A Highly Specific Probe for Sensing Hydrogen Sulfide in Live Cells Based on Copper-Initiated Fluorogen with Aggregation-Induced Emission Characteristics

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SUPPORTING INFORMATION

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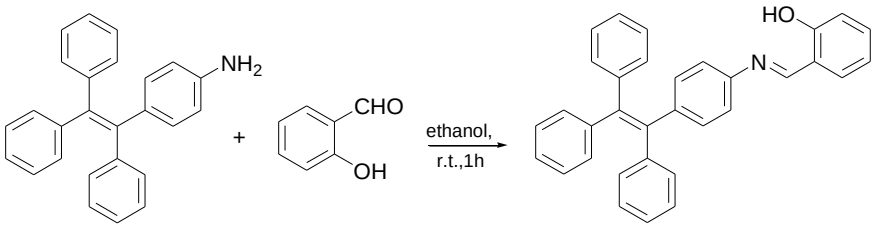
General Experimental

Unless otherwise mentioned, all the chemicals and reagents were from commercial supplies and used without further purification. Tetraphenylethene aniline was prepared according to literature procedures.[1] Dry ethanol was distilled form sodium. Isolated yields refer to spectroscopically (^{1}H NMR) homogeneous materials. Reactions were monitored by thin-layer chromatography (TLC) carried out on Silica gel 60 F254 plates supplied by Qingdao Puke Separation Material Corporation using UV light as the visualizing agent. Flash column chromatography was performed using 200-300 mesh silica gel supplied by Qingdao Marine Chemical Factory, Qingdao, China. ^{1}H-NMR spectra were recorded on a Bruker Fourier transform spectrometer (400 MHz) at 25 °C. ^{13}C-NMR spectra were recorded on a Bruker Fourier transform spectrometer (100 Hz) spectrometer and were calibrated using residual undeuterated solvent as an internal reference ($CDCl_3$: ^{1}H NMR = 7.26, ^{13}C NMR = 77.16). All chemical shifts were given in ppm and coupling constants (*J*) in Hz. The following abbreviations or combinations thereof were used to explain the multiplicities: s = singlet, t = triplet, m = multiplet. IR spectra were recorded on a Bruker Vector 22 spectrophotometer as KBr pellets. High resolution mass spectra (HRMS) were recorded on an Agilent 6224 TOF LC/MS spectrometer using ESI-TOF (electrospray ionization-time of flight). Absorption spectra were acquired using a Hitachi U-3010 spectrophotometer. Fluorescence measurements were carried out on a PE LS45 fluorescence spectrometer.

[1] X. Duan, J. Zeng, J. Lu, Z. Zhang, ***J. Org. Chem.***, 2006, ***71***, 9873-9876.

Scheme S1. Synthesis of AIE-S1



To a stirred solution of tetraphenylethene aniline (200 mg, 0.576 mmom) in dry ethanol was added salicylaldehyde (3.00 mL, 28.7 mmol). The reaction was allowed going at ambient temperature under N_2 atmosphere for 1 h. A yellow precipitate was formed gradually which was isolated by filtration and further purified by flash column chromatography on silica gel (petroleum ether /CH_2Cl_2, 5:1) to afford **AIE-S1** as a yellow powder (234 mg, 90% yield).

Rf = 0.45 (10:1 petroleum ether:EtOAc)

δ_H (400 MHz, $CDCl_3$): 13.30 (1 H, s), 8.59 (1 H, s), 7.37 (2 H, t, *J* 7.0), 7.15-7.01 (20 H, m), 6.93 (1 H, t, *J* 7.4).

δ_C (100 MHz, $CDCl_3$): 162.07, 161.29, 146.43, 143.75, 143.72, 143.67, 142.87, 141.54, 140.29, 133.16, 132.57, 132.30, 131.50, 131.47, 131.45, 127.98, 127.90, 127.80, 126.76, 126.73, 126.66, 120.68, 119.38, 119.15, 117.36

IR (cm^{-1}): 3420, 3025, 1618, 1567, 1493, 1450, 1279, 1181, 753, 696.

ESI-HRMS (m/z): $[M+H]^+$ calc'd. for $C_{33}H_{26}NO$: 452.2014; found 452.2018.

Fluorometric analysis

All fluorescence measurements were carried out at room temperature in HEPES (10 mmol, pH 7.4) which was prepared with DI water and purged with nitrogen for 5 minutes before use. The probes were dissolved in DMSO to make a 1.0 mM stock solution, which was diluted to the required concentrations with HEPES for measurements. $CuCl_2$ and Na_2S were dissolved in the above mentioned deoxygenated HEPES to make a stock solution of 1.0 mM. The spectrometer slit width of excitation and emission was adjusted to 5 and 10 nm, respectively, for measurements. All fluorometric experiments were performed in triplicate.

Cell lines and imaging experiments

HeLa cells were cultured in DMEM (Invitrogen, Carlsbad, CA), supplemented with 10% fetal bovine serum in a humidified atmosphere of 5% CO_2 at 37°C. For imaging experiments, **e**xponentially growing cells (at a density of 20000-40000 cells per well, respectively) were seeded in 24-well plate. Cells were cultured at 37°C in a 5% CO_2 atmosphere for 24 h before they were exposed to reagents. After the staining steps as described in figure captions, the images were collected upon excitation using the corresponding filters for DAPI (blue).

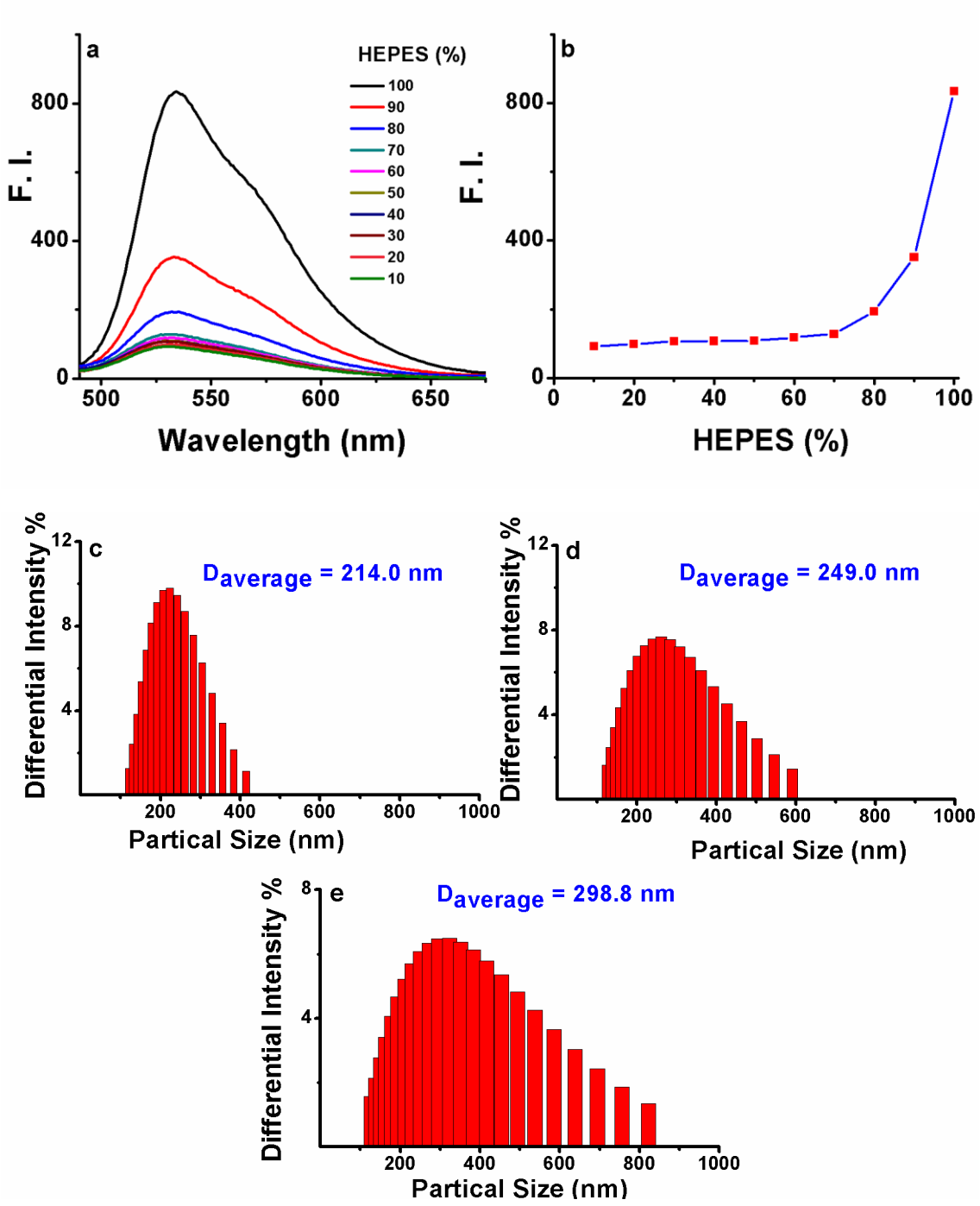


Fig. S1 Fluorescent property of **AIE-S** in a mixture solution of THF and HEPES buffer. (a) Spectra profiles of **AIE-S** in the medium with different fractions of HEPES buffer (λex 350 nm). (b) Correlation between the fluorescence intensity at 533 nm (y) and the fractions of HEPES buffer (x). (c-e) Particle size distribution of **AIE-S** in the mixture solution with HEPES buffer fraction being 70% (c), 90% (d) and 100% (e).

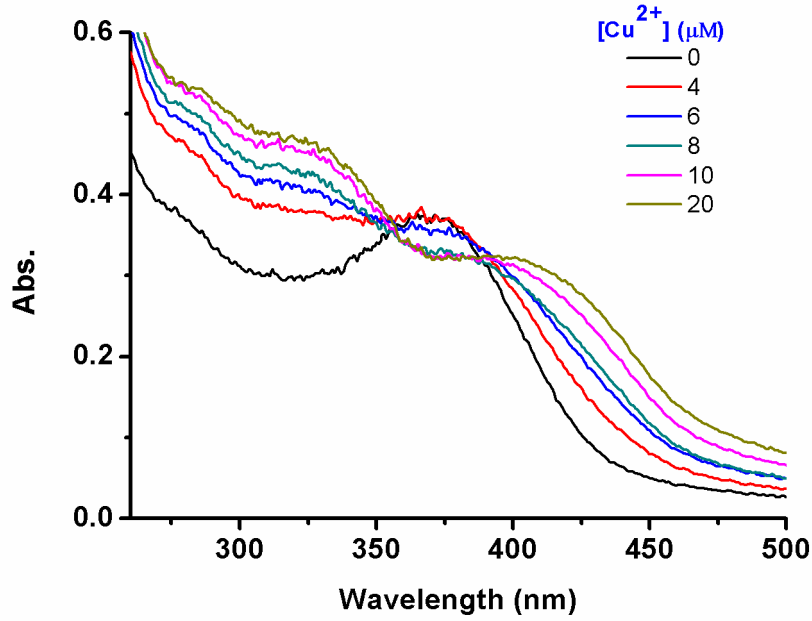


Fig. S2 The UV-vis spectra of **AIE-S** (20 μM) in HEPES (10 mM, pH 7.4, 25°C, 10% THF) when various concentrations of $CuCl_2$ (0-20 μM) were added.

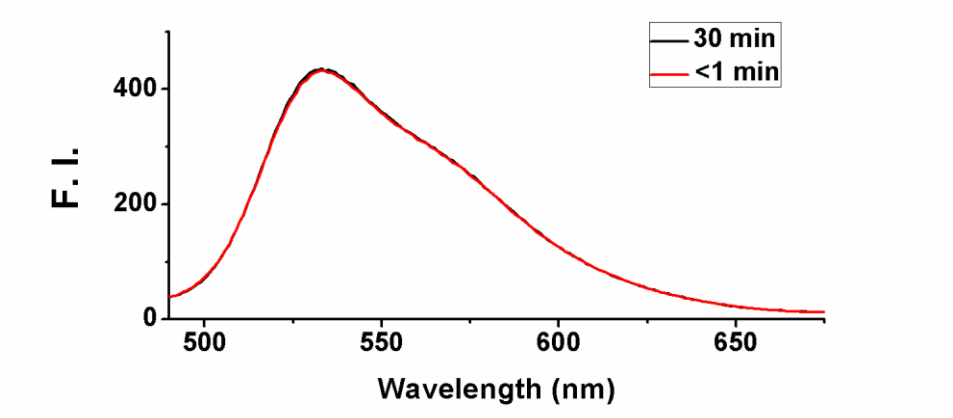
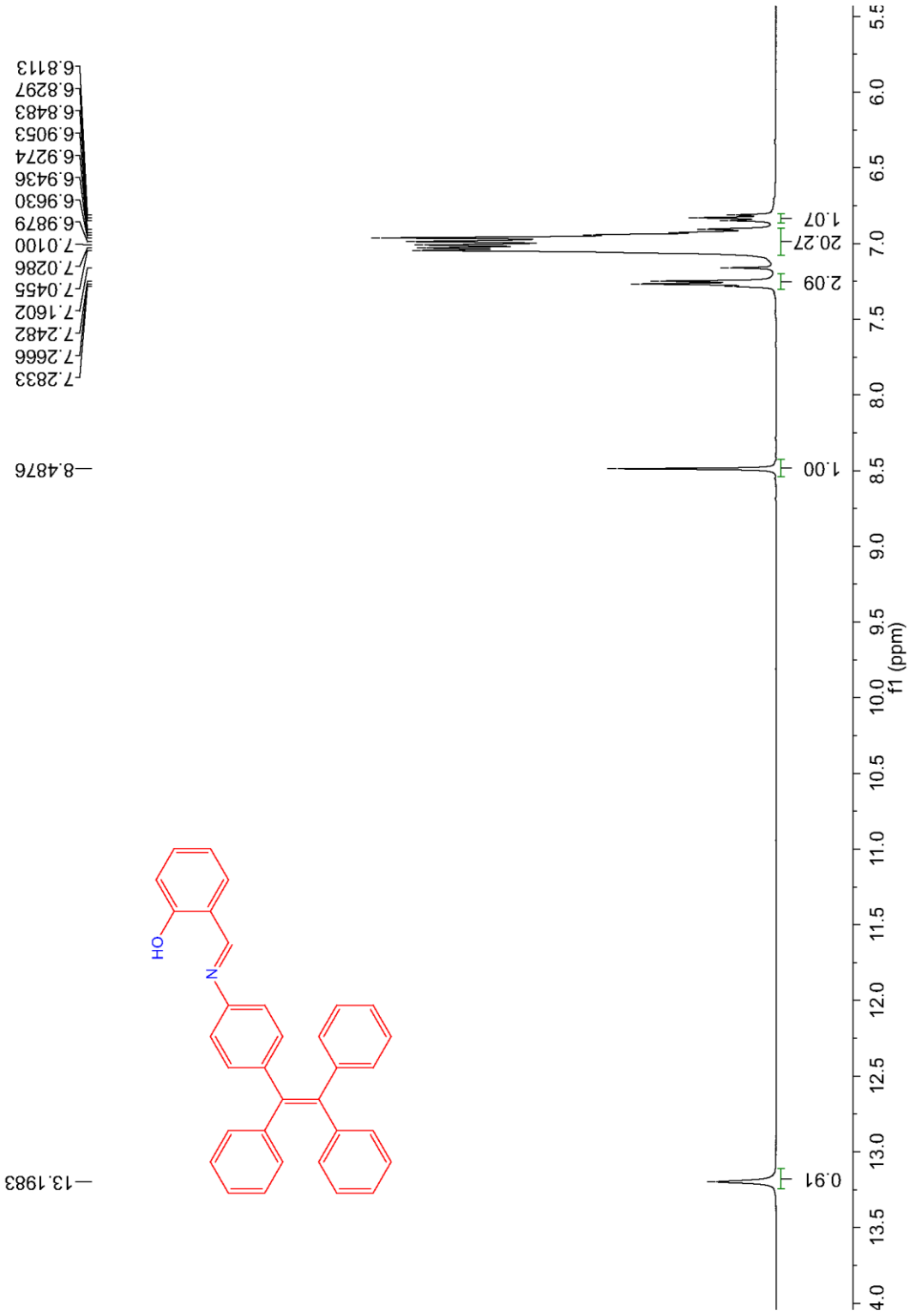
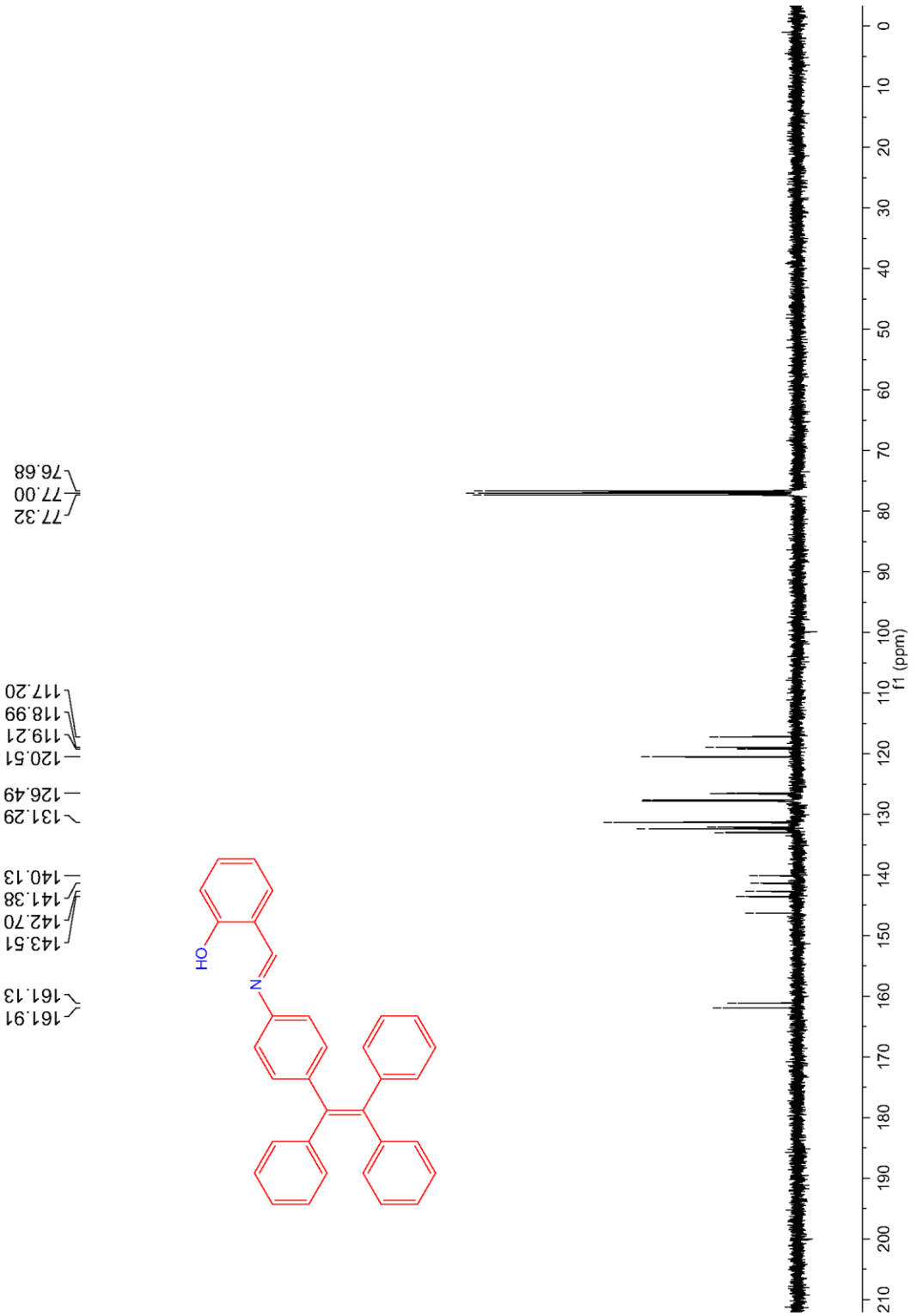


Fig. S3 Fluorescence spectra of **AIE-S-Cu (**20 μM**)** after being treated with Na_2S (25 μM) for less than 1 minute and 30 min. Spectra were taken in HEPES (10 mM, pH 7.4, 25°C, 10% THF).

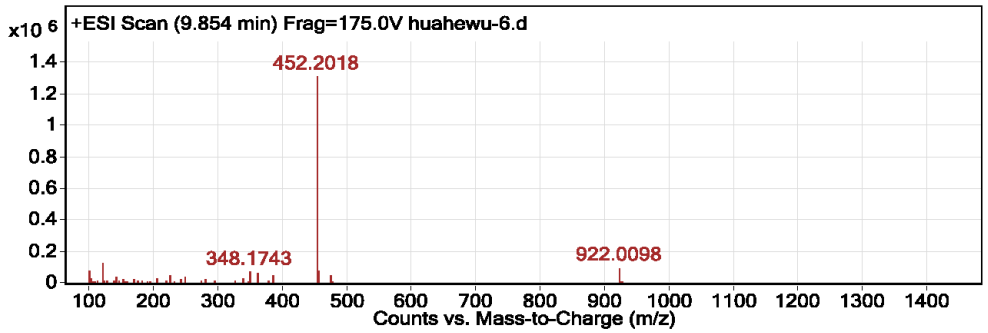


^{1}H NMR of **AIE-S**



^{13}C NMR of **AIE-S**

Qualitative Analysis Report



Peak List

m/z	z	Abund
100.112		86516.3
120.1017		129189.1
121.0509		133240.9
348.1743		75824.6
360.3242		74476.1
452.2018	1	1317913.3
453.2051	1	499596.8
454.208	1	84141.6
922.0098		102816.5

HRMS spectra of **AIE-S**