Supporting information

A multifunctional perylenediimide derivative as recyclable specific Hg^{2+} ion sensor and efficient DNA delivery carrier

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Materials

1, 6, 7, 12-Tetrachloroperylene-3, 4, 9, 10-tetracar-boxylic acid dianhydride was obtained from Beijing Wenhaiyang Perylene Chemistry (Beijing, China). 4-Hydroxybenzaldehyde (98%) and 2-aminoethanethiol hydrochloride (＞95.0%) were purchased from Alfa Aesar and TCI, respectively. All other solvents and reagents were purchased from commercial suppliers and used as received. S2 cells were propagated in Schneider Drosophila Medium supplemented with 10% FBS, 100 U/mL penicillin and 100 μg/mL streptomycin at 25 °C without CO_2.

Instruments

Nuclear magnetic resonance (NMR) spectra were recorded on Bruker 400 (400 MHz ^1H; 101 MHz ^{13}C) spectrometer using D_2O, $CDCl_3$ and MeOD as solvent at room temperature. Chemical shifts were reported downfield from 0.00 ppm using TMS as internal reference. Matrix-assisted laser-desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) were determined on AXIMA-CFR plus MALDI-TOF mass spectrometer. The UV-Vis absorption spectra were recorded on a

spectrophotometer (Cintra 20, GBC, and Australia). The corrected Fluorescence spectroscopic studies were performed on a fluorescence spectrophotometer (Horiba Jobin Yvon FluoroMax-4 NIR, NJ, USA) at room temperature (25 ^{o}C). Fluorescence quantum yields (FQYs) were measured at room temperature by using cresyl violet in methanol as reference ($Ø_f$ =0.54).[1]

Cytotoxicity Assay

Cell viability was monitored using Tali^{TM} viability kit-Dead Cell Green (Invitrogen, Catalog A10787) that was a green-fluorescent nuclear and chromosome stain. It does not penetrate intact membranes, but easily penetrate compromised membranes characteristic of dead cells. The measurement was performed at 48 h post-incubation of **DTPDI**. Replace the fresh cell medium after 48 h of incubation and then add 1 μL Dead Cell Green into 100 μL cell medium for 0.5 h incubation.

Cellular Hg^{2+} response

HeLa cells were purchased from Gibco by Life Technologies. HeLa cells were incubated in Dulbeccos Modified Eagle Medium (DMEM 1X) which was supplemented with 10% fetal bovine serum (FBS, GIBCO) and 1% penicillin streptomycin (PS). Cells were grown onto 35 mm × 35 mm cell culture dish at a density of 1×10^5 cells/well at 37 ^{o}C under 5% CO_2. After 2 days, two groups of cells were incubated with **DTPDI** (2.5 μM) for 2 h and then washed three times with Phosphate Buffered Saline(PBS) to remove excess probe, and one group of cells were incubated with Hg^{2+} (7 μM) for 2 h and then washed with the PBS buffer to remove excess Hg^{2+}. Then added the fresh cell culture media (DMEM 1X) and observed the cell imaging under the fluorescence microscopy.

Cellular Uptake

HeLa cells were purchased from Gibco by Life Technologies. HeLa cells were incubated in Dulbeccos Modified Eagle Medium (DMEM 1X) which was supplemented with 10% fetal bovine serum (FBS, GIBCO) and 1% penicillin

streptomycin (PS). Cellular uptake experiment was performed in 35 mm × 35 mm cell culture dish, 2.5×10^5 cells per well. After 8 h of cell seeding, making the HeLa cells adhere to the bottom of the dish, add the **DTPDI** to the cell culture dish. Cellular uptake was imaged by fluorescence microscope. The fluorescence intensity of **DTPDI** inside cells was calculated by Image-J Program.

In vitro Transfection Efficiency

Drosophila S2 cells were propagated in Schneider's *Drosophila* Medium (Sigma) supplemented with 10% FBS, 100 U/mL penicillin and 100 μg/mL streptomycin at 25 °C without CO_2. Single-strand DNA (20 bp, 100 μM) was labeled with 30 μM CXR Reference Dye (Promega, Catalog C5411) that could bind with DNA at a final concentration of 0.3-0.5 μM. DNA and **DTPDI** at different N/P ratios of 4:1, 8:1 and 16:1 were pre-incubated in culture medium at room temperature and then treated with cells. The fluorescence images of the distribution of **DTPDI** and DNA were obtained under a fluorescence microscope.

In vivo Transfection assay

Immediately after hatching from eggs, Black Cutworm larvae were picked out and individually fed with an artificial diet mixed with dsRNA alone or complex of DTPDI/dsRNA. dsRNA targeting *wg* was synthesized using T7 RiboMAX expression RNAi system (Promega). For oral feeding, 4 μg of dsRNA was mixed with 4 μg of **DTPDI**, and then mixed with 16 mg of fresh artificial diet. The newly-hatched larvae were individually fed with above diet. The larvae fed with dsRNA alone were used as controls. After 6 days, dsRNA-contained food was replaced with fresh normal food. Then photos were taken for comparison.

The sequence of wg-dsRNA is:

TGACAACATTGGCTTCGGCTTCCGATTCAGCAGAGAATTCGTCGACACCGGAGAAAG
AGGCAAAACCCTCAGAGAAAAAATGAACCTACACAACAACGAAGCCGGCCGAGCGC
ACGTGCAAACGGAGATGCGCCAGGAGTGCAAGTGCCACGGCATGTCAGGCTCGTGCA
CAGTCAAGACCTGTTGGATGAGGCTGCCCAGCTTCCGCTCCGTGGGCGACTCACTGA
AGGACCGGTTCGACGGCGCGTCCCGGGTCATGATGCCTAACACTGACCTGGAACCGC
CAGCGCAGCGGAACGACGCGGCGCCACACAGAGTGCCGCGAAGAGACCGATACCGG

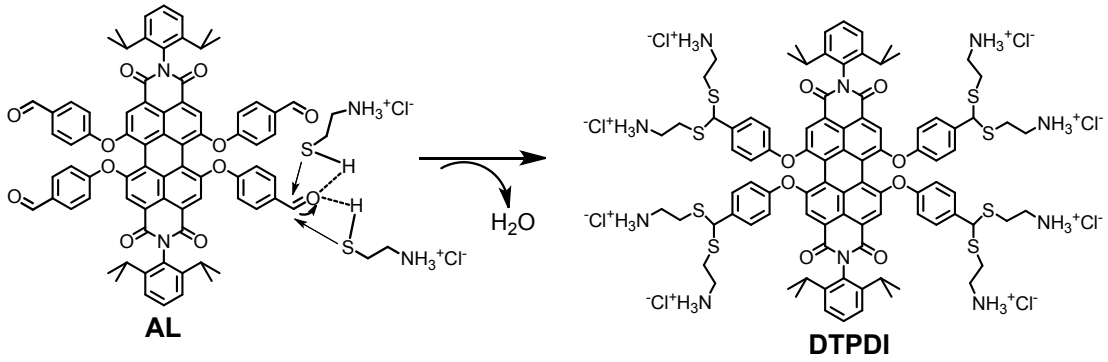
TTTCAACTGCGACCTCACAATCCTGACCATAAATCGCCGGGGGCGAAAGACTTGGTCT
ACCTAGAATCATCGCCGGGTTTCTGTGAAAAGAACCCGAGGCTGG

The primers for dsRNA synthesis are:

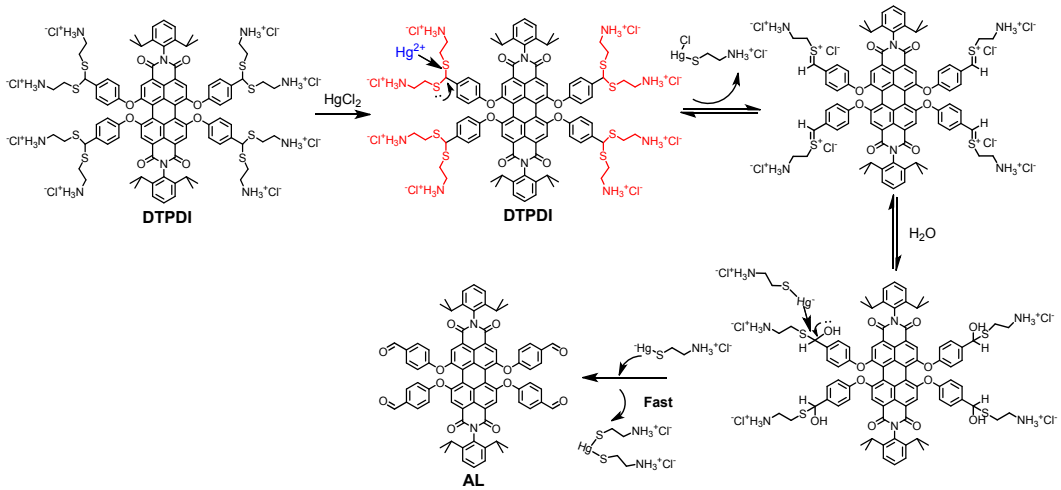
FT7: TAATACGACTCACTATAGGTGACAACATTGGCTTCGG

RT7: TAATACGACTCACTATAGGCCAGCCTCGGGTTC

Synthesis Schemes



Scheme S1. The mechanism for the conversion of **AL** to **DTPDI**



Scheme S2 Hg^{2+}-promoted conversion mechanism of **DTPDI** to aldehyde **AL** in presence of $HgCl_2$

UV-Vis Absorption and Fluorescence Emission Properties

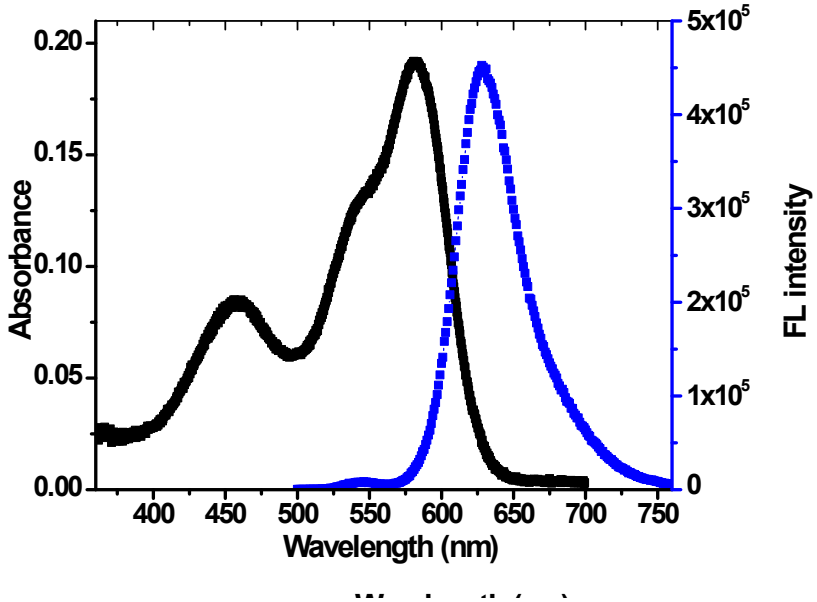


Fig. S1 UV-vis absorption and fluorescence (λ_{ex} = 450 nm) of **DTPDI** (5 μM) in distilled water.

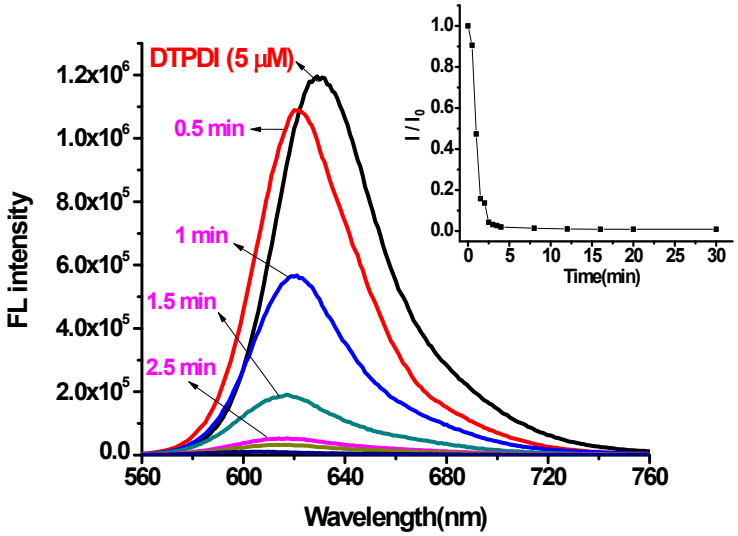


Fig. S2 Fluorescence changes of **DTPDI** (5 μM) upon addition of Hg^{2+} (10 eq) in distilled water from 0 min to 30 min (λ_{ex} = 450 nm). Inset: Plot of the intensity maximum of **DTPDI** versus Hg^{2+} (10 eq) at different time.

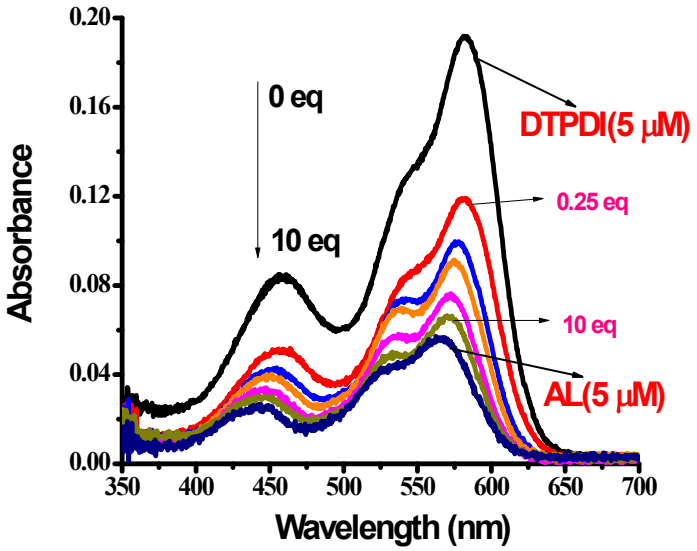


Fig. S3 UV-vis absorption changes of **DTPDI** (5 μM) upon addition of Hg^{2+} (0 - 10 eq) in distilled water

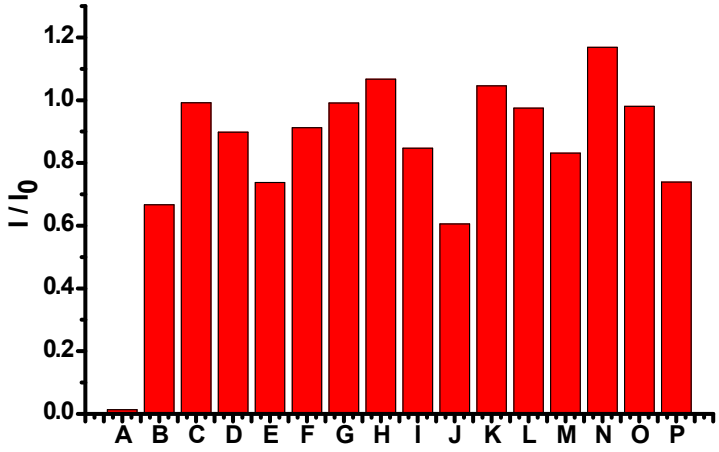


Fig. S4 Fluorescence intensity ratio (I_0 /I) of **DTPDI** (5 μM) in distilled water at 25 ^oC upon addition of 10 eq of different metal ions, measured after 2 min (λ_{ex} = 450 nm): (A) Hg^{2+}, (B) Fe^{2+}, (C) Na^+, (D) K^+, (E) Zn^{2+}, (F) Cu^+, (G) Cu^{2+}, (H) Fe^{3+}, (I) Ca^{2+}, (J) Mg^{2+}, (K) Cr^{2+}, (L) Mn^{2+}, (M) Cd^{2+}, (N) Pb^{2+}, (O) Ni^{2+}, (P) Ag^+.

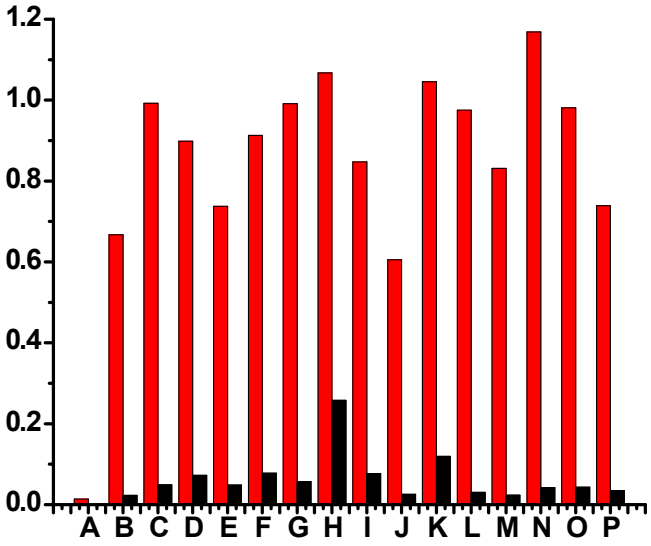


Fig. S5 Fluorescence intensity ratio (I_0 /I) of **DTPDI** (5 μM) of the competition experiments upon addition of each metal ion (200 μM, red bars) followed by Hg^{2+} (200 μM, black bars) in water at 25 °C, measured after 2 min (λ_{ex} = 450 nm): (A) Hg^{2+}, (B) Fe^{2+}, (C) Na^{+}, (D) K^{+}, (E) Zn^{2+}, (F) Cu^{+}, (G) Cu^{2+}, (H) Fe^{3+}, (I) Ca^{2+}, (J) Mg^{2+}, (K) Cr^{2+}, (L) Mn^{2+}, (M) Cd^{2+}, (N) Pb^{2+}, (O) Ni^{2+}, (P) Ag^{+}.

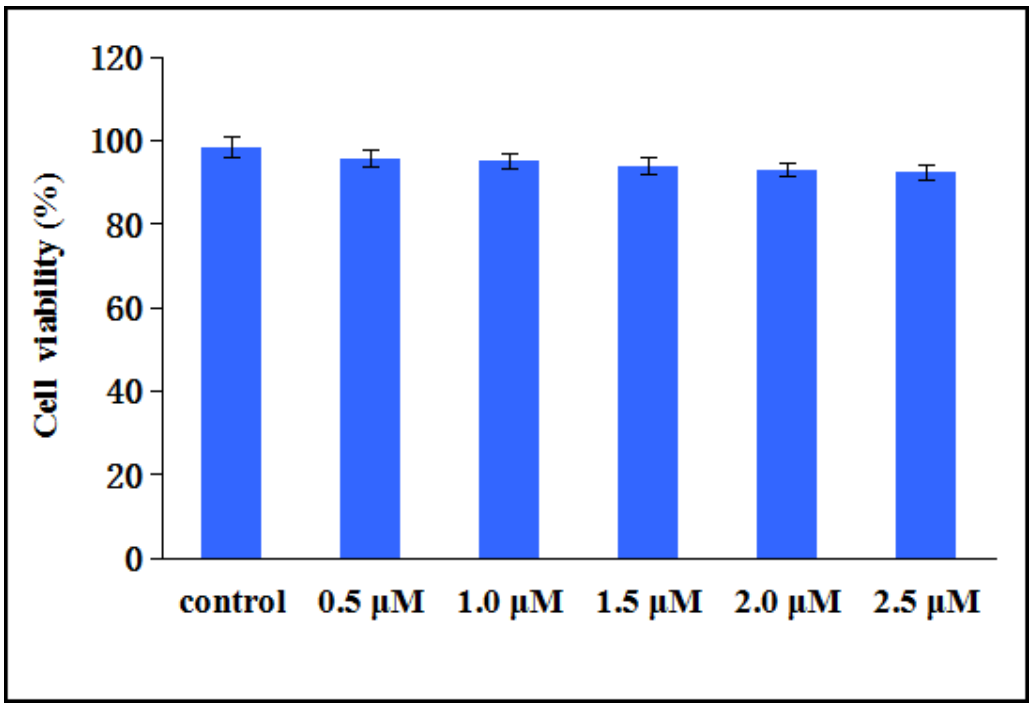


Fig. S6 Cell viability of cells treated with **DTPDI** at various concentrations for 48 h. The data were mean±SEM.

The DNA-binding affinity of "**DTPDI**" in water was determined using an isothermal titration calorimetry (ITC) as well as agarose gel electrophoresis. The association constant (Ka of "**DTPDI**" / DNA) is 1.153×10^6 M^{-1} (Fig. S7). "**DTPDI**" could compact DNA completely at a N : P ratio of above 1 (Fig. S8). These data demonstrate the interactions between "**DTPDI**" and DNA.

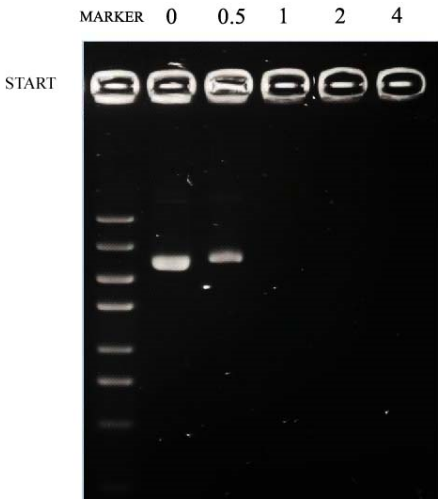
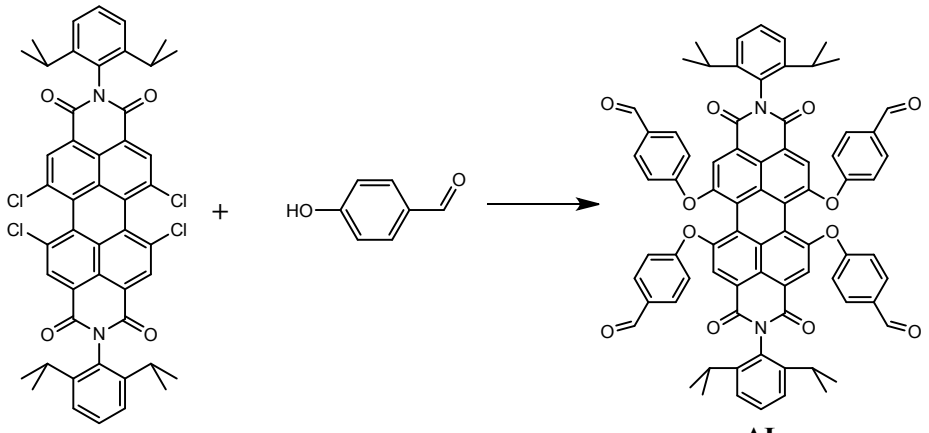


Fig. S7 Agarose gel Electrophoresis of pDNA/**DTPDI** complexes at various N : P ratios.

Synthesis and characterizations

1. Synthesis of AL



Scheme S3 Synthesis approach for **AL**

Under an atmosphere of argon, **4Cl-PDI** (0.1698 g, 0.2 mmol), 4-hydroxybenzaldehyde (0.244 g, 2 mmol) and K_2CO_3 (0.11 g, 0.8 mmol) were added into NMP (20 mL) with stirring. The reaction was carried out 80 ^oC under stirring for 36 h. After cooled down to room temperature, the mixture solution was treated with diluted hydrochloric acid and filtered to remove excess 4-hydroxy benzaldehyde. The residue was dissolved in dichloromethane and then washed with distilled water until the solution was neutral. The solvent was removed under reduced pressure, then the solid was purified by column chromatography with dichloromethane as the eluting solvent to give a reddish black solid **AL** (0.119 g, 50%). ^1H NMR (400 MHz, $CDCl_3$) δ 9.95 (s, 4H), 8.35 (s, 4H), 7.85 (d, J = 8.7 Hz, 8H), 7.48 (t, J = 7.8 Hz, 2H), 7.32 (d, J = 7.8 Hz, 4H), 7.09 (d, J = 8.6 Hz, 8H), 2.70 (dt, J = 13.6, 6.8 Hz, 4H), 1.15 (d, J = 6.8 Hz, 24H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 190.37, 162.66, 160.29, 154.91, 145.49, 132.97, 132.07, 130.02, 129.81, 124.10, 123.74, 121.76, 121.35, 119.66, 29.44, 24.02. MS (MALDI-TOF, m/z) Calc. for $C_{76}H_{58}N_2O_{12}$: 1191.28, found: 1192.547 ($M+H^+$).

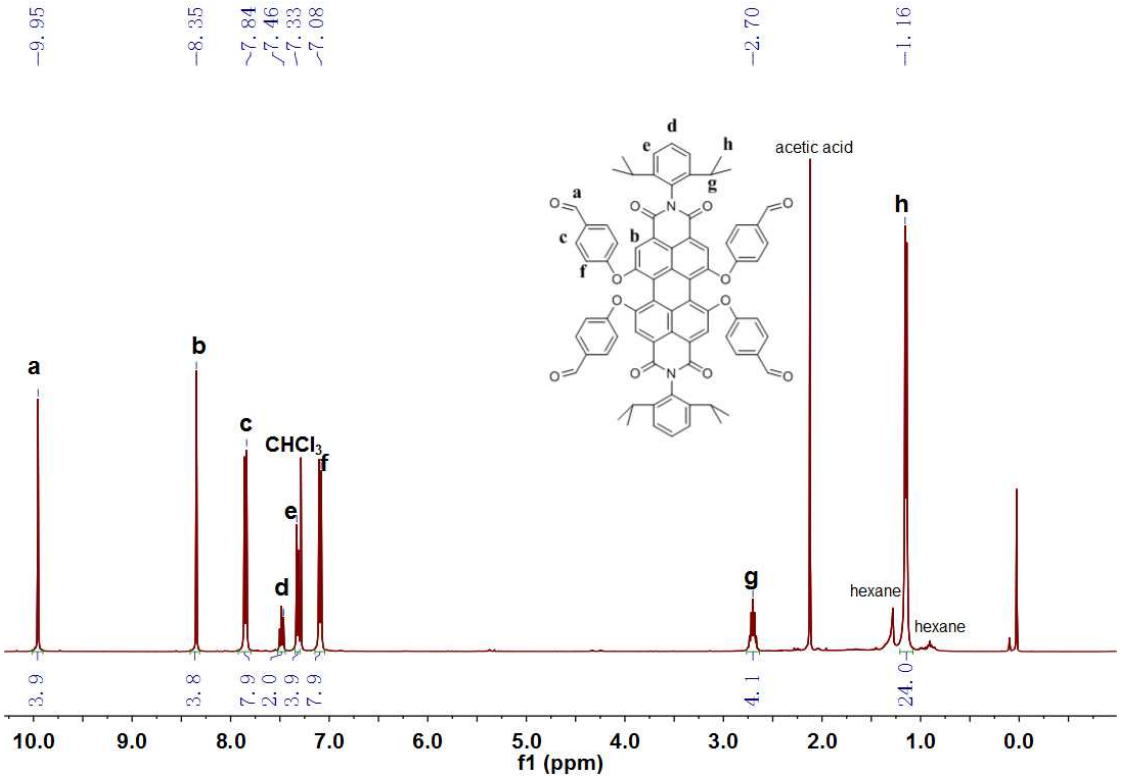


Fig. S8 ^{1}H NMR spectrum of **AL** in $CDCl_3$

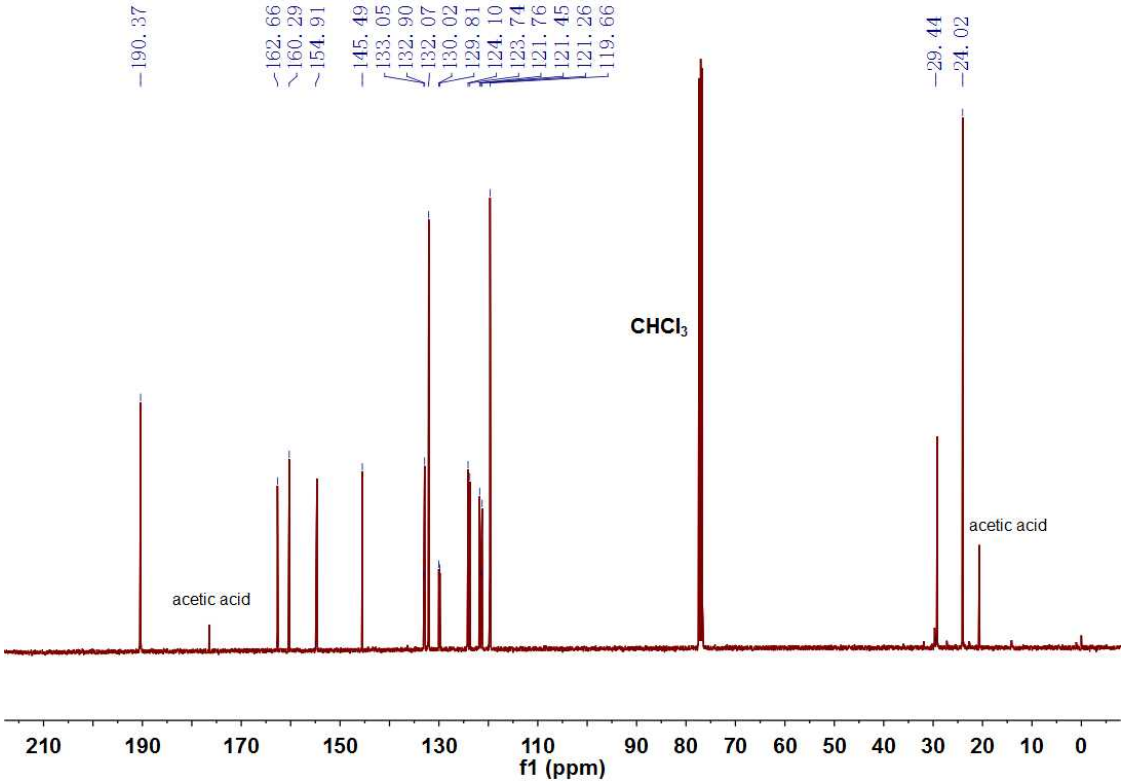
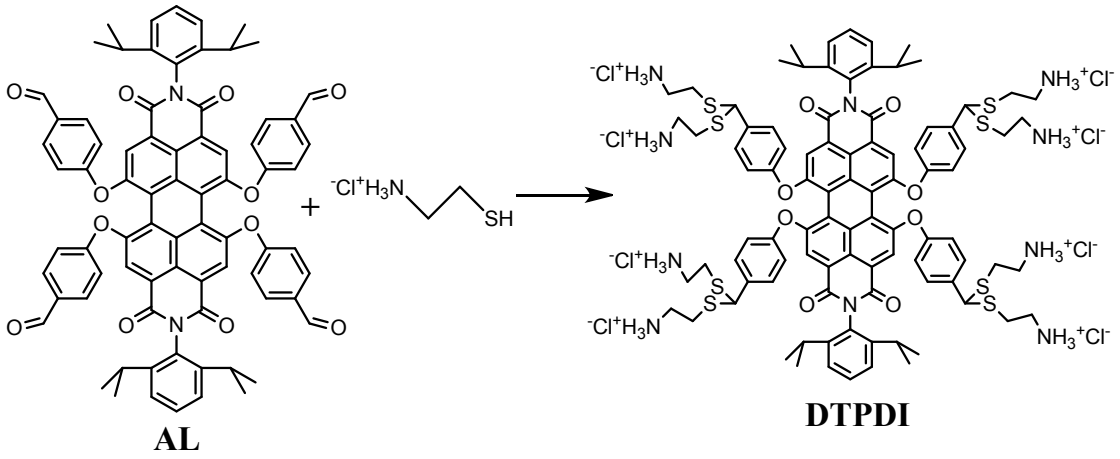


Fig. S9 ^{13}C NMR spectrum of **AL** in $CDCl_3$

2. Synthesis of DTPDI



Scheme S4 Synthesis approach for **DTPDI**

A solution of $BF_3 \cdot Et_2O$ (102 mg, 0.72 mmol) in dry dichloromethane (3 mL) was slowly added into a mixture solution of **AL** (39 mg, 0.033 mmol) and 2-aminoethanethiol hydrochloride (63 mg, 0.55 mmol) in DMF (3 mL) under nitrogen atmosphere at 0 °C. After one day, the temperature of reaction was raised to 37 °C. After stirred for 4 days at 37 °C, the solution was redissolved in deionized water (0.5 mL) and washed with diethyl ether for several times. The further dialysis in 1 mM HCl and lyophilization afforded a purple solid **AL**. Yield: 96.5 %. ^{1}H NMR (400 MHz, D_2O) δ 8.21 (s, 4H), 7.43 (d, J = 40.5 Hz, 16H), 7.02 (s, 6H), 5.19 (s, 4H), 2.86 (dd, J = 116.4, 65.5 Hz, 36H), 1.01 (s, 24H). ^{13}C NMR (101 MHz, MeOD) δ 165.01, 157.38, 156.64, 147.20, 137.90, 134.48, 131.77, 131.15, 130.81, 125.18, 123.96, 122.27, 121.60, 121.20, 52.87, 40.31, 30.72, 30.36, 24.33. MS (MALDI-TOF, m/z) Calc. for $C_{92}H_{106}N_{10}O_8S_8$: 1736.41, found: 1736.91 ($M+H^+$).

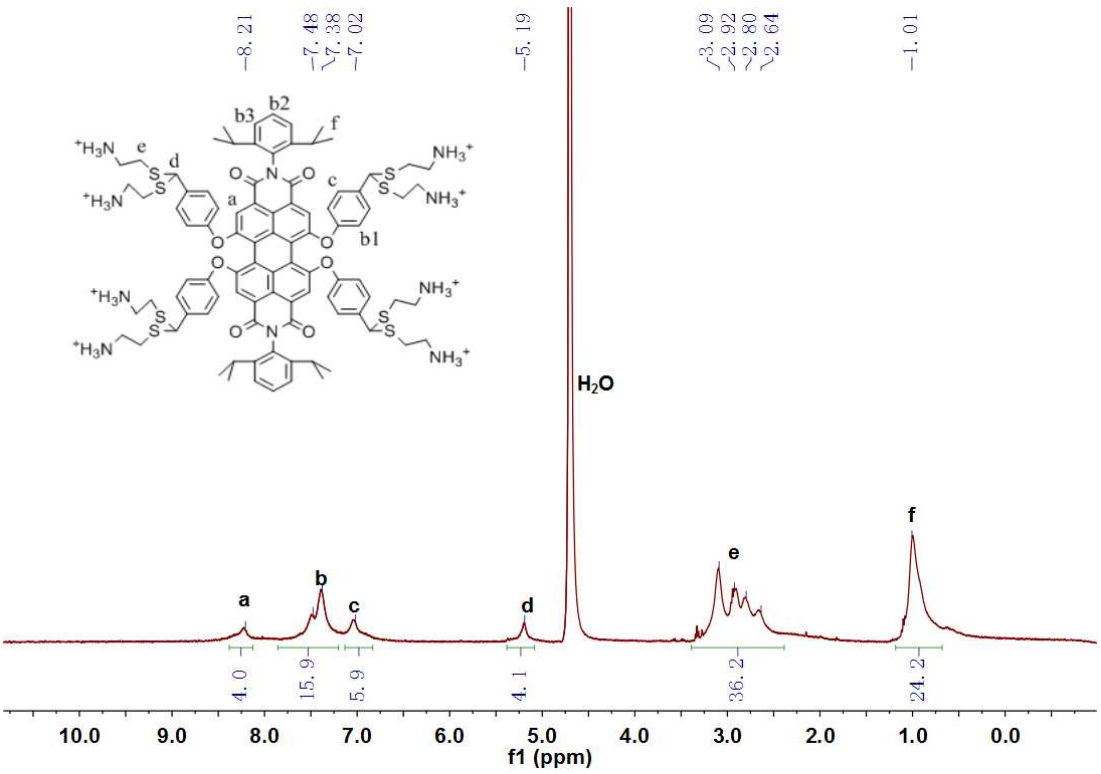


Fig. S10 ^{1}H NMR spectrum of **DTPDI** in D_2O

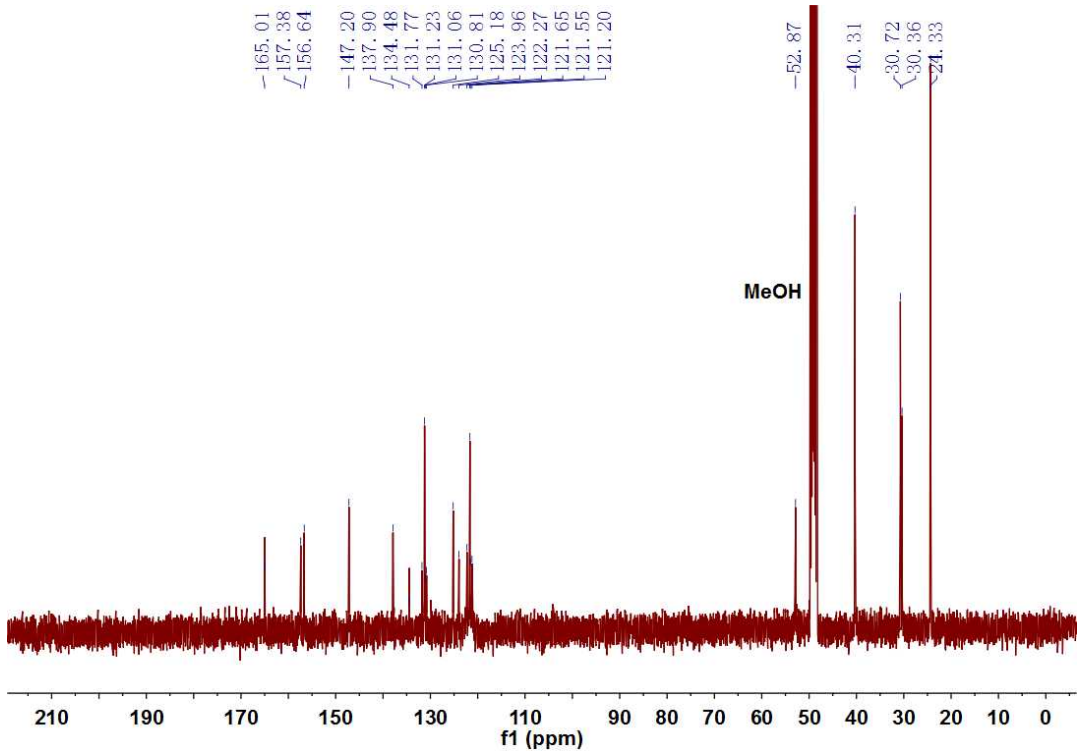


Fig. S11 ^{13}C NMR spectrum of **DTPDI** in MeOD

[1] C. Kohl, T. Weil, J. Qu and K. Müllen, *Chem. Eur. J.,* 2004, **10**, 5297.