Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

A general strategy to develop cell membrane fluorescent probes with location- and target-specific fluorogenicities: a case of Zn^{2+} probe with cellular selectivity

Fei Deng,[a,b] Limin Liu,[a] Qinglong Qiao,[*,b] Chunfang Huang,[a] Lu Miao,[b] Zhaochao Xu [*,b]

[a]*School of Chemistry and Chemical Engineering, Jinggangshan University, Ji'an, Jiangxi 343009, China.*

[b]*Key Laboratory of Separation Science for Analytical Chemistry, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian 116023, China.*

[*]Corresponding author: Qinglong Qiao and Z. C. Xu, E-mail: qqlqiao@dicp.ac.cn and zcxu@dicp.ac.cn.

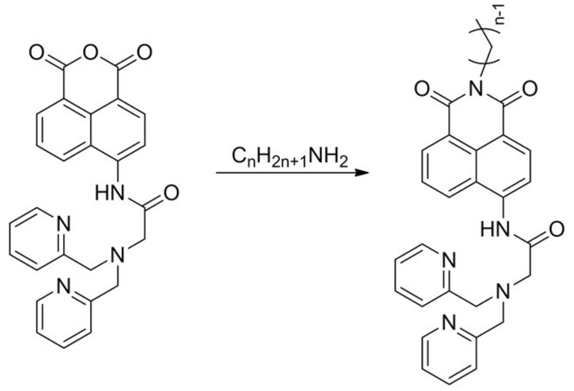
1. Materials and Instruments

Unless otherwise stated, all reagents and solvents for synthesis and detection were purchased from commercial suppliers and used without further purification. All water used was from a Millipore water purification system with a minimum resistivity of 18.0 MΩ cm.

^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker 400 spectrometer, using TMS as an internal standard. Chemical shifts were given in ppm and coupling constants (J) in Hz. Mass spectrometry data were obtained with a HP1100LC/MSD mass spectrometer and a LC/Q-TOF MS spectrometer. UV-Vis absorption spectra were collected on an Agilent Cary 60 UV-Vis Spectrophotometer. Fluorescence measurements were performed on an Agilent CARY Eclipse fluorescence spectrophotometer (Serial No.FL0812-M018). The distributions of particle size were recorded by the laser particle size analyzer (Zetasizer Nano, Malvern). The TEM imaging was performed by using JEM-2100 high revolution translation electron microscopy.

HT-29 cells were cultured in Dulbecco's modified Eagle's medium (DMEM, Invitrogen) containing 10% fetal bovine serum (FBS) under standard culture conditions (atmosphere of 5% CO_2 and 95% air at 37 ^{0}C) for 48 h before imaging performed by using ANDOR™ living cell laser scanning confocal microscope (Revolution WD).

2. Synthesis



n = 8: **ZTRS-C$_8$**
n = 10: **ZTRS-C$_{10}$**
n = 12: **ZTRS-C$_{12}$**
n = 14: **ZTRS-C$_{14}$**
n = 16: **ZTRS-C$_{16}$**
n = 18: **ZTRS-C$_{18}$**

1 **ZTRS-alkyl**

Scheme S1. Synthesis of Compounds **ZTRS-alkyl**.

Compound **1** was synthesized according to the previous literature[1].

Compound **1** (45 mg, 0.1 mmol) and corresponding alkylamine (0.2 mmol, 2 eq.) were dissolved in 20 mL ethanol. After stirred and refluxed for 8h, the solvent was removed under reduced pressure. The crude product was purified by column chromatography with CH_2Cl_2/CH_3OH (50:1) and afforded pure product.

ZTRS-C$_8$: 40 mg, 70% yield; ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.75 (s, 1H), 9.09 (d, J = 8.1 Hz, 1H), 8.68 (d, J = 6.7 Hz, 1H), 8.60 (dd, J = 30.9, 8.0 Hz, 2H), 8.46 (s, 2H), 7.84 (t, J = 7.2 Hz, 1H), 7.62 (s, 2H), 7.32 (d, J = 7.1 Hz, 2H), 7.16 (s, 2H), 4.17 (s, 2H), 4.08 (s, 4H), 3.63 (s, 2H), 1.74 (s, 2H), 1.27 (s, 10H), 0.87 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.80, 164.40, 163.78, 157.67, 149.60, 139.81, 136.73, 132.72, 131.06, 129.09, 128.19, 126.14, 123.54, 123.39, 123.10, 122.72, 117.73, 117.12, 60.67, 59.15, 40.44, 31.83, 29.36, 29.24, 28.16, 27.18, 22.64, 14.11; MS (ESI) m/z: calcd for $C_{34}H_{38}N_5O_3$ $[M+H]^+$ 564.2975, observed 564.2977.

ZTRS-C$_{10}$: 46 mg, 78% yield; ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.75 (s, 1H), 9.09 (d, J = 8.3 Hz, 1H), 8.68 (d, J = 7.0 Hz, 1H), 8.61 (dd, J = 30.3, 8.1 Hz, 2H), 8.46 (s, 2H), 7.84 (t, J = 7.6 Hz, 1H), 7.62 (t, J = 7.4 Hz, 2H), 7.32 (d, J = 7.5 Hz, 2H), 7.15 (s, 2H), 4.21 – 4.14 (m, 2H), 4.08 (s, 4H), 3.63 (s, 2H), 1.74 (s, 2H), 1.26 (s, 14H), 0.87 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.81, 164.42, 163.79, 157.67, 149.60, 139.81, 136.73, 132.74, 131.06, 129.10, 128.19, 126.14, 123.55, 123.38, 123.11, 122.72, 117.74, 117.13, 60.65, 59.13, 40.45, 31.90, 29.58, 29.41, 29.31, 28.17, 27.19, 22.69, 14.14; MS (ESI) m/z: calcd for $C_{36}H_{42}N_5O_3$ $[M+H]^+$ 592.3288, observed 592.3300.

ZTRS-C$_{12}$: 45 mg, 73% yield; ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.73 (s, 1H), 9.08 (d, J = 8.6 Hz, 1H), 8.69 (d, J = 7.2 Hz, 1H), 8.61 (dd, J = 23.5, 8.2 Hz, 2H), 8.45 (d, J = 4.2 Hz, 2H), 7.89 – 7.78 (m, 1H), 7.62 (td, J = 7.6, 1.6 Hz, 2H), 7.31 (d, J = 7.6 Hz, 2H), 7.15 (dd, J = 7.4, 5.0 Hz, 2H), 4.22 – 4.12 (m, 2H), 4.08 (s, 4H), 3.62 (s, 2H), 1.77 – 1.69 (m, 2H), 1.25 (s, 18H), 0.87 (t, J = 6.7 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.81, 164.42, 163.80, 157.66, 149.61, 139.80, 136.74, 132.73, 131.07, 129.11, 128.19, 126.15, 123.57, 123.40, 123.12, 122.73, 117.77, 117.18, 60.70, 59.14, 40.45, 31.92, 29.65, 29.63, 29.58, 29.42, 29.35, 28.17, 27.19, 22.69, 14.13; MS (ESI) m/z: calcd for $C_{38}H_{46}N_5O_3$ $[M+H]^+$ 620.3601, observed 620.3599.

ZTRS-C$_{14}$: 48 mg, 74% yield; ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.73 (s, 1H), 9.08 (d, J = 8.5 Hz, 1H), 8.68 (d, J = 7.2 Hz, 1H), 8.60 (dd, J = 27.5, 8.3 Hz, 2H), 8.45 (d, J = 4.5 Hz, 2H), 7.88 – 7.80 (m, 1H), 7.62 (td, J = 7.6, 1.4 Hz, 2H), 7.32 (d, J = 7.7 Hz, 2H), 7.15 (dd, J = 6.9, 5.3 Hz, 2H), 4.20 – 4.13 (m, 2H), 4.08 (s, 4H), 3.62 (s, 2H), 1.76 – 1.71 (m, 2H), 1.25 (s, 22H), 0.87 (t, J = 6.7 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.81, 164.41, 163.79, 157.66, 149.60, 139.80, 136.75, 132.72, 131.07, 129.09, 128.19, 126.15, 123.56, 123.40, 123.11, 122.73, 117.76, 117.17, 60.69, 59.14, 40.45, 31.92, 29.70, 29.66, 29.63, 29.58, 29.42, 29.36, 28.17, 27.19, 22.69, 14.14; MS (ESI) m/z: calcd for $C_{40}H_{50}N_5O_3$ $[M+H]^+$ 648.3914, observed 648.3930.

ZTRS-C$_{16}$: 48 mg, 71% yield; ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.76 (s, 1H), 9.09 (d, J = 8.5 Hz, 1H), 8.69 (d, J = 7.2 Hz, 1H), 8.62 (dd, J = 28.3, 8.3 Hz, 2H), 8.45 (d, J = 4.6 Hz, 2H), 7.84 (t, J = 7.9 Hz, 2H), 7.62 (dd, J = 10.9, 4.4 Hz, 2H), 7.31 (d, J = 7.7 Hz, 2H), 7.15 (dd, J = 7.0, 5.2 Hz, 2H), 4.21 – 4.15 (m, 2H), 4.08 (s, 4H), 3.62 (s, 2H), 1.73 (d, J = 7.3 Hz, 2H), 1.25 (s, 26H), 0.88 (t, J = 6.7 Hz, 3H); ^{13}C NMR (101

MHz, $CDCl_3$) δ 170.79, 164.41, 163.79, 157.68, 149.61, 139.81, 136.72, 132.73, 131.05, 129.10, 128.18, 126.13, 123.55, 123.38, 123.12, 122.71, 117.75, 117.13, 60.68, 59.15, 40.45, 31.93, 29.70, 29.42, 29.36, 28.18, 27.20, 22.70, 14.13; MS (ESI) m/z: calcd for $C_{42}H_{54}N_5O_3$ $[M+H]^+$ 676.4227, observed 676.4246.

ZTRS-C_{18}: according to previous reports.[1]

3. Supplementary Figures

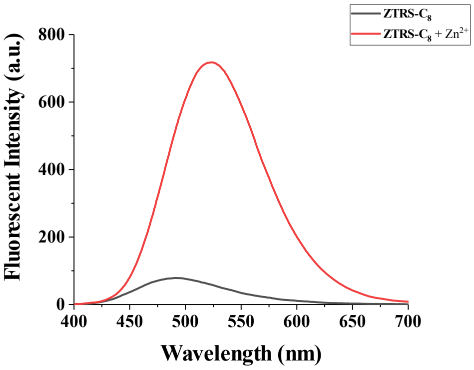


Fig. S1 Fluorescence spectra of 10 μM **ZTRS-C_8** in the presence of 30 μM Zn^{2+} in 20 mM HEPES (pH = 7.4).

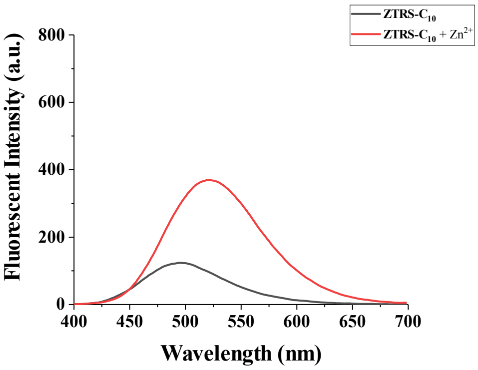


Fig. S2 Fluorescence spectra of 10 μM **ZTRS-C_{10}** in the presence of 30 μM Zn^{2+} in 20 mM HEPES (pH = 7.4).

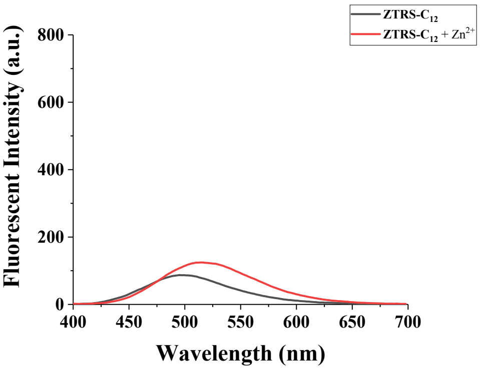


Fig. S3 Fluorescence spectra of 10 μM **ZTRS-C_{12}** in the presence of 30 μM Zn^{2+} in 20 mM HEPES (pH = 7.4).

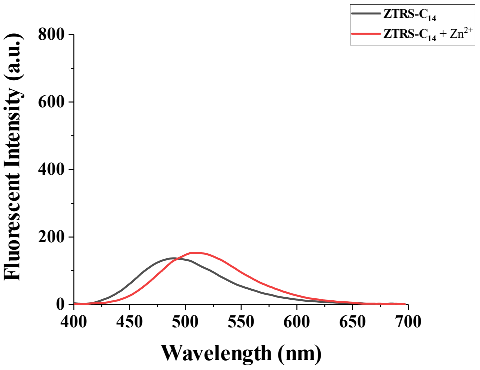


Fig. S4 Fluorescence spectra of 10 μM **ZTRS-C_{14}** in the presence of 30 μM Zn^{2+} in 20 mM HEPES (pH = 7.4).

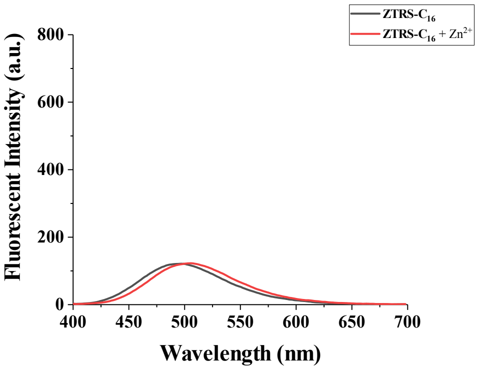


Fig. S5 Fluorescence spectra of 10 μM **ZTRS-C_{16}** in the presence of 30 μM Zn^{2+} in 20 mM HEPES (pH = 7.4).

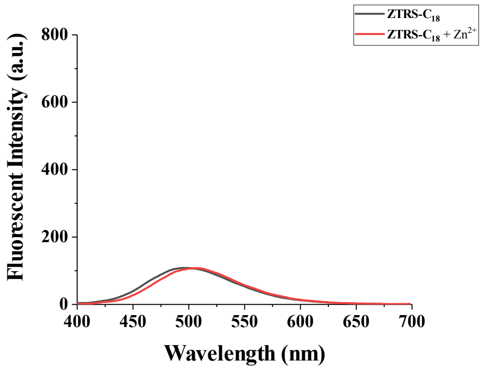


Fig. S6 Fluorescence spectra of 10 μM **ZTRS-C_{18}** in the presence of 30 μM Zn^{2+} in 20 mM HEPES (pH = 7.4).

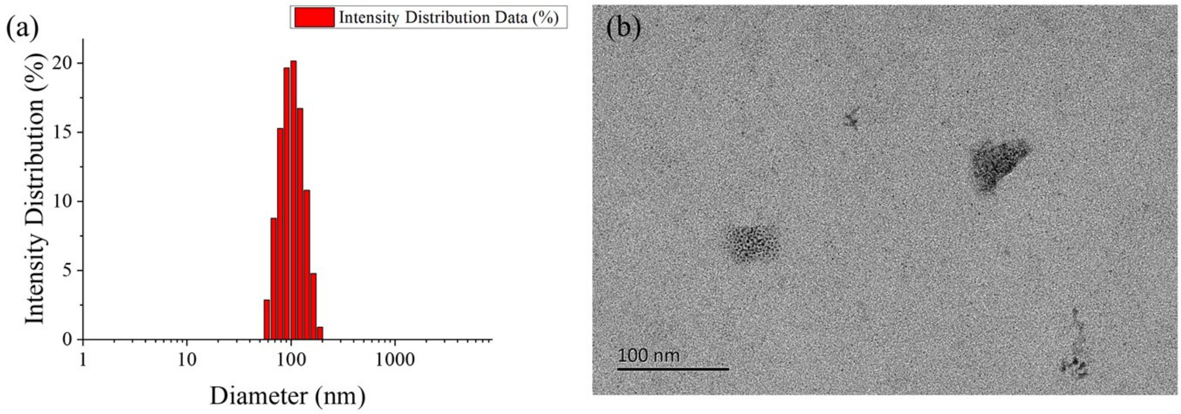


Fig. S7 (a) DLS analysis of particle-size distribution of the self-assembled **ZTRS-C_{12}** (10 μM) in 20 mM HEPES (pH 7.4). (b) TEM image of the self-assembled **ZTRS-C_{12}**.

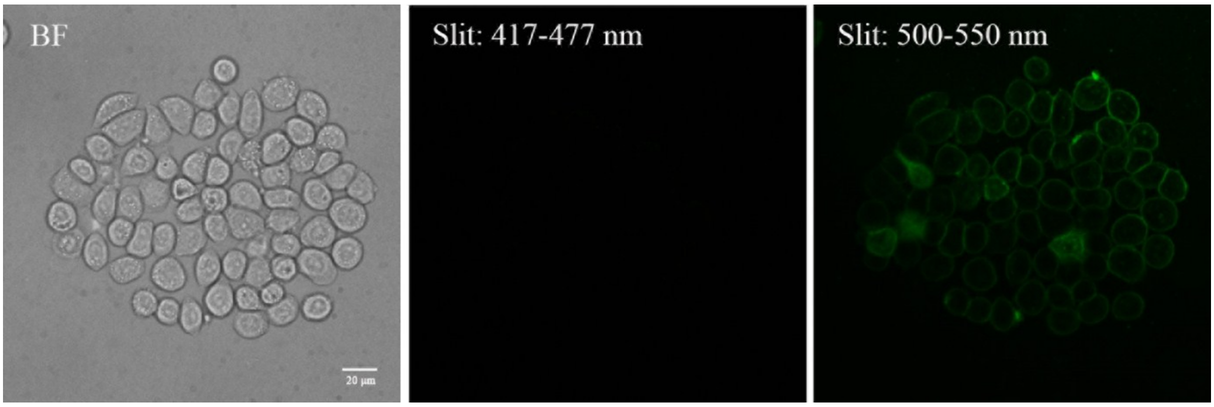


Fig. S8 Fluorescence imaging of **ZTRS-C_{12}-Zn(II)** at the plasma membrane of HT-29 cells. (a) Bright field; (b) Ex: 405 nm, Slit: 417-477 nm; (c) Ex: 405 nm, Slit: 500-550 nm. Scale bar: 20 μm.

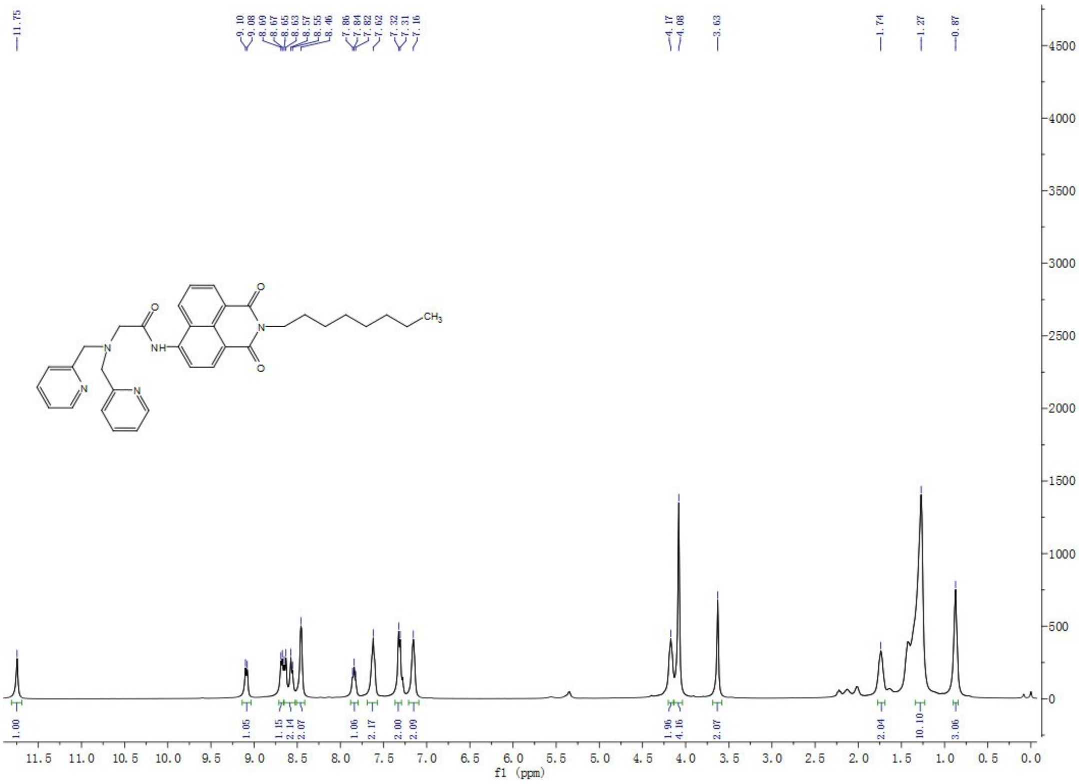


Fig. S9 ^{1}H NMR spectra of **ZTRS-C$_8$** in CDCl$_3$.

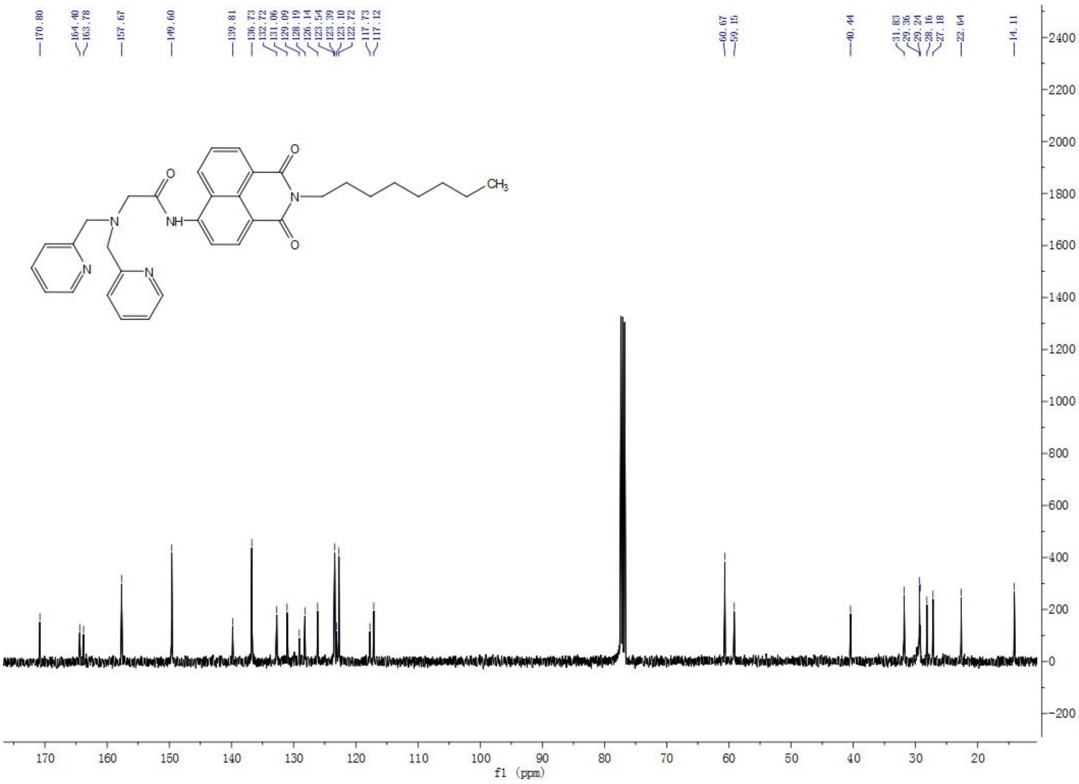


Fig. S10 ^{13}C NMR spectra of **ZTRS-C$_8$** in CDCl$_3$.

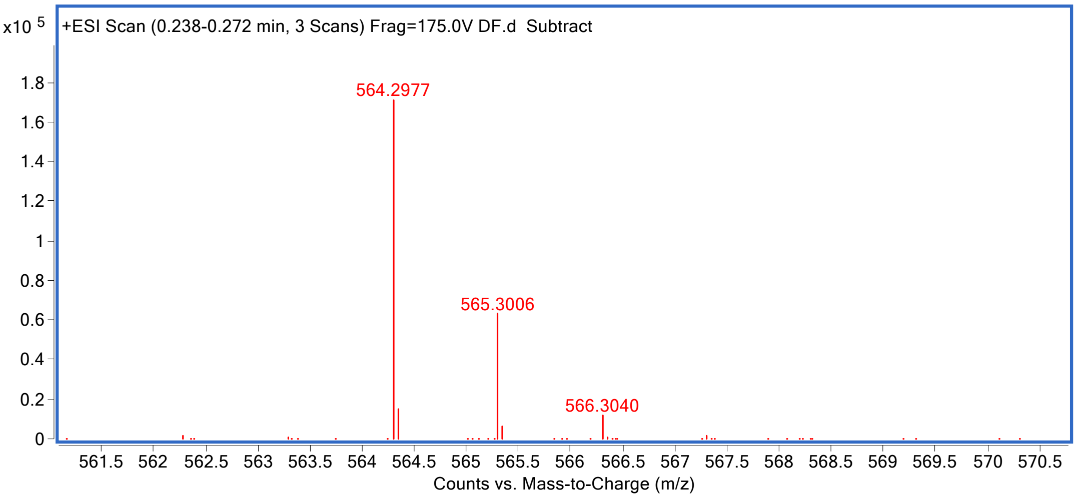


Fig. S11 MS spectrum of **ZTRS-C$_8$**.

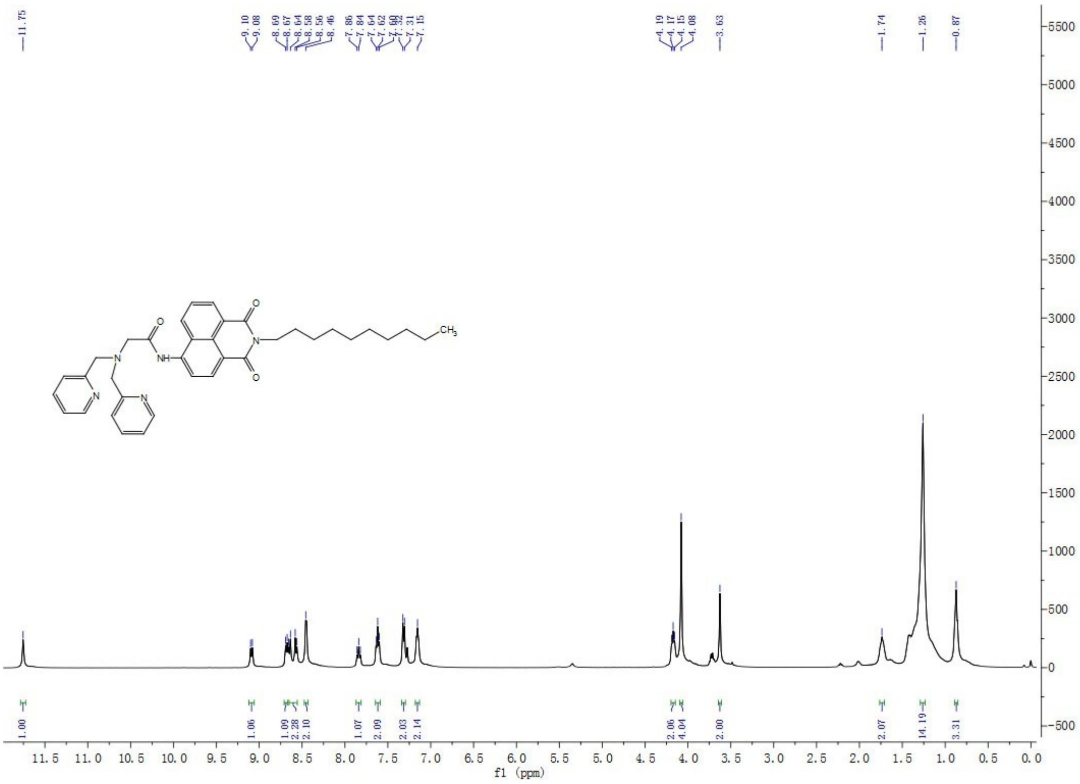


Fig. S12 ^{1}H NMR spectra of **ZTRS-C$_{10}$** in CDCl$_3$.

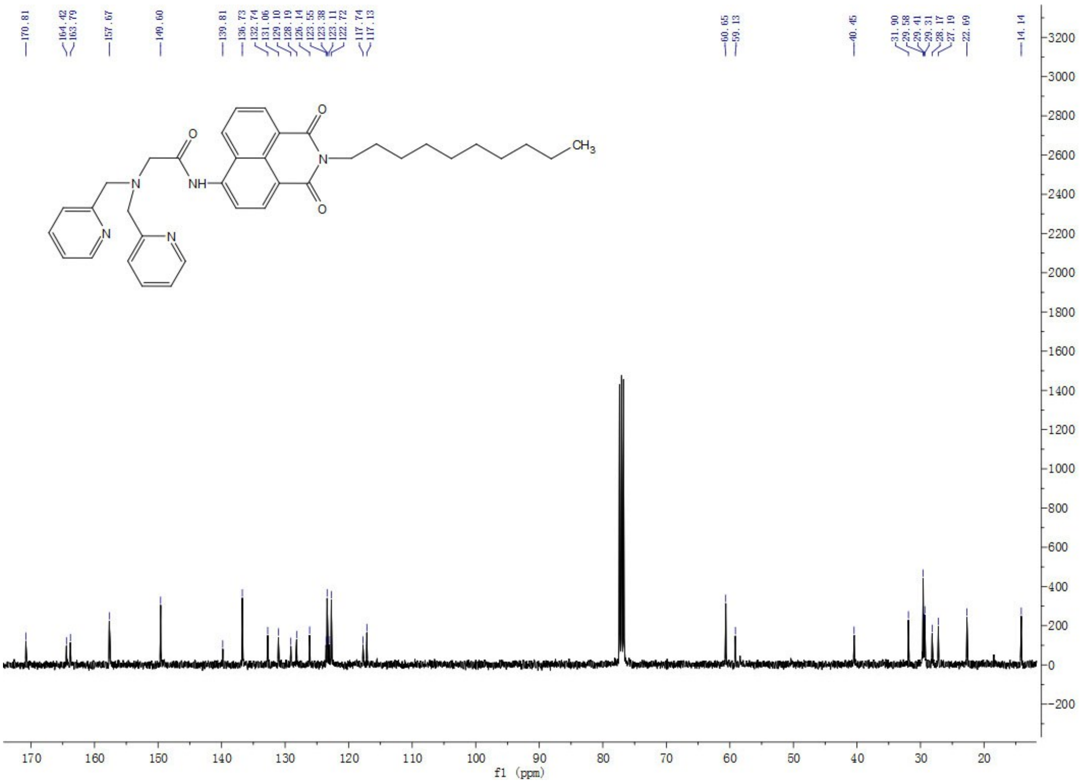


Fig. S13 ^{13}C NMR spectra of **ZTRS-C$_{10}$** in CDCl$_3$.

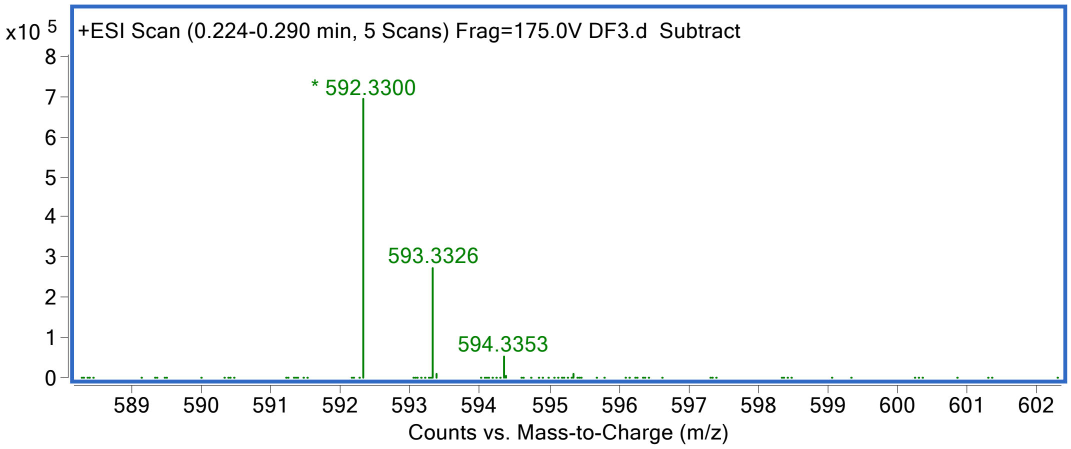


Fig. S14 MS spectrum of **ZTRS-C$_{10}$**.

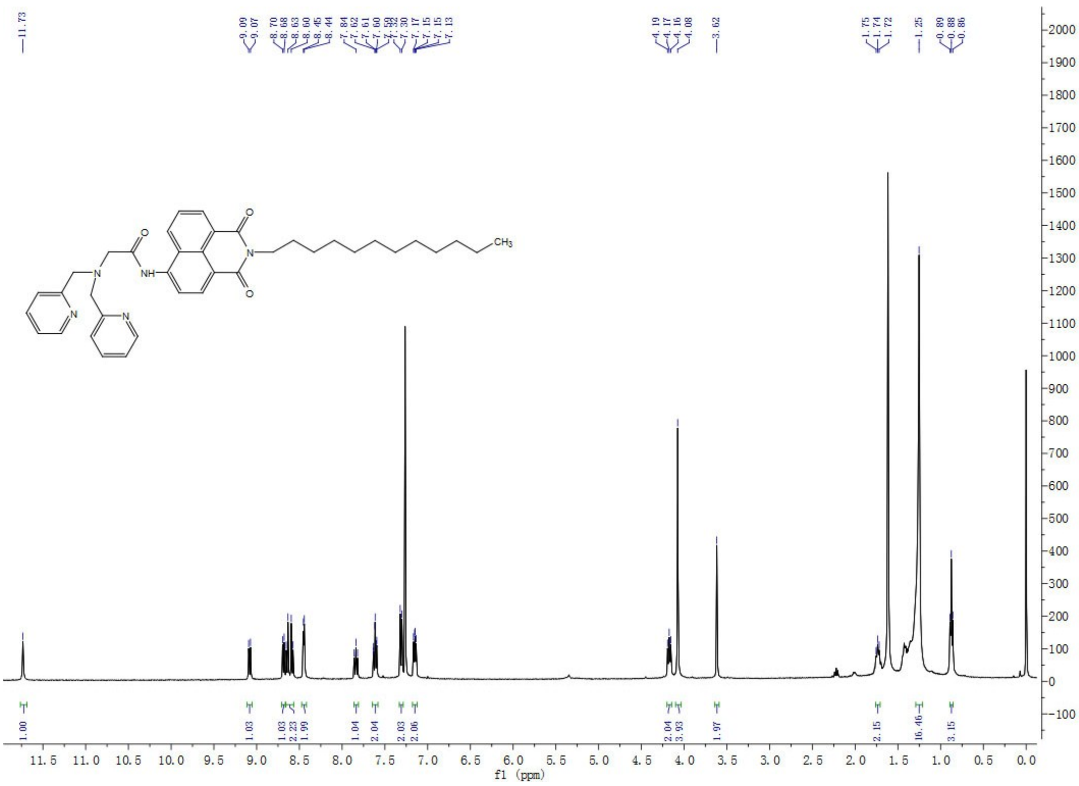


Fig. S15 ^{1}H NMR spectra of **ZTRS-C$_{12}$** in CDCl$_3$.

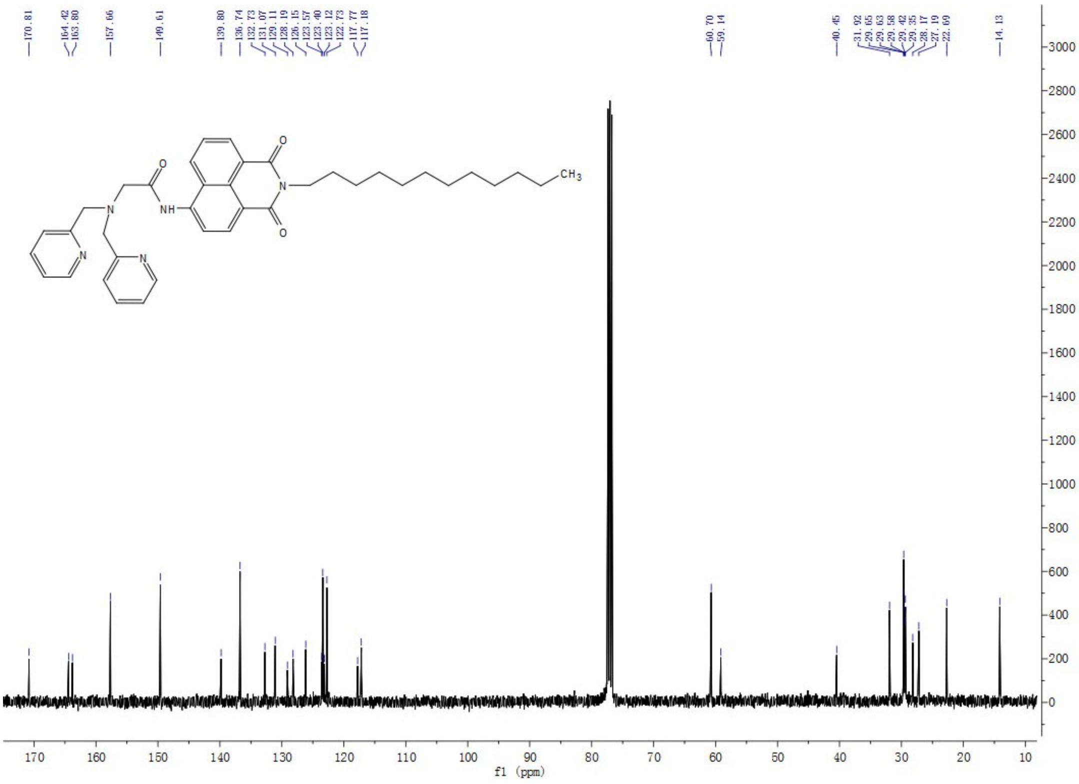


Fig. S16 ^{13}C NMR spectra of **ZTRS-C$_{12}$** in CDCl$_3$.

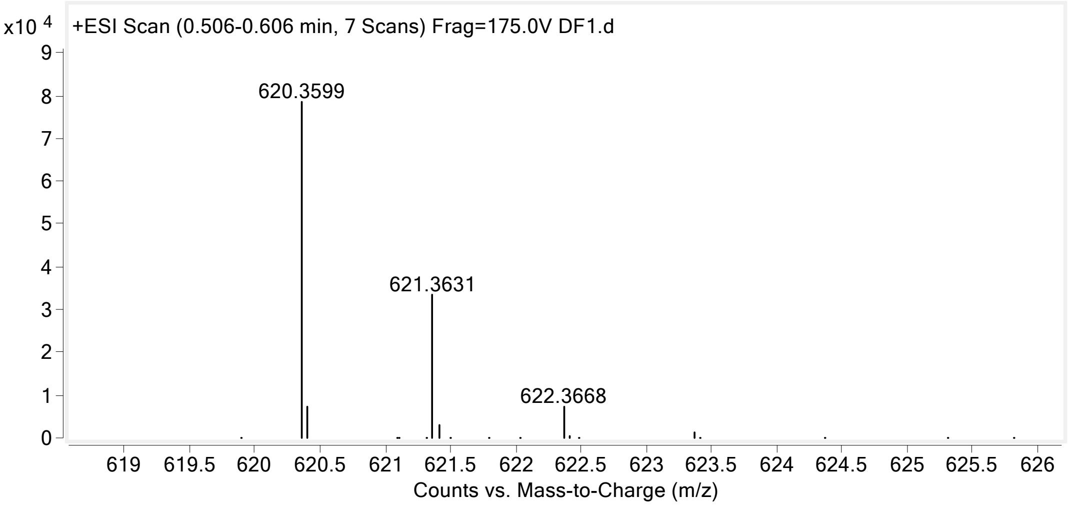


Fig. S17 MS spectrum of **ZTRS-C_{12}**.

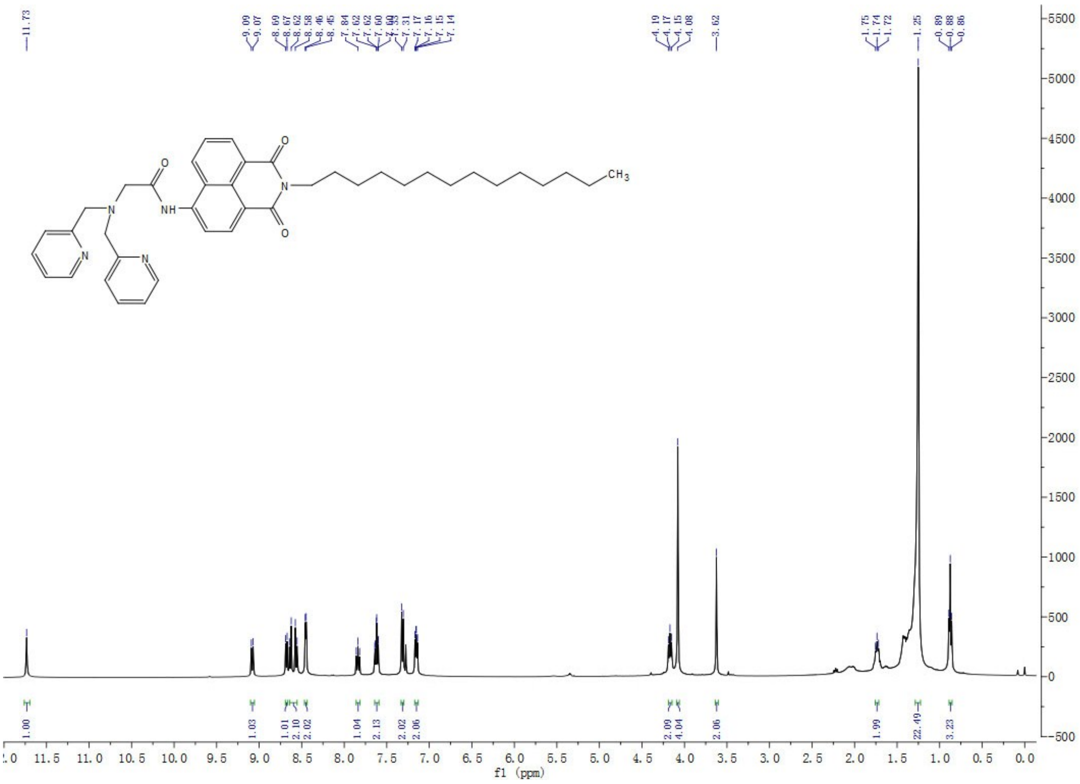


Fig. S18 ^{1}H NMR spectra of **ZTRS-C_{14}** in $CDCl_3$.

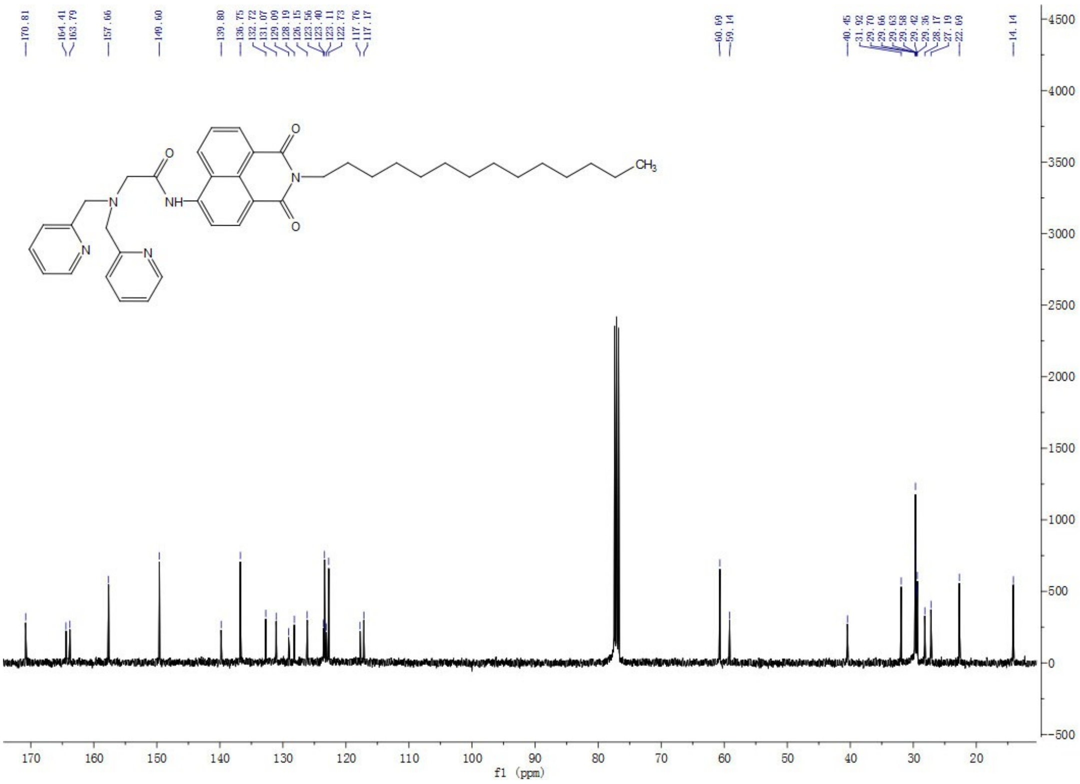


Fig. S19 ^{13}C NMR spectra of **ZTRS-C$_{14}$** in CDCl$_3$.

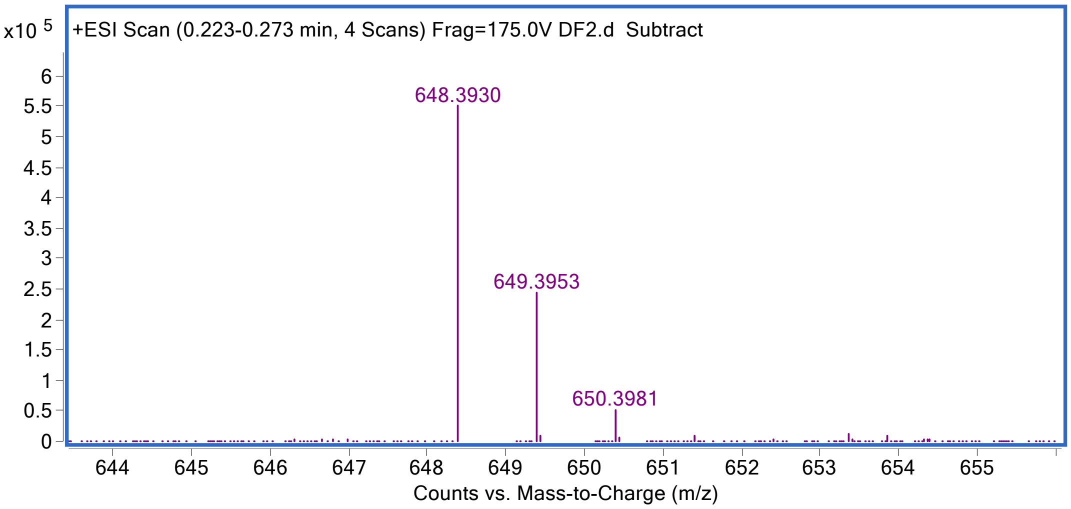


Fig. S20 MS spectrum of **ZTRS-C$_{14}$**.

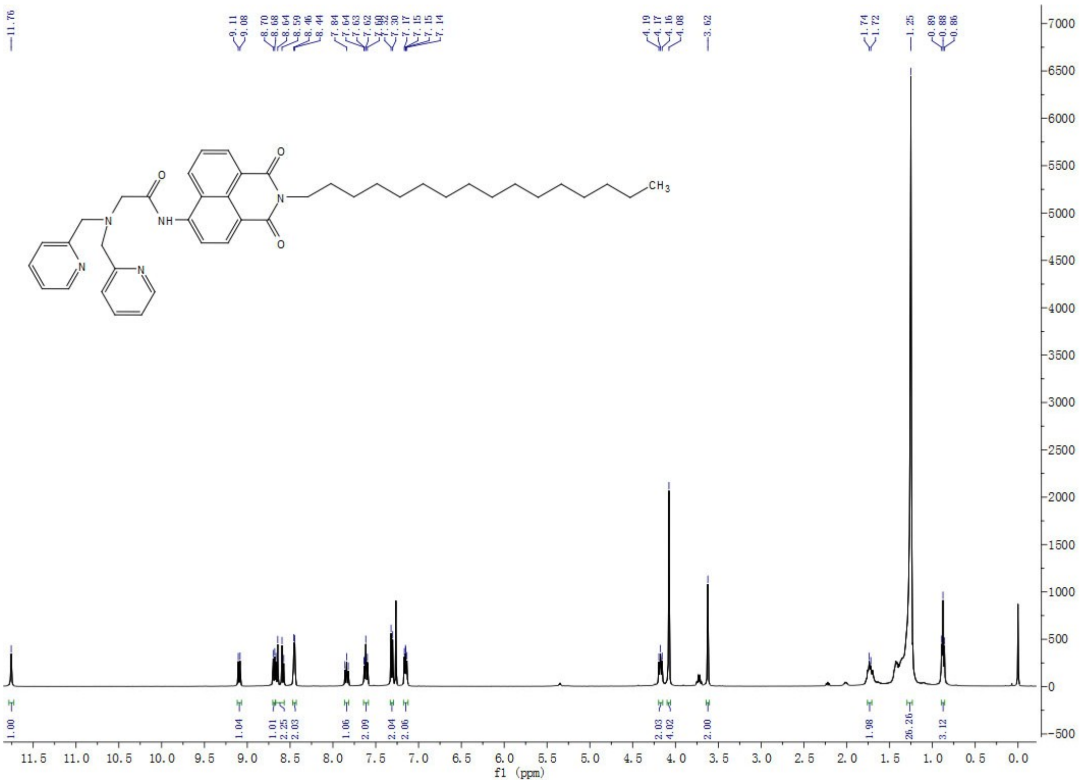


Fig. S21 ^{1}H NMR spectra of **ZTRS-C$_{16}$** in CDCl$_3$.

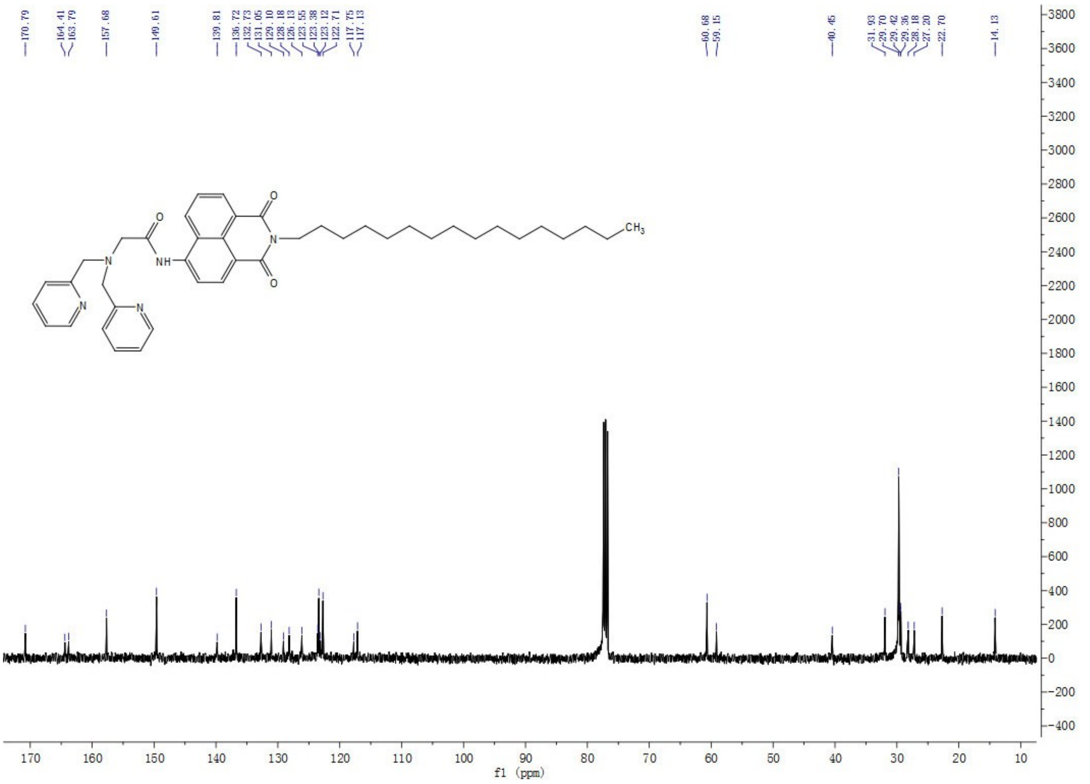


Fig. S22 ^{13}C NMR spectra of **ZTRS-C$_{16}$** in CDCl$_3$.

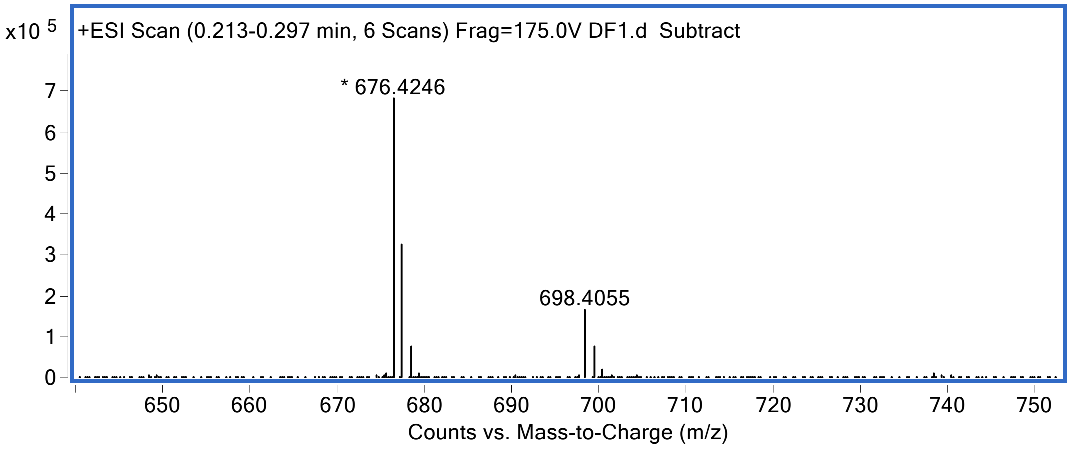


Fig. S23 MS spectrum of **ZTRS-C$_{16}$**.

4. References

1. F. Deng, S. S. Long, Q. L. Qiao and Z. C. Xu, *Chem. Commun.*, **2018**, 54, 6157.