

## Supporting Information

### **Si-Rhodamine derivative with large Stokes shift for ELISA-based detection of SARS-CoV-2**

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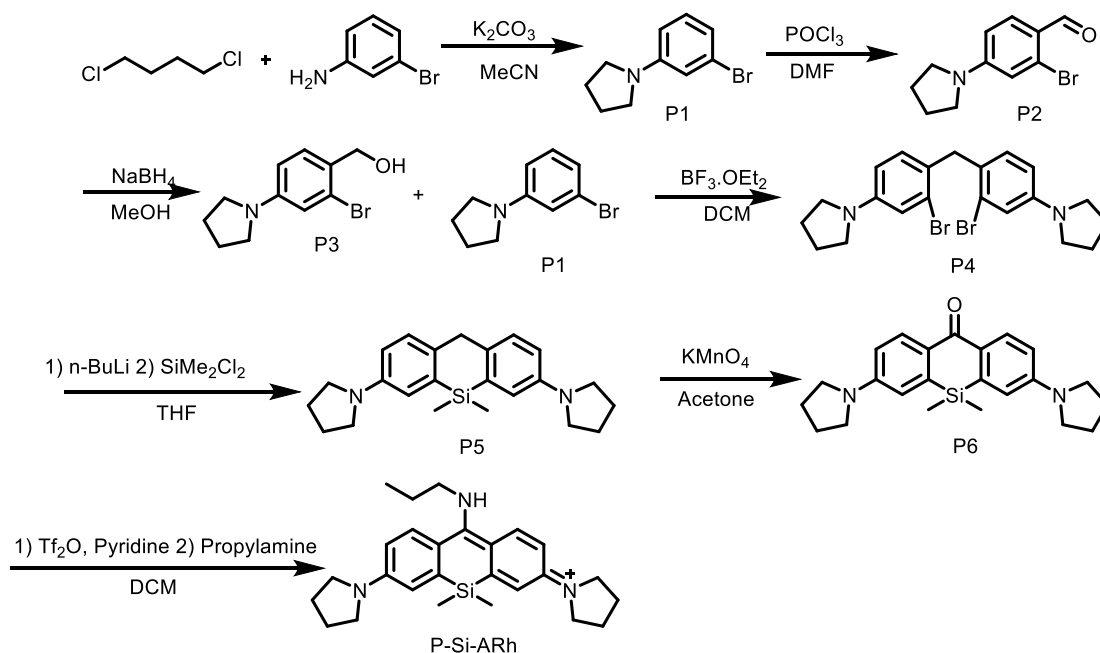
## Materials and instruments

Unless otherwise noted, all chemical reagents were purchased from commercial suppliers and used without further purification. All solvents were dried according to the standard methods prior to use. In the optical spectroscopic studies, all of the solvents were either HPLC or spectroscopic grade. Thin layer chromatography (TLC) was performed on silica gel plates, and spots were visualized under UV light. Column chromatography was carried out using 200-300 mesh silica gel (Qingdao Ocean Chemicals). NMR spectra were recorded on a Bruker AMX-400 spectrometer at 25°C (<sup>1</sup>H NMR: 400 MHz, <sup>13</sup>C NMR: 101 MHz) and chemical shifts ( $\lambda$ ) are expressed in parts per million (ppm) using the internal standard tetramethylsilane or the deuterated solvent (CDCl<sub>3</sub>, CD<sub>3</sub>OD) as reference. Spin multiplicities in <sup>1</sup>H NMR are reported as singlet (s), doublet (d), double doublet (dd), double double doublet (ddd), triplet (t), triplet of triplet (tt), multiplet/overlapping peaks (m) or broad (br). The High-resolution mass spectra (HRMS) were obtained on a Finnigan LCQDECA. The pH values were determined by a pH-3c digital pH-meter (Shanghai Lei Ci Device Works, Shanghai, China) with a combined glass-calomel electrode. UV absorption spectra were recorded on a Persee TU-1901 UV-visible spectrophotometer. Fluorescence spectra were measured on a Hitachi F-7000 fluorescence spectrophotometer. Cell imaging was performed on a Zeiss LSM 780 confocal laser scanning microscope.

## Methods/Experimental

All experiments were performed in accordance with the principles of the Declaration of Helsinki, and approved by the ethics committee at the Institutional Review Board and Biomedical Ethics Committee of West China Hospital of Sichuan University (WCH/SCU) (2020, no. 126). Informed consents were obtained from human participants of this study.

Serum sample test producer: Patients serum samples and normal serum samples were diluted by using PBS buffer (pH=7.4) for different proportions, and then stained with the immunoglobulin solution that labeled with P-Si-MRh for 30 min. The test were finished in glass bottom 96-well plates on a Spark 20M (Tecan) microplate reader at 25 °C.



**Scheme S1.** Synthesis of **P-Si-ARh**.

#### Synthesis of **P1**:

The 1, 4-Dibromobutane ( 12.9 g, 60 mmol ) and 3-Bromoaniline ( 8.49 ml, 78 mmol ) were dissolved in 100 ml acetonitrile, and the potassium carbonate ( 18.2 g, 132 mmol ) was added to the solution. The mixture was heated to reflux for 12 h. The potassium carbonate was filtered, and the solution was concentrated under vacuum. The mixture was used without purification.

#### Synthesis of **P2**:

The  $\text{POCl}_3$  ( 8.4 ml, 90 mmol ) was added dropwise to DMF ( 20 ml ) at 0 °C and stirred for 0.5 h, and the solution of P1 ( 13.5 g, 60 mmol ) in 50 ml DMF was added to the mixture. The mixture was stirred at 80 °C for 8 h. After the reaction was completed, the solution was poured into the ice water and extracted with DCM. The organic layers were collected, dried over anhydrous  $\text{Na}_2\text{SO}_4$ , and concentrated under vacuum. The residue was purified by column chromatography on silica gel (petroleum ether: ethyl acetate 10: 1) to afford **P2** (15.6 g, 83% yield).  $^1\text{H NMR}$  ( 400 MHz,  $\text{CDCl}_3$  )  $\delta$  (ppm): 10.03 ( s, 1H ), 7.75 ( d,  $J = 8.8$  Hz, 1H ), 6.63 ( d,  $J = 2.4$  Hz, 1H ), 6.46 ( d,  $J = 9.2$  Hz, 1H ), 3.33 ( t,  $J = 4$  Hz, 4H ), 2.02 ( t,  $J = 4$  Hz, 4H ).  $^{13}\text{C NMR}$  (101 MHz,  $\text{CDCl}_3$  )  $\delta$  (ppm): 190.24, 152.11, 131.15, 129.79, 121.49, 114.84,

110.87, 47.75, 25.35. ESI (+)-HRMS (m/z): [M + H]<sup>+</sup>calcd. for: 254.0181, found: 254.0176

#### Synthesis of **P3**:

The compound **P2** (6.3 g, 25 mmol) was dissolved in 50 ml MeOH, and NaBH<sub>4</sub> (1.2 g, 30 mmol) was added to the solution at 0 °C and stirred for 4 h. After the reaction was completed, the mixture was washed with saturated NaCl solution and extracted with DCM. The organic layer was collected, dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, and concentrated under vacuum to afford **P3** (8.2 g, 99% yield). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ (ppm): 7.19 (s, 1H), 6.71 (d, J = 2.5 Hz, 1H), 6.44 (dd, J = 8.4 Hz, 1H), 4.61 (d, J = 6.2 Hz, 2H), 3.27 – 3.20 (m, 4H), 1.99 (t, J = 6.6 Hz, 4H). <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) δ (ppm): 148.54, 130.67, 125.83, 124.53, 115.18, 110.71, 65.25, 47.60, 25.43.

#### Synthesis of **P4**:

The compound **P3** (6.35 g, 25 mmol) and **P1** (5.6 g, 25 mmol) was dissolved in 50 ml DCM, and the boron trifluoride etherate (5 ml, 40 mmol) was added dropwise to the solution. After the reaction was completed, the mixture was concentrated under vacuum. The residue was purified by column chromatography on silica gel (petroleum ether: ethyl acetate 4: 1) to afford **P4** (7.5 g, 65% yield). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ (ppm): 6.82 (d, J = 8.5 Hz, 2H), 6.77 (d, J = 2.5 Hz, 2H), 6.40 (dd, J = 8.5 Hz, 2H), 3.98 (s, 2H), 3.22 (t, J = 6.6 Hz, 8H), 2.02 – 1.92 (m, 8H). <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) δ (ppm) :147.31, 130.83, 125.91, 125.61, 115.16, 110.92, 47.61, 39.90, 25.45. ESI (+)-HRMS (m/z): [M + H]<sup>+</sup>calcd. for: 463.0384, found 463.0374.

#### Synthesis of **P5**:

To a 50 mL well-dried flask flushed with argon, **P4** (4.62 g, 10.0 mmol) and dry THF (50 mL) were added. The solution was cooled to -78 °C, n-BuLi (2.5 M in n-hexane, 13.67 mL, 22.0 mmol) was added and the reaction mixture was stirred at -78 °C for 1h. Dichlorodimethylsilane (1.06 mL, 11 mmol) was added dropwise at -78 °C for 60 min and the reaction mixture was slowly warmed to room temperature, then stirred overnight. The

resulting solution was extracted with DCM. The organic layers were collected, dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, and concentrated under vacuum. The crude product was used in the next step without further purification.

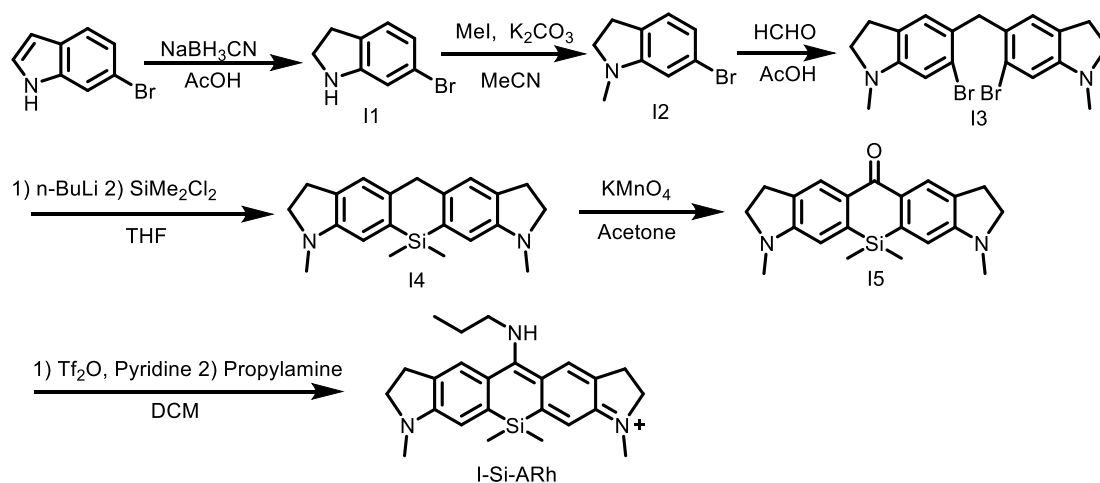
#### Synthesis of **P6**:

The mixture of **P5** in 150 ml acetone was cooled to 0 °C, and the KMnO<sub>4</sub> (4.74 g, 30 mmol) was added to the mixture. The solution was stirred at 0 °C for 3 h. The manganese(IV) oxide was filtered off through a pad of silica gel, and the solid was thoroughly washed with DCM. Mixed filtrates were concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (petroleum ether: dichloromethane 1: 1) to afford **P6** (940 mg, 25% yield). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ (ppm): 8.39 (d, *J* = 8.9 Hz, 2H), 6.68 (dd, *J* = 8.9, 2.6 Hz, 2H), 6.63 (d, *J* = 2.6 Hz, 2H), 3.43 – 3.36 (t, 8H), 2.05 – 2.00 (t, 8H), 0.45 (s, 6H). <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) δ (ppm): 185.18, 148.93, 140.60, 131.73, 129.13, 114.14, 113.08, 47.45, 25.48, -0.97. ESI (+)-HRMS (*m/z*): [M + H]<sup>+</sup>calcd. for: 377.2049, found: 377.2048

#### Synthesis of **P-Si-ARh**:

The compound **P6** (188 mg, 0.5 mmol) and pyridine (0.32 ml, 4 mmol) was dissolved in 20 ml DCM and stirred at 0 °C for 15 min. Then, the Tf<sub>2</sub>O (0.42 ml, 2.5 mmol) was added dropwise to the solution and stirred at 0 °C for 1 h. The propylamine (0.41 ml, 5 mmol) was added to the solution and stirred at room temperature for 2 h. After the reaction was completed, the solution was concentrated under vacuum. The residue was purified by column chromatography on silica gel (DCM: MeOH) to afford **P-Si-ARh** (109 mg, 52% yield). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ (ppm): 8.07 (d, *J* = 8.9 Hz, 1H), 7.47 (d, *J* = 8.9 Hz, 1H), 6.78 (d, *J* = 2.5 Hz, 1H), 6.72 (dd, *J* = 9.0, 2.4 Hz, 1H), 6.65 (d, *J* = 2.5 Hz, 1H), 6.60 (dd, *J* = 8.9, 2.5 Hz, 1H), 3.91 (q, *J* = 6.5 Hz, 2H), 3.40 (m, 8H), 2.04 (m, 8H), 1.92 (q, *J* = 7.3 Hz, 2H), 0.86 (t, *J* = 7.4 Hz, 3H), 0.45 (s, 6H). <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) δ (ppm): 173.49, 149.38, 149.07, 142.37, 138.43, 131.57, 129.75, 124.62, 119.57, 116.47, 115.25,

113.87, 111.42, 52.16, 47.57, 25.40, 22.69, 11.07, -2.20. ESI (+)-HRMS (m/z):  $[M]^+$  calcd. for: 418.2673, found: 418.2672



**Scheme S2.** Synthesis of I-Si-ARh.

#### Synthesis of **I1**:

The 6-Bromo-1H-indole (4.8 g, 24.5 mmol) and sodium cyanoborohydride (6.16 g, 98.1 mmol) were dissolved in 100 ml AcOH. The mixture was stirred at room temperature for 6 h. The solution was adjusted to alkaline and extracted with DCM. The organic layers were collected, dried over anhydrous  $\text{Na}_2\text{SO}_4$ , and concentrated under vacuum. The residue was purified by column chromatography on silica gel (petroleum ether: ethyl acetate 10: 1) to afford **I1** (6.74 g, 85% yield).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  6.94 (d,  $J = 7.7$  Hz, 1H), 6.79 (d,  $J = 7.8$  Hz, 1H), 6.70 (s, 1H), 3.72 (s, 1H), 3.54 (t,  $J = 8.2$  Hz, 2H), 2.95 (t,  $J = 8.2$  Hz, 2H).

#### Synthesis of **I2**:

The compound **I1** (1.96 g, 10 mmol), potassium carbonate (2.76 g, 20 mmol) and iodomethane (0.93 ml, 15 mmol) were dissolved in 100 ml acetonitrile and heated to reflux. After the reaction was completed, the potassium carbonate was filtered and the solution was concentrated under vacuum. The mixture was purified by column chromatography to afford **I2** (10.2 g, 55% yield).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  (ppm): 6.88 (d,  $J = 7.7$  Hz, 1H),

6.74 (d,  $J = 7.8$  Hz, 1H), 6.53(s, 1H), 3.32 (t,  $J = 8.2$  Hz, 2H), 2.87 (t,  $J = 8.2$  Hz, 2H), 2.72(s, 1H).  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$  (ppm): 154.75, 129.23, 125.21, 121.01, 120.01, 110.00, 56.09, 35.63, 28.20. ESI (+)-HRMS ( $m/z$ ):  $[\text{M} + \text{H}]^+$  calcd. for: 443.1888, found: 443.1907

#### Synthesis of **I3**:

The compound **I2** (16.6 g, 79 mmol) and 37% formaldehyde (3.85 g, 47.5 mmol) were dissolved in 100 ml AcOH, the solution were stirred at room temperature for 12 h. After the reaction was completed, the solution was adjusted to alkaline and extracted with DCM. The organic layers were collected, dried over anhydrous  $\text{Na}_2\text{SO}_4$ , and concentrated under vacuum. The residue was purified by column chromatography on silica gel (petroleum ether: ethyl acetate 10: 1) to afford **I3** (7.4 g, 42% yield).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  (ppm): 6.70(s, 2H), 6.64(s, 2H), 3.96(s, 2H), 3.27(t,  $J = 8.1$  Hz, 1H), 2.81(t,  $J = 7.9$  Hz, 1H), 2.72(s, 6H).  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$  (ppm): 153.00, 130.21, 128.21, 126.02, 123.00, 110.84, 56.29, 40.56, 36.06, 28.37. ESI (+)-HRMS ( $m/z$ ):  $[\text{M} + \text{H}]^+$  calcd. for: 435.0071, found: 435.0059.

#### Synthesis of **I4**:

To a 50 mL well-dried flask flushed with argon, **I3** (1.86 g, 4.27 mmol) and dry THF (50 mL) were added. The solution was cooled to  $-78$  °C,  $n\text{-BuLi}$  (2.5 M in  $n\text{-hexane}$ , 3.76 mL, 9.4 mmol) was added and the reaction mixture was stirred at  $-78$  °C for 1h. Dichlorodimethylsilane (0.45 mL, 4.98 mmol) was added dropwise at  $-78$  °C for 60 min and the reaction mixture was slowly warmed to room temperature, then stirred overnight. The solution was extracted with DCM. The organic layers were collected, dried over anhydrous  $\text{Na}_2\text{SO}_4$ , and concentrated under vacuum. The crude product was used in the next step without further purification.

#### Synthesis of **I5**:

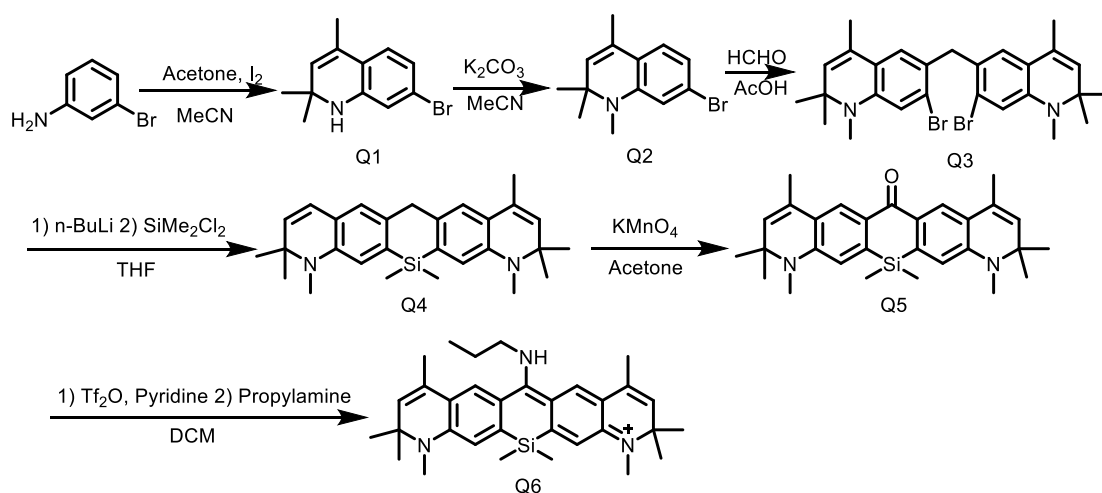
The mixture of **I4** in 150 ml acetone was cooled to  $0$  °C, and the  $\text{KMnO}_4$  (2.02 g, 12.8 mmol)

was added to the mixture. The solution was stirred at 0 °C for 3 h. The manganese(IV) oxide was filtered off through a pad of silica gel, and the solid was thoroughly washed with DCM. Mixed filtrates were concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (petroleum ether: dichloromethane 1: 1) to afford **I5** (283 mg, 19% yield). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ (ppm): 8.19 (s, 2H), 6.49 (s, 2H), 3.45 (t, J = 8.4 Hz, 4H), 3.03 (t, J = 8.4, 1.3 Hz, 4H), 2.88 (s, 6H), 0.43 (s, 6H). <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) δ (ppm): 185.13, 154.75, 140.00, 132.14, 131.47, 126.06, 107.90, 54.84, 34.61, 28.05, -1.09.

#### Synthesis of **I-Si-ARh**:

The compound **I5** (110 mg, 0.32 mmol) and pyridine (0.22 ml, 2.52 mmol) was dissolved in 20 ml DCM and stirred at 0 °C for 15 min. Then, the Tf<sub>2</sub>O (0.26 ml, 1.58 mmol) was added dropwise to the solution and stirred at 0 °C for 1 h. The propylamine (0.26 ml, 3.2 mmol) was added to the solution and stirred at room temperature for 2 h. After the reaction was completed, the solution was concentrated under vacuum. The residue was purified by column chromatography on silica gel (DCM: MeOH) to afford **I-Si-ARh** (47 mg, 38% yield). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ (ppm): 7.84 (s, 1H), 7.22 (s, 1H), 6.60 (s, 1H), 6.46 (s, 1H), 3.89 (t, J = 7.1 Hz, 2H), 3.55 (t, J = 23.6, 8.5 Hz, 4H), 3.15 (t, J = 8.5 Hz, 2H), 3.07 (t, J = 8.7 Hz, 2H), 2.93 (s, 3H), 2.88 (s, 3H), 1.91 (q, J = 7.2 Hz, 2H), 0.85 (t, J = 7.4 Hz, 3H), 0.43 (s, 6H). <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) δ (ppm): 174.12, 155.45, 155.07, 142.61, 138.40, 133.57, 130.69, 126.86, 125.49, 123.85, 121.55, 109.78, 108.34, 54.59, 54.39, 52.26, 33.92, 33.86, 27.74, 27.49, 22.56, 11.06, -2.22. ESI (+)-HRMS (m/z): [M + H]<sup>+</sup> calcd. for: 390.2360, found: 390.2361.





**Scheme S3.** Synthesis of **Q-Si-ARh**.

### Synthesis of **Q1**:

The 3-Bromoaniline (10 g, 105 mmol) were dissolved in 100 ml acetone, and the potassium carbonate (18.2 g, 132 mmol) was added to the solution. The mixture was heated to reflux for 12 h. The mixture was concentrated under vacuum, and the residue was purified by column chromatography on silica gel (PE: EA) to afford **Q1**.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  (ppm): 8.01 (s, 2H), 7.14 (d,  $J = 1.9$  Hz, 1H), 7.02 (dd,  $J = 8.2, 1.9$  Hz, 1H), 6.97 (d,  $J = 8.2$  Hz, 1H), 5.43 (q,  $J = 1.5$  Hz, 1H), 1.95 (d,  $J = 1.4$  Hz, 3H), 1.34 (s, 6H).  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$  (ppm): 137.83, 129.29, 128.44, 125.50, 125.11, 123.90, 121.75, 120.49, 54.09, 27.86, 18.30. ESI (+)-HRMS ( $m/z$ ):  $[\text{M} + \text{H}]^+$  calcd. for: 456.2199, found: 456.2200.

### Synthesis of **Q2**:

The compound **Q1** (5 g, 20 mmol), potassium carbonate (5.5 g, 40 mmol) and Iodomethane (1.5 ml, 24 mmol) were dissolved in 50 ml acetonitrile and heated to reflux. After the reaction was completed, the potassium carbonate was filtered and the solution was concentrated under vacuum. The mixture was purified by column chromatography to afford **Q2** (3.3 g, 62% yield).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  (ppm): 6.85 (d,  $J = 8.0$  Hz, 1H), 6.73 (dd,  $J = 8.1$  Hz, 1H), 6.59 (d,  $J = 1.9$  Hz, 1H), 5.28 (d,  $J = 1.5$  Hz, 1H), 2.76 (s, 3H), 1.94 – 1.92 (d,  $J = 1.2$  Hz, 3H), 1.28 (d,  $J = 0.6$  Hz, 6H).  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$  (ppm): 130.15, 128.71, 127.50, 124.34, 122.48, 122.01, 118.71, 113.31, 56.52, 30.73, 27.37, 18.50. ESI (+)-HRMS ( $m/z$ ):  $[\text{M} + \text{H}]^+$  calcd. for: 266.0544, found: 266.0533.

#### Synthesis of **Q3**:

The compound **Q2** (21.2 g, 80 mmol) and 37% formaldehyde (3.85 g, 47.5 mmol) were dissolved in 100 ml AcOH, the solution were stirred at room temperature for 12 h. After the reaction was completed, the solution was adjusted to alkaline and extracted with DCM. The organic layers were collected, dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, and concentrated under vacuum. The residue was purified by column chromatography on silica gel (petroleum ether: ethyl acetate 10: 1) to afford **Q3** (11.3 g, 26 % yield). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ (ppm): 6.75 (s, 2H), 6.69 (s, 2H), 5.26 (d, *J* = 1.3 Hz, 2H), 3.96 (s, 2H), 2.75 (s, 6H), 1.82 (s, 6H), 1.26 (s, 12H). <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) δ (ppm): 144.62, 130.45, 127.80, 126.69, 125.16, 124.50, 122.84, 114.36, 56.31, 39.88, 30.70, 26.99, 18.39. ESI (+)-HRMS (*m/z*): [M + H]<sup>+</sup> calcd. for: 543.1010, found: 543.1007.

#### Synthesis of **Q4**:

To a 50 mL well-dried flask flushed with argon, **Q3** (1.63 g, 3 mmol) and dry THF (50 mL) were added. The solution was cooled to -78 °C, n-BuLi (2.5 M in n-hexane, 2.52 mL, 6.3 mmol) was added and the reaction mixture was stirred at -78 °C for 1h. Dichlorodimethylsilane (0.15 mL, 1.1 mmol) was added dropwise at -78 °C for 60 min and the reaction mixture was slowly warmed to room temperature, then stirred overnight. The solution was extracted with DCM. The organic layers were collected, dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, and concentrated under vacuum. The crude product was used in the next step without further purification.

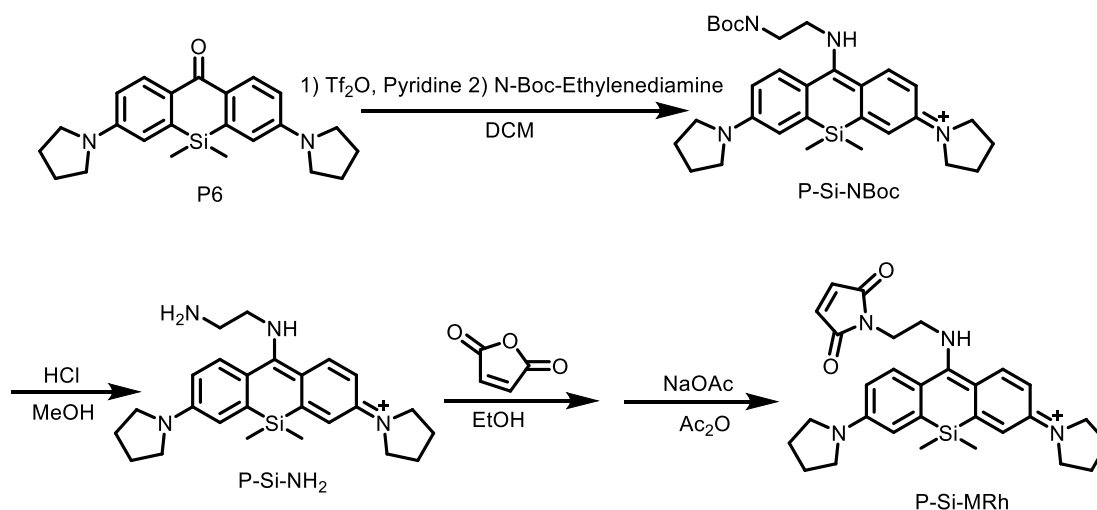
#### Synthesis of **Q5**:

The mixture of **Q4** in 150 ml acetone was cooled to 0 °C, and the KMnO<sub>4</sub> (1.42 g, 9 mmol) was added to the mixture. The solution was stirred at 0 °C for 3 h. The manganese(IV)

oxide was filtered off through a pad of silica gel, and the solid was thoroughly washed with DCM. Mixed filtrates were concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (petroleum ether: dichloromethane 1: 1) to afford **Q5** (547 mg, 40% yield). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ (ppm): 8.16 (s, 2H), 6.55 (s, 2H), 5.30 (s, 2H), 2.92 (s, 6H), 2.06 (d, J = 1.2 Hz, 6H), 1.36 (s, 12H), 0.44 (s, 6H). <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) δ (ppm): 185.19, 146.79, 140.71, 129.81, 129.64, 127.98, 124.78, 123.22, 112.40, 57.19, 31.04, 28.63, 18.72, -1.05. ESI (+)-HRMS (m/z): [M + H]<sup>+</sup> calcd. for: 457.2675, found: 457.2675

#### Synthesis of **Q-Si-ARh**:

The compound **Q5** (114 mg, 0.25 mmol) and pyridine (0.16 ml, 2 mmol) was dissolved in 20 ml DCM and stirred at 0 °C for 15 min. Then, the Tf<sub>2</sub>O (0.21 ml, 1.25 mmol) was added dropwise to the solution and stirred at 0 °C for 1 h. The propylamine (0.21 ml, 2.5 mmol) was added to the solution and stirred at room temperature for 2 h. After the reaction was completed, the solution was concentrated under vacuum. The residue was purified by column chromatography on silica gel (DCM: MeOH) to afford **Q-P-ARh** (85 mg, 68% yield). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ (ppm): 7.39 (s, 2H), 6.64 (s, 2H), 5.34 (s, 2H), 3.94 (t, J = 13.5 Hz, 2H), 2.98 (s, 6H), 2.01 (s, 6H), 1.95 (q, 2H), 1.62 (s, 6H), 1.40 (s, 12H), 0.98 (t, J = 7.4 Hz, 3H). <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) δ (ppm): 173.21, 148.62, 147.58, 137.01, 130.79, 127.04, 126.13, 125.08, 124.55, 124.18, 121.74, 121.35, 121.00, 120.80, 119.42, 118.16, 117.82, 114.96, 114.64, 113.63, 58.18, 52.10, 31.22, 29.00, 23.12, 18.33, 10.87, -2.19. ESI (+)-HRMS (m/z): [M]<sup>+</sup> calcd. for: 498.3299, found: 498.3300.



**Scheme S4.** Synthesis of **P-Si-MRh**.

#### Synthesis of **P-Si-Boc**:

The compound **P6** (188 mg, 0.5 mmol) and pyridine (0.32 ml, 4 mmol) was dissolved in 20 ml DCM and stirred at 0 °C for 15 min. Then, the  $\text{Tf}_2\text{O}$  (0.42 ml, 2.5 mmol) was added dropwise to the solution and stirred at 0 °C for 1 h. The N-Boc-Ethylenediamine (800 mg, 5 mmol) was added to the solution and stirred at room temperature for 2 h. After the reaction was completed, the solution was concentrated under vacuum. The residue was purified by column chromatography on silica gel (DCM: MeOH) to afford **P-Si-Boc** (181 mg, 70% yield). <sup>1</sup>H NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  (ppm): 7.82 (d,  $J = 8.7$  Hz, 1H), 7.55 (d,  $J = 8.5$  Hz, 1H), 6.75 (s, 1H), 6.70 – 6.55 (m, 2H), 5.95 (d,  $J = 5.6$  Hz, 1H), 4.06 (s, 2H), 3.57 (s, 3H), 3.39 (m, 8H), 2.04 (m, 8H), 1.33 (s, 9H), 0.44 (s, 6H). <sup>13</sup>C NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$  (ppm): 173.49, 157.95, 149.41, 142.68, 139.14, 132.21, 129.44, 124.41, 122.18, 119.00, 118.64, 116.72, 115.61, 113.41, 111.81, 52.52, 47.65, 39.66, 29.66, 28.23, 25.36. ESI (+)-HRMS ( $m/z$ ):  $[\text{M}]^+$  calcd. for: 519.3150, found: 519.3151.

#### Synthesis of **P-Si-NH<sub>2</sub>**:

The compound **P-Si-Boc** (181 mg, 0.35 mmol) was dissolved in 30 ml methanol hydrogen chloride solution for 4 h. After the reaction was completed, the solution was concentrated under vacuum to afford **P-Si-NH<sub>2</sub>** (85 mg, 68% yield). <sup>1</sup>H NMR (400 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  (ppm): 7.82 (d,  $J = 8.7$  Hz, 1H), 7.55 (d,  $J = 8.5$  Hz, 1H), 6.75 (s, 1H), 6.70 – 6.55 (m, 2H), 5.95 (d,  $J = 5.6$  Hz, 1H), 4.06 (s, 2H), 3.57 (s, 3H), 3.39 (m, 8H), 2.04 (m, 8H), 1.33 (s, 9H), 0.44

(s, 6H).  $^{13}\text{C}$  NMR (101 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  (ppm): 173.49, 157.95, 149.41, 142.68, 139.14, 132.21, 129.44, 124.41, 122.18, 119.00, 118.64, 116.72, 115.61, 113.41, 111.81, 52.52, 47.65, 39.66, 29.66, 28.23, 25.36. ESI (+)-HRMS (m/z):  $[\text{M}]^+$  calcd. for: 519.3150, found: 519.3151.

#### Synthesis of **P-Si-MRh**:

The compound **P-Si-NH<sub>2</sub>** (85 mg, 0.2 mmol) was dissolved in 30 ml ethanol, and the maleic anhydride (39 mg, 0.4 mmol) was added to the solution. The mixture was stirred at room temperature for 6 h. And the solution was concentrated under vacuum, then 30 ml acetic anhydride and sodium acetate (54 mg, 0.4 mmol) were added to the mixture, the solution was heated to 70 °C for 2 h. After the reaction was completed, the solution was concentrated under vacuum. The residue was purified by column chromatography on silica gel (DCM: MeOH) to afford **P-Si-MRh** (46 mg, 48% yield).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  (ppm): 8.03 (d,  $J = 8.5$  Hz, 1H), 7.46 (d,  $J = 8.6$  Hz, 1H), 6.75 (s, 1H), 6.64 (s, 2H), 6.62 (s, 2H), 6.58 (d,  $J = 7.9$  Hz, 1H), 4.15 (t,  $J = 5.9$  Hz, 2H), 4.00 (t,  $J = 5.9$  Hz, 2H), 3.37 (d,  $J = 17.0$  Hz, 8H), 2.02 (d,  $J = 18.2$  Hz, 8H), 0.43 (s, 6H).  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$  (ppm): 173.77, 170.34, 169.28, 162.42, 162.08, 161.74, 161.40, 135.75, 134.22, 118.26, 115.34, 53.46, 48.21, 47.54, 45.71, 37.08, 25.29, 8.44, -2.21. ESI (+)-HRMS (m/z):  $[\text{M}]^+$  calcd. for: 499.2524, found: 499.2523.

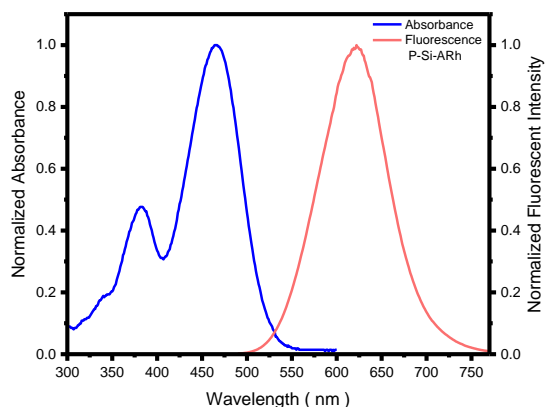


Figure S1. The normalized absorbance spectrum and fluorescence spectrum of P-Si-ARh in PBS at 25 °C.

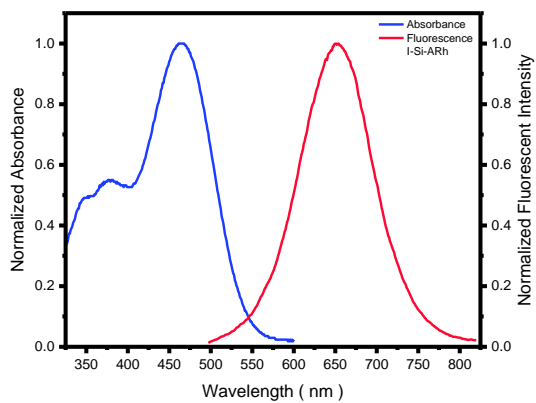


Figure S2. The normalized absorbance spectrum and fluorescence spectrum of I-Si-ARh in PBS at 25 °C.

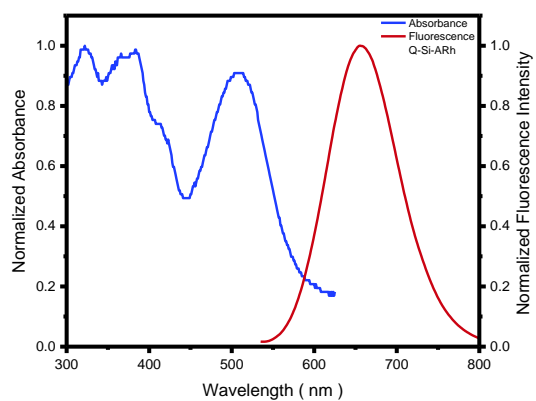


Figure S3. The normalized absorbance spectrum and fluorescence spectrum of Q-Si-ARh in PBS at 25 °C.

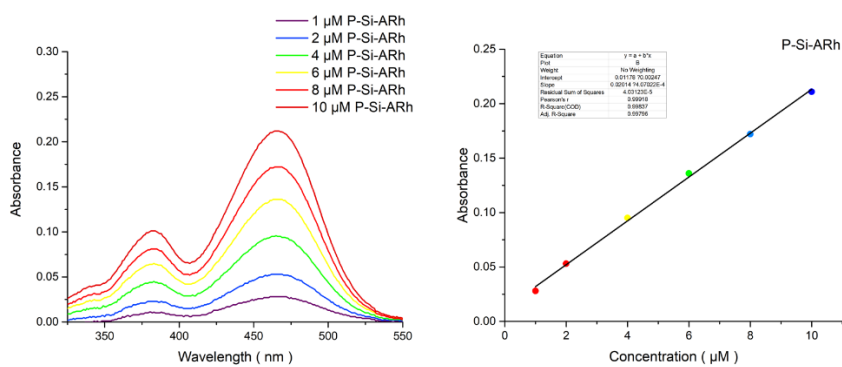


Figure S4. UV spectra of P-Si-ARh at different concentrations and the absorption-concentration curve of P-Si-ARh.

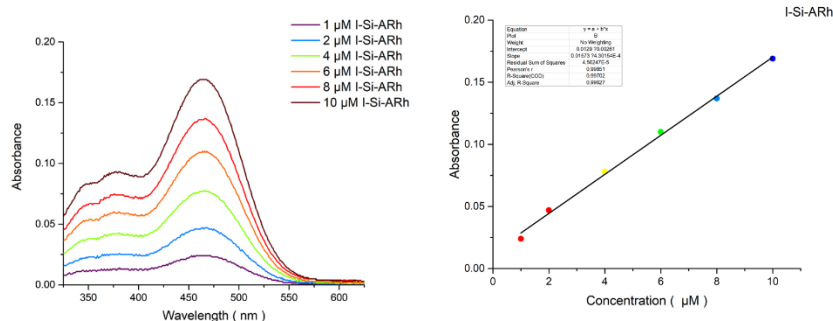


Figure S5. UV spectra of I-Si-ARh at different concentrations and the absorption-concentration curve of I-Si-ARh.

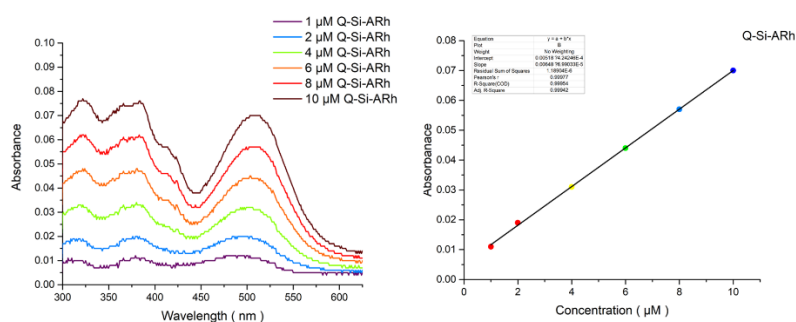


Figure S6. UV spectra of Q-Si-ARh at different concentrations and the absorption-concentration curve of Q-Si-ARh.

Structure	$\lambda_{\text{abs}}/\lambda_{\text{fl}}$ (nm)	$\Phi_{\text{fl}}$	Reference
	646/660	0.31	<i>J. Am. Chem. Soc.</i> <b>2012</b> , 134, 5029–5031
	691/712	0.12	<i>J. Am. Chem. Soc.</i> <b>2012</b> , 134, 5029–5031

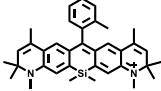
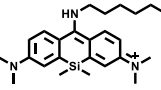
	721/740	0.05	<i>J. Am. Chem. Soc.</i> <b>2012</b> , 134, 5029–5031
	455/617	0.09	<i>Biomaterials</i> <b>2018</b> , 158, 10–22
This work	465 – 508/ 622 – 655	0.22-0.1	-----

Table S1. The structures and optical data of reported Si-rhodamines derivatives.

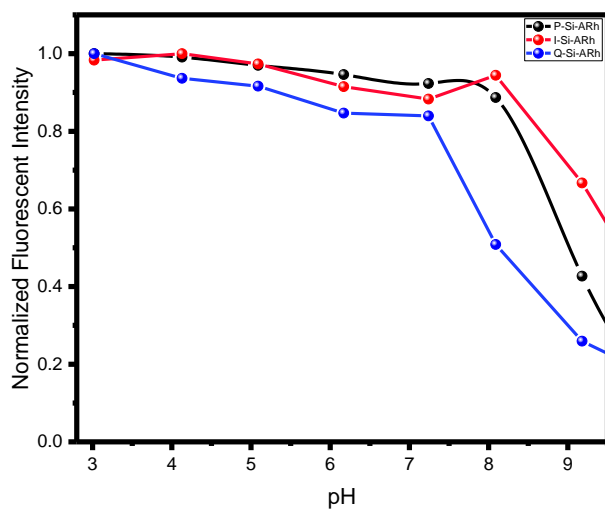


Figure S7. Normalized fluorescence intensities of Si-ARh derivatives (10  $\mu$ M) at various pH values in B-R buffer at 25  $^{\circ}$ C.



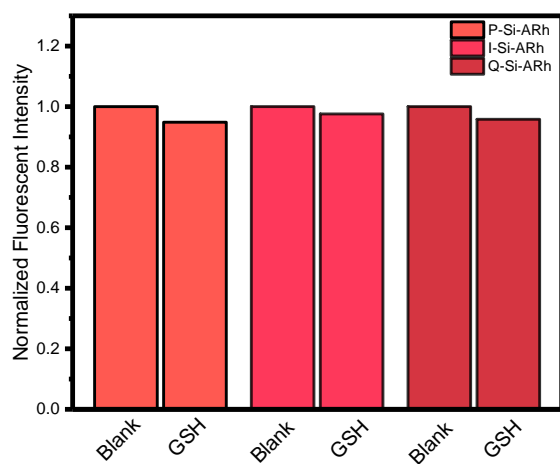


Figure S8. The effects of GSH (1 mM) and on the fluorescence of Si-ARh derivatives (10  $\mu$ M) in PBS solution pH 7.4 at 25°C.

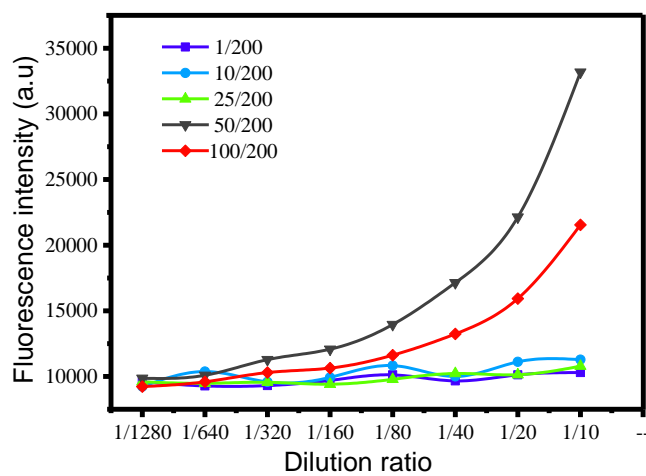


Figure S9. UV spectra of P-Si-MRh at different concentrations and the absorption-concentration curve of P-Si-MRh.

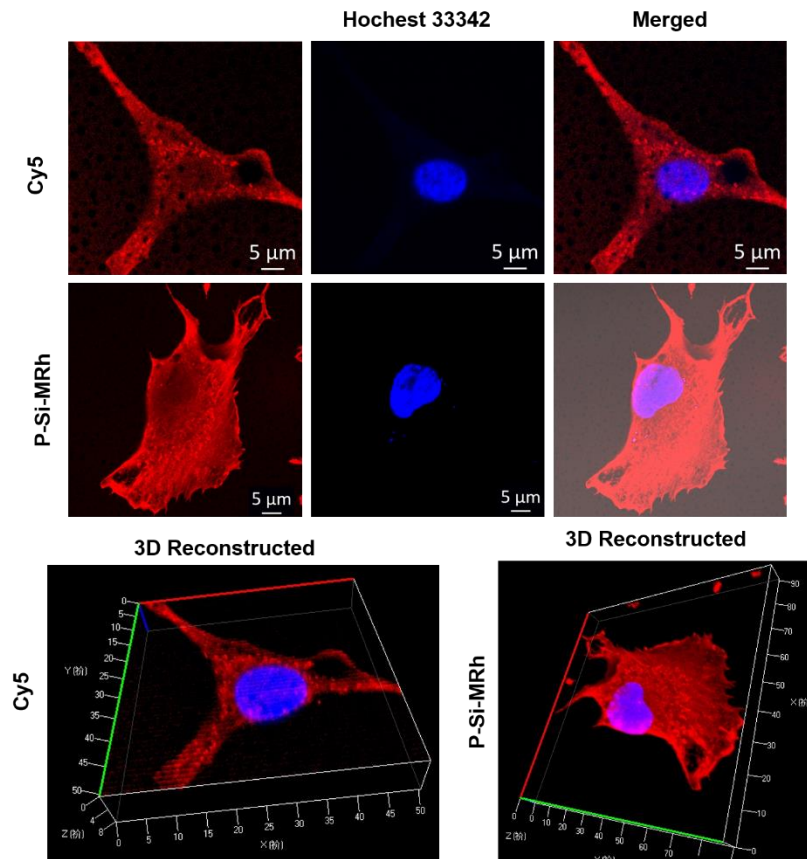


Figure s10. The fluorescent images of F-actin in A549 cells which were stained by Cy5 and P-Si-MRh respectively

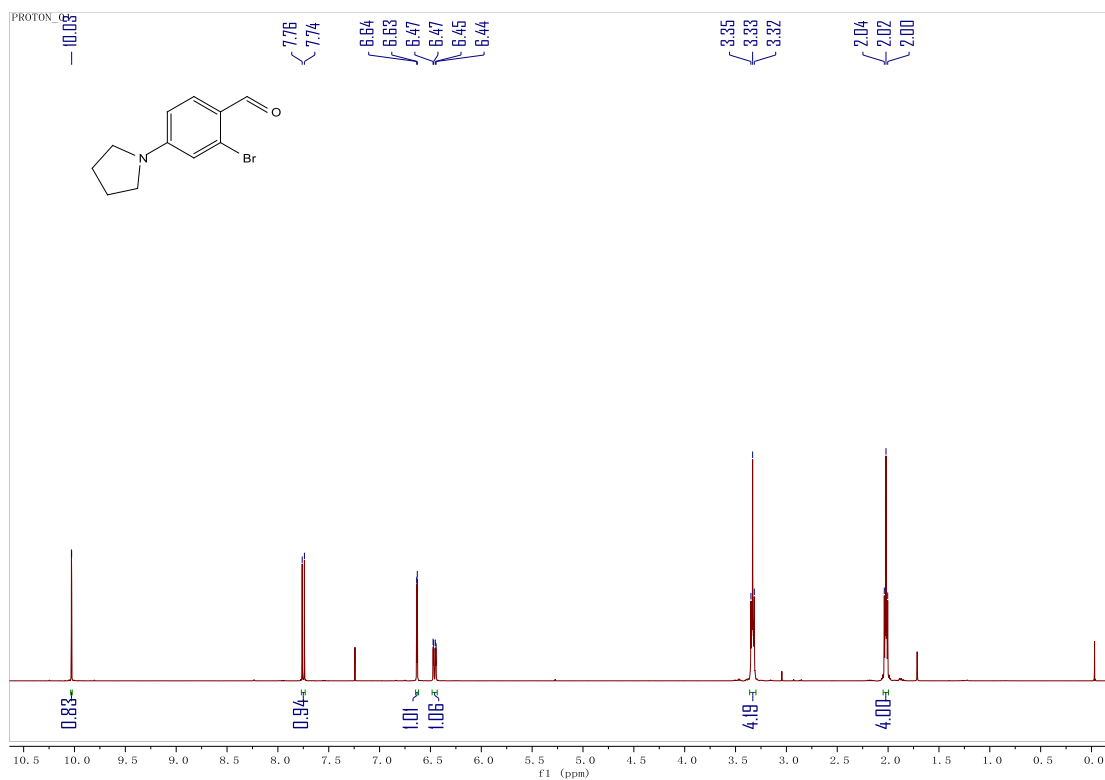


Figure S11. The  $^1\text{H}$  NMR spectra of P2 in  $\text{CDCl}_3$ .

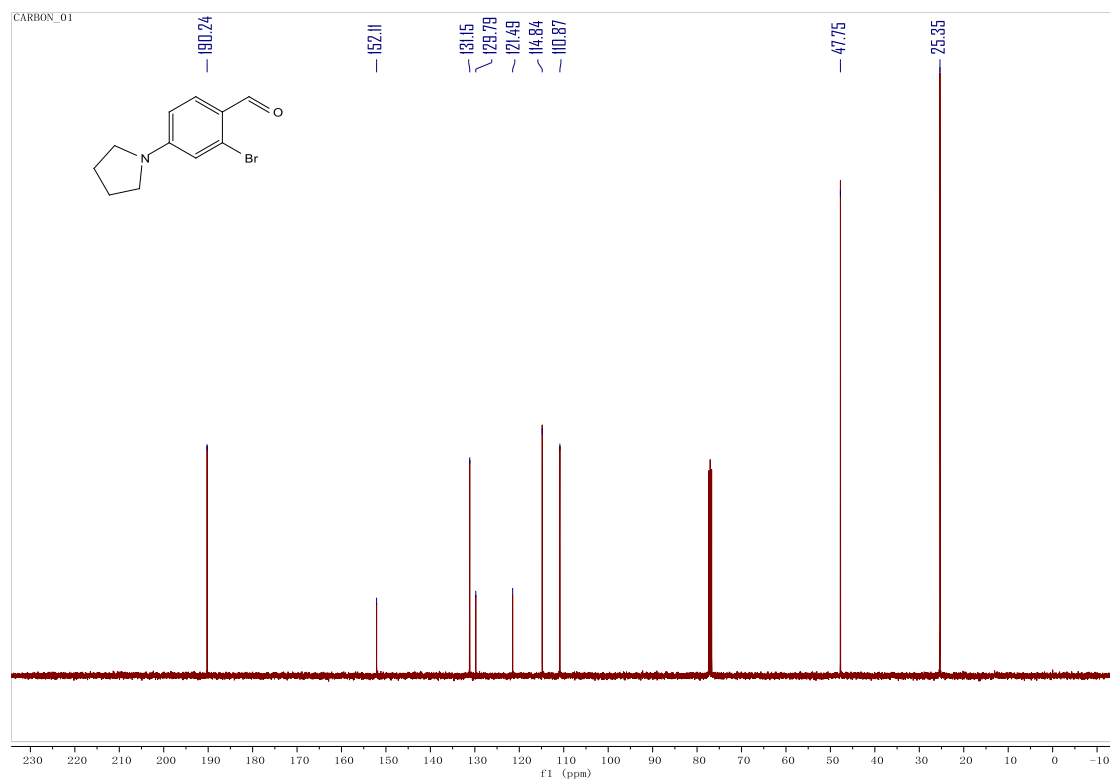


Figure S12. The  $^{13}\text{C}$  NMR spectra of P2 in  $\text{CDCl}_3$ .

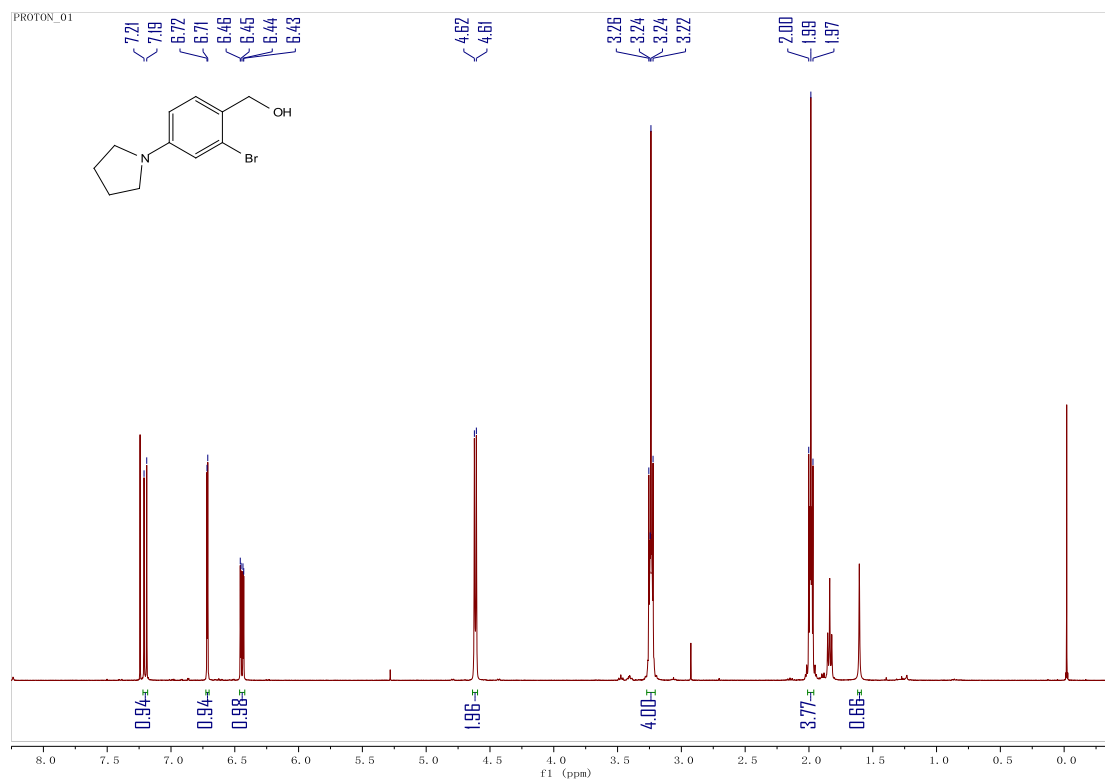


Figure S13. The  $^1\text{H}$  NMR spectra of **P3** in  $\text{CDCl}_3$ .

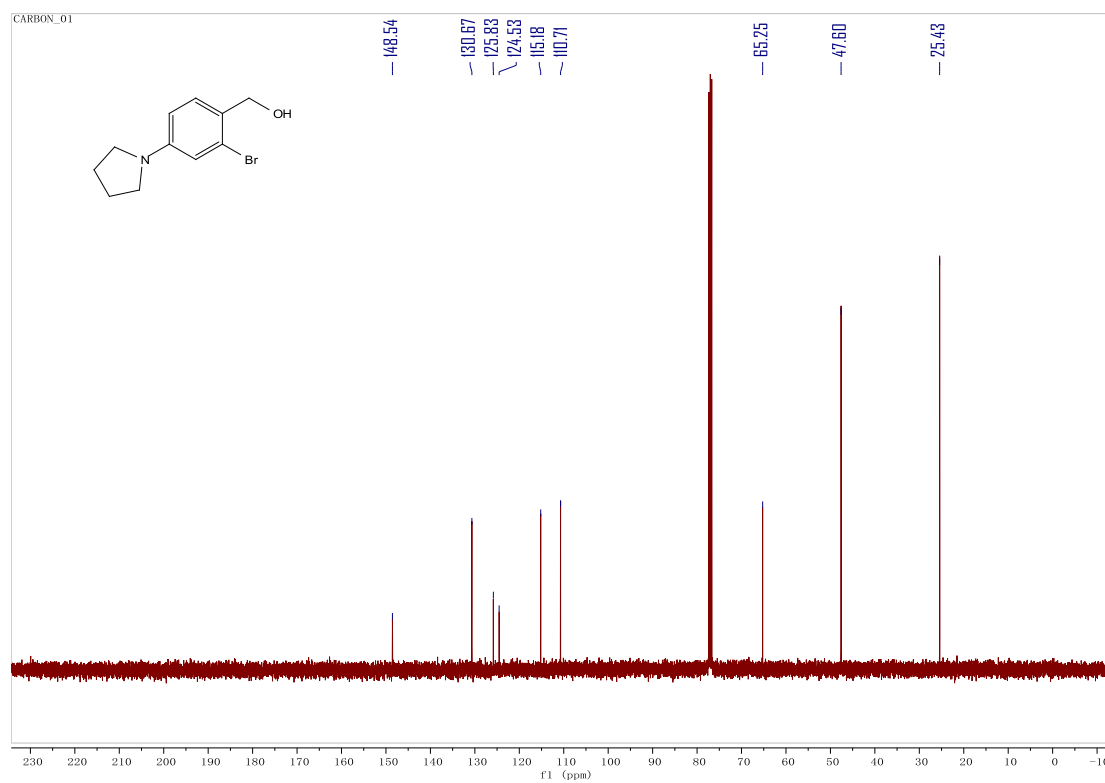


Figure S14. The  $^{13}\text{C}$  NMR spectra of **P3** in  $\text{CDCl}_3$ .

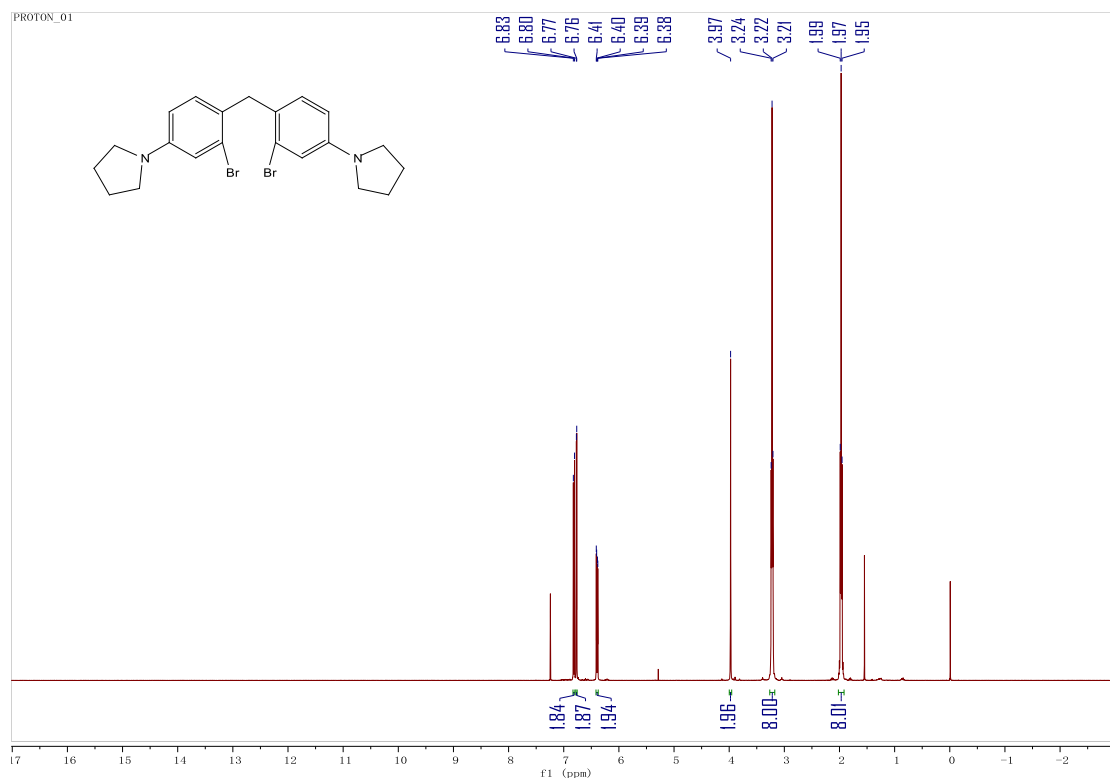


Figure S15. The  $^1\text{H}$  NMR spectra of P4 in  $\text{CDCl}_3$ .

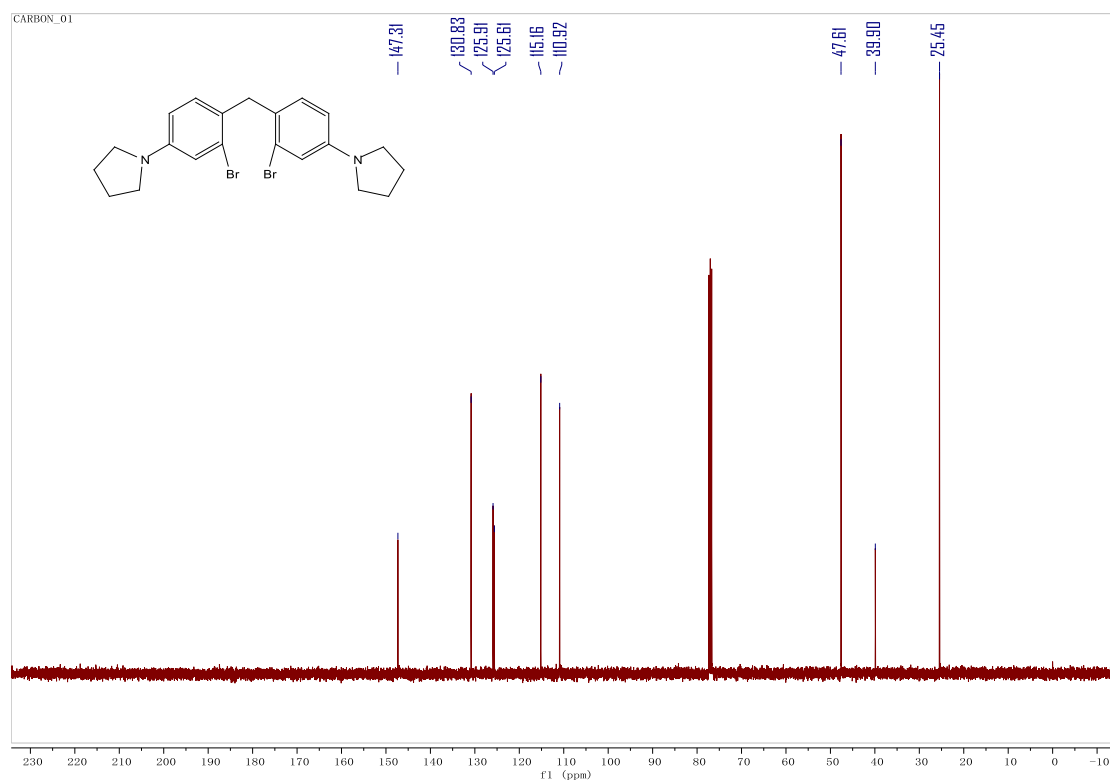


Figure S16. The  $^{13}\text{C}$  NMR spectra of P4 in  $\text{CDCl}_3$ .

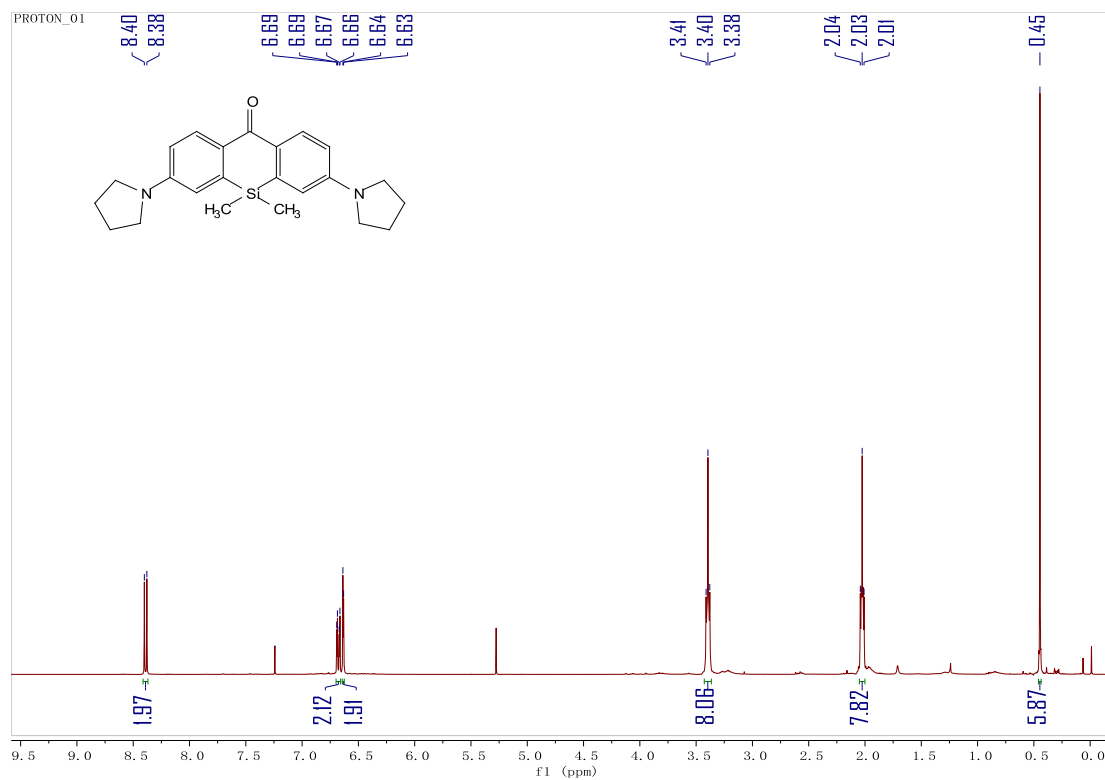


Figure S17. The  $^1\text{H}$  NMR spectra of P6 in  $\text{CDCl}_3$ .

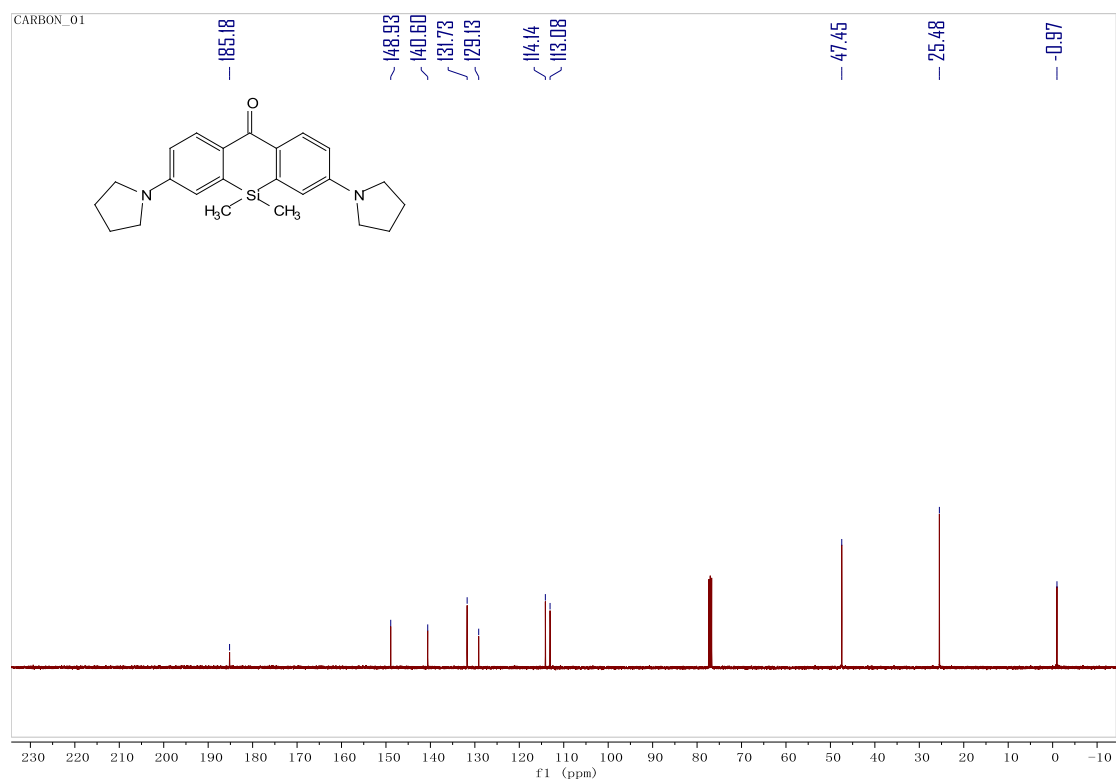


Figure S18. The  $^{13}\text{C}$  NMR spectra of P6 in  $\text{CDCl}_3$ .

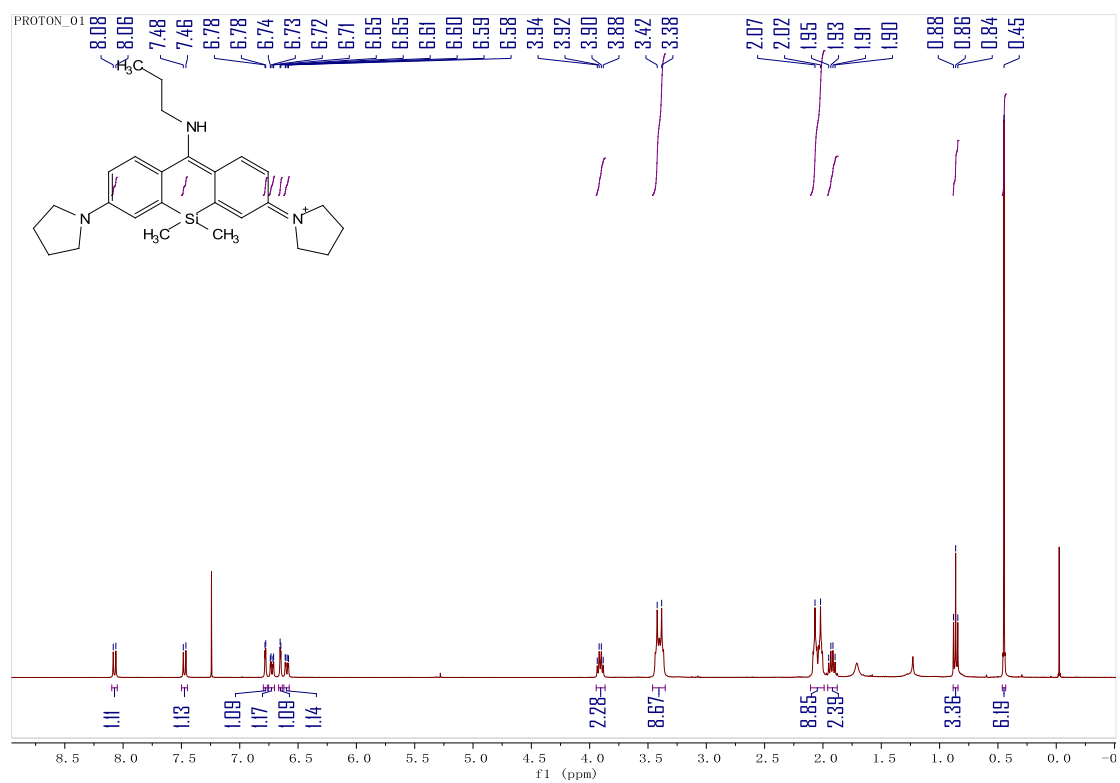


Figure S19. The  $^1\text{H}$  NMR spectra of P-Si-ARh in  $\text{CDCl}_3$ .

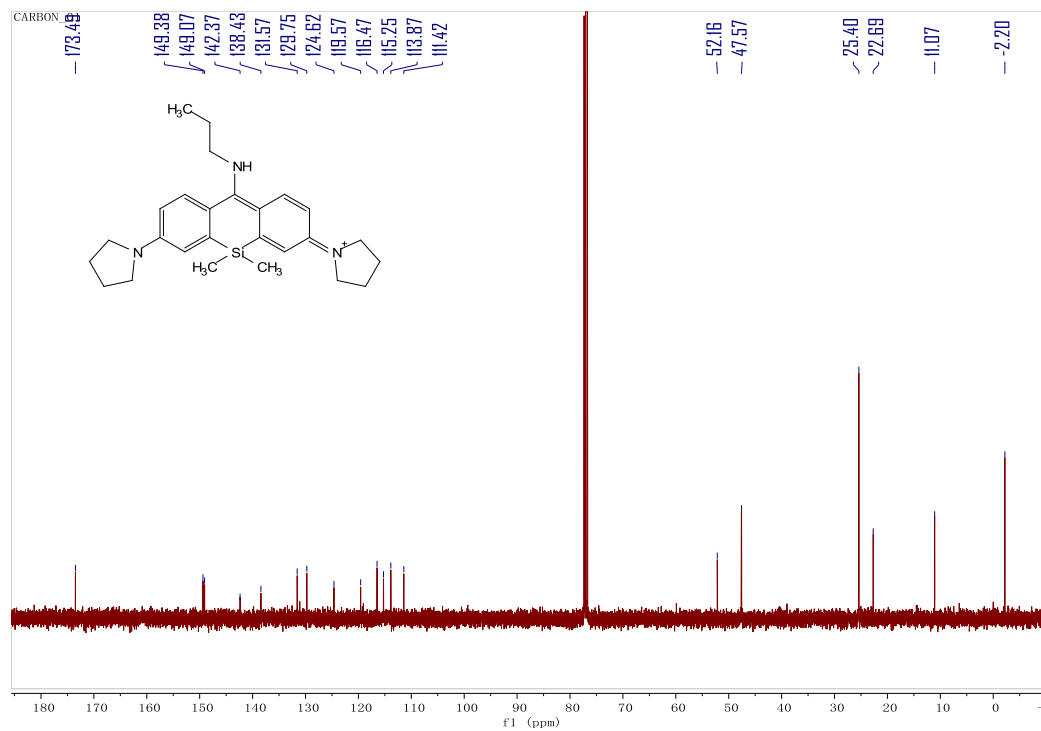


Figure S20. The  $^{13}\text{C}$  NMR spectra of P-Si-ARh in  $\text{CDCl}_3$ .

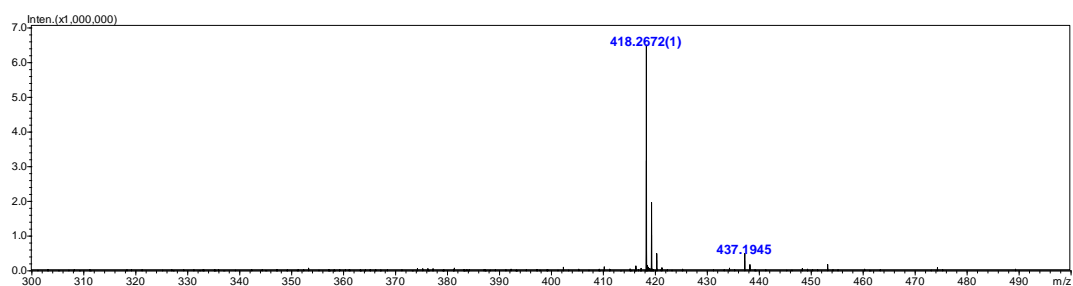


Figure S21. The ESI-MS spectra of P-Si-ARh.

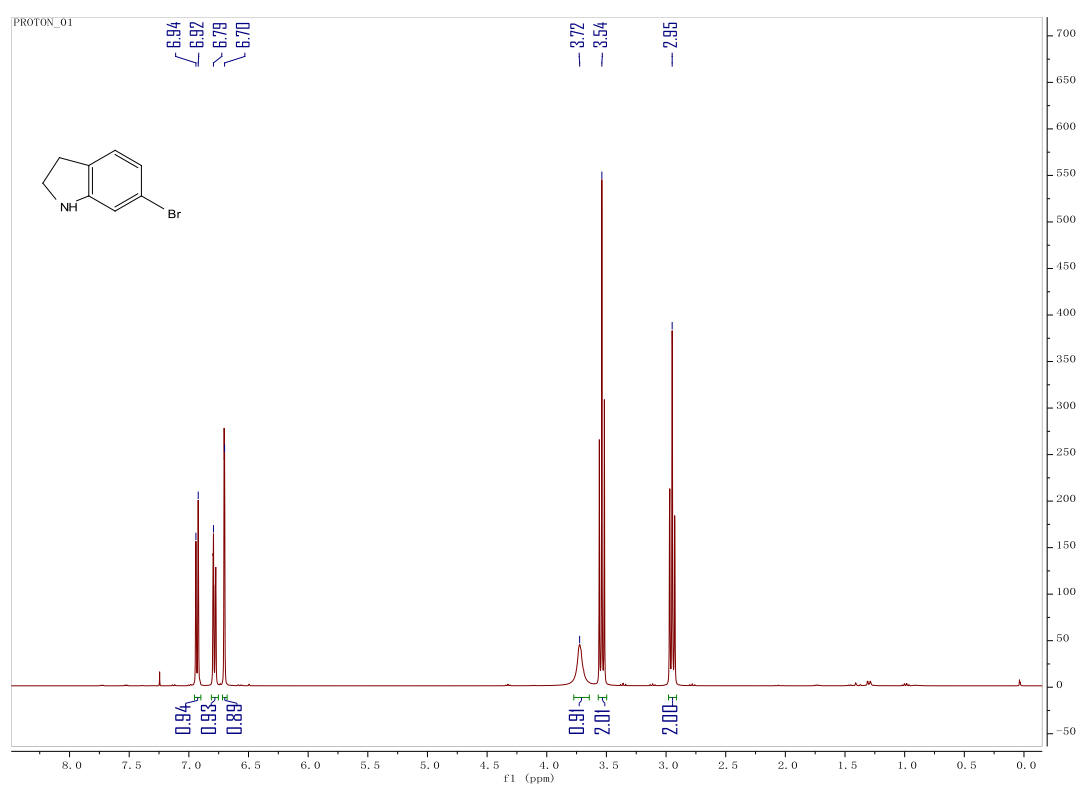


Figure S22. The <sup>1</sup>H NMR spectra of I1 in CDCl<sub>3</sub>.



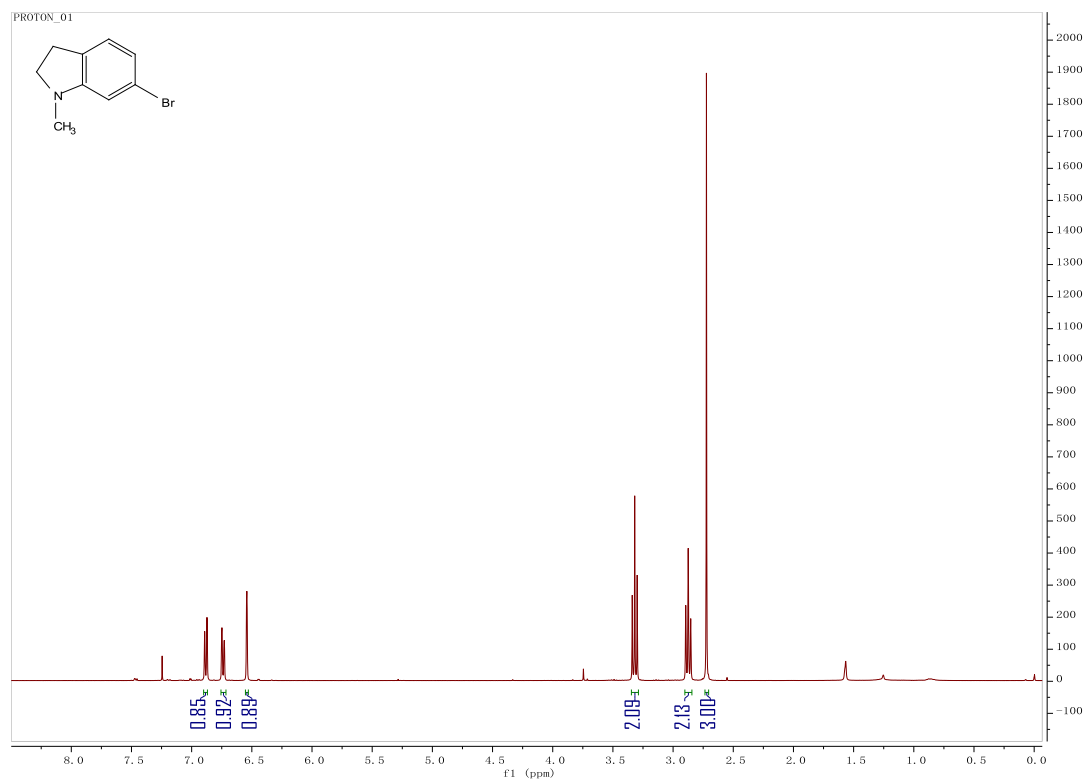


Figure S23. The  $^1\text{H}$  NMR spectra of **I2** in  $\text{CDCl}_3$ .

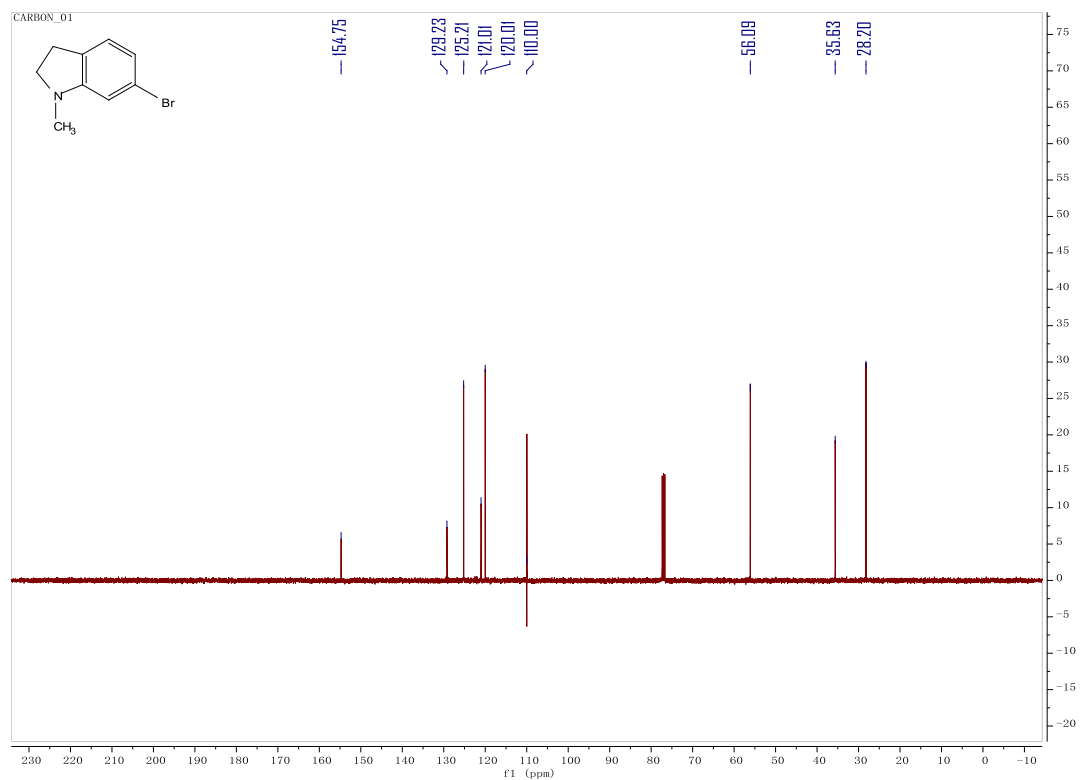


Figure S24. The  $^{13}\text{C}$  NMR spectra of **I2** in  $\text{CDCl}_3$

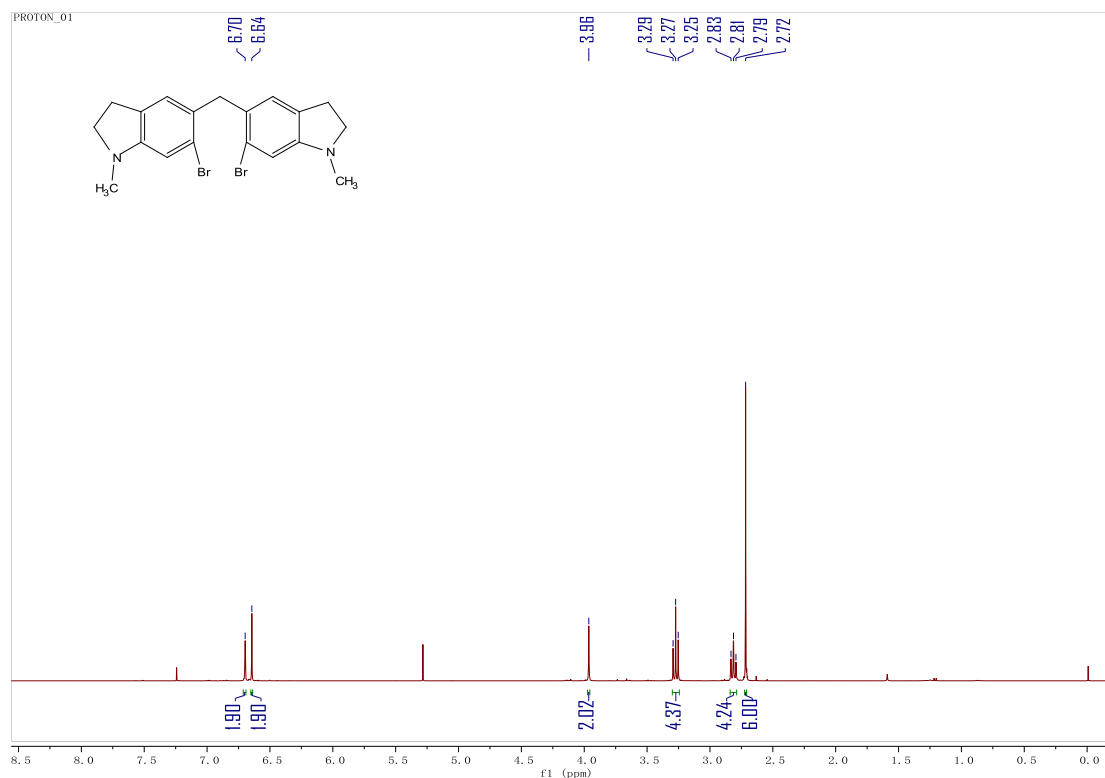


Figure S25. The  $^1\text{H}$  NMR spectra of I3 in  $\text{CDCl}_3$ .

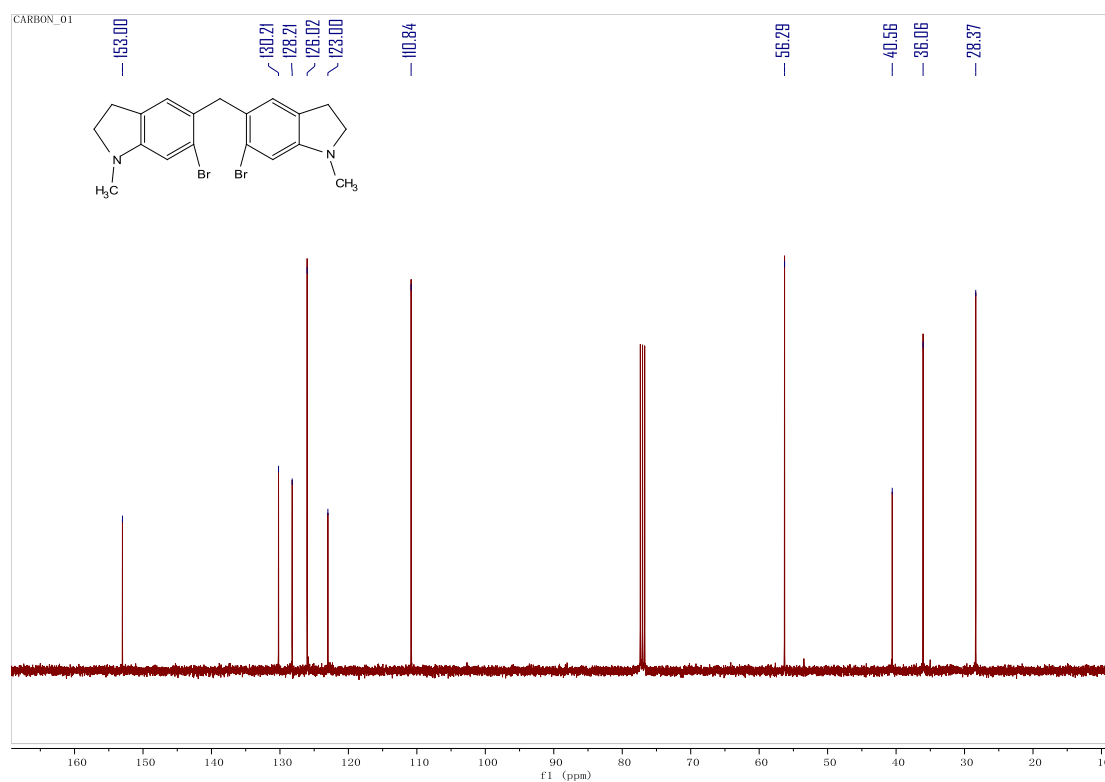


Figure S26. The  $^{13}\text{C}$  NMR spectra of I3 in  $\text{CDCl}_3$

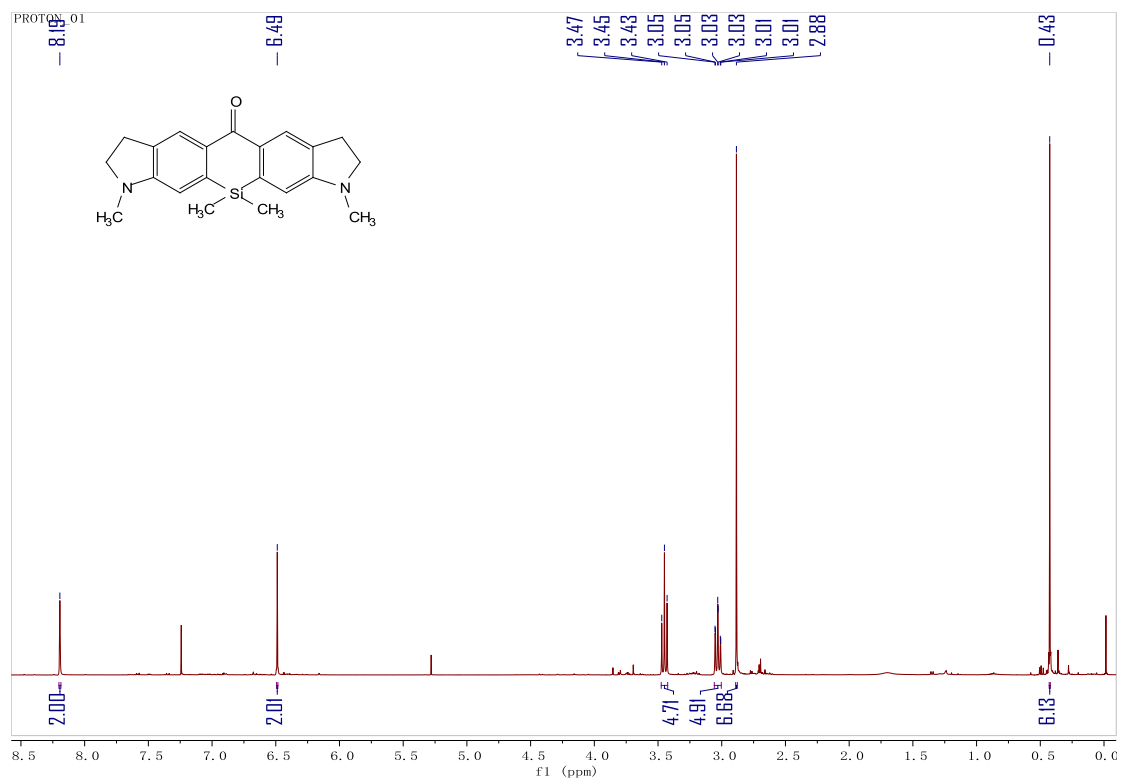


Figure S27. The  $^1\text{H}$  NMR spectra of **I5** in  $\text{CDCl}_3$ .

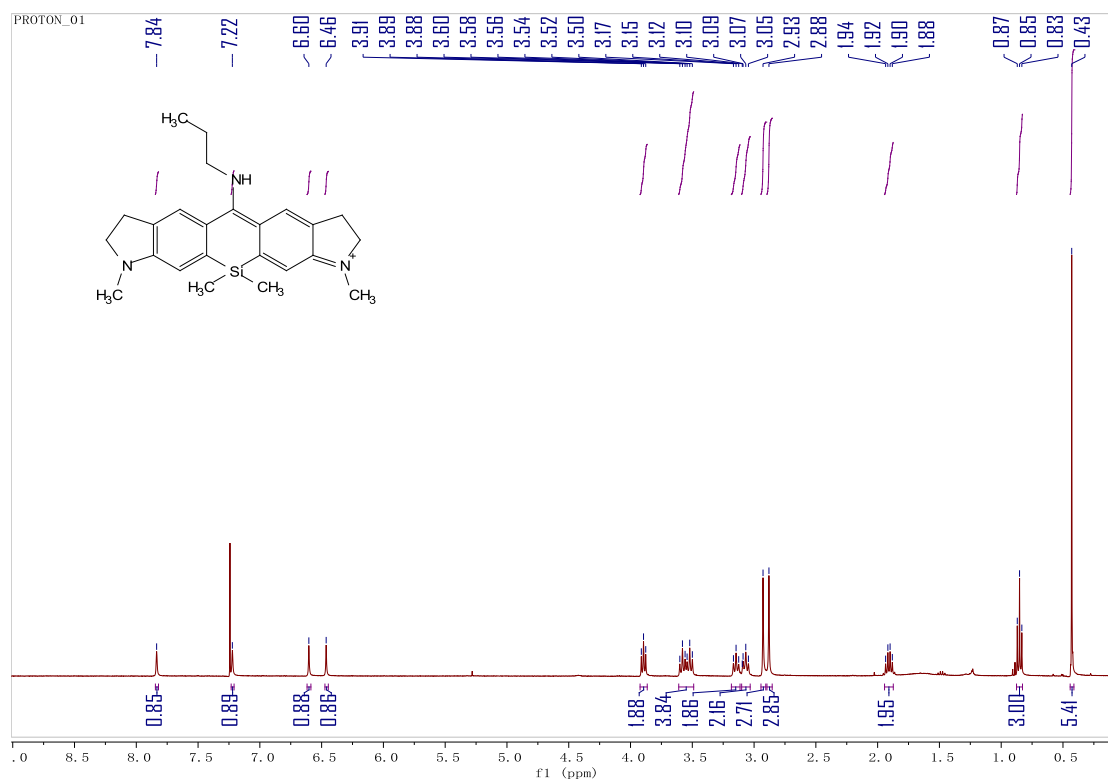


Figure S28. The  $^1\text{H}$  NMR spectra of **I-Si-ARh** in  $\text{CDCl}_3$ .

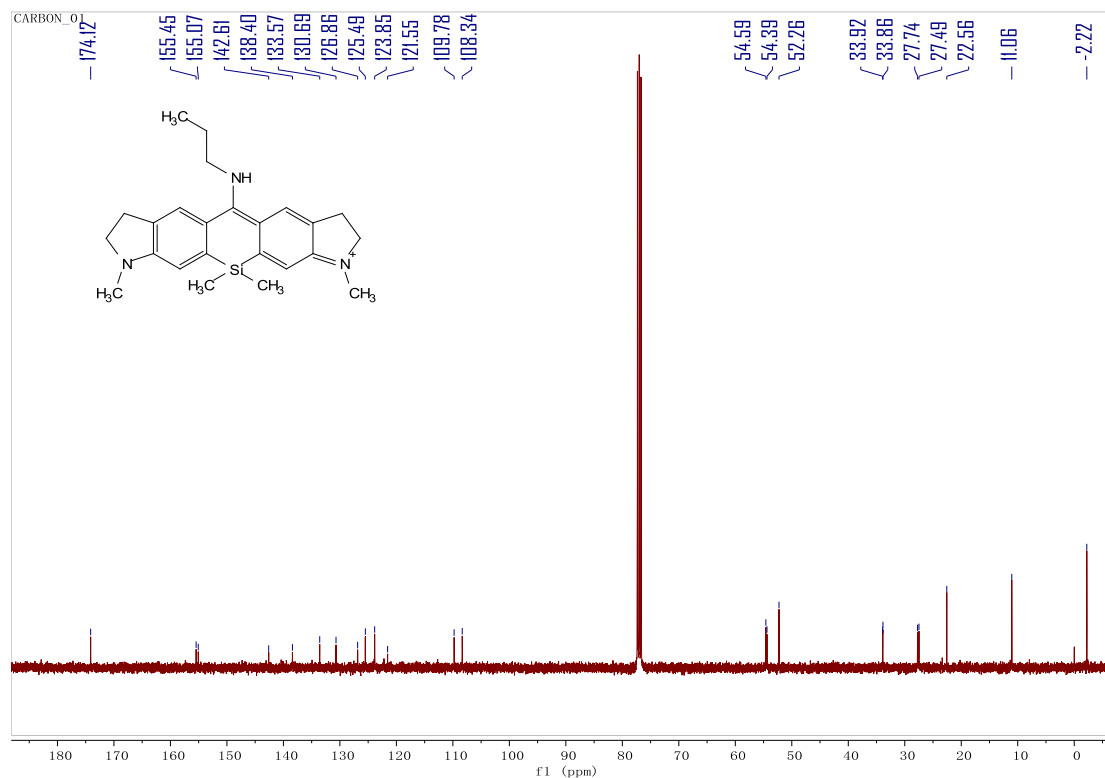


Figure S29. The  $^{13}\text{C}$  NMR spectra of I-Si-ARh in  $\text{CDCl}_3$

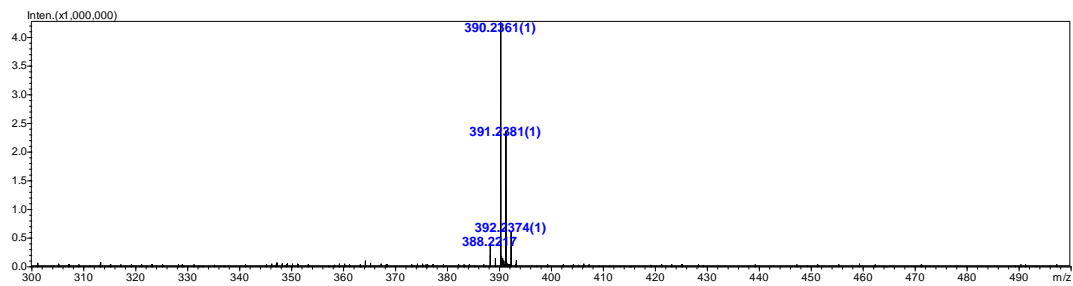
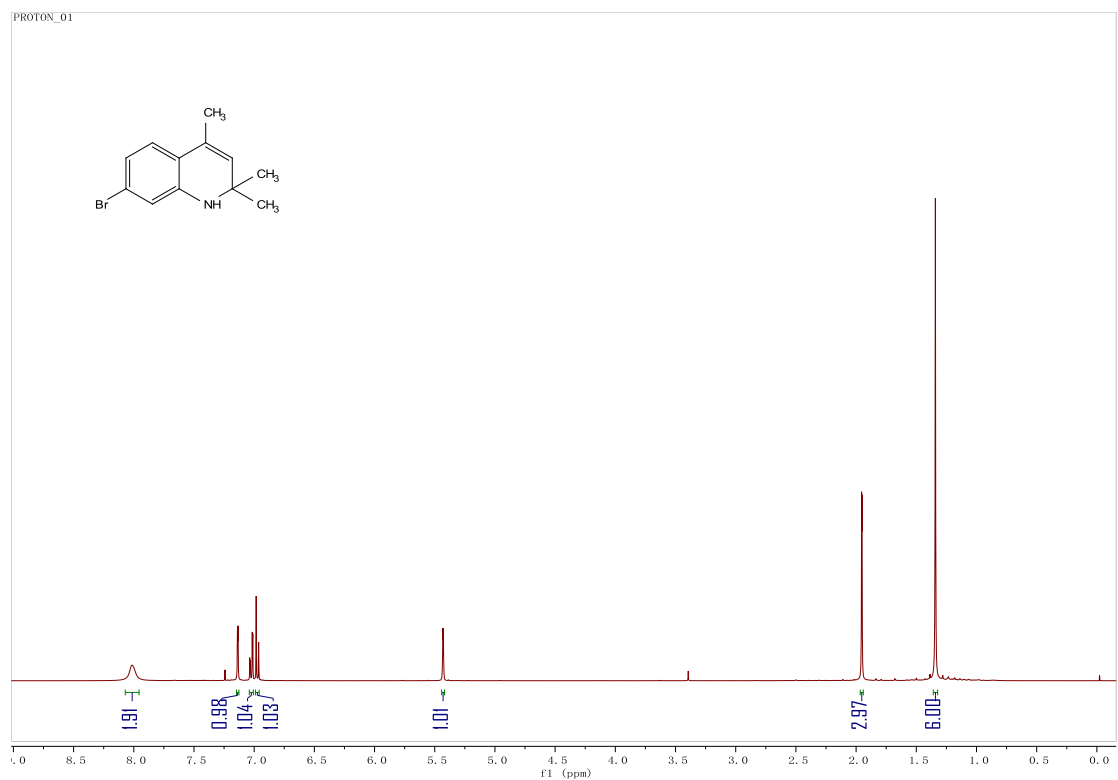
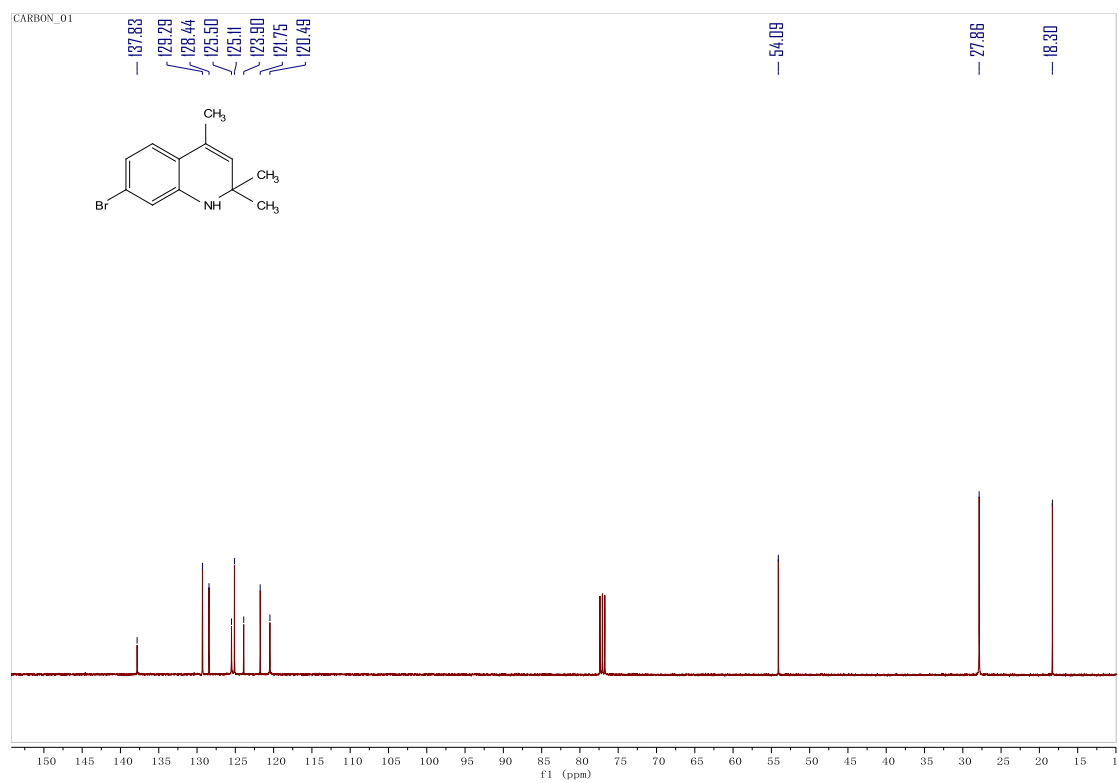


Figure S30. The ESI-MS spectra of I-Si-ARh.



**Figure S31.** The  $^1\text{H}$  NMR spectra of **Q1** in  $\text{CDCl}_3$ .



**Figure S32.** The  $^{13}\text{C}$  NMR spectra of **Q1** in  $\text{CDCl}_3$ .

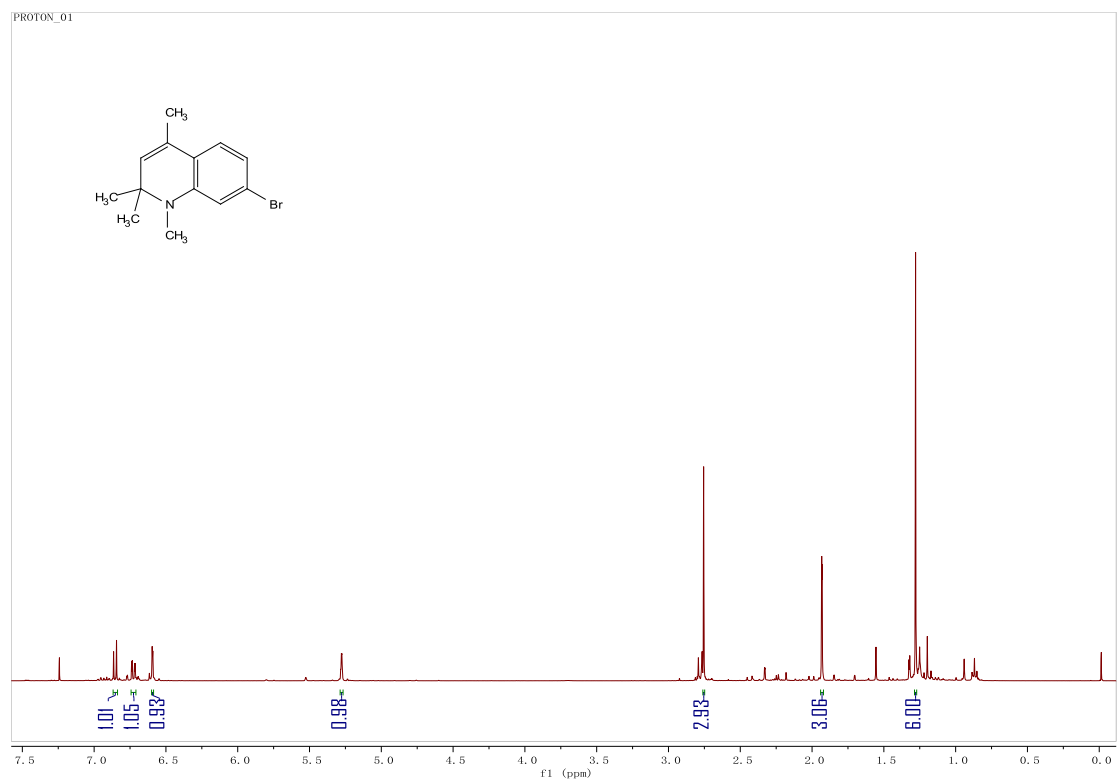


Figure S33. The <sup>1</sup>H NMR spectra of **Q2** in CDCl<sub>3</sub>.

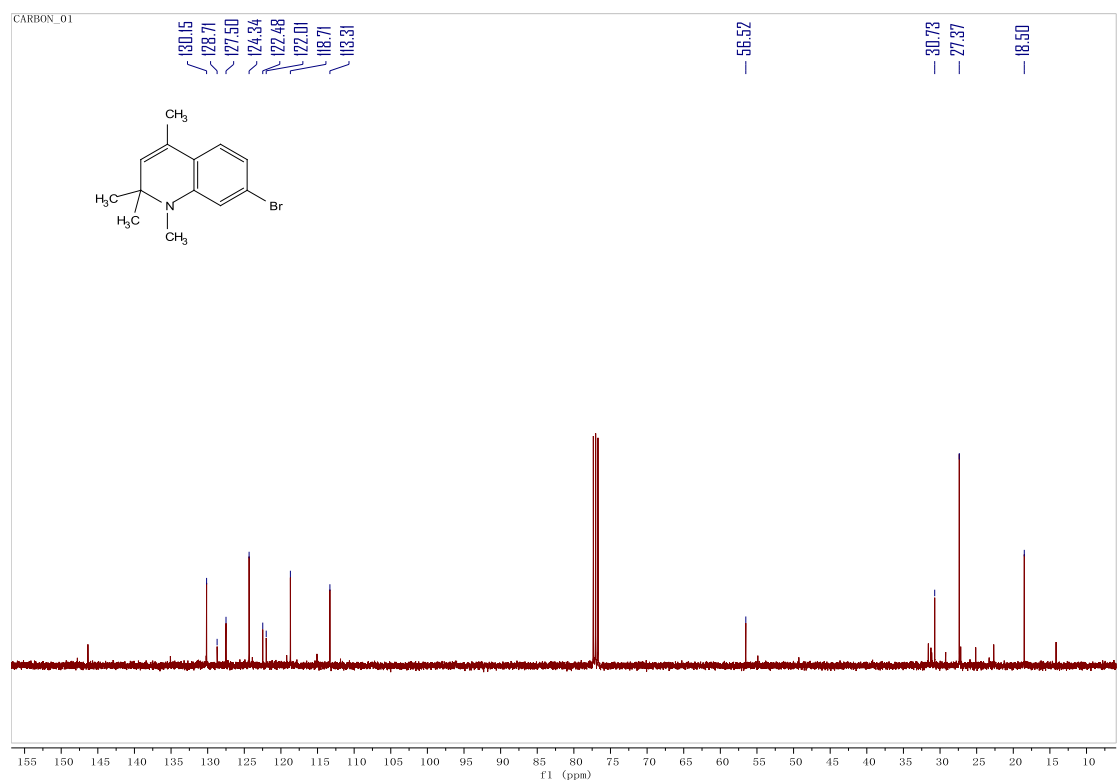
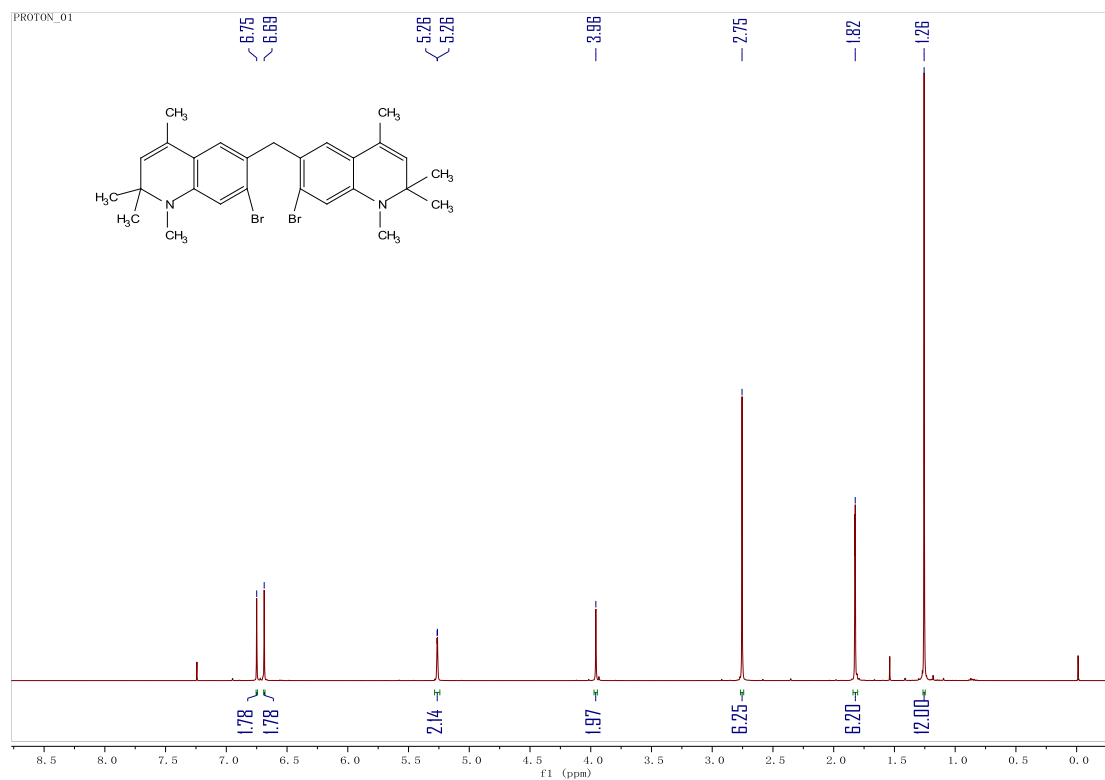
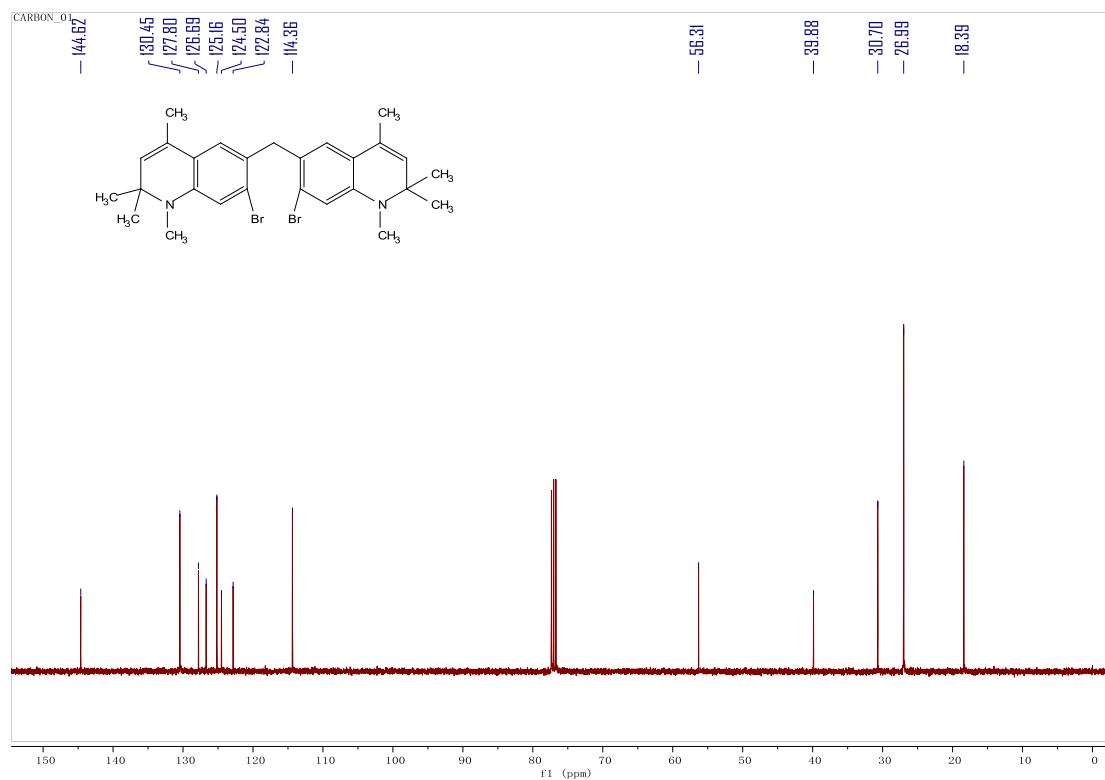


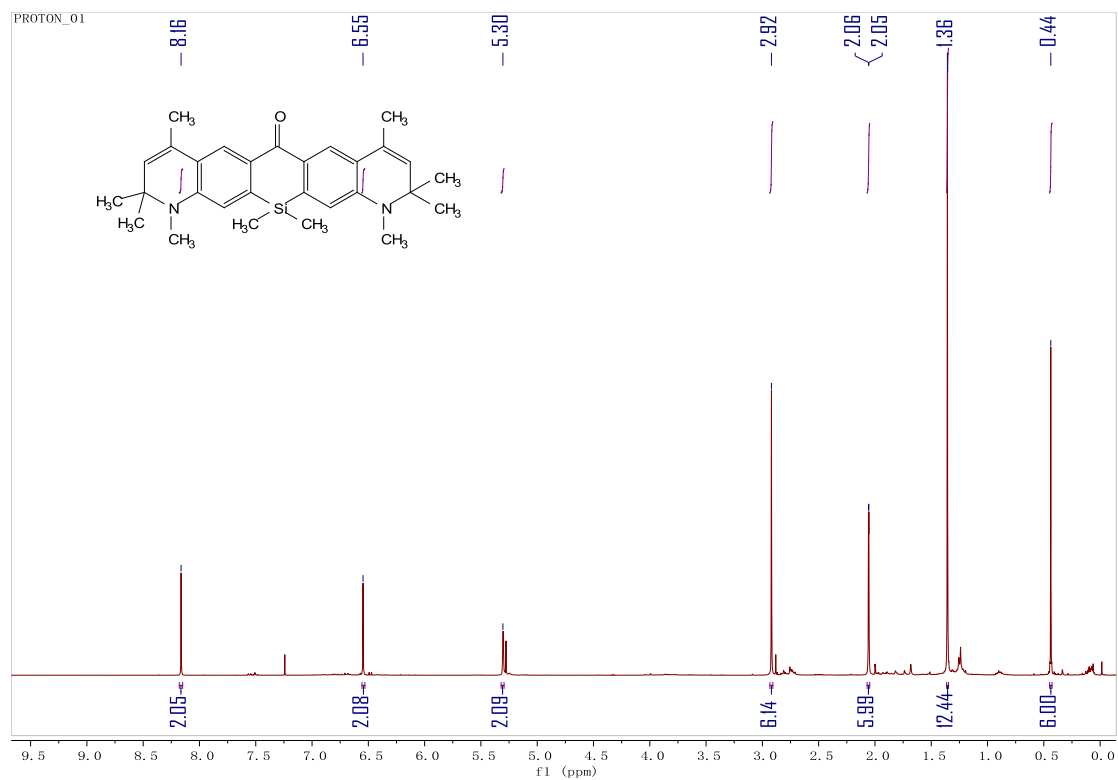
Figure S34. The <sup>13</sup>C NMR spectra of **Q2** in CDCl<sub>3</sub>.



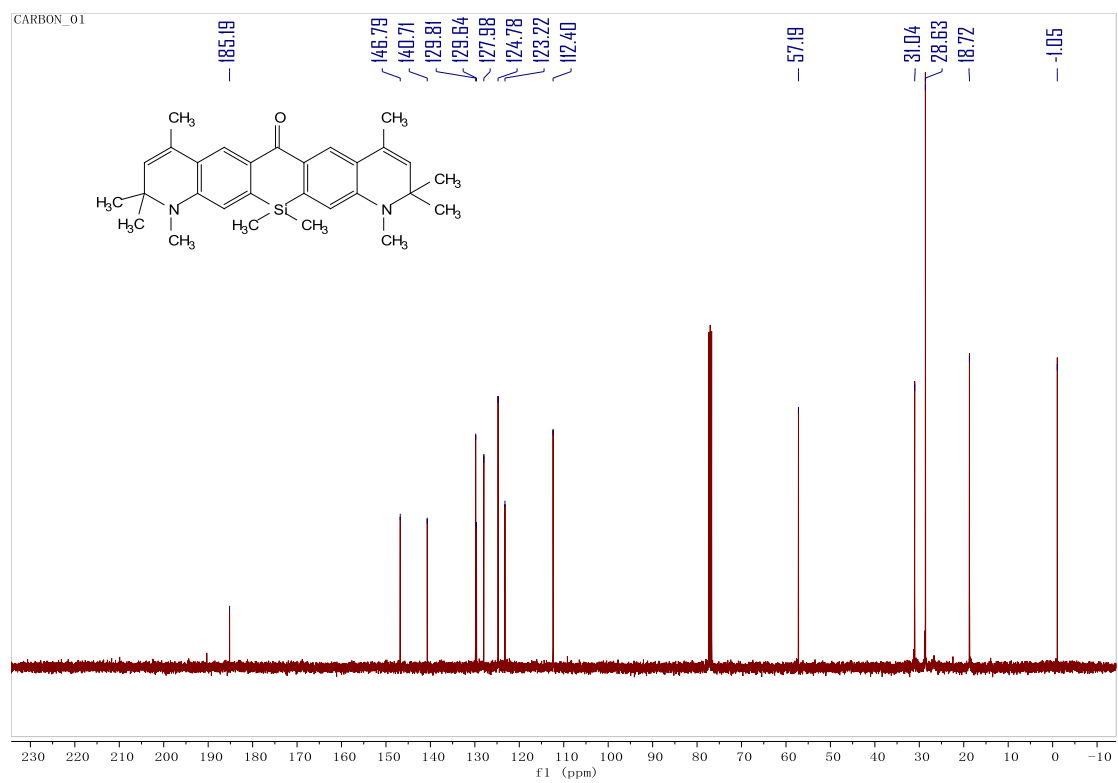
**Figure S35.** The  $^1\text{H}$  NMR spectra of **Q3** in  $\text{CDCl}_3$ .



**Figure S36.** The  $^{13}\text{C}$  NMR spectra of **Q3** in  $\text{CDCl}_3$ .



**Figure S37.** The  $^1\text{H}$  NMR spectra of **Q5** in  $\text{CDCl}_3$ .



**Figure S38.** The  $^{13}\text{C}$  NMR spectra of **Q5** in  $\text{CDCl}_3$ .



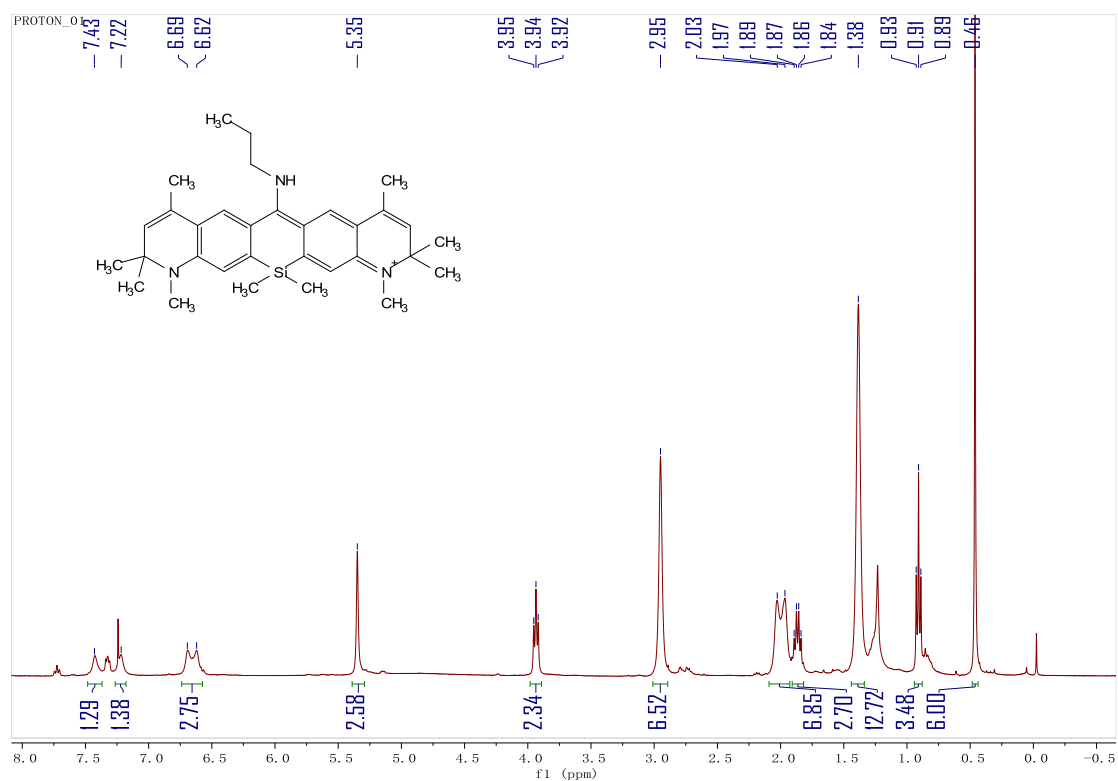


Figure S39. The <sup>1</sup>H NMR spectra of Q-Si-ARh in CDCl<sub>3</sub>.

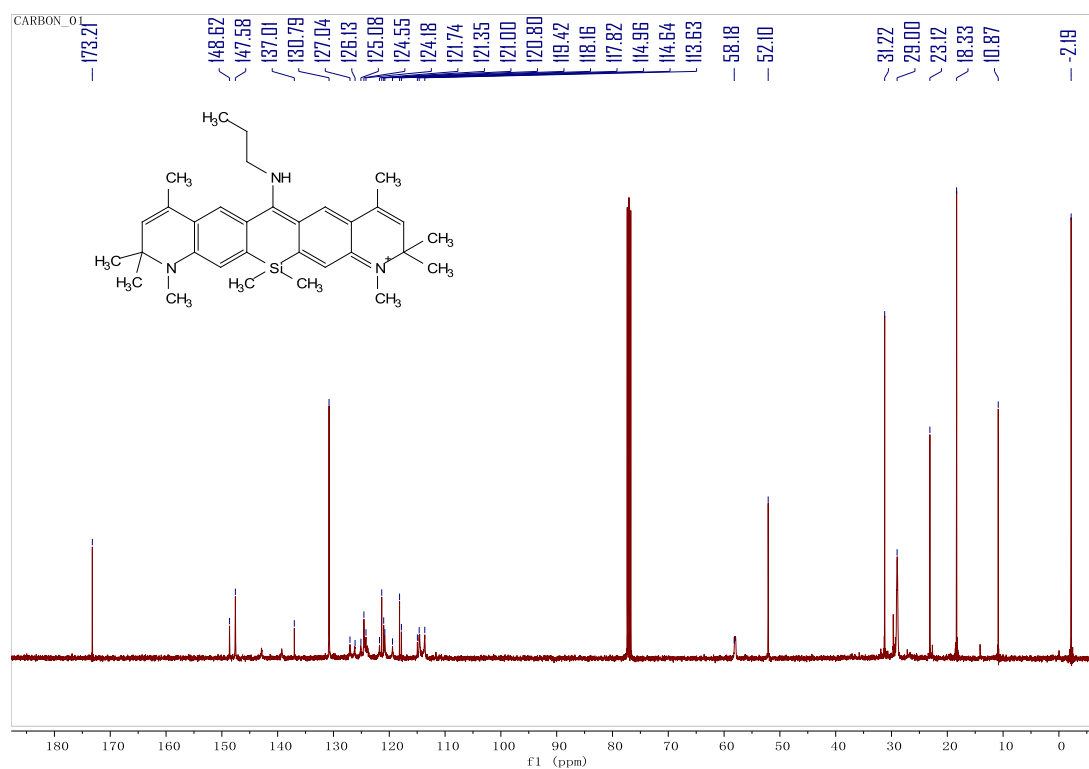


Figure S40. The <sup>13</sup>C NMR spectra of Q-Si-ARh in CDCl<sub>3</sub>.

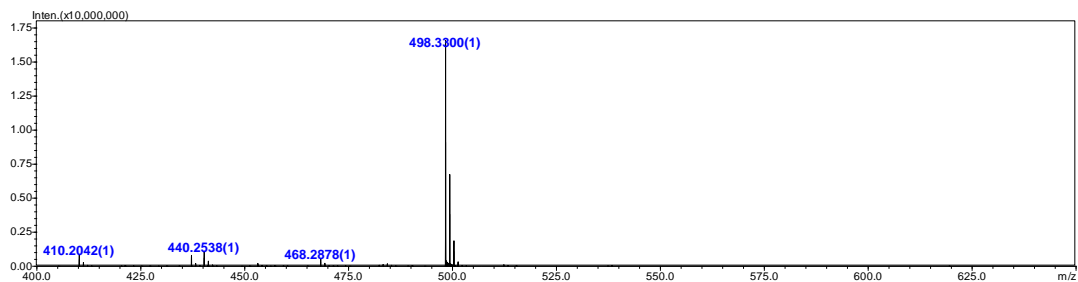


Figure S41. The ESI-MS spectra of Q-Si-ARh.

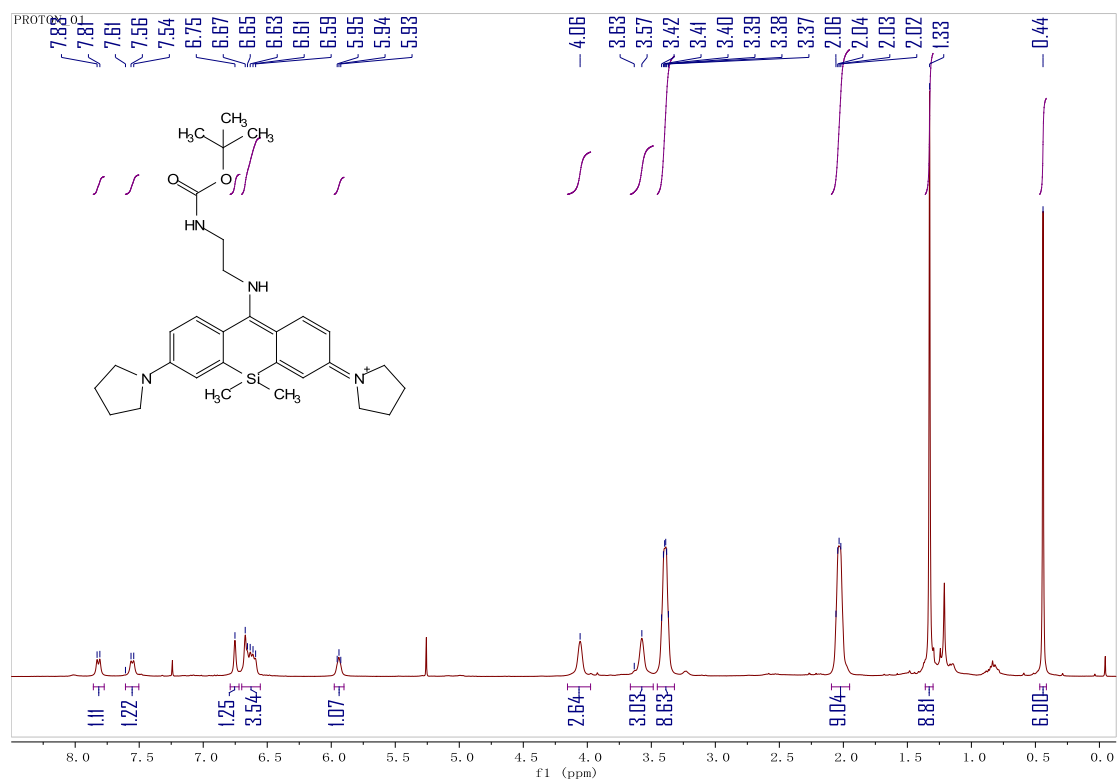


Figure S42. The <sup>1</sup>H NMR spectra of Q-Si-NBoc in CDCl<sub>3</sub>.

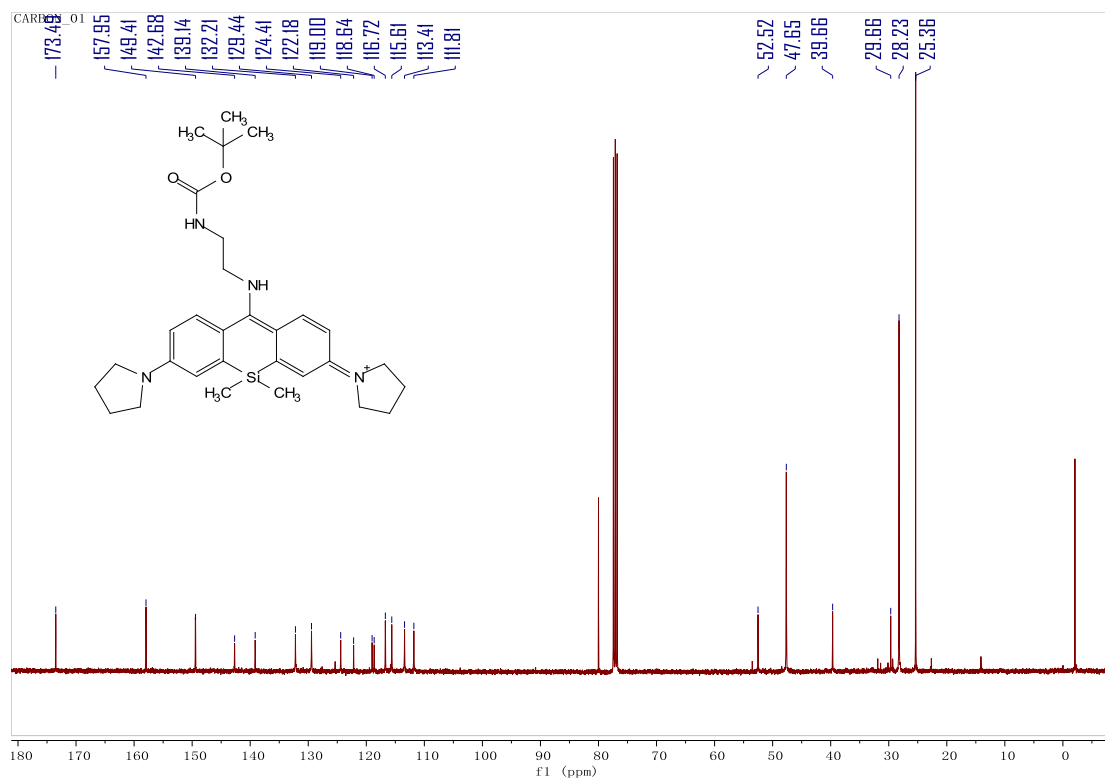


Figure S43. The  $^{13}\text{C}$  NMR spectra of P-Si-NBoc in  $\text{CDCl}_3$ .

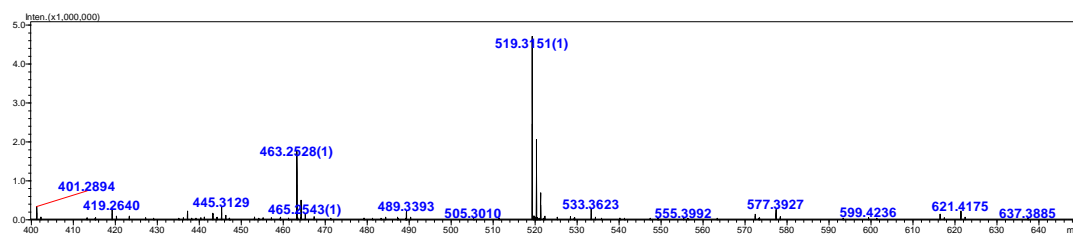


Figure S44. The ESI-MS spectra of P-Si-NBoc.

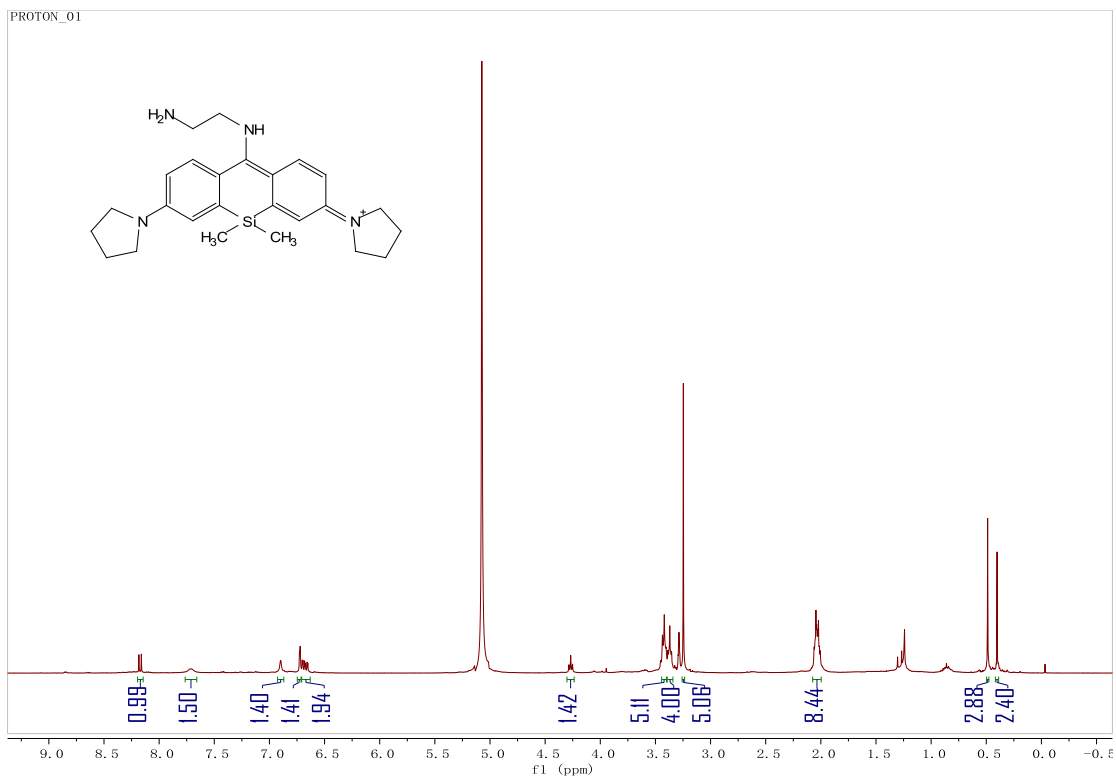


Figure S45. The <sup>1</sup>H NMR spectra of P-Si-NH<sub>2</sub> in CD<sub>3</sub>OD.

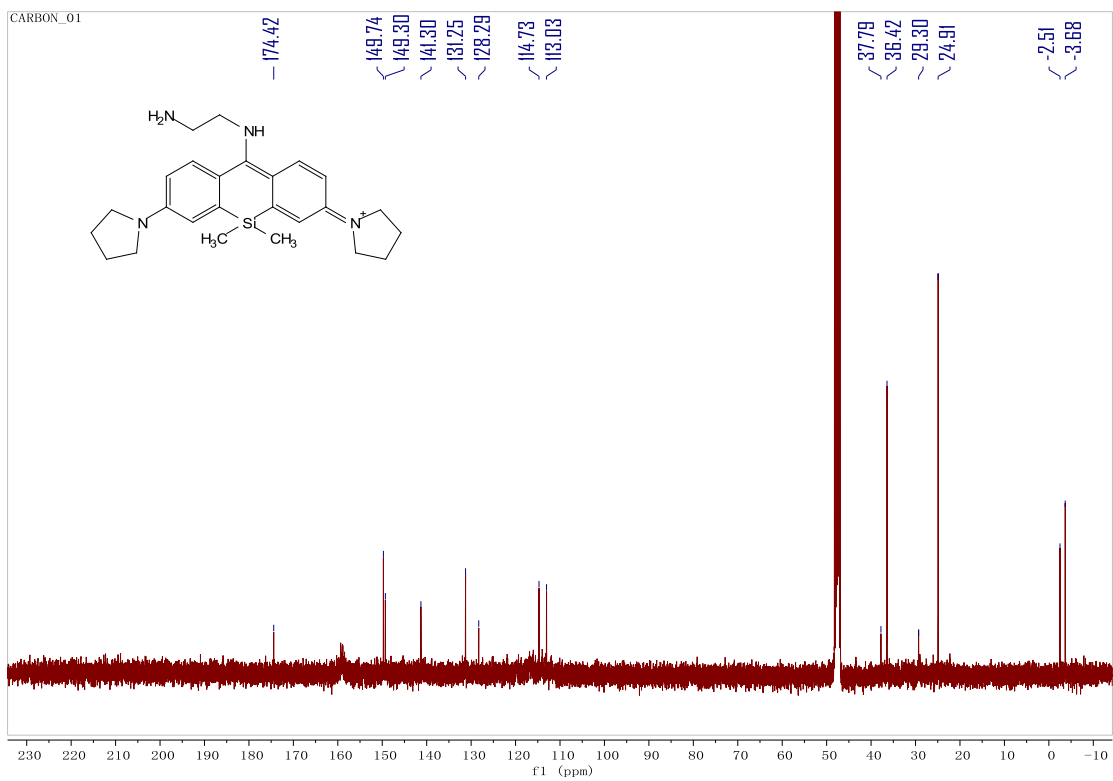


Figure S46. The <sup>13</sup>C NMR spectra of P-Si-NH<sub>2</sub> in CD<sub>3</sub>OD.

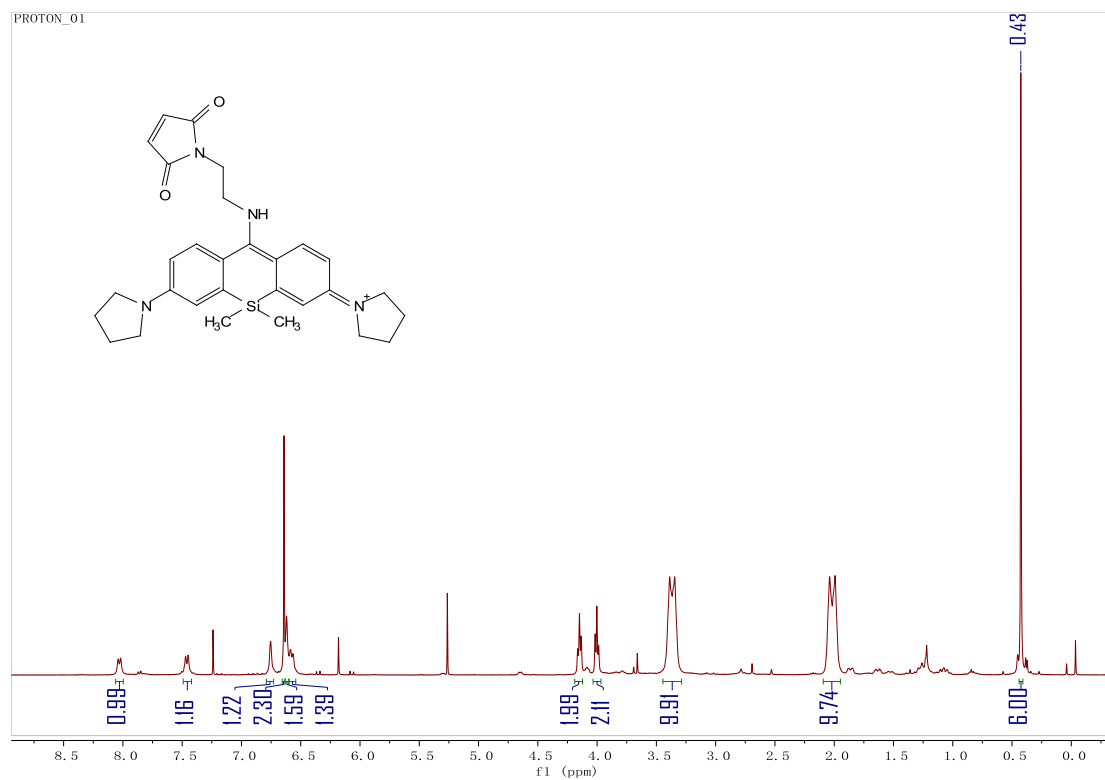


Figure S47. The <sup>1</sup>H NMR spectra of P-Si-MRh in CDCl<sub>3</sub>.

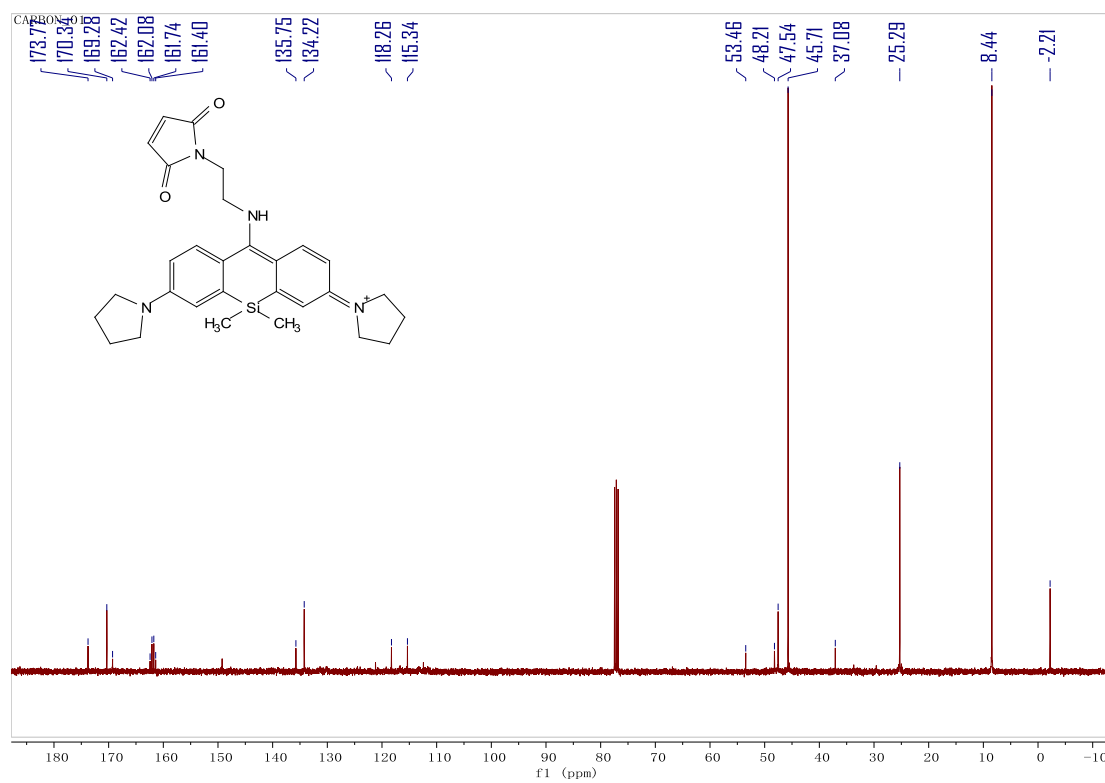
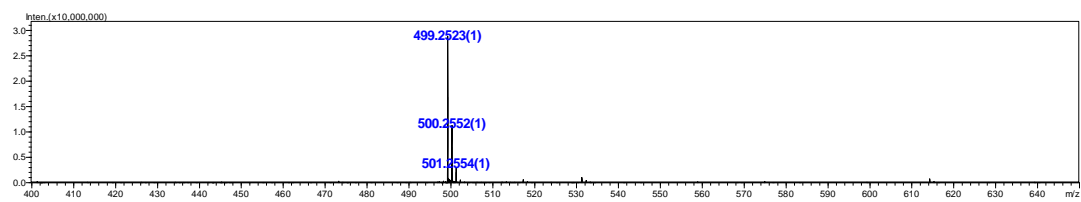


Figure S48. The <sup>13</sup>C NMR spectra of P-Si-MRh in CDCl<sub>3</sub>.



**Figure S49.** The ESI-MS spectra of **P-Si-MRh**.