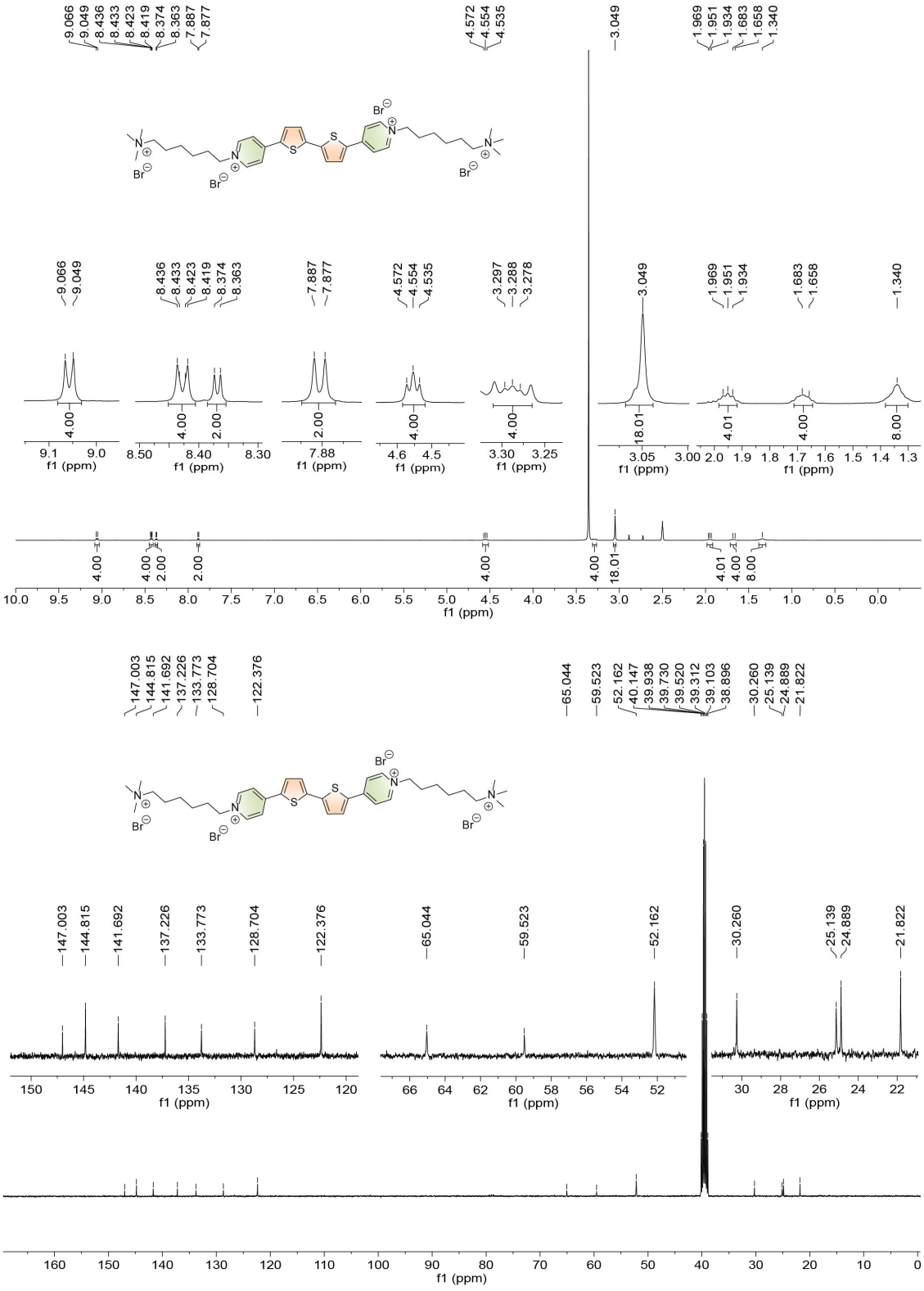


$^{1}$H NMR (DMSO-$d_6$, 400 MHz) and $^{13}$C NMR (DMSO-$d_6$, 100 MHz) spectra of **2TPyC$_6$**.

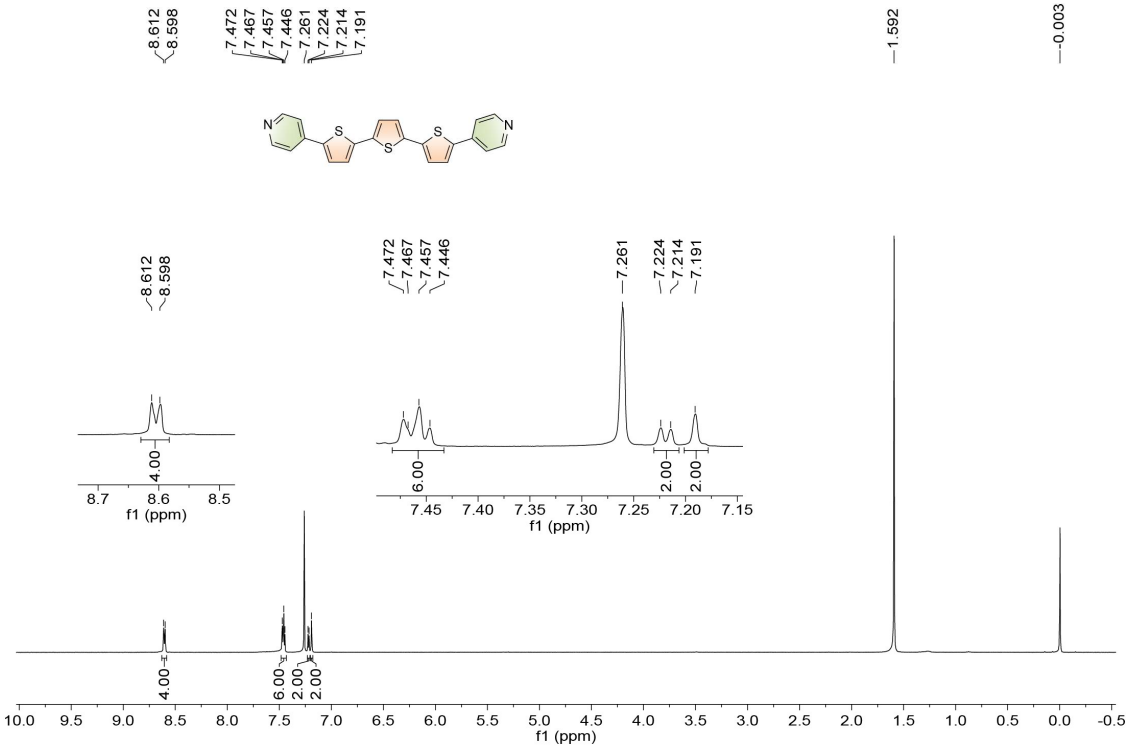


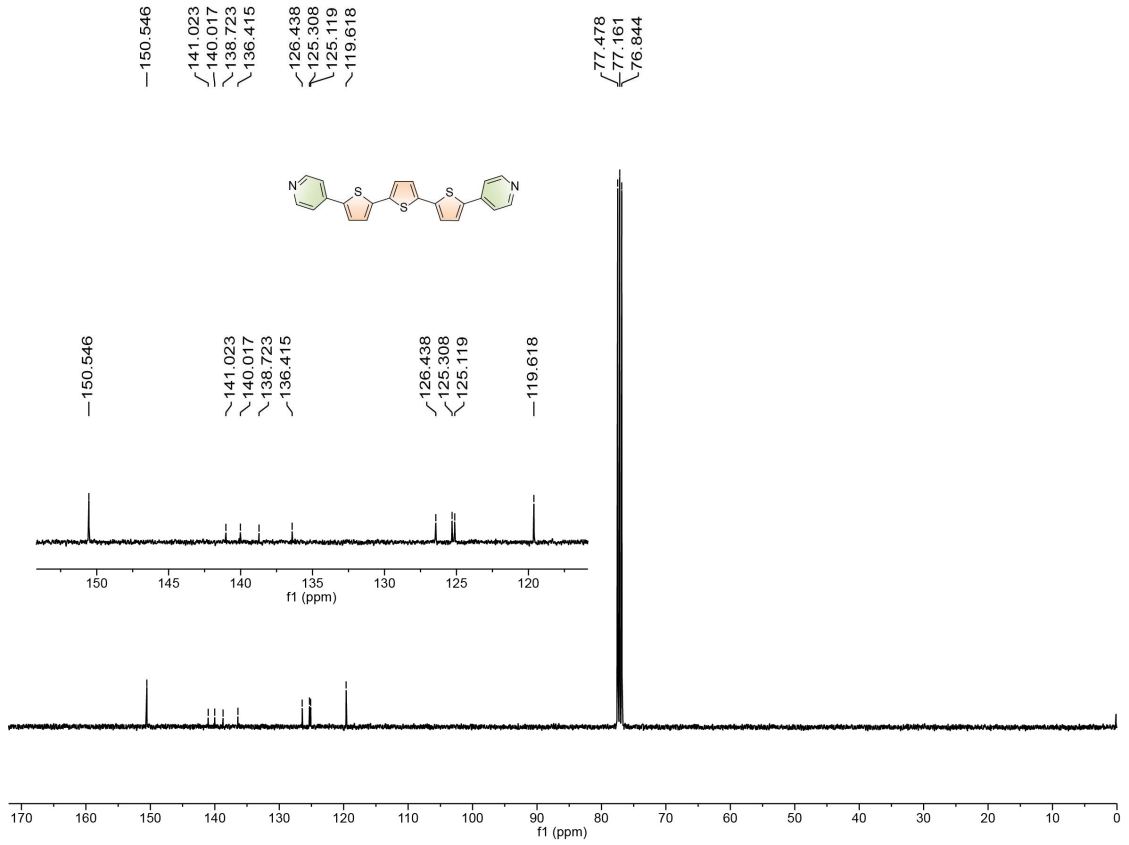



$^{1}$H NMR (DMSO-$d_6$, 400 MHz) and $^{13}$C NMR (DMSO-$d_6$, 100 MHz) spectra of **2TPyQA**.

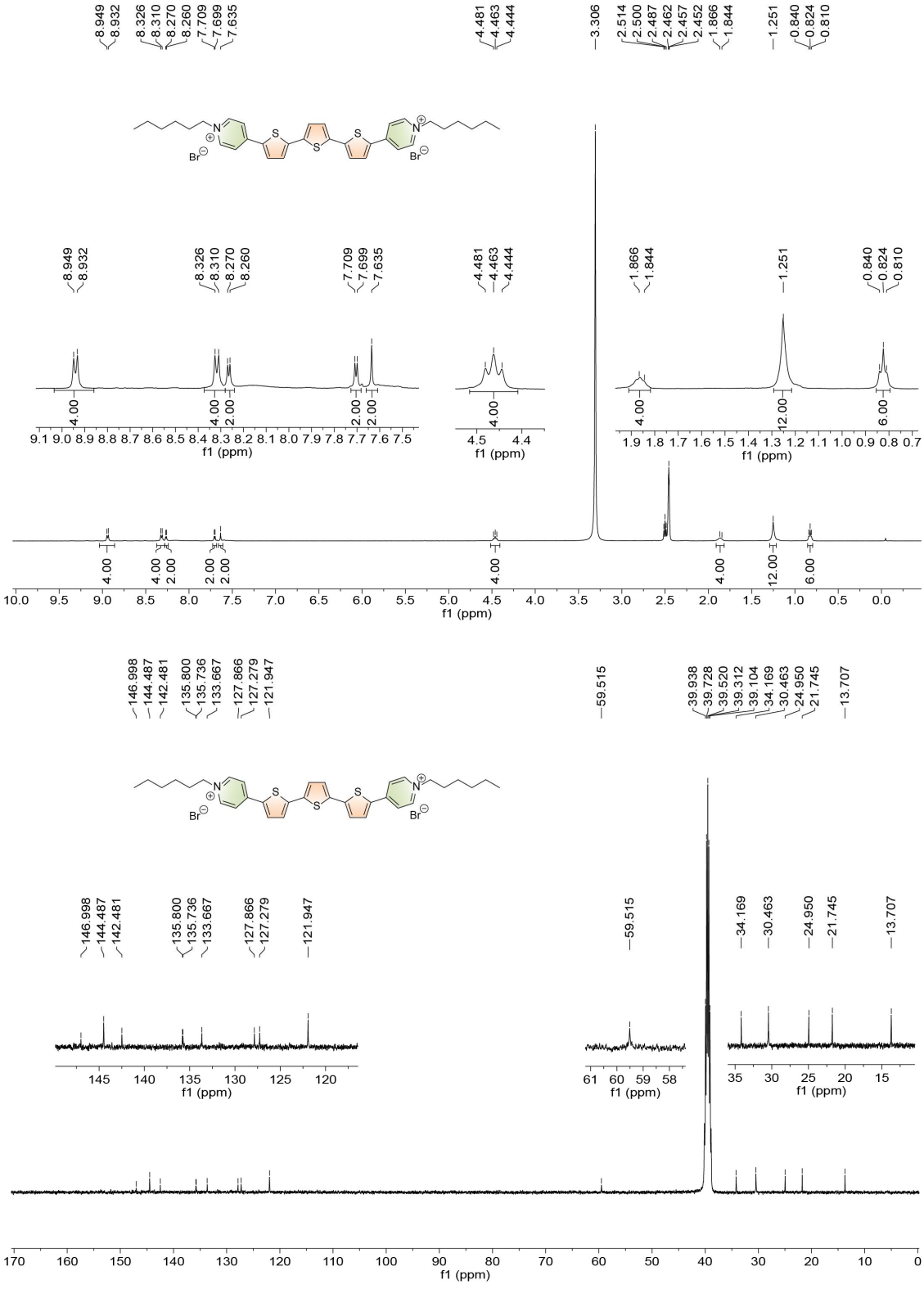


$^{1}$H NMR (DMSO-$d_6$, 400 MHz) and $^{13}$C NMR ($CDCl_3$, 100 MHz) spectra of **3TPy**.

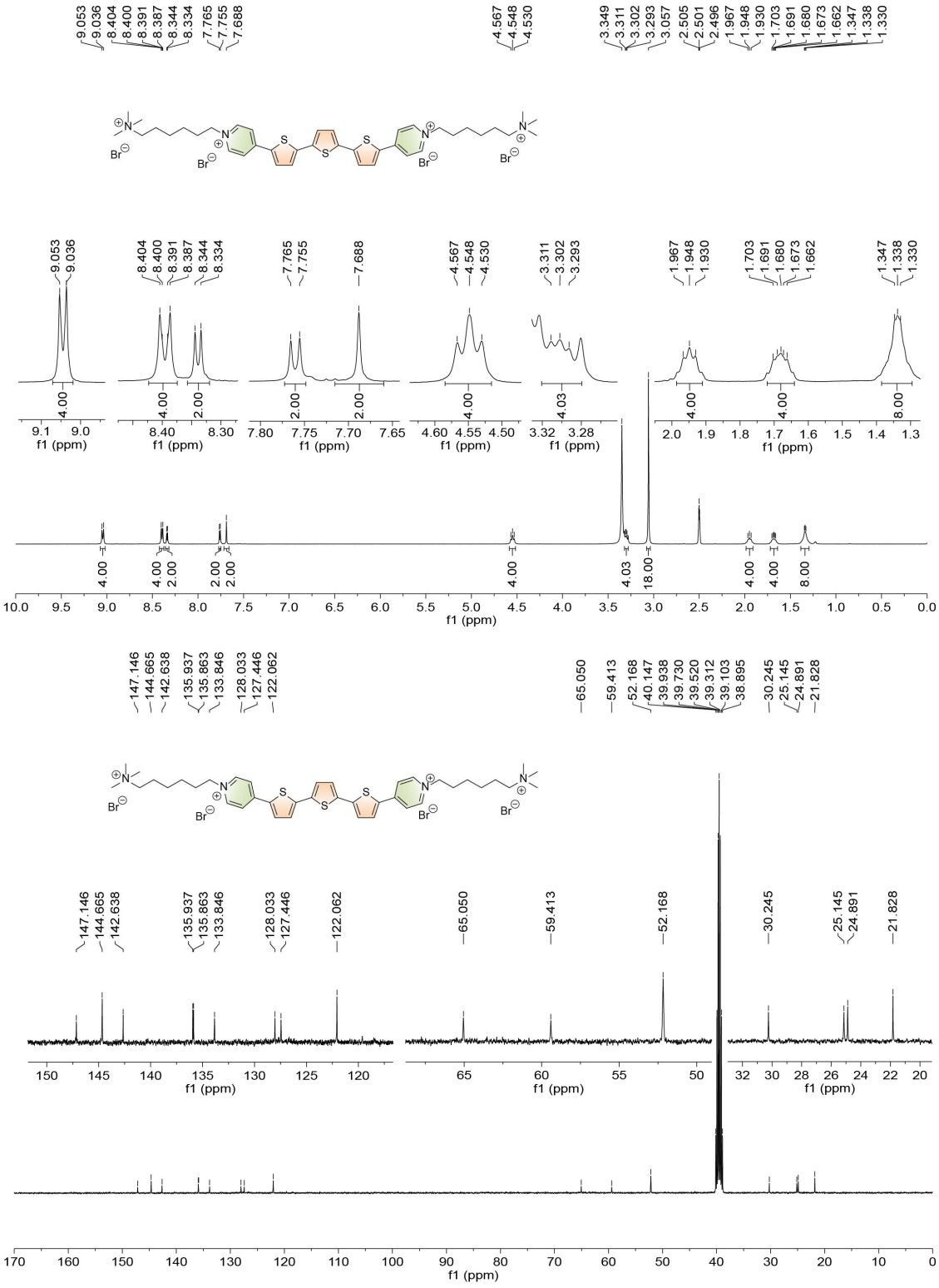


$^{1}$H NMR (DMSO-$d_6$, 400 MHz) and $^{13}$C NMR (DMSO-$d_6$, 100 MHz) spectra of **3TPyC$_6$**.

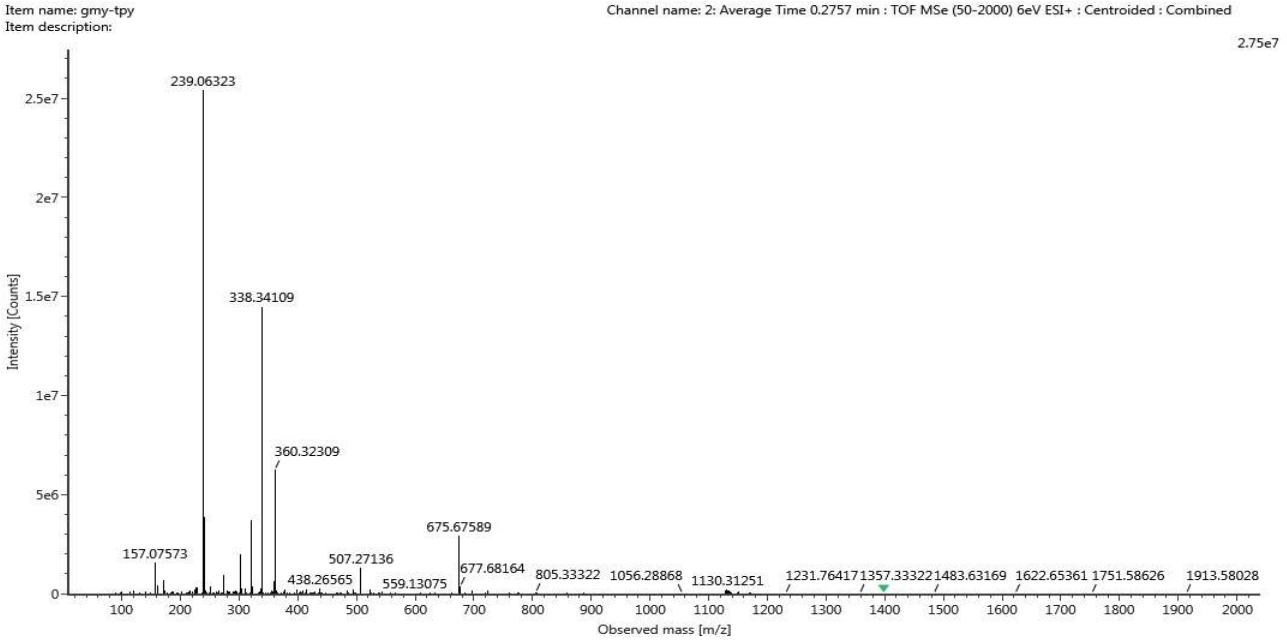


$^{1}$H NMR (DMSO-$d_6$, 400 MHz) and $^{13}$C NMR (DMSO-$d_6$, 100 MHz) spectra of **3TPyQA**.

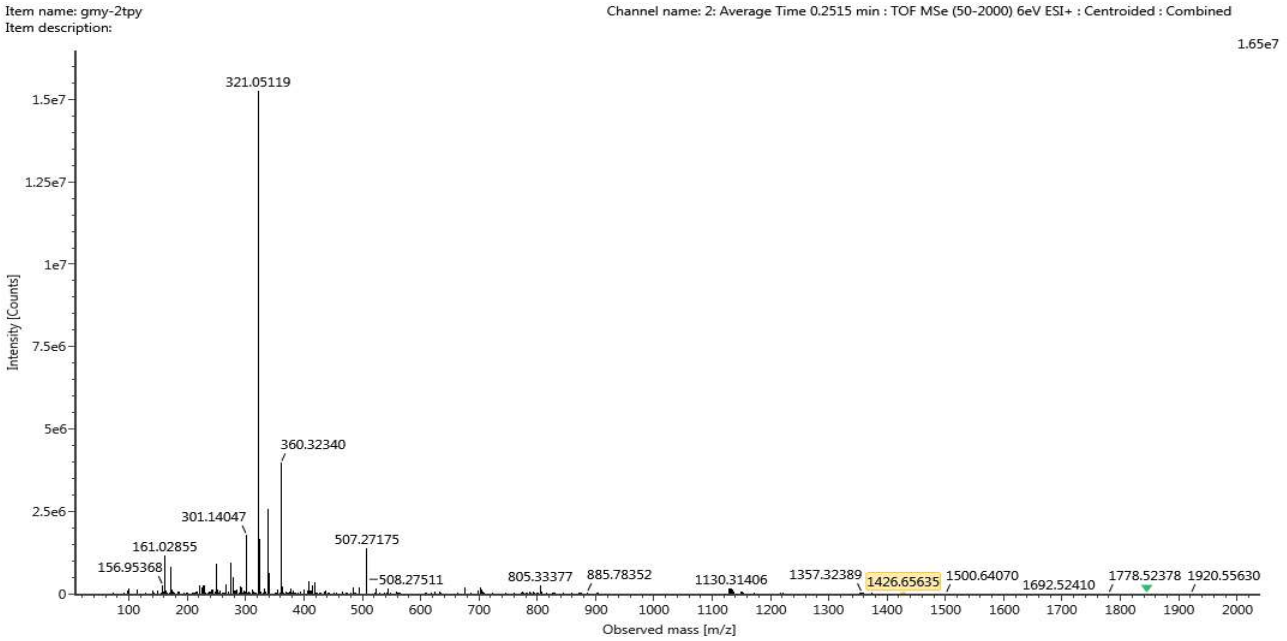


## 10. Mass spectra

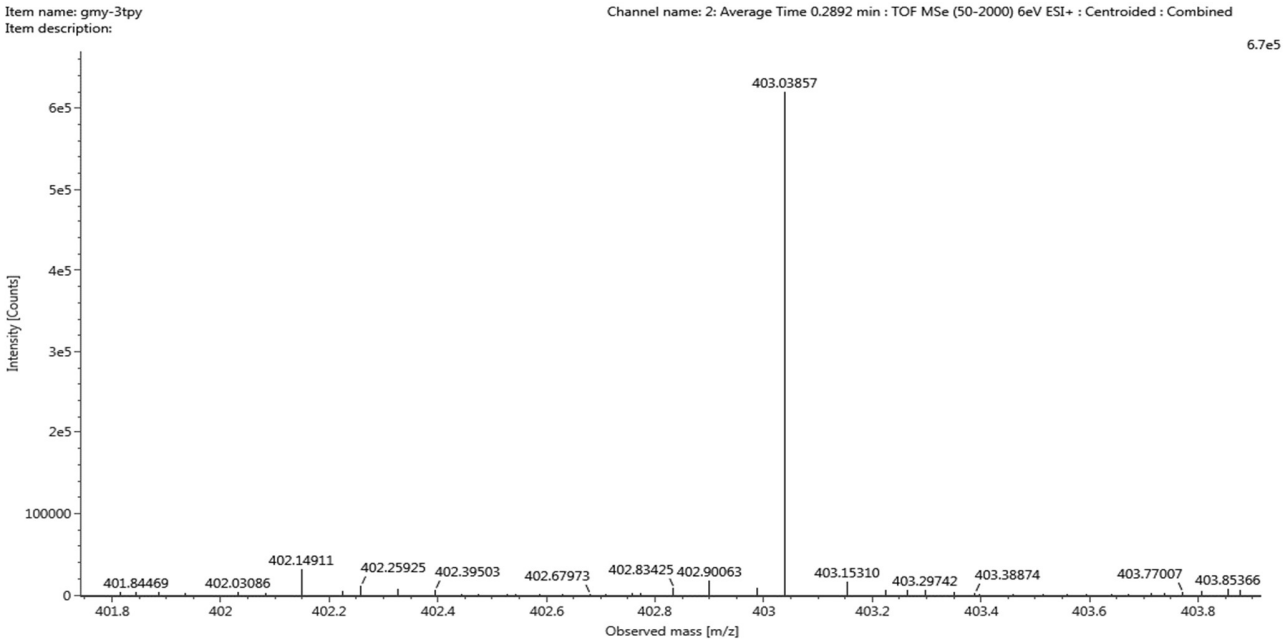
Mass spectra of **TPy**.



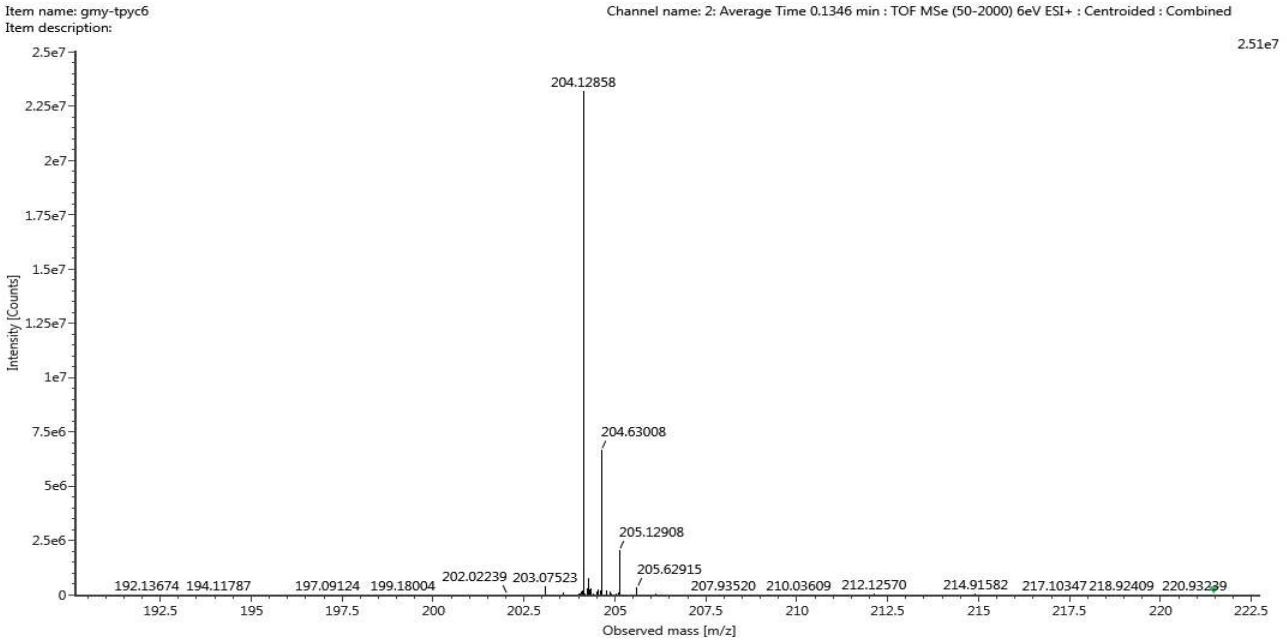

Mass spectra of **2TPy**.



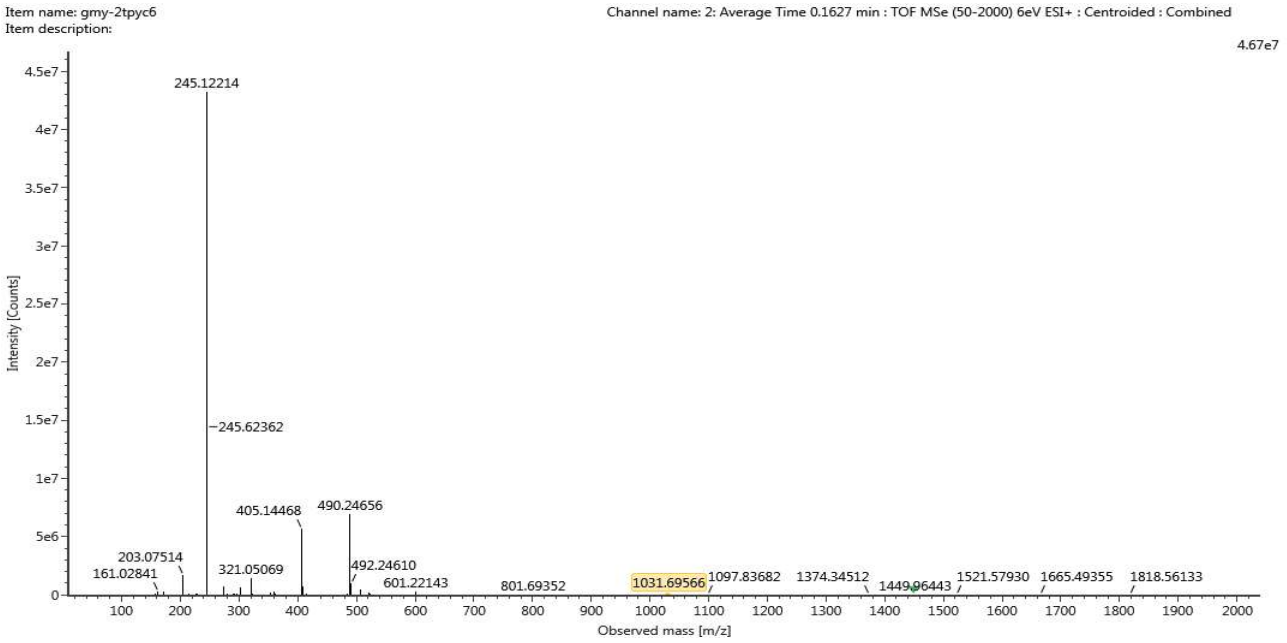
Mass spectra of **3TPy**.



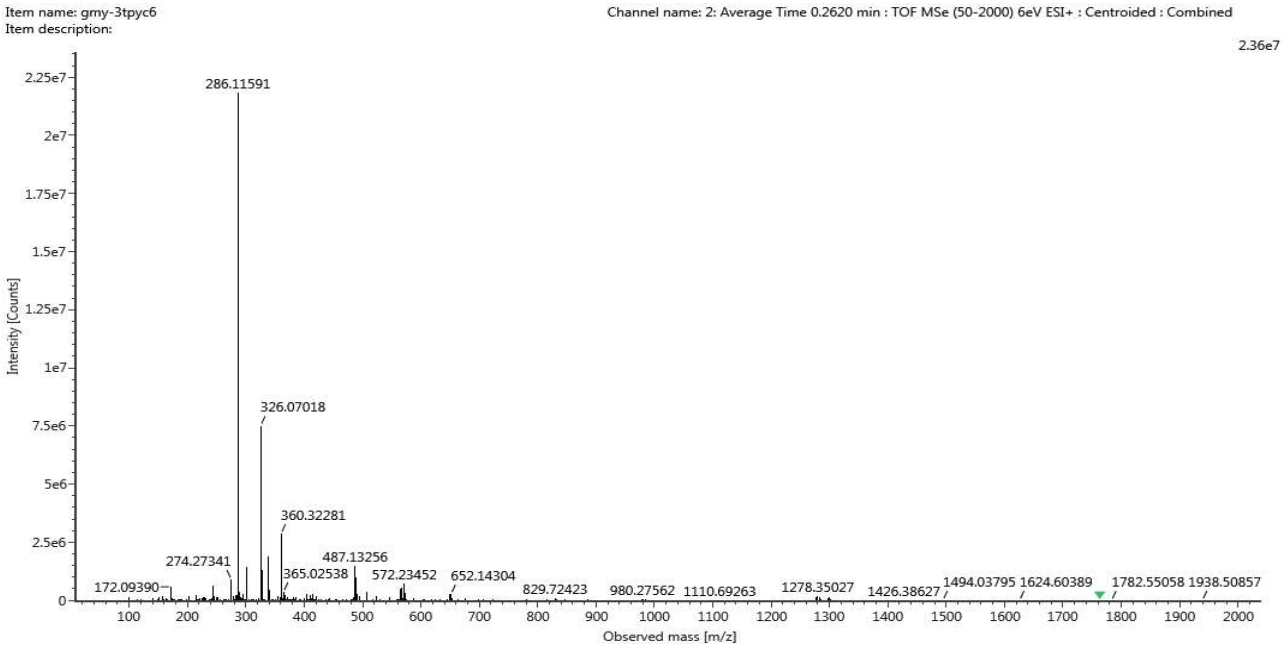

Mass spectra of **TPyC$_6$**.



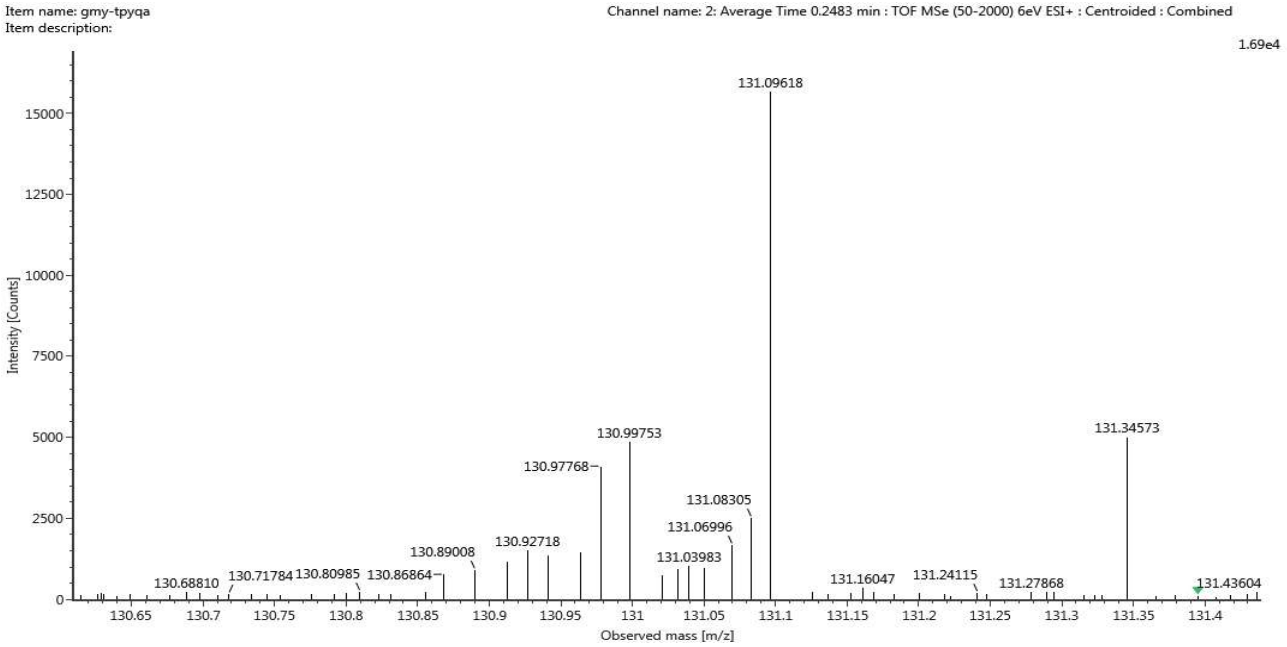
Mass spectra of **2TPyC$_6$**.



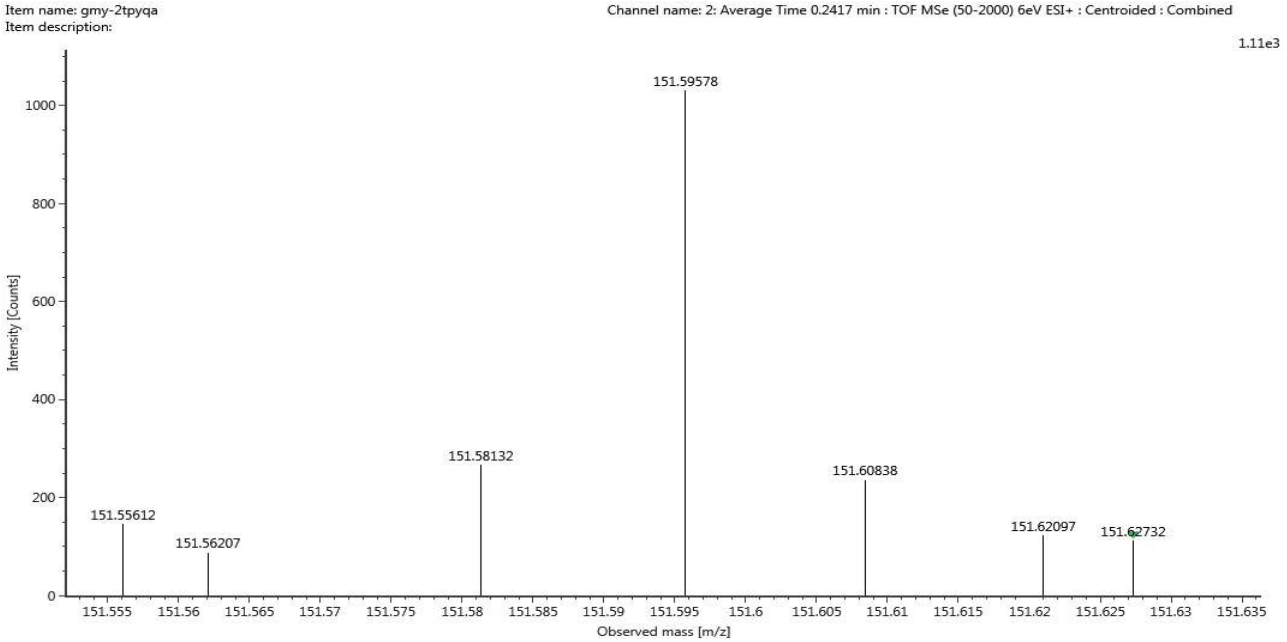

Mass spectra of **3TPyC$_6$**.



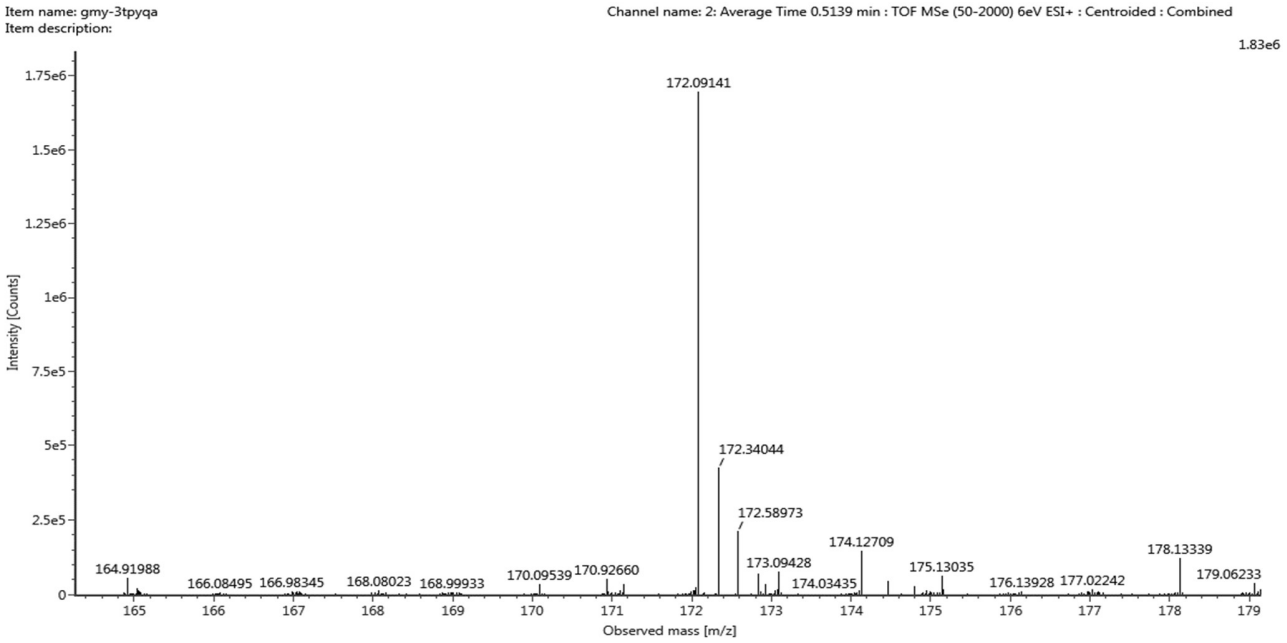
Mass spectra of **TPyQA**.




Mass spectra of **2TPyQA**.



Mass spectra of **3TPyQA**.



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