

Pyrene and triphenylamine substituted cyanostyrene and cyanostilbene derivatives with dual-state emission for high contrast mechanofluorochromism and cell imaging

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Materials and instruments

^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a JNM-ECZ400s (JEOL, Tokyo, Japan) spectrometer, using CDCl_3 as solvent. The UV-vis absorption spectra were recorded using a TU-1901 (Shimadzu, Kyoto, Japan) spectrometer with a 1 cm quartz cell. Fluorescence measurements were performed on a Hitachi FL-7000 spectrofluorimeter (Hitachi High Technologies Corporation, Tokyo, Japan). All spectroscopic studies were carried out at an ambient temperature and concentrations of the solutions are 1.0×10^{-5} M. Infrared spectra were taken on a Nicolet NEXUS 380 (Thermo Fisher Scientific, Waltham, America) spectrometer ($4000\text{-}400\text{ cm}^{-1}$, KBr pellets). The absolute fluorescence quantum yield was determined by an integrating sphere on HORIBA FluoroMax-4 spectrofluorometer. The mass spectra were obtained by LC-MS (ThermoFisher, America).

The absolute fluorescence quantum yields (Φ) and fluorescence lifetimes in the solid state were obtained using a HORIBA FluoroMax-4 spectrofluorometer (Paris, French) in the oxygen environment through the absolute method using an integrating sphere. All fluorescence emission and excitation spectra were corrected for the characteristics of the wavelength dependence of the detector and the light source characteristics. Absolute errors of $\leq 2\%$ are included. Powder X-ray diffraction (XRD) experiments were carried out on a Bruker SMART APEX-II Single-crystal diffractometer (Japan).

Theoretical Calculations

The molecular geometries were optimized using density functional theory (DFT)

calculations on the B3LYP/6-31G(d)^{S1-S4} level by the Gaussian 09 program,^{S5} in which the effects of solvent were considered using polarized continuum model (PCM)^{S6-S7} with tetrahydrofuran (THF) as solvent. Vertical excitation energies were computed using time-dependent DFT (TD-DFT) using the long-range corrected CAM-B3LYP functional^{S8} with 6-31G(d)^{S2-S4}.

Relative Quantum yield measurements

The relative fluorescence quantum yield was measured in diluted solutions using the following equation:

$$\Phi_x = \Phi_s \cdot (F_x/F_s) \cdot (A_s/A_x) \cdot (\eta_x/\eta_s)^2 \quad (1)$$

The reference system used was 9,10-diphenylanthracene ($\Phi = 0.95$ in cyclohexane). Φ represents the quantum yield, F stands for the integrated area under the corrected emission spectrum, A is the absorbance at the excitation wavelength, η is the refractive index of the solution, and the subscripts x and s refer to the unknown and the standard, respectively.

Lippert-Mataga equation

The relationship between the solvent polarity parameter (Δf) and the Stokes shift ($\Delta\nu$) according to the Lippert-Mataga equation was investigated.

$$\Delta f = \frac{\varepsilon - 1}{2\varepsilon + 1} - \frac{n^2 - 1}{2n^2 + 1} \quad (2)$$

$$\Delta\nu = \nu_e - \nu_g = \frac{2(\mu_e - \mu_g)^2}{4\pi\varepsilon_0 hca^3} \Delta f + C \quad (3)$$

Where Δf is the orientation polarizability of the solvent, ε and n are the dielectric constant and the refractive index of the solvent, $\Delta\nu$ is the Stokes shift between the

absorption and emission maximum, μ_e and μ_g are the dipolar moments in the excited and ground states, h is Planck's constant, c is the speed of light, and a is the radius of the molecule cavity, C is Planck's constant.

Mechanofluorochromism testing

The ground powders were obtained by grinding the as-prepared powder with a pestle in the mortar. The fumed samples were prepared by solvent exposure the grinding powder with dichloromethane (DCM) for 5 min. Fluorescence spectra of the pristine, ground, and recovered samples were recorded on a fluorescence spectrophotometer. Test strips were prepared by soaking Whatman filter paper in the solution of **PYTPA** or **PYPHTPA** in EtOH (1×10^{-3} M) and drying in air at room temperature for 30 min.

Cell imaging

HeLa cells were grown in confocal dishes at 37 °C for 24 h. For long-time living-cell imaging, **PYTPA** (10 μ M) or **PYPHTPA** (10 μ M) was incubated with cells for 30 min. Finally, after rinsing three times with PBS, the visualization of the cells was carried out by Leica TCS SP8X confocal laser scanning microscope (Leica, Germany) at 405 nm. The green fluorescence channel was 500-600 nm.

Synthetic experimental

Scheme S1 The synthetic route of PYTPA and PYPHTPA.

2-(4'-(diphenylamino)biphenyl-4-yl)acetonitrile (1)

2-(4-bromophenyl)acetonitrile (0.98 g, 5.0 mmol), (4-(diphenylamino)phenyl)boronic acid (1.74 g, 6.0 mmol), Pd-132 (0.5 mol%), K₂CO₃ (2.0 M, 10 mL), and THF (30 mL) was stirred at 90 °C for 8 h. After cooling, DCM (3 × 30mL) was added to the mixture. The organic portion was separated and washed with brine before drying over anhydrous Na₂SO₄. The solvent was evaporated, and the solid residues were purified by column chromatography using DCM/petroleum ether (1:1, v/v) as an eluent to afford 1.35 g of compound **1** (white powder) with a yield of 75.1%. IR (KBr pellet, cm⁻¹): 3026, 2888, 2244, 1957, 1914, 1589, 1490, 1330, 1277, 1208, 1178, 1157, 917, 849, 805, 766, 753, 731, 697. ¹H NMR (400 MHz, CDCl₃, δ): 7.57 (d, *J* = 8.3, 2H), 7.44 (d, *J* = 8.7, 2H), 7.36 (d, *J* = 8.32, 2H), 7.24~7.28 (m, 3H),

7.12 (d, $J = 8.1$, 6H), 7.03 (t, $J = 7.32$, 2H), 3.77 (s, 2H).

4-(pyren-1-yl)benzaldehyde (2)

A mixture of 4-formylphenylboronic acid (1.74 g, 6.0 mmol), 1-bromopyrene (0.90 g, 5.0 mmol), Pd-132 (0.5 mol%), and K_2CO_3 (2.0 M, 10 mL) in THF (30 mL) was stirred at 90 °C for 8 h. After cooling, DCM (3×30 mL) was added to the mixture. The organic portion was separated and washed with brine before drying over anhydrous Na_2SO_4 . The solvent was evaporated, and the solid residues were purified by column chromatography using DCM/petroleum ether (1:1, v/v) as an eluent to afford 1.11 g of compound **2** (white powder) with a yield of 72.3%. IR (KBr pellet, cm^{-1}): 2826, 2724, 1691, 1597, 1480, 1453, 1382, 1299, 1205, 1001, 854, 817, 772, 726, 688. 1H NMR (400 MHz, $CDCl_3$, δ): 10.15 (s, 1H), 8.18~8.25 (m, 3H), 8.01~8.14 (m, 7H), 7.98 (d, $J = 7.9$, 1H), 7.81 (d, $J = 8.1$, 2H).

(Z)-2-(4'-(diphenylamino)biphenyl-4-yl)-3-(pyren-1-yl)acrylonitrile (PYTPA)

A mixture of pyrene-1-carbaldehyde (230 mg, 1.0 mmol), 2-(4'-(diphenylamino)biphenyl-4-yl)acetonitrile (432 mg, 1.2 mmol) and potassium *t*-BuOK (224 mg, 2.0 mmol) in ethanol (15 mL) was stirred and refluxed overnight under nitrogen. After cooling to room temperature, the mixture was filtered and washed with ethanol. The solid residues were purified by column chromatography using DCM/petroleum ether (1.5:1, v/v) as an eluent to afford the pure coupled products **PYTPA** as a yellow-green solid (421 mg, 73.6%). IR (KBr pellet, cm^{-1}): 2213, 1589, 1492, 1400, 1383, 1325, 1291, 1276, 846, 818, 753, 715, 695 cm^{-1} . 1H NMR (600 MHz, $CDCl_3$, δ): 8.66 (d, $J = 8.0$, 1H), 8.61 (s, 1H), 8.03~8.29 (m, 8H), 7.88 (d, $J = 8.2$, 2H), 7.71 (d, $J = 8.3$, 2H), 7.54 (d, $J = 8.5$, 2H), 7.26~7.29 (m, 4H), 7.14~7.17 (m, 6H), 7.04~7.06 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$, δ): 147.94, 147.62, 141.78, 139.66, 133.57, 132.96, 132.89, 131.39, 130.81, 129.95, 129.47, 128.83, 128.15, 127.80, 127.55, 127.24, 126.70, 126.44, 126.33, 126.26, 126.10, 125.10, 124.84, 124.78, 124.67, 123.68, 123.34, 122.70, 118.32, 114.56. HR-MS (APCI-MS): $m/z = 573.2312$. calcd. for $[C_{43}H_{29}N_2]^+ = 573.2331$

(Z)-2-(4'-(diphenylamino)biphenyl-4-yl)-3-(4-(pyren-1-yl)phenyl)acrylonitrile (PYPHTPA)

A mixture of 4-(pyren-1-yl)benzaldehyde (306 mg, 1.0 mmol), 2-(4'-(diphenylamino)biphenyl-4-yl)acetonitrile (432 mg, 1.2 mmol) and potassium t-BuOK (224 mg, 2.0 mmol) in ethanol (15 mL) was stirred and refluxed overnight under nitrogen. After cooling to room temperature, the mixture was filtered and washed with ethanol. The solid residues were purified by column chromatography using DCM/petroleum ether (2:1, v/v) as an eluent to afford the pure coupled products PYTPA as a yellow-green solid (487 mg, 75.2%). IR (KBr pellet, cm^{-1}): 2214, 1591, 1492, 1404, 1385, 1324, 1274, 850, 848, 818, 753, 719, 692 cm^{-1} . ^1H NMR (600 MHz, CDCl_3 , δ): 8.23 (d, $J = 7.8$, 1H), 8.17~8.21 (m, 3H), 8.09~8.11 (m, 4H), 8.05 (d, $J = 9.24$, 1H), 7.99~8.03 (m, 2H), 7.78 (d, $J = 8.4$, 2H), 7.75 (d, $J = 8.2$, 2H), 7.68 (d, $J = 7.3$, 2H), 7.51 (d, $J = 8.6$, 2H), 7.26~7.28 (m, 4H), 7.13~7.15 (m, 6H), 7.04 (t, $J = 7.3$, 2H). ^{13}C NMR (100 MHz, CDCl_3 , δ): 147.91, 147.62, 143.67, 141.64, 141.14, 136.60, 133.57, 132.92, 132.87, 131.57, 131.30, 131.07, 131.05, 129.46, 128.53, 127.98, 127.86, 127.78, 127.51, 127.49, 127.20, 126.52, 126.25, 125.45, 125.16, 125.11, 125.00, 124.97, 124.85, 124.78, 123.67, 123.32, 118.29, 111.43. HR-MS (APCI-MS): $m/z = 647.4600$. calcd. for $[\text{C}_{49}\text{H}_{31}\text{N}_2] = 647.2487$.

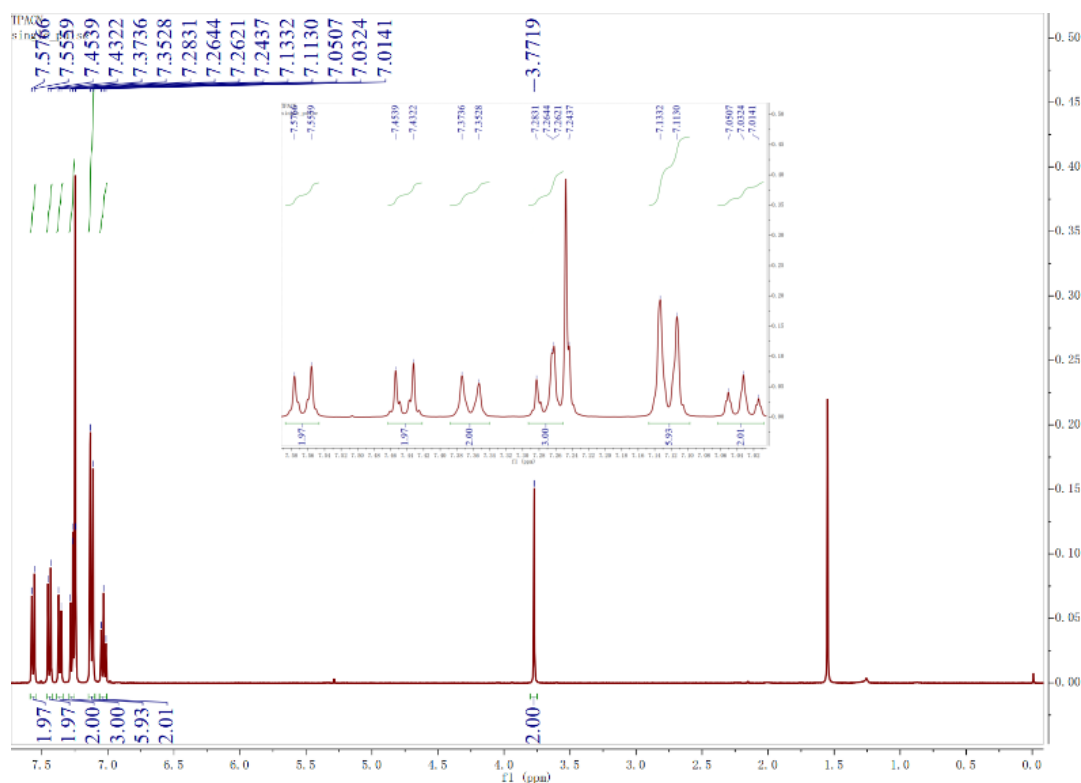


Fig S1. ^1H NMR spectrum of compound **1**.

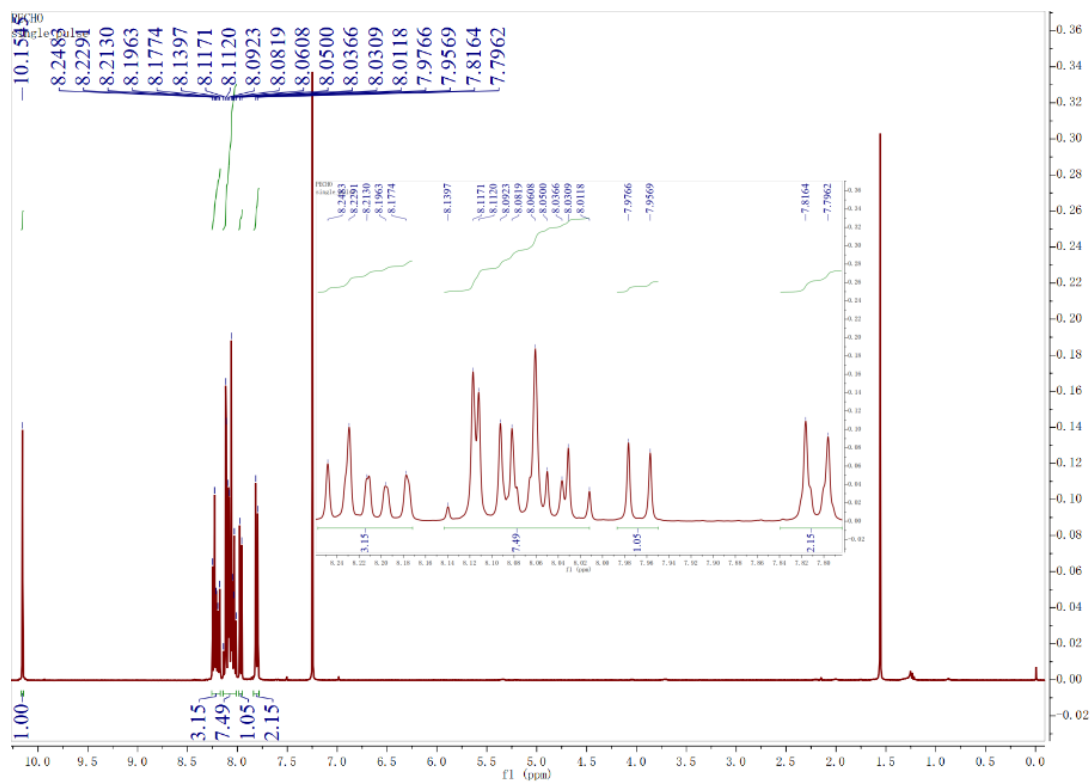


Fig S2. ^1H NMR spectrum of compound 2.

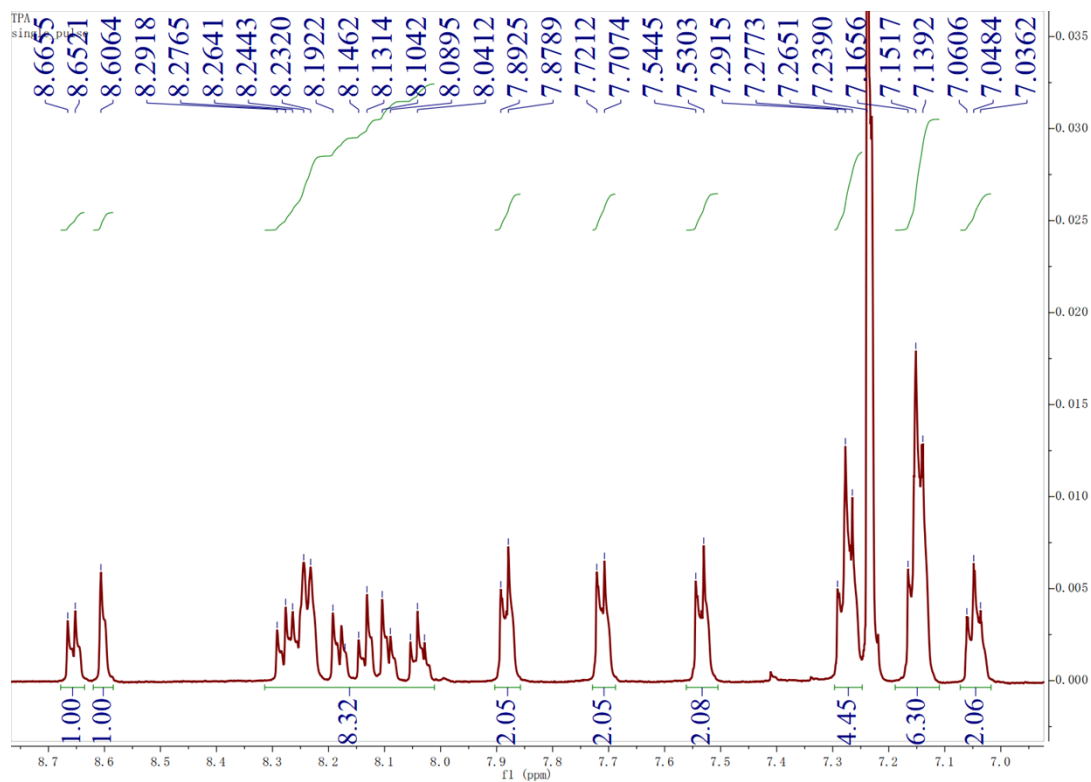


Fig. S3 ^1H NMR spectrum of PYTPA.

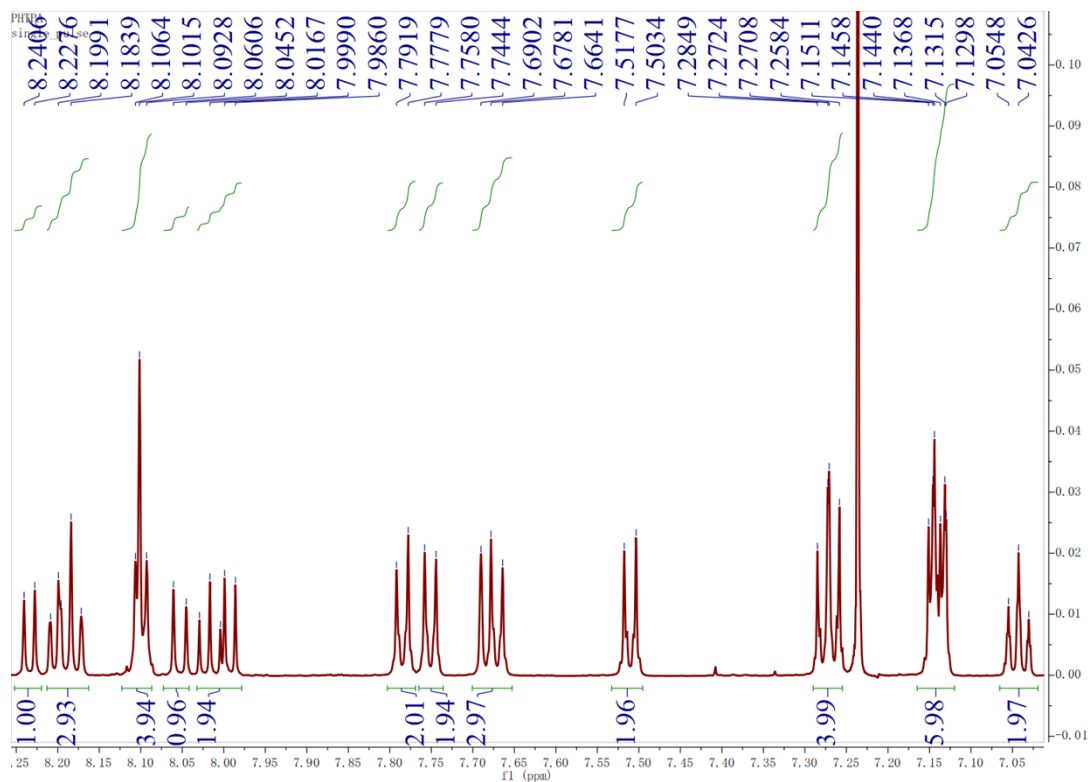


Fig. S4 ^1H NMR spectrum of PYPHTPA.

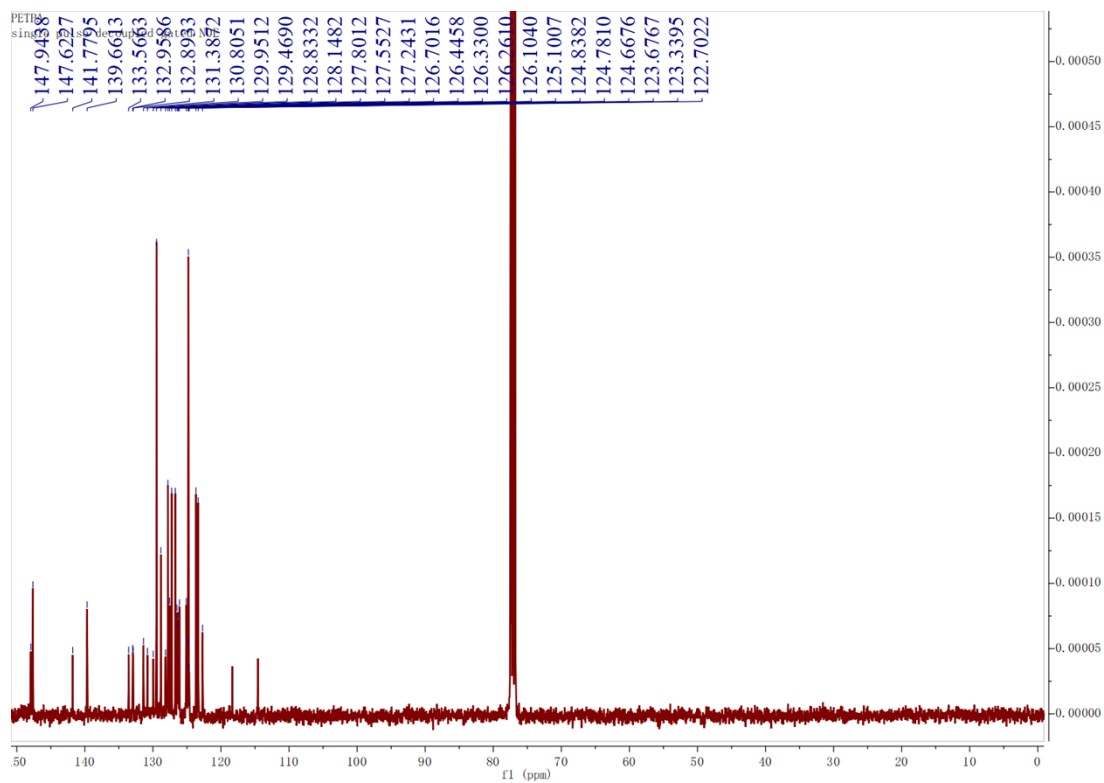


Fig. S5 ^{13}C NMR spectrum of PYTPA.

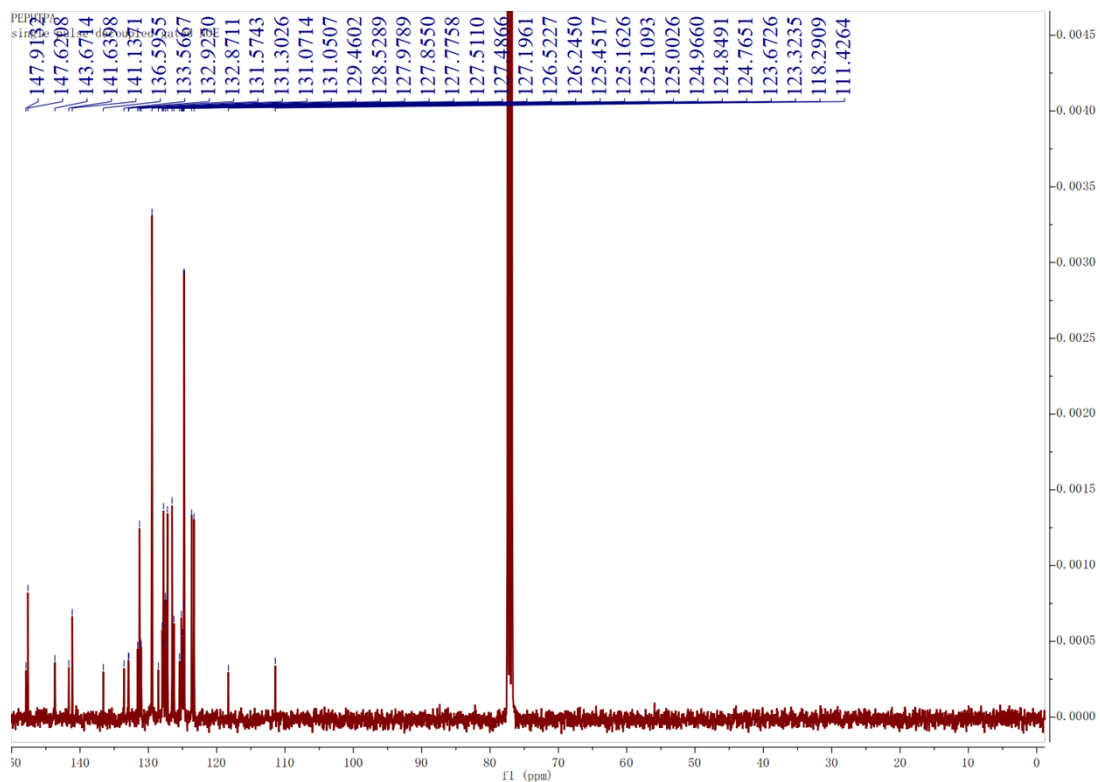


Fig. S6 ^{13}C NMR spectrum of PYPHTPA.

PETPA_211222161456 #11 RT: 0.10 AV: 1 SB: 1 0.08 NL: 3.27E6
T: FTMS + c APCI corona Full ms [80.00-1000.00]

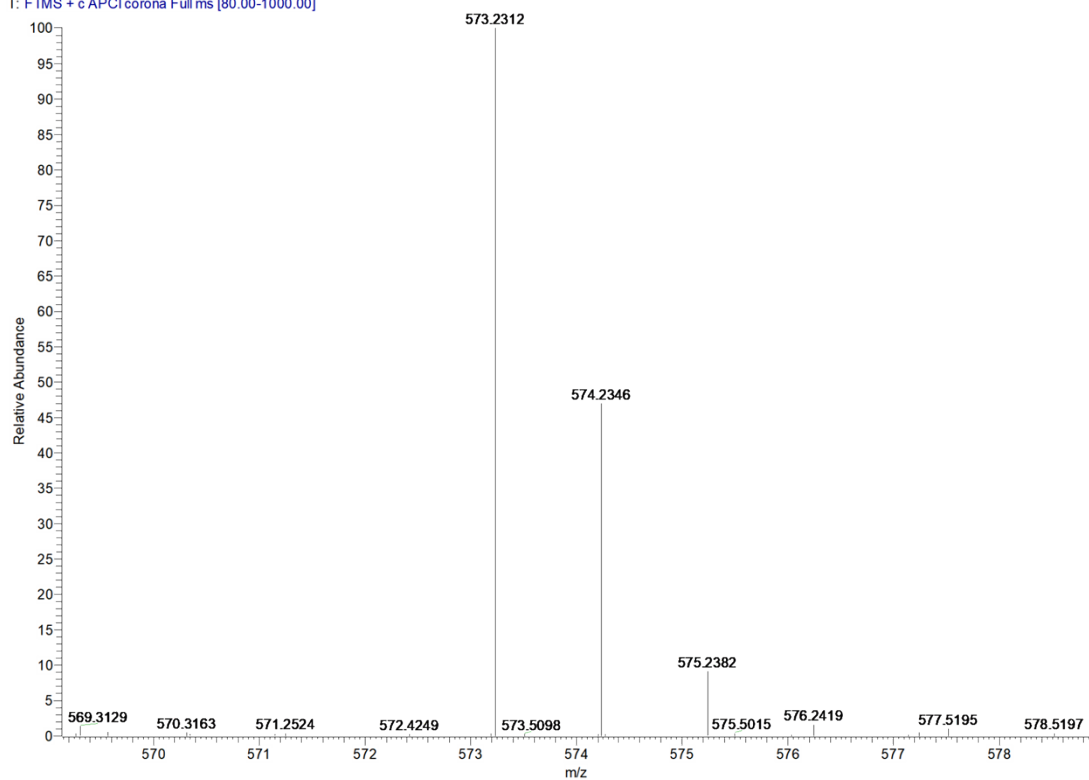


Fig. S7 Mass spectrum of PYTPA.

PEPHTPA_211223141236#19 RT: 0.17 AV: 1 SB: 1 0.04 NL: 2.14E6
 T: FTMS + c APCI corona Full ms [100.00-1000.00]

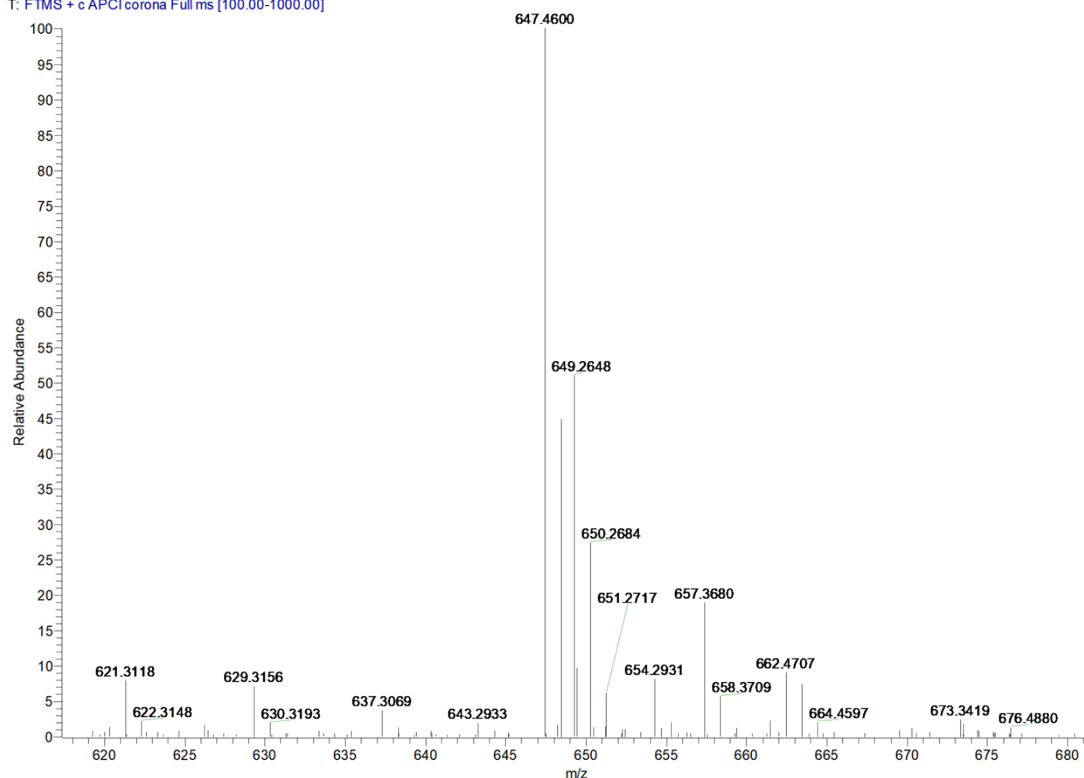
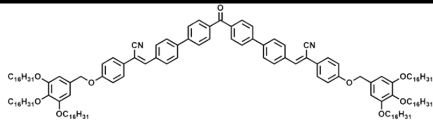
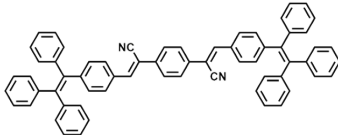
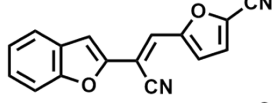
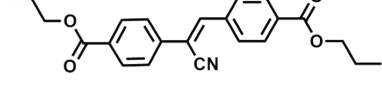
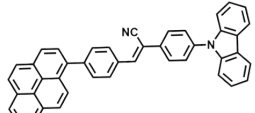
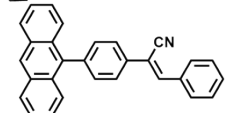
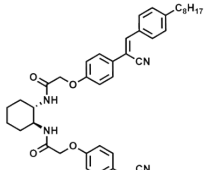
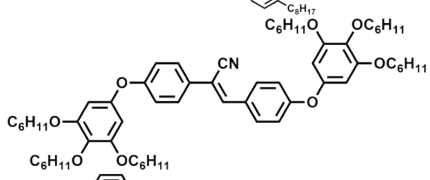
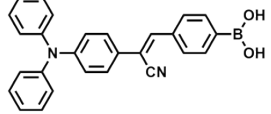
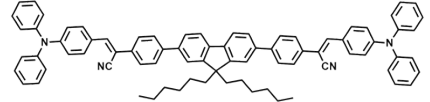


Fig. S8 Mass spectrum of PYPHTPA.

Table S1. A comparison of quantum yields (Φ) by this work and other reports.

Compound	Φ_{liquid}	$\Phi_{\text{solid/film/aggregate state}}$	Ref
	0.16(THF)	0.20($f_w=90\%$)	S9
	≤ 0.005 (THF)	0.35($f_w=90\%$)	S10
	0.38(CHCl_3)	0.02(film)	S11
	0.005(DMS O)	0.84(film)	S12
	0.30(THF)	0.36(film)	S13
	0.12(THF)	0.55(solid)	S14

	≤ 0.01 (THF)	0.48(film)	S15
	0.005(THF)	0.59(solid)	S16
	0.75(THF)	0.40(solid)	S17
	0.20(THF)	0.04(fw=99%)	S18
PYTPA	0.49(THF)	0.95(solid)	This work
PYPHTPA	0.77(THF)	0.51($f_w=90\%$)	
		0.97(solid)	This work
		0.79($f_w=90\%$)	

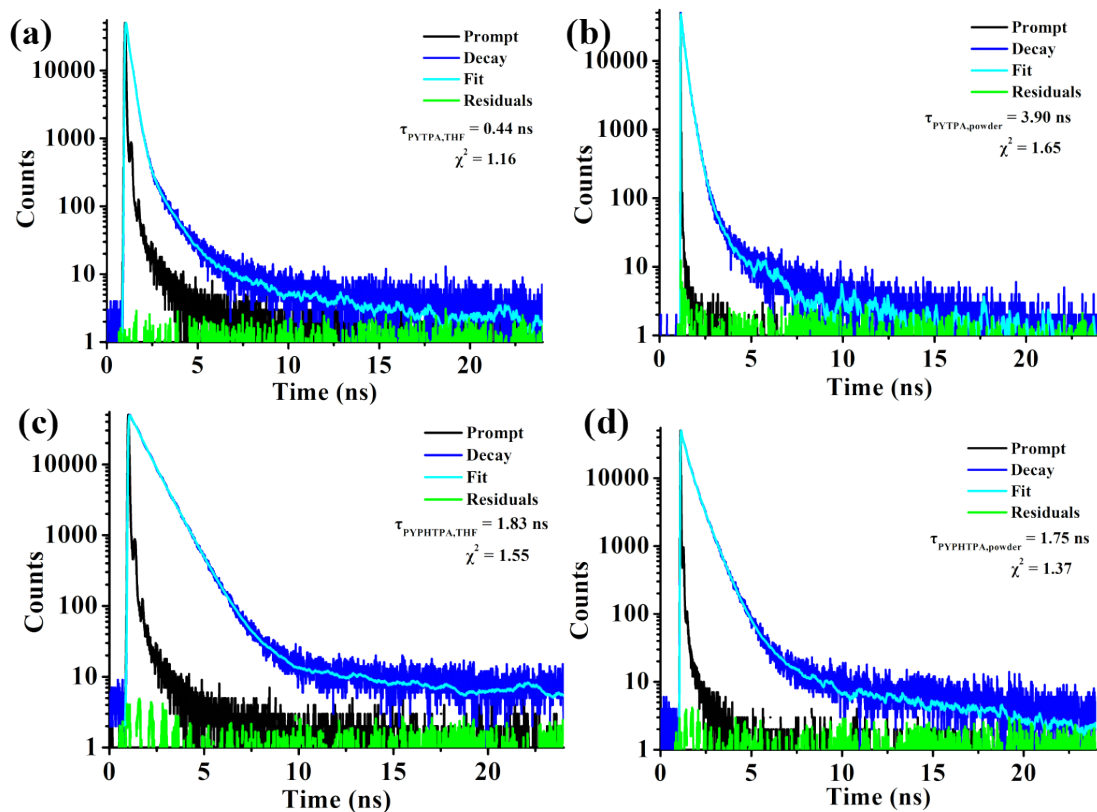


Fig S9. Time-resolved FL decay curves of (a and b) **PYTPA** and (c and d) **PYPHTPA** in THF solution and the powder state.

Table S2 Emission maxima (λ_{em}), quantum yields (Φ), and fluorescence lifetime (τ) of **PYTPA** and **PYPHTPA** samples in solution before and after grinding.

Compound	state	Φ	$\lambda_{max}^{[a]}$	$E^{[b]}$ (eV)	τ (ns)	$k_r(10^7 s^{-1})^{[c]}$	$k_{nr}(10^7 s^{-1})$
PYTPA	pristine	0.95	534	2.322	3.90	24.2	1.4
	ground	0.69	584	2.123	4.44	15.5	7.0
	solution (THF)	0.49	564	2.199	0.44	111.4	115.9
PYPHTPA	pristine	0.97	498	2.490	1.75	55.3	1.7
	ground	0.91	553	2.242	4.96	18.3	1.8
	solution (THF)	0.77	550	2.271	1.83	42.1	12.6

[a] The maximum emission wavelength. [b] $E = 1240/\lambda_{max}$ [c] k_r = radiative decay rate (Φ/τ). k_{nr} = nonradiative decay rate ($1/\tau - k_r$).

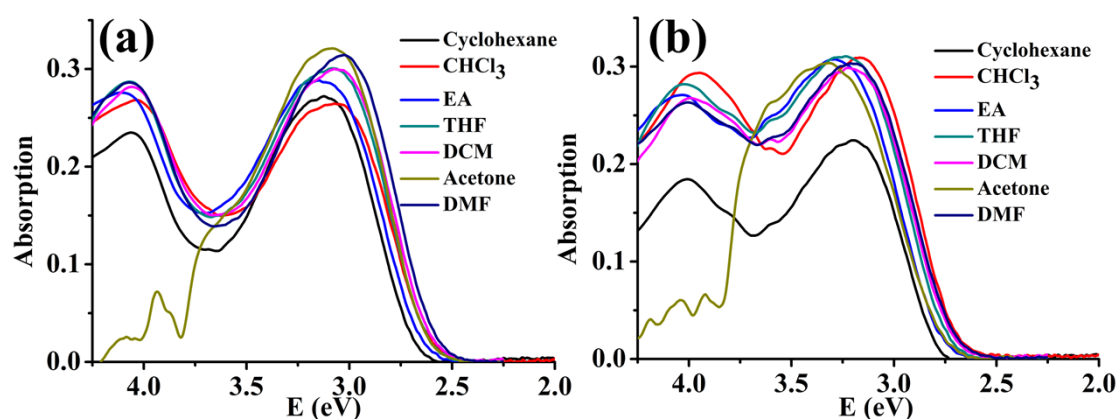


Fig S10. UV-vis spectra of **PYTPA** (a) and **PYPHTPA** (b) in various solvents ($1.0 \times 10^{-5}M$).

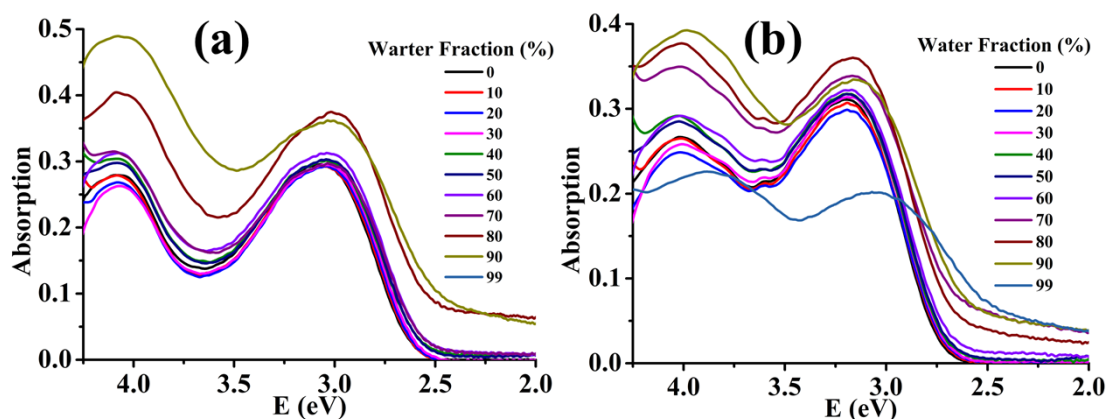


Fig S11. The emission spectra of (a) **PYTPA** and (b) **PYPHTPA** in THF/water mixtures with different fractions of water ($1.0 \times 10^{-5}M$).

Table S3. Photophysical data of **PYTPA** and **PYPHTPA** in different solvent.

Compound	Solvents	$\lambda^{[a]}_{\text{abs}}$	$E^{[b]}_{\text{abs}}$	$\lambda^{[c]}_{\text{em}}$	E_{em}	$\epsilon(10^4)^{[d]}$	$\Delta\lambda^{[e]}$	$\Delta\nu^{[f]}$	$\Phi^{[g]}$
d			(eV)		(eV)				
PYTPA	Cyclohexane	397	3.123	494	2.510	2.72	97	4946	0.85
	CHCl ₃	403	3.077	558	2.222	2.64	155	6893	0.33
	EA	395	3.139	560	2.214	2.89	165	7459	0.51
	THF	400	3.100	564	2.199	3.01	164	7270	0.49
	DCM	406	3.054	573	2.164	3.01	167	7179	0.29
	Acetone	402	3.085	600	2.067	3.21	198	8209	0.02
	+	DMF	410	3.024	610	2.033	3.15	200	7997
PYPHTPA A	Cyclohexane	386	3.212	485	2.557	2.25	99	5288	0.92
	CHCl ₃	390	3.179	540	2.296	3.09	150	7122	0.85
	EA	377	3.289	546	2.271	3.07	169	8210	0.78
	THF	383	3.238	550	2.255	3.11	167	7928	0.77
	DCM	385	3.221	559	2.218	2.99	174	8085	0.51
	Acetone	375	3.307	567	2.187	3.03	192	9030	0.05
		DMF	388	3.196	590	2.102	3.02	202	8824

[a] The maximum absorption wavelength. [b] $E = 1240/\lambda$. [c] The maximum emission wavelength. [d] extinction coefficient. [e] $\Delta\lambda = \lambda_{\text{em}} - \lambda_{\text{abs}}$. [f] $\Delta\nu = 1/\lambda_{\text{abs}} - 1/\lambda_{\text{em}}$, Stokes shifts (cm^{-1}). [g] fluorescence quantum yields.

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