Supporting information

A Thiophen-Thiooxorhodamine Conjugate Fluorescent Probe for Detecting Mercury in Aqueous Media and Living Cells

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1. Materials and general methods

All the solvents were of analytic grade. The salts used in stock solutions of metal ions were $NaNO_3$, KNO_3, $Mg(NO_3)_2$, $Ca(NO_3)_2$, $FeCl_2{\cdot}4H_2O$, $MnCl_2$, $Ni(NO_3)_2{\cdot}6H_2O$, $Co(NO_3)_2{\cdot}6H_2O$, $CuSO_4$, $Zn(NO_3)_2{\cdot}2H_2O$, $CdCl_2{\cdot}H_2O$, $AgNO_3$, $Hg(ClO_4)_2$, $Pb(NO_3)_2$ and $CrCl_3{\cdot}6H_2O$. Water was re-distilled. 2-thiophenecarboxaldehyde was purchased from Sigma-Aldrich. The other reagents were purchased from Taiyuan RHF Reagents Ltd. Rhodamine B hydrozide was synthesized according to the reference.

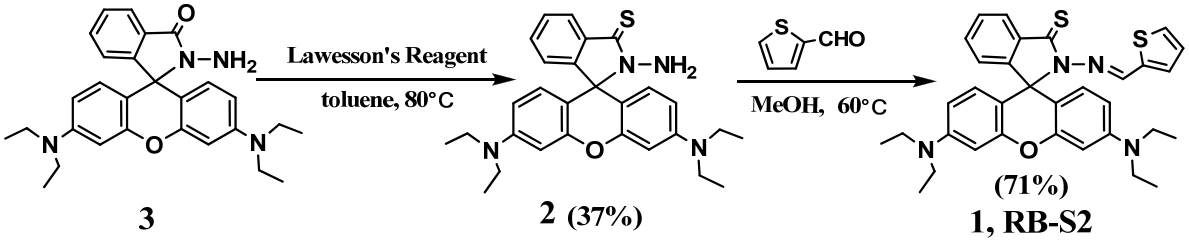
General instruments

^{1}H-NMR and ^{13}C-NMR were measured on a BrukerAV-500 or BrukerAV-300 spectrometer with chemical shifts reported in ppm (in $CDCl_3$ or DMSO-d_6; TMS as internal standard). UV-visible spectra were recorded on a Shimadzu UV-2550 spectrometer. Electrospray ionization mass spectra (ESI-MS) were measured on a Micromass LCTTM system. All pH measurements were made with a Sartorius basic pH-Meter PB-10. Fluorescence measurements were performed at room temperature on Perkin-Elmer LS 50B fluorescence spectrophotometer. Melting points were determined on a hot-plate melting point apparatus XT4-100A and uncorrected.

Cell Culture and Fluorescence Imaging

MCF-7 cells (human breast carcinoma cells) were passed and plated on a 24-well plate at a density of 2×10^3 cells per well in Dulbecco's Modified Eagle Medium (DMEM) (Gibco) supplemented with 10% fetal bovine serum (FBS, Sigma), penicillin (100 μg mL^{-1}), and streptomycin (100 μg mL^{-1}) at 37 °C in a humidified amosphere with 5% CO_2 and 95% air for 24 h prior to staining. Experiments to assess Hg(II) uptake were performed in the same media supplemented with 5 μM $Hg(ClO_4)_2$ for 30 min. 5 μM **RB-S2** in the culture media containing ethanol/PBS (1:49, v/v) was added to the cells, which were incubated for 30 min at 37 °C. After washing twice with PBS (phosphate buffered saline, pH=7.2, Gibco) to remove the remaining sensor, the treated cells were imaged by fluorescence microscopy (BX51, olympus, Japan).

2. Synthesis



3 **2** (37%) **1, RB-S2**

Rhodamine-B hydrazide **3**: Rhodamine B hydrazide was synthesis according to reported method.[5f] ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 7.93 (d, J= 5.4 Hz, 1H, Ar-H), 7.44 (d, J= 3.6 Hz, 1H, Ar-H), 7.42 (d, J= 3.6 Hz, 1H, Ar-H), 7.10 (d, J= 5.6 Hz, 1H, Ar-H), 6.46 (d, J = 8.0 Hz, 2H, Xanthene-H), 6.42(d, J = 3.2 Hz, 2H, Xanthene-H), 6.29 (m, 2H, Xanthene-H), 3.60 (s, 2H, NH_2), 3.34 (q, 7.0 Hz, 8H, NCH_2CH_3), δ = 1.16 (t, J = 7.0 Hz, 12H, NCH_2CH_3).

Thiooxorhodamine-B hydrazide **2**: Rhodamine B hydrazide (5.0 mmol, 2.28 g) and Lawesson's Reagent (5.0 mmol, 2.03 g) were dissolved in dry toluene (60 ml), the reaction mixture were heated at 80°C for 24 h under N_2 atmosphere. After removal of toluene, the residue was stirred with K_2CO_3 concentrated for 2 h, and then extracted by CH_2Cl_2. After removal of CH_2Cl_2, the residue was purified by flash chromatography (CH_2Cl_2 / petroleum, 4:1, R_f = 0.4) as eluent to afford Thiooxorhodamine B hydrazide (0.87 g, yield: 37%). M.p. 185.4~186.7 °C. R_f = 0.4 (SiO_2; CH_2Cl_2 / petroleum, 4:1). ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 8.09 (d, J= 7.2 Hz, 1H, Ar-H), 7.46 (m, 2H, Ar-H), 7.10 (d, J = 7.2Hz, 1H, Ar-H), 6.43 (s, 2H, xanthene-H), 6.35 (d, J = 8.8Hz, 2H, xanthene-H), 6.27 (m, 2H, xanthene-H), 4.81 (s, 2H, NH_2), 3.34 (q, J = 7.0Hz, 8H, NCH_2CH_3), δ 1.16 (t, J = 7.0Hz, 12H, NCH_2CH_3). ^{13}C-NMR (75 MHz, $CDCl_3$): 182.93, 153.45, 149.26, 149.20, 136.36, 131.95, 128.50, 128.02, 124.53, 123.05, 108.02, 103.34, 97.98, 44.34, 12.55. Anal. Calcd for $C_{28}H_{32}N_4OS$: C, 71.15; H, 6.82; N, 11.85. Found: C, 71.24; H, 6.86; N, 11.77. TOF-MS: m/z 473.16 $[M+1]^+$.

Chemosensor **1** (RB-S2): Thiooxorhodamine B hydrazide (1.0 mmol, 0.47 g) and 2-thiophenecarboxaldehyde (1.50 mmol, 0.17 g) were dissolved in dry boiling methanol (10 ml), the reaction mixture were heated at 60 °C for 12 h under N_2 atmosphere. The yellow precipitates were filtered and washed with cold methanol. The crude product recrystallized from hot EtOH to afford a yellowish solid RB-S2 (0.40 g, yield: 71%). M.p. 166.2~167.2 °C. ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 8.30 (d, J= 7.0 Hz, 1H, Ar-H), 8.15 (s, 1H, Ar-H), 7.69 (d, J= 4.8 Hz, 1H, Ar-H),

7.42 (m, 3H, Ar-H), 7.10 (t, J= 7.6 Hz, 2H, Ar-H), 6.80 (d, J= 8.5 Hz, 2H, xanthene-H), 6.31 (s, 2H, xanthene-H), 6.27 (m, 2H, xanthene-H). 3.32 (q, J = 10.4Hz, 8H, NCH_2CH_3), δ 1.16 (t, J = 10.4Hz, 12H, NCH_2CH_3). ^{13}C-NMR (75 MHz, $CDCl_3$): 170.21, 155.53, 151.72, 148.26, 147.96, 135.31, 134.84, 133.27, 132.10, 130.42, 130.20, 127.83, 126.92, 126.32, 123.10, 110.21, 108.25, 97.48, 44.34, 12.61. Anal. Calcd for $C_{33}H_{34}N_4OS_2$: C, 69.93; H, 6.05; N, 9.89. Found: C, 69.99; H, 6.08; N, 9.81. TOF-MS: m/z 567.14 $[M+1]^+$, 589.16 $[M+Na]^+$.

3. Supplementary spectra data

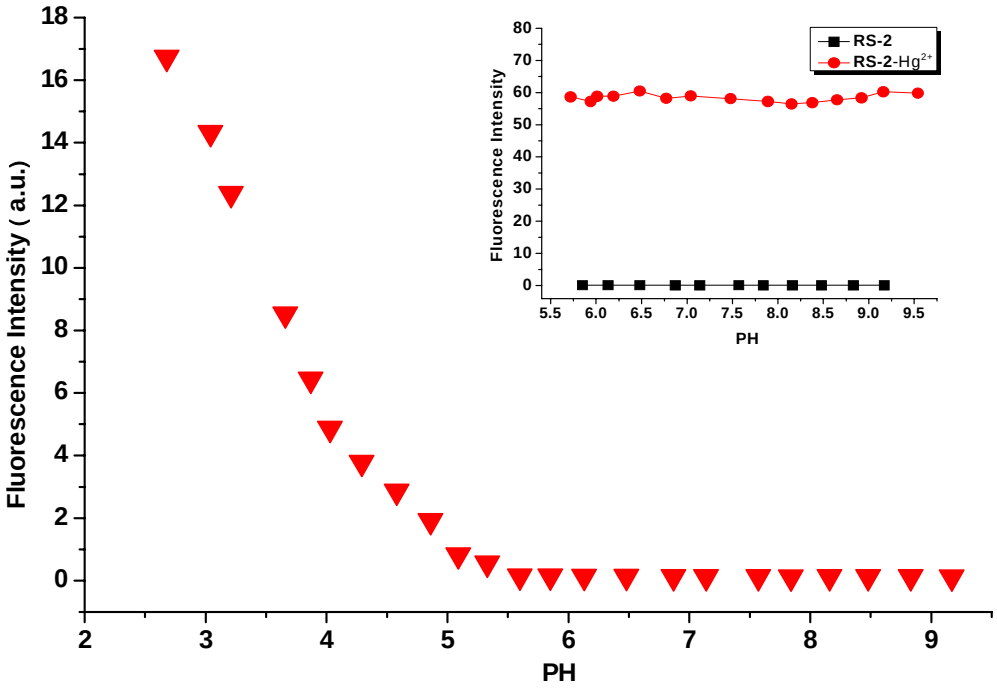


Figure S1: Fluorescence responses of probe **RB-S2** (1 μM) at different pH.

Table S1: Spectral property of **RB-S2** (1 μM) and **RB-S2**/Hg^{2+} (1 μM/1 μM) in ethanol/H_2O

Compd.	λ_{ab} (nm)	ε ($L \cdot mol^{-1} \cdot cm^{-1}$)	λ_{em} (nm)	Φ
RB-S2	567	6.82×10^2	593	0.00037
RB-S2/Hg^{2+}	567	1.48×10^5	593	0.47

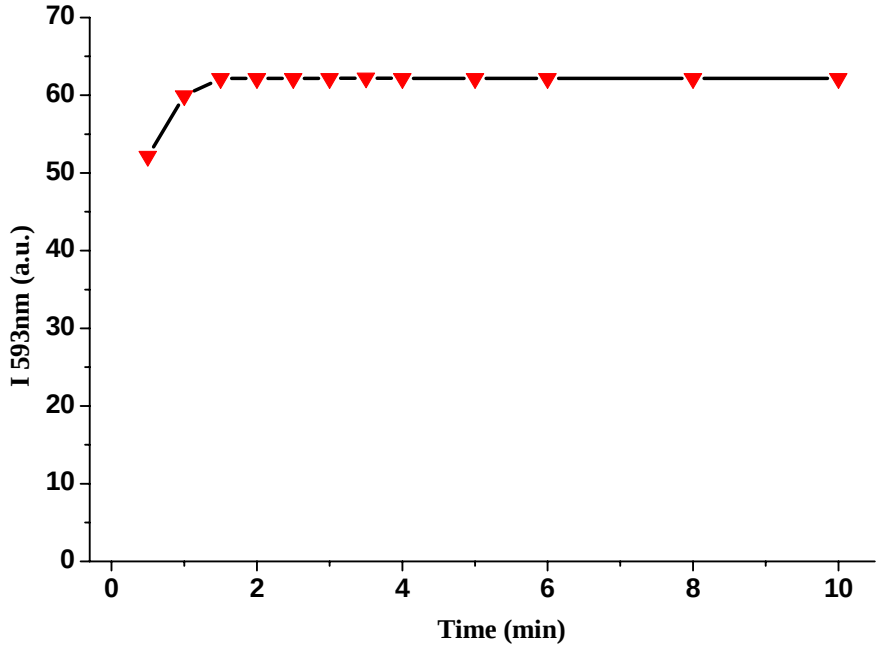


Figure S2: Time evolution of **RB-S2** (5 μM) in aqueous ethanol (50 mM, pH 7.0, 50:50, v/v) in the presence of 2.0 equiv of Hg^{2+} ion.

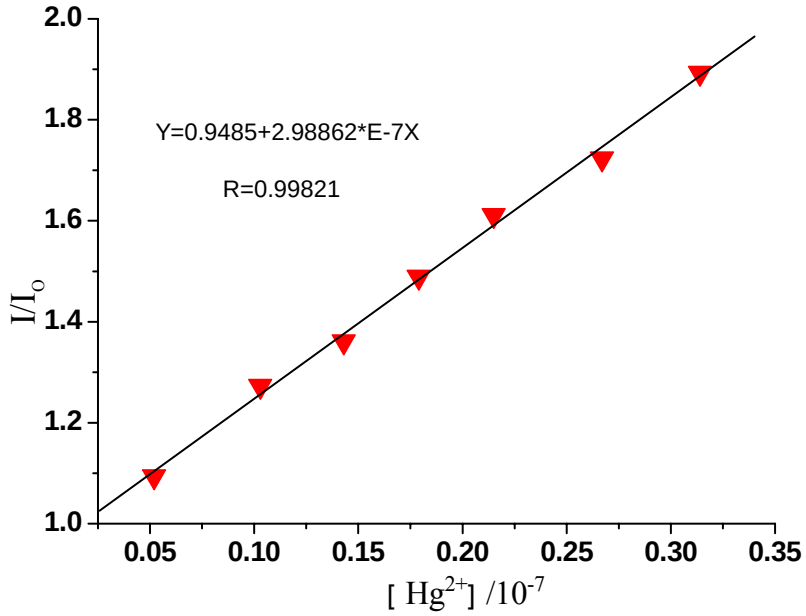


Figure S3: The fluorescence at 593 nm of **RB-S2** (0.1 μM) as a function of the Hg^{2+} concentration (0.5–3.14 × 10^{-8} M).

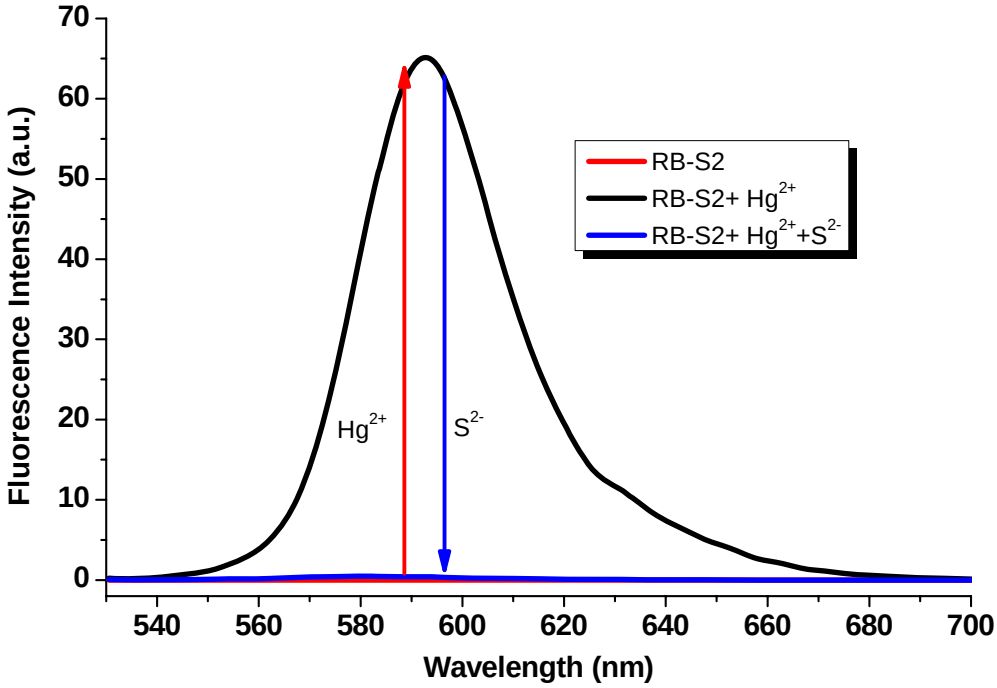


Figure S4. Reversibility of Hg^{2+} ions to **RB-S2** (1 μM) by Na_2S. Red line: free **RB-S2** (1 μM), black line: **RB-S2** + 2 equiv of Hg^{2+}, Blue line: **RB-S2** + 2 equiv of Hg^{2+} + 0.2 mL of Na_2S (0.1 mM).

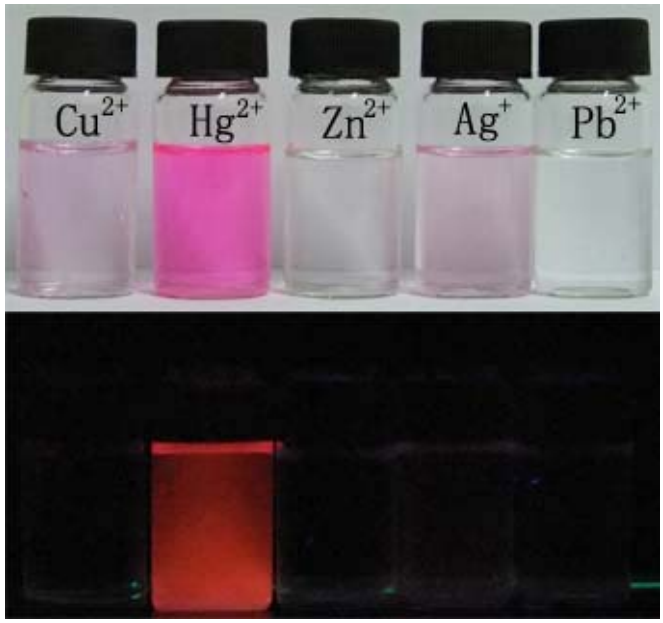


Figure S5: Change in (top) color and (bottom) fluorescence of **RB-S2** (5 μM) in aqueous ethanol (50 mM, pH 7.0, 50:50, v/v) with different ions.

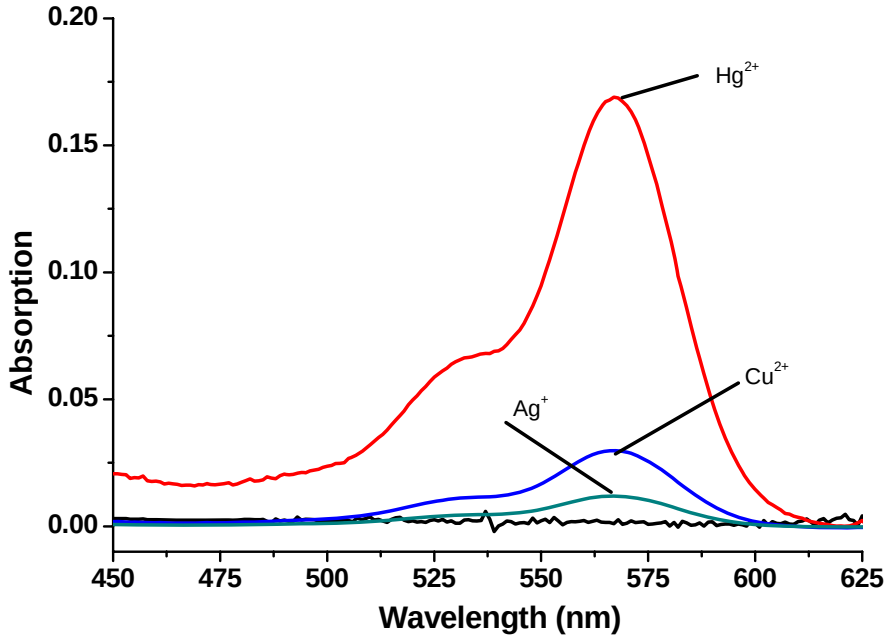


Figure S5: Absorption spectra of 5 μM **RB-S2** in aqueous ethanol (50 mM, pH 7.0, 50:50, v/v) upon addition of of Hg^{2+}, Ag^{+}, and Cu^{2+}ions.

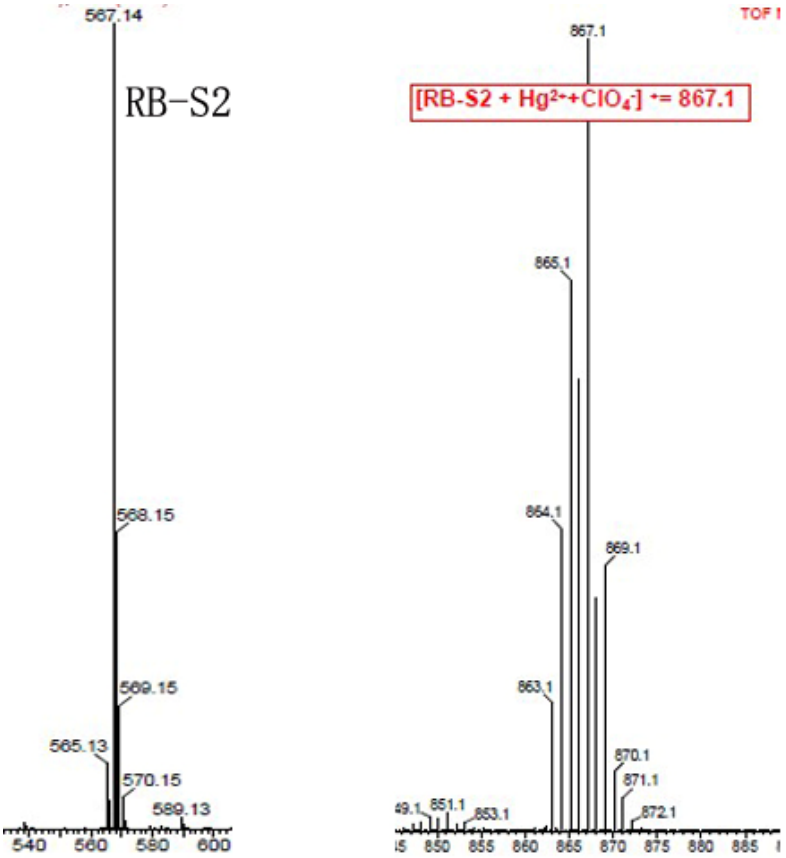


Figure S7: ESI mass spectrum

4. Cytotoxicity assay

To ascertain the cytotoxic effect of $Hg(ClO_4)_2$ treatment over a 24 h, the MTT assay was performed. MCF-7 cells (5×10^4) were passed and plated to 70% confluence in 96-well plates 24 h before treatment. $(0.2–25)\times10^{-6}$ mol/L RB-S2 was added to the cells and incubated at 37°C for 24 h. Cytotoxicity was then determined by the method of Thiazolyl Blue Tetrazolium Bromide (MTT) assay (Cell Proliferation Kit; keygen biological products, Nanjing, China), following the instructions of the kit. Subsequently, the cells incubated with 5 mg/mL MTT reagent at 37°C for 4 h, the absorbance of each well was measured by a microplate reader (SPECTRA SLT; Labinstruments, Salzburg, Austria). The excitation wavelength was 492 nm, and the emission was read at 690 nm. Each treatment was done in six wells, and the experiments were repeated three times. Cytotoxicity was calculated relative to the absorbance of the control for each treatment. Data were expressed as means. SD. The reported percent cell survival values are relative to untreated control cells.

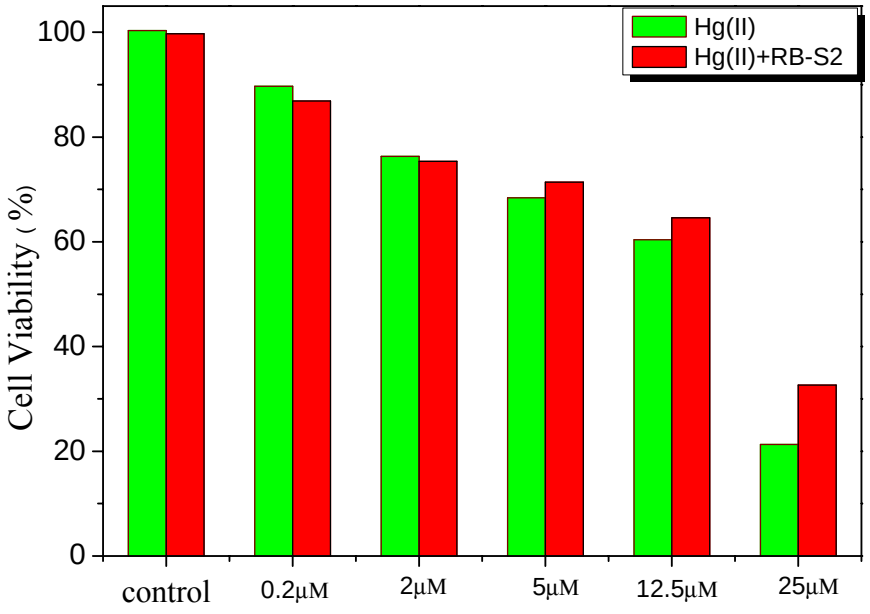
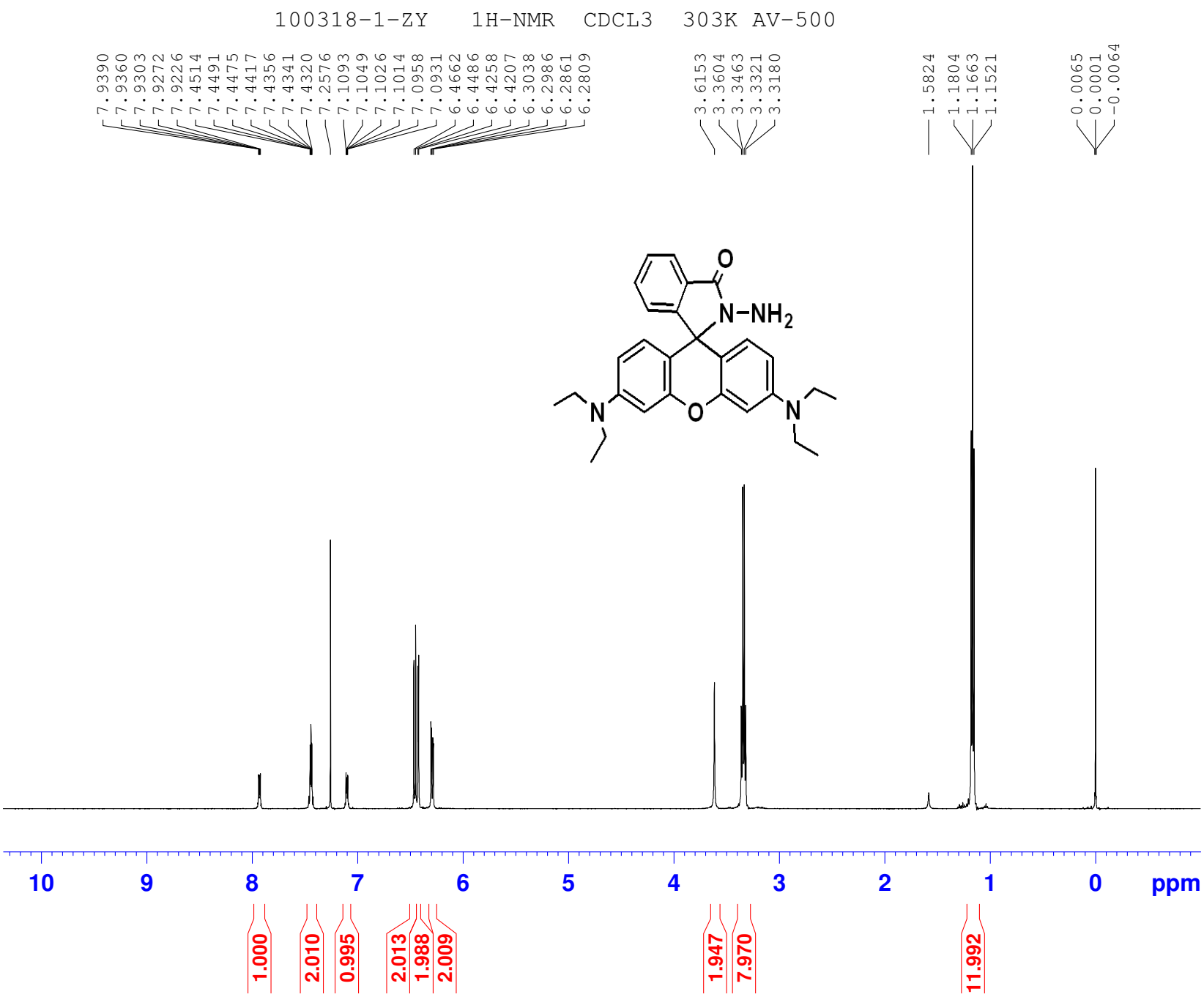
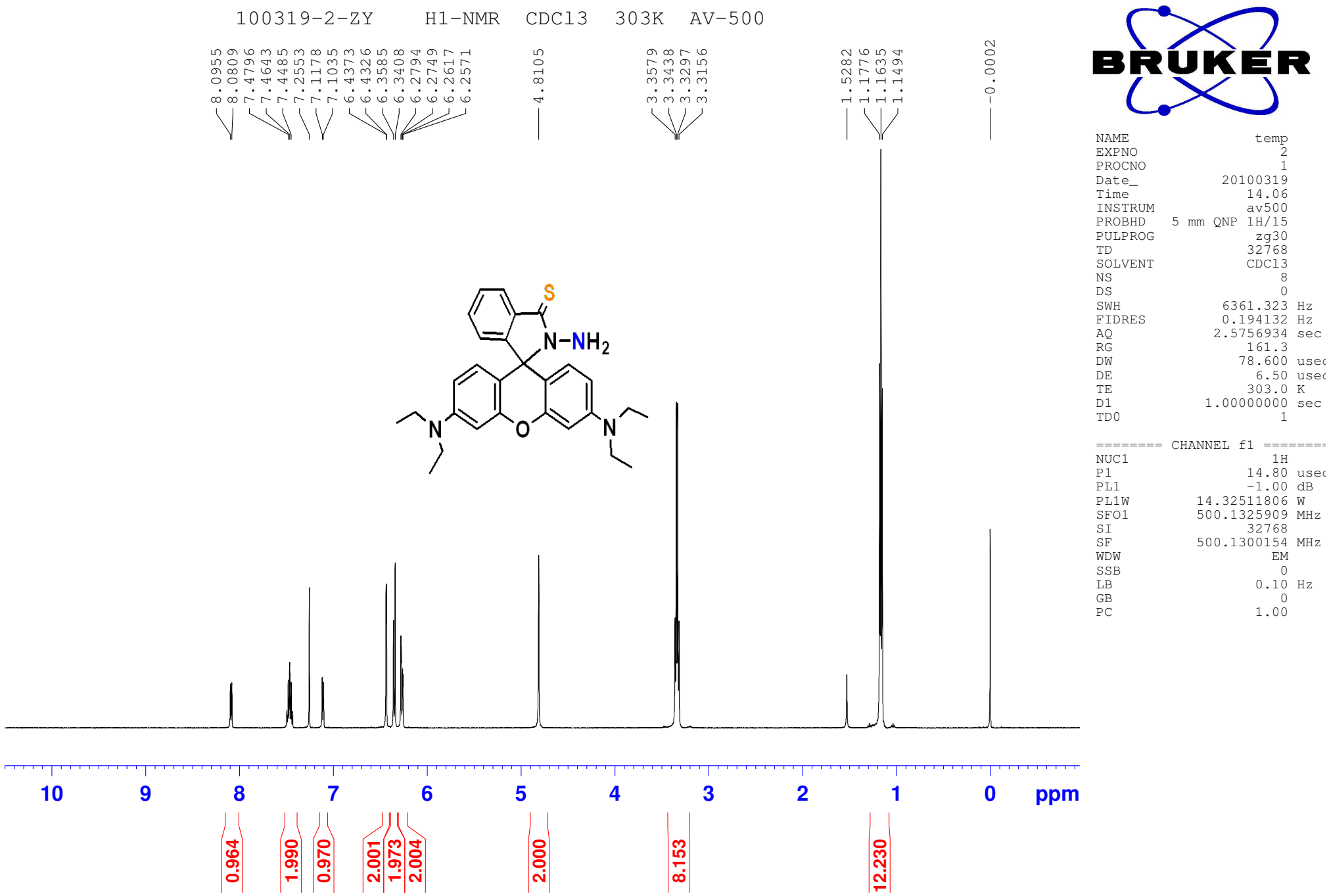


Figure S8: Cell viability was quantified by the MTT assay (MCF-7, 24h)



NAME	temp
EXPNO	1
PROCNO	1
Date_	20100319
Time	14.01
INSTRUM	av500
PROBHD	5 mm QNP 1H/15
PULPROG	zg30
TD	65536
SOLVENT	CDCl3
NS	8
DS	0
SWH	5681.818 Hz
FIDRES	0.086698 Hz
AQ	5.7673059 sec
RG	114
DW	88.000 usec
DE	6.50 usec
TE	303.0 K
D1	2.00000000 sec
TD0	1
======== CHANNEL f1 ========	
NUC1	1H
P1	14.80 usec
PL1	-1.00 dB
PL1W	14.32511806 W
SFO1	500.1323559 MHz
SI	32768
SF	500.1300143 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00



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PROCNO	1
Date_	20100319
Time	14.06
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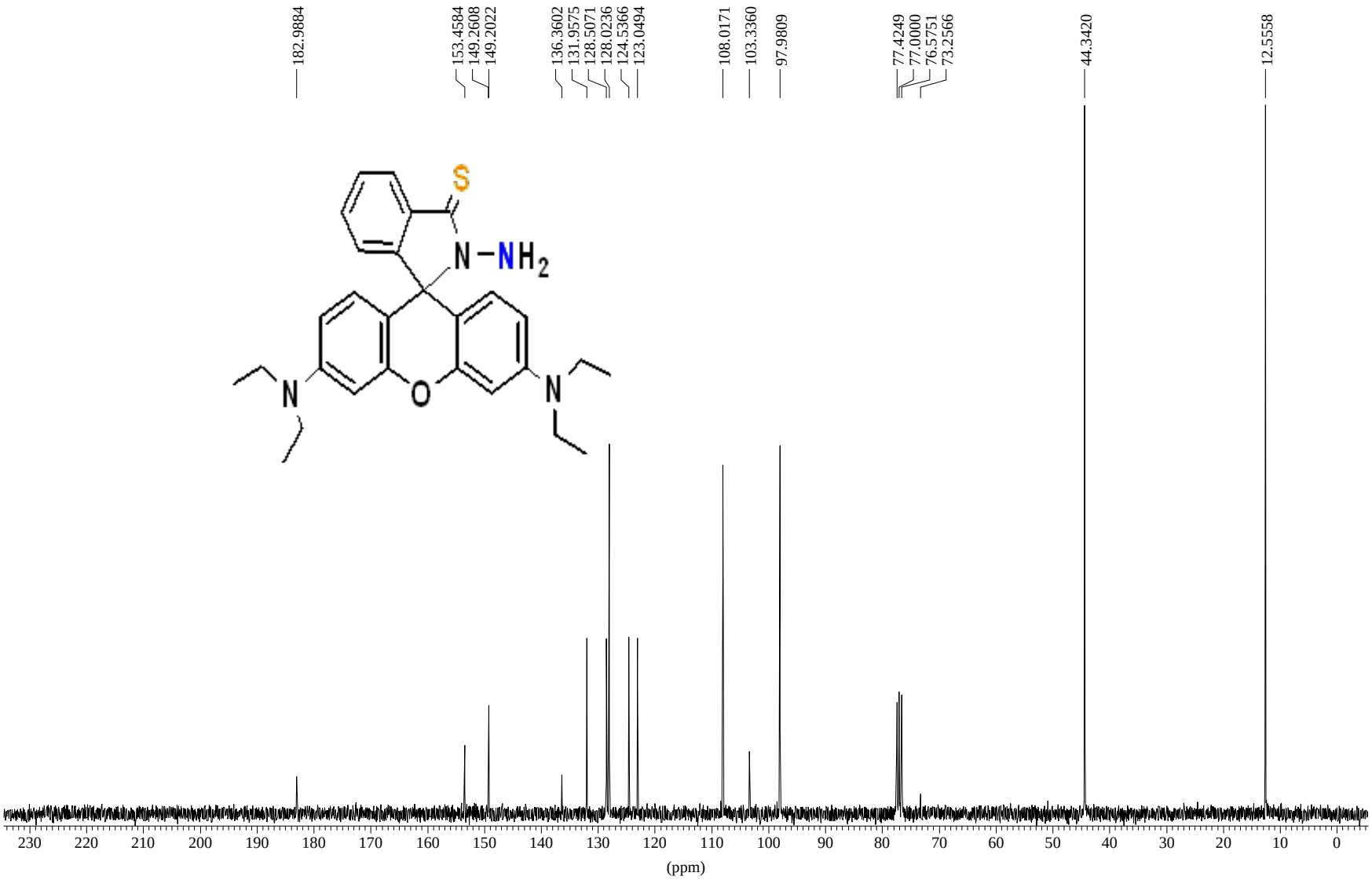
PROBHD	5 mm QNP 1H/15
PULPROG	zg30
TD	32768
SOLVENT	CDCl3
NS	8
DS	0
SWH	6361.323 Hz
FIDRES	0.194132 Hz
AQ	2.5756934 sec
RG	161.3
DW	78.600 usec
DE	6.50 usec
TE	303.0 K
D1	1.00000000 sec
TD0	1
======== CHANNEL f1 ========	
NUC1	1H
P1	14.80 usec
PL1	-1.00 dB
PL1W	14.32511806 W
SFO1	500.1325909 MHz
SI	32768
SF	500.1300154 MHz
WDW	EM
SSB	0
LB	0.10 Hz
GB	0
PC	1.00

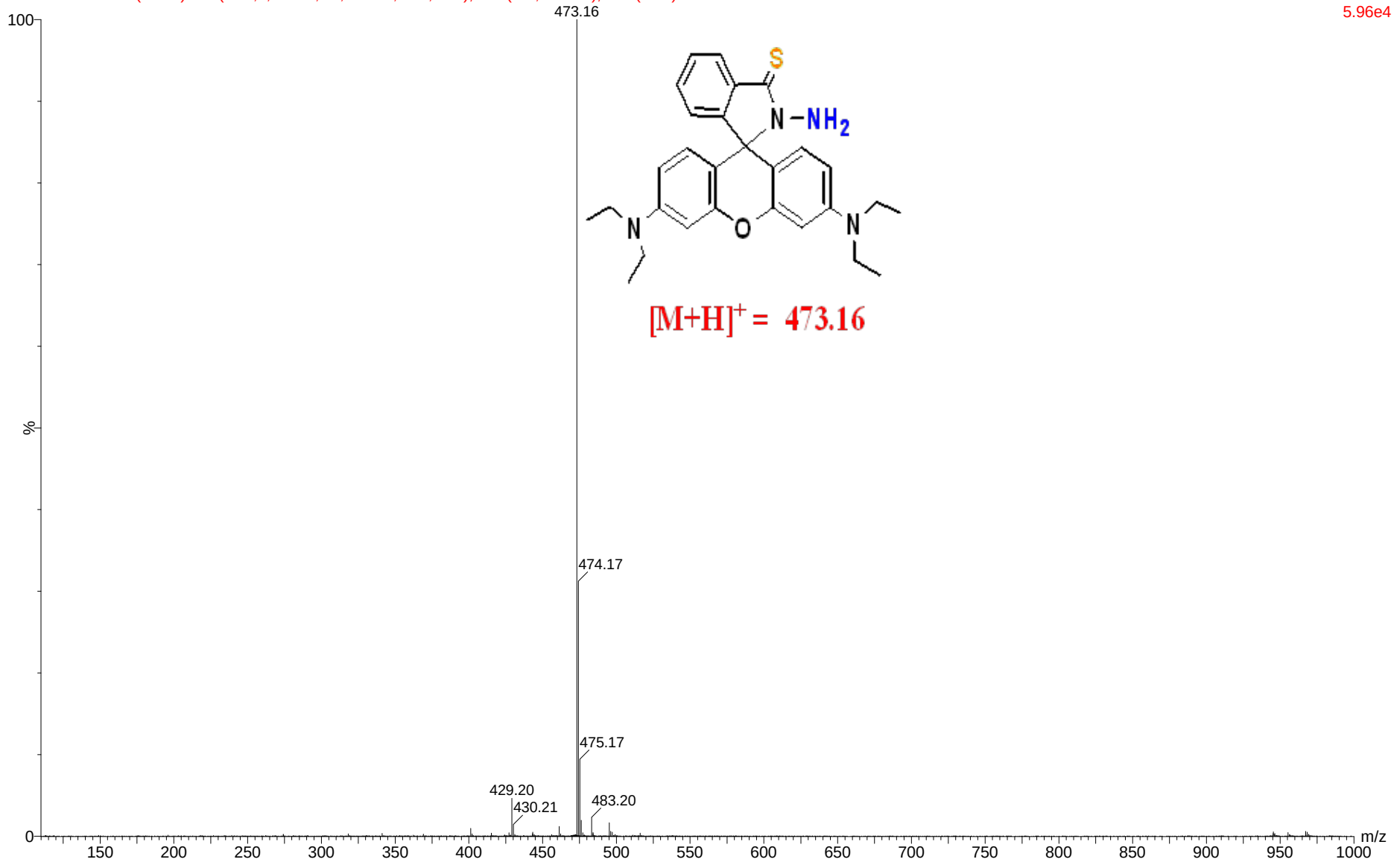
100321-1#-ZY 13C-NMR CDCl3 300K AV-300



1

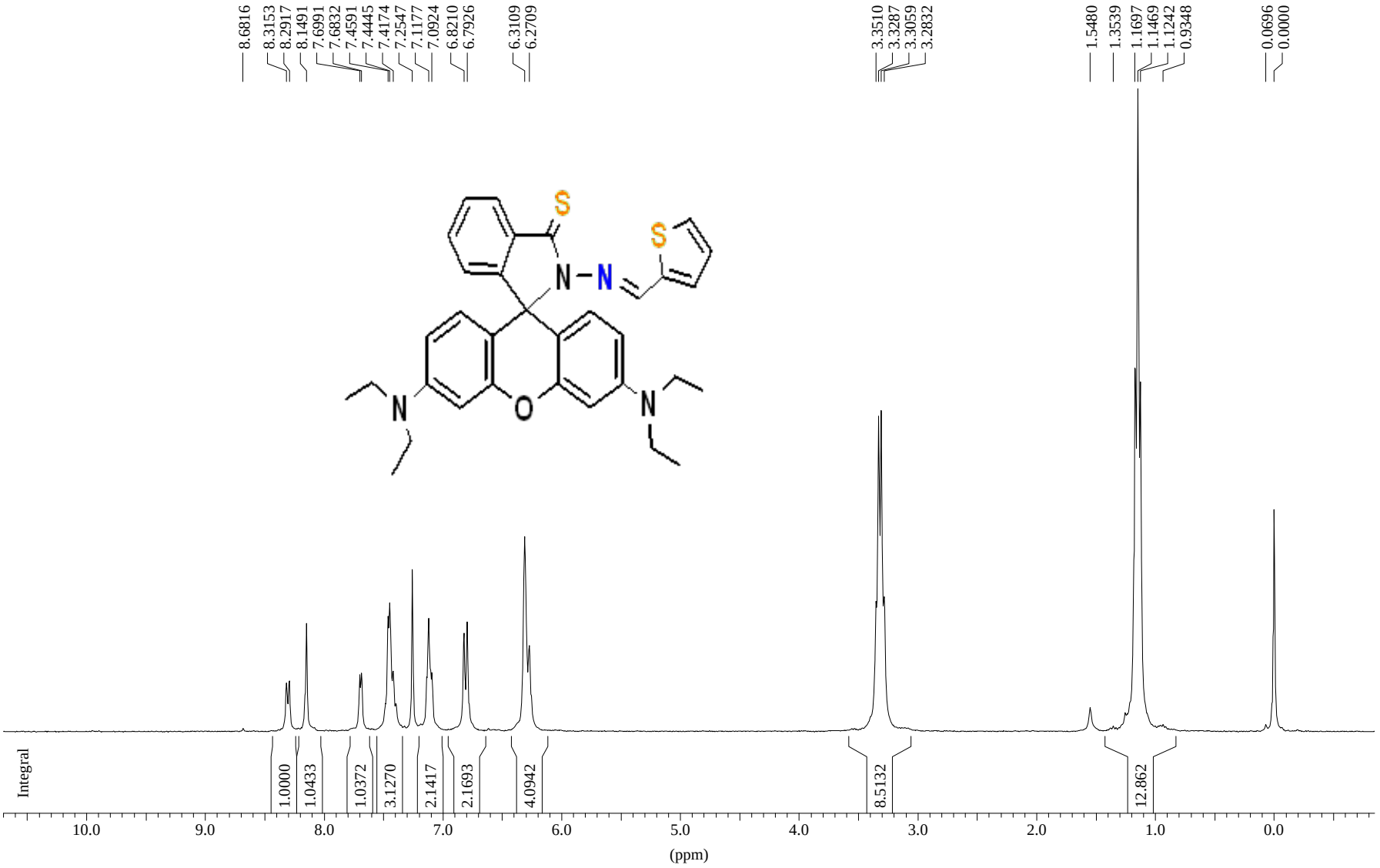
MA-100323-05 10 (0.125) AM (Cen,2, 80.00, Ar,5000.0,0.00,0.70); Sm (SG, 2x3.00); Cm (5:37)

TOF MS ES+
5.96e4

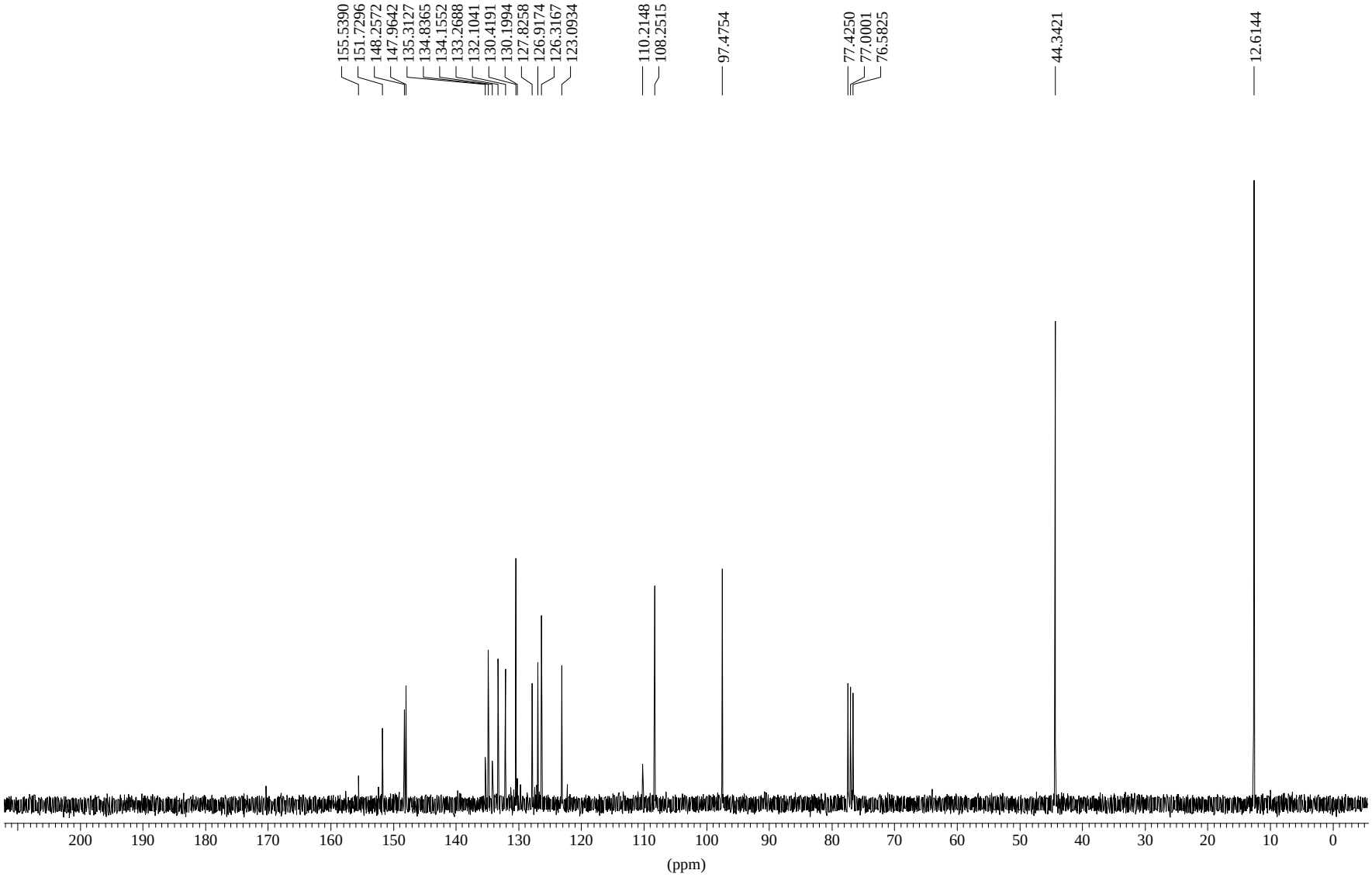


$[M+H]^+ = 473.16$

100322-zhouyi 1H-NMR CDCl3 300K AV-300



2#-ZY 13C-NMR CDCl3 300K AV-300



2

MA-100323-06 35 (0.442) AM (Cen,2, 80.00, Ar,5000.0,0.00,0.70); Sm (SG, 2x3.00); Cm (7:36)

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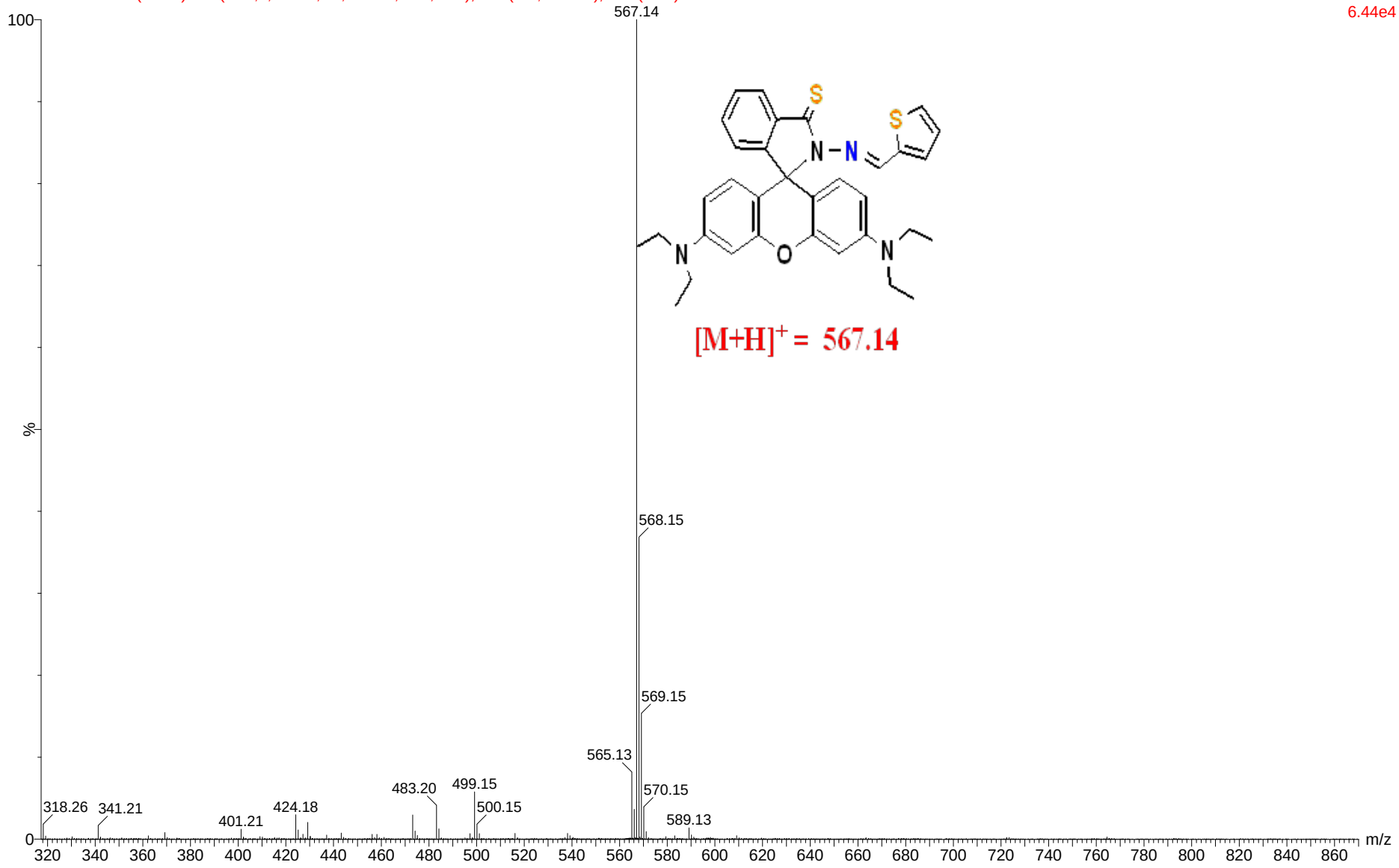
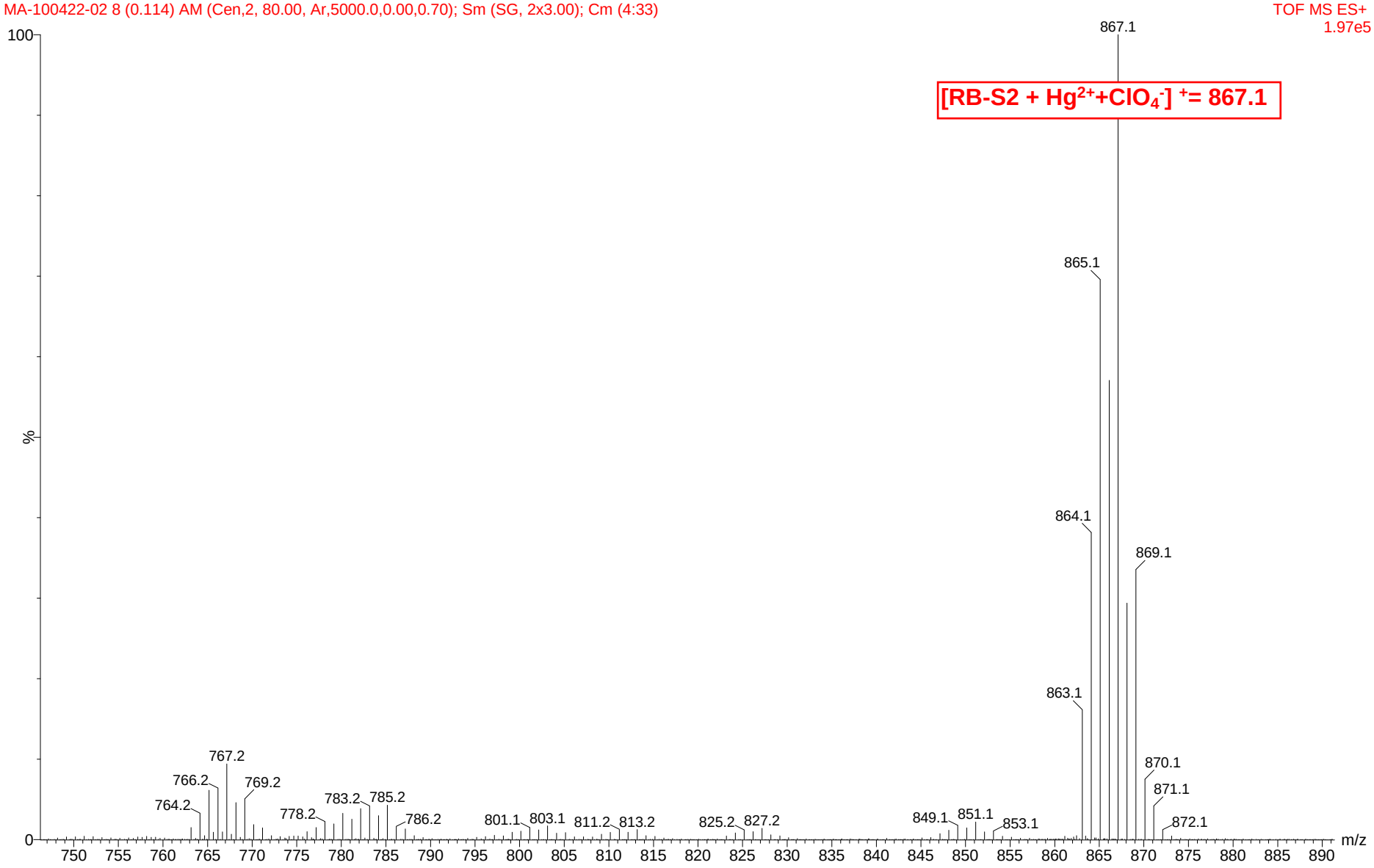
6.44e4



$[M+H]^+ = 567.14$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TOF MS ES+
1.97e5
[RB-S2 + Hg2+ClO4-] += 867.1
867.1
865.1
864.1
869.1
863.1
870.1
871.1
872.1
767.2
766.2
769.2
764.2
783.2
785.2
778.2
786.2
801.1
803.1
811.2
813.2
825.2
827.2
849.1
851.1
853.1
100
%
0
m/z
750 755 760 765 770 775 780 785 790 795 800 805 810 815 820 825 830 835 840 845 850 855 860 865 870 875 880 885 890