Electronic Supplementary Information

for

Towards A New FRET System via Combination of Pyrene and Perylene Bisimide: Synthesis, Self-assembly and Fluorescence Behavior

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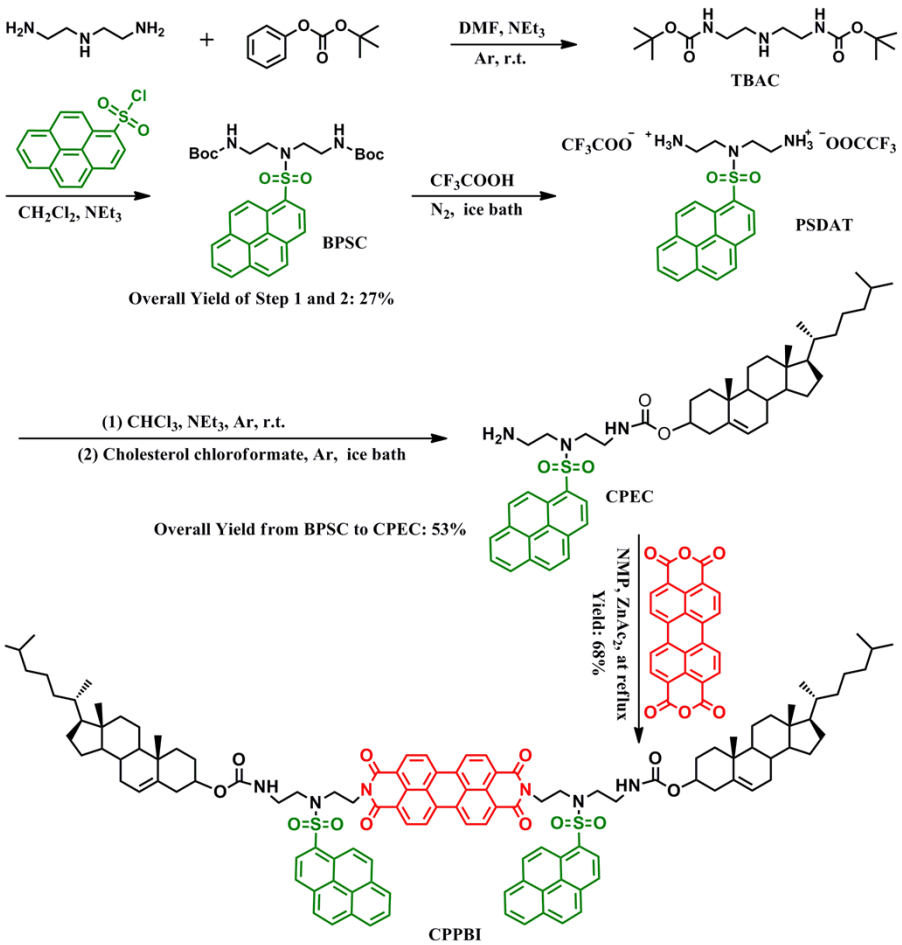
Contents

1. Synthesis route of the compound (CPPBI)



Scheme S1. The synthesis route of CPPBI

2. Synthesis and characterization

Preparation of di-tert-butyl (azanediylbis(ethane-1,2-diyl))dicarbamate (TBAC)

To a solution of the diethylenetriamine (2.1 mL) in DMF containing 2.2 eq. of tert-butyl-phenyl carbonate was added a catalytic amount of triethylamine. The mixture was stirred at room temperature overnight. After that, the reaction mixture was poured into phosphate buffer (500 mL, 0.025 M K_2HPO_4 and 0.025 M NaH_2PO_4). The pH of the solution as obtained was adjusted to ~3 with 2 M H_2SO_4 under vigorous stirring. The mixture was

extracted with ether (3×150 mL, the extracts discarded), then the aqueous layer was basified to pH ~10 with 4.5 M aqueous NaOH, and finally the system was extracted with DCM (3×150 mL) for three times. The organic phase was collected and dried over anhydrous Na_2SO_4, filtered and concentrated by rotary evaporation under reduced pressure [S1]. The mixture was used successfully in the subsequent reaction, and further purification is not necessary.

Preparation of di-tert-butyl(((pyren-1-ylsulfonyl)azanediyl)bis(ethane-2,1-diyl)) dicarbamate (BPSC)

Triethylamine (8.2 mL, 58.8 mmol) was slowly injected into a stirred suspension of PSC (5.5 g, 18.3 mmol) and TBAC in $CHCl_3$ (90 mL) to give a yellow solution under argon atmosphere at room temperature, and the stirring was continued overnight. Then, the solvent was removed by rotary evaporation under reduced pressure, the residue was subjected to two successive column chromatography on silica gel column eluting with ethyl acetate/petroleum ether (v/v, 3:1) and ethyl acetate/petroleum ether (v/v, 3:1) respectively. The crude product was recrystallized from CH_2Cl_2/ether (v/v, 1:1) to yield a pure product. Overall yield from diethylenetriamine to BPSC was 27% (Scheme S1). M.p. 122.2-122.8 ˚C, ^{1}H NMR ($CDCl_3$/Me_4Si, 400 MHz, Fig. S19), δ (ppm): 8.99 (d, *J*=9.2, 1H, pyrene), 8.60 (d, *J*=6.8, 1H, pyrene), 8.10-8.33 (m, 7H, pyrene), 4.93 (s, 2H, N*H*), 3.42 (t, J=5.2, 4H, CH_2N), 3.31 (m, 4H, CH_2NH), 1.32 (s, 18 H, CH_3), FTIR (KBr), ν_{max} (cm^{-1}): 3383 (NH), 2983 (CH_2), 1689 (C=O),1519 (C=C), 1150 (S=O); MS (m/z), and Calcd for [(M+Na)$^+$]: 590.2301, found: 590.2304.

Preparation of 2,2'-((pyren-1-ylsulfonyl)azanediyl)diethanaminium trifluoroacetate (PSDAT)

Trifluoroacetic acid (4.0 mL, 51.4 mmol) was slowly injected into a stirred solution of BPSC (0.67 g, 1.2 mmol) in CH_2Cl_2 (8 mL) under nitrogen atmosphere in an ice bath, and the stirring was continued for overnight. TLC with CH_2Cl_2/CH_3OH (v/v, 20:l) exhibited the disappearance of the starting materials (BPSC, R_f = 0.6). The reaction mixture was concentrated, yielding a green viscous liquid. To the liquid, 10 mL of distilled water was added, producing a white precipitate immediately. The

precipitate was filtered, and the solid as obtained was dried in air dry oven at 50 °C for 12 h to give a yellow solid (0.7 g), which was directly used for subsequent synthesis.

Preparation of 3β-cholest-5-en-3yl-(2-(N-(2-aminoethyl)pyrene-1-sulfonamido)ethyl) carbamate (CPEC)

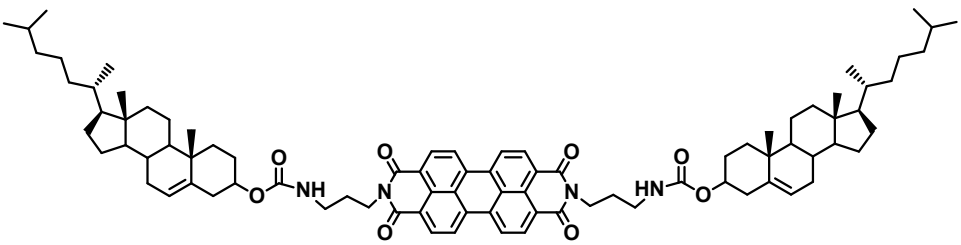
Triethylamine (1.0 mL, 7.2 mmol) and PSDAT were added to CH_2Cl_2 (150 mL), and stirred rapidly for 1 h under argon atmosphere at room temperature. Cholesterol chloroformate (0.53 g, 1.2 mmol) was dissolved in CH_2Cl_2 (100 mL) and added dropwise to the colorless solution as obtained in an ice bath under stirring. After the addition (~9 h), stirring was continued overnight. The reaction mixture was extracted with saturated brine (3×200 mL, the extracts discarded), dried over Na_2SO_4, filtered. The solvent was removed by rotary evaporation under reduced pressure. The residue as obtained was purified by column chromatography on silica gel column with CH_2Cl_2/CH_3OH (v/v, 30:l) to yield the pure product. Overall yield from BPSC to CPEC step 3 and 4 was 53% (Scheme S1). M.p. 112-122.5 ˚C; ^{1}H NMR ($CDCl_3/Me_4Si$, 400 MHz, Fig. S20), δ (ppm): 9.02(d, *J*=9.2, 1H, pyrene) 8.63 (d, *J*=8.4, 1H, pyrene), 8.11-8.32 (m, 7H, pyrene), 5.43 (s, 1H, *H*-C=C), 5.30 (s, 1H, N*H*), 4.36 (m, 1H, OC*H*), 3.43 (m, 6H, CH_2N and CH_2NH), 2.94 (m, 2H, CH_2NH$_2$), 0.66-2.19 (m, 45H, NH_2 and cholesteryl protons); FTIR (KBr), ν_{max} (cm^{-1}): 3420 (NH, NH$_2$), 3044 (ArH), 2934 (CH$_2$), 1720 (C=O), 1526 (C=C), 1162 (S=O); MS (m/z), Calcd for [(M+H)$^+$]: 780.4774, found: 780.4784.

Preparation of N,N'-(N-(2-(3β-cholest-5-en-3yl-formamido)ethyl)pyrene-1-sulfon-amido)ethyl perylene-3,4:9,10-tetracarboxyllic acid bisimide (CPPBI)

CPEC (0.3 g, 0.39 mmol), 3,4:9,10-perylenetetracarboxylic dianhydride (0.076 g, 0.19 mmol) and zinc acetate (0.023 g, 0.13 mmol) were added to 1-methyl-2-pyrrolidinone (10 mL), and stirred rapidly under argon atmosphere at reflux for 6 h, and then cooled to room temperature. The solvent was removed by rotary evaporation under reduced pressure. Subsequently, the mixture was washed with methanol and purified by column chromatography on silica gel column with CH_2Cl_2/CH_3OH (v/v, 30:l) to give red solid product, yield 68%. M.p. 200.2-201.0 ˚C; ^{1}H NMR ($CDCl_3/Me_4Si$, 600 MHz, Fig. S21), δ (ppm): 8.60 (d, *J*=7.8, 2H, pyrene), 8.40 (d, *J*=9.6, 2H, pyrene), 7.97 (d, *J*=7.2, 4H,

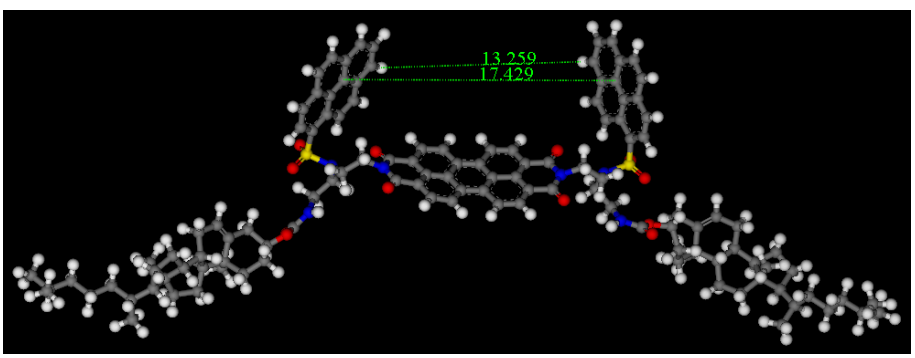
perylene), 7.92 (d, *J*=7.8, 2H, pyrene), 7.76 (d, *J*=7.2, 4H, perylene), 7.72 (d, *J*=9.6, 2H, pyrene), 7.59 (d, *J*=7.8, 2H, pyrene), 7.51 (d, *J*=7.8, 2H, pyrene), 7.15 (d, *J*=7.8, 2H, pyrene), 6.91 (t, *J*=7.8, 2H, pyrene), 6.76 (d, *J*=7.8, 2H, pyrene), 5.42 (s, 2H, *H*-C=C), 5.28 (s, 2H, N*H*), 4.60 (m, 2H, OC*H*), 3.75-4.31 (m, 16H, CH_2N and CH_2NH), 0.68-2.43 (m, 86H, cholesteryl protons); IR (KBr), ν_{max} (cm^{-1}): 3438 (NH), 2934 (CH_2), 1696 (O=C-O), 1659 (O=C-N), 1593 (C=C), 1161 (S=O); MS (m/z), Calcd for [(M+ $Na)^+$]: 1937.94, found: 1937.84.

3. Molecular structure of the corresponding molecule (PTCDI-Chol)



Scheme S2. Molecular structure of the corresponding molecule (PTCDI-Chol).

4. Energy-optimized structural model of CPBBI



Scheme S3. Energy-optimized structural model of CPPBI (A result from Molecular Dynamics Modelling).

5. UV-Vis absorption spectra of CPPBI

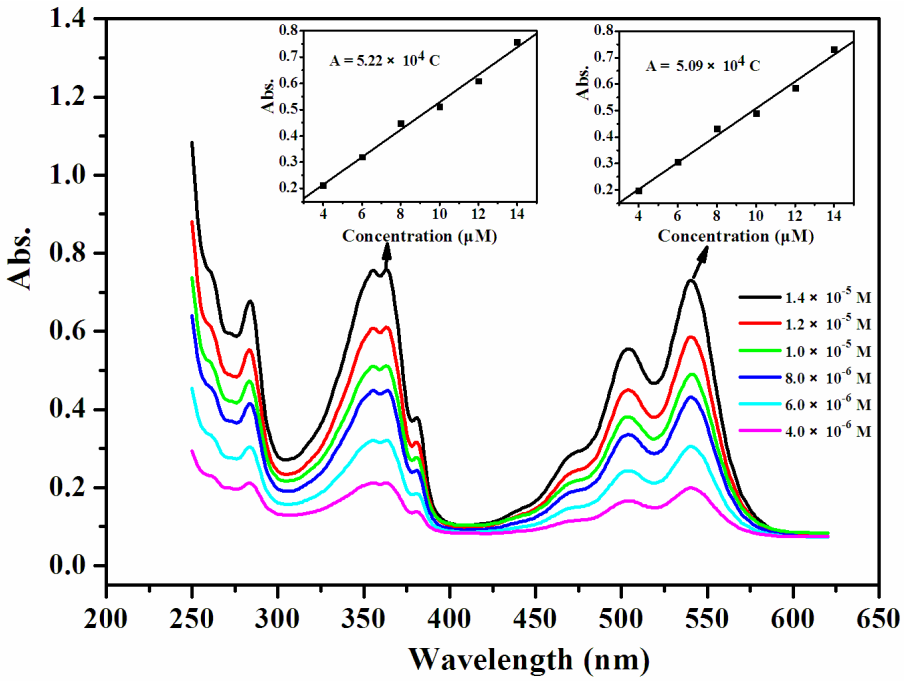


Fig. S1 UV-Vis absorption spectra of CPPBI in chloroform at room temperature for concentrations from 1.4×10^{-5} mol/L to 4.0×10^{-6} mol/L. Insets are the change of the absorbance with increasing in the concentration, recorded at 364 and 541 nm, respectively. In the equation, "A" represents for the absorbance and "C" is the concentration of the samples under study.

6. Fluorescence emission and absorption spectra of CPPBI and reference molecules

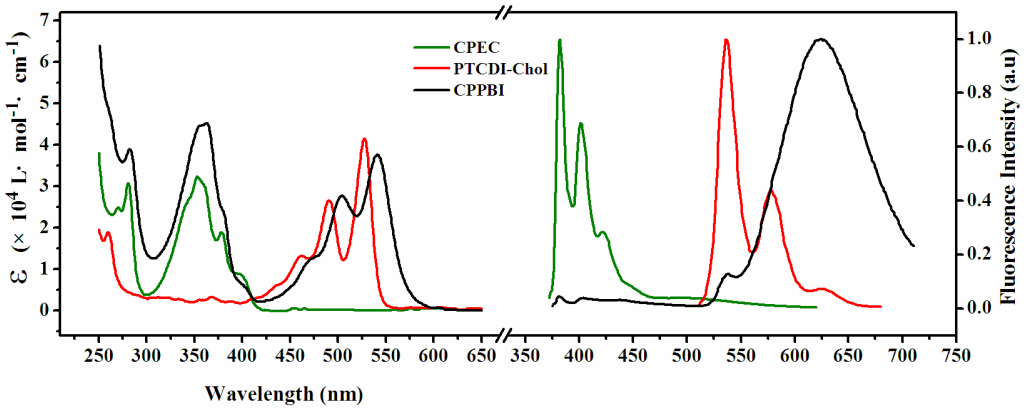


Fig. S2 Normalized fluorescence emission (right) and absorption spectra (left) of CPPBI, CPEC, PTCDI-Chol in chloroform at a concentration of 5.0×10^{-6} mol/L, the excitation wavelengths of CPPBI, PTCDI-Chol and CPEC are 364 nm, 490 nm and 350 nm, respectively.

7. Normalized absorption of CPPBI and emission spectra of CPEC

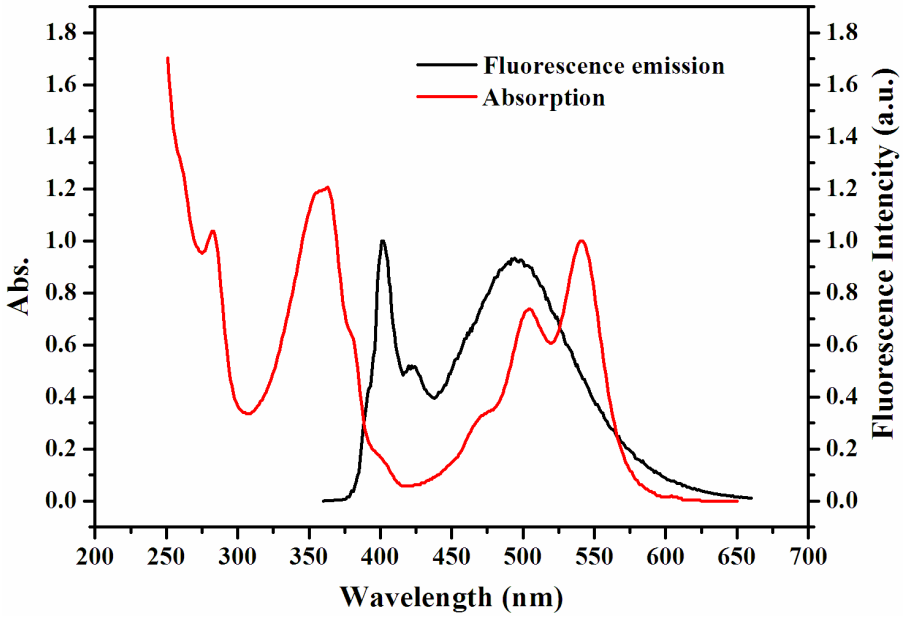


Fig. S3 Normalized absorption (red) of CPPBI in chloroform (5.0×10^{-6} mol/L) and emission spectra (black) of CPEC excited at 340 nm in chloroform (2.5×10^{-3} mol/L).

8. Fluorescence excitation and emission spectra of PTCDI-Chol

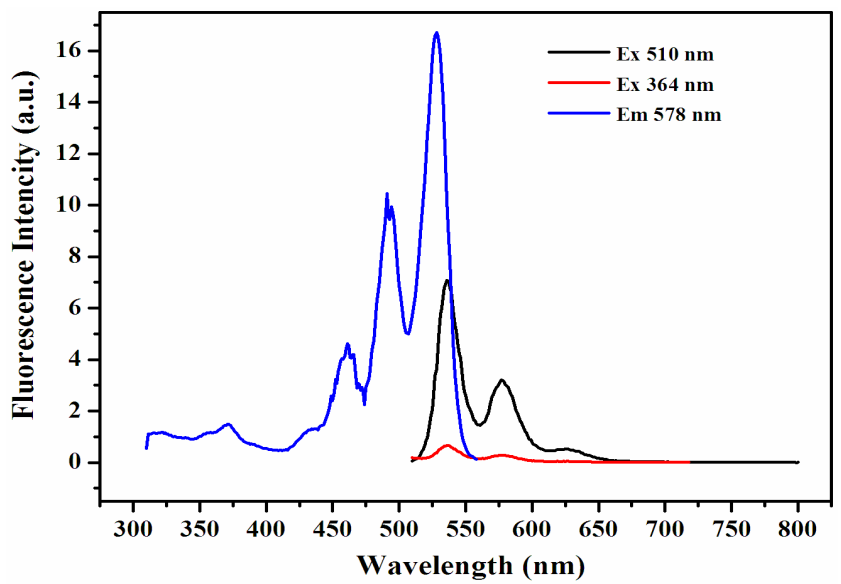


Fig. S4 Fluorescence excitation and emission spectra of PTCDI-Chol in chloroform at a concentration of 5.0×10^{-6} mol·L^{-1}. The detected wavelength was set at 578 nm, the excited wavelength was 364, 509, respectively.

9. Summary of the photophysical properties of CPPBI and reference compounds in chloroform

Table S1. Summary of the fluorescence quantum yields of CPPBI, CPEC, PTCDI-Chol in chloroform

Fluorophore	CPPBI	CPEC	PTCDI-Chol
Fluorescence quantum yield	18.4% (Φ_{pe}, λ_{ex} = 364 nm) 15.6% (Φ_{pe}, λ_{ex} = 505 nm)	28.8%	~1

Note: λ_{ex} represents for the excitation wavelength, Φpe the fluorescence quantum yield of perylene bisimide of CPPBI determined through using integrating sphere method. The fluorescence quantum yield of CPEC and PTCDI-Chol were obtained using quinine in 0.1 M sulfuric acid solution (0.55) [S2] and rhodamine B in ethanol (0.45) [S3] as quantum counter, repectively.

Table S2. Summary of the lifetimes of CPPBI in chloroform (λ_{ex}=375 nm, 1.0×10^{-6} mol/L)

Emission wavelength (λ/ nm)	436	536	624
Lifetime (ns)	τ = 1.6	τ_1 = 2.2 (62%) τ_2 = 6.9 (38%)	τ = 12.0
χ^2	1.36	1.50	1.28

Note: Chi-square (χ^2) is a parameter to quantify the fitting quality.

10. Fluorescence excitation and emission spectra of CPPBI in chloroform at a concentration of 1.0×10^{-8} mol/L

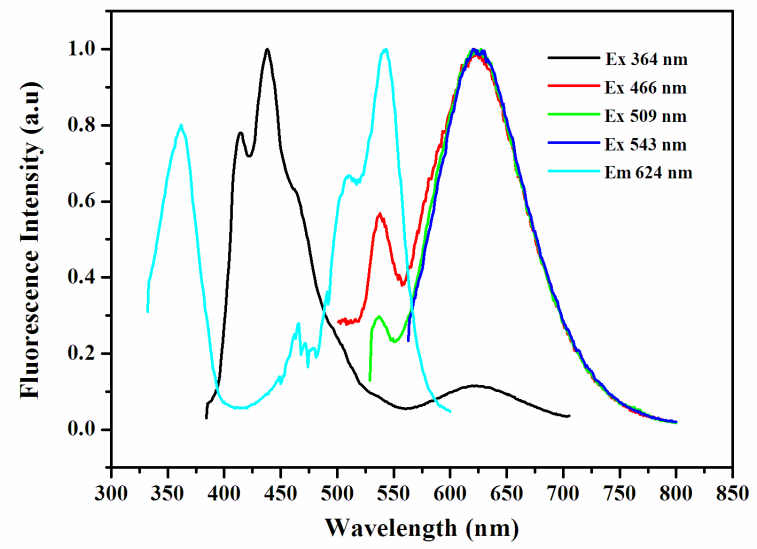


Fig. S5 Normalized fluorescence excitation and emission spectra of CPPBI in chloroform at a concentration of 1.0×10^{-8} mol/L. The detected wavelength was set at 624 nm, the excited wavelength was 364, 46, 509, and 543 nm, respectively.

11. Fluorescence excitation and emission spectra of CPPBI in chloroform at a concentration of 1.0×10^{-9} mol/L

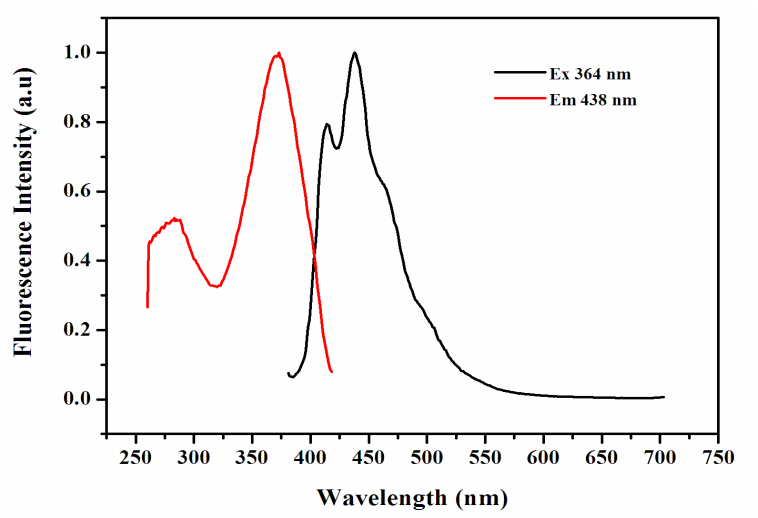


Fig. S6 Normalized fluorescence excitation and emission spectra of CPPBI in chloroform at a concentration of 1.0×10^{-9} mol/L.

12. Temperature-dependent fluorescence emission spectra of CPPBI in chloroform

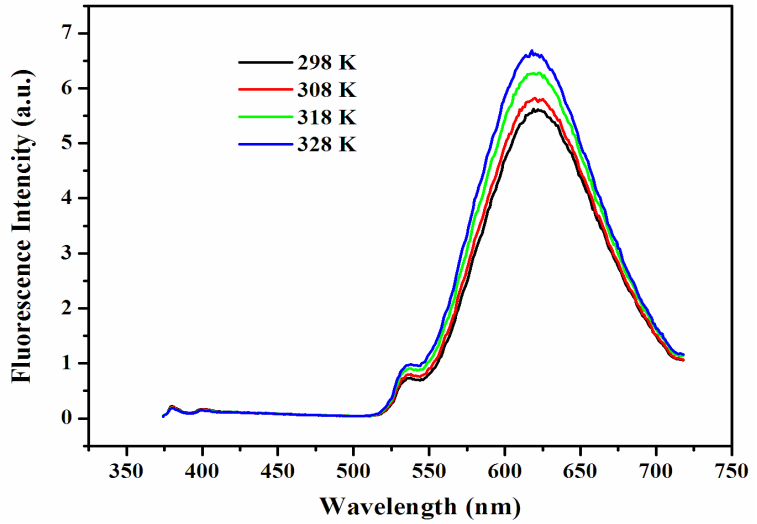


Fig. S7 Temperature-dependent fluorescence emission spectra of CPPBI in chloroform at a concentration of 5.0×10^{-6} mol/L from 298 K to 328 K excited at 364 nm.

13. Temperature-dependent fluorescence emission spectra of CPPBI in tetrachloroethane

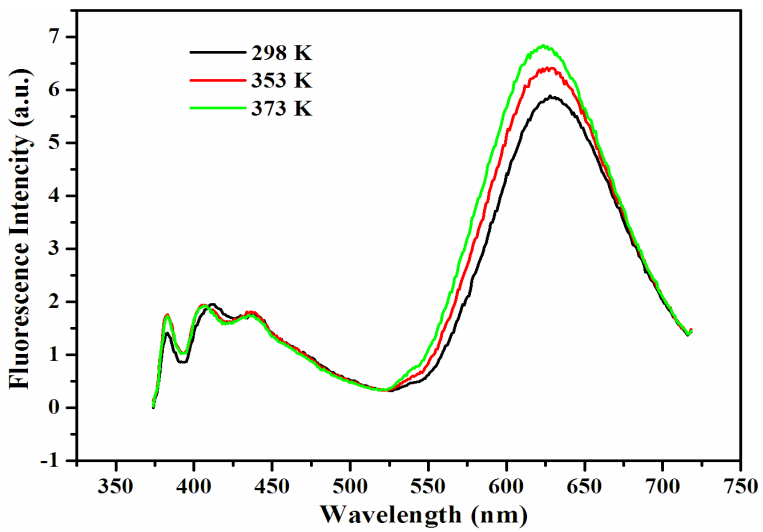


Fig. S8 Temperature-dependent fluorescence emission spectra of CPPBI in tetrachloroethane at a concentration of 5.0×10^{-6} mol/L from 298 K to 373 K excited at 364 nm.

14. Lifetime of CPEC in chloroform

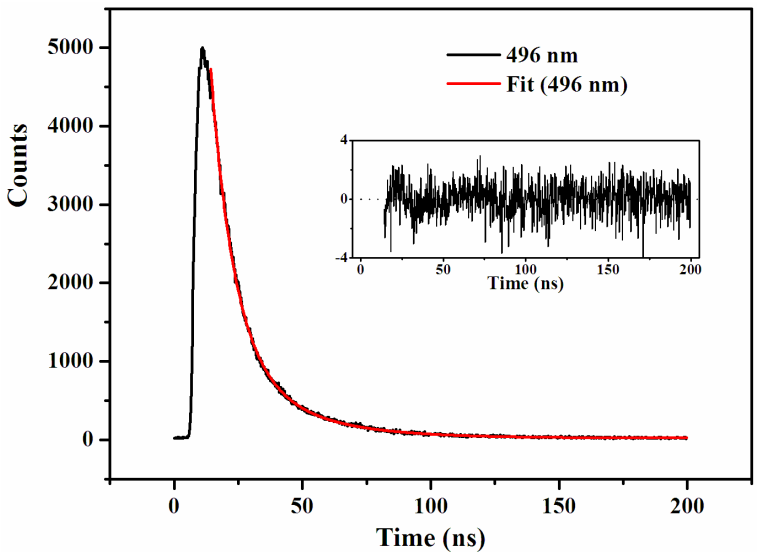


Fig. S9 Lifetime at 496 nm of CPEC in chloroform at a concentration of 1.0×10^{-3} mol/L using pico-second pulsed diode laser (EPLED-340) as an excitation source, τ_1 = 9.3 ns (52.5%), τ_2 = 27.6 ns (47.5%), τ_D = $\tau_1\times52.5\%+\tau_2\times47.5\%$ = 18.0 ns. Inset is the corresponding residual-distributions, the χ^2 value is 1.16.

15. Lifetime at different monitoring wavelengths of CPPBI in chloroform

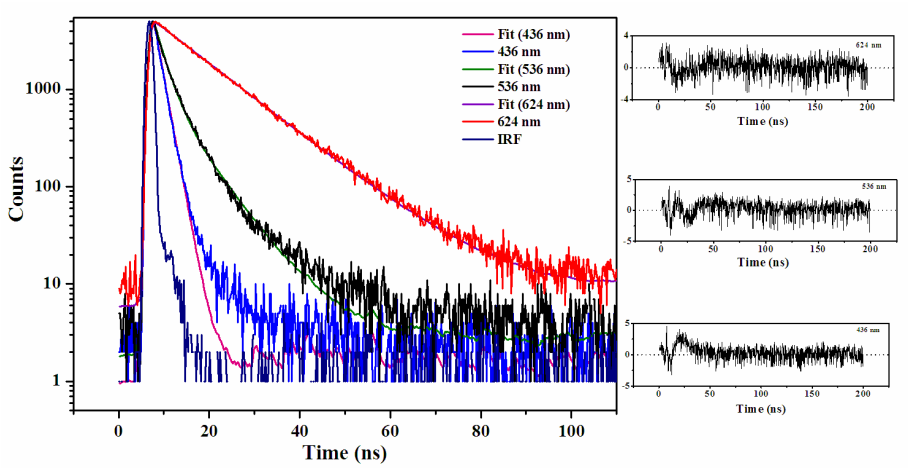


Fig. S10 Lifetime at different monitoring wavelengths of CPPBI in chloroform at a concentration of 1.0×10^{-6} mol/L using pico-second pulsed diode laser (EPL-375) as an excitation source. The left are the corresponding residual-distributions.

16. The three dimensional AFM image of the aggregated CPPBI from its chloroform solution

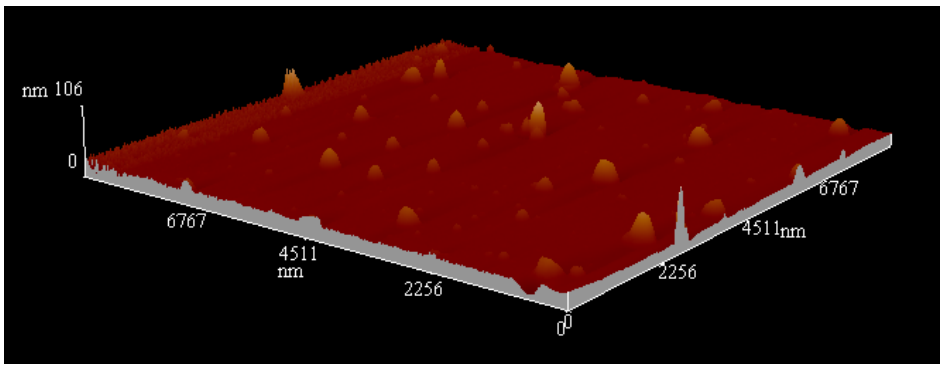


Fig. S11 The three dimensional AFM image of the aggregated CPPBI from its chloroform solution at a concentration of 1.0×10^{-6} mol/L.

17. ^{1}H-^{1}H chemical shift correlation spectroscopy (COSY) of CPPBI in $CDCl_3$

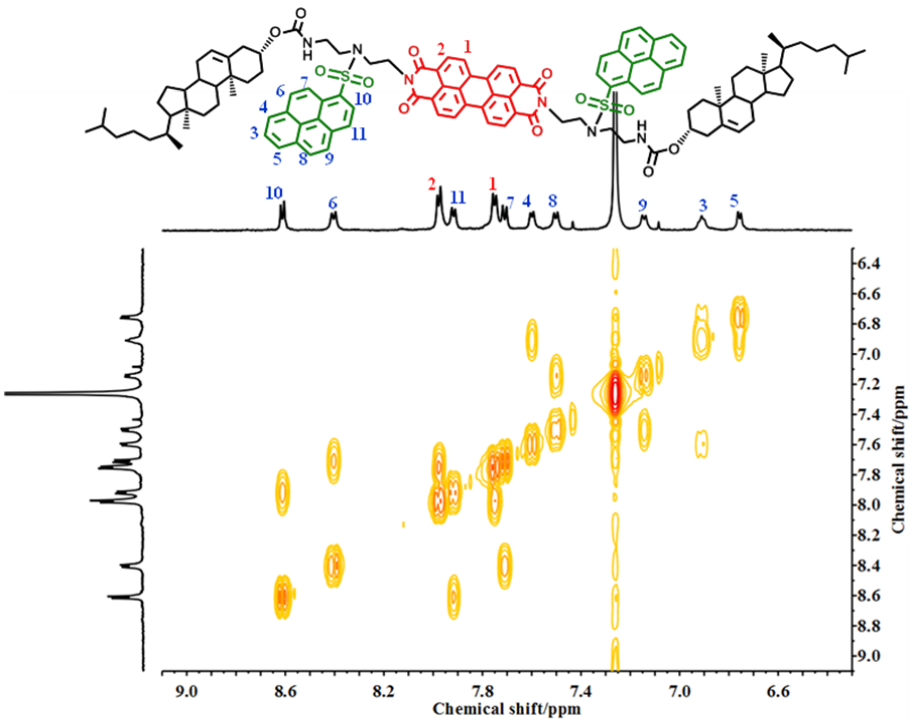


Fig. S12 ^{1}H-^{1}H chemical shift correlation spectroscopy (COSY) of CPPBI in $CDCl_3$.

18. Temperature-dependent ^{1}H NMR spectra of CPPBI in deuterated tetrachloroethane

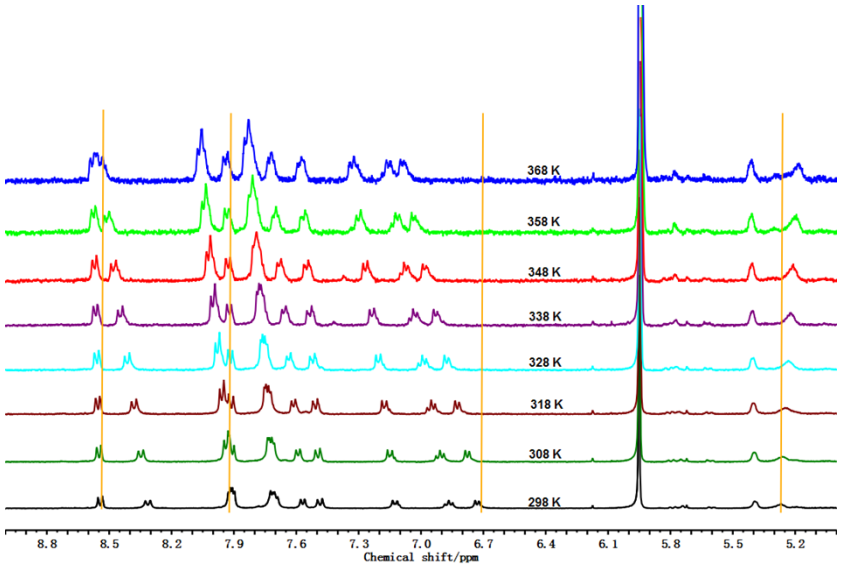


Fig. S13 Temperature-dependent ^{1}H NMR spectra of CPPBI in deuterated tetrachloroethane.

19. Time-resolved emission spectra of CPEC in chloroform

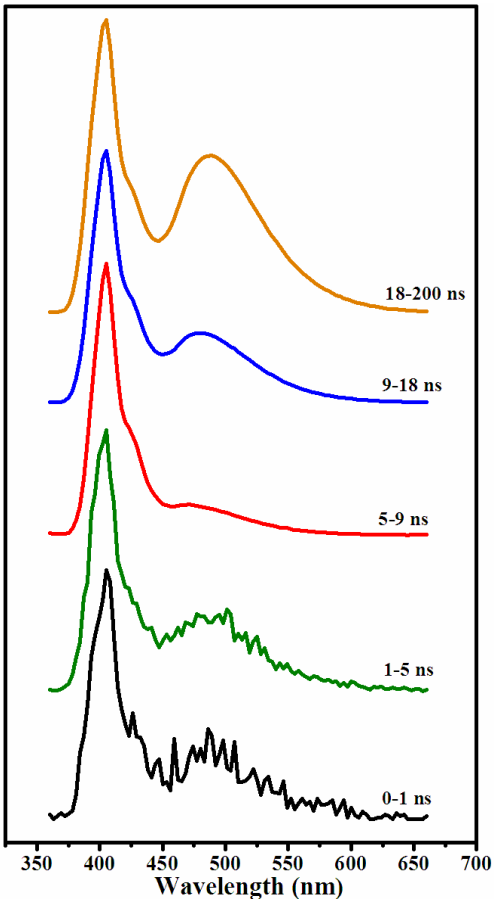


Fig. S14 Time-resolved emission spectra of CPEC in chloroform at a concentration of 1.0×10^{-3} mol·L^{-1} using pico-second pulsed diode laser (EPL-340) as an excitation source.

20. Fluorescence excitation and emission spectra of the sensing film

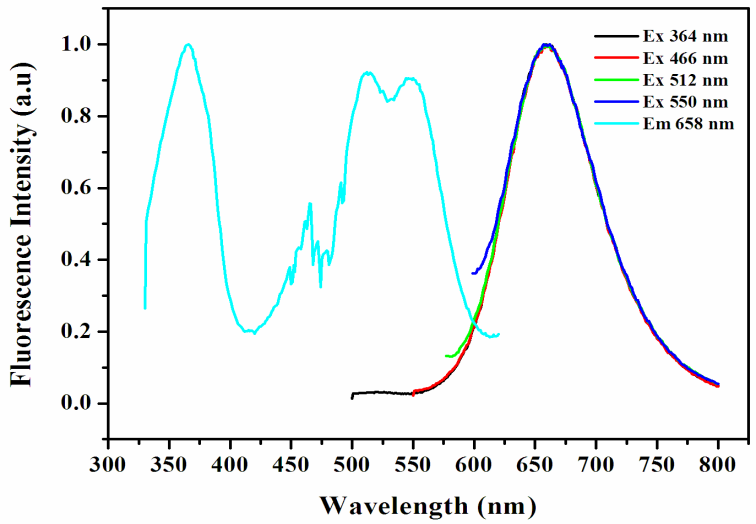


Fig. S15 Normalized fluorescence excitation and emission spectra of the sensing film prepared by dip-coating CPPBI on a glass plate surface.

21. Fluorescence emission intensities recorded of CPPBI in film state

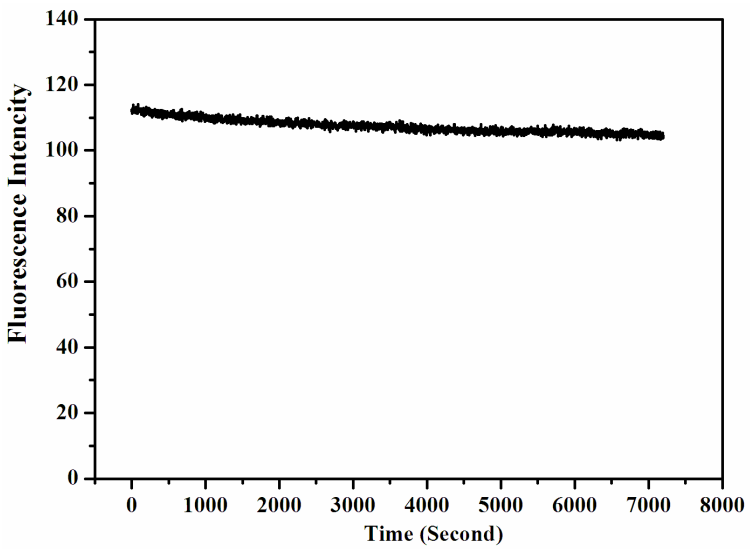


Fig. S16 Fluorescence emission intensities recorded of CPPBI in film state excited at 364 nm, the monitored wavelength was 658 nm.

22. Fluorescence response of the film to the saturated vapor of various reagents

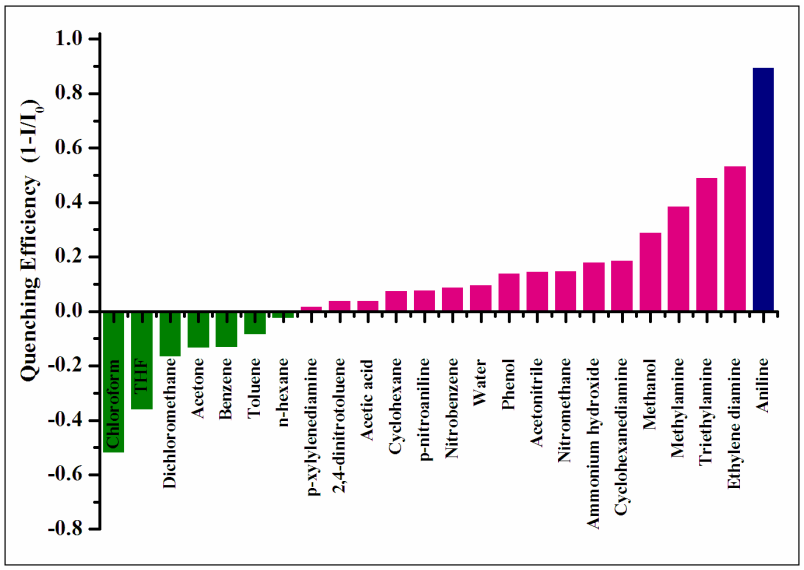


Fig. S17 Fluorescence response of the film to various reagents with injection of 5 mL saturated vapor of various reagents into the quartz cell (~4.5 mL).

23. Fluorescence emission spectra of the CPPBI functionalized film recorded at different vapor pressures of aniline

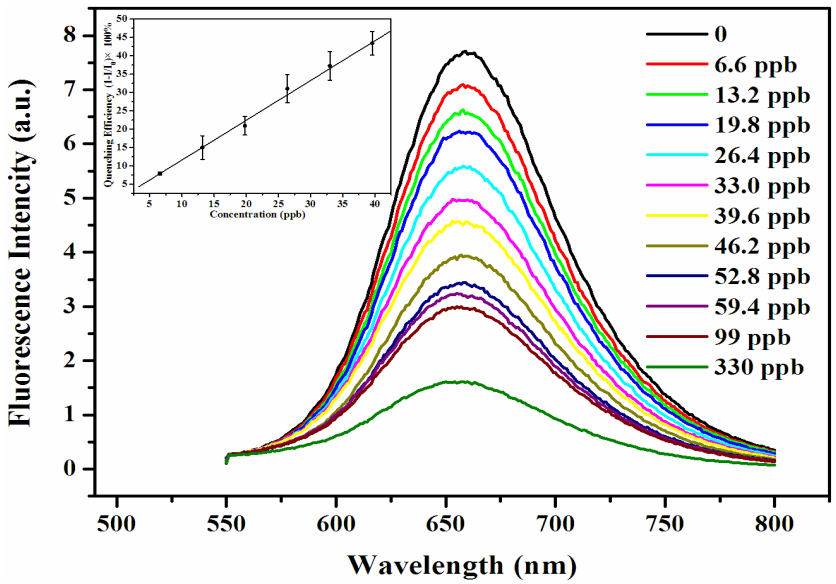


Fig. S18 Fluorescence emission spectra of the CPPBI functionalized film recorded at different vapor pressures of aniline (λ_{ex}= 364 nm). Inset is the plot of quenching efficiency against analyte concentration, of which the results utilized are from three parallel measurements.

24. Calculation of the Förster radius for CPPBI system

The Förster distance R_0 (distance at which the energy transfer efficiency is 50%) can be calculated according to the following equation:

$$R_0^6 = \frac{9000(\ln 10)\kappa^2 \Phi_D}{125\pi^5 N_{Avo} n^4} J(\lambda) = 8.79\times10^{-5}\kappa^2\Phi_D n^{-4} J(\lambda)$$

where Φ_D refers to the fluorescence quantum yield of the donor in the absence of the acceptor (CPEC, $\Phi_D = 0.288$), N_{Avo} is Avogadro's number, n is the refractive index of the medium, and the value for chloroform is 1.4476, κ^2 is the orientation factor, and generally assumed as 2/3, $J(\lambda)$ is the overlap integral describing the degree of overlap between the donor fluorescence emission spectrum (reference compound, CPEC at a concentration of 2.5×10^{-3} mol/L in chloroform) and the acceptor (CPPBI) absorption spectrum (Figure S3), calculated to be 9.35×10^{16} M^{-1} cm^{-1} nm^4.

25. ^{1}H NMR spectra of intermediates and target molecule

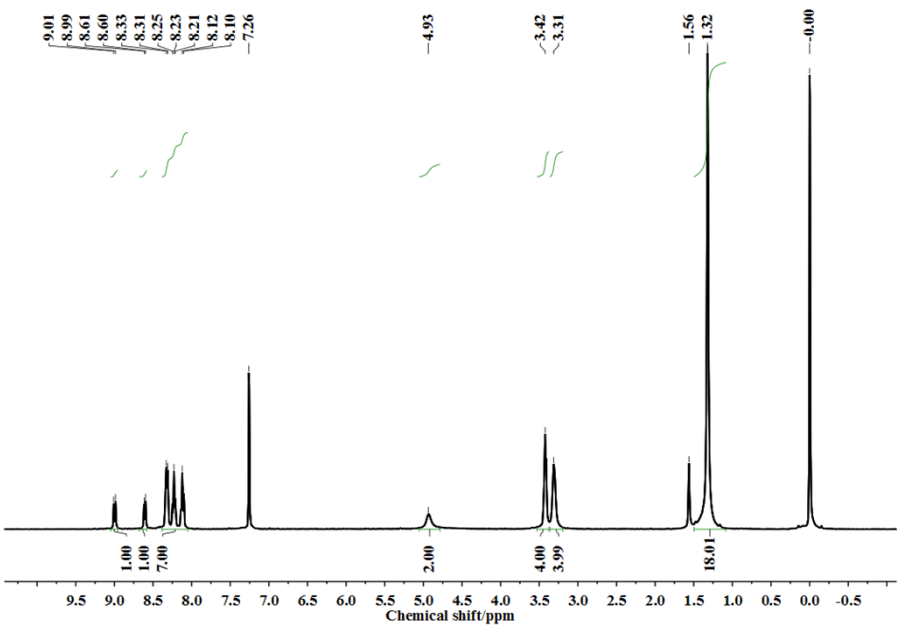


Fig. S19 ^{1}HNMR spectrum of BPSC in $CDCl_3$

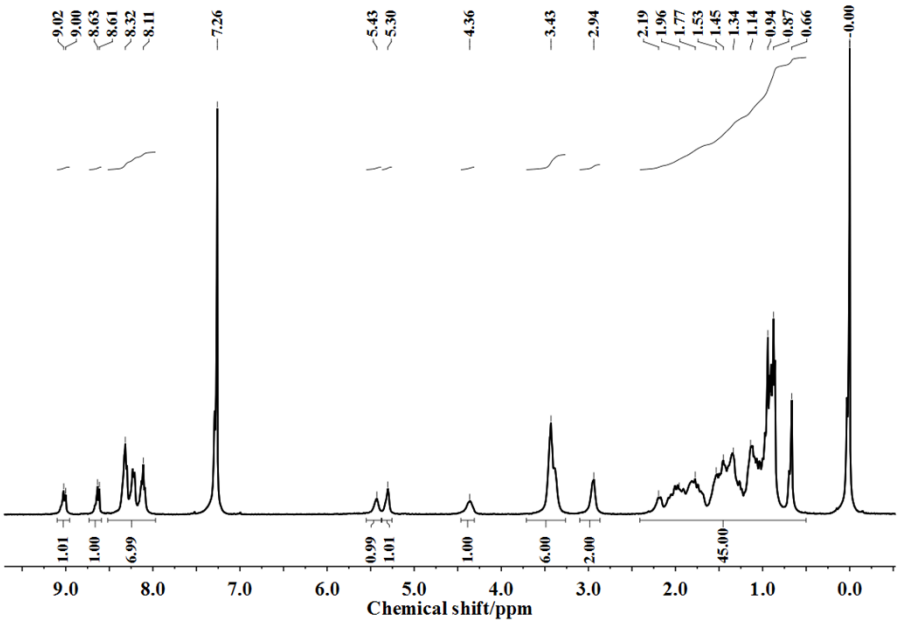


Fig. S20 ^{1}HNMR spectrum of CPEC in $CDCl_3$

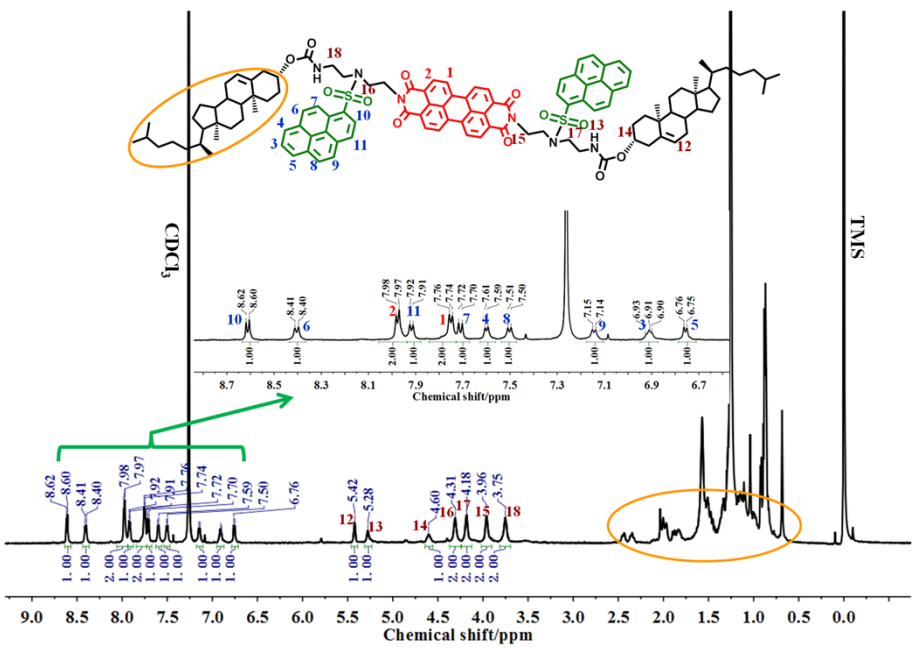


Fig. S21 ^{1}HNMR spectrum of CPPBI in $CDCl_3$

References

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S2 J. N. Dernas and G. A. Crosby, *J. Phys. Chem.*, 1971, **75**, 991-1024.

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