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Supporting Information

Cationic tetraphenylethylene-based AIE-active acrylonitriles: Investigating the regioisomeric effect, mechanochromism, and wash-free bioimaging

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Materials and Methods

General Information

Unless otherwise stated, all chemicals were purchased from commercial suppliers and used directly without further purification. Deionized water was used in all experimental procedures. Reactants were purchased from Bide Pharmatech Ltd. Thin layer analysis was performed on silica gel plates, and silica gel column chromatography separation was performed using a SepaBean U200 machine from Changzhou Santai Technology Co. MTT was purchased from Genview. MitoTracker Deep Red (MTDR) was purchased from Beyotime Biotechnology. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were performed on a Bruker AVANCE III HD 400 MHz spectrometer. High-resolution mass spectra were tested on a Bruker Impact II mass spectrometer. UV-visible absorption and fluorescence spectra were recorded on a U-2900 spectrophotometer and an F-2700 FL spectrophotometer, respectively. Fluorescence quantum yields were determined on an Edinburgh FLS920/LP920 fluorescence spectrophotometer with a calibrated integrating sphere. Dynamic light scattering data were recorded with a Zetasizer Nano instrument (Malvern Instruments Ltd). X-ray diffractometer data were tested by a PANalytical X'Pert3 Powder diffractometer equipped with an Anton Paar XRK-900 chamber. The orbital structures of the molecules were calculated at the B3LYP/6-31G (d,p) level using the Gaussian 09 program package.

Mechanochromism

The mechanochromic behaviors of o-TPE-ANPy^{+}, m-TPE-ANPy^{+}, and p-TPE-ANPy^{+} were first pre-checked on filter paper rubbed with hands, then further verified by mechanical grinding with an agate mortar. Finally, the emission changes were recorded on a fluorescence spectrophotometer. The reversible mechanochromic behavior of o-TPE-ANPy^{+} was determined as follows: An appropriate amount of dichloromethane (about 60 mL) was added to a 100-mL open beaker. The beaker was heated at 50 ℃. The ground o-TPE-ANPy^{+} powder was placed flat on the filter paper, and the filter paper was covered over the open beaker for 10 min. The orange emission sample returned to its initial yellow emission color upon fuming by dichloromethane vapor. Then the fluorescence was recorded to confirm the recovery of the ground o-TPE-ANPy^{+} powder.

Cell Culture and Fluorescence Imaging

HeLa cells were cultured in H-DMEM culture flasks containing 10% fetal bovine serum and 1% penicillin and streptomycin at 37°C and 5% CO_2 in an incubator. Cells were inoculated and grown in confocal culture dishes for 24 h before incubation of dyes. HeLa cells were stained with 8 μM o-TPE-ANPy$^+$, m-TPE-ANPy$^+$, and p-TPE-ANPy$^+$, and incubated at 37°C and 5% CO_2 for 2 h. In co-localization experiments, the pre-stained HeLa cells with o-TPE-ANPy$^+$, m-TPE-ANPy$^+$, and p-TPE-ANPy$^+$ were further incubated with 100 nM MTDR for 15 min. The stained HeLa cells were directly imaged without washing by confocal laser scanning microscope (CLSM). Fluorescence imaging data were obtained using the Olympus FV1200 confocal fluorescence microscope. Imaging conditions: for o-TPE-ANPy$^+$ and m-TPE-ANPy$^+$, excitation wavelength: 405 nm, emission collection: 420-520 nm; for p-TPE-ANPy$^+$, excitation wavelength: 473 nm, emission collection: 540-640 nm; for MTDR, excitation wavelength: 635 nm, emission collection: 640-700 nm.

Cytotoxicity Assay

The cytotoxicity effect of the AIEgens (o-TPE-ANPy$^+$, m-TPE-ANPy$^+$, and p-TPE-ANPy$^+$) on HeLa cell was detected by standard MTT assay. HeLa cells were inoculated into 96-well plates and placed in a cell culture incubator for 24 h. After pouring out the culture medium, the AIEgens with different concentrations (2, 4, 6, 8 and 10 μM) diluted with the culture medium were added into the wells, and only the culture medium was added to the control group. After incubation for 24 hours, the medium was removed and 20 μL MTT and 100 μL medium were added to each well. After incubation for another 4 h, the culture medium in the wells was poured off and 100 μL DMSO was added. The absorbance for all the wells was measured at 570 nm. Finally, the cell viability was calculated using the following method: Cell viability = $(A_{sample} - A_{blank})/(A_{control} - A_{blank})$.

Synthetic Details

Synthesis of Compound 3a: Compound **1** (1.336 g, 4 mmol), compound **2a** (928 mg, 4 mmol), and $Pd(PPh_3)_4$ (230 mg, 0.2 mmol) were added to the toluene solution (24 mL) in a 250 mL Schlenk flask. Then 6 mL K_2CO_3 saturated solution (1.5 M) was added. The mixture was stirred to reflux for 24 h at 95 ℃ under nitrogen protection. After cooling to room temperature, the mixture was extracted

with CH_2Cl_2 and washed with water. The obtained organic layer was dried over anhydrous Na_2SO_4, then concentrated via rotary evaporation, and further purified by column chromatography using n-hexane/ethyl acetate (8:1, *v*/*v*) mixture as eluent to afford compound **3a** as a pale yellow solid (924 mg, 64% yield). ^{1}H NMR (400 MHz, DMSO-d_6), δ (ppm): 10.27 (s, 1H), 7.63 (dd, J_1 = 7.8 Hz, J_2 = 1.2 Hz, 1H), 7.57-7.51 (m, 1H), 7.35 (t, J = 7.5 Hz, 1H), 7.26 (dd, J_1 = 7.6 Hz, J_2 = 0.6 Hz, 1H), 7.22-7.03 (m, 11H), 7.03-6.98 (m, 2H), 6.92-6.86 (m, 2H). HRMS-ESI *m*/z: calcd for [$C_{27}H_{21}O^+$] 361.1587, found 361.1531 ([M+H]$^+$).

Synthesis of Compound 3b: Compound **1** (1.336 g, 4 mmol), compound **2b** (928 mg, 4 mmol), and $Pd(PPh_3)_4$ (230 mg, 0.2 mmol) were added to the toluene solution (24 mL) in a 250 mL Schlenk flask. Then 6 mL K_2CO_3 saturated solution (1.5 M) was added. The mixture was stirred to reflux for 24 h at 95 ℃ under nitrogen protection. After cooling to room temperature, the mixture was extracted with CH_2Cl_2 and washed with water. The obtained organic layer was dried over anhydrous Na_2SO_4, then concentrated via rotary evaporation, and further purified by column chromatography using n-hexane/ethyl acetate (8:1, *v*/*v*) mixture as eluent to afford compound **3b** as a pale yellow solid (1.032g, 72% yield). ^{1}H NMR (400 MHz, DMSO-d_6), δ (ppm): 9.82 (s, 1H), 7.66 (dt, J_1 = 7.5 Hz, J_2 = 1.2 Hz, 1H), 7.49 (s, 1H), 7.37 (t, J = 7.6 Hz, 1H), 7.33-7.28 (m, 1H), 7.20-7.08 (m, 9H), 7.05-6.94 (m, 6H). HRMS-ESI m/z: calcd for [$C_{27}H_{21}O^+$] 361.1587, found 361.1584 ([M+H]$^+$).

Synthesis of Compound 3c: Compound **1** (1.336 g, 4 mmol), compound **2c** (928 mg, 4 mmol), and $Pd(PPh_3)_4$ (230 mg, 0.2 mmol) were added to the toluene solution (24 mL) in a 250 mL Schlenk flask. Then 6 mL K_2CO_3 saturated solution (1.5 M) was added. The mixture was stirred to reflux for 24 h at 95 ℃ under nitrogen protection. After cooling to room temperature, the mixture was extracted with CH_2Cl_2 and washed with water. The obtained organic layer was dried over anhydrous Na_2SO_4, then concentrated via rotary evaporation, and further purified by column chromatography using n-hexane/ethyl acetate (8:1, *v*/*v*) mixture as eluent to afford compound **3c** as a pale yellow solid (994 mg, 69% yield). ^{1}H NMR (400 MHz, DMSO-d_6), δ (ppm): 9.89 (s, 1H), 7.68 (dd, J_1 = 8.1 Hz, J_2 = 1.6 Hz, 2H), 7.23-7.08 (m, 11H), 7.04-6.93 (m, 6H). HRMS-ESI m/z: calcd for [$C_{27}H_{21}O^+$] 361.1587, found 361.1538 ([M+H]$^+$).

Synthesis of Compound 5a: Compound **4** (354 mg, 3 mmol) and t-BuOK (420 mg, 3.75 mmol) were successively added to anhydrous EtOH (25 mL) in a round-bottom flask and stirred at room temperature for 10 min. Compound **3a** (865 mg, 2.40 mmol) was then added to the solution and stirred at room temperature for 4 h. The solvent is then removed by rotary evaporation, and purified by column chromatography using CH_2Cl_2/MeOH (10:1, *v*/*v*) mixture as eluent to afford compound **5a** as a bright yellow solid (448 mg, 41%). ^{1}H NMR (400 MHz, DMSO-d_6), δ (ppm): 8.64 (dd, J_1 = 4.7 Hz, J_2 = 1.4 Hz, 2H), 8.29 (s, 1H), 7.7-7.67 (m, 1H), 7.55 (dd, J_1 = 4.75 Hz, J_2 = 1.6 Hz, 2H), 7.43-7.33 (m, 2H), 7.24-7.19 (m, 1H), 7.18-7.12 (m, 3H), 7.12-7.03 (m, 6H), 7.03-6.96 (m, 4H), 6.72 (d, J = 7.1 Hz, 2H). HRMS-ESI m/z: calcd for [$C_{34}H_{25}N_2^+$] 461.2012, found 461.2021 ([M+H]$^+$).

Synthesis of Compound 5b: Compound **4** (354 mg, 3 mmol) and t-BuOK (420 mg, 3.75 mmol) were successively added to anhydrous EtOH (25 mL) in a round-bottom flask and stirred at room temperature for 10 min. Compound **3b** (850 mg, 2.36 mmol) was then added to the solution and stirred at room temperature for 10 min. The solvent is then removed by rotary evaporation, and purified by column chromatography using CH_2Cl_2/MeOH (10:1, *v*/*v*) mixture as eluent to afford compound **5b** as a yellow solid (413 mg, 38% yield). ^{1}H NMR (400 MHz, DMSO-d_6), δ (ppm): 8.64 (dd, J_1 = 4.6 Hz, J_2 = 1.6 Hz, 2H), 8.15 (s, 1H), 7.80 (d, J = 7.9 Hz, 1H), 7.70-7.62 (m, 3H), 7.29 (t, J = 7.8 Hz, 1H), 7.19-7.06 (m, 10H), 7.04-6.96 (m, 6H). HRMS-ESI m/z: calcd for [$C_{34}H_{25}N_2^+$] 461. 2012, found 461.2015 ([M+H]$^+$).

Synthesis of Compound 5c: Compound **4** (354 mg, 3 mmol) and t-BuOK (420 mg, 3.75 mmol) were successively added to anhydrous EtOH (25 mL) in a round-bottom flask and stirred at room temperature for 10 min. Compound **3c** (856 mg, 2.38 mmol) was then added to the solution and stirred at room temperature for 10 min. The solvent is then removed by rotary evaporation, and purified by column chromatography using CH_2Cl_2/MeOH (10:1, *v*/*v*) mixture as eluent to afford compound **5c** as an orange solid (526 mg, 48% yield). ^{1}H NMR (400 MHz, DMSO-d_6), δ (ppm): 8.68 (dd, J_1 = 4.6 Hz, J_2 = 1.6 Hz, 2H), 8.21 (s, 1H), 7.78 (d, J = 8.4 Hz, 2H), 7.70 (dd, J_1 = 4.6 Hz, J_2 = 1.6 Hz, 2H), 7.22-7.09 (m, 11H), 7.05-6.96 (m, 6H). HRMS-ESI *m*/z: calcd for [$C_{34}H_{25}N_2^+$] 461. 2012, found 461.2074 ([M+H]$^+$).

Synthesis of Compound o-TPE-ANPy$^+$: The mixture of **5a** (455 mg, 0.99 mmol) and CH_3I (320 mg, 2.25 mmol) was reflux at 90 °C in CH_3CN (15 mL) for 12 h. After cooling to room temperature,, Et_2O (35 mL) was added to the solution. The mixture was filtered to remove the solvent and the resulting solid was dried under vacuum. The resulting solid was used directly in the next step without further purification. The solid was then dissolved in acetone (20 mL) and the saturated solution of KPF_6 (4 mL) was further added. The mixture was stirred at room temperature for 24 h. The solvent was then removed by rotary evaporation, and purified by column chromatography using CH_2Cl_2/MeOH (10:1, *v*/*v*) mixture as eluent to afford o-TPE-ANPy$^+$ as a bright yellow solid (315 mg, 51% yield).^{1}H NMR (400 MHz, DMSO-d_6), δ (ppm): 8.96 (d, J = 7.0 Hz, 2H), 8.69 (s, 1H), 8.26 (d, J = 7.0 Hz, 2H), 7.76 (d, J = 7.4 Hz, 1H), 7.51-7.40 (m, 2H), 7.25-7.21 (dd, J_1 = 7.6 Hz, J_2 = 1.0 Hz, 1H), 7.17-7.03 (m, 9H), 7.03-6.95 (m, 4H), 6.68-6.61 (m, 2H), 4.30 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6), δ (ppm): 152.10, 148.47, 145.97, 145.47, 144.75, 143.30, 142.84, 142.28, 137.59, 132.38, 132.33, 131.60, 130.94, 130.91, 130.47, 128.28, 127.89, 127.83, 127.65, 127.61, 127.38, 127.20, 126.85, 122.73, 114.97, 108.24, 47.43. HRMS-ESI *m*/z: calcd for [$C_{35}H_{27}N_2^+$] 475.2169, found 475.2175 ([M]$^+$).

Synthesis of Compound m-TPE-ANPy$^+$: The mixture of **5b** (427 mg, 0.93 mmol) and CH_3I (315 mg, 2.22 mmol) was reflux at 90 °C in CH_3CN (15 mL) for 12 h. After cooling to room temperature,, Et_2O (35 mL) was added to the solution. The mixture was filtered to remove the solvent and the resulting solid was dried under vacuum. The resulting solid was used directly in the next step without further purification. The solid was then dissolved in acetone (20 mL) and the saturated solution of KPF_6 (4 mL) was further added. The mixture was stirred at room temperature for 24 h. The solvent was then removed by rotary evaporation, and purified by column chromatography using CH_2Cl_2/MeOH (10:1, *v*/*v*) mixture as eluent to afford m-TPE-ANPy$^+$ as a yellow solid (186 mg, 32% yield). ^{1}H NMR (400 MHz, DMSO-d_6), δ (ppm): 9.01 (d, J = 7.0 Hz, 2H), 8.56 (s, 1H), 8.36 (d, J = 7.0 Hz, 2H), 7.92 (d, J = 8.0 Hz, 1H), 7.74 (s, 1H), 7.41 (t, J = 7.8 Hz, 1H), 7.29 (d, J = 7.8 Hz, 1H), 7.20-7.09 (m, 9H), 7.05-6.97 (m, 6H), 4.32 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6), δ (ppm): 151.27, 148.77, 145.80, 144.47, 142.68, 142.58, 142.36, 141.88, 139.31, 135.41, 133.03, 132.24,

130.70, 130.57, 130.54, 129.00, 128.18, 128.09, 128.00, 127.94, 126.89, 126.86, 123.23, 116.03, 105.60, 47.36. HRMS-ESI *m*/z: calcd for [$C_{35}H_{27}N_2^+$] 475.2169, found 475.2180 ([M]$^+$).

Synthesis of Compound p-TPE-ANPy$^+$: The mixture of **5c** (442 mg, 0.96 mmol) and CH_3I (338 mg, 2.38 mmol) was reflux at 90 °C in CH_3CN (15 mL) for 12 h. After cooling to room temperature,, Et_2O (35 mL) was added to the solution. The mixture was filtered to remove the solvent and the resulting solid was dried under vacuum. The resulting solid was used directly in the next step without further purification. The solid was then dissolved in acetone (20 mL) and the saturated solution of KPF_6 (4 mL) was further added. The mixture was stirred at room temperature for 24 h. The solvent was then removed by rotary evaporation, and purified by column chromatography using CH_2Cl_2/MeOH (10:1, *v*/*v*) mixture as eluent to afford p-TPE-ANPy$^+$ as an orange red solid (173 mg, 29% yield). ^{1}H NMR (400 MHz, DMSO-d_6), δ (ppm): 9.04 (d, J = 7.0 Hz, 2H), 8.63 (s, 1H), 8.39 (d, J = 7.0 Hz, 2H), 7.89 (d, J = 8.4 Hz, 2H), 7.28-7.10 (m, 11H), 7.07-6.96 (m, 6H), 4.33 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6), δ (ppm): 150.96, 148.98, 148.53, 145.81, 142.66, 142.62, 142.40, 139.53, 131.67, 130.72, 130.58, 130.46, 130.23, 128.08, 127.89, 127.10, 126.96, 123.06, 104.74, 47.33. HRMS-ESI *m*/z: calcd for [$C_{35}H_{27}N_2^+$] 475.2169, found 475.2167 ([M]$^+$).

NMR Spectra

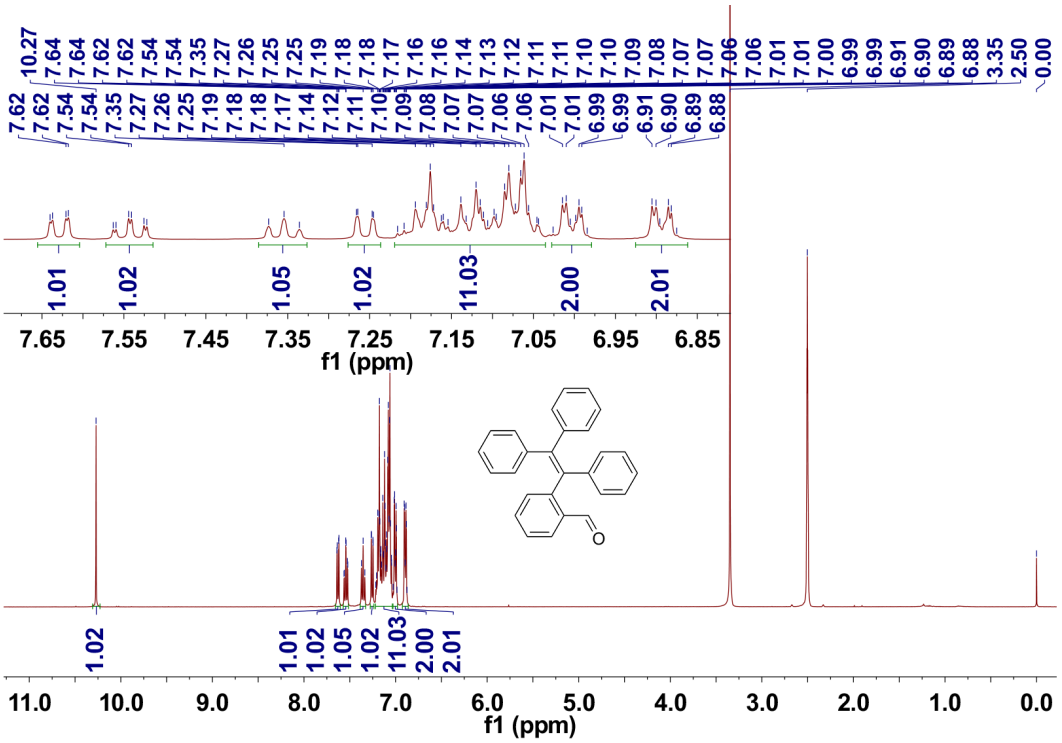

Fig. S1 ^{1}H NMR spectrum of compound **3a** in DMSO-d_6.

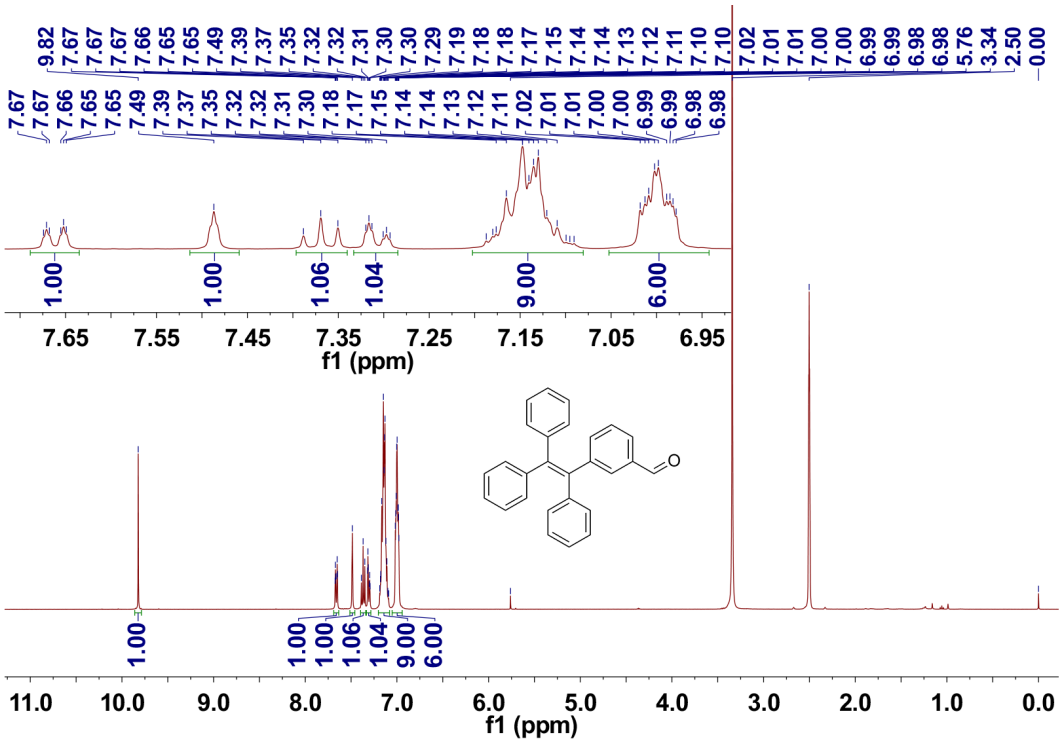

Fig. S2 ^{1}H NMR spectrum of compound **3b** in DMSO-d_6.

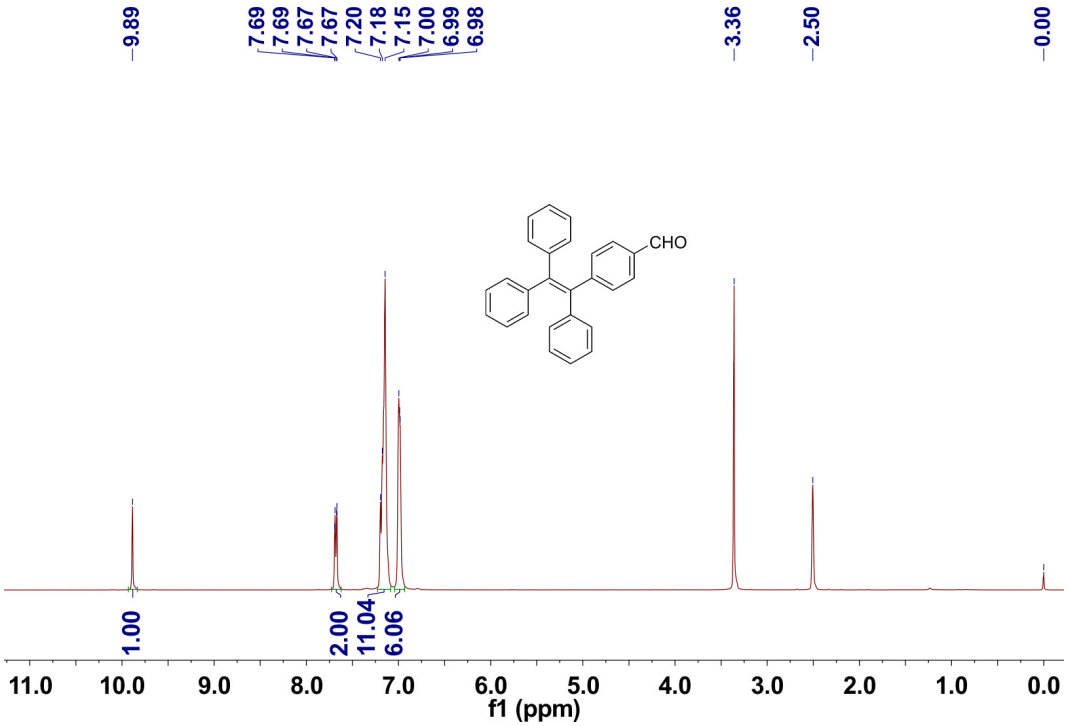

Fig. S3 ^{1}H NMR spectrum of compound **3c** in DMSO-d_6.

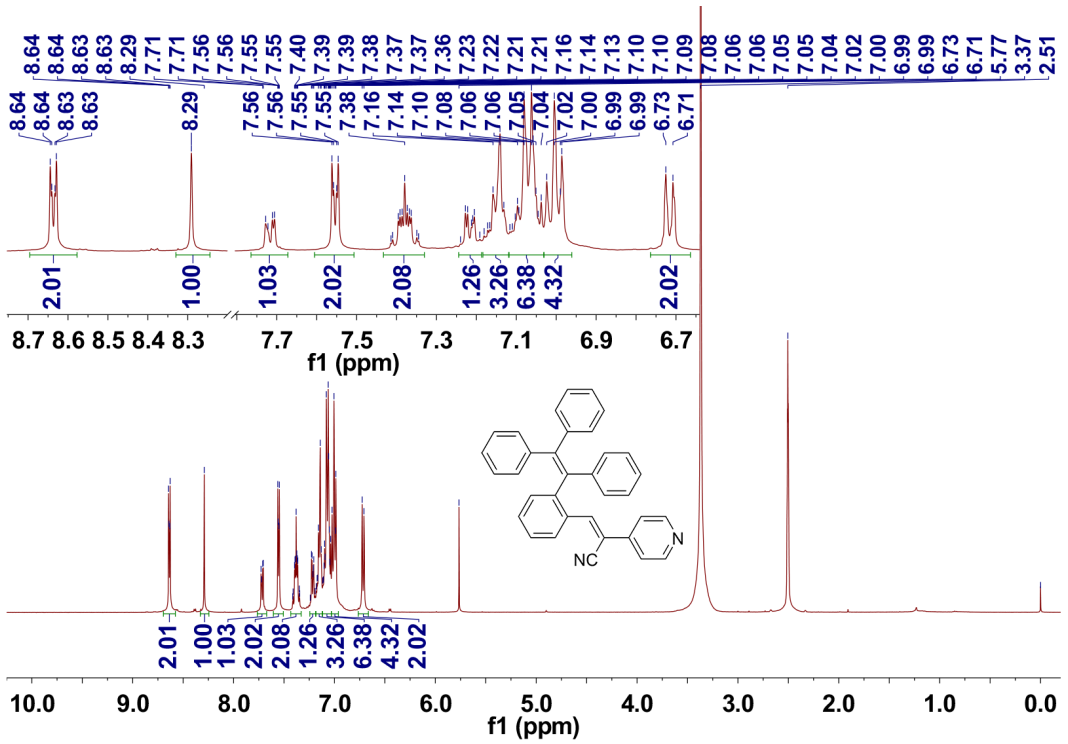

Fig. S4 ^{1}H NMR spectrum of compound **5a** in DMSO-d_6.

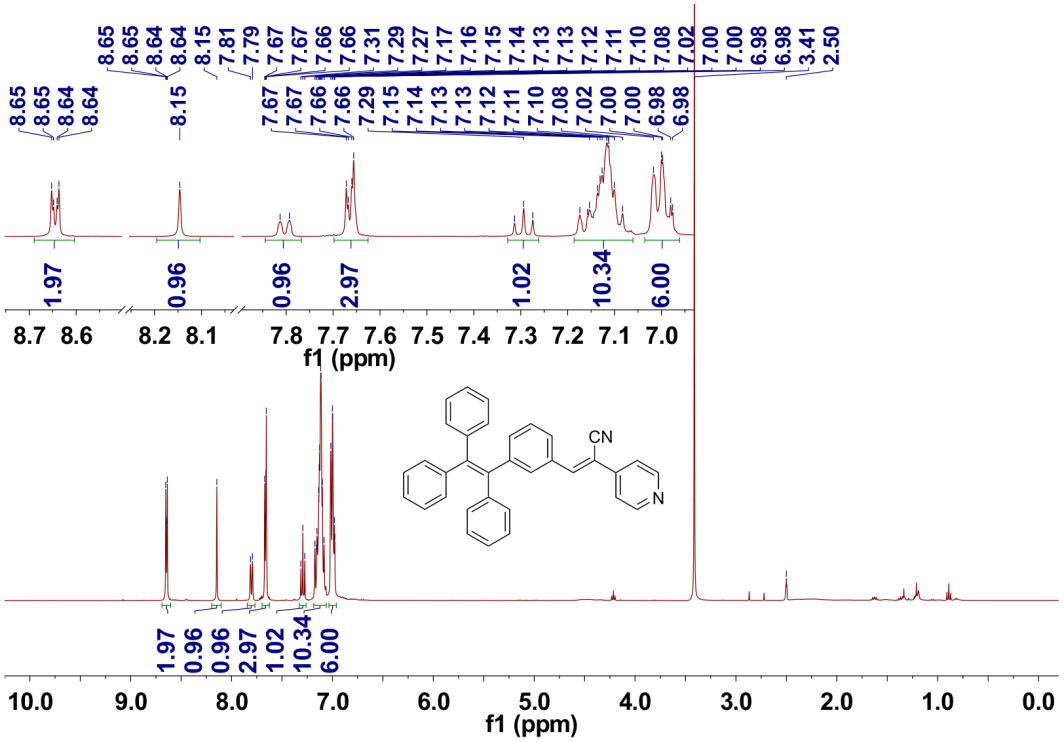

Fig. S5 ^{1}H NMR spectrum of compound **5b** in DMSO-d_6.

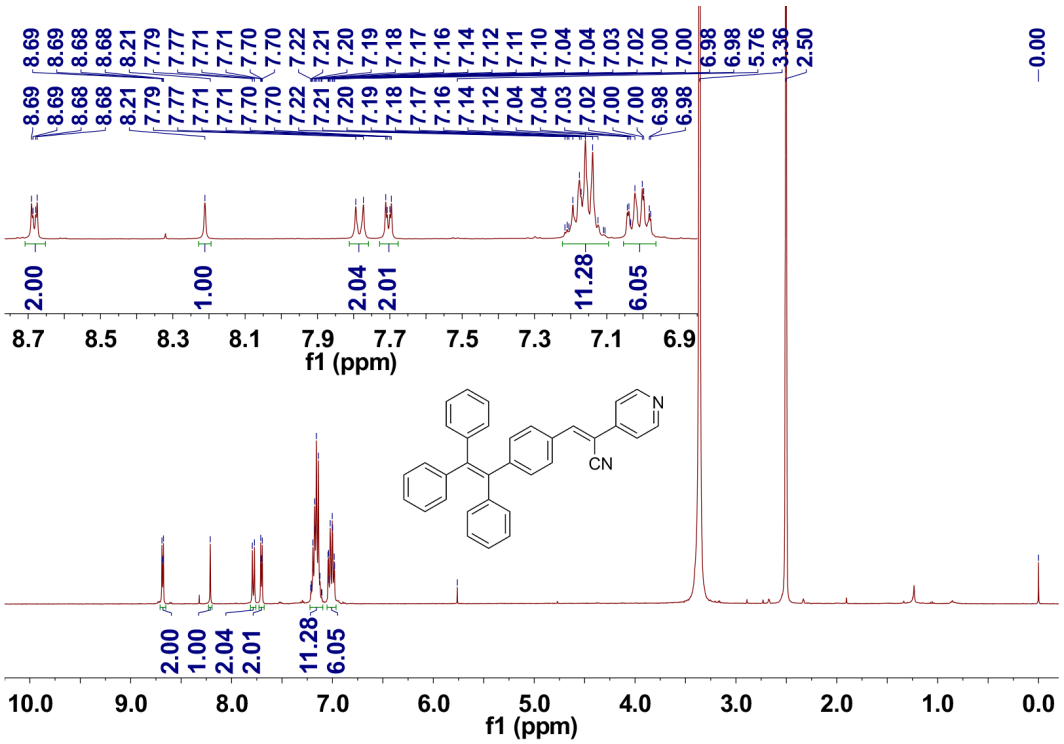

Fig. S6 ^{1}H NMR spectrum of compound **5c** in DMSO-d_6.

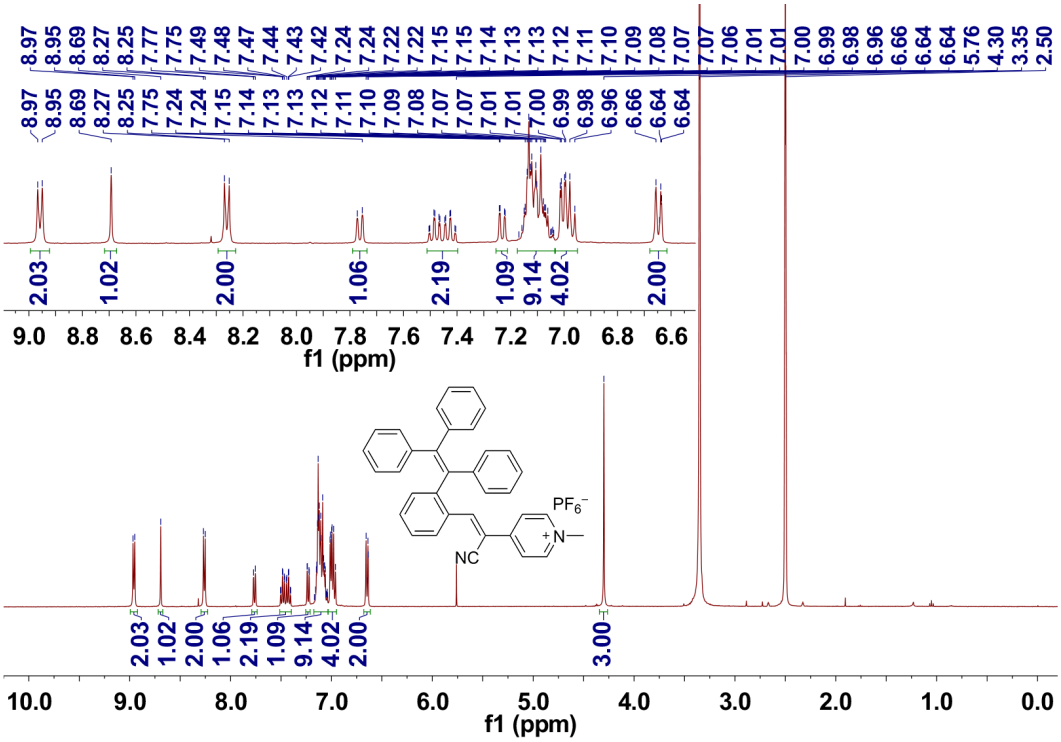

Fig. S7 ^{1}H NMR spectrum of o-TPE-ANPy^{+} in DMSO-d_6.

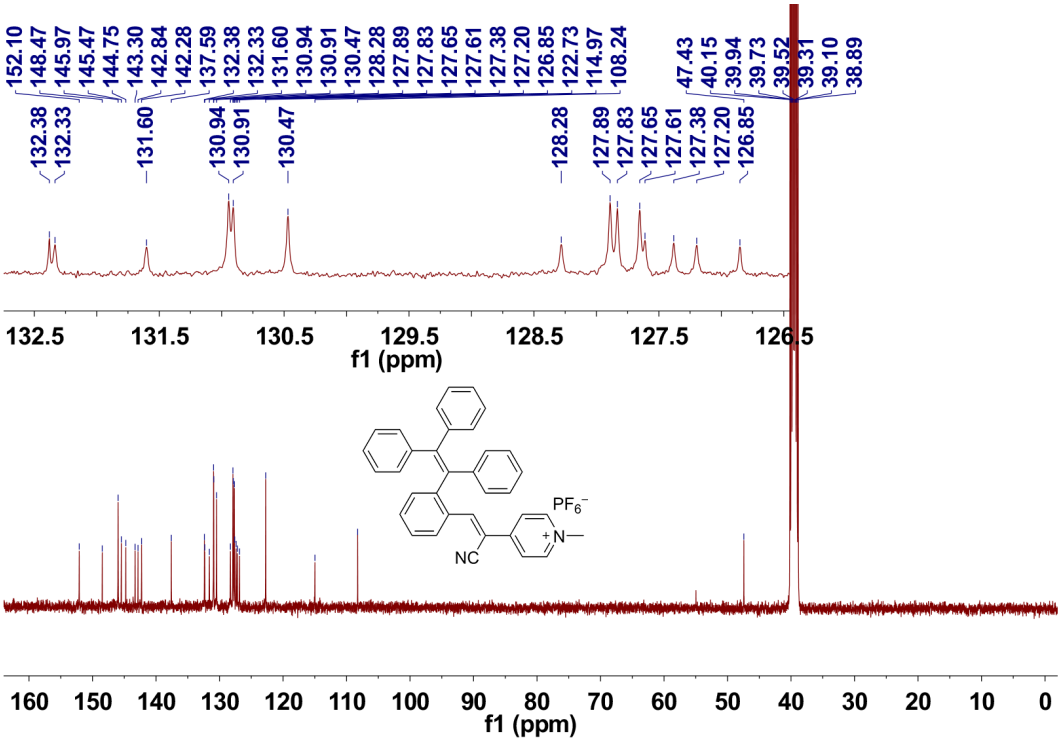

Fig. S8 ^{13}C NMR spectrum of o-TPE-ANPy^{+} in DMSO-d_6.

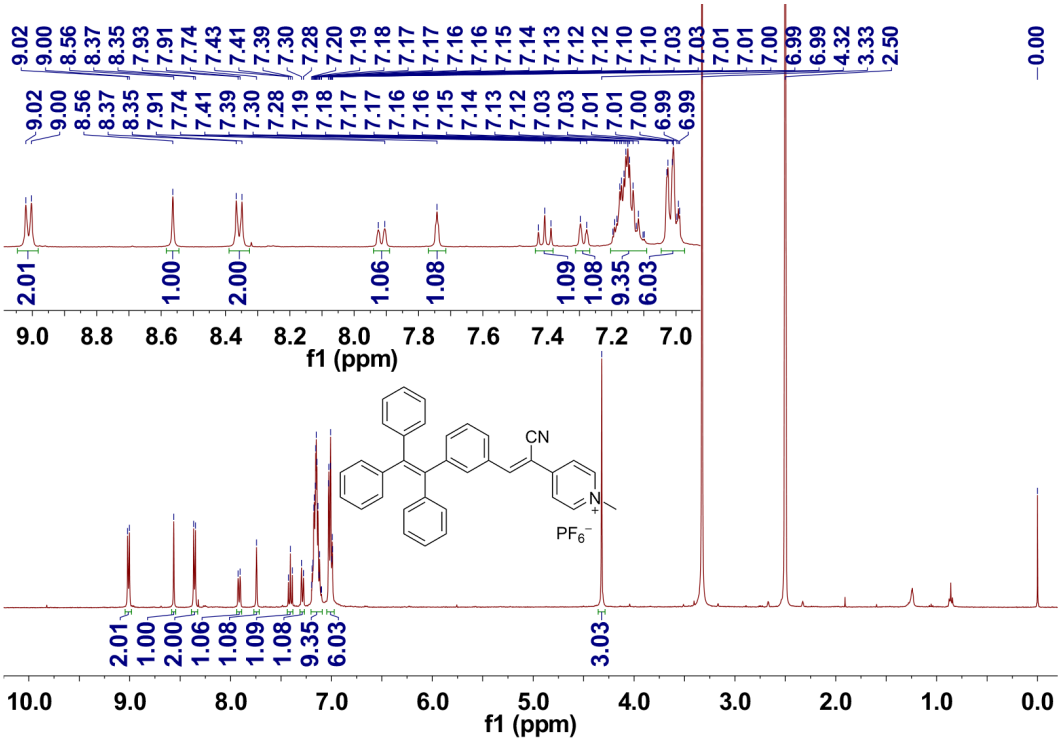

Fig. S9 ^{1}H NMR spectrum of m-TPE-ANPy^{+} in DMSO-d_6.

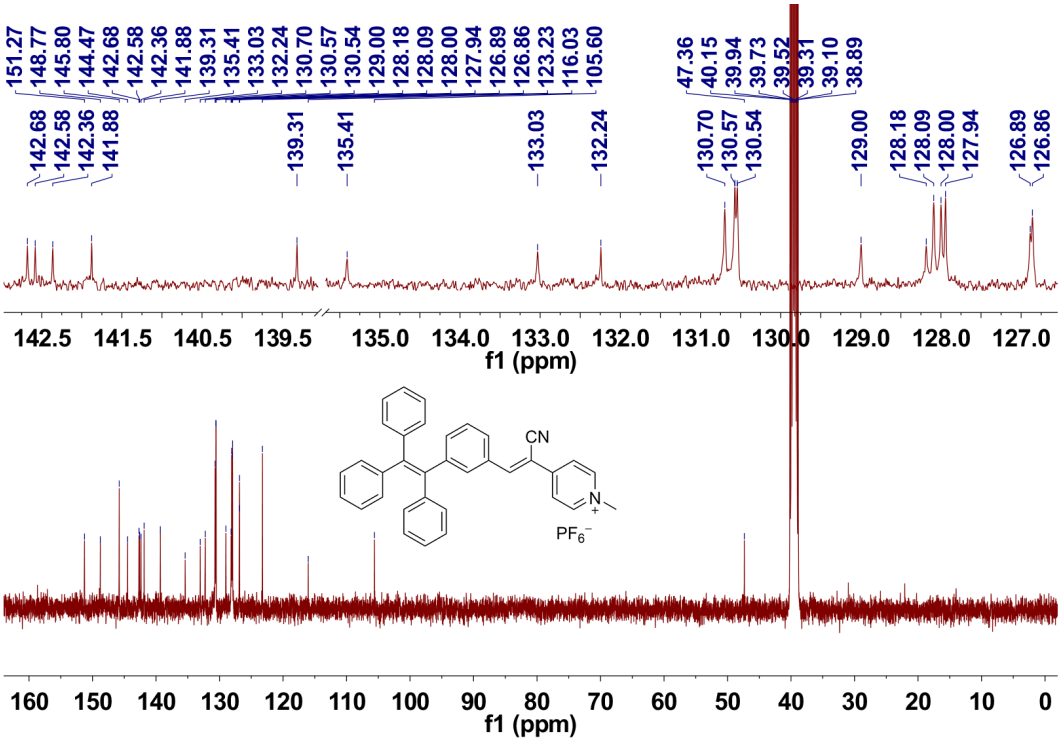

Fig. S10 ^{13}C NMR spectrum of m-TPE-ANPy^{+} in DMSO-d_6.

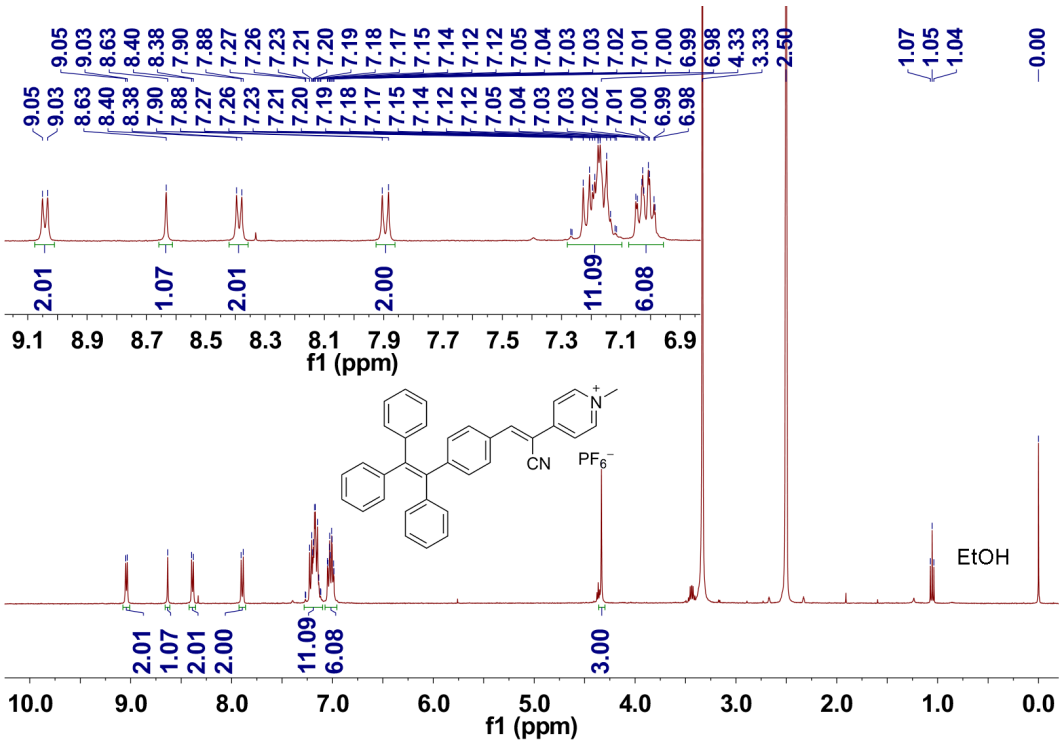

Fig. S11 ^{1}H NMR spectrum of p-TPE-ANPy^{+} in DMSO-d_6.

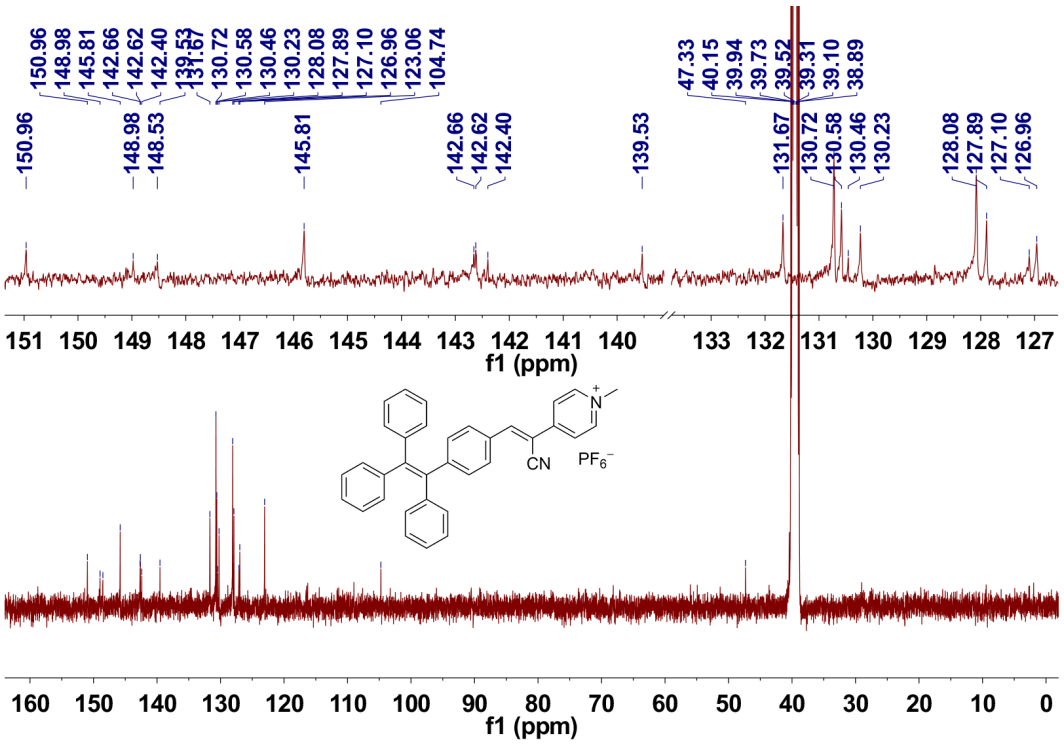

Fig. S12 ^{13}C NMR spectrum of p-TPE-ANPy^{+} in DMSO-d_6.

HRMS Spectra

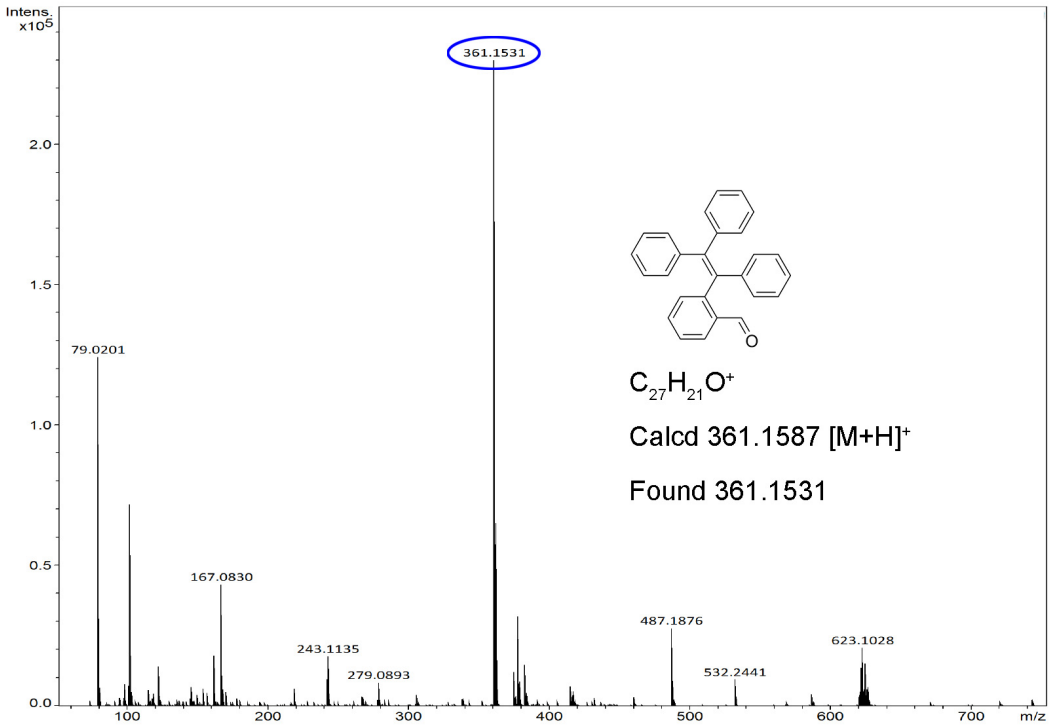

Fig. S13 HRMS spectrum of compound **3a**.

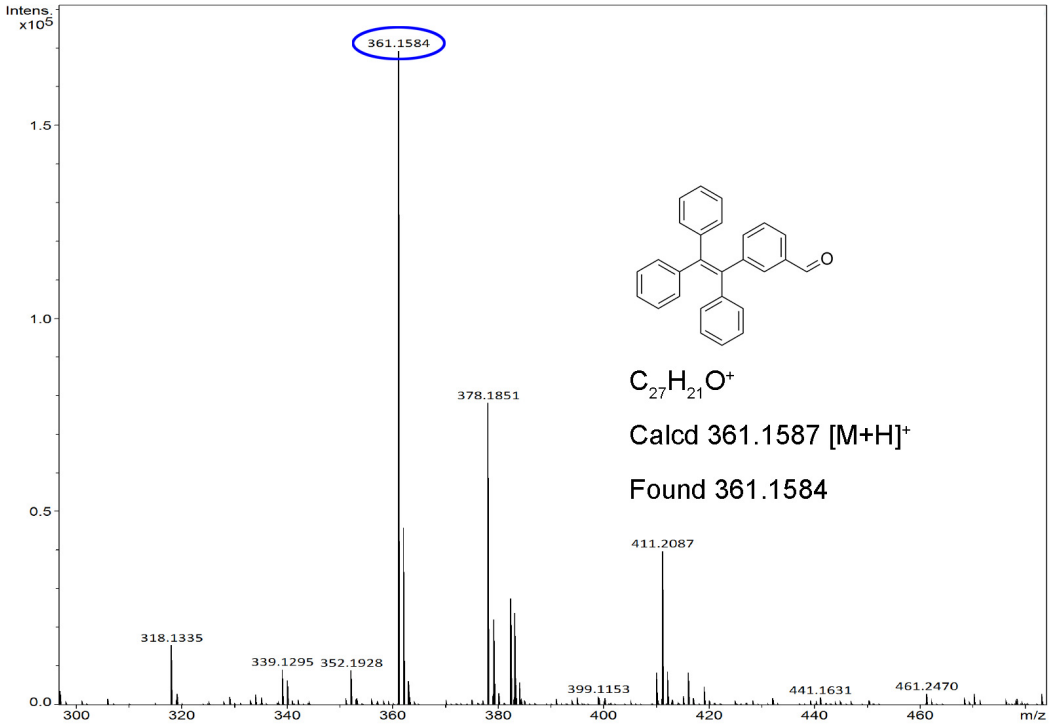

Fig. S14 HRMS spectrum of compound **3b**.

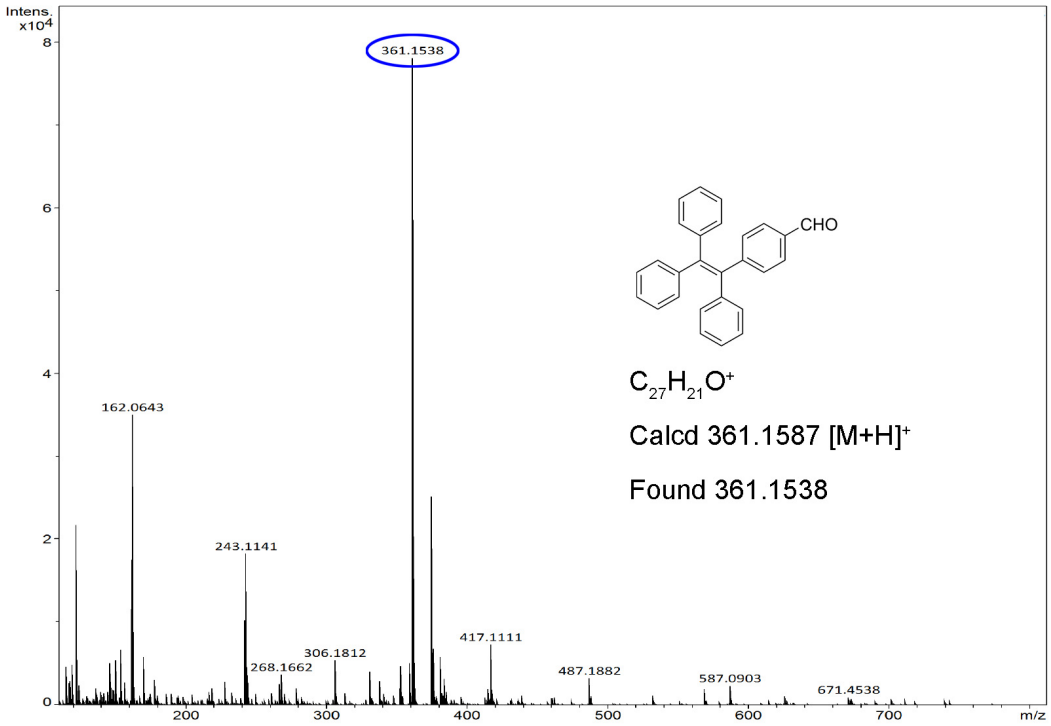

Fig. S15 HRMS spectrum of compound **3c**.

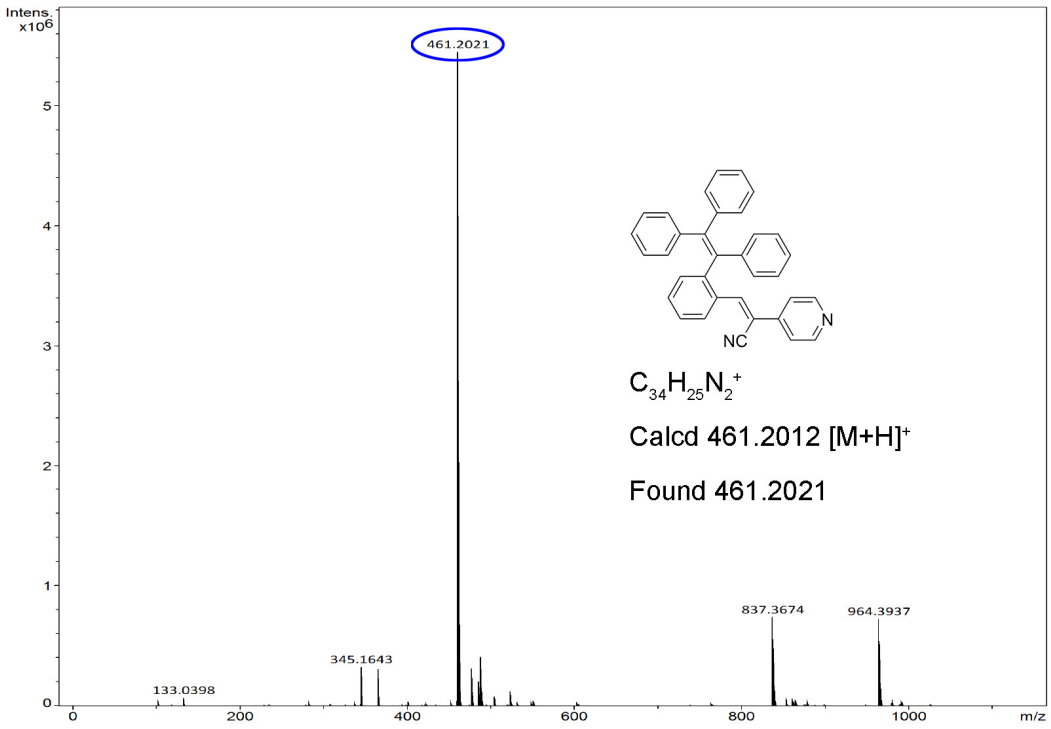

Fig. S16 HRMS spectrum of compound **5a**.

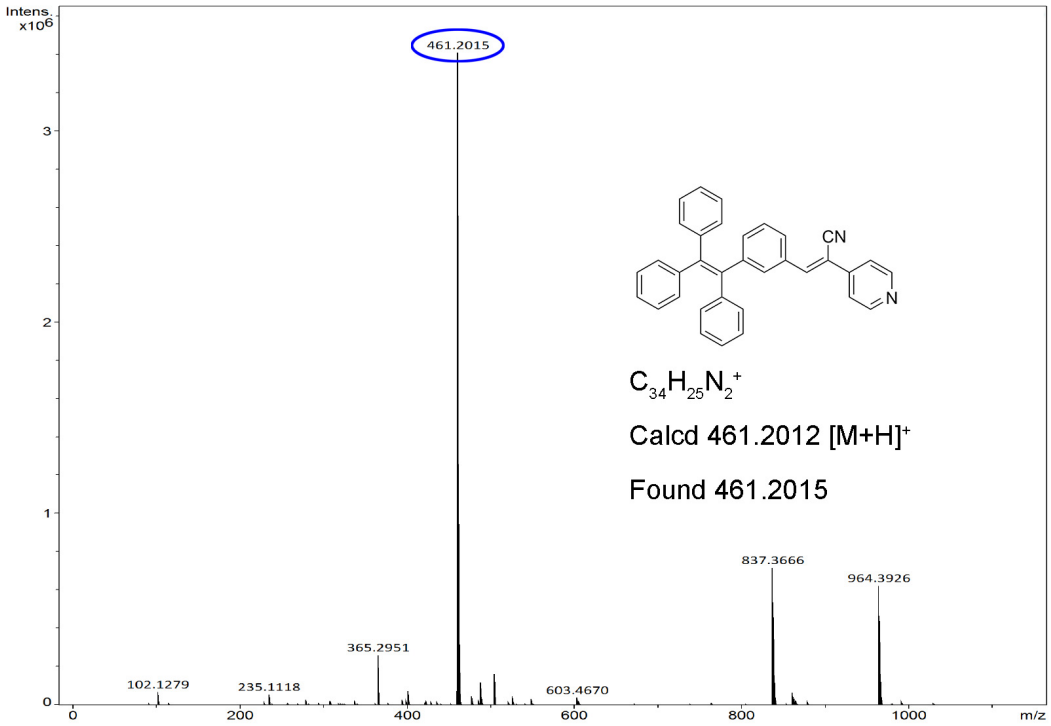

Fig. S17 HRMS spectrum of compound **5b**.

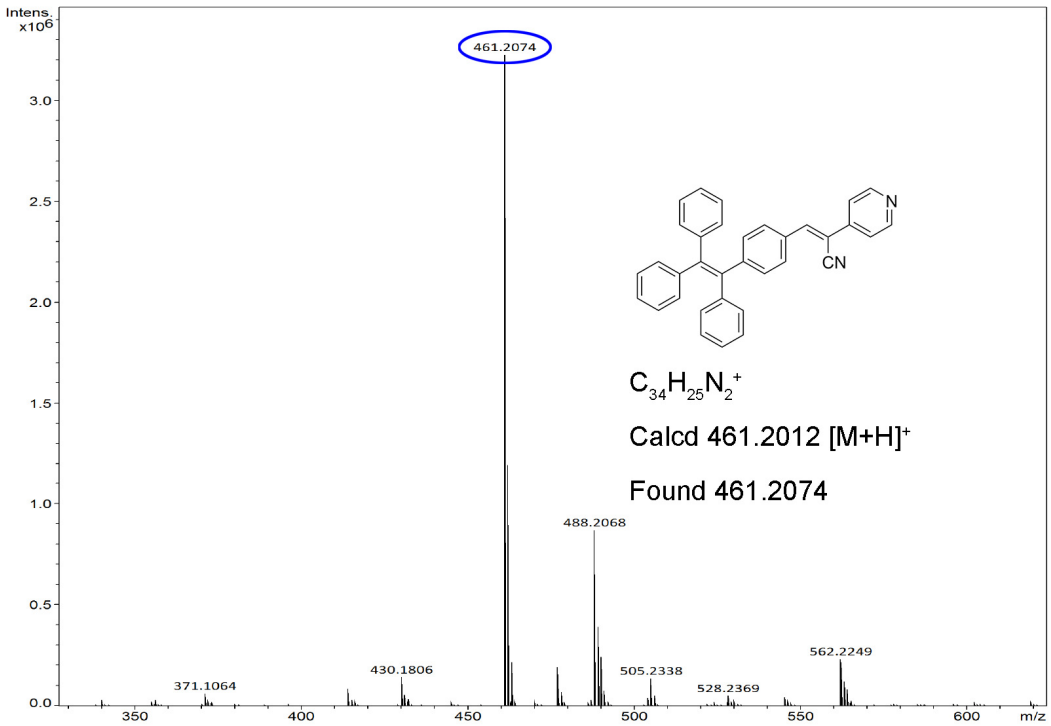

Fig. S18 HRMS spectrum of compound **5c**.

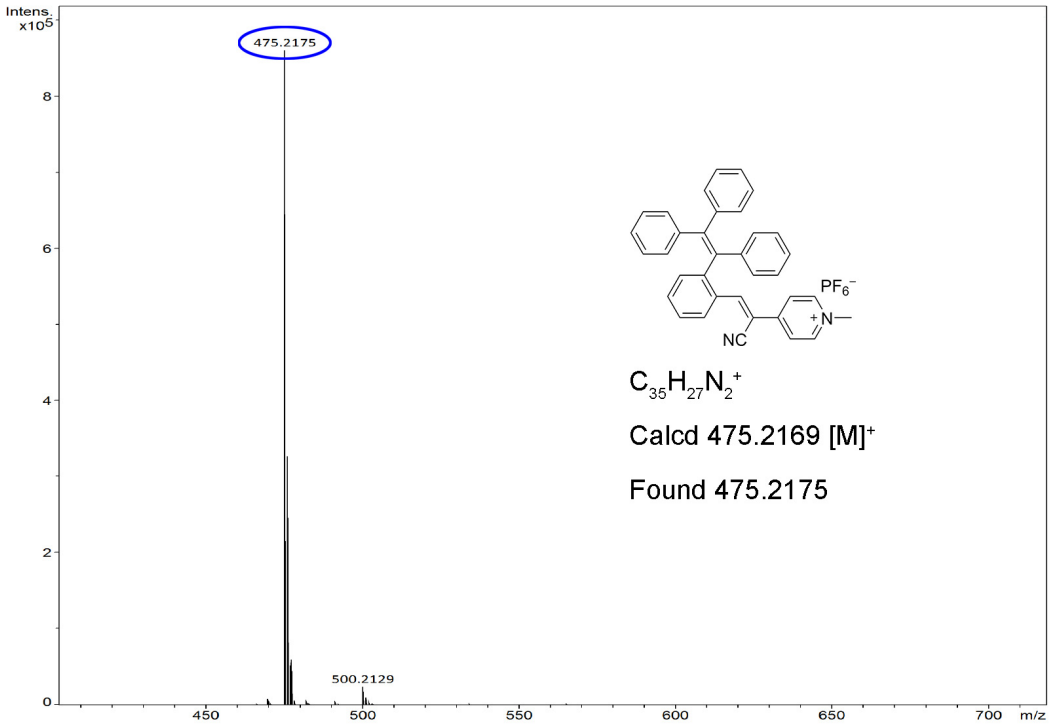

Fig. S19 HRMS spectrum of o-TPE-ANPy^{+}.

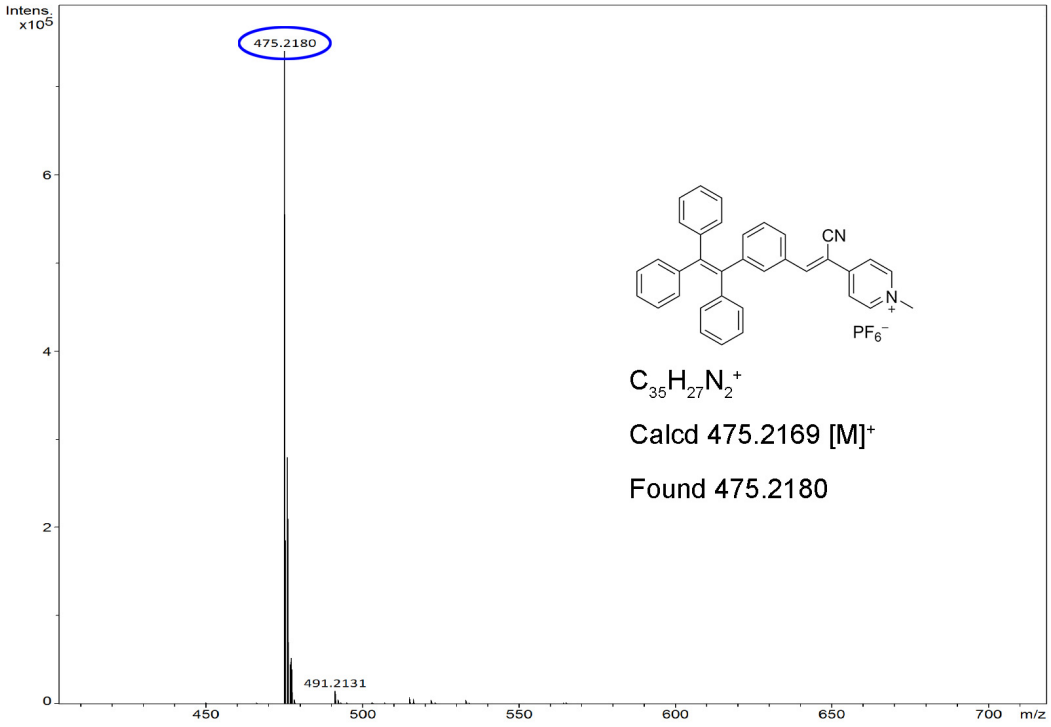

Fig. S20 HRMS spectrum of m-TPE-ANPy^{+}.

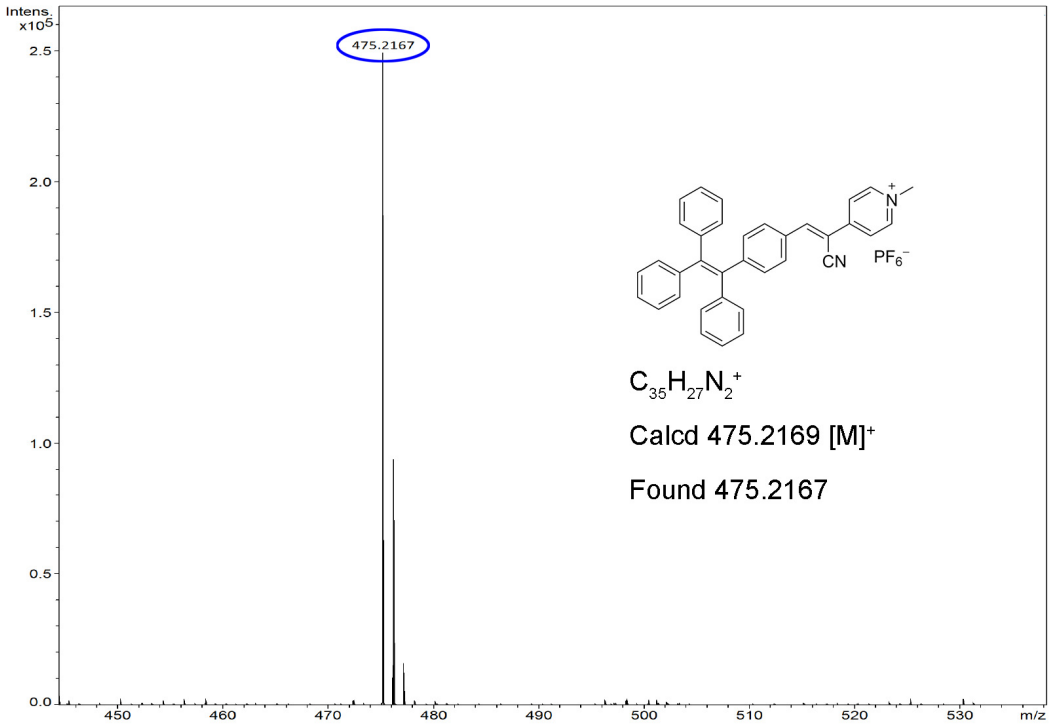

Fig. S21 HRMS spectrum of p-TPE-ANPy^{+}.

Crystallographic Data

Table S1 Crystal data and structure refinement for o-TPE-ANPy^{+}.

```
Bond precision:   C-C = 0.0036 A          Wavelength=1.54184

Cell:             a=12.1185(8)     b=16.6865(10)        c=30.588(2)
                  alpha=90         beta=100.899(3)      gamma=90
Temperature:      173 K

                 Calculated                Reported
Volume           6073.8(7)                 6073.7(7)
Space group      C 2/c                     C 1 2/c 1
Hall group       -C 2yc                    -C 2yc
Moiety formula   C35 H27 N2, F6 P          2(F3 P0.5), C35 H27 N2
Sum formula      C35 H27 F6 N2 P           C35 H27 F6 N2 P
Mr               620.56                    620.55
Dx,g cm-3        1.357                     1.357
Z                8                         8
Mu (mm-1)        1.367                     1.367
F000             2560.0                    2560.0
F000'            2571.07
h,k,lmax         14,19,35                  14,19,35
Nref             5176                      5138
Tmin,Tmax        0.936,0.960               0.644,0.753
Tmin'            0.921

Correction method= # Reported T Limits: Tmin=0.644 Tmax=0.753
AbsCorr = MULTI-SCAN

Data completeness= 0.993           Theta(max)= 65.089

R(reflections)= 0.0572( 4213)                wR2(reflections)=
                                             0.1573( 5138)
S = 1.045                  Npar= 577
```

Table S2 Crystal data and structure refinement for m-TPE-ANPy^{+}.

Bond precision:	C-C = 0.0052 A	Wavelength=1.54178	
Cell:	a=35.838(9)	b=9.307(2)	c=21.453(6)
	alpha=90	beta=117.610(9)	gamma=90
Temperature:	173 K		

	Calculated	Reported
Volume	6341(3)	6341(3)
Space group	C 2/c	C 1 2/c 1
Hall group	-C 2yc	-C 2yc
Moiety formula	2(C35 H27 N2), 2(F6 P), C H2 Cl2	2(F6 P), 2(C35 H27 N2), C H2 Cl2
Sum formula	C71 H56 Cl2 F12 N4 P2	C71 H56 Cl2 F12 N4 P2
Mr	1326.04	1326.03
Dx,g cm-3	1.389	1.389
Z	4	4
Mu (mm-1)	2.102	2.102
F000	2728.0	2728.0
F000'	2742.08	
h,k,lmax	42,11,25	42,11,25
Nref	5645	5578
Tmin,Tmax	0.618,0.828	0.501,0.753
Tmin'	0.456	

Correction method= # Reported T Limits: Tmin=0.501 Tmax=0.753
AbsCorr = MULTI-SCAN

Data completeness= 0.988 Theta(max)= 67.064

R(reflections)= 0.0689(4141) wR2(reflections)= 0.2123(5578)

S = 1.053 Npar= 468

Table S3 Crystal data and structure refinement for p-TPE-ANPy^{+}.

Bond precision:	C-C = 0.0041 A	Wavelength=1.54178	
Cell:	a=8.3266(4)	b=21.8990(12)	c=32.8375(18)
	alpha=90	beta=95.772(3)	gamma=90
Temperature:	100 K		

	Calculated	Reported
Volume	5957.4(5)	5957.4(5)
Space group	P 21/n	P 1 21/n 1
Hall group	-P 2yn	-P 2yn
Moiety formula	C35 H27 N2, F6 P	F6 P, C35 H27 N2
Sum formula	C35 H27 F6 N2 P	C35 H27 F6 N2 P
Mr	620.56	620.55
Dx,g cm-3	1.384	1.384
Z	8	8
Mu (mm-1)	1.394	1.394
F000	2560.0	2560.0
F000'	2571.07	
h,k,lmax	9,26,39	9,26,39
Nref	10572	10507
Tmin,Tmax	0.739,0.894	0.580,0.753
Tmin'	0.610	

Correction method= # Reported T Limits: Tmin=0.580 Tmax=0.753
AbsCorr = MULTI-SCAN

Data completeness= 0.994 Theta(max)= 66.894

R(reflections)= 0.0568(7487) wR2(reflections)= 0.1646(10507)

S = 1.041 Npar= 851

Photophysical Data

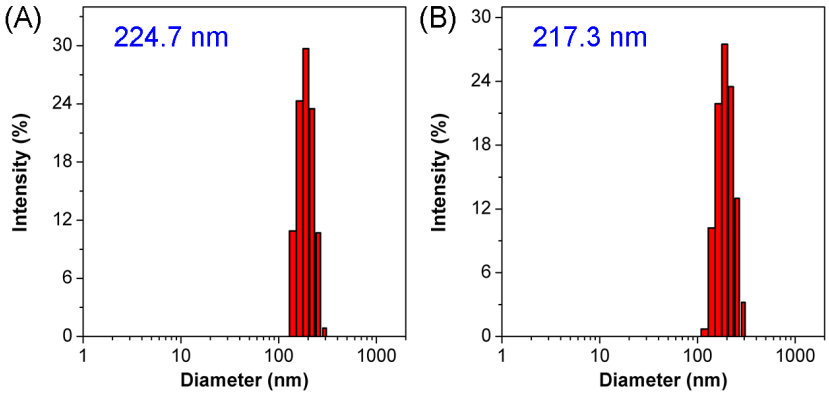

Fig. S22 DLS analysis of (A) m-TPE-ANPy$^+$ and (B) p-TPE-ANPy$^+$ in the THF/Et_2O mixture containing 99% Et_2O. Concentration: 10 μM.

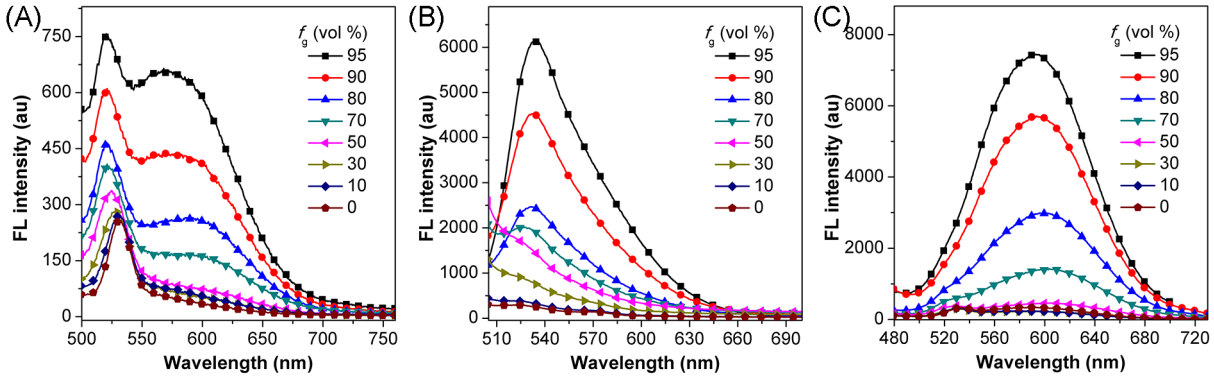

Fig. S23 Fluorescence spectra of (A) o-TPE-ANPy$^+$, (B) m-TPE-ANPy$^+$, and (C) p-TPE-ANPy$^+$ in water/glycerol mixtures (containing 0.25% DMSO) with different glycerol fractions (f_g). Concentration: 10 μM.

Table S4 Calculated DFT data of o-TPE-ANPy$^+$, m-TPE-ANPy$^+$, and p-TPE-ANPy$^+$ at the optimized ground states.

Compound	Transition type	f [a]	λ_{ca} [b] (nm)
o-TPE-ANPy$^+$	$S_0 \rightarrow S_2$	0.3068	369.5
m-TPE-ANPy$^+$	$S_0 \rightarrow S_2$	0.9126	375.7
p-TPE-ANPy$^+$	$S_0 \rightarrow S_1$	0.9527	510.2

[a] f = The oscillator strength; [b] λ_{ca} = The calculated absorption wavelength.

Table S5 Reorganization energy data of o-TPE-ANPy$^+$, m-TPE-ANPy$^+$, and p-TPE-ANPy$^+$.

Compound	λ_{gs}[a] (eV)	λ_{es}[b] (eV)	λ[c] (eV)
o-TPE-ANPy$^+$	0.33	0.33	0.66
m-TPE-ANPy$^+$	**0.38**	**0.38**	**0.76**
p-TPE-ANPy$^+$	0.21	0.21	0.42

[a] λ_{gs} = The ground-state reorganization energy; [b] λ_{es} = The excited-state reorganization energy; [c] λ = The reorganization energy of ground and excited state.

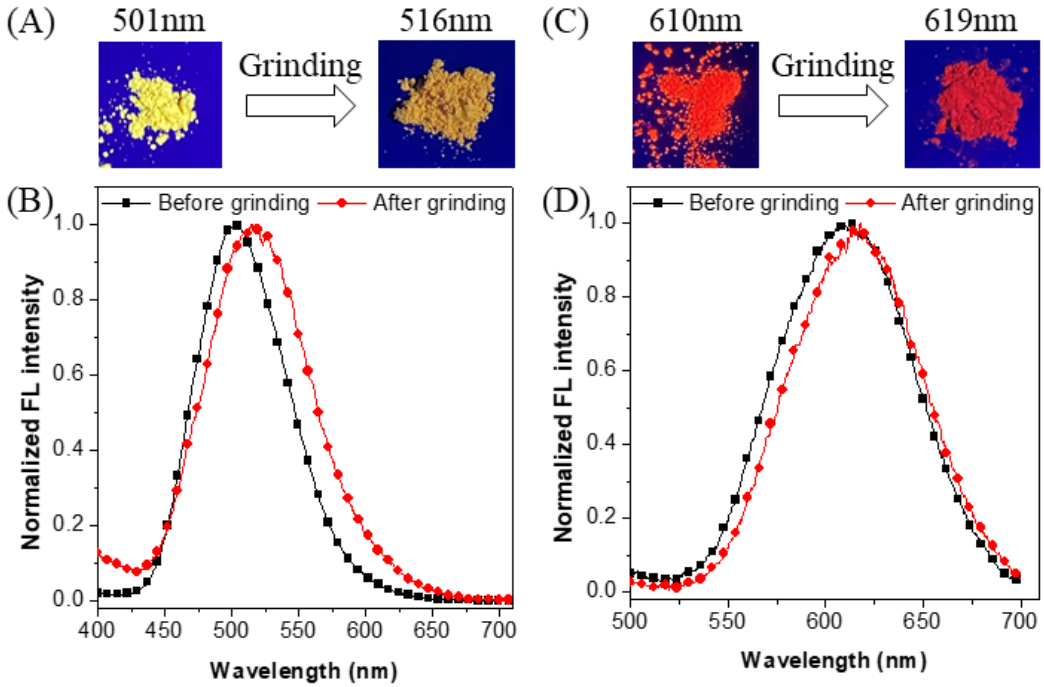

Fig. S24 (A) Solid-state fluorescent photos and (B) normalized fluorescence spectra of m-TPE-ANPy$^+$ before and after grinding. (C) Solid-state fluorescent photos and (D) normalized fluorescence spectra of p-TPE-ANPy$^+$ before and after grinding.

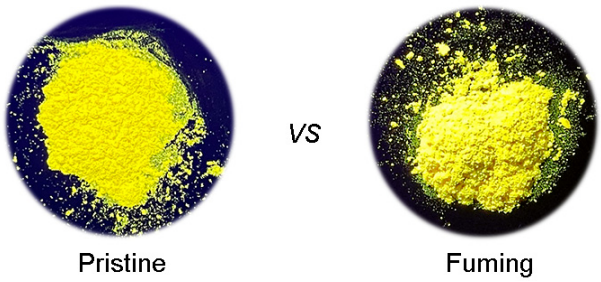

Fig. S25 Fluorescent photos of pristine solid of o-TPE-ANPy$^+$ and dichloromethane vapor-fumed solid of grinding o-TPE-ANPy$^+$.

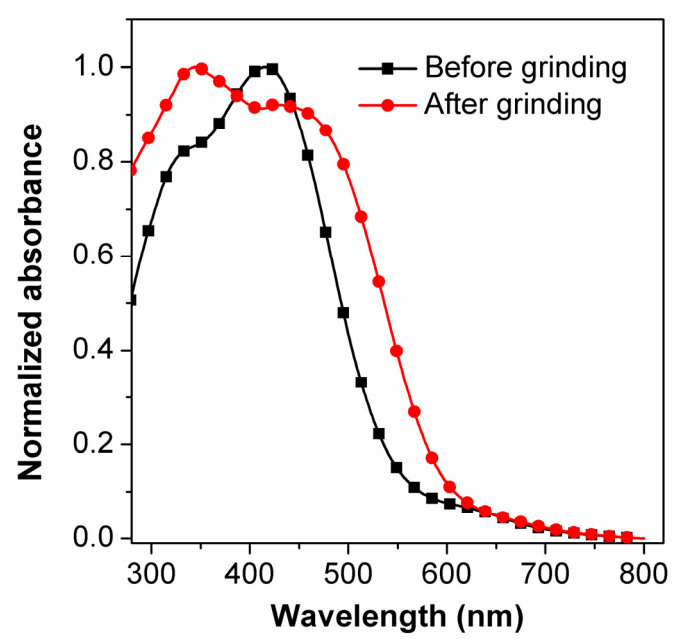

Fig. S26 Normalized absorption spectra of solid samples of o-TPE-ANPy^{+} before and after grinding.

Imaging Data

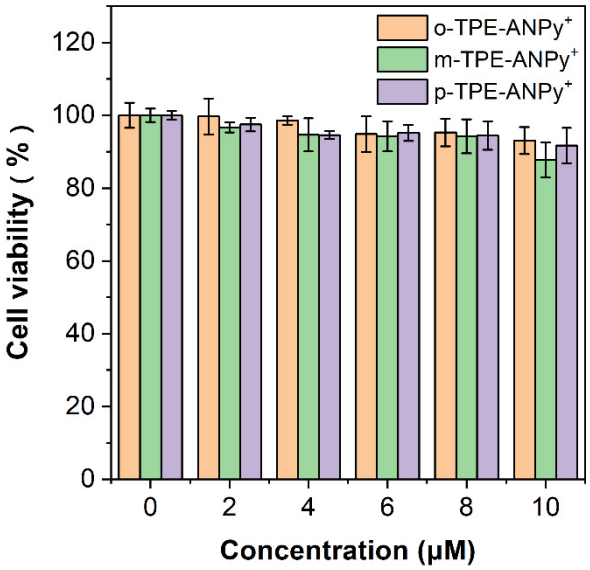

Fig. S27 Cytotoxicity of AIEgens in HeLa cells at different concentrations.

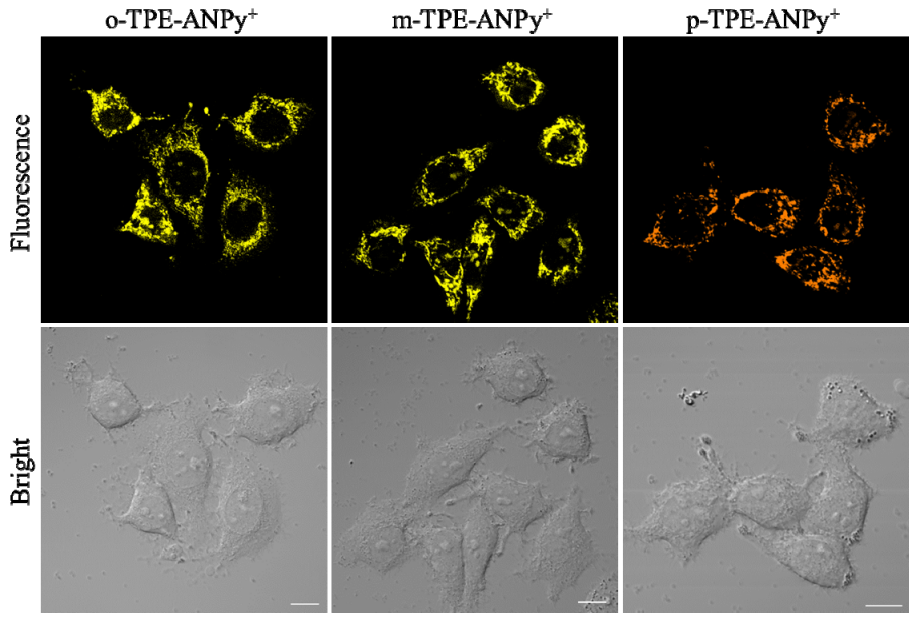

Fig. S28 CLSM images of HeLa cells incubated with o-TPE-ANPy$^+$, m-TPE-ANPy$^+$, or p-TPE-ANPy$^+$ (8 μM). Scale bar: 10 μm.