Supramolecular coordination complexes (SCCs) with aggregation-induced emission for *in vitro* photodynamic therapy

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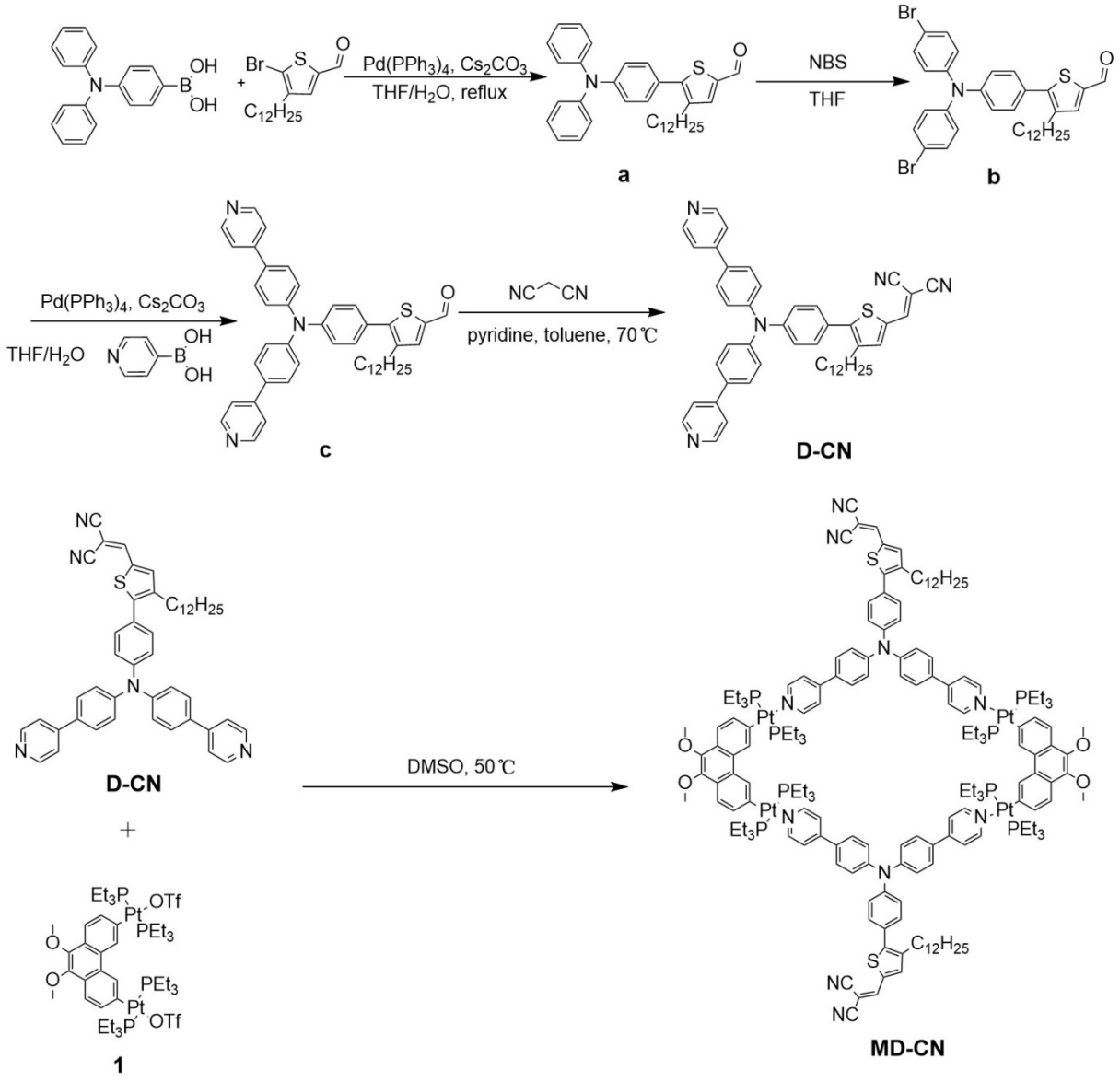
Supporting Information

1. Materials and methods

Alkyl thiophenal was purchased from Nanjing Zhiyan Technology Co., LTD. All other reagents were purchased from Energy Chemical Company and used without further purification. Dulbecco's Modified Eagle Medium (DMEM) medium and Penicillin-Streptomycin were purchased from Gibco-BRL (Burlington, Canada). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), trypsine-EDTA, Calf serum (CS) and Dio were purchased from Biomics Biotechnologies Co. Ltd. (Nantong, Jiangsu, China). Reaction Oxygen Species Assay Kit and Calcein/PI Live/Dead Viability were purchased from Beyotime Biotechnology (Shanghai, China).

^{1}H, ^{13}C, and ^{31}P NMR spectra were collected on a Bruker Advance 500 MHz spectrometer with $CDCl_3$ as deuterated solvent. Mass spectra were recorded on an Agilent Technologies 6530 Accurat-Mass Q-TOF LC/MS instrument. UV-vis absorption spectra were performed on a Hitachi U-5300 absorption spectrophotometer. The fluorescent emission spectra were conducted on an Edinburgh FLS 980 or Hitachi F-7000 fluorescence spectrophotometer. Transmission electron microscopy (TEM) was performed on a Hitachi S-4800 and images were recorded on a Hitachi F-7700. Electron spin resonance (ESR) test was performed with JEOL JES-FA200. Cytotoxicity test data were obtained using a Perlong-DNM-9602 plate Reader. Flow cytometry was measured on CytoFLEX S. Cellular fluorescence images were taken using a Nexcope inverted fluorescence microscope and a ZEISS LSM 710 confocal laser scanning microscope (CLSM).

2. Synthetic procedures and characterization data



Scheme S1 Synthetic routes of **D-CN** and **MD-CN**.

2.1 Synthesis of a

A mixture of 5-bromothiophene-2-carbaldehyde (0.20 g, 0.56 mmol), triphenyl-amino boronic acid (0.18 g, 0.61 mmol), cesium carbonate (0.36 g, 1.12 mmol), [$Pd(PPh_3)_4$] (0.06 g, 0.05 mmol), THF (18 mL), and water (2 mL) was degassed for 0.5 h and heated to reflux for 12 h under an argon atmosphere. After cooling down, the solvent was removed with rotary evaporator, and the residue was extracted with CH_2Cl_2 for three times. The organic layer was dried over $MgSO_4$ and evaporated. The residue was purified by chromatography (silica gel, CH_2Cl_2/PE, 1:8, *v*/*v*) to get compound **a**. ^{1}H NMR (500 MHz, $CDCl_3$, 298K) δ (ppm): 9.83 (s, 1H), 7.63 (s, 1H), 7.31−7.27 (m, 6H), 7.16−7.14 (m, 4H), 7.09−7.07 (m, 4H). ^{13}C {^{1}H} NMR (126 MHz, $CDCl_3$, 298K) δ (ppm): 182.7, 148.9, 148.4, 147.2, 140.4, 139.7, 138.8, 129.8, 129.5, 126.5, 125.1, 123.7, 122.2, 31.9, 30.8, 29.7, 29.7, 29.6, 29.5, 29.4, 28.7, 22.7, 14.2.

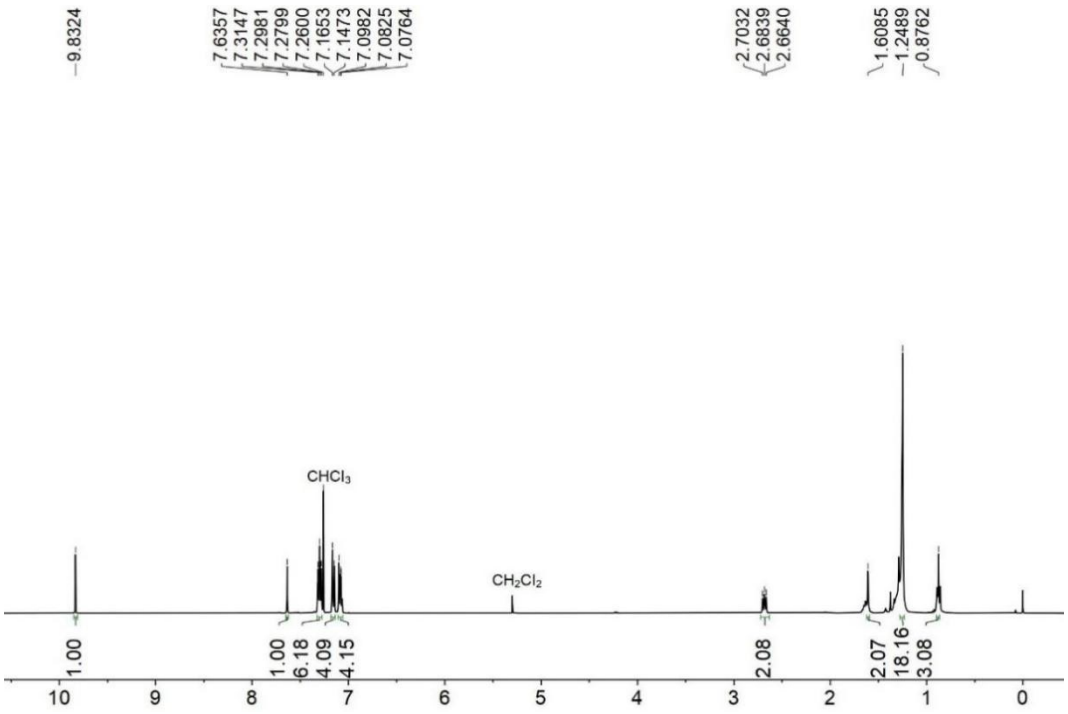


Figure S1 ^{1}H NMR spectrum (500 MHz, $CDCl_3$, 298 K) of **a**.

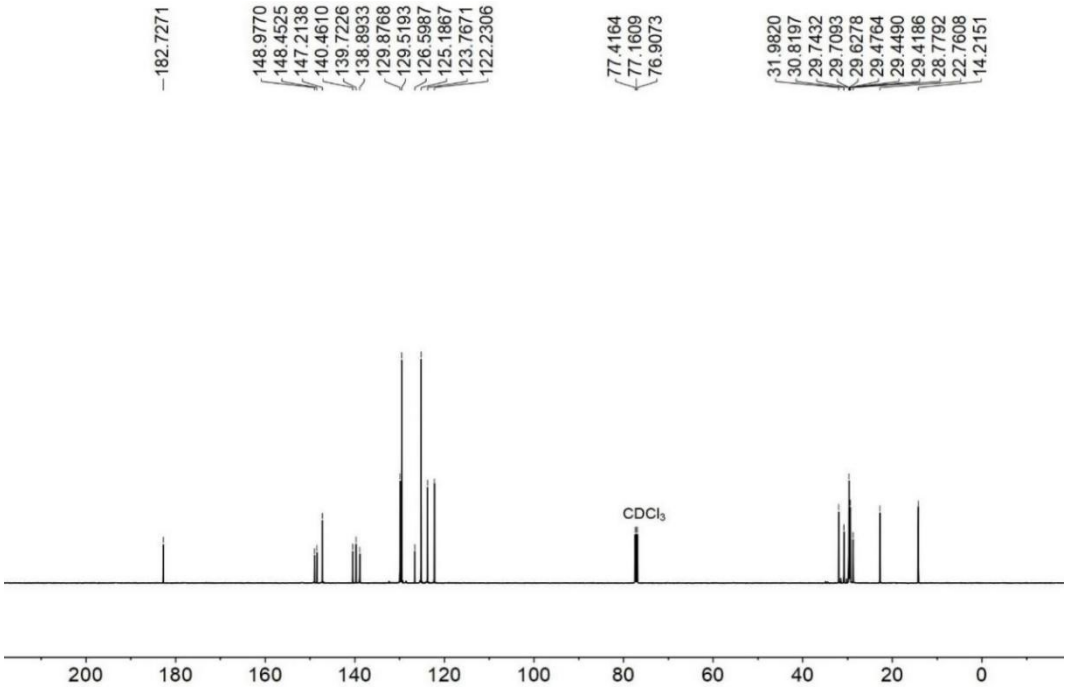


Figure S2 ^{13}C $\{^{1}$H$\}$ NMR spectrum (126 MHz, $CDCl_3$, 298 K) of **a**.

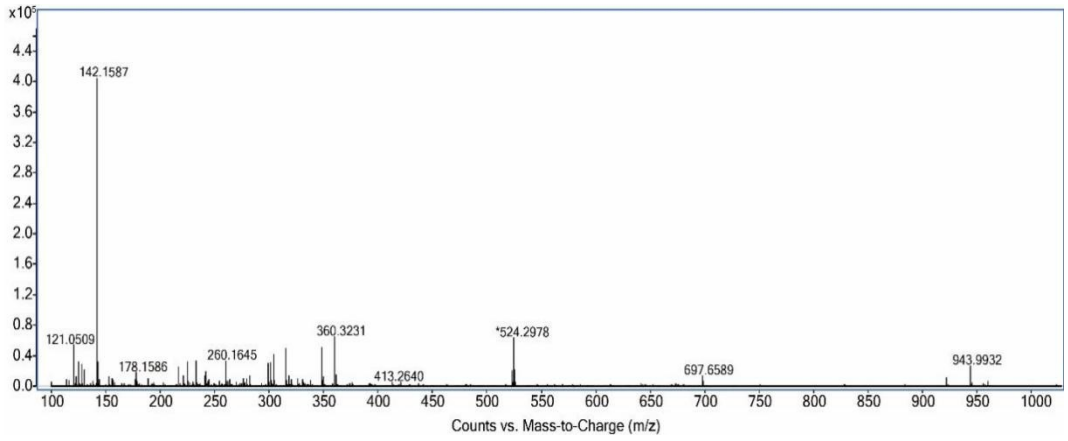


Figure S3 ESI-HRMS spectrum of **a**.

2.2 Synthesis of b

a (0.27 g, 0.52 mmol) was dissolved in THF (25 mL) and the solution was cooled to 0 °C. NBS (0.19 g, 1.08 mmol) was added and the mixture was stirred for 1 h at 0 °C. Then the mixture was stirred for another 1.5 h at room temperature. The solvent was removed with rotary evaporator, and the residue was extracted with CH_2Cl_2 for three times. The organic layer was dried over $MgSO_4$ and evaporated. The residue was purified by chromatography (silica gel, CH_2Cl_2/PE, 1:10, *v*/*v*) to get compound **b**. ^{1}H NMR (500 MHz, $CDCl_3$, 298K) δ (ppm): 9.83 (s, 1H), 7.63 (s, 1H), 7.40 (d, J = 10.9 Hz, 4H), 7.33 (d, J = 10.8 Hz, 2H), 7.08 (d, J = 10.7 Hz, 2H), 7.01 (d, J = 11.0 Hz, 4H), 2.66 (t, J = 9.7 Hz, 2H), 1.64−1.62 (m, 2H), 1.24 (s, 18H), 0.87 (t, J = 8.2 Hz, 3H). ^{13}C {^{1}H} NMR (126 MHz, $CDCl_3$, 298K) δ (ppm): 182.9, 148.4, 147.5, 146.1, 140.8, 140.1, 138.9, 132.8, 130.3, 128.0, 126.4, 123.2, 116.7, 32.0, 30.9, 29.8, 29.7, 29.6, 29.5, 29.5, 29.4, 28.8, 22.8, 14.2. ESI-HRMS [**b** + H]$^+$: calcd for: $[C_{35}H_{40}Br_2NOS]^+$ 680.1192, found 680.1175.

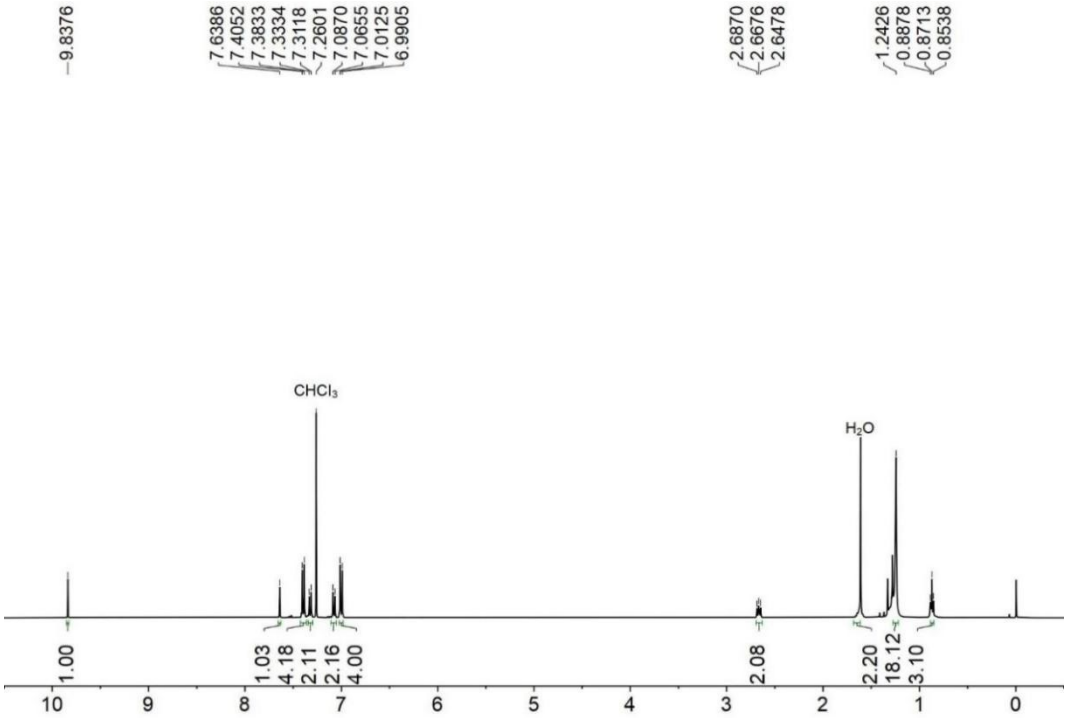


Figure S4 ^{1}H NMR spectrum (500 MHz, $CDCl_3$, 298 K) of **b**.

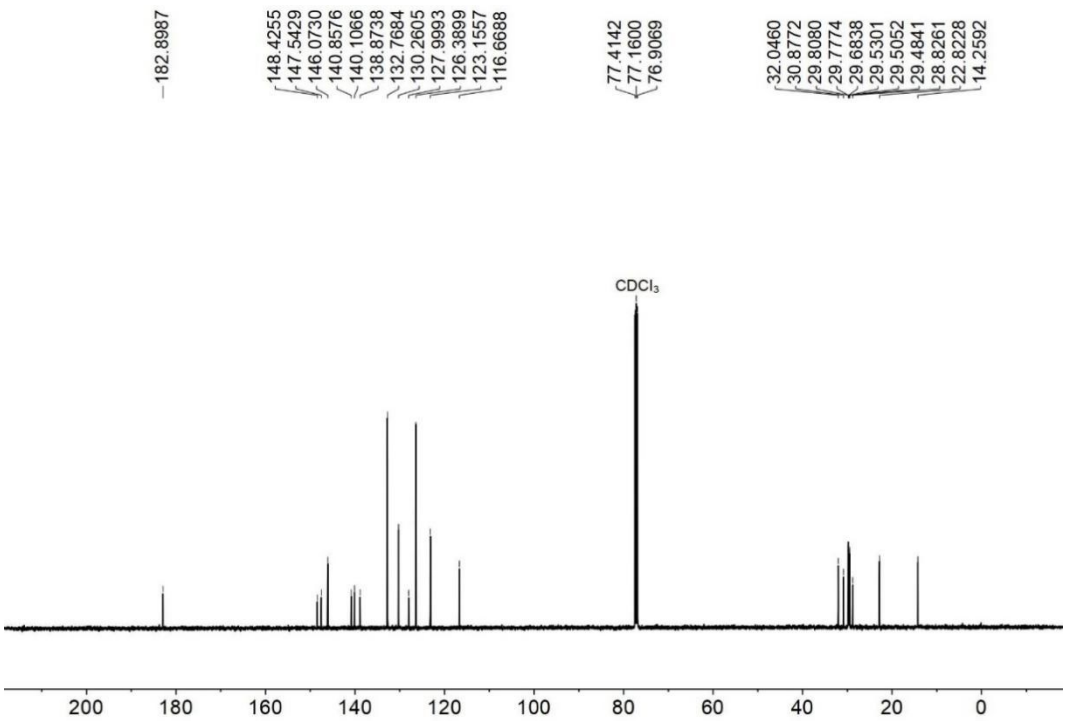


Figure S5 ^{13}C $\{^{1}H\}$ NMR spectrum (126 MHz, $CDCl_3$, 298 K) of **b**.

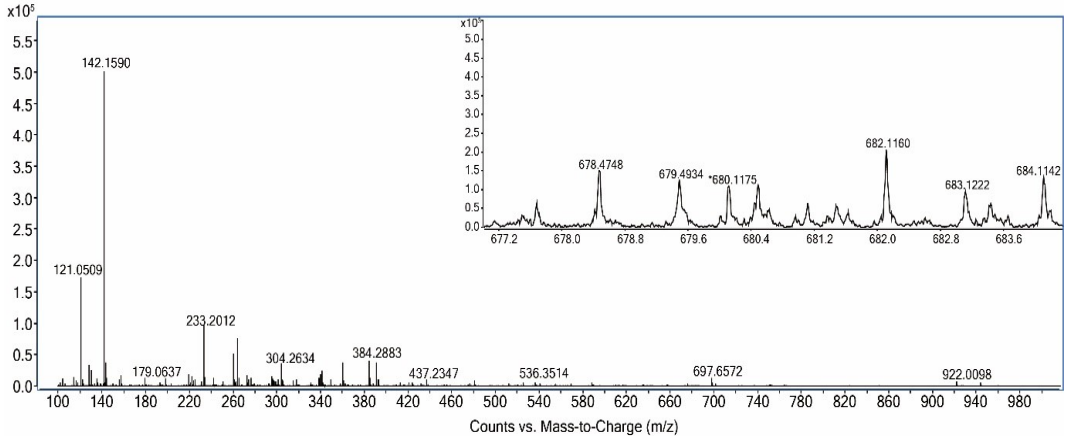


Figure S6 ESI-HRMS spectrum of **b**.

2.3 Synthesis of c

A mixture of **b** (0.33 g, 0.48 mmol), pyridine-4-boronic acid (0.59 g, 4.84 mmol), cesium carbonate (0.31 g, 0.97 mmol), $[Pd(PPh_3)_4]$ (0.11 g, 0.09 mmol), THF (30 mL), and water (6 mL) was degassed for 0.5 h and heated to reflux for 12 h under an argon atmosphere. After cooling down, the solvent was removed with rotary evaporator, and the residue was extracted with CH_2Cl_2 for three times. The organic layer was dried over $MgSO_4$ and evaporated. The residue was purified by chromatography (silica gel, CH_2Cl_2/CH_3OH, 100:1, *v*/*v*) to get compound **c**. ^{1}H NMR (500 MHz, $CDCl_3$, 298K) δ (ppm): 9.84 (s, 1H), 8.64 (d, J = 5.1 Hz, 4H), 7.65 (s, 1H), 7.61 (d, J = 8.2 Hz, 4H), 7.50 (d, J = 5.1 Hz, 4H), 7.40 (d, J = 8.2 Hz, 2H), 7.28 (d, J = 8.2 Hz, 4H), 7.23 (d, J = 8.2 Hz, 2H), 2.70 (t, J = 7.8 Hz, 2H), 1.64 (t, J = 7.4 Hz, 2H), 1.24 (s, 18H), 0.85 (t, J = 7.0 Hz, 3H). ^{13}C $\{^{1}H\}$ NMR (126 MHz, $CDCl_3$, 298K) δ (ppm): 182.8, 150.4, 148.2, 147.8, 147.4, 140.8, 140.1, 138.8, 133.0, 130.3, 128.5, 128.2, 124.9, 124.1, 121.1, 31.9, 30.8, 29.7, 29.7, 29.7, 29.6, 29.4, 29.4, 29.3, 28.8, 22.7, 14.2. ESI-

HRMS [**c** + H]$^+$: calcd for: $[C_{45}H_{48}N_3OS]^+$ 678.3513, found 678.3501.

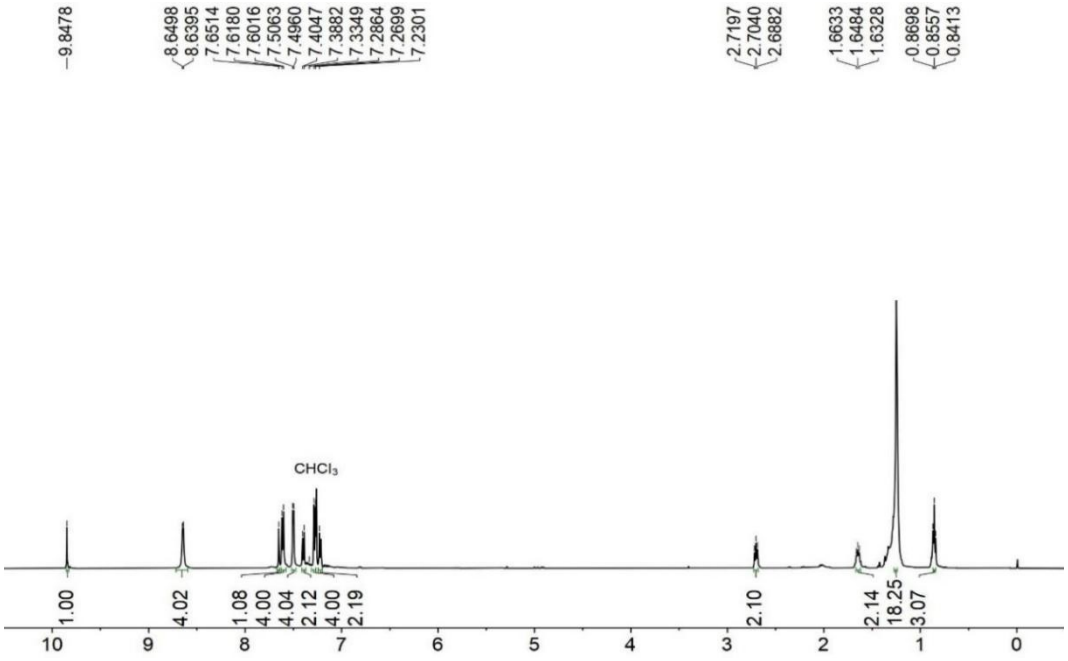


Figure S7 ^{1}H NMR spectrum (500 MHz, $CDCl_3$, 298 K) of **c**.

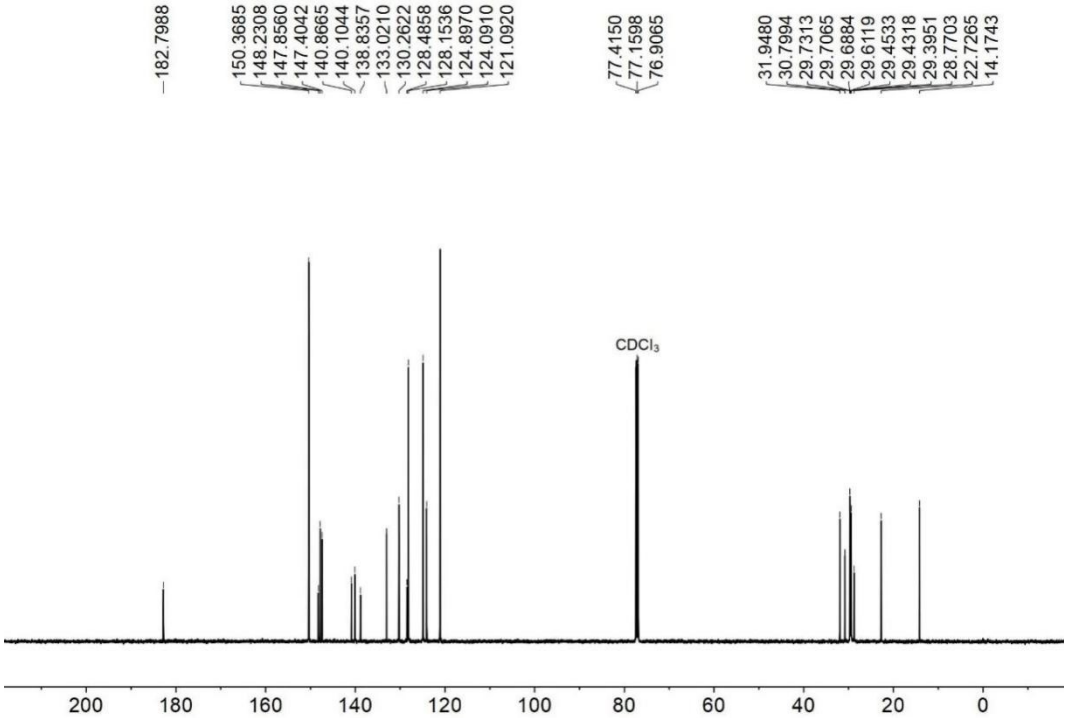


Figure S8 ^{13}C {^{1}H} NMR spectrum (126 MHz, $CDCl_3$, 298 K) of **c**.

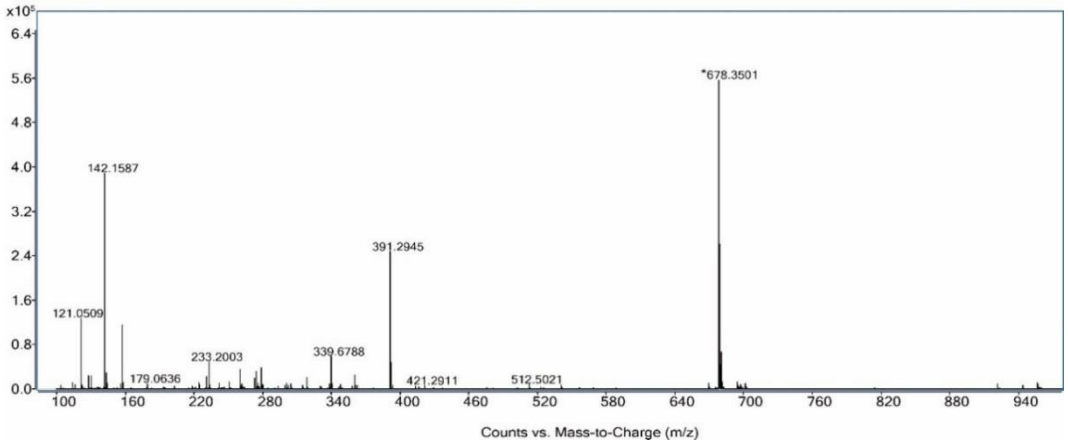


Figure S9 ESI-HRMS spectrum of **c**.

2.4 Synthesis of D-CN

A mixture of **c** (0.18 g, 0.26 mmol), malononitrile (0.17 g, 2.65 mmol), pyridine (0.3 mL) was dissolved in toluene (15 mL) and heated to reflux for 12 h under an argon atmosphere. After cooling down, the solvent was removed with rotary evaporator, and the residue was extracted with CH_2Cl_2 for three times. The organic layer was dried over $MgSO_4$ and evaporated. The residue was purified by chromatography (silica gel, CH_2Cl_2/CH_3OH, 100:1, *v*/*v*) to get compound **D-CN**. ^{1}H NMR (500 MHz, $CDCl_3$, 298K) δ (ppm): 8.66 (d, J = 6.2 Hz, 4H), 7.74 (s, 1H), 7.63 (d, J = 8.6 Hz, 4H), 7.60 (s, 1H), 7.51 (d, J = 6.2 Hz, 4H), 7.42 (d, J = 8.6 Hz, 2H), 7.29 (d, J = 8.6 Hz, 4H), 7.23 (d, J = 8.6 Hz, 2H), 2.73 (t, J = 7.8 Hz, 2H), 1.64 (t, J = 6.9 Hz, 2H),1.24 (s, 18H), 0.87 (t, J = 7.8 Hz, 3H). ^{13}C {^{1}H} NMR (126 MHz, $CDCl_3$, 298K) δ (ppm): 182.8, 150.4, 148.2, 147.8, 147.4, 140.8, 140.1, 138.8, 133.0, 130.3, 128.5, 128.2, 124.9, 124.1, 121.1, 31.9, 30.8, 29.7, 29.7, 29.7, 29.6, 29.4, 29.4, 29.3, 28.8, 22.7, 14.2. ESI-HRMS [**D-CN** + H]$^+$: calcd for: $[C_{48}H_{47}N_5S]^+$ 726.3625, found 726.3637.

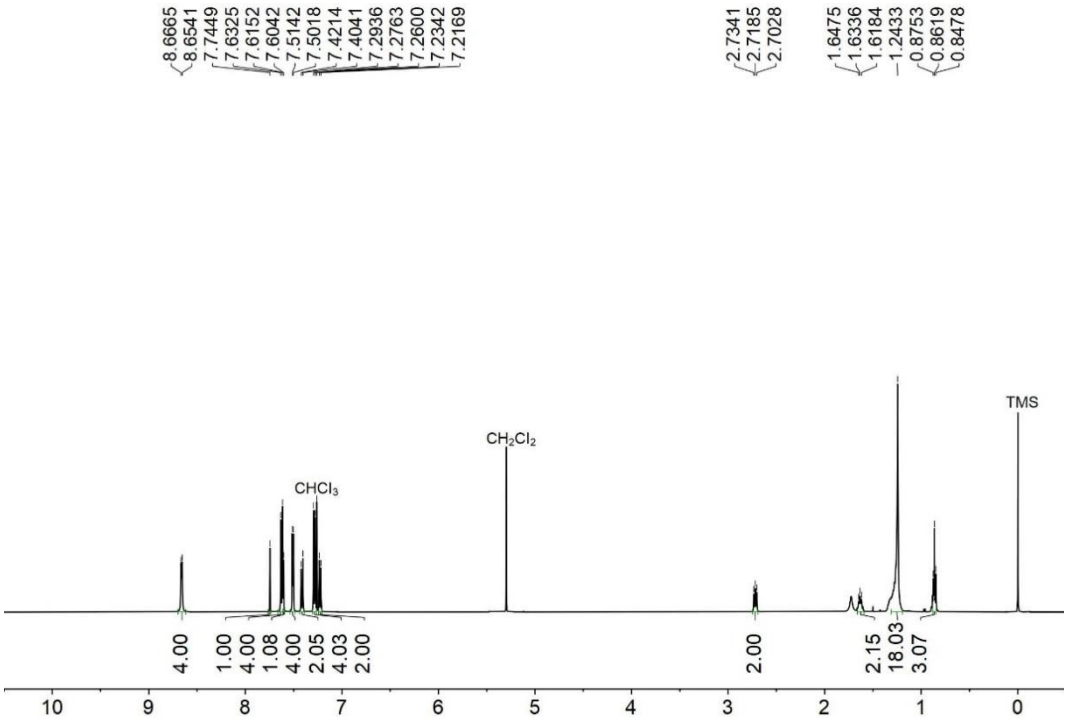


Figure S10 ^{1}H NMR spectrum (500 MHz, $CDCl_3$, 298 K) of **D-CN**.

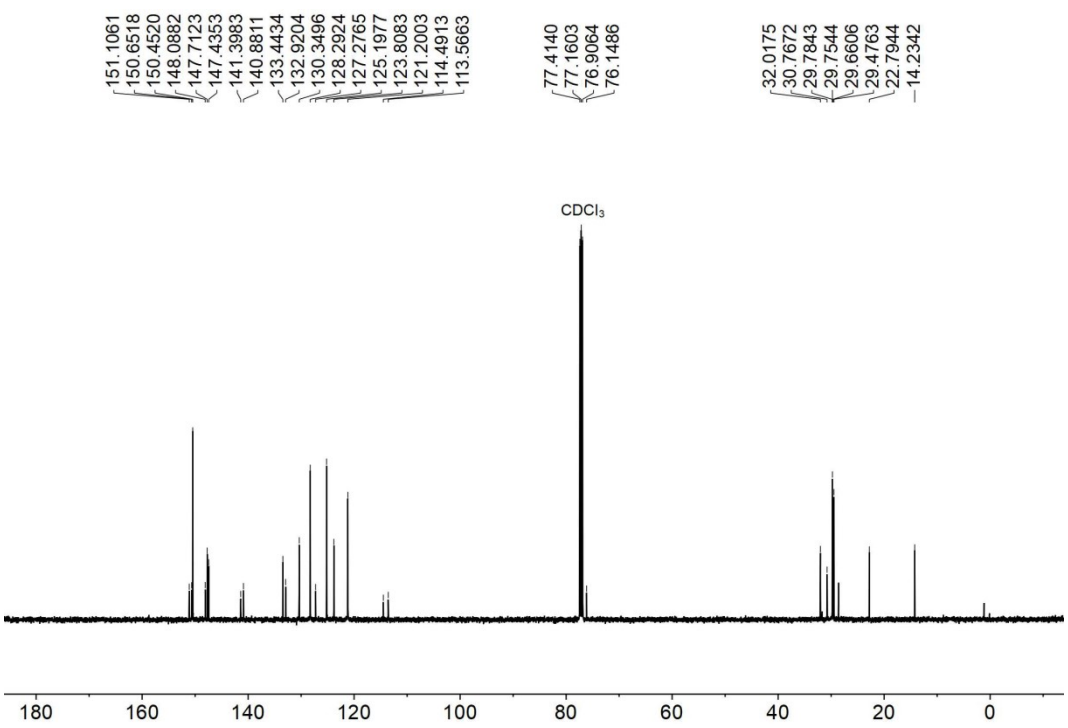


Figure S11 ^{13}C $\{^{1}H\}$ NMR spectrum (126 MHz, $CDCl_3$, 298 K) of **D-CN**.

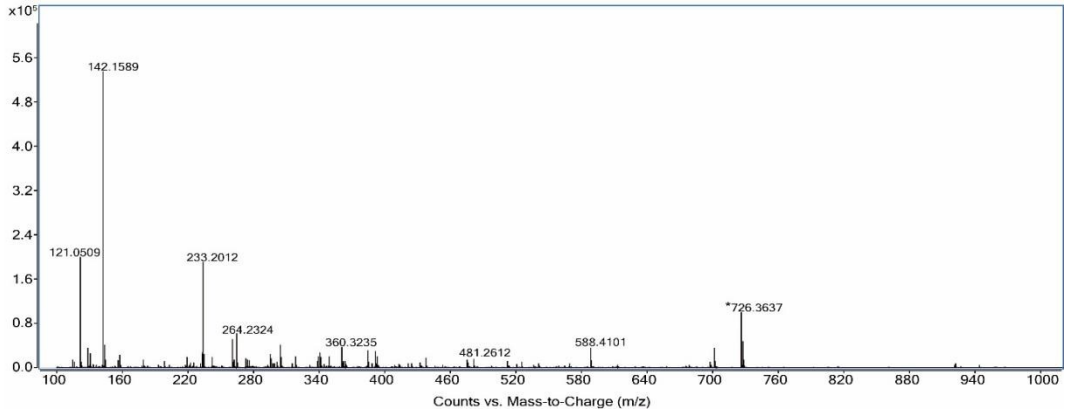


Figure S12 ESI-HRMS spectrum of **D-CN**.

2.5 Synthesis of 1

1 was synthesized according to literature procedure.[1] ^{1}H NMR (500 MHz, $CDCl_3$, 298K) δ (ppm): 8.47 (s, 2H), 7.73 (d, J = 8.3 Hz, 2H), 7.59 (d, J = 8.3 Hz, 2H), 4.08 (s, 6H), 1.68−1.66 (m, 24H), 1.08−1.05 (m, 36H). ^{31}P $\{^{1}H\}$ NMR (202 MHz, $CDCl_3$, 298 K) δ (ppm): 19.45(s, ^{195}Pt satellites, $^{1}J_{Pt-P}$ = 2825.56 Hz).

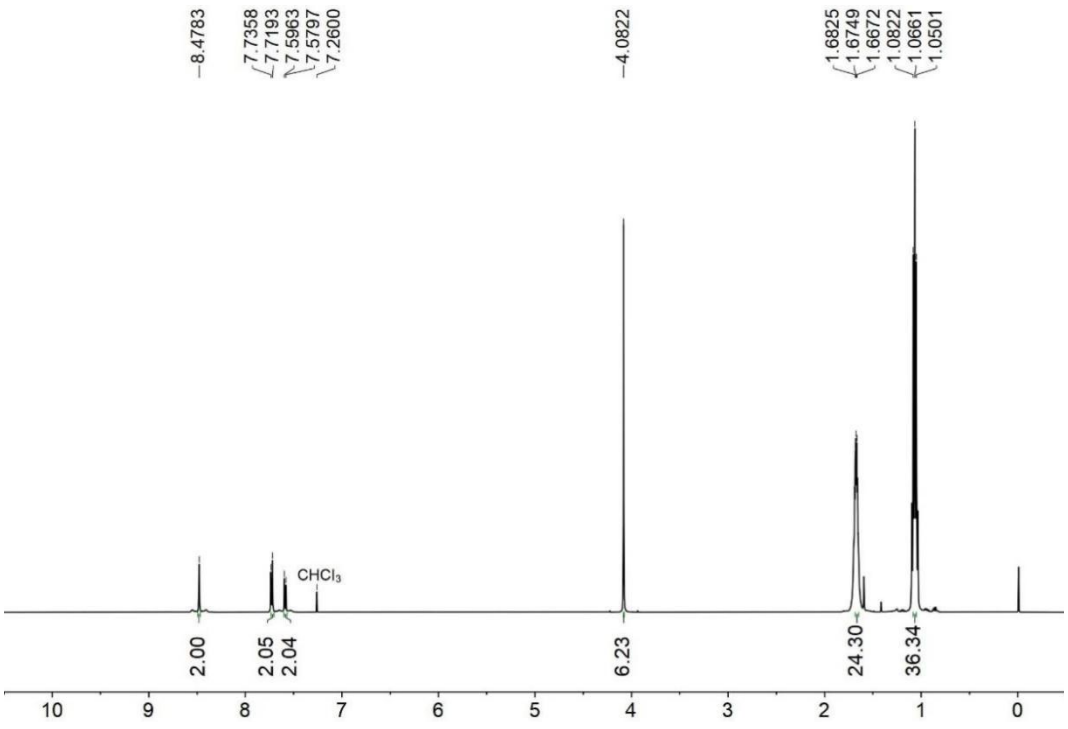


Figure S13 ^{1}H NMR spectrum (500 MHz, $CDCl_3$, 298 K) of **1**.

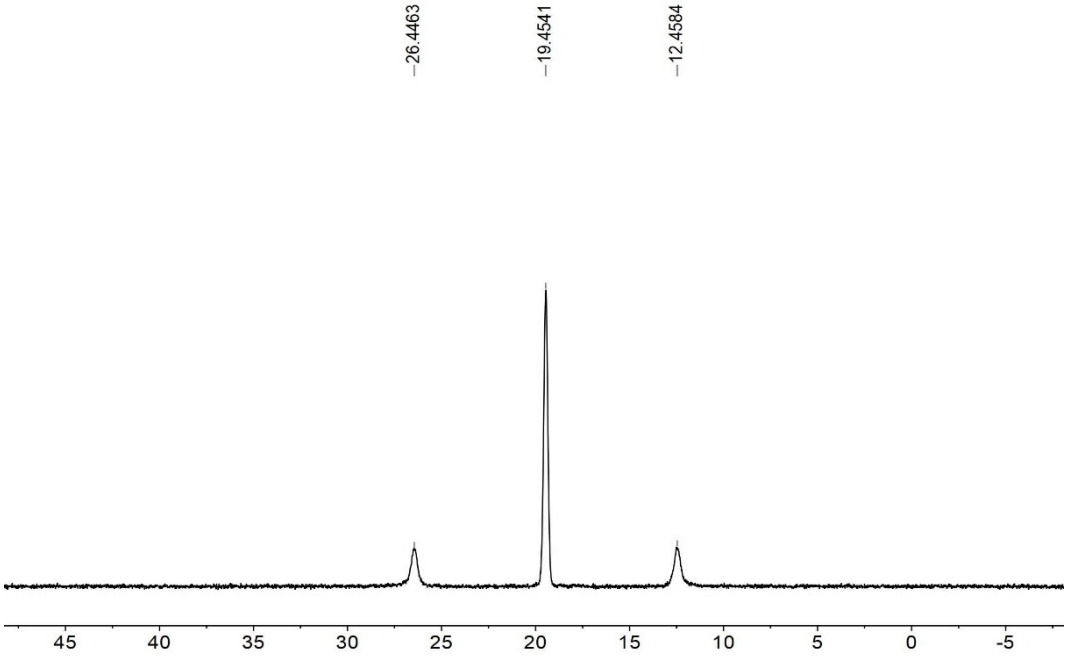


Figure S14 ^{31}P $\{^{1}H\}$ NMR spectrum (202 MHz, $CDCl_3$, 298 K) of **1**.

2.6 Synthesis of MD-CN

D-CN (10.085 mg, 0.012 mmol) and **1** (20.012 mg, 0.012 mmol) were dissolved in anhydrous DMSO (0.5 mL) and the mixture was stirred at 50 ^{0}C for 12 h. After cooling down, diethyl ether (7.5 mL) were added to obtain a red solid. ^{1}H NMR (500 MHz, $CDCl_3$, 298K) δ (ppm): 9.23 (d, J = 5.6 Hz, 4H), 8.75 (s, 4H), 8.71 (d, J = 5.7 Hz, 4H), 7.98 (m, 8H), 7.92 (d, J = 8.2 Hz, 4H), 7.83 (m, 10H), 7.65 (s, 2H), 7.62 (d, J = 8.2 Hz, 4H), 7.51 (d, J = 8.5 Hz, 4H), 7.35 (d, J = 8.7 Hz, 8H), 7.32 (d, J = 8.5 Hz,

4H), 4.12 (s, 12H), 2.80 (t, J = 7.7 Hz, 4H), 1.69 (t, J = 6.9 Hz, 4H), 1.39−1.38 (m, 48H), 1.25 (s, 36H), 1.17−1.15 (m, 72H), 0.87 (t, J = 6.8 Hz, 6H). ^{31}P {^{1}H} NMR (202 MHz, DMSO, 298 K) δ (ppm): 13.81 (s, ^{195}Pt satellites, $^{1}J_{Pt-P}$ = 2659.93 Hz). ESI-TOF-MS: *m/z* 1265.47 [**MD-CN**– 3OTf]$^{3+}$.

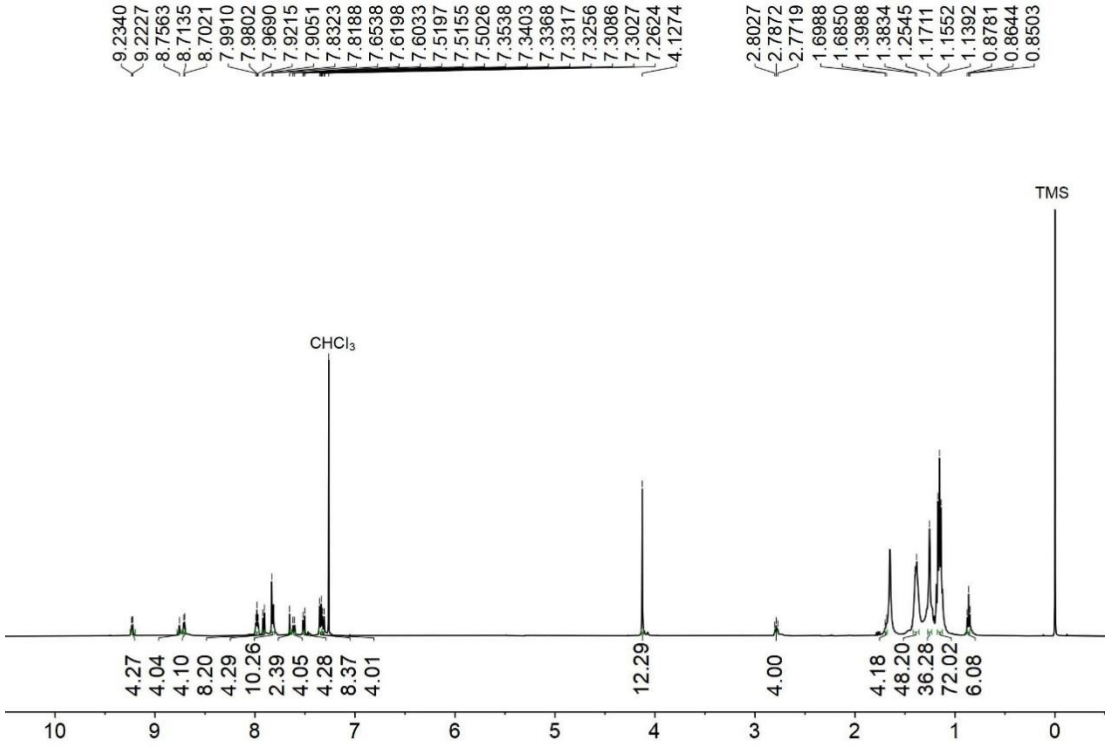


Figure S15 ^{1}H NMR spectrum (500 MHz, $CDCl_3$, 298 K) of **MD-CN**.

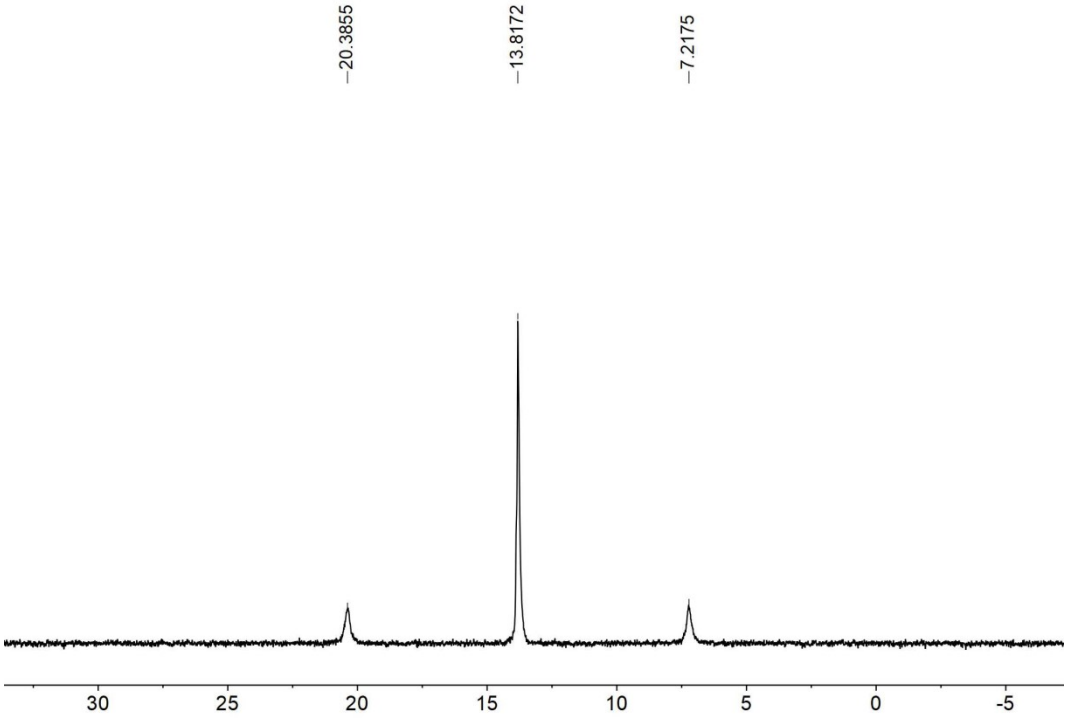


Figure S16 ^{31}P {^{1}H} NMR spectrum (202 MHz, DMSO, 298 K) of **MD-CN**.

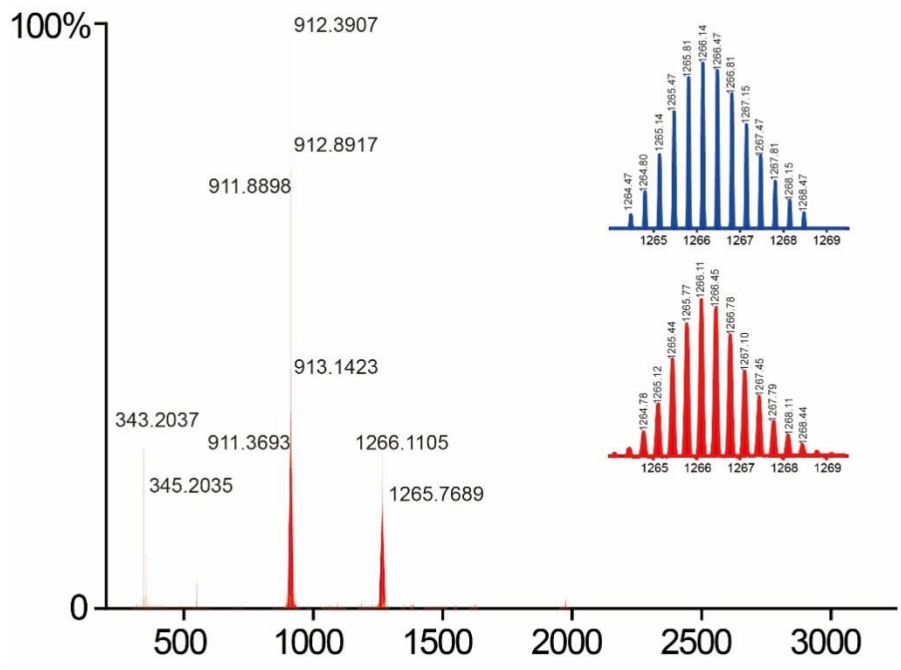


Figure S17 ESI-TOF-MS spectrum of **MD-CN**.

3. Preparation of nanoparticles

To prepare **D-CN** NPs / **MD-CN** NPs, Pluronic F127 (10.0 mg) was dissolved in deionized water (10 mL), and stirred for 20 minutes. **D-CN** or **MD-CN** (1.0 mg) in THF (2 mL) was added dropwise to the aqueous solution of Pluronic F127 under magnetic stirring. After THF was completely volatilized, the solution was filtered with a disposable filter (220 nm) to obtain **D-CN** NPs / **MD-CN** NPs.

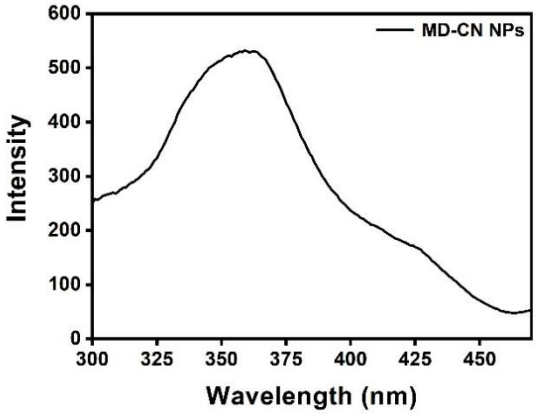


Figure S18 Excitation spectra of **MD-CN** NPs emission.

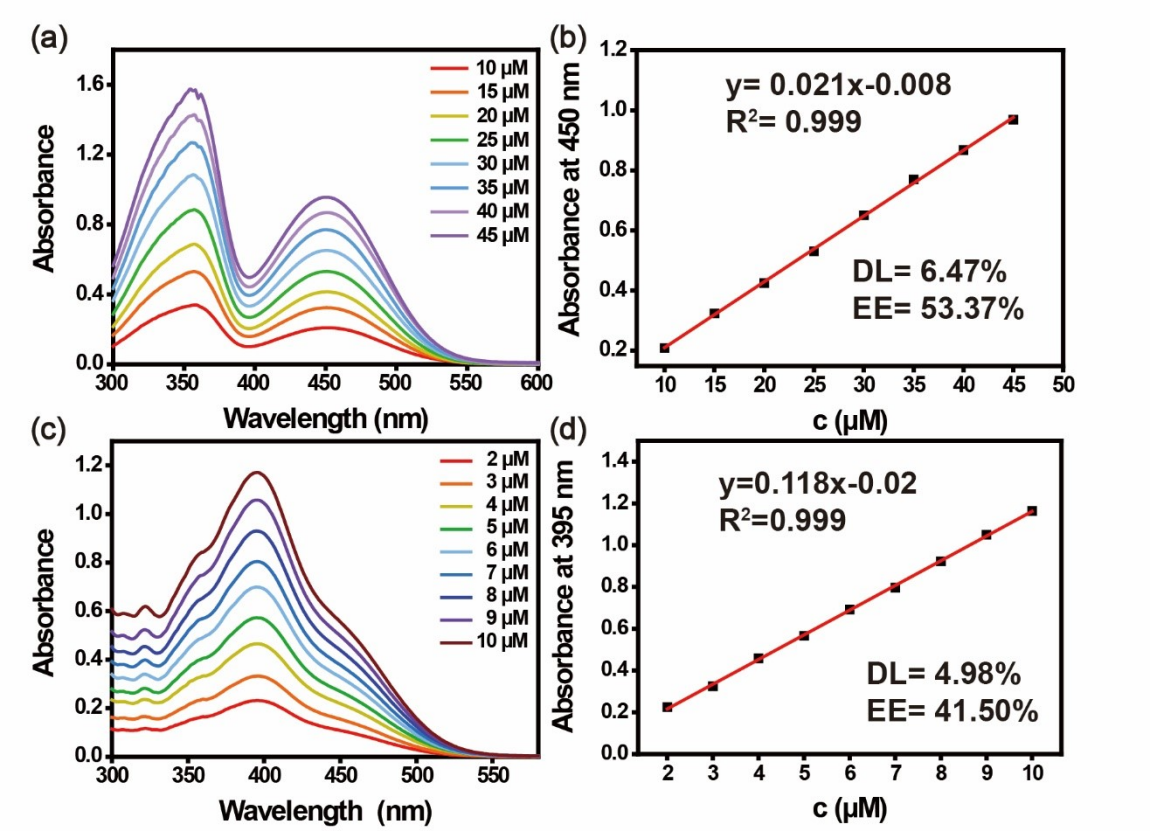


Figure S19 (a) UV-vis absorption spectra of **D-CN** at various concentration in THF and (b) the maximum absorbance at 450 nm *versus* the concentration of **D-CN**. (c) UV-vis absorption spectra of **MD-CN** at various concentration in THF and (d) the maximum absorbance at 395 nm *versus* the concentration of **MD-CN**.

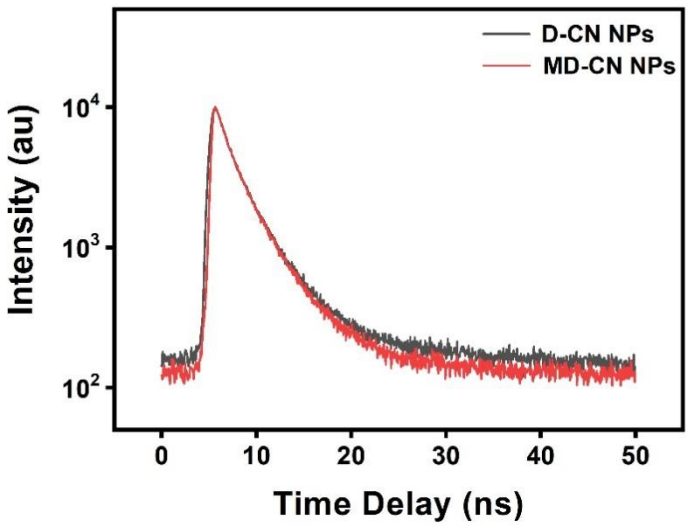


Figure S20 Fluorescence decay curves of **D-CN** NPs and **MD-CN** NPs in solid states.

4. Photodynamic performance of D-CN and MD-CN

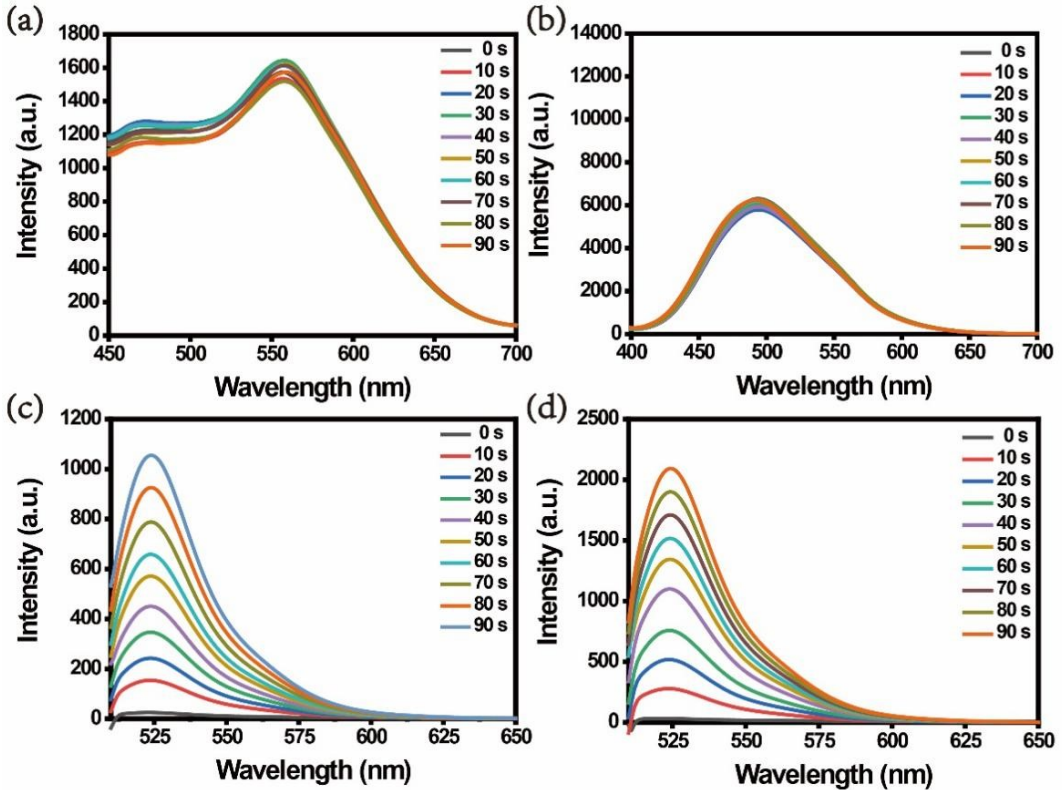


Figure S21 (a) Fluorescence (FL) spectra of **D-CN** NPs under 450 nm irradiation (80 mW/cm^2). (b) FL spectra of **MD-CN** NPs under 450 nm irradiation (80 mW/cm^2). (c) FL spectra of DCFH in the presence of **D-CN** NPs (10 μM) under 450 nm irradiation (80 mW/cm^2). (d) FL spectra of DCFH in the presence of **MD-CN** NPs (10 μM) under 450 nm irradiation (80 mW/cm^2).

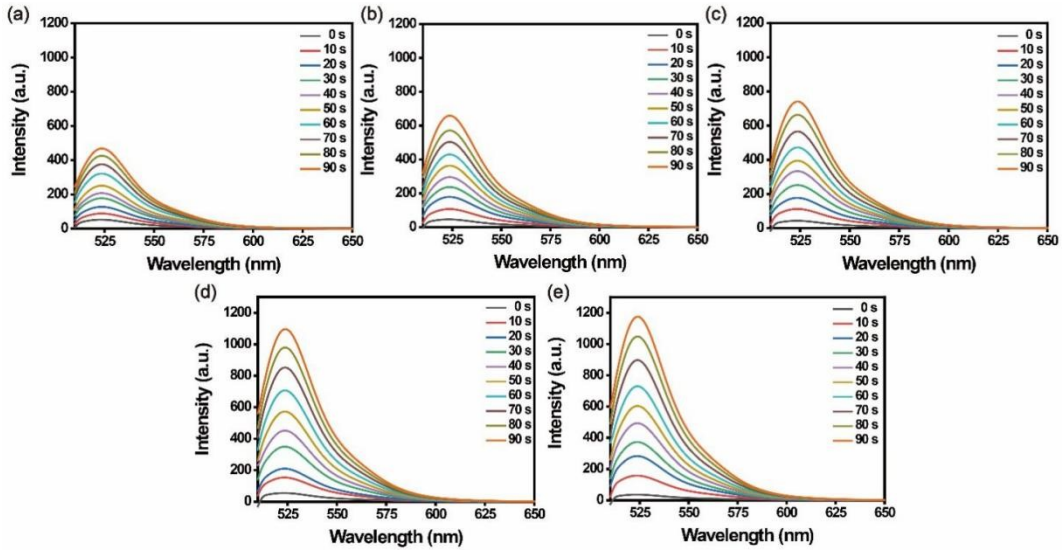


Figure S22 ROS generation by **D-CN** NPs. (a-e) FL spectra of DCFH in the presence of **D-CN** NPs (2, 4, 6, 8, 10 μM) under 450 nm irradiation (80 mW/cm^2).

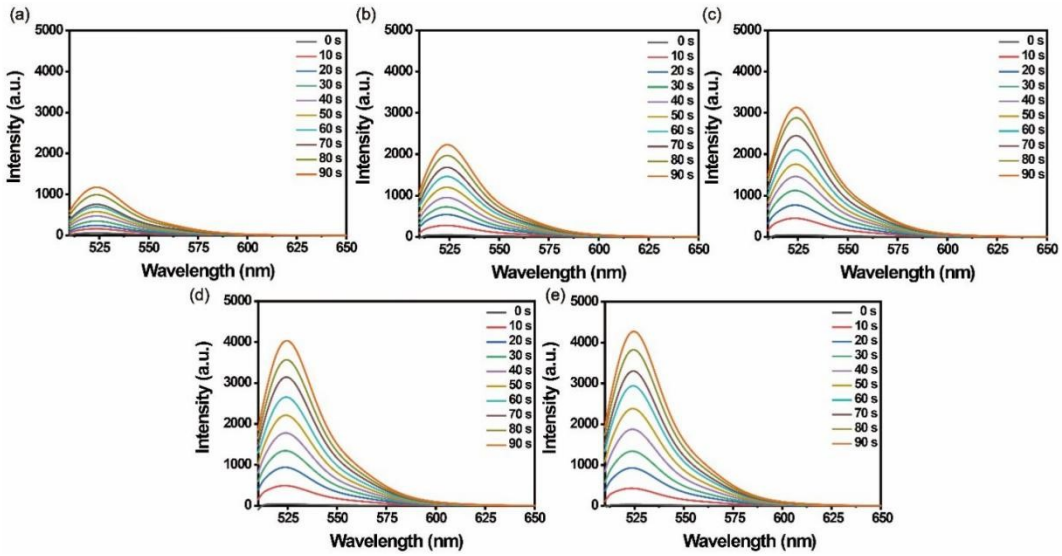


Figure S23 ROS generation by **MD-CN** NPs. (a-e) FL spectra of DCFH in the presence of **MD-CN** NPs (2, 4, 6, 8, 10 μM) under 450 nm irradiation (80 mW/cm^2).

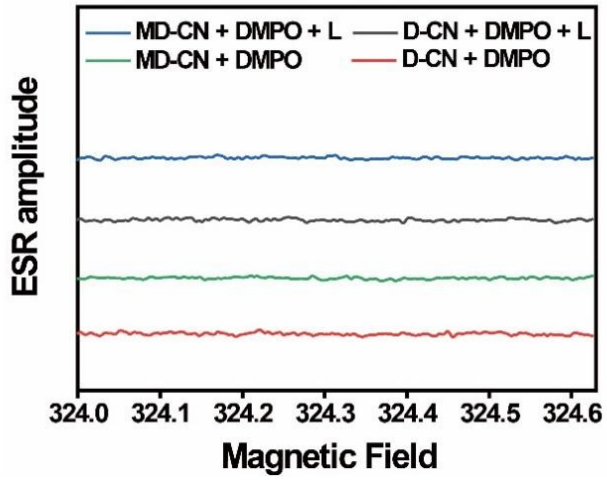


Figure S24 ESR signals of DMPO for type-I ROS characterization in the presence of **D-CN** or **MD-CN** before and after light irradiation (450 nm, 80 mW/cm^2).

5. Cell culture

The U87 cells were cultured in Dulbecco's modified Eagle medium (DMEM) containing 10% Newborn Calf Serum (NCS), 1% penicillin and streptomycin under a humidified atmosphere with 5% CO_2 at 37 ℃.

6. MTT assay

The cytotoxicities of **D-CN** NPs and **MD-CN** NPs were assessed by MTT assay. U87 cells were seeded in two 96-well plates at a density of 1×10^4 cells/well. After 18 h, the medium was replaced by

the fresh DMEM with different concentration of **D-CN**/**MD-CN** NPs. After 4 h, one plate was irradiation with 450 nm laser irradiation (80 mW/cm^2), and another plate were kept in the dark as control. After 24 h incubation, the MTT solution (5 mg/mL) was replaced the medium for another 4 h, then each well was replaced with 100 μL of DMSO and measured spectrophotometrically on Perlong-DNM-9602 plate reader at a wavelength of 570 nm. Cell viability values were calculated by the following formula:

Cell viability (%) = absorbance of experimental group/the absorbance of control group × 100%.

7. Intracellular ROS detection

Intracellular ROS generation was detected by 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA) as an indicator. Following incubation with PBS, **D-CN** NPs, **MD-CN** NPs for 24 h, DCFH-DA was loaded into the cells. After 30 min incubation, cells were washed twice with PBS, and then treated with or without irradiation (450 nm, 80 mW/cm^2, 5 min). The fluorescence images were acquired by CLSM.

8. Live/dead cell staining assay

Live/dead cell staining assay was detected by calcein acetoxymethyl ester (calcein AM) and propidium iodide (PI). U87 cells were seeded in 96-well plate at a density of 1×10^4 cells/well. After 18 h, the medium was replaced by the fresh DMEM with PBS, **D-CN** NPs, **MD-CN** NPs. After 4 h, light groups were irradiation with 450 nm laser irradiation (80 mW/cm^2, 5 min), and other wells were kept in the dark as control. After 24 h incubation, the cells were rinsed with PBS and stained by calcein AM and PI, the residual dyes were washed out by PBS for two times. CLSM was applied to observe the green fluorescence of calcein AM and the red fluorescence of PI, which indicated live and dead cells, respectively.

9. Flow cytometry assay

U87 cells were seeded in a 12-well plate and incubated at 37℃ and 5% CO_2 for 18 h. After 1, 2, 4, 6 and 8 h, the medium containing materials **D-CN** NPs, **MD-CN** NPs were added and incubated. After removing the medium containing materials, the cells were washed three times with PBS, and the cell suspension was collected and filtered for detection by flow cytometry.

10. Cellular uptake study

U87 cells were seeded in 24-well plates containing cell slides, each well density was 5×10^4 cells/well, incubated at 37℃ with 5% CO_2. After 18 h, **D-CN** NPs, **MD-CN** NPs were added for co-incubation. After 4 h, the material solution was abandoned and the cells were fixed with 300 μL 4% paraformaldehyde for 7 min. The paraformaldehyde solution was sucked out, washed twice with high glucose DMEM, and stained with 300 μL Dio probe solution for 8 min. Blot out the dye and repeat the washing operation. The cell slides were removed, the anti-fluorescence quench agent was added and fixed on the slides.

11. References

1. Y. Li, X. Yuan, J. Yu, Y. Fan, T. He, S. Lu, X. Li, H. Qiu, S. Yin, *ACS Appl. Bio Mater.* **2020**, *3*, 8061−8068.