**Supporting Information**
***for***

# AIEgens for dark through-bond energy transfer: design, synthesis, theoretical study and its application in ratiometric $Hg^{2+}$ sensing

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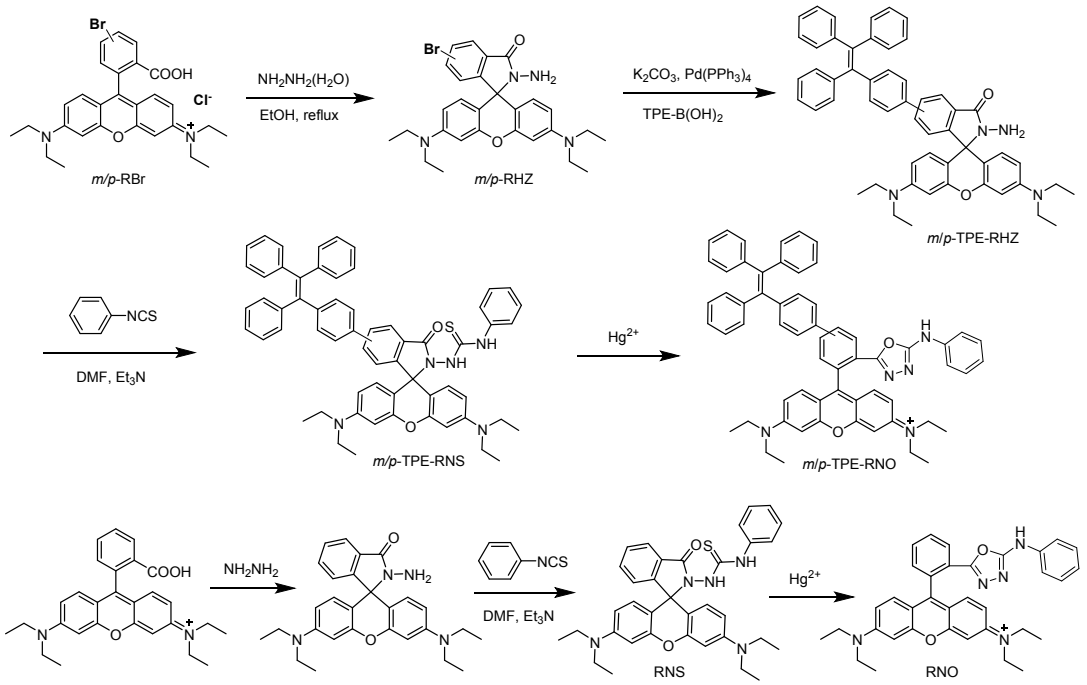
## 1. Synthetic Routes and Characterizations.




***Scheme S1.*** Synthetic route of *m*/*p*-TPE-RNS, *m*/*p*-TPE-RNO and control compounds RNS, RNO.

**Synthesis of *p*-RHZ** Compound *p*-RBr were prepared according the reported procedure.[i] Compound *p*-RBr (0.535 g, 1.0 mmol) was dissolved in 20 mL ethanol and 2 mL Hydrazine monohydrate was added. The reaction mixture was refluxed with stirring for 6 h and then evaporated in vacuo. The residue was purified by column chromatography on silica gel (Hexane/ ethyl acetate, 5:1 to 3:1, v/v) to give *p*-RHZ (0.35 g) as white powder, yield, 64%. $^1$H NMR (400 MHz, $CDCl_3$): δ 7.82 (d, *J* = 8.0 Hz, 1H), 7.60 (dd, *J* = 1.6 Hz, *J* = 8.0 Hz, 1H), 7.26 (d, *J* = 1.6 Hz, 1H), 6.48 (d, *J* = 8.8 Hz, 2H), 6.44 (d, *J* = 1.6 Hz, 2H), 6.32 (dd, *J* = 1.6 Hz, *J* = 8.8 Hz, 2H), 3.62 (s, 2H), 3.37 (q, *J* = 7.2 Hz, 8H), 1.20 (t, *J* = 7.2 Hz, 12H); $^{13}$C NMR (100 MHz, $CDCl_3$): δ 165.3, 153.8, 153.3, 149.1, 131.7, 128.8, 128.0, 127.2, 124.6, 108.1, 103.6, 98.0, 65.8, 44.4, 12.6; HRMS: Calc. for [M+H$^+$] 535.1703, found 535.1729.

**Synthesis of m-RHZ** Synthetic procedure is similar to that of *p*-RHZ using *m*-RBr as the starting material, yield, 68%. $^1$H NMR (400 MHz, $CDCl_3$): δ 8.09 (d, *J* = 2.0 Hz, 1H), 7.59 (dd, *J* = 1.0 Hz, *J* = 7.6 Hz, 1H), 7.01 (d, *J* = 7.6 Hz, 1H), 6.47 (d, *J* = 8.4 Hz, 2H), 6.43 (d, *J* = 2.4 Hz, 2H), 6.32 (dd, *J* = 2.4 Hz, *J* = 8.4 Hz, 2H), 3.63 (s, 2H), 3.36 (q, *J* = 7.2 Hz, 8H), 1.19 (t, *J* = 7.2 Hz, 12H); $^{13}$C NMR (100 MHz, $CDCl_3$): δ 164.6, 153.8, 150.2, 149.0, 135.5, 132.0, 128.0, 126.1, 125.6, 122.2, 108.1, 103.7, 98.0, 66.0, 44.4, 12.6; HRMS: Calc. for [M] 534.1630, found 534.1635.

**Synthesis of *p*-TPE-RHZ** Compounds *p*-RHZ (268 mg, 0.5 mmol), TPE-B(OH)$_2$ (188 mg, 0.5 mmol), Pd(PPh$_3$)$_4$ (20 mg, 0.017 mmol) and $K_2CO_3$ (138 mg, 1.0 mmol) were placed in a 100 mL two neck flask. After vacuum and filling $N_2$ for 3 times, 25 mL of THF and 10 mL $H_2O$ were added.

The reaction mixture was left refluxing overnight. The THF solvent was removed under vacuum, distract with DCM and $H_2O$. After removing DCM, the resulting residue was purified by column chromatography on silica gel (Hexane/$CH_2Cl_2$/ethyl acetate, 2:1:1 v/v/v) to give *p*-TPE-RHZ (324 mg, 82%). $^1$H NMR (400 MHz, $CDCl_3$): δ 7.95 (d, *J* = 8.0 Hz, 1H), 7.64 (dd, *J* = 1.6 Hz, *J* = 8.0 Hz, 1H), 7.25 (d, *J* = 1.6 Hz, 1H), 6.98-7.07 (m, 19H), 6.49 (d, *J* = 8.8 Hz, 2H), 6.42 (d, *J* = 2.4 Hz, 2H), 6.30 (dd, *J* = 2.4 Hz, *J* = 8.8 Hz, 2H), 3.61 (s, 2H), 3.35 (q, *J* = 6.8 Hz, 8H), 1.17 (t, *J* = 6.8 Hz, 12H); $^{13}$C NMR (100 MHz, $CDCl_3$): δ 166.0, 153.8, 152.2, 148.9, 145.1, 143.6, 141.3, 140.2, 137.8, 131.8, 131.4, 131.3, 131.2, 128.9, 128.2, 127.8, 127.7, 127.6, 127.0, 126.6, 126.5, 123.3, 122.1, 108.1, 104.5, 97.9, 66.1, 44.4, 12.6; HRMS: Calc. for [M] 786.3934, found 786.3986.

**Synthesis of** *m*-TPE-RHZ Synthetic procedure is similar to that of *p*-TPE-RHZ using *m*-RHZ as the starting material, yield, 85%. $^1$H NMR (400 MHz, $CDCl_3$): δ 8.12 (d, *J* = 2.4 Hz, 1H), 7.65 (dd, *J* = 2.4 Hz, *J* = 8.0 Hz, 1H), 7.39 (d, *J* = 8.0 Hz, 2H), 7.04-7.11 (m, 19H), 6.50 (d, *J* = 8.8 Hz, 2H), 6.43 (d, *J* = 2.4 Hz, 2H), 6.30 (dd, *J* = 2.4 Hz, *J* = 8.8 Hz, 2H), 3.63 (s, 2H), 3.35 (q, *J* = 6.8 Hz, 8H), 1.17 (t, *J* = 6.8 Hz, 12H); $^{13}$C NMR (100 MHz, $CDCl_3$): δ 166.1, 153.9, 150.3, 148.9, 143.7, 143.6, 143.5, 143.3, 141.4, 141.0, 140.4, 137.9, 131.9, 131.4, 131.3, 131.2, 130.6, 128.2, 127.8, 127.7, 127.6, 127.0, 126.6, 126.5, 126.4, 126.3, 124.1, 121.2, 108.1, 104.5, 98.0, 65.9, 44.4, 12.6; HRMS: Calc. for [M] 786.3934, found 786.3918.

**Synthesis of *p*-TPE-RNS** Isothiocyanatobenzene (135 mg, 1.0 mmol), *p*-TPE-RHZ (197 mg, 0.25 mmol), and TEA (0.1 mL) were dissolved in 10 mL DMF, the reaction mixture was stirred at room temperature under $N_2$ protection for 8 hours. The solvent were removed under vacuum, and the resulting residue was purified by column chromatography on silica gel (Hexane/$CH_2Cl_2$/ethyl acetate, 2:1:1 v/v/v) to give *p*-TPE-RNS (210 mg, 91%). $^1$H NMR (400 MHz, $CDCl_3$): δ 8.02 (d, *J* = 8.0 Hz, 1H), 7.77 (dd, *J* = 1.6 Hz, *J* = 8.0 Hz, 1H), 7.53 (s, 1H), 7.42 (d, *J* = 1.6 Hz, 1H), 7.30 (d, *J* = 8.0 Hz, 2H), 7.19 (t, *J* = 3.6 Hz, 2H), 6.97-7.10 (m, 21 H), 6.53 (d, *J* = 8.8 Hz, 2H), 6.45 (d, *J* = 2.8 Hz, 2H), 6.30 (dd, *J* = 2.8 Hz, *J* = 8.8 Hz, 2H), 3.35 (q, *J* = 6.8 Hz, 8H), 1.17 (t, *J* = 6.8 Hz, 12H); $^{13}$C NMR (100 MHz, $CDCl_3$): δ 182.8, 167.1, 154.3, 150.9, 149.4, 147.0, 144.2, 143.5, 143.4, 141.6, 140.1, 137.7, 137.3, 132.0, 131.4, 131.3, 128.3, 127.9, 127.7, 127.6, 126.6, 126.5, 126.1, 125.2, 124.2, 122.8, 108.4, 104.2, 98.3, 67.3, 44.4, 12.6; HRMS: Calc. for [M] 921. 4076, found 921. 4098.

**Synthesis of *m*-TPE-RNS** Synthetic procedure is similar to that of *p*-TPE-RNS using *m*-TPE-RHZ as the starting material, yield, 92%. $^1$H NMR (400 MHz, $CDCl_3$): δ 8.20 (s, 1H), 7.85 (d, *J* = 8.0 Hz, 1H), 7.53 (s, 1H), 7.45 (d, *J* = 8.0 Hz, 2H), 7.31 (d, *J* = 8.0 Hz, 2H), 7.06-7.21 (m, 22 H), 6.98 (s, 1H), 6.54 (d, *J* = 8.8 Hz, 2H), 6.47 (d, *J* = 2.0 Hz, 2H), 6.31 (dd, *J* = 2.0 Hz, *J* = 8.8 Hz, 2H), 3.36 (q, *J* = 7.2 Hz, 8H), 1.19 (t, *J* = 7.2 Hz, 12H); $^{13}$C NMR (100 MHz, $CDCl_3$): δ 182.7, 167.2, 154.3, 149.4, 148.8, 143.8, 143.6, 143.5, 142.0, 141.6, 140.2, 137.7, 137.3, 133.0, 132.1, 131.4, 131.3, 131.2, 129.7, 129.6, 128.3, 127.9, 127.8, 127.7, 127.6, 126.7, 126.6, 126.4, 126.1, 125.8, 125.1, 125.0, 124.2, 121.9, 108.4, 104.2, 98.4, 67.2, 44.4, 12.6; HRMS: Calc. for [M] 921. 4076, found 921. 4036.

**Synthesis of *p*-TPE-RNO** Compound *p*-TPE-RNS (92 mg, 0.1 mmol) was dissolved in 5 mL $CH_3CN$, $HgCl_2$ (54 mg, 0.2 mmol) was added and the mixture was left stirring for 6h at room

temperature. After removing the solvent, the residue was purified by column chromatography on silica gel (DCM/MeOH, 20:1, v/v) to give *p*-TPE-RNO (80 mg, 90%). $^1$H NMR (400 MHz, $CD_2Cl_2$): δ 11.20 (br, 1H), 8.30 (s, *J* = 8.0 Hz, 1H), 7.88 (d, *J* = 8.4 Hz, 1H), 7.73 (d, *J* = 8.4 Hz, 2H), 7.48 (s, 1H), 7.42 (d, *J* = 8.0 Hz, 2H), 7.02-7.21 (m, 21 H), 6.89 (t, *J* = 7.6 Hz, 2H), 6.76 - 6.78 (m, 4H), 3.56 (q, *J* = 7.2 Hz, 8H), 1.28 (t, *J* = 7.2 Hz, 12H); $^{13}$C NMR (100 MHz, $CDCl_3$): δ 160.5, 158.1, 157.7, 156.0, 155.6, 144.4, 143.6, 143.5, 143.4, 142.1, 141.8, 140.1, 139.0, 136.3, 132.0, 131.4, 131.2, 131.1, 131.0, 130.5, 129.2, 128.6, 128.0, 127.8, 127.7, 127.6, 126.6, 126.2, 122.2, 121.7, 117.7, 114.1, 113.9, 96.3, 46.0, 12.3; HRMS: Calc. for [M] 888. 4272, found 888. 4253.

**Synthesis of *m*-TPE-RNO** Synthetic procedure is similar to that of *p*-TPE-RNO using *m*-TPE-RNS as the starting material, yield, 95%. $^1$H NMR (400 MHz, $CDCl_3$): δ 10.99 (br, 1H), 8.49 (s, 1H), 7.79 (d, *J* = 8.0 Hz, 1H), 7.73 (d, *J* = 8.0 Hz, 2H), 7.54 (d, *J* = 8.0 Hz, 2H), 7.27 (d, *J* = 8.0 Hz, 1H), 7.06-7.20 (m, 21 H), 6.86 (t, *J* = 7.2 Hz, 1H), 6.79 (d, *J* = 9.6 Hz, 2H), 6.72 (s, 2H), 3.52 (q, *J* = 6.8 Hz, 8H), 1.28 (t, *J* = 6.8 Hz, 12H); $^{13}$C NMR (100 MHz, $CDCl_3$): δ 160.7, 157.9, 157.4, 156.1, 155.4, 144.4, 143.6, 143.4, 143.0, 141.6, 140.3, 138.8, 136.2, 132.2, 131.5, 131.4, 131.3, 131.2, 130.9, 128.6, 128.5, 127.9, 127.8, 127.7, 126.8, 126.7, 126.6, 126.5, 124.2, 121.7, 118.0, 114.2, 113.8, 96.5, 46.1, 12.7; HRMS: Calc. for [M] 888. 4272, found 888. 4244.

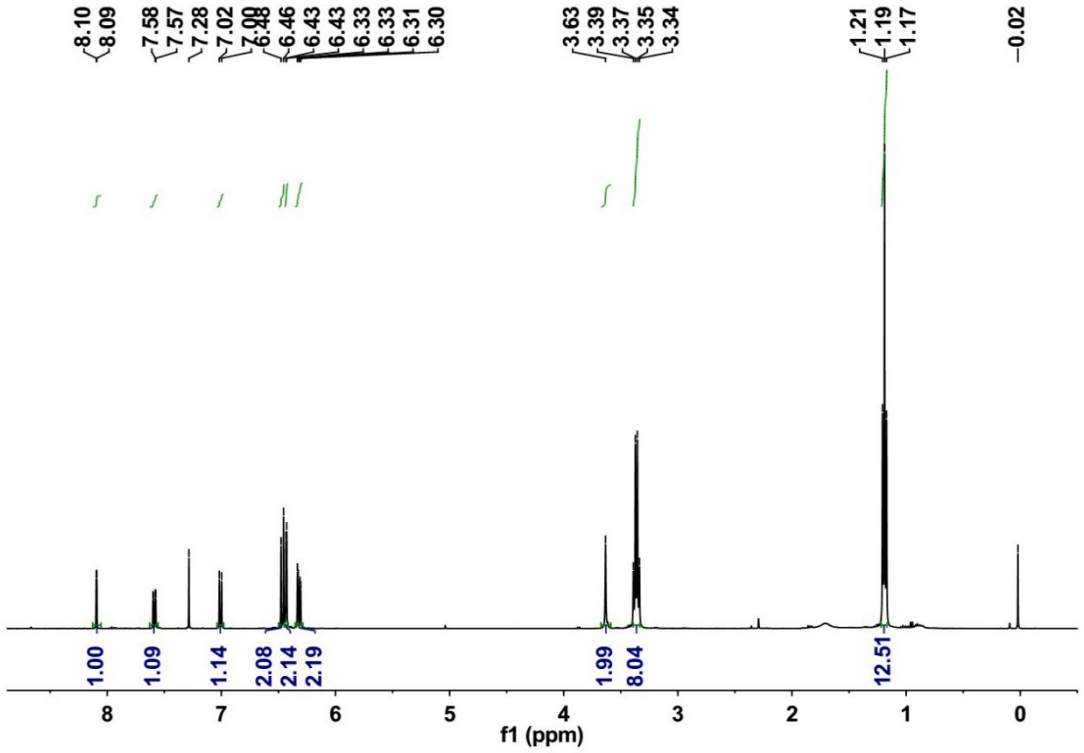



***Figure S1.*** $^1$H NMR spectrum of *m*-Br-RHZ in $CDCl_3$.

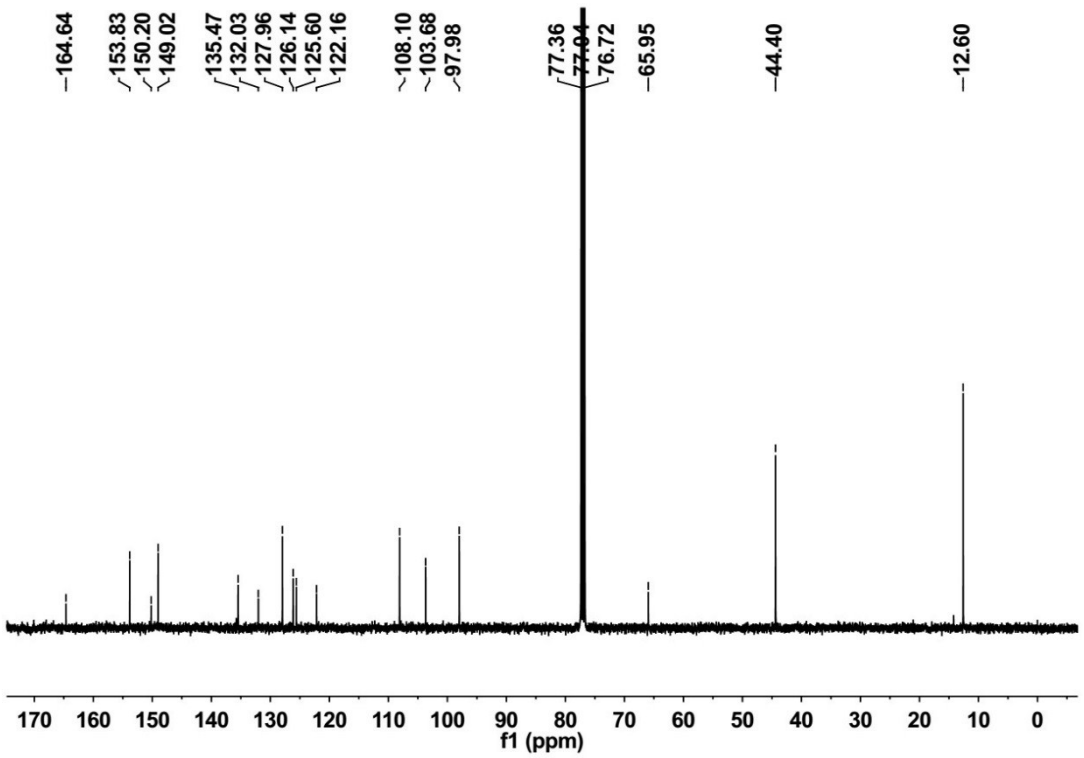



***Figure S2.*** $^{13}C$ NMR spectrum of *m*-Br-RHZ in $CDCl_3$.

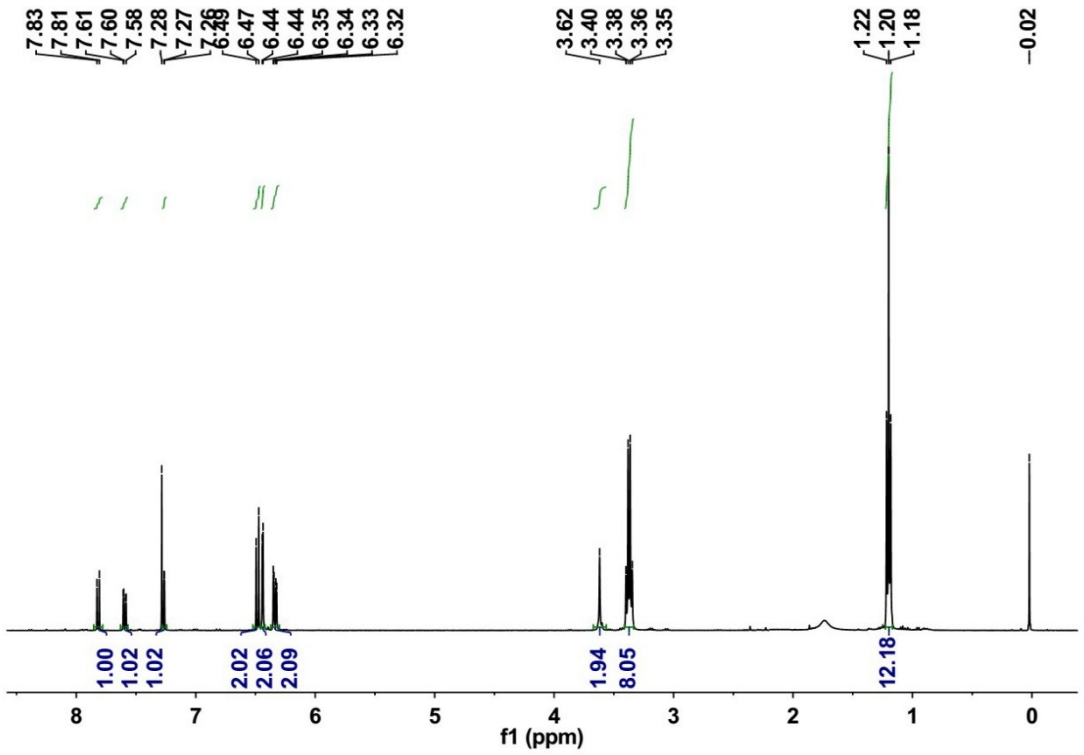



***Figure S3.*** $^{1}H$ NMR spectrum of ***p*-Br-RHZ** in $CDCl_3$.

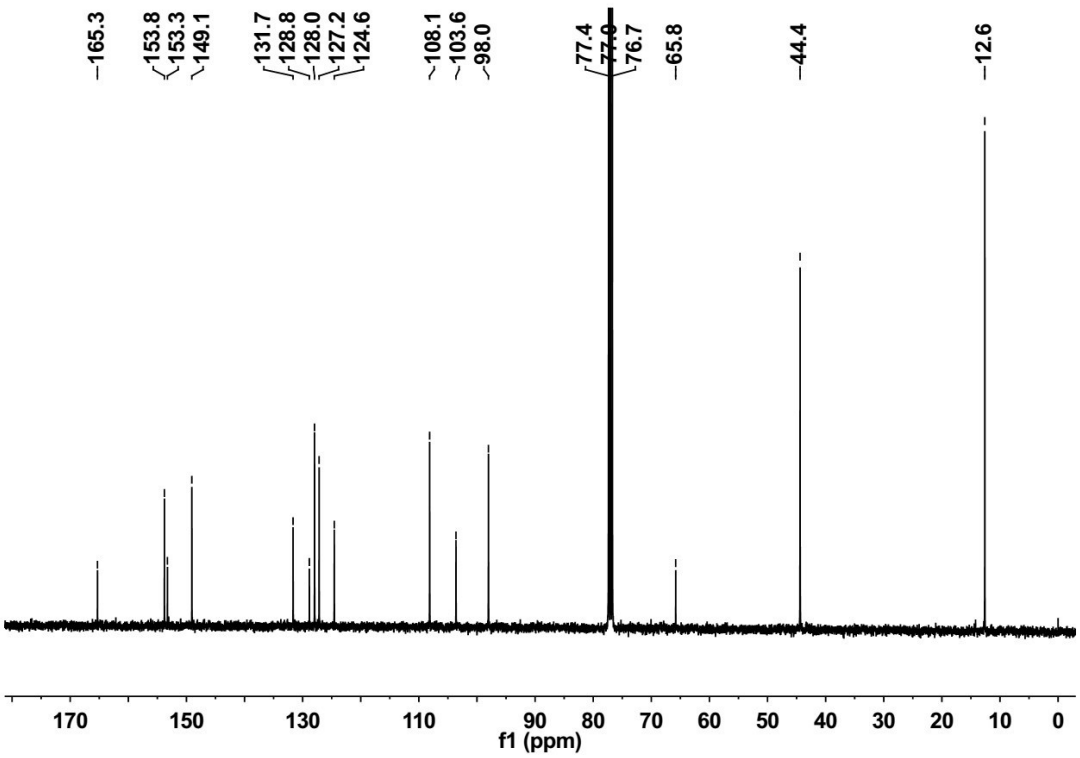



***Figure S4.*** $^{13}C$ NMR spectrum of *p*-Br-RHZ in $CDCl_3$.

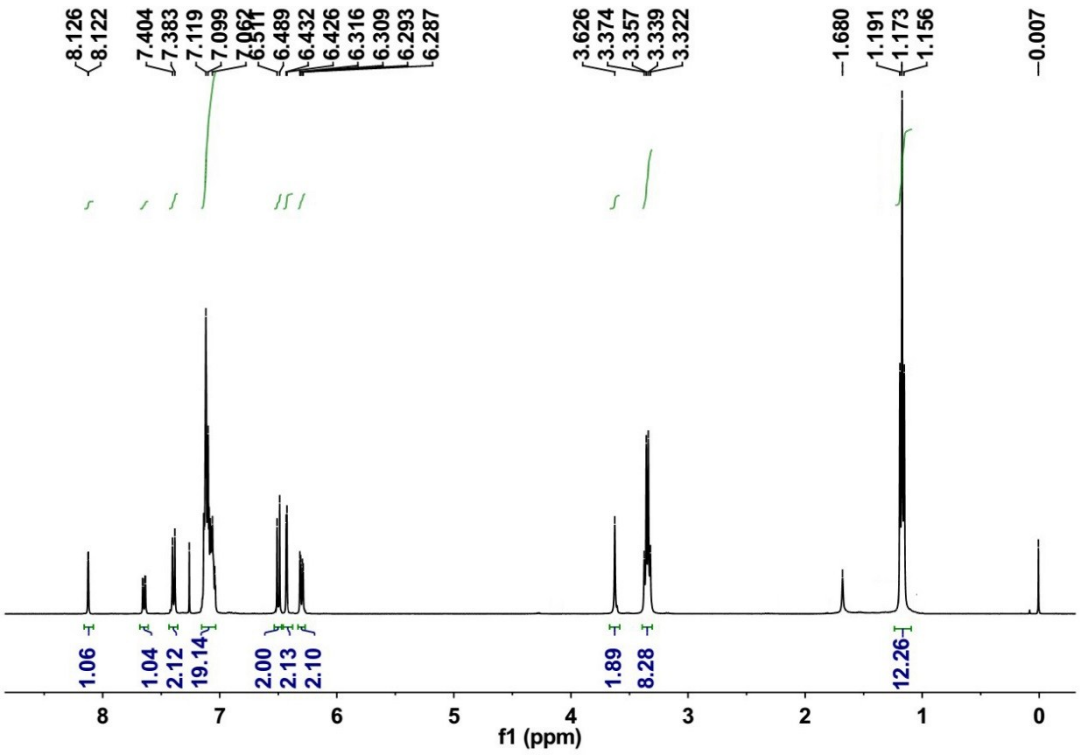



***Figure S5.*** $^{1}H$ NMR spectrum of *m*-TPE-RHZ in $CDCl_3$.

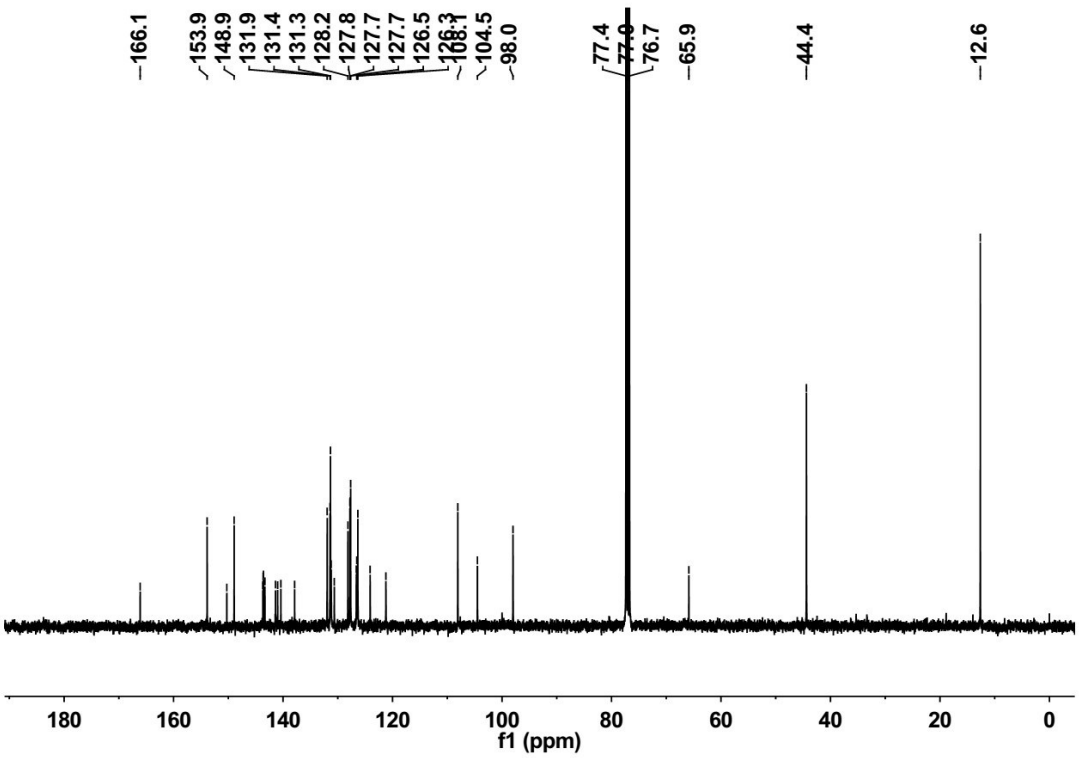



***Figure S6.*** $^{13}C$ NMR spectrum of *m*-TPE-RHZ in $CDCl_3$.

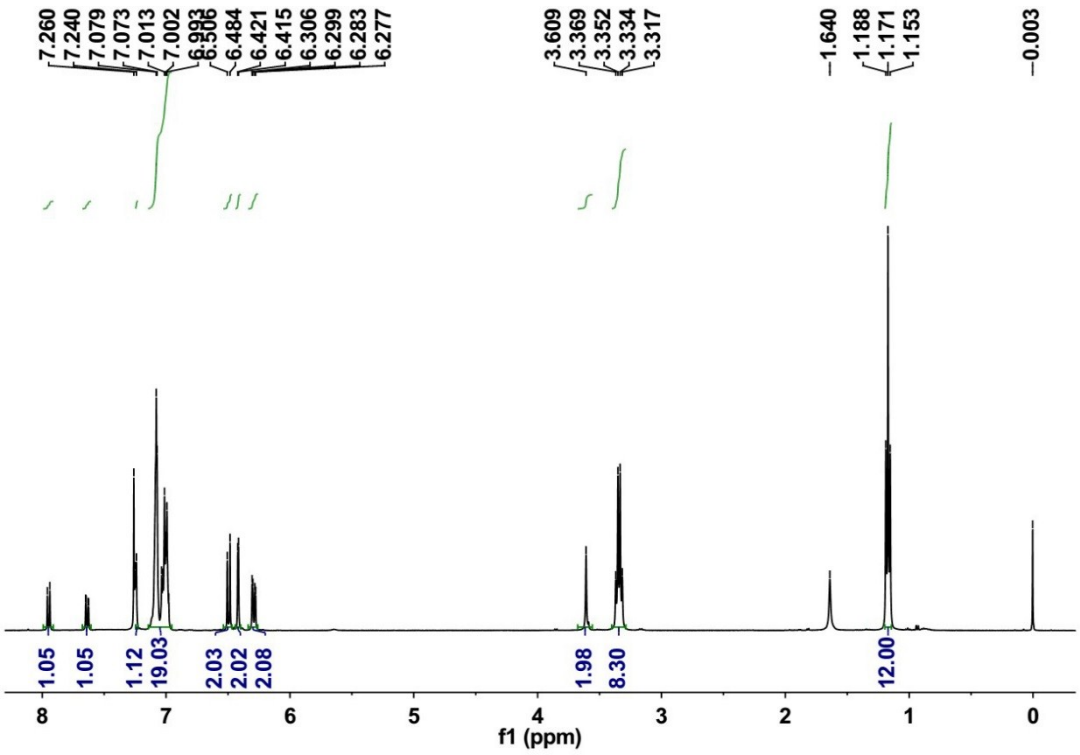



***Figure S7.*** $^{1}H$ NMR spectrum of *p*-TPE-RHZ in $CDCl_3$.

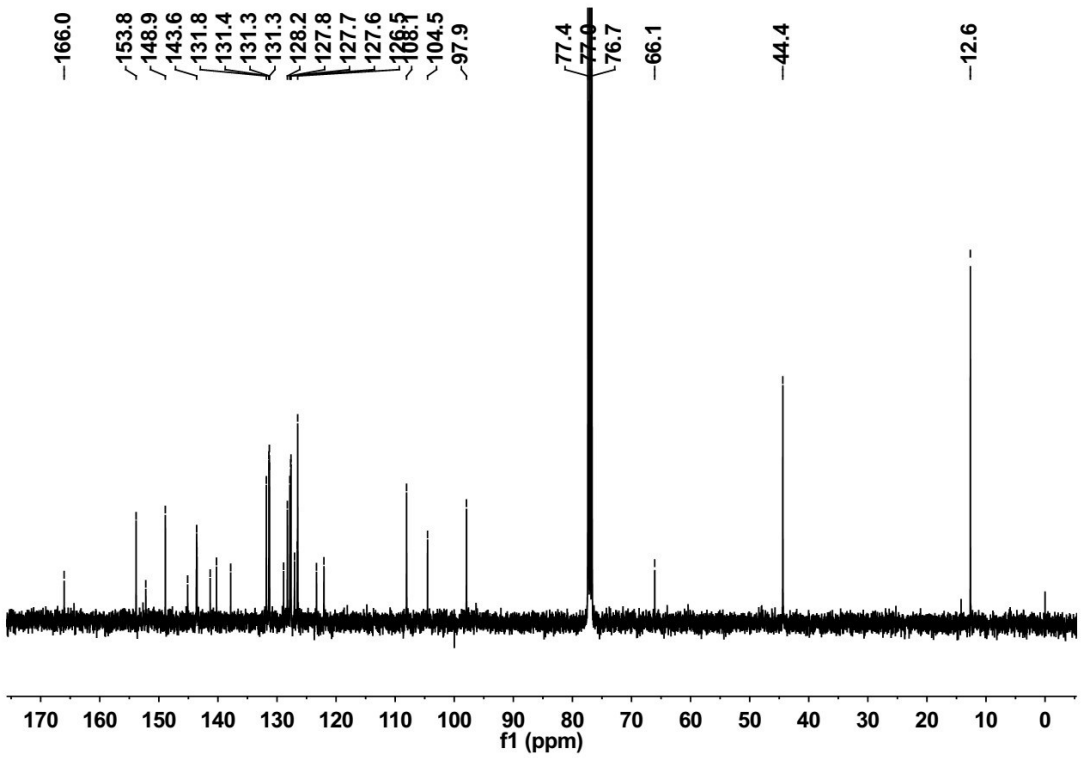



***Figure S8.*** $^{13}C$ NMR spectrum of *p*-TPE-RHZ in $CDCl_3$.

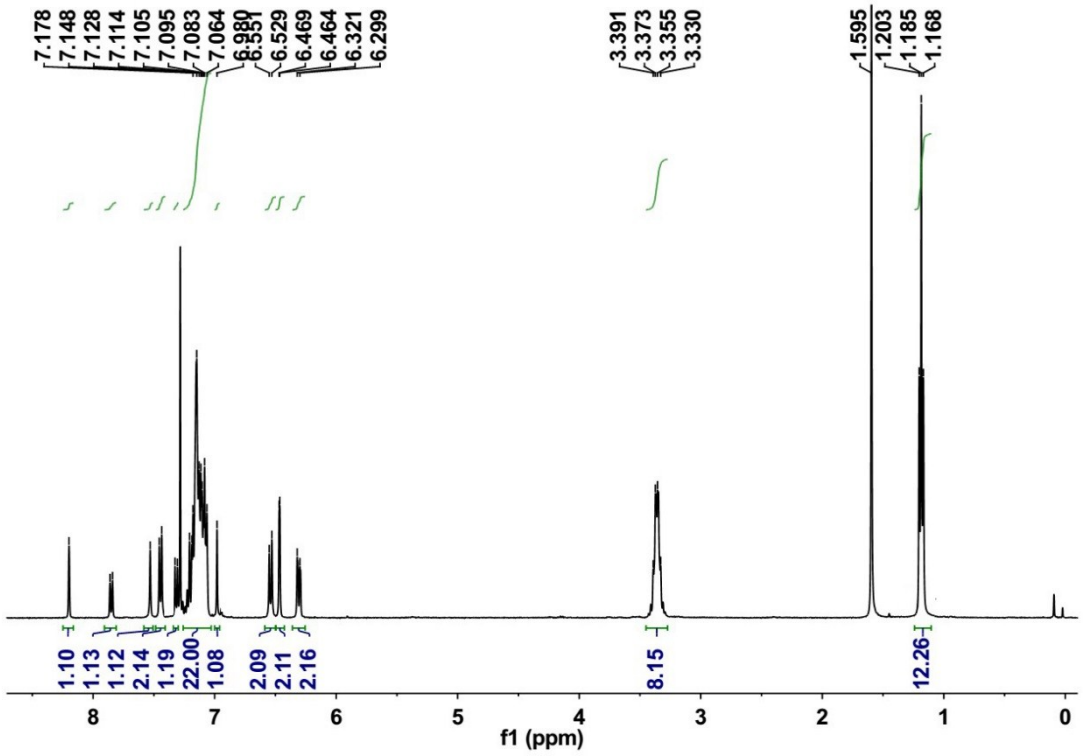



***Figure S9.*** $^{1}H$ NMR spectrum of *m*-TPE-RNS in $CDCl_3$.

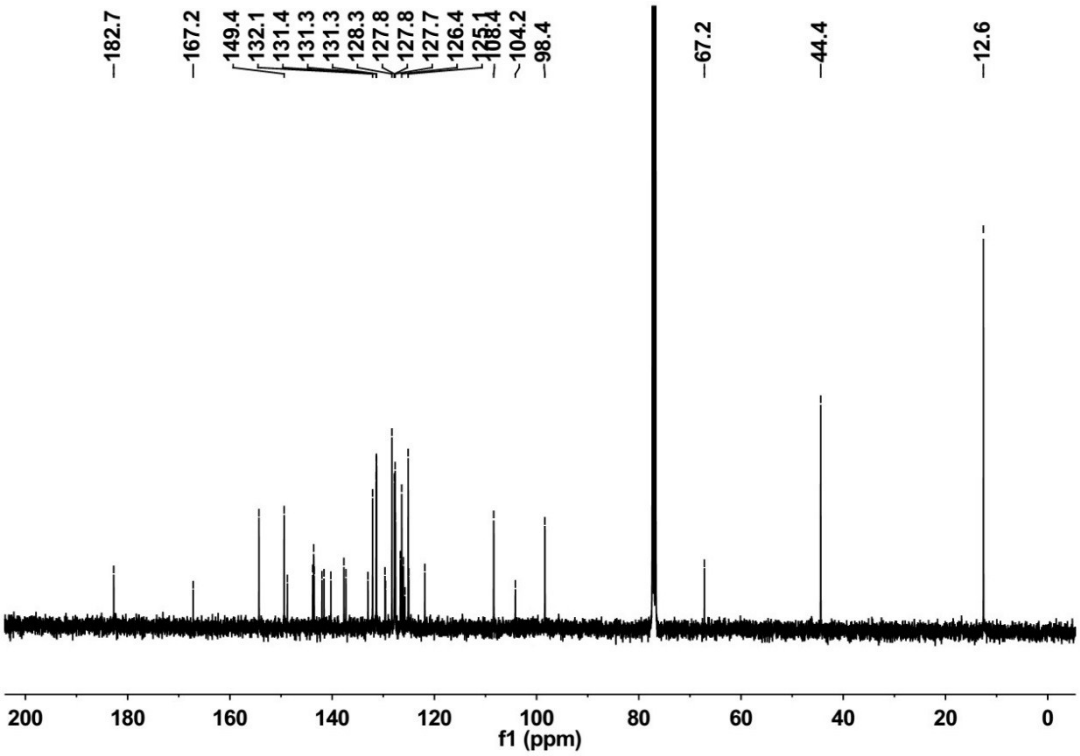



***Figure S10.*** $^{13}C$ NMR spectrum of *m*-TPE-RNS in $CDCl_3$.

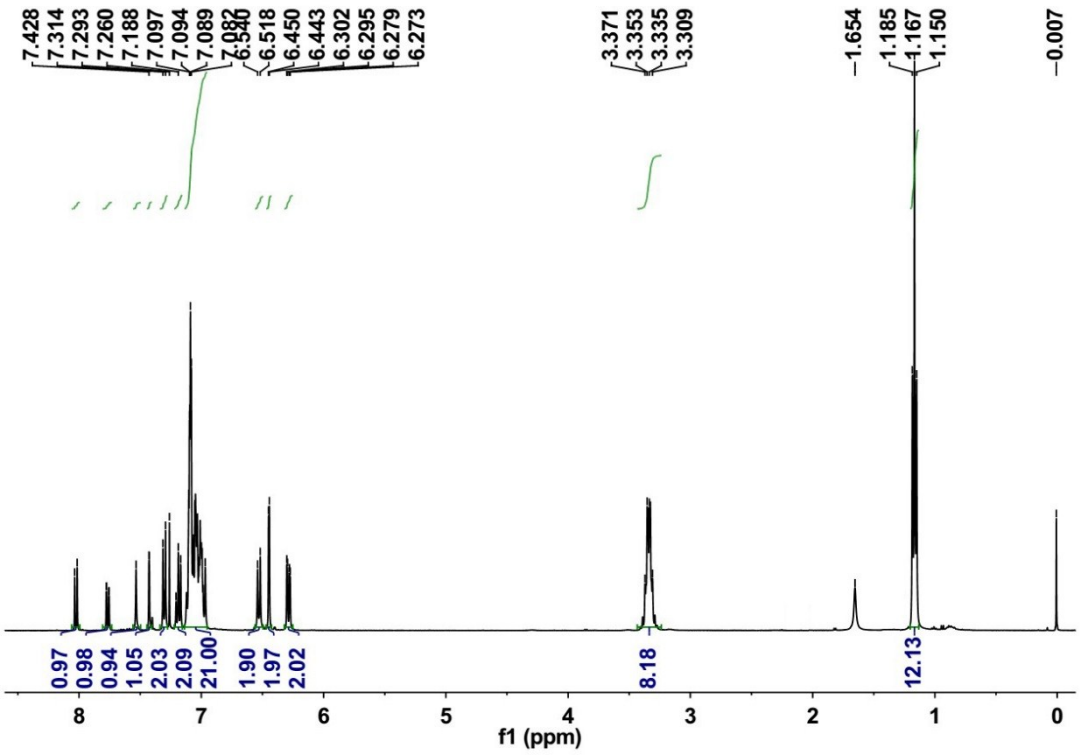



***Figure S11.*** $^{1}H$ NMR spectrum of *p*-TPE-RNS in $CDCl_3$.

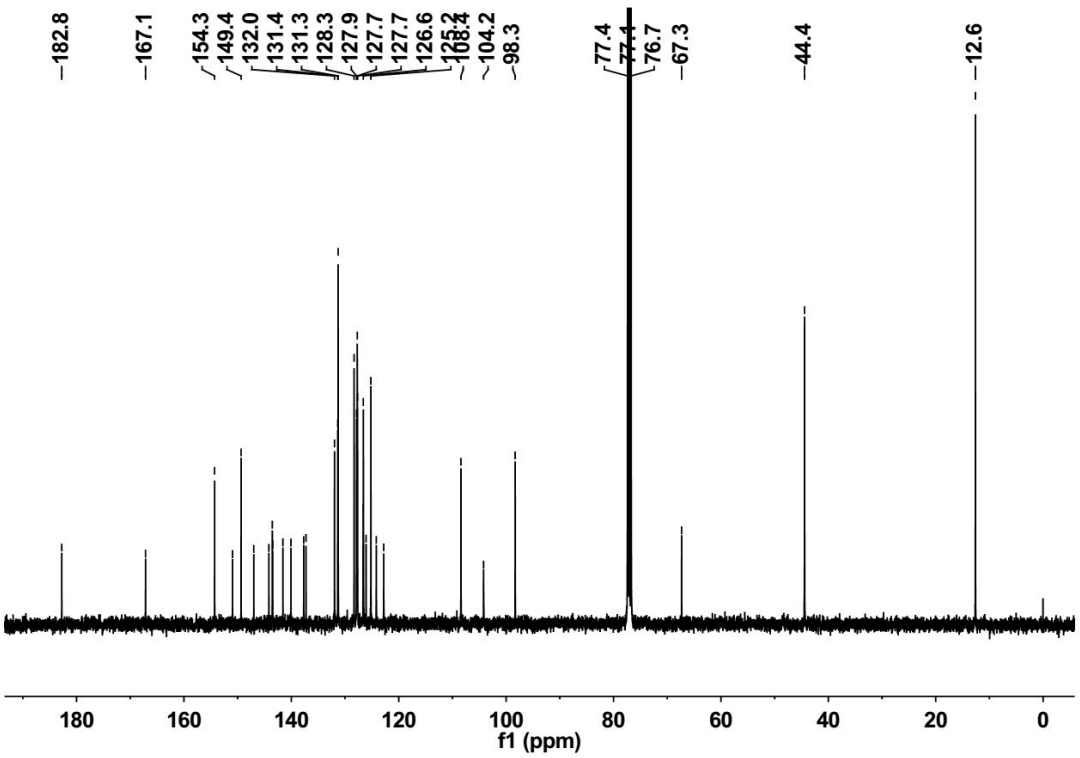



***Figure S12***. $^{13}C$ NMR spectrum of *p*-TPE-RNS in $CDCl_3$.

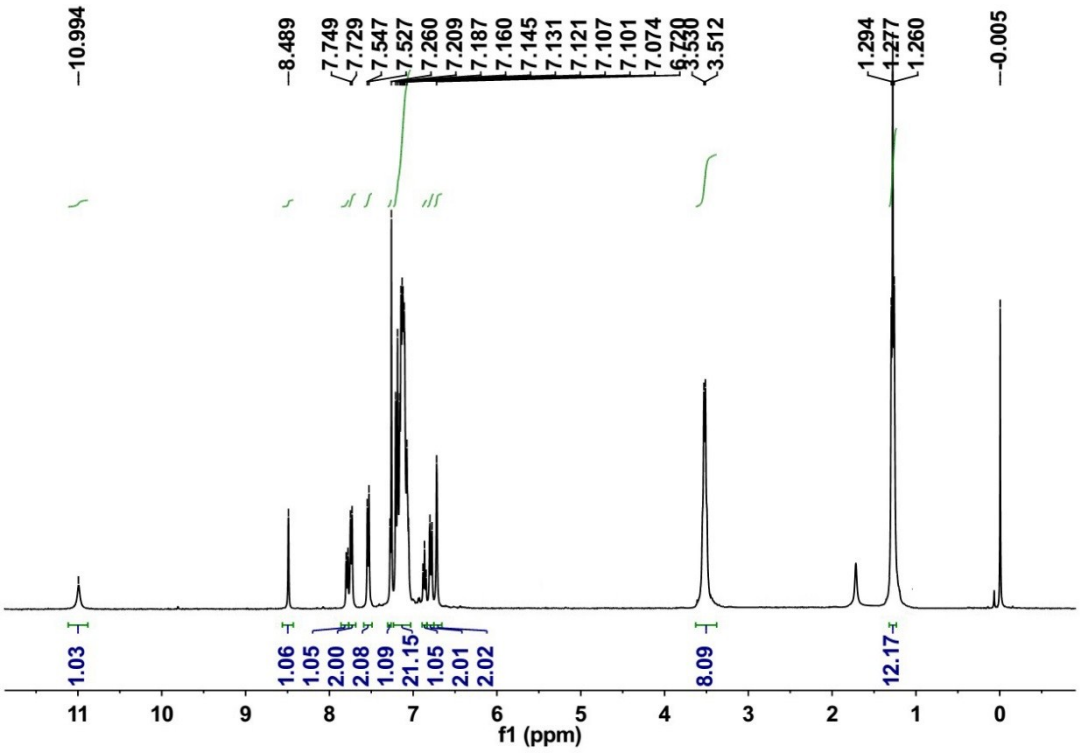



***Figure S13***. $^{1}H$ NMR spectrum of *m*-TPE-RNO in $CDCl_3$.

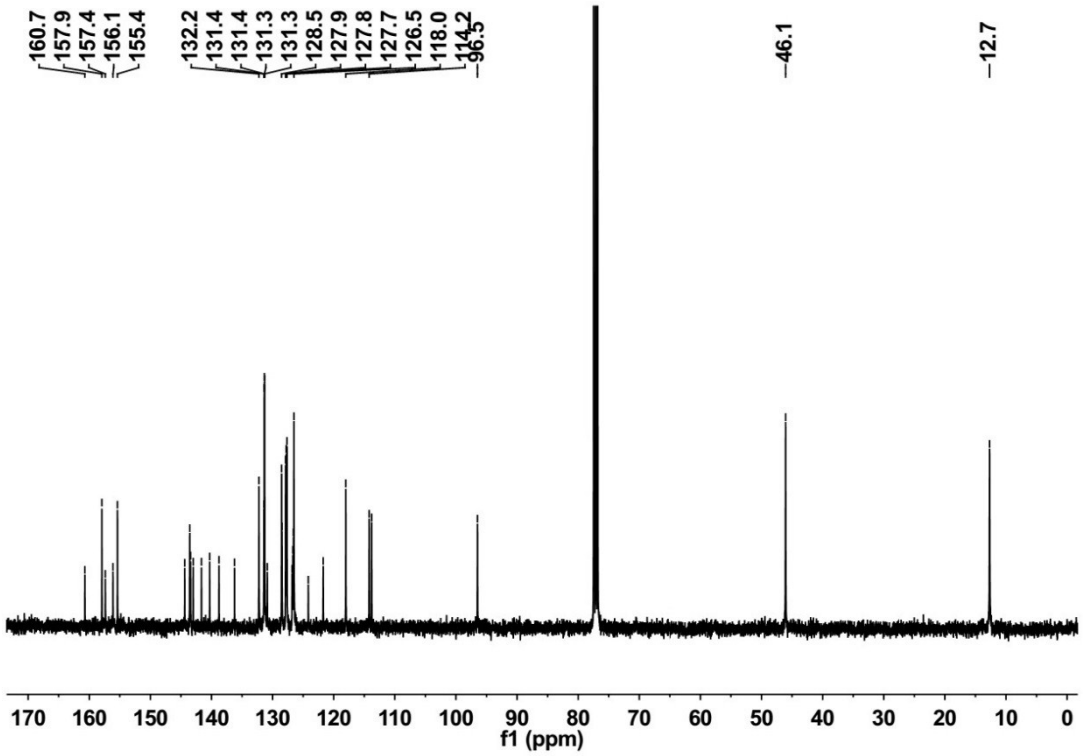



***Figure S14.*** $^{13}C$ NMR spectrum of *m*-TPE-RNO in $CDCl_3$.

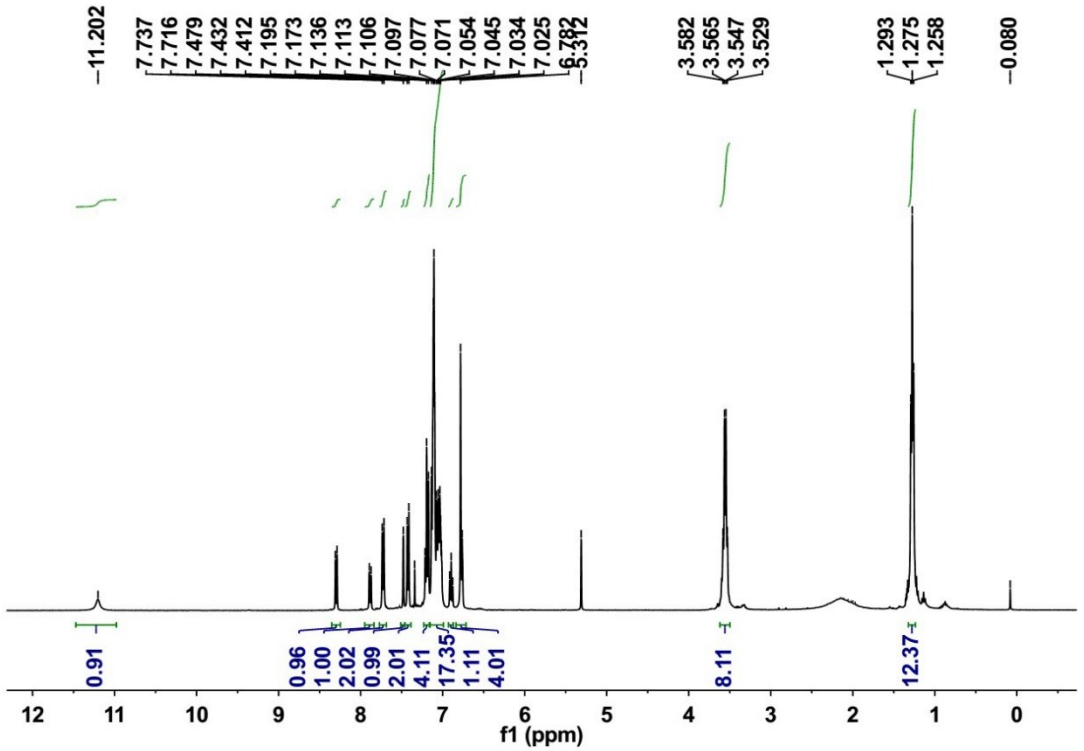



***Figure S15.*** $^{1}H$ NMR spectrum of *p*-TPE-RNO in $CD_2Cl_2$.

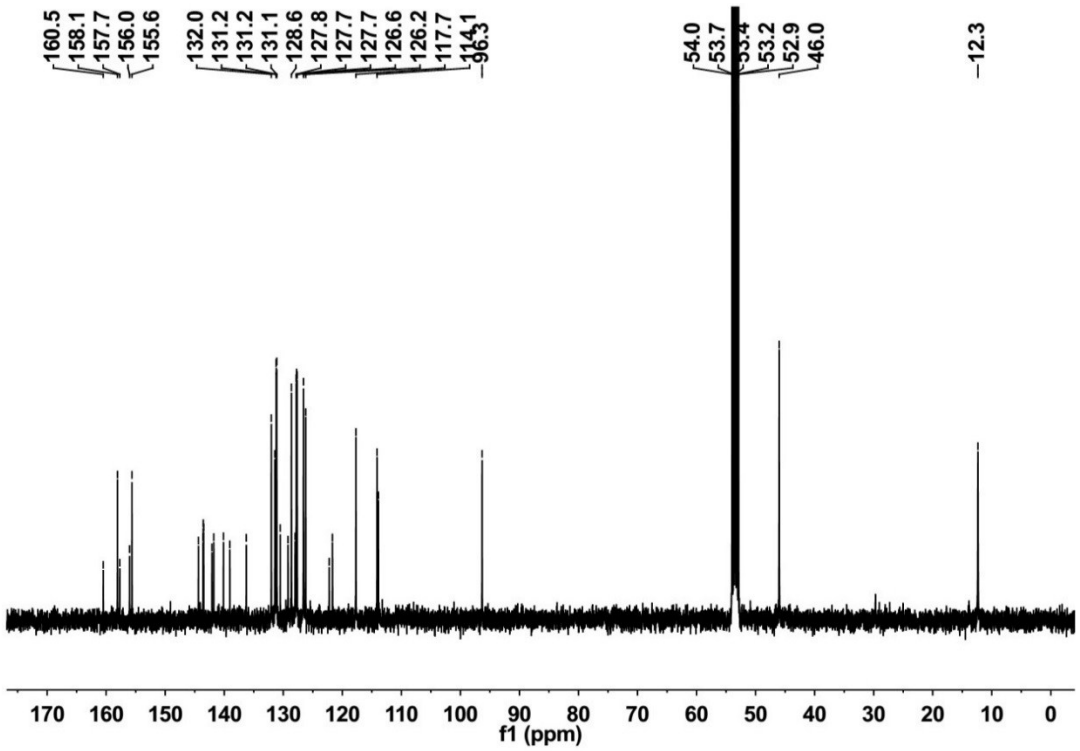



***Figure S16.*** $^{13}C$ NMR spectrum of *p*-TPE-RNO in $CD_2Cl_2$.

## 2. Theoretical Calculations

***Table S1.*** Selected parameters for the vertical excitation (UV-vis absorptions) of the named compounds Calculated by TDDFT//B3LYP/6-31G(d). (THF was employed as solvent in all the calculations). H stands for HOMO and L stands for LUMO, contributions were calculated from the CI coefficients of the wave functions for each excitations.

| Compounds | Electronic Transition | Energy | Oscillator Strength ( *f* ) | Composition | Contribution |
|---|---|---|---|---|---|
| RNO | $S_0 \rightarrow S_1$ | 2.347 eV | 0.0256 | H → L | 48.4% |
| | | | | H-1 → L | 51.6% |
| | $S_0 \rightarrow S_2$ | 2.615 eV | 0.9941 | H → L | 52.0% |
| | | | | H-1 → L | 48.0% |
| *p*-TPE-RNO | $S_0 \rightarrow S_1$ | 2.093 eV | 0.0017 | H → L | 92.7% |
| | $S_0 \rightarrow S_2$ | 2.377 eV | 0.0190 | H-1 → L | 52.6% |
| | | | | H-2 → L | 39.9% |
| | $S_0 \rightarrow S_3$ | 2.613 eV | 0.9558 | H-1 → L | 44.0% |
| | | | | H-2 → L | 56.0% |
| | $S_0 \rightarrow S_4$ | 3.035 eV | 0.8363 | H → L+1 | 96.7% |
| *m*-TPE-RNO | $S_0 \rightarrow S_1$ | 2.198 eV | 0.0296 | H → L | 97.7% |
| | $S_0 \rightarrow S_2$ | 2.360 eV | 0.0240 | H-1 → L | 43.4% |
| | | | | H-2 → L | 52.7% |
| | $S_0 \rightarrow S_3$ | 2.612 eV | 0.9649 | H-1 → L | 55.0% |
| | | | | H-2 → L | 45.0% |
| | $S_0 \rightarrow S_6$ | 3.241 eV | 0.4584 | H → L+1 | 92.6% |

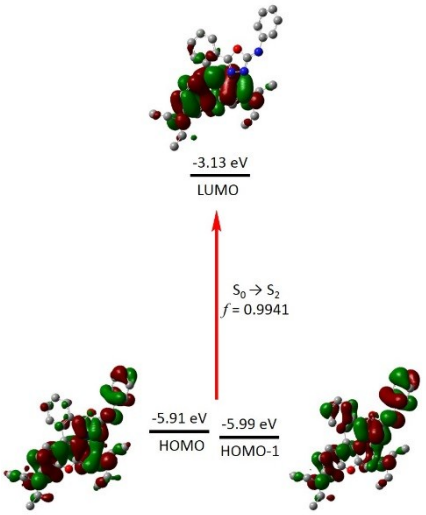



***Figure S17.*** Time dependent B3LYP/6-31G(d) calculated frontier molecular orbitals, energy levels, allowed electronic transition ($S_0$-$S_2$) and oscillator strength of RNO. Hydrogen atoms are omitted for clarity.

## 3. AIE Curves

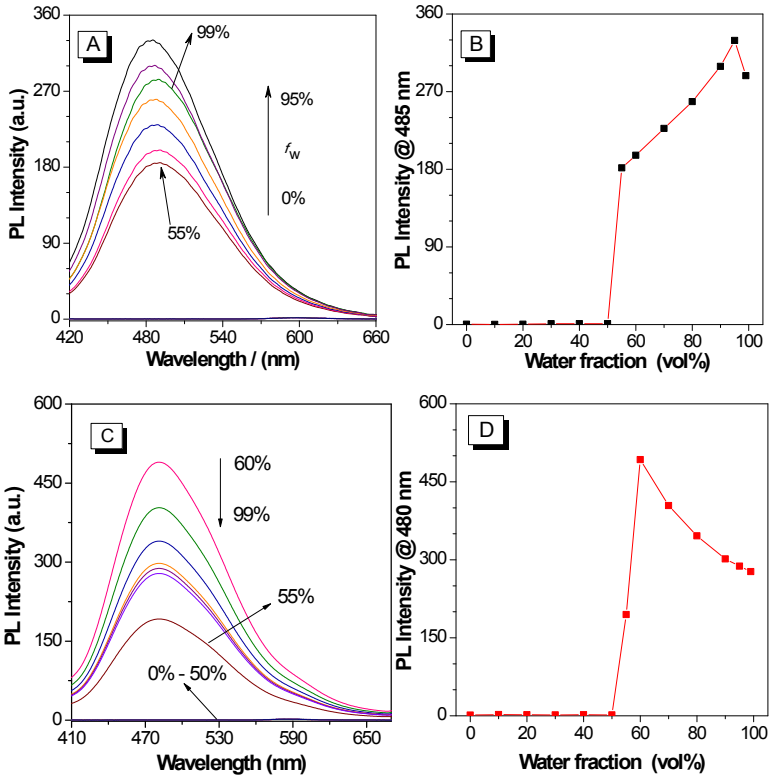



**Figure S18.** PL spectra of 10 μM *p*-TPE-RNS (A) and 10 μM *m*-TPE-RNS (C) in $CH_3CN$-water mixture with different water fraction; PL intensity profiles of 10 μM *p*-TPE-RNS (B) and 10 μM *m*-TPE-RNS (D) in $CH_3CN$-water mixture with different water fraction at 485 nm and 480 nm, respectively. Excitation, 355 nm.

## 4. Particle Size.

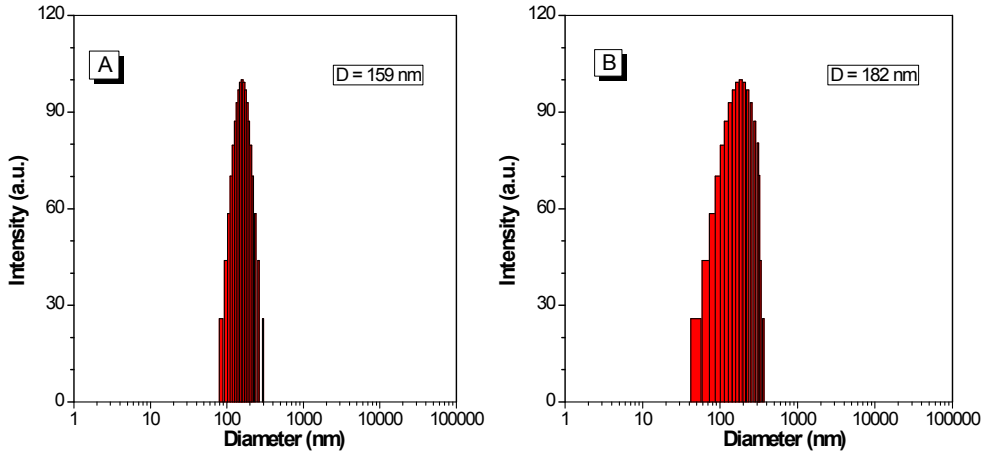



***Figure S19.*** Particle sizes of the nano-aggregates of 10 μM *m*-TPE-RNS (A) and 10 μM *p*-TPE-RNS (B) in $CH_3CN$-water mixture with 60% water fraction measured by DLS.

## 5. AIE Property and Response to $Hg^{2+}$

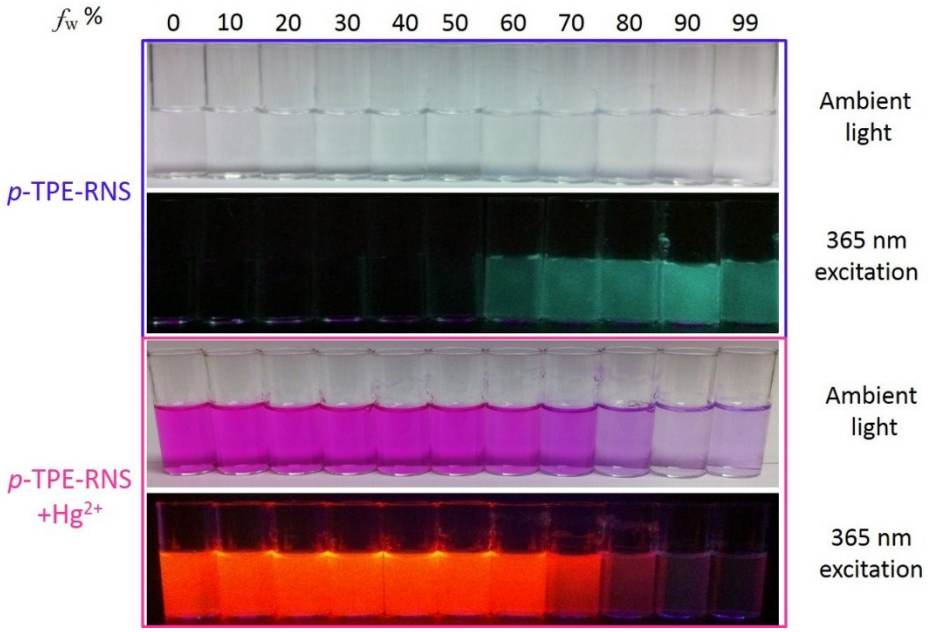



***Figure S20.*** Photos of 10 μM *p*-TPE-RNS in $CH_3CN$-water mixture with different water fraction in the absence (row 1, row 2) and presence (row 3, row 4) of 2 equiv. of $Hg^{2+}$. Row1, row 3: under ambient light; row2, row 4: under excitation of a UV lamp at 365 nm.

## 6. Detect limit

The spectra of free sensors (0.1 μM) in $CH_3CN$:$H_2O$ (3:7, v/v) mixture were collected for 10 times to determine the background noise $\sigma$. Then the solution was treated with various concentration of $Hg^{2+}$ from 0-10.0 ppb, all fluorescence spectra were collected after mixing for 30 s. Linear regression curves was then fitted according to the data in the range of [$Hg^{2+}$] from 0 to 3.0 ppb, and the slopes of the curves was obtained (Figure S21). The detection limits ($3\sigma$ /slope) were then determined to be 0.3 ppb and 1.2 ppb for *m*-TPE-RNS and *p*-TPE-RNS, respectively.

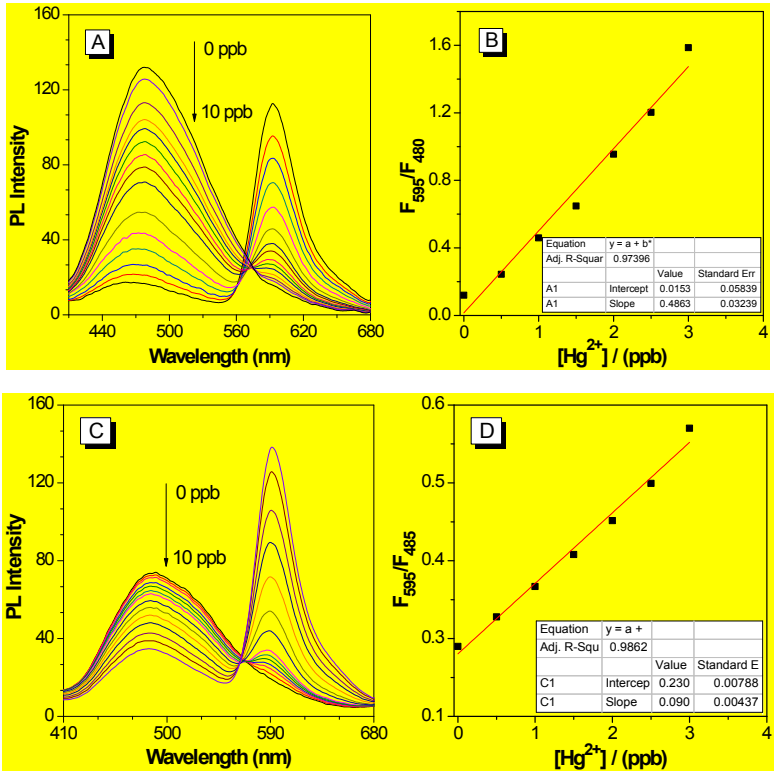



***Figure S21.*** Fluorescent spectra of 0.1 μM *m*-TPE-RNS (A) and *p*-TPE-RNS (C) in $CH_3CN$-water mixture with 70% water fraction in the presence of 0-10 ppb of $Hg^{2+}$. The linear fit plots of 0.1 μM *m*-TPE-RNS (B) and *p*-TPE-RNS (D) in the range of 0-3 ppb of $Hg^{2+}$.

## 7. Cell Viability Test

Cells were seeded in 96-well plates at density of 5000 cells/well. After overnight culture, medium in each wells were replaced by fresh medium containing different concentrations of *p*-TPE-RNS. After 24 hours treatment, into each well, 10 μL MTT solution (5 mg/mL in phosphate buffer solution) was added. After 4 hours incubation at 37 °C, 100 μL SDS-HCl solution (10% SDS and 0.01 M HCl) was added to each well. After 6 hours incubation at 37 °C, the absorbance of each wells at 570 nm was recorded by the plate reader (Perkin-Elmer Victor3™).

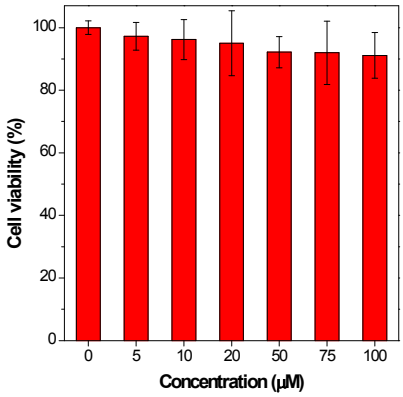



***Figure S22.*** Cytotoxicity of *p*-TPE-RNS evaluated on HeLa cells by MTT assay.

## References:

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