

# Supporting Information

## A Six-Membered-Ring Incorporated Si-Rhodamine for Imaging of Copper(II) in Lysosomes

Baogang Wang,<sup>a</sup> Xiaoyan Cui,<sup>b</sup> Zhiqiang Zhang,<sup>a</sup> Xiaoyun Chai,<sup>a</sup> Hao Ding,<sup>a</sup> Qiuye  
Wu,<sup>a</sup> Zhongwu Guo,<sup>a,c\*</sup> and Ting Wang<sup>a\*</sup>

<sup>a</sup>Department of Organic Chemistry, College of Pharmacy, Second Military Medical  
University, Shanghai 200433, China

<sup>b</sup>Department of Chemistry, New York University, New York, New York 10003, USA

<sup>c</sup>Department of Chemistry, Wayne State University, 5101 Cass Avenue, Detroit,  
Michigan 48202, USA

E-mail: wangting1983927@gmail.com, zwguo@chem.wayne.edu

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# 1. Synthesis

## Synthesis of R-Cu-1

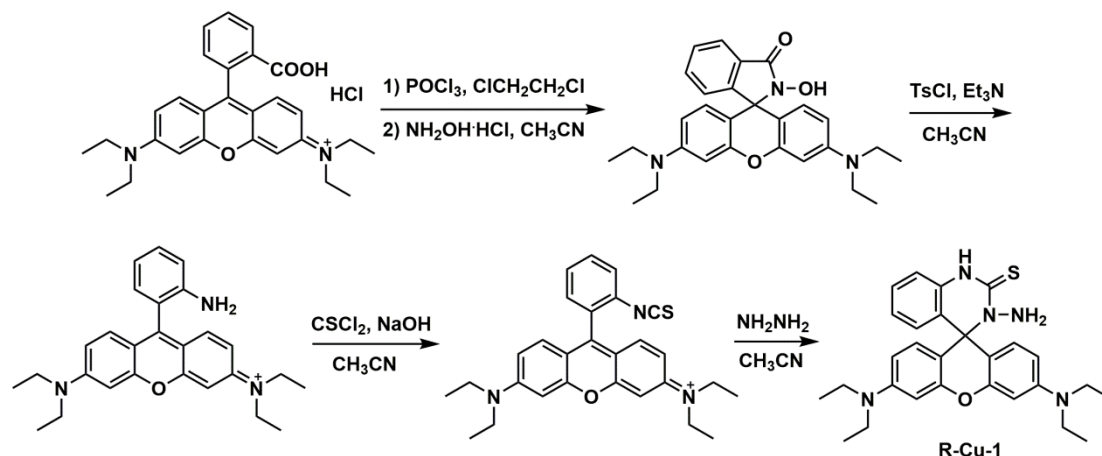


Fig. S1 Synthesis of R-Cu-1.

**R-Cu-1.** The compound was synthesized according to the reported procedure. <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>): δ 1.17 (t, 12H, *J* = 7.2 Hz), 3.34 (q, 8H, *J* = 7.2 Hz), 4.53 (s, 2H), 6.33-7.14 (m, 10H); <sup>13</sup>C NMR (150 MHz, CDCl<sub>3</sub>): δ 12.67, 44.37, 65.45, 97.01, 108.08, 111.46, 112.87, 123.58, 127.64, 128.15, 130.10, 130.56, 132.21, 148.68, 152.15, 171.56; HRMS (ESI) calcd. for C<sub>28</sub>H<sub>34</sub>N<sub>5</sub>OS<sup>+</sup> [M+H]<sup>+</sup>: 488.2479, found:488.2483.

## Synthesis of R-Cu-2

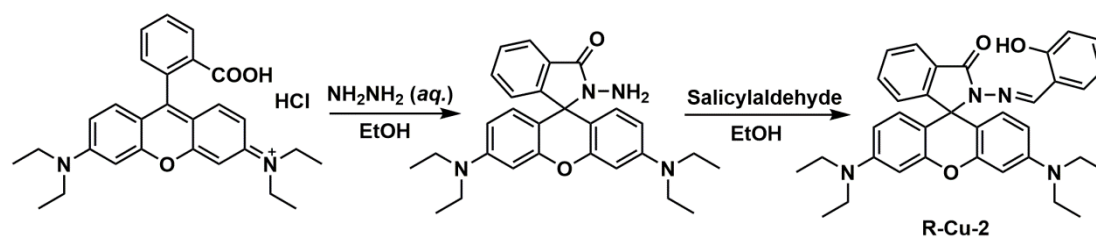


Fig. S2 Synthesis of R-Cu-2

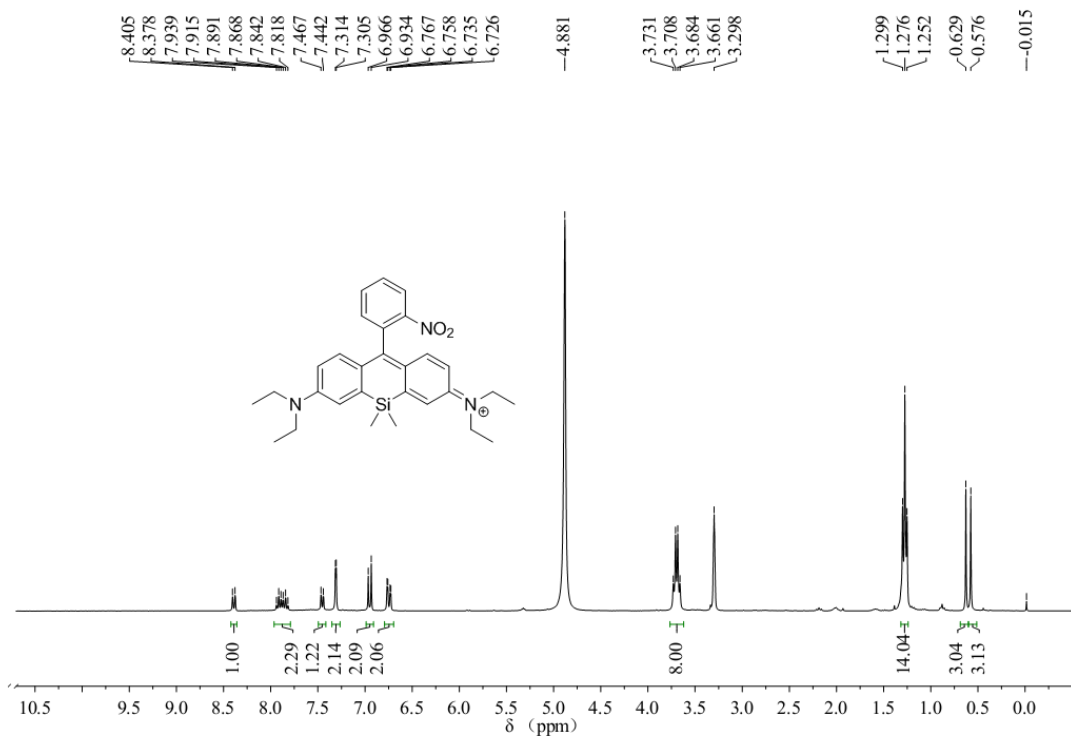
**R-Cu-2.** The compound was synthesized according to the reported procedure. <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>): δ 1.17 (t, 12H, *J* = 7.2 Hz), 3.34 (q, 8H, *J* = 7.2 Hz), 4.53 (s, 2H), 6.27-8.00 (m, 14H), 9.25 (s, 1H), 10.85 (s, 1H); <sup>13</sup>C NMR (150 MHz, CDCl<sub>3</sub>): δ 12.63, 44.36, 66.50, 97.99, 105.42, 108.13, 117.00, 118.66, 118.88, 123.32, 124.20, 128.12, 128.58, 130.08, 131.45, 131.22, 133.47, 149.10, 150.76, 152.96, 153.61, 158.67, 164.23; HRMS (ESI) calcd. for C<sub>35</sub>H<sub>37</sub>N<sub>4</sub>O<sub>3</sub><sup>+</sup> [M+H]<sup>+</sup>: 561.2860, found:561.2870.

## 2. X-Ray Crystallography

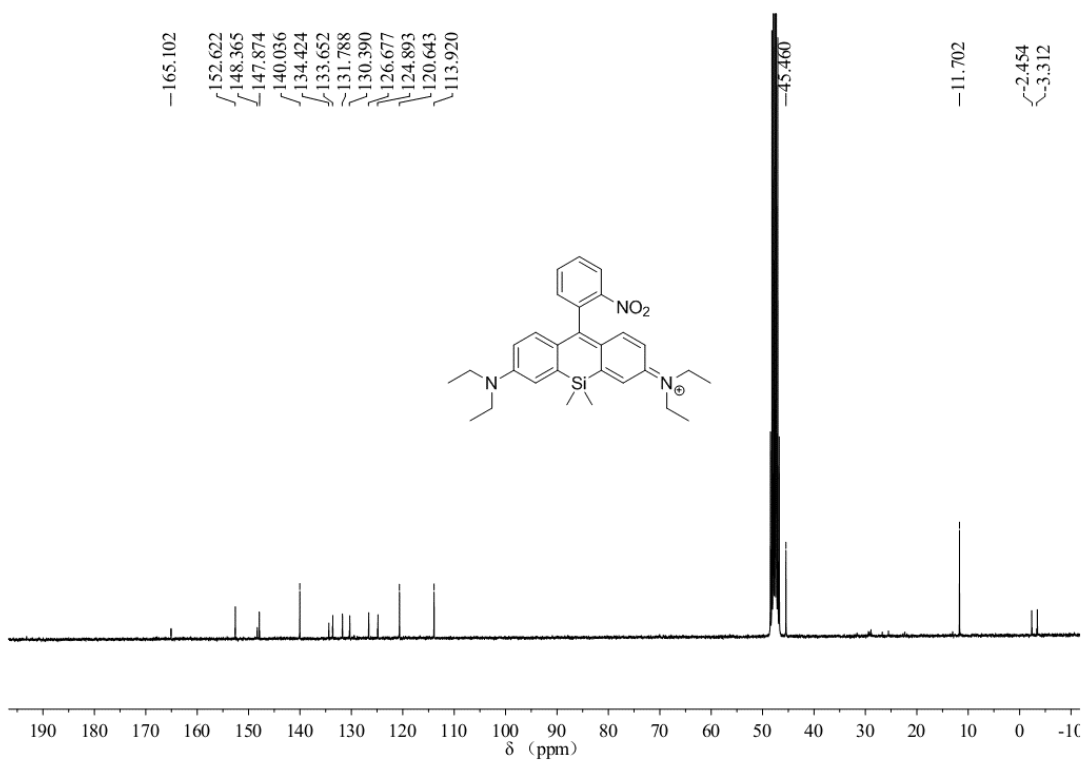
**Table S1** Crystal data and structure refinement for mo\_50629a.

Identification code	mo_50629
Empirical formula	C <sub>30</sub> H <sub>39</sub> Cl <sub>2</sub> N <sub>5</sub> SSi
Formula weight	529.81
Temperature	203(2) K
Wavelength	0.71073 Å
Crystal system	Triclinic
Space group	P -1
Unit cell dimensions	a = 11.709(6) Å      α = 105.979(8)° b = 17.325(10) Å     β = 103.227(9)° c = 17.761(10) Å     γ = 99.347(9)°
Volume	3273(3) Å <sup>3</sup>
Z, Density (calculated)	4, 1.075 Mg/m <sup>3</sup>
Absorption coefficient	0.160 mm <sup>-1</sup>
F (000)	1136
Crystal size	0.380 x 0.210 x 0.200 mm <sup>3</sup>
Theta range for data collection	1.245 to 25.009 °
Index ranges	-11 ≤ h ≤ 13, -20 ≤ k ≤ 20, -20 ≤ l ≤ 21
Reflections collected	18829
Independent reflections	11266 [R(int) = 0.0542]
Completeness to theta = 25.242 °	95.2 %
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.746 and 0.628
Refinement method	Full-matrix least-squares on F <sup>2</sup>
Data / restraints / parameters	11266 / 84 / 678
Goodness-of-fit on F <sup>2</sup>	1.475
Final R indices [I > 2σ(I)]	R1 = 0.1530, wR2 = 0.3885
R indices (all data)	R1 = 0.1954, wR2 = 0.4280
Extinction coefficient	0.0027(11)
Largest diff. peak and hole	2.579 and -0.813 e.Å <sup>-3</sup>

### 3. $^1\text{H}$ NMR, $^{13}\text{C}$ NMR and HRMS Spectra



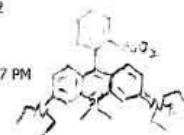
$^1\text{H}$  NMR of compound **SiR-NO<sub>2</sub>**



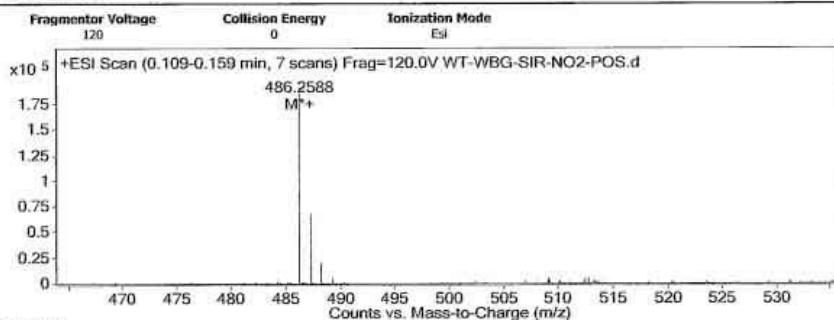
$^{13}\text{C}$  NMR of compound **SiR-NO<sub>2</sub>**

## Qualitative Analysis Report

Data Filename	WT-WBG-SIR-NO2-POS.d	Sample Name	WT-WBG-SIR-NO2
Sample Type	Sample	Position	P1-E5
Instrument Name	Instrument 1	User Name	
Acq Method	TEST-POS-01-WL.m	Acquired Time	6/25/2013 4:04:27 PM
IRM Calibration Status	Success	DA Method	Default.m
Comment			



### User Spectra



### Peak List

m/z	z	Abund	Formula	Ion
173.1654	1	182310		
274.2751	1	82935		
415.2126	1	99380		
430.1958	1	97094		
453.1683	1	52134		
458.2272	1	105415		
486.2588	1	190266	C29 H36 N3 O2 Si	M*+
487.2612	1	68179	C29 H36 N3 O2 Si	M*+
579.5366	1	90727		
607.5679	1	72183		

### Formula Calculator Element Limits

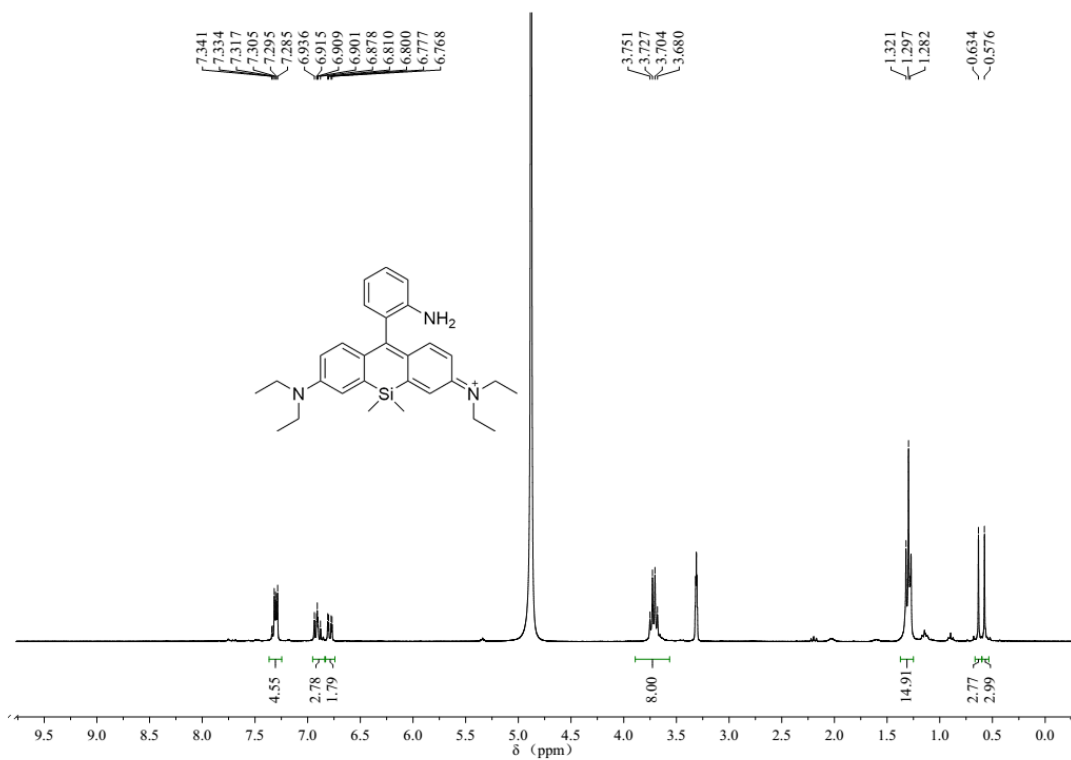
Element	Min	Max
C	3	50
H	0	300
O	0	2
N	0	10
Si	1	1

### Formula Calculator Results

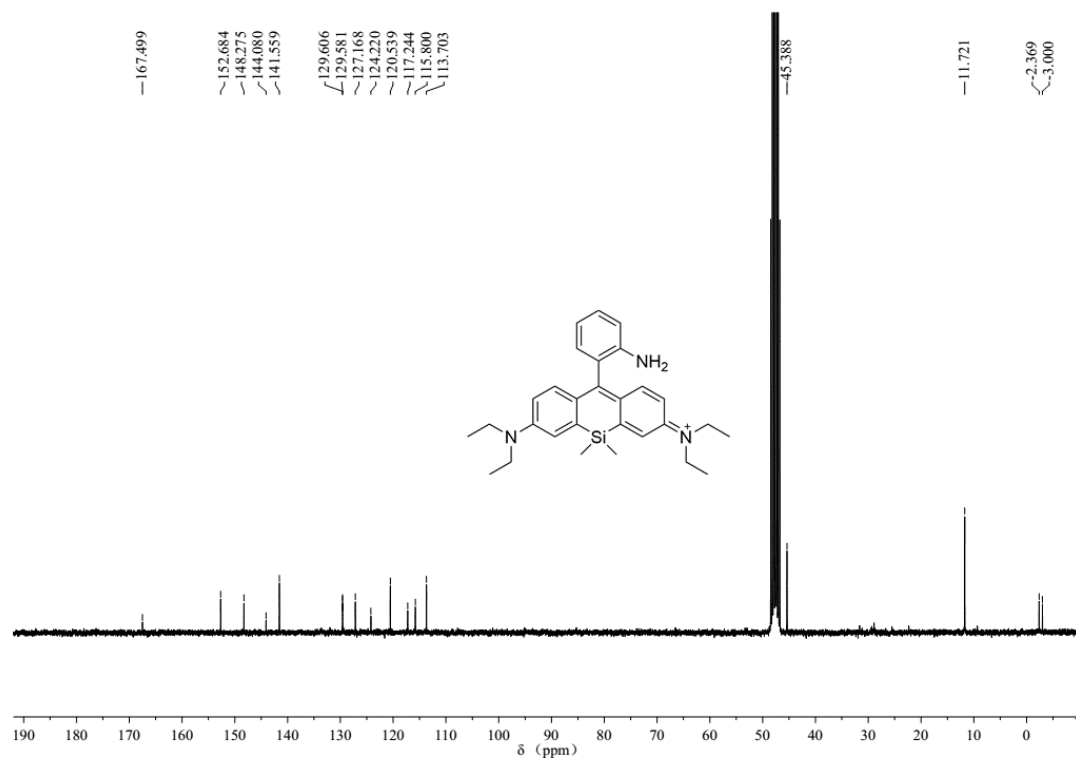
Formula	Best	Mass	Tgt Mass	Diff (ppm)	Ion Species	Score
C29 H36 N3 O2 Si	TRUE	486.2594	486.2577	-3.53	C29 H36 N3 O2 Si	81.47

--- End Of Report ---

HRMS spectra of compound **SiR-NO<sub>2</sub>**

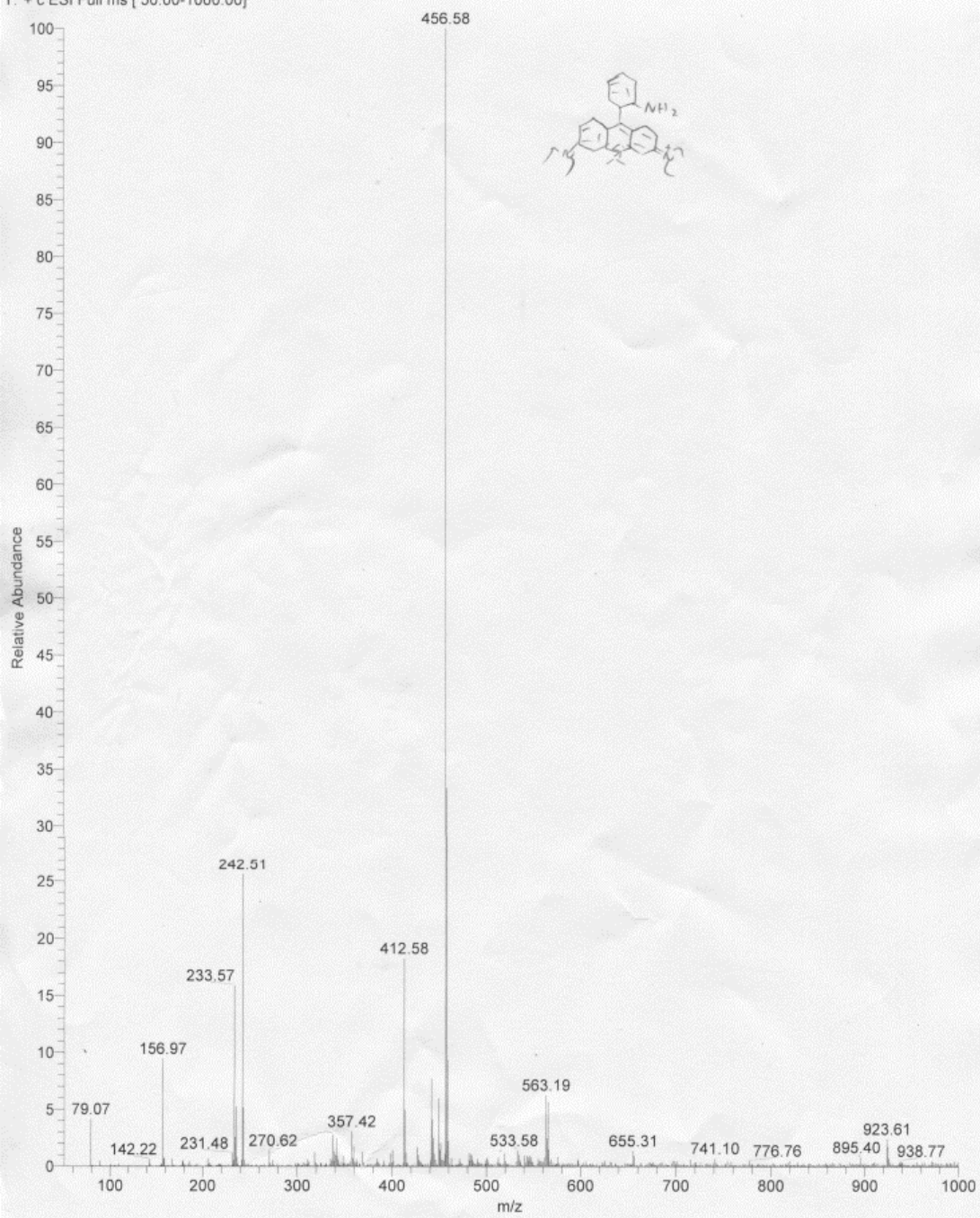


<sup>1</sup>H NMR of compound SiR-NH<sub>2</sub>



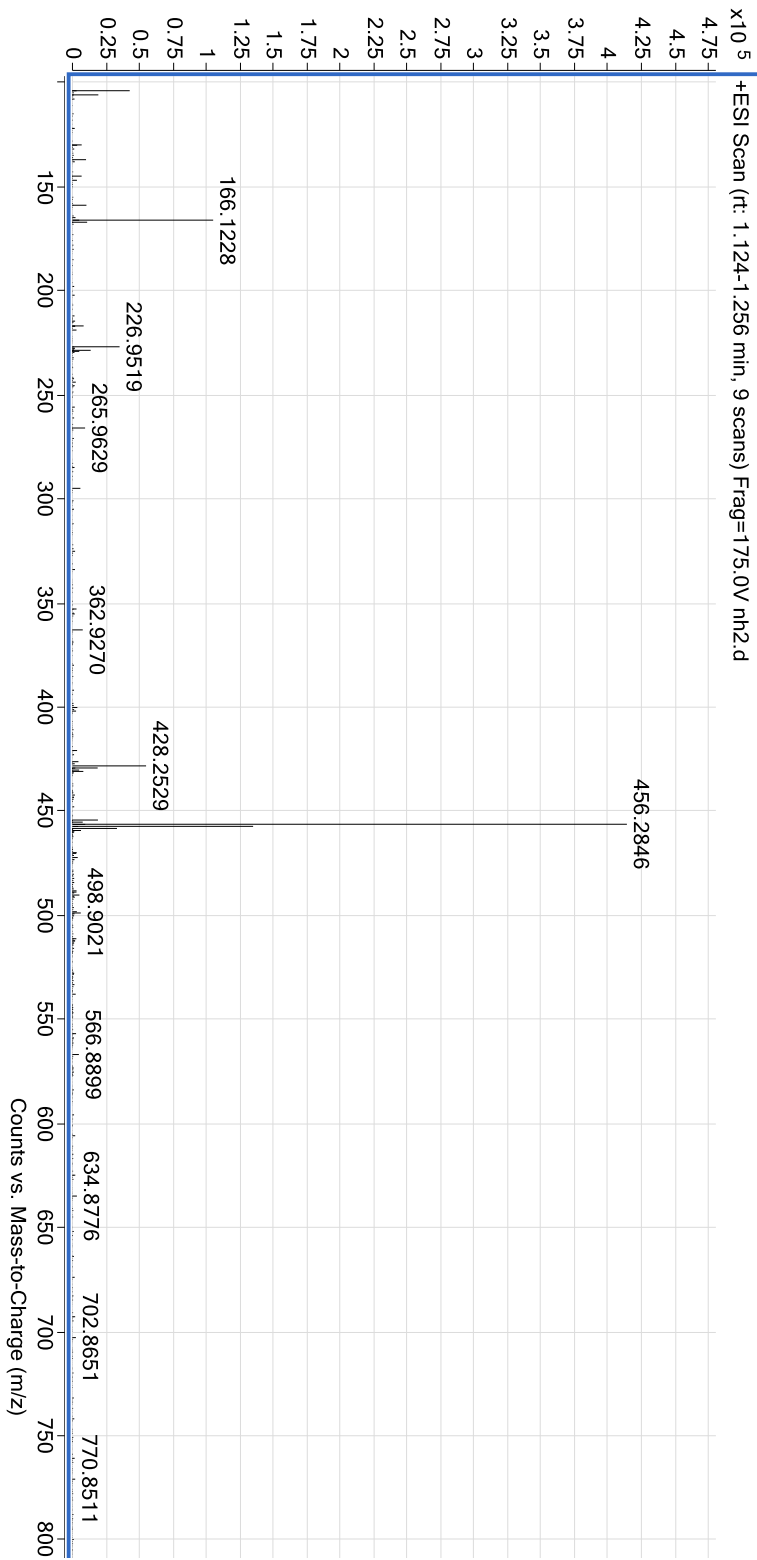
<sup>13</sup>C NMR of compound SiR-NH<sub>2</sub>

WT-WBG-SiR-NH2 #3-12 RT: 0.07-0.32 AV: 5 NL: 7.07E8  
T: + c ESI Full ms [ 50.00-1000.00]

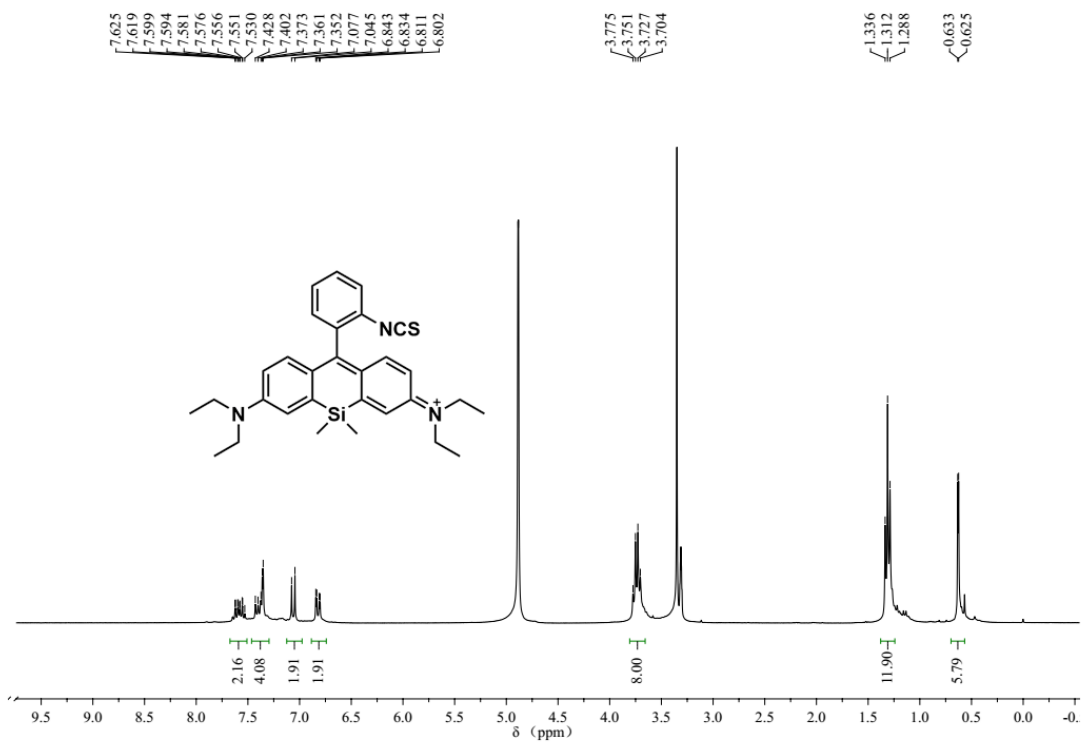


MS spectra of compound **SiR-NH<sub>2</sub>**

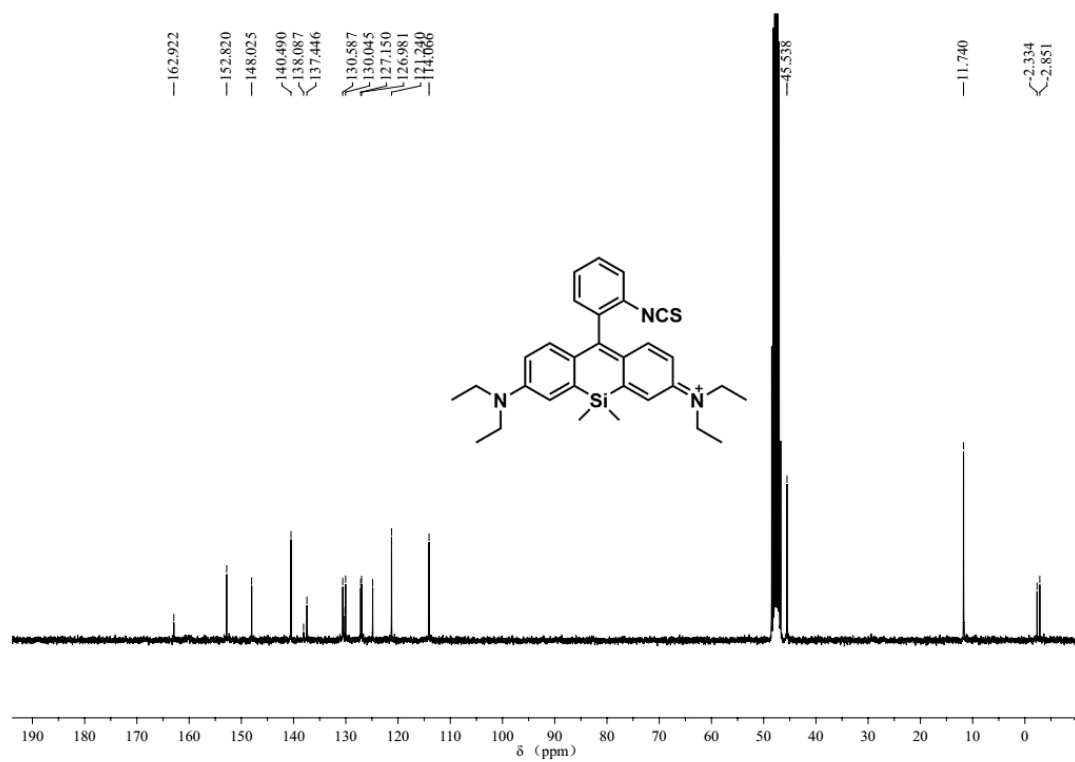




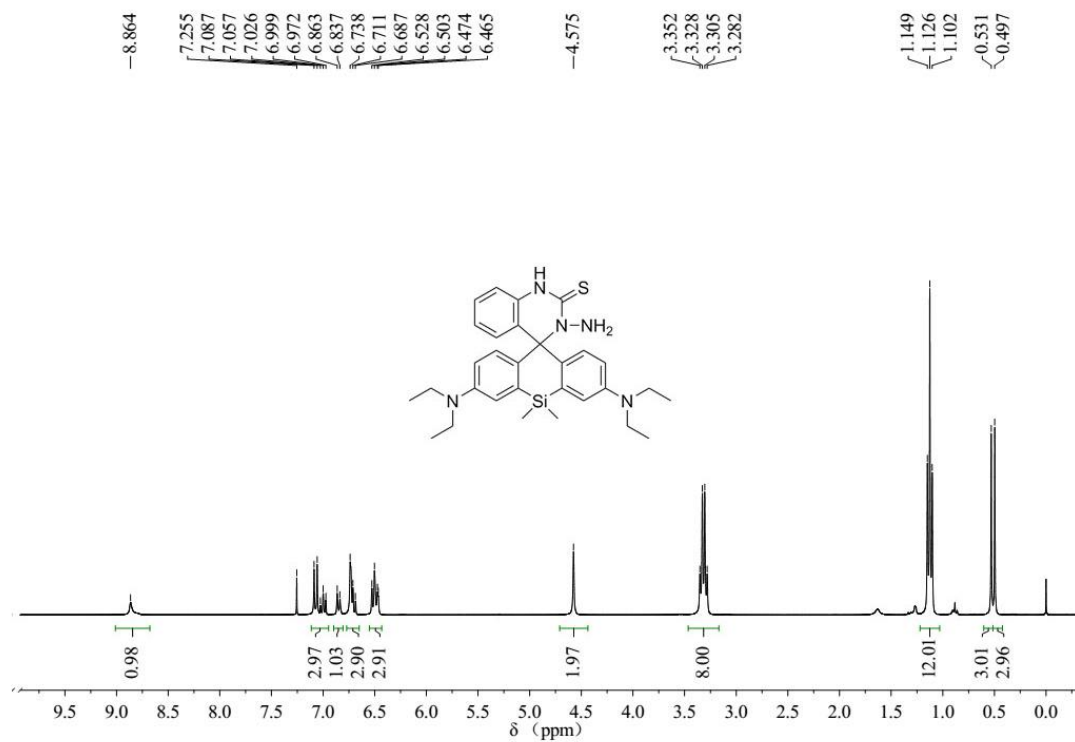
HRMS spectra of compound **SiR-NH<sub>2</sub>**



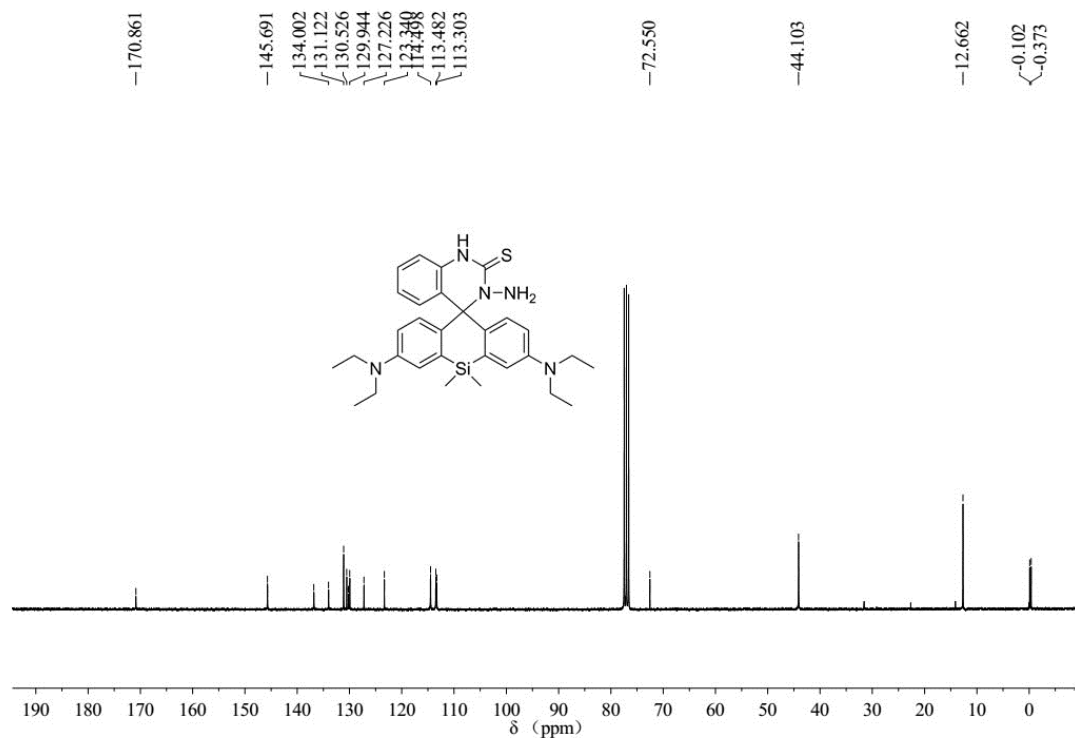
$^1\text{H NMR}$  of compound **SiR-NCS**



$^{13}\text{C NMR}$  of compound **SiR-NCS**



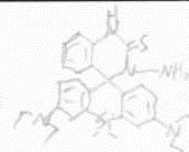
<sup>1</sup>H NMR of compound **SiRB-Cu**



<sup>13</sup>C NMR of compound **SiRB-Cu**

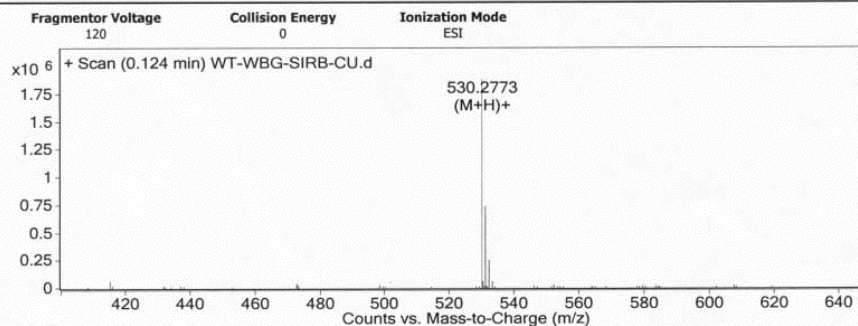
## Qualitative Analysis Report

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Sample Type	Sample	Position	P1-A1
Instrument Name	Instrument 1	User Name	
Acq Method	TEST-POS-WL.m	Acquired Time	9/16/2015 4:58:53 PM
IRM Calibration Status	Success	DA Method	Default.m



Sample Group      Info.

### User Spectra



#### Peak List

m/z	z	Abund	Formula	Ion
265.6447	2	5631299.5		
266.1443	2	2993447		
530.2773		1883524.5	C30 H40 N5 S Si	(M+H)+

#### Formula Calculator Element Limits

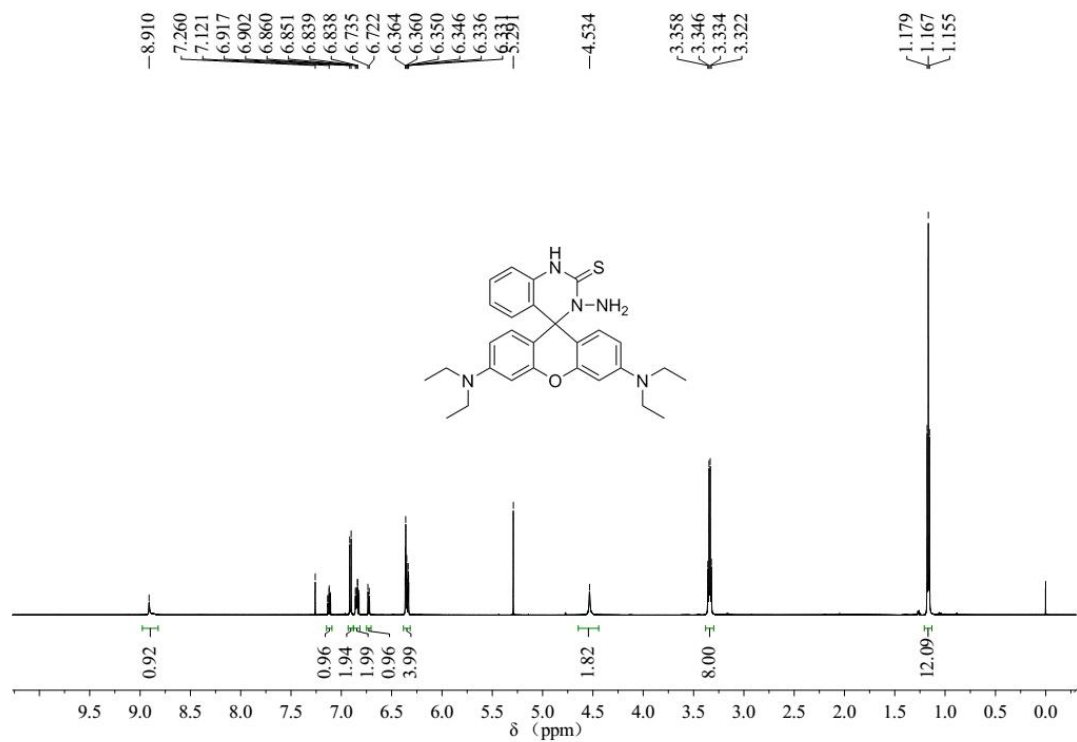
Element	Min	Max
C	20	40
H	0	600
O	0	25
N	0	10
Si	0	1
S	0	1

#### Formula Calculator Results

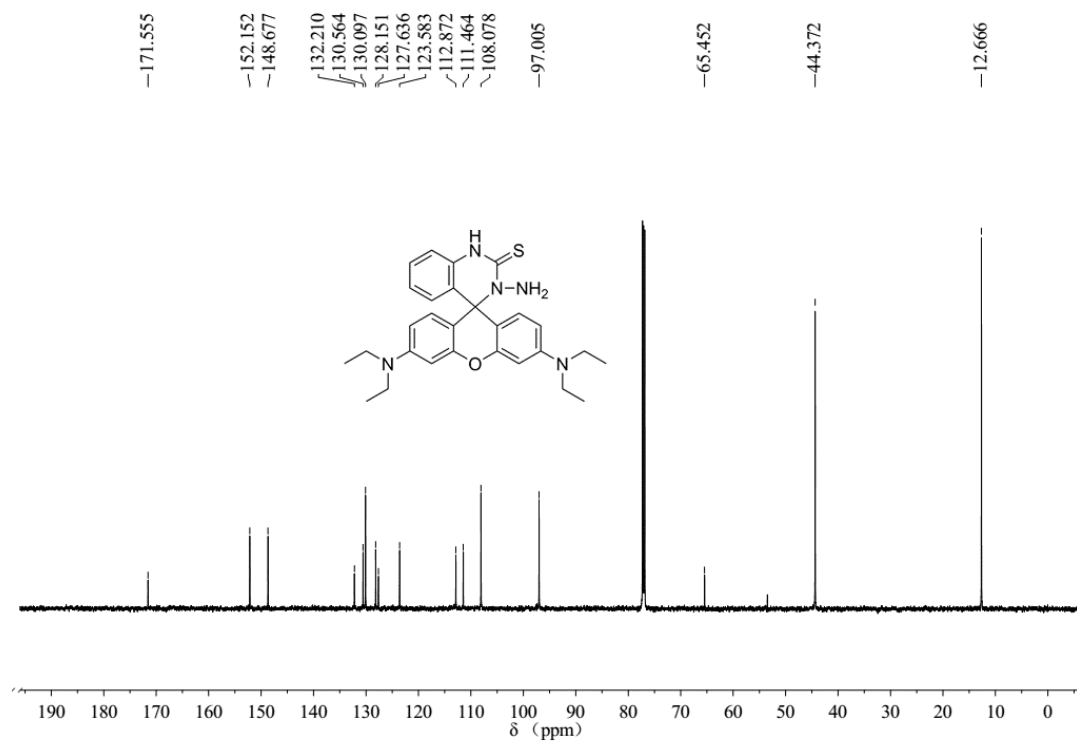
Formula	Best	Mass	Tgt Mass	Diff (ppm)	Ion Species	Score
C30 H39 N5 S Si	TRUE	529.2701	529.2695	-0.98	C30 H40 N5 S Si	98.27

--- End Of Report ---

HRMS spectra of compound **SiRB-Cu**



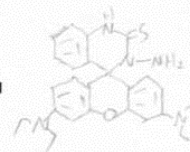
<sup>1</sup>H NMR of compound **R-Cu-1**



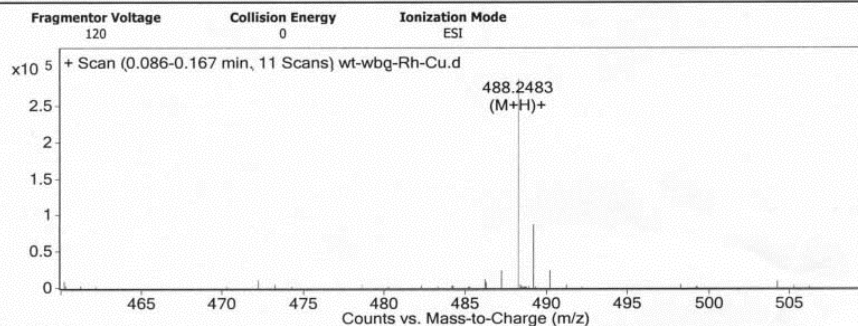
<sup>13</sup>C NMR of compound **R-Cu-1**

## Qualitative Analysis Report

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Sample Type	Sample	Position	P1-A1
Instrument Name	Instrument 1	User Name	
Acq Method	TEST-POS-WL.m	Acquired Time	9/18/2015 6:14:16 PM
IRM Calibration Status	Success	DA Method	Default.m
Comment			
Sample Group	Info.		



### User Spectra



#### Peak List

m/z	z	Abund	Formula	Ion
141.1135		48564.8		
244.6296	2	72259.3		
274.2744		71643.7		
318.3005		37098.1		
338.3423		80286		
415.2122		62591.6		
437.1936		38521.2		
488.2483	1	287316	C28 H34 N5 O S	(M+H)+
489.2516	1	88007.7	C28 H34 N5 O S	(M+H)+
555.3182		45102.4		

#### Formula Calculator Element Limits

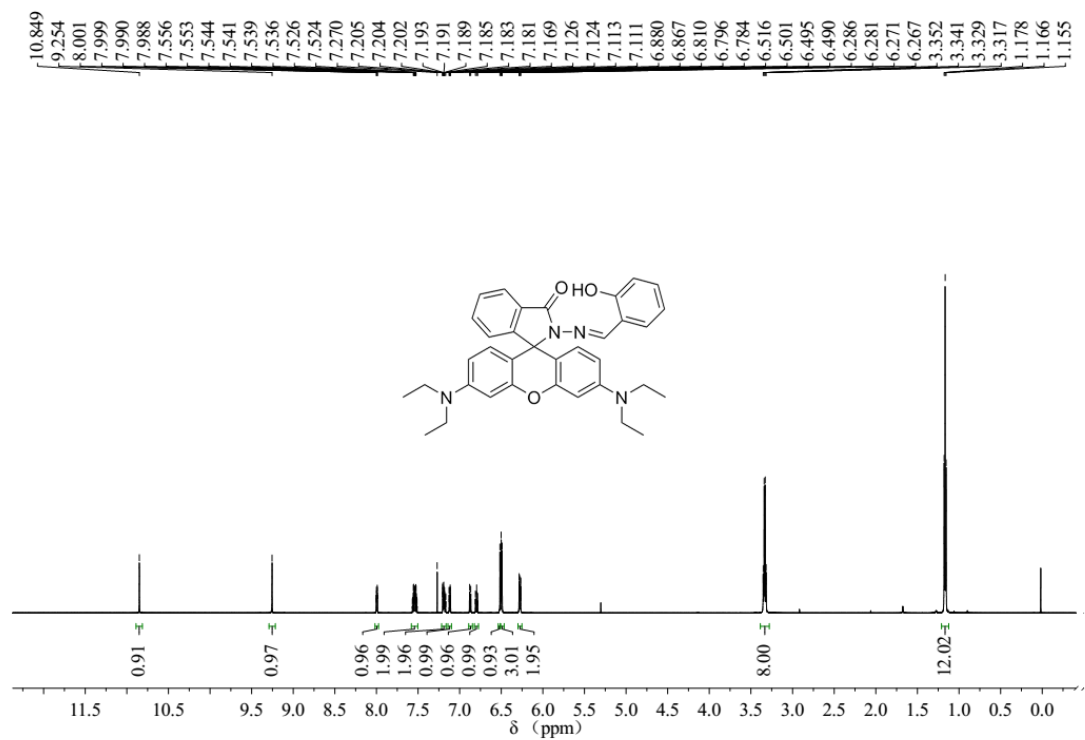
Element	Min	Max
C	0	150
H	0	300
O	0	39
N	0	43
S	0	1

#### Formula Calculator Results

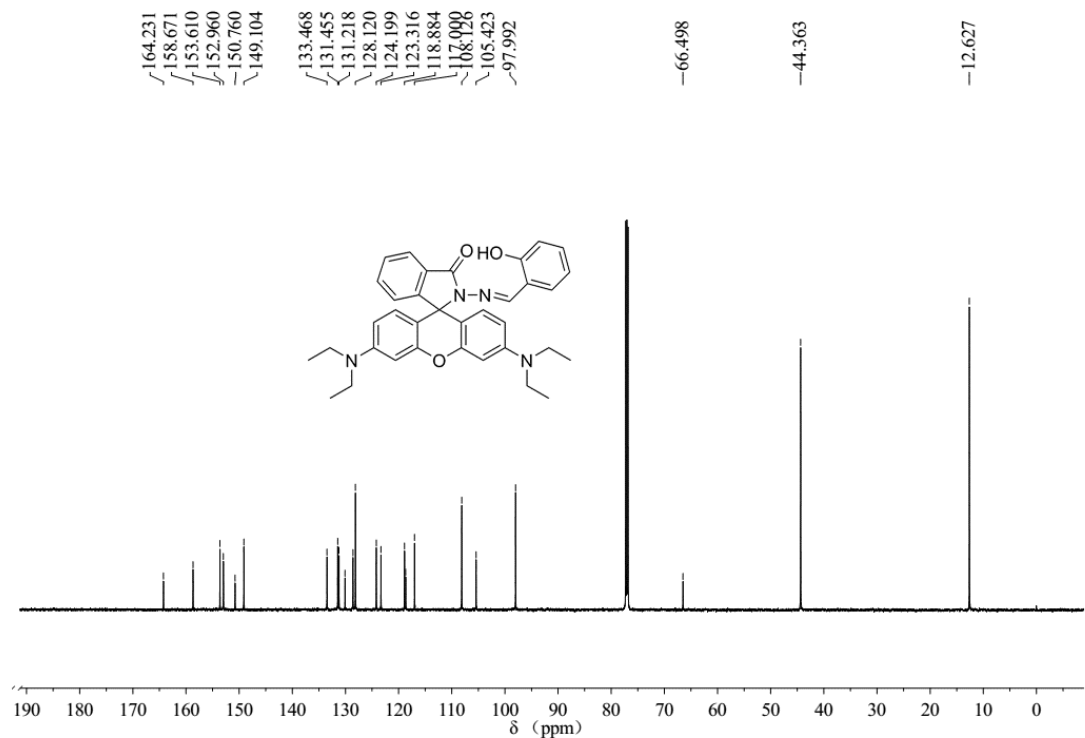
Formula	Best	Mass	Tgt Mass	Diff (ppm)	Ion Species	Score
C28 H33 N5 O S	TRUE	487.2411	487.2406	-0.97	C28 H34 N5 O S	97.91

--- End Of Report ---

HRMS spectra of compound **R-Cu-1**



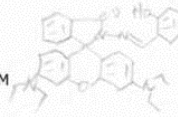
<sup>1</sup>H NMR of compound **R-Cu-2**



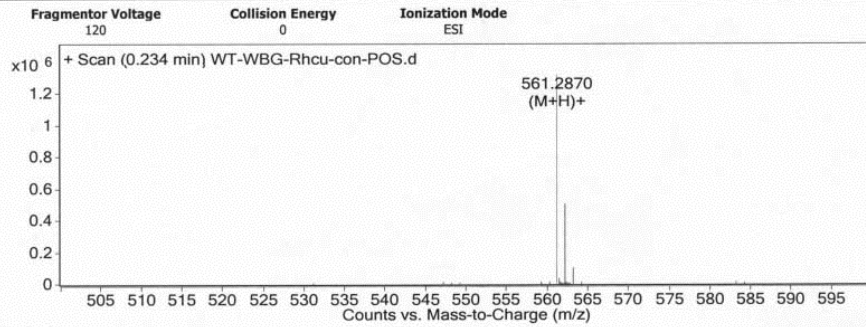
<sup>13</sup>C NMR of compound **R-Cu-2**

## Qualitative Analysis Report

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<b>Sample Type</b>	Sample	<b>Position</b>	P1-D1
<b>Instrument Name</b>	Instrument 1	<b>User Name</b>	
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<b>IRM Calibration Status</b>	Success	<b>DA Method</b>	Default.m
<b>Comment</b>			
<b>Sample Group</b>	<b>Info.</b>		



### User Spectra



#### Peak List

m/z	z	Abund	Formula	Ion
281.1477	2	2177331.8		
281.6491	2	886908.4		
561.287		1316686.1	C35 H37 N4 O3	(M+H)+

#### Formula Calculator Element Limits

Element	Min	Max
C	3	330
H	0	600
O	0	100
N	0	10

#### Formula Calculator Results

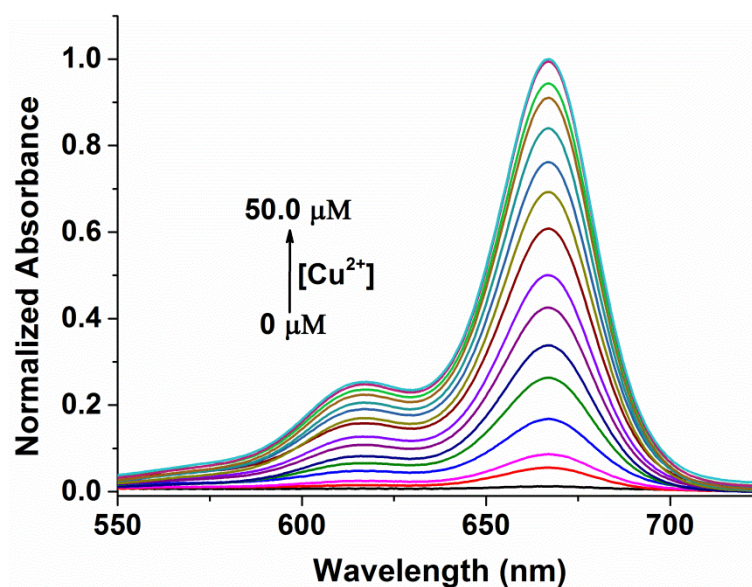
Formula	Best	Mass	Tgt Mass	Diff (ppm)	Ion Species	Score
C35 H36 N4 O3	TRUE	560.2797	560.2787	-1.8	C35 H37 N4 O3	97.92

--- End Of Report ---

HRMS spectra of compound **R-Cu-2**

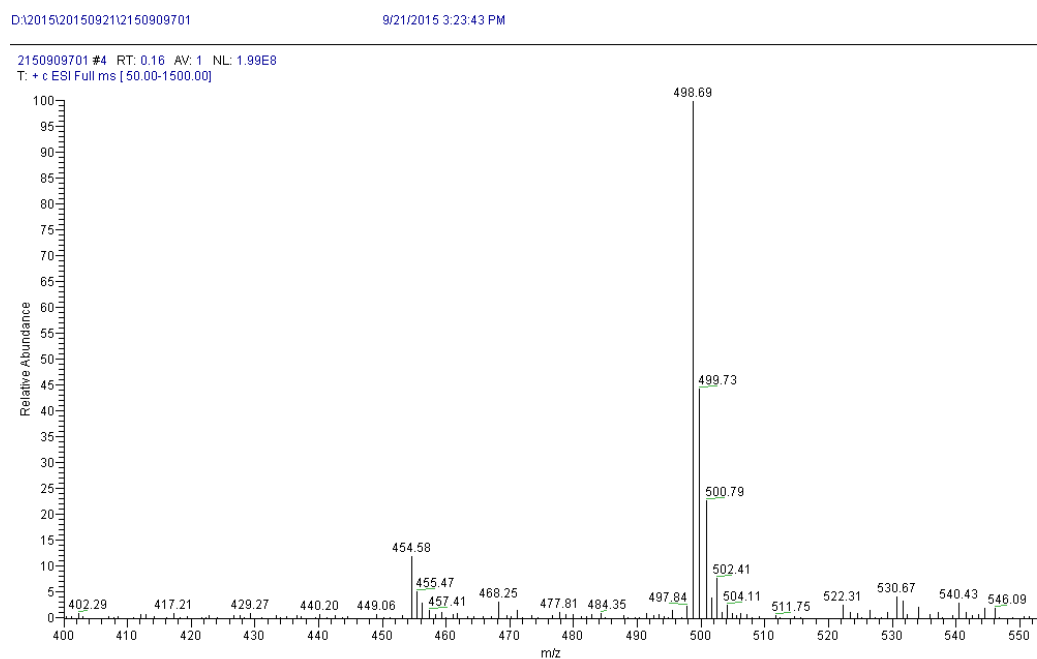


#### 4. Absorption Spectra of Probe SiRB-Cu in HEPES (pH = 7.4)



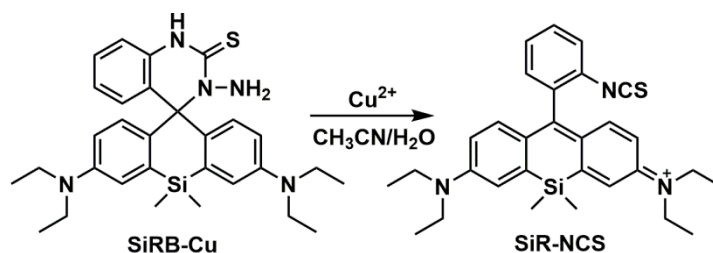
**Fig. S3** Absorption spectra of probe SiRB-Cu (2.5 μM) in the presence of different concentration of Cu<sup>2+</sup> at pH 7.4 HEPES buffer solution.

## 5. The Response Mechanism of Probe SiRB-Cu Reacted with Cu<sup>2+</sup>



**Fig. S4.** ESI-MS of the solution which probe **SiRB-Cu** (10.0  $\mu\text{M}$ ) react with  $\text{Cu}^{2+}$  (20.0  $\mu\text{M}$ ).

## Synthesis of SiR-NCS by reaction of probe SiRB-Cu with Cu<sup>2+</sup>



**Scheme S3** Synthesis of **SiR-NCS** by reaction of probe **SiRB-Cu** with Cu<sup>2+</sup>

**SiR-NCS.** To a solution of **SiRB-Cu** (53 mg, 0.10 mmol) in acetonitrile (10 mL), copper chloride dihydrate (51 mg) in H<sub>2</sub>O (0.5 mL) was added and the reaction mixture was stirred for 30 min at room temperature. After removal of the solvent under reduced pressure, the residue was dissolved in dichloride (15 mL). After washed with water (10 mL × 3) and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, the solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography (CH<sub>2</sub>Cl<sub>2</sub>/CH<sub>3</sub>OH, v/v, 20/1) to afford compound **SiR-NCS** as a blue solid (30 mg, 57% yield). <sup>1</sup>H NMR (600 MHz, CD<sub>3</sub>OD): δ 0.62 (s, 3H), 0.63 (s, 3H), 1.31 (t, 12H, *J* = 7.2 Hz), 3.74 (q, 8H, *J* = 7.2 Hz), 6.81-7.63 (m, 10H); <sup>13</sup>C NMR (150 MHz, CD<sub>3</sub>OD): δ -2.85, -2.34, 11.74, 45.54, 114.08, 121.25, 124.87, 126.99, 127.15, 130.05, 130.46, 130.60, 137.46, 138.10, 140.50, 148.04, 152.84, 162.95; HRMS (ESI) calcd. for C<sub>30</sub>H<sub>36</sub>N<sub>3</sub>SSi<sup>+</sup> [M]<sup>+</sup>: 498.2394, found:498.2397.

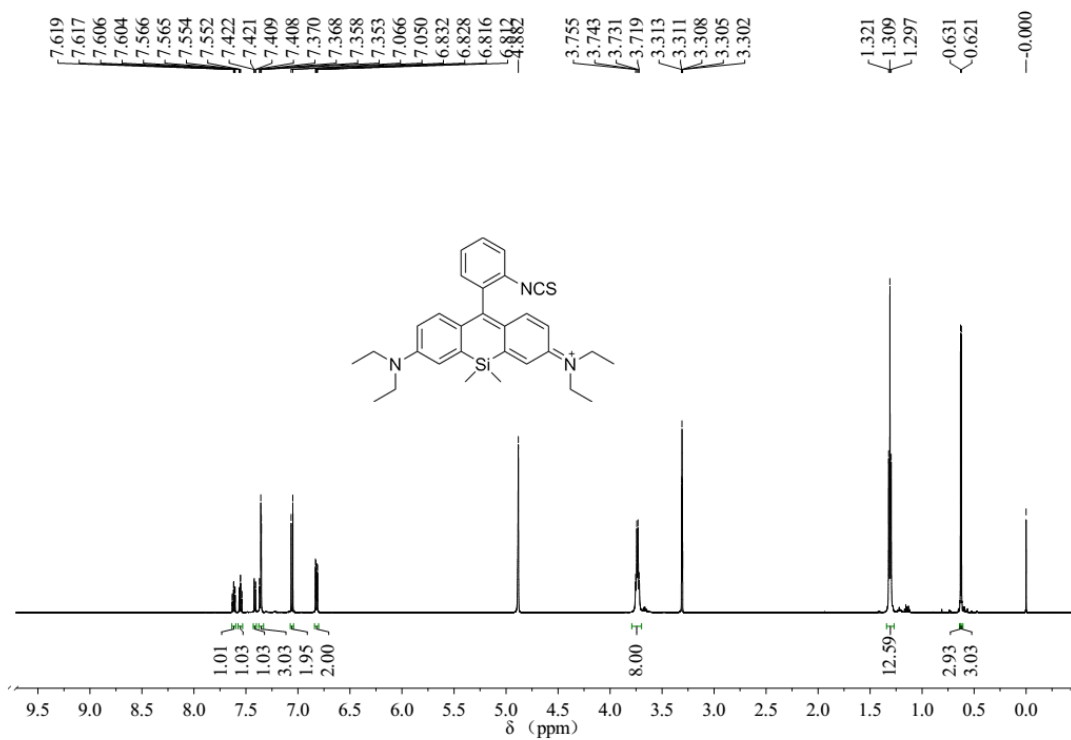


Figure S5  $^1\text{H}$  NMR of compound SiR-NCS

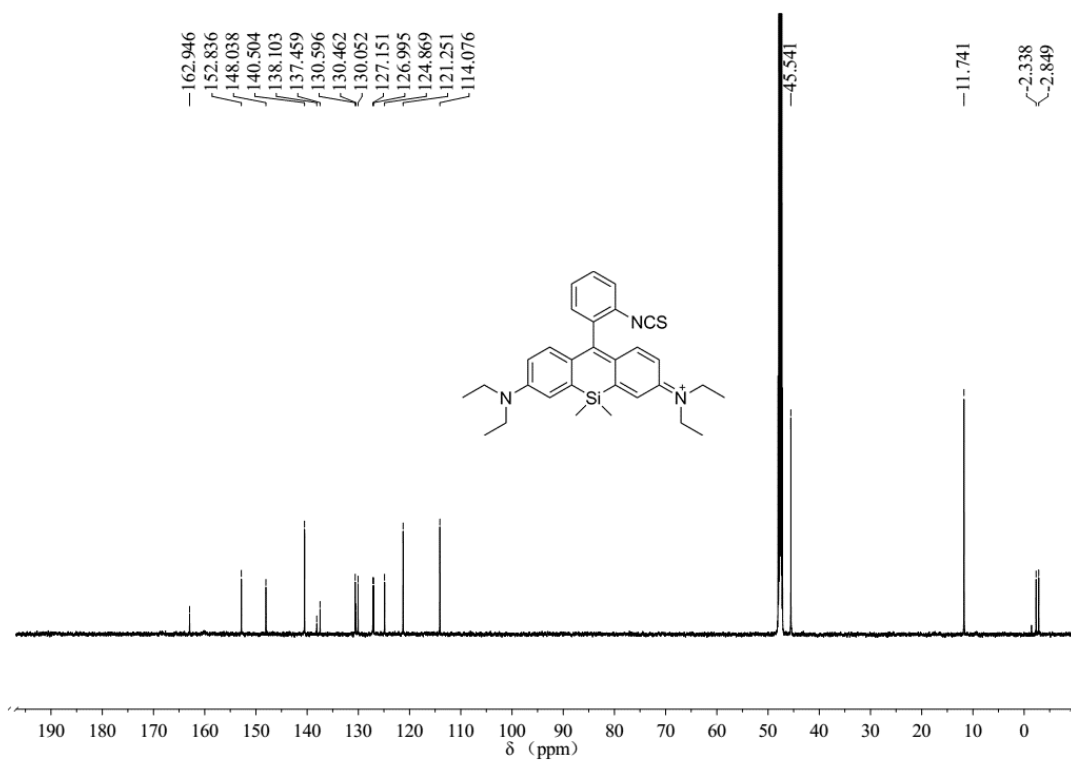
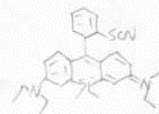


Figure S6  $^{13}\text{C}$  NMR of compound SiR-NCS

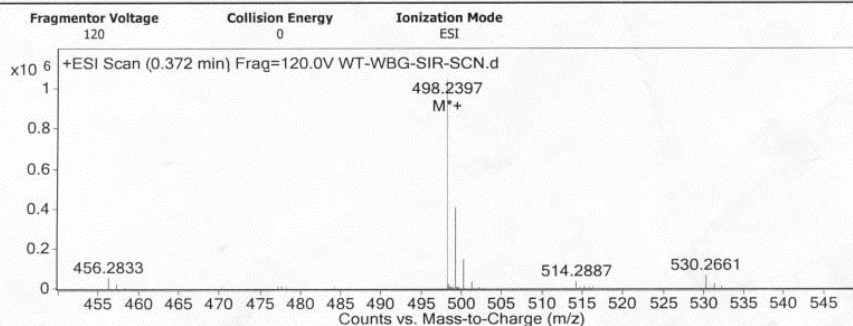
## Qualitative Analysis Report

Data Filename	WT-WBG-SIR-SCN.d	Sample Name	WT-WBG-SIR-SCN
Sample Type	Sample	Position	P1-D1
Instrument Name	Instrument 1	User Name	
Acq Method	TEST-POS-WL.m	Acquired Time	7/15/2015 4:04:38 PM
IRM Calibration Status	Success	DA Method	Default.m



Sample Group      Info.

### User Spectra



m/z	z	Abund	Formula	Ion
102.0133		68152.3		
116.9764		137625.1		
130.0078		513582		
143.0397		136016.3		
158.0027	1	1184670.8		
158.0591		71546.6		
179.9905		75961.6		
498.2397	1	1044865.6	C30 H36 N3 S Si	M*+
499.2428	1	407815.7	C30 H36 N3 S Si	M*+
500.2444	1	143466.3	C30 H36 N3 S Si	M*+

#### Formula Calculator Element Limits

Element	Min	Max
C	3	60
H	0	120
N	0	8
S	0	1
Si	0	1

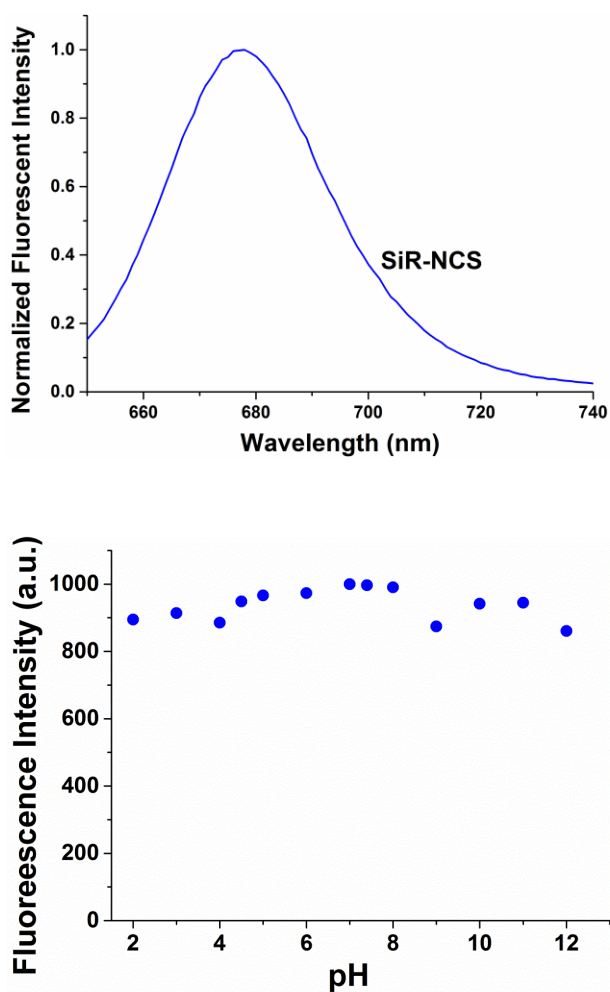
#### Formula Calculator Results

Formula	Best	Mass	Tgt Mass	Diff (ppm)	Ion Species	Score
C30 H36 N3 S Si	TRUE	498.2403	498.2399	-0.71	C30 H36 N3 S Si	94.66

--- End Of Report ---

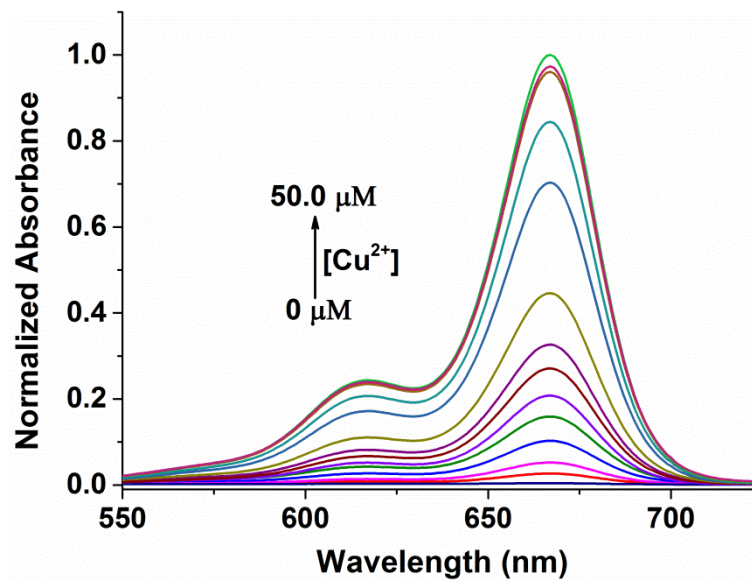
**Fig. S7** HRMS spectra of compound **SiR-NCS**

## 6. Effect of pH on The Fluorescence Intensity of SiR-NCS



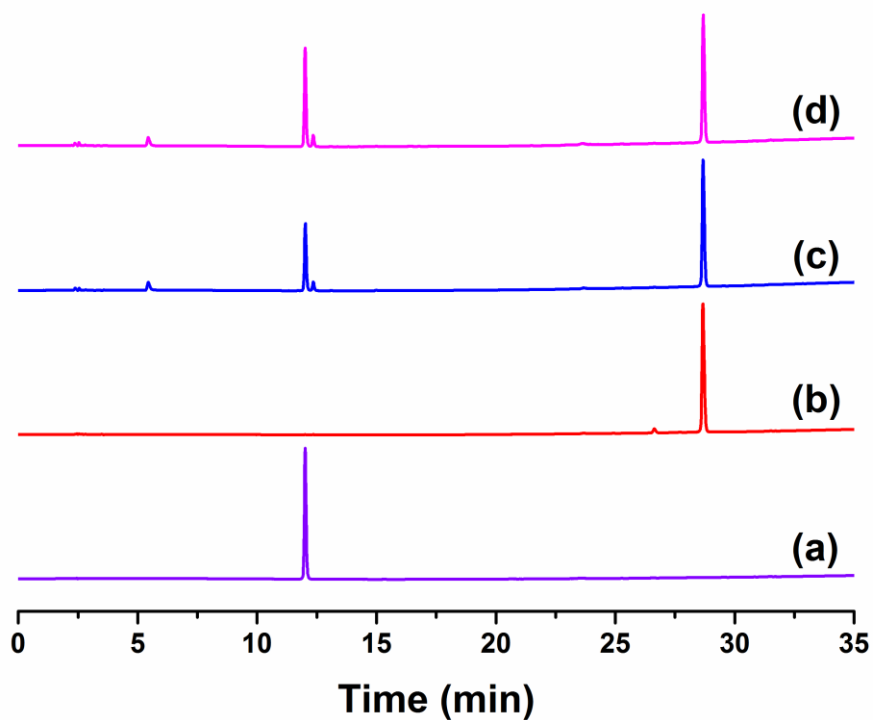
**Fig. S8.** The fluorescence emission of compound **SiR-NCS** and the effect of pH (2.0-12.0) on fluorescence intensity of **SiR-NCS** (2.5  $\mu$ M).

## 7. Absorption Spectra of Probe SiRB-Cu in HEPES (pH = 5.0)



**Fig. S9.** Absorption spectra of probe SiRB-Cu (2.5 μM) in the presence of different concentrations of Cu<sup>2+</sup> at pH 5.0 HEPES buffer

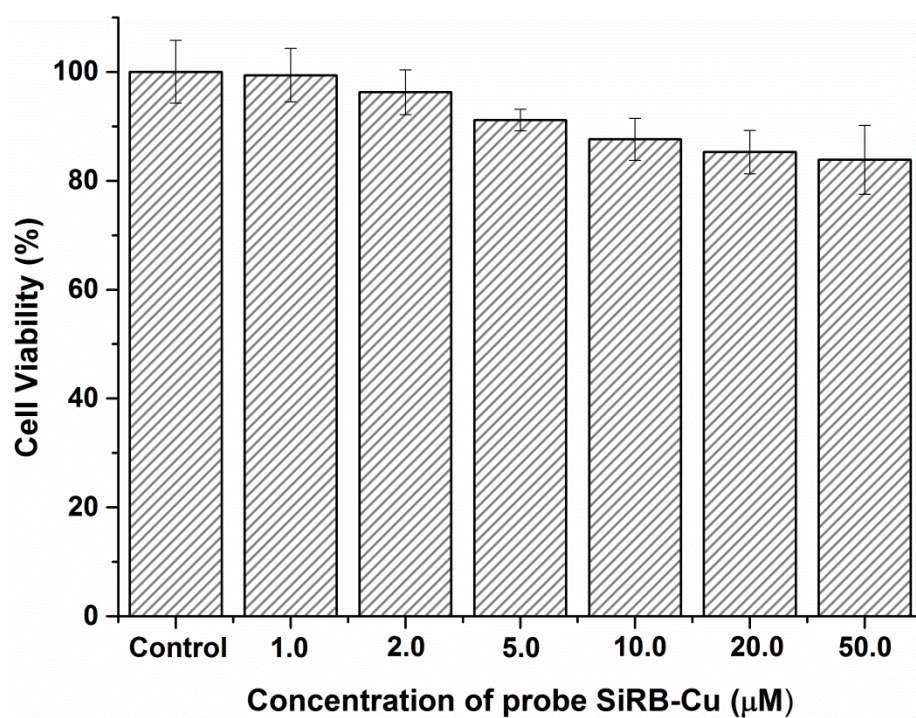
## 8. HPLC Analysis of The Reaction Mixture of Probe SiRB-Cu with Cu<sup>2+</sup>.



**Fig. S10.** HPLC analysis of reaction mixture of probe **SiRB-Cu** with Cu<sup>2+</sup>. (a) Probe **SiRB-Cu**; (b) Fluorescent product **SiR-NCS**; (c) the reaction mixture of probe **SiRB-Cu** and Cu<sup>2+</sup> at pH 7.4 HEPES buffer solution; (d) the reaction mixture of probe **SiRB-Cu** and Cu<sup>2+</sup> at pH 5.0 HEPES buffer solution.



## 9. Cytotoxic Effects of Probe SiRB-Cu on MCF-7 Cells.



**Fig. S11.** Cytotoxic effects of probe SiRB-Cu on MCF-7 cells.

## 10. Living Cell Imaging Experiments

For co-staining experiment between probe **SiRB-Cu**, LysoTracker Green DND-26 (for lysosomal staining) or rhodamine 123 (for mitochondrion staining) and Hoechst 33342 (for nucleus staining), the cells were washed with PBS for one time and fresh culture medium containing probe **SiRB-Cu** (5.0  $\mu\text{M}$ ) was added. After incubated for 40 min, the cells were washed with fresh PBS for three times and treated with  $\text{Cu}^{2+}$  (200  $\mu\text{M}$ ) for 3 h at 37  $^{\circ}\text{C}$ . The cells were washed with PBS for three times and stained with LysoTracker Green DND-26 (1.0  $\mu\text{M}$ ) or rhodamine 123 (1.0  $\mu\text{M}$ ) and Hoechst 33342 (5.0  $\mu\text{M}$ ) for 30 min. After washed three times with fresh PBS, the cells were imaged by confocal fluorescence microscopy.

For co-staining experiment between **SiRB-Cu** and **R-Cu-2**, after the cells were washed with PBS for one time, fresh culture medium containing probe **SiRB-Cu** (5.0  $\mu\text{M}$ ) and **R-Cu-2** (5.0  $\mu\text{M}$ ) were added and the cells were incubated for 40 min. After washed with PBS for three times, the cells were further treated with  $\text{Cu}^{2+}$  for 3 h at 37  $^{\circ}\text{C}$  and stained with Hoechst 33342 (5.0  $\mu\text{M}$ ) for 30 min. After washed three times with fresh PBS, the cells were imaged by confocal fluorescence microscopy.

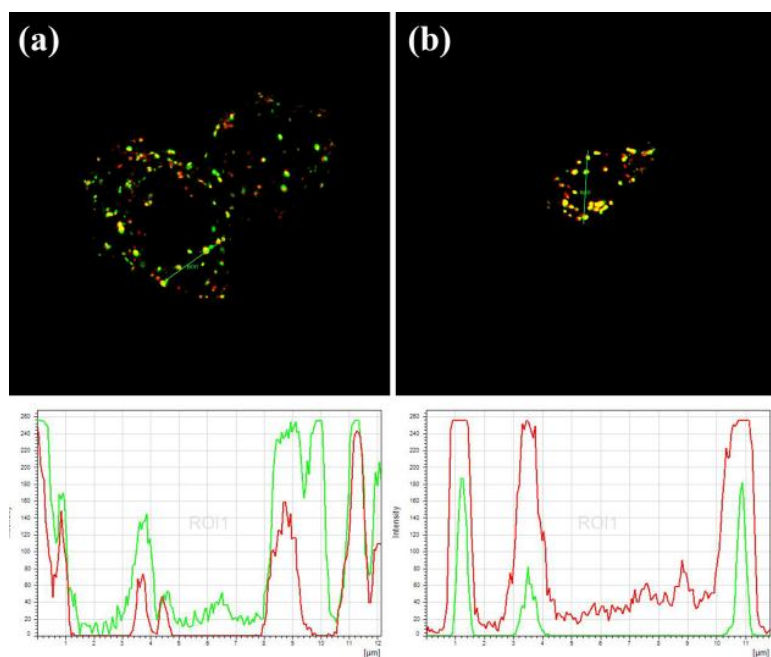
For co-staining experiment between **SiR-NCS** and LysoTracker Green DND-26 or rhodamine 123, after the cells were washed with PBS for one time, fresh culture medium containing **SiR-NCS** (5.0  $\mu\text{M}$ ) and LysoTracker Green DND-26 (1.0  $\mu\text{M}$ ) or rhodamine 123 (1.0  $\mu\text{M}$ ) were added and the cells were incubated for 30 min. After washed three times with fresh PBS, the cells were imaged by confocal fluorescence microscopy.

For fluorescent imaging experiment of probe **SiRB-Cu** responding to various  $\text{Cu}^{2+}$  concentrations, the cells were washed with PBS for one time and fresh culture medium containing **SiRB-Cu** (5.0  $\mu\text{M}$ ) were added. After incubated for 40 min and washed with PBS for three times, the cells were further treated with different concentrations of  $\text{Cu}^{2+}$  (0, 20.0, 50.0, 100.0, 200.0  $\mu\text{M}$ ) for 3 h. After washed three times with fresh PBS, the cells were imaged by confocal fluorescence microscopy.

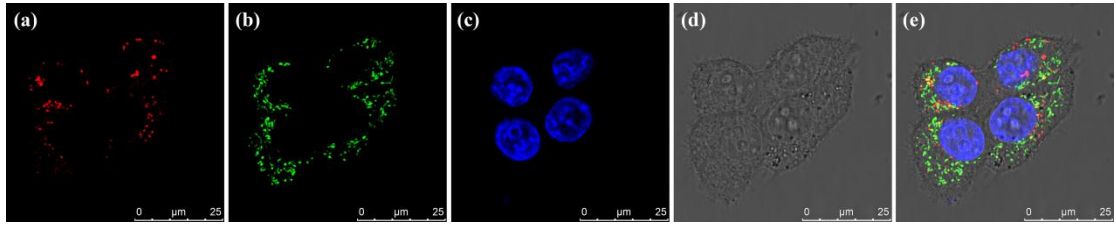
To verify the effect of the addition of AA to fluorescence intensity, the cells were washed with PBS for one time and fresh culture medium containing **SiRB-Cu** (5.0  $\mu\text{M}$ ) were added. After incubated for 40 min and washed with PBS for three times, the cells were further incubated with AA (1.0 mM) for 2 h. In the control experiment, the

cells stained with probe **SiRB-Cu** (5.0  $\mu\text{M}$ ) were incubated with fresh culture medium for the same time. Then the cells were washed with PBS for three times and treated with  $\text{Cu}^{2+}$  (200.0  $\mu\text{M}$ ) for 3 h. After washed three times with fresh PBS, the cells were imaged by confocal fluorescence microscopy.

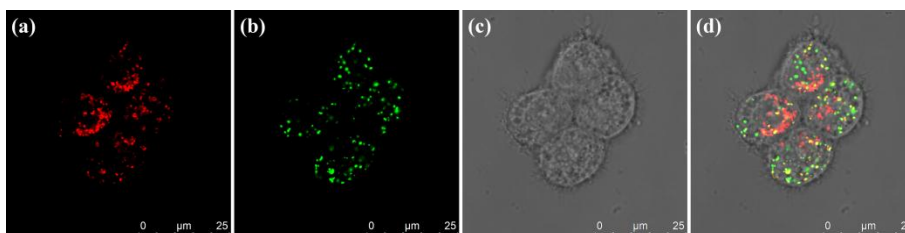
To identify the effect of addition of PDTC to fluorescence intensity, the cells were washed with PBS for one time and fresh culture medium containing **SiRB-Cu** (5.0  $\mu\text{M}$ ) were added. After incubated for 40 min and washed with PBS for three times, the cells were further incubated with PDTC (100  $\mu\text{M}$ ) for 3 h. In the control experiment, the cells stained with probe **SiRB-Cu** (5.0  $\mu\text{M}$ ) were incubated with fresh culture medium for the same time. After washed three times with fresh PBS, the cells were imaged by confocal fluorescence microscopy.



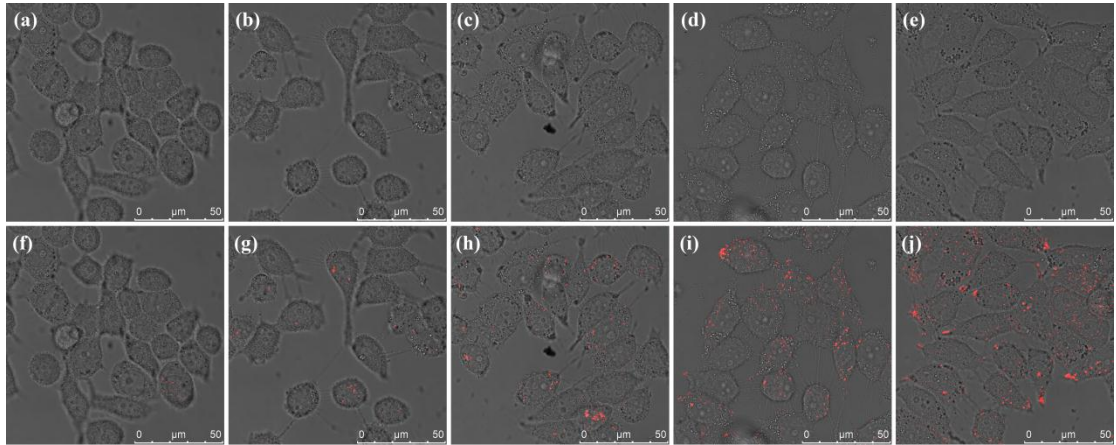
**Fig. S12** Intensity profiles of region of interest across the cells stained with  $\text{Cu}^{2+}$ -activated **SiRB-Cu** and LysoTracker Green DND-26 in (a) MCF-7 and (b) A549 cells.



**Fig. S13** Co-staining experiments in MCF-7 cells. (a) Fluorescent image of cells incubated with probe **SiRB-Cu** ( $5.0 \mu\text{M}$ ) for 40 min and supplemented with  $\text{Cu}^{2+}$  ( $200.0 \mu\text{M}$ ) for 3 h. (b) Fluorescence image of cells incubated with R123 ( $1.0 \mu\text{M}$ ) for 20 min. (c) Fluorescence image of cells incubated with Hoechst 33342 ( $5.0 \mu\text{M}$ ) for 20 min. (d) DIC image. (e) Overlay image of panel (a), (b) (c) (d) and (e).



**Fig. S14** Co-staining experiments in MCF-7 cells. (a) Fluorescent images of cells incubated with probe **SiR-NCS** ( $5.0 \mu\text{M}$ ) for 40 min. (b) Fluorescence image of cells incubated with LysoTracker Green DND-26 ( $1.0 \mu\text{M}$ ) ( $1.0 \mu\text{M}$ ) for 20 min. (c) DIC images. (d) Overlay image of panel (a), (b) and (c).



**Fig. S15** DIC images (a-e) and overlay images (f-j) of MCF-7 cells incubated with probe **SiRB-Cu** ( $5.0 \mu\text{M}$ ) after supplemented with different  $\text{Cu}^{2+}$  concentration (0, 20.0, 50.0, 100.0, 200.0  $\mu\text{M}$ ) for 3.0 h.