Supporting Information

Asymmetric Si-rhodamine Scaffold: Rational Design of pH-Durable Protease-Activated NIR Probes *in vivo*

Min Li,[a]† Chuanfeng Wang,[a]† Ting Wang,[b]* Mengting Fan,[a] Ning Wang,[a] Danying Ma,[a] Ting Hu[a] and Xiaoyan Cui [a]*

[a] Department of Chemistry, School of Chemistry and Molecular Engineering, East China Normal University, Shanghai, 200241, P. R. China

[b] College of Pharmacy, Second Military Medical University, Shanghai, 200433, P.R. China

† Both authors contributed equally to the work.

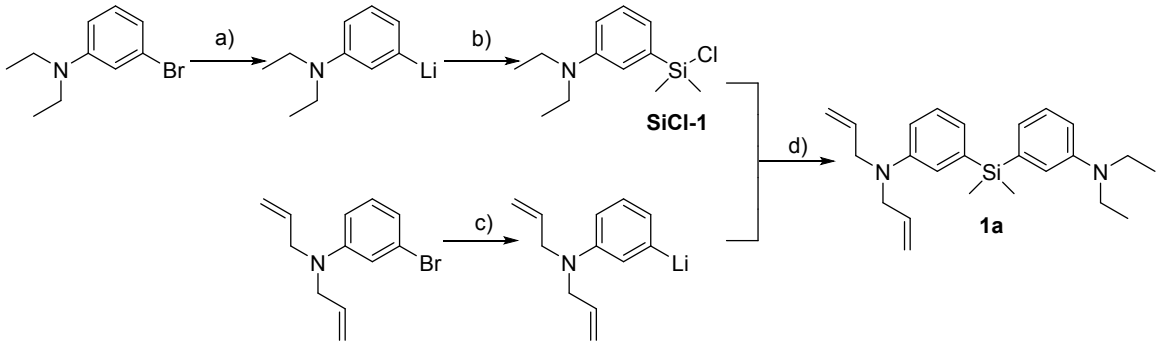
Table of Contents

1. Synthesis

Materials. General chemicals were of the best grade available, supplied by Adamas-beta, Shanghai Chemical Reagent Co., Tokyo Chemical Industries (TCI), J&K chemical LTD., and Acros Organics. Unless otherwise stated, all commercial reagents were used without additional purification. All solvents were freshly distilled according to standard procedures prior to use.

Apparatus. All reactions were monitored by thin-layer chromatography (TLC) on gel F254 plates. Flash chromatography was carried out on silica gel (200-300 mesh; Qingdao Ocean Chemicals). The condensation reactions were performed in sealable pressure tubes (Beijing Synthware Glass) behind a blast shield. NMR spectra were recorded on a Bruker AC-300P spectrometer at 300 MHz for ^{1}H NMR and at 75 MHz for ^{13}C NMR or on a Bruker AC-600P spectrometer at 600 MHz for ^{1}H NMR and at 150 MHz for ^{13}C NMR. δ values are given in ppm relative to tetramethylsilane. Mass spectra (MS) were measured with an API-3000 MS spectrometer using electrospray ionization (ESI). High-resolution mass spectra (HRMS) were recorded on an Aglilent Technologies 6538 UHD Accurate-Mass Q-TOF MS spectrometer using ESI. UV-visible spectra were obtained on an Analytikjena Specord 210 PLUS UV−vis spectrophotometer. Fluorescence spectroscopic studies were performed on a Hitachi F-7000. HPLC analysis was performed on a Diamonsil C18 (4.6 × 250 mm) column (Dikma technologies) using an HPLC system composed of a pump (LC-20AD, Shimadzu) and a detector (SPD-M20A, Shimadzu).

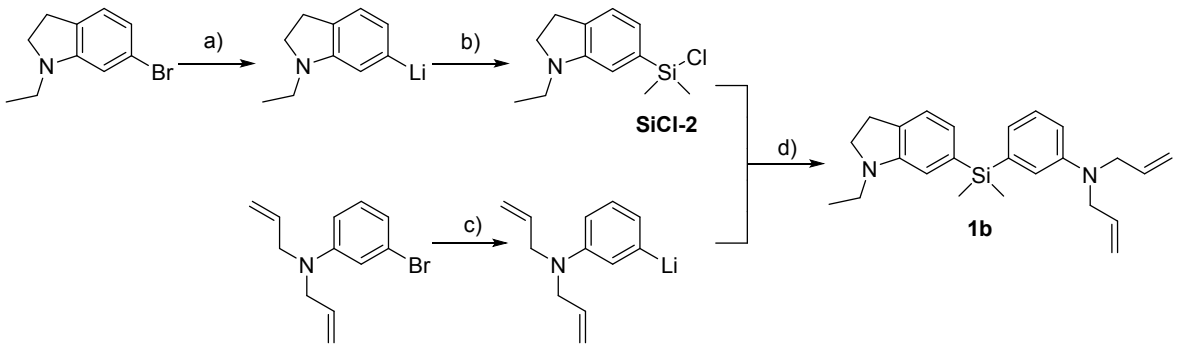
Synthesis



SiCl-1. To a 100 mL well-dried flask flushed with argon, 3-bromo-N,N-diethylaniline (2.74 g, 12.0 mmol) and diethyl ether (10 mL) were added. After the solution was cooled to 0 °C, *n*-BuLi (1.6 M in *n*-hexane, 7.9 mL, 12.0 mmol) was added. After the reaction mixture was stirred at the same temperature for 2 h, the resulting solution was transferred via cannula to a solution of dichlorodimethylsilane (7.3 mL, 60.0 mmol) in diethyl ether (10 mL) at 0 °C. The reaction was slowly warmed to room temperature and stirred for another 2 h. The solvent and the redundant dichlorodimethylsilane were removed under reduced pressure to give the crude **SiC-1**, which was used without further purification.

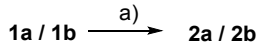
1a. To a 50 mL well-dried flask flushed with argon, 6-bromo-1-ethylindoline (1.36 g, 6.0 mmol) and diethyl ether (10 mL) were added. After the solution was cooled to 0 °C, *n*-BuLi (1.6 M in *n*-hexane, 4.0 mL, 6.4 mmol) was added and the reaction mixture was stirred at 0 °C for 2 h. This resulting mixture was transferred via cannula into the solution of compound **SiCl-1** in diethyl ether (10 mL) at 0 °C. The reaction mixture was slowly warmed to room temperature and stirred overnight. The reaction was quenched with water (30 mL) and extracted with diethyl ether. After filtration and removal of the solvent under reduced pressure, the residue was purified by column chromatography on silica gel to give **1a** (1.12 g, 53% yield) as light yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.25-7.18 (m, 2H), 6,92-6.84 (m, 4H), 6.72 (dd, *J* = 8.2, 2.1 Hz, 1H), 5.91-5.79 (m, 2H), 5.20-5.13 (m, 4H), 3.91 (d, *J* = 4.9 Hz, 2H), 3.34 (q, *J* = 7.0

Hz, 2H), 1.14 (t, J = 7.0 Hz, 3H), 0.53 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 148.0, 147.0, 139.0, 139.0, 134.2, 128.7, 128.4, 122.3, 121.4, 118.2, 117.8, 116.1, 113.3, 112.8, 52.9, 44.4, 12.6, -2.3; MS (ESI) calcd. for $C_{24}H_{34}N_2Si$ $[M+H]^+$: 379.3, found: 379.5.



1b. Compound **1b** was synthesized by following the similar method described above. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.21 (dd, J = 8.1, 7.3 Hz, 1H), 7.10 (d, J = 7.1 Hz, 1H), 6.92 (d, J = 2.6 Hz, 1H), 6.87 (dd, J = 7.0, 3.7 Hz, 2H), 6.73 (dd, J = 8.0, 2.2 Hz, 1H), 6.67 (s, 1H), 5.92-5.80 (m, 2H), 5.21-5.13 (m, 4H), 3.92 (d, J = 5.0 Hz, 4H), 3.34 (t, J = 8.2 Hz, 2H), 3.15 (q, J = 7.2 Hz, 2H), 2.97 (t, J = 8.2 Hz, 2H), 1.20 (t, J = 7.2 Hz, 3H), 0.51 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 151.8, 147.9, 139.1, 136.9, 134.3, 131.7, 128.5, 124.0, 123.9, 122.3, 118.2, 116.1, 113.3, 112.3, 52.9, 52.2, 43.2, 28.6, 12.1, -2.1. MS (ESI) calcd. for $C_{24}H_{32}N_2Si$ $[M+H]^+$: 377.2, found: 377.1.

General procedure for synthesis of 2.



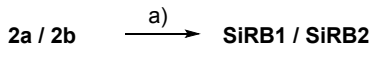
2. To a 15 mL sealable pressure tube charged with a magnetic stir bar were added the intermediate **1** (1.0 mmol, 1.0 equiv.), 2-formylbenzoic acid (5.0 mmol, 5.0 equiv.) and copper(II) bromide (0.1 mmol, 0.1 equiv.). The tube was sealed tightly and heated at 140 °C for 5 h. **[Caution:** To avoid potential danger, all the reaction tubes were placed behind a blast shield.**]** After cooling to room temperature, the reaction mixture was dissolved in 5 mL dichloromethane and purified by column chromatography on silica

gel to give the desired compounds.

2a. Compound **1a** was added and the reaction mixture was stirred for 5 h. The mixture was purified by column chromatography on silica gel to give **2a** (183 mg, 36% yield) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.97 (d, *J* = 7.6 Hz, 1H), 7.67 (t, J = 7.2 Hz, 1H), 7.56 (t, *J* = 7.4 Hz, 1H), 7.37 (d, *J* = 7.6 Hz, 1H), 6.97 (d, *J* = 2.8 Hz, 1H), 6.92 (s, 1H), 6.72 (dd, *J* = 8.8, 3.8 Hz, 2H), 6.50 (dd, *J* = 8.9, 2.9 Hz, 2H), 5.90-5.78 (m, 2H), 5.19-5.14 (m, 4H), 3.93 (d, *J* = 4.7 Hz, 4H), 3.36 (q, *J* = 7.0 Hz, 4H), 1.16 (t, *J* = 7.0 Hz, 6H), 0.61 (s, 3H), 0.60 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.5, 153.9, 147.5, 146.6, 137.4, 137.2, 133.7, 133.4, 131.94, 130.7, 128.7, 128.4, 128.1, 127.5, 125.6, 124.9, 116.8, 116.2, 116.0, 113.0, 112.4, 92.2, 52.77, 44.3, 12.5, 0.4, -1.9. MS (ESI) calcd. for $C_{32}H_{36}N_2O_2Si$ $[M+H]^+$: 509.3, found: 509.4.

2b. Compound **1b** was added and the reaction mixture was stirred for 5 h. The mixture was purified by column chromatography on silica gel to give **2b** (147 mg, 29% yield) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.96 (d, *J* = 7.5 Hz, 1H), 7.61 (t, *J* = 7.3 Hz, 1H), 7.52 (t, *J* = 7.4 Hz, 1H), 7.26 (d, *J* = 7.4 Hz, 2H), 6.94 (d, *J* = 1.8 Hz, 1H), 6.74 (d, *J* = 8.9 Hz, 1H), 6.68 (s, 1H), 6.63 (s, 1H), 6.52 (dd, *J* = 8.8, 2.1 Hz, 1H), 5.90-5.77 (m, 2H), 5.18 (d, *J* = 5.6 Hz, 2H), 5.14 (s, 2H), 3.92 (d, *J* = 4.6 Hz, 4H), 3.31 (t, *J* = 8.2 Hz, 2H), 3.20 (q, *J* = 7.1 Hz, 2H), 2.80 (t, *J* = 7.6 Hz, 2H), 1.20 (t, *J* = 7.2 Hz, 3H), 0.58 (s, 3H), 0.56 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.9, 155.1, 151.3, 147.4, 136.4, 134.9, 133.7, 133.7, 132.3, 128.5, 128.17, 126.7, 125.6, 124.4, 123.6, 116.6, 116.5, 116.3, 113.4, 106.0, 92.1, 52.8, 51.7, 42.7, 28.4, 12.0, 0.2, -1.2. MS (ESI) calcd. for $C_{32}H_{34}N_2O_4Si$ $[M+H]^+$: 507.3, found: 507.4.

General procedure for synthesis of SiRB.



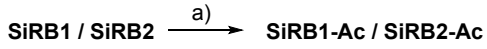
To a dried flask flushed with argon, $Pd(PPh_3)_4$ (35.0 mg, 0.03 mmol) and 1,3-dimethylbarbituric acid (160 mg, 1.0 mmol) were added. Compound **SiRB** (0.25 mmol)

dissolved in CH_2Cl_2 (10 mL) was further added and the solution was stirred at 35 °C for 16 h. The organic layer was washed with Na_2CO_3 solution and brine, and dried over Na_2SO_4. After filtration and removal of the solvent under reduced pressure, the residue was purified by column chromatography on silica gel.

SiRB1. Compound **2a** was added. The mixture was purified by column chromatography on silica gel to give **SiRB1** (86mg, 80% yield) as a light blue solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.97 (d, *J* = 7.6 Hz, 1H), 7.66 (t, *J* = 7.4 Hz, 1H), 7.55 (t, *J* = 7.4 Hz, 1H), 7.33 (d, *J* = 7.6 Hz, 1H), 6.97 (d, *J* = 2.5 Hz, 1H), 6.92 (d, *J* = 2.2 Hz, 1H), 6.77-6.69 (m, 2H), 6.51-6.47 (m, 2H), 3.74 (s, 2H), 3.36 (q, *J* = 7.1 Hz, 4H), 1.16 (t, *J* = 7.0 Hz, 7H), 0.62 (s, 3H), 0.59 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.6, 154.1, 146.6, 145.6, 137.7, 136.9, 134.5, 133.6, 130.5, 128.7, 128.4, 128.3, 127.1, 125.7, 124.7, 119.7, 115.9, 115.9, 112.5, 91.8, 44.2, 12.5, 0.3, -1.7. HRMS (ESI) calcd. for $C_{26}H_{28}N_2O_2Si$ $[M+H]^+$: 429.1993, found: 429.1986.

SiRB2. Compound **2b** was added. The mixture was purified by column chromatography on silica gel to give **SiRB2** (83 mg, 78% yield) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.96 (d, J = 7.5 Hz, 1H), 7.60 (t, *J* = 7.4 Hz, 1H), 7.51 (t, *J* = 7.4 Hz, 1H), 7.23 (d, *J* = 7.6 Hz, 1H), 6.94 (d, *J* = 2.2 Hz, 1H), 6.73 (d, *J* = 8.6 Hz, 1H), 6.66 (s, 2H), 6.51 (dd, *J* = 8.6, 2.2 Hz, 1H), 3.75 (s, 2H), 3.31 (t, *J* = 8.3 Hz, 2H), 3.20 (q, *J* = 7.2 Hz, 2H), 2.80 (t, *J* = 9.6 Hz, 2H), 1.20 (t, *J* = 7.2 Hz, 3H), 0.59 (s, 3H), 0.56 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.0, 155.3, 151.5, 145.6, 136.9, 134.3, 133.9, 133.0, 132.5, 128.6, 128.3, 126.2, 125.6, 124.2, 123.5, 119.4, 116.3, 110.2, 91.7, 51.7, 42.7, 28.4, 12.0, 0.1, -1.0. HRMS (ESI) calcd. for $C_{26}H_{26}N_2O_4Si$ $[M+H]^+$: 427.1836, found: 427.1822.

General procedure for synthesis of SiRB-Ac.

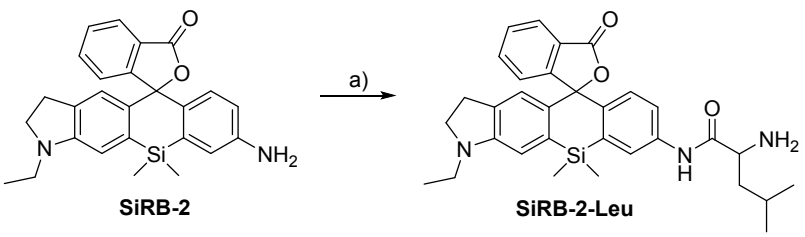


To a solution of compound **SiRB** (0.1 mmol) in 5 mL of CH_2Cl_2 was added 100.0 μL

of pyridine. Then 27 μL of acetic anhydride (30 mg, 0.3 mmol) was slowly added. The reaction mixture was stirred overnight at room temperature. After removal of the solvent under reduced pressure, the residue was purified by column chromatography on silica gel.

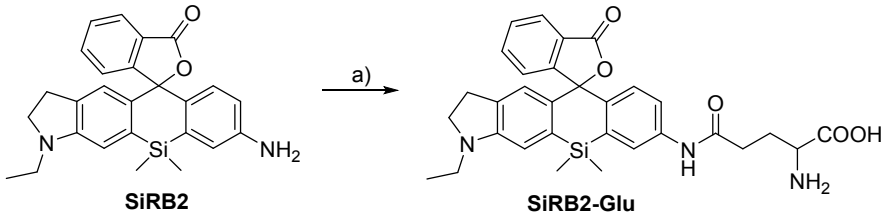
SiRB1-Ac. Compound **SiRB1** was added. The mixture was purified by column chromatography on silica gel to give **SiRB1-Ac** (35 mg, 74%) as light white solid. NMR (300 MHz, $CDCl_3$) δ 7.97 (d, *J* = 7.5 Hz, 1H), 7.90-7.87 (m, 1H), 7.66 (t, *J* = 7.4 Hz, 1H), 7.56 (t, *J* = 7.4 Hz, 1H), 7.30 (d, *J* = 7.5 Hz, 2H), 6.90 (d, *J* = 2.5 Hz, 1H), 6.83-6.79 (m, 2H), 6.51 (dd, *J* = 8.9, 2.6 Hz, 1H), 3.36 (q, *J* = 7.0 Hz, 4H), 2.17 (s, 3H), 1.15 (t, *J* = 7.0 Hz, 6H), 0.63 (s, 3H), 0.54 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ. 171.0, 168.7, 154.2, 146.7, 140.0, 137.6, 137.3, 136.5, 134.0, 129.7, 129.0, 128.5, 127.5, 126.4, 125.8, 124.6, 124.5, 120.7, 115.8, 112.7, 91.4, 44.3, 24.6, 12.5, 0.1, -1.5. HRMS (ESI) calcd. for $C_{28}H_{28}N_2O_4Si$ $[M+H]^+$: 469.1942, found: 469.1921.

SiRB2-Ac. Compound **SiRB2** was added. The mixture was purified by column chromatography on silica gel to give **SiRB2-Ac** (36 mg, 78%) as light white solid. ^1H NMR (300 MHz, $CDCl_3$) δ 7.99-7.96 (m, 1H), 7.91 (d, *J* = 2.4 Hz, 1H), 7.86 (s, 1H), 7.64-7.51 (m, 2H), 7.31-7.25 (m, 1H), 7.21 (d, *J* = 7.6 Hz, 1H), 6.86 (d, *J* = 8.7 Hz, 1H), 6.70 (s, 1H), 6.66 (s, 1H), 3.34 (t, *J* = 8.3 Hz, 2H), 3.22 (q, *J* = 7.2 Hz, 2H), 2.85-2.80 (m, 2H), 2.18 (s, 3H), 1.21 (t, *J* = 7.2 Hz, 3H), 0.62 (s, 3H), 0.52 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.3, 168.7, 155.3, 151.5, 139.9, 137.5, 136.6, 134.3, 134.0, 132.7, 132.21, 128.8, 127.5, 125.7, 125.6, 124.4, 124.08, 123.4, 120.9, 110.2, 91.3, 51.6, 42.6, 28.34, 24.5, 12.0, -0.1, -0.9. HRMS (ESI) calcd. for $C_{28}H_{28}N_2O_4Si$ $[M+H]^+$: 469.1942, found: 469.1921.



SiRB-2 **SiRB-2-Leu**

SiRB2-Leu. Compound **SiRB2** (43 mg, 0.1 mmol), HATU (152 mg, 0.4 mmol) and DIEA (66 μL, 0.4 mmol) were dissolved in DMF (4 mL) and the mixture was stirred at 0 °C under an Ar atmosphere for 10 min. Then, Boc-Leu-OH (92 mg, 0.4 mmol) in DMF (0.5 mL) was slowly added. The reaction mixture was stirred at room temperature for 8h. After evaporation of the solvent, the residue was dissolved in CH_2Cl_2 and TFA (2 mL/2 mL). The reaction mixture was stirred at room temperature for 2h. After removal of the solvent under reduced pressure, the residue was purified by column chromatography on silica gel to give **SiRB2-Leu** (38 mg, 70%) as light white solid. ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.57 (d, *J* = 5.7 Hz, 1H), 7.96-7.94 (m, 2H), 7.59-7.38 (m, 3H), 7.19 (dd, *J* = 7.7, 3.5 Hz, 1H), 6.94 (dd, *J* = 8.7, 5.8 Hz, 1H), 6.70 (d, *J* = 5.1 Hz, 1H), 6.64 (d, *J* = 1.9 Hz, 1H), 3.53-3.51 (m, 1H), 3.31 (t, *J* = 8.3 Hz, 2H), 3.19 (q, *J* = 7.2 Hz, 2H), 2.84-2.76 (m, 2H), 1.83-1.79 (m, 2H), 1.44-1.40 (m, 1H), 1.18 (t, *J* = 7.2 Hz, 3H), 0.97 (d, *J* = 6.1 Hz, 3H), 0.94 (d, *J* = 6.3 Hz, 3H), 0.62 (s, 3H), 0.57 (3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 173.6, 171.1, 155.4, 155.27, 151.6, 140.1, 137.2, 136.7, 136.6, 134.1, 134.1, 134.0, 132.7, 132.7, 132.4, 128.7, 127.6, 127.6, 125.8, 124.0, 123.9, 123.9, 123.4, 123.4, 120.5, 120.5, 110.1, 91.1, 53.9, 51.7, 43.8, 42.6, 28.4, 25.0, 23.4, 21.3, 12.0, 0.0, -0.8. . HRMS (ESI) calcd. for $C_{32}H_{37}N_3O_3Si$ $[M+H]^+$: 540.2677, found: 540.2673.

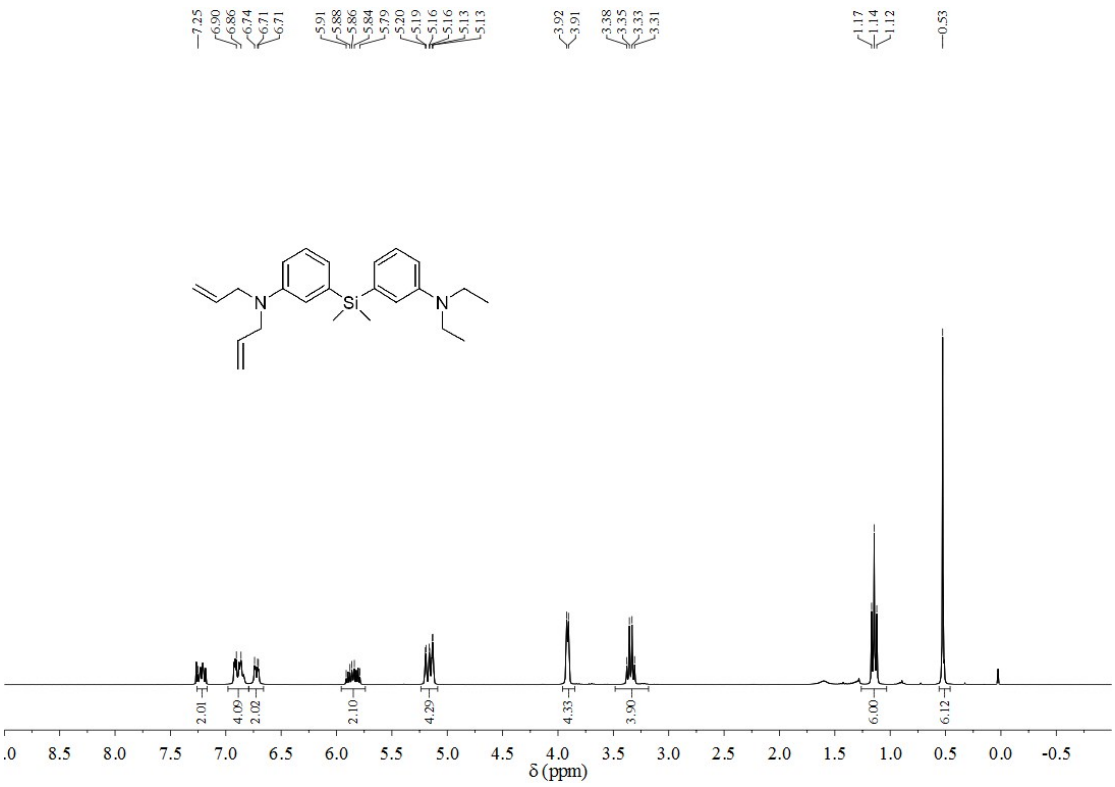


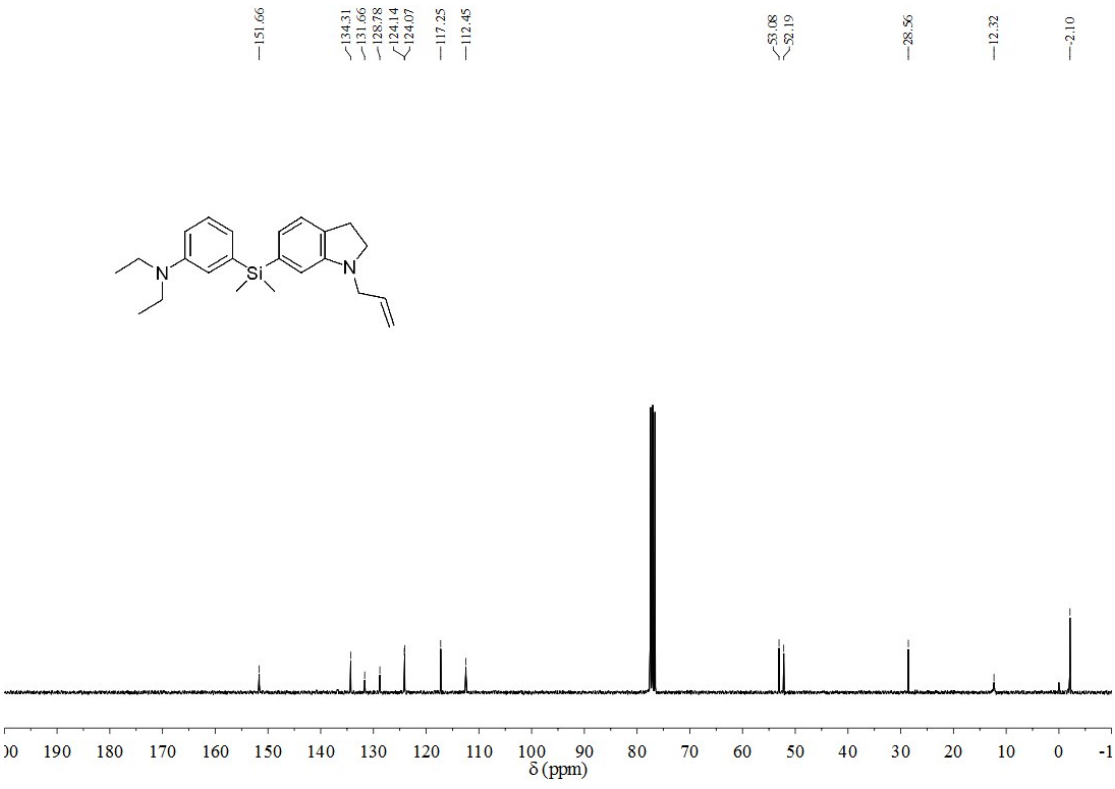
SiRB2 **SiRB2-Glu**

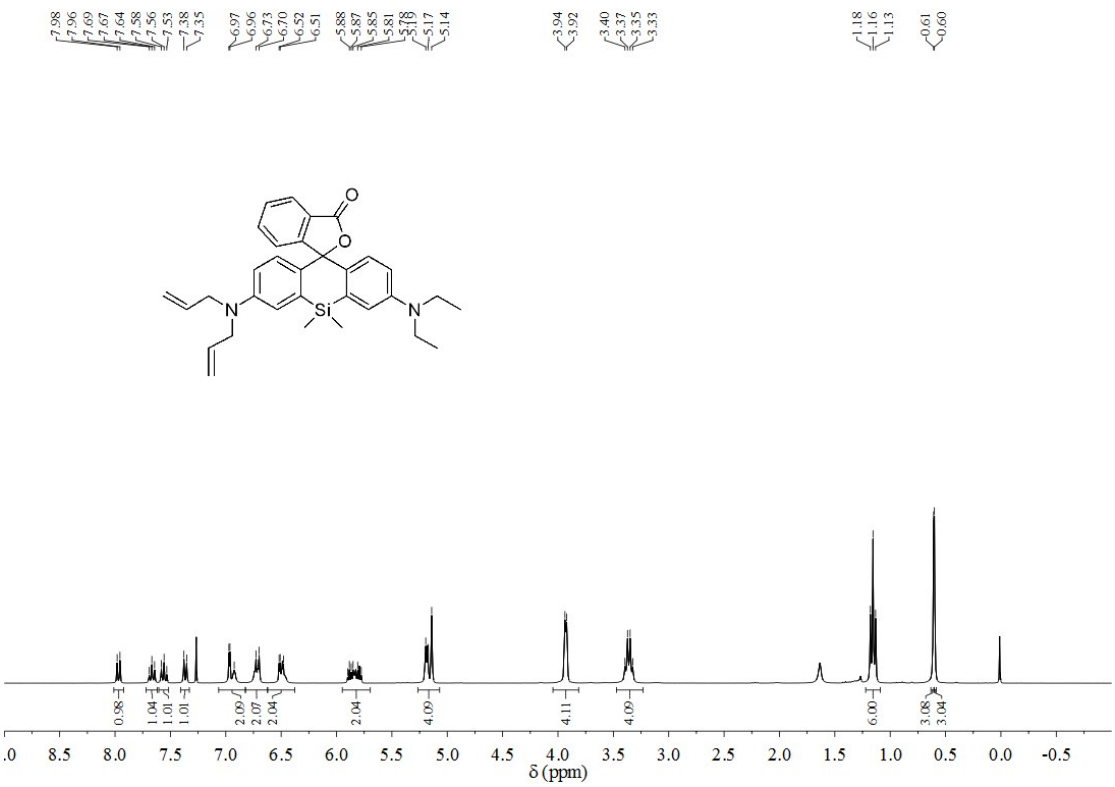
SiRB2-Glu. Compound **SiRB2** (43 mg, 0.1 mmol), HATU (152 mg, 0.4 mmol) and DIEA (66 μL, 0.4 mmol) were dissolved in DMF (4 mL) and the mixture was stirred at 0 °C under an Ar atmosphere for 10 min. Then, Boc-Glu-OtBu (121 mg, 0.4 mmol) in DMF (0.5 mL) was slowly added. The reaction mixture was stirred at room temperature for 8h. After evaporation of the solvent, the residue was dissolved in CH_2Cl_2 and TFA

(2 mL/2 mL). The reaction mixture was stirred at room temperature for 2h. After removal of the solvent under reduced pressure, the residue was purified by column chromatography on silica gel to give **SiRB2-Glu** (37 mg, 67%) as light white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.03-8.01 (m, 2H), 7.77 (t, *J* = 7.3 Hz, 1H), 7.67 (t, *J* = 7.3 Hz, 1H), 7.52 (d, *J* = 8.6 Hz, 1H), 7.28 (d, *J* = 7.5 Hz, 1H), 7.01 (s, 1H), 6.92 (d, *J* = 8.7 Hz, 1H), 6.73 (s, 1H), 4.11 (t, *J* = 6.0 Hz, 1H), 3.47 (t, *J* = 6.0 Hz, 2H), 2.88 (t, *J* = 9.0 Hz, 2H), 2.73 (t, *J* = 6.7 Hz, 2H), 2.49-2.10 (m, 2H), 1.26 (t, *J* = 7.0 Hz, 3H), 0.67 (s, 1H), 0,60 (s, 1H); ^{13}C NMR (75 MHz, MeOD) δ 171.3, 170.1, 139.4, 138.3, 136.8, 134.3, 133.8, 129.1, 127.7, 125.7, 124.3, 120.7, 112.7, 52.0, 51.7, 43.4, 31.8, 27.5, 25.5, 10.5, -1.4, -2.6. HRMS (ESI) calcd. for $C_{31}H_{34}N_3O_5Si$ $[M+H]^+$: 556.2262, found: 556.2242.

2. ^{1}H NMR and ^{13}C NMR Spectra.



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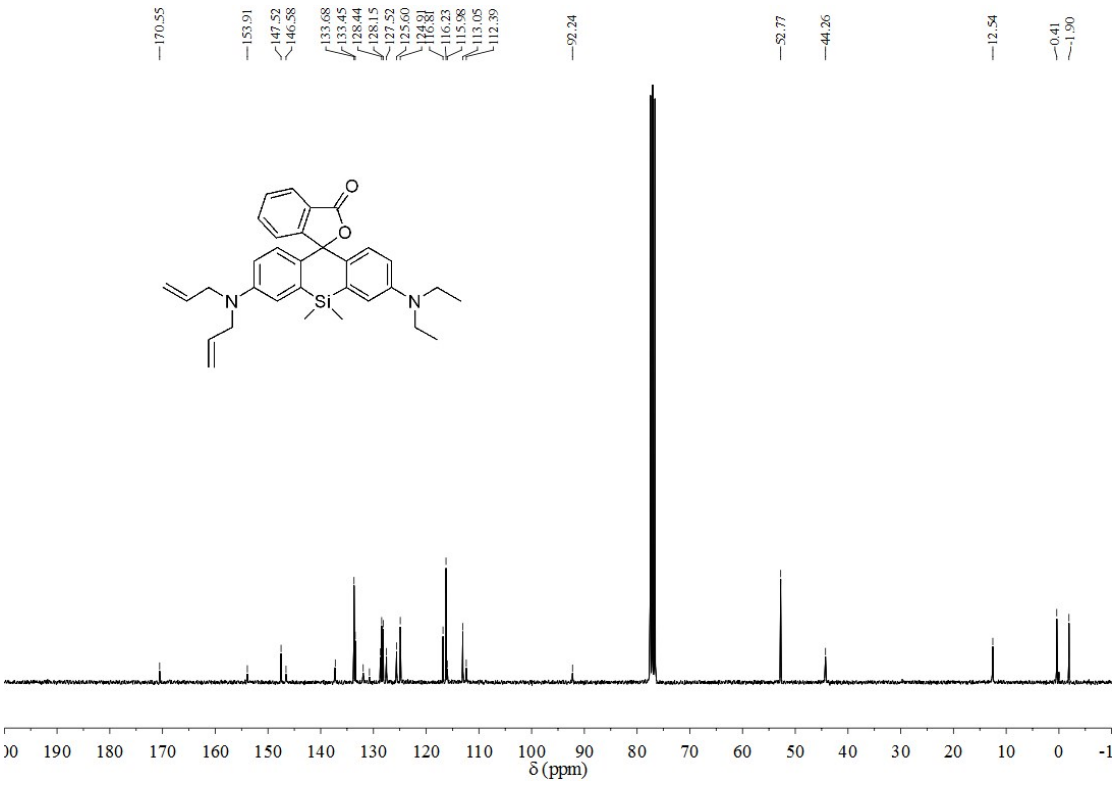
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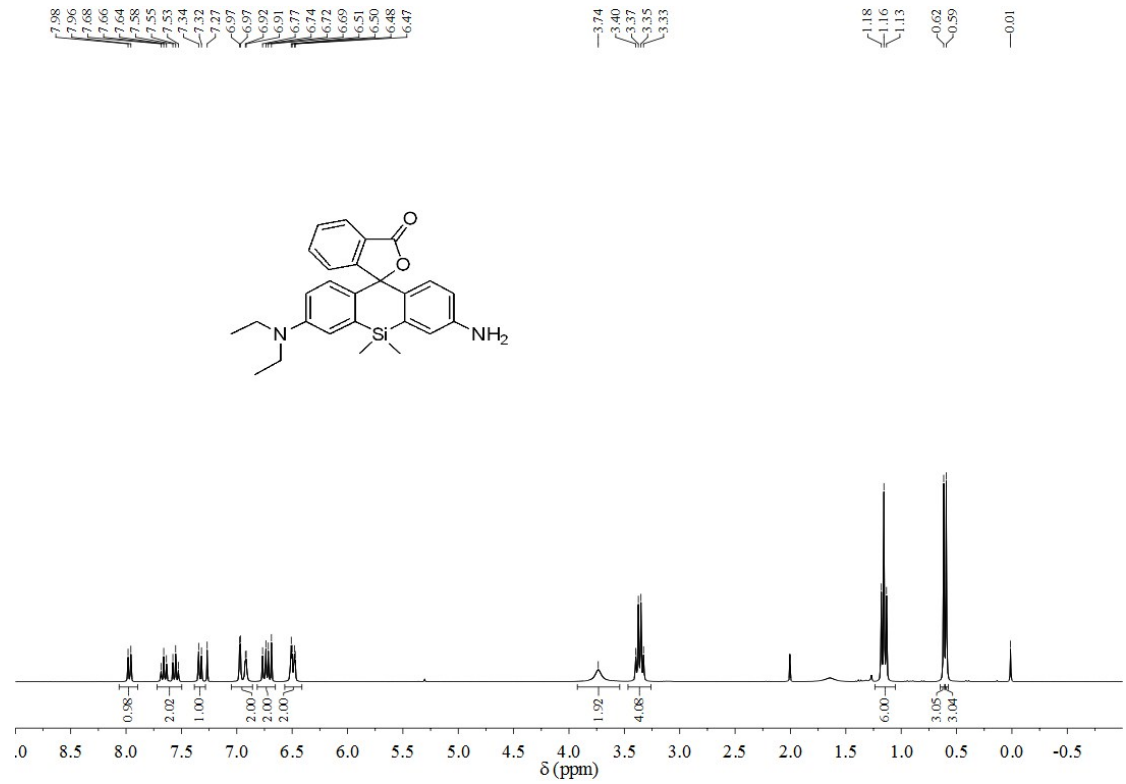
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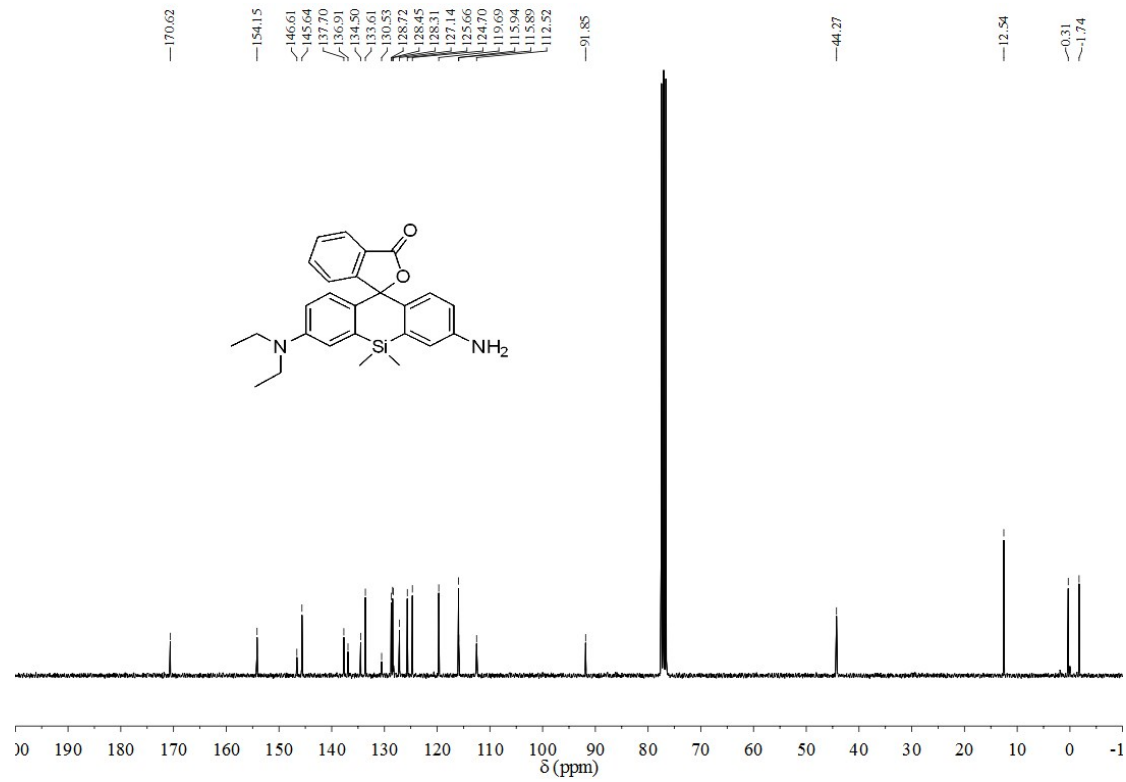
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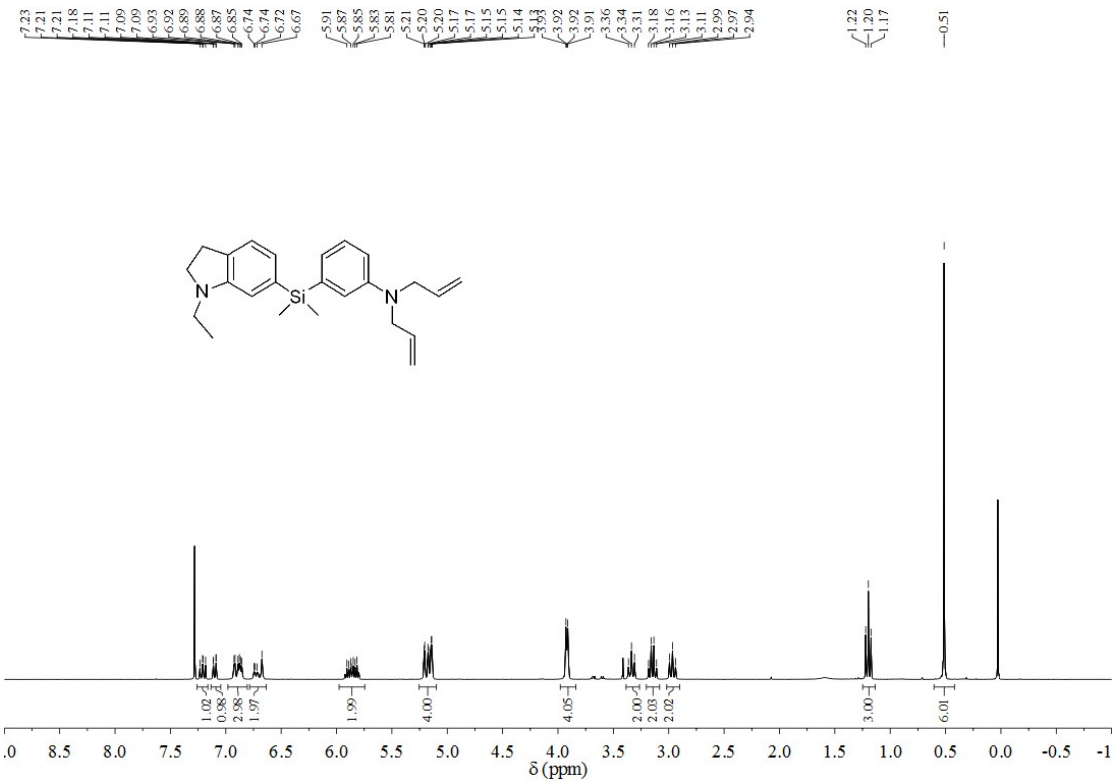
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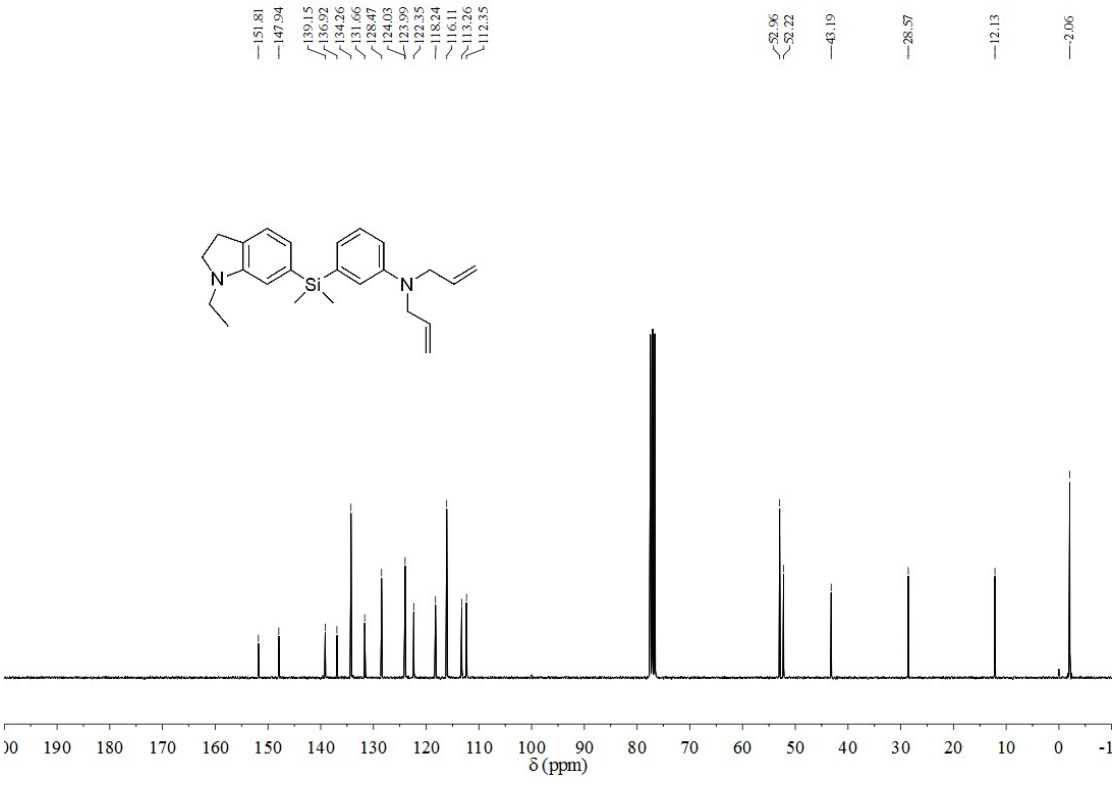
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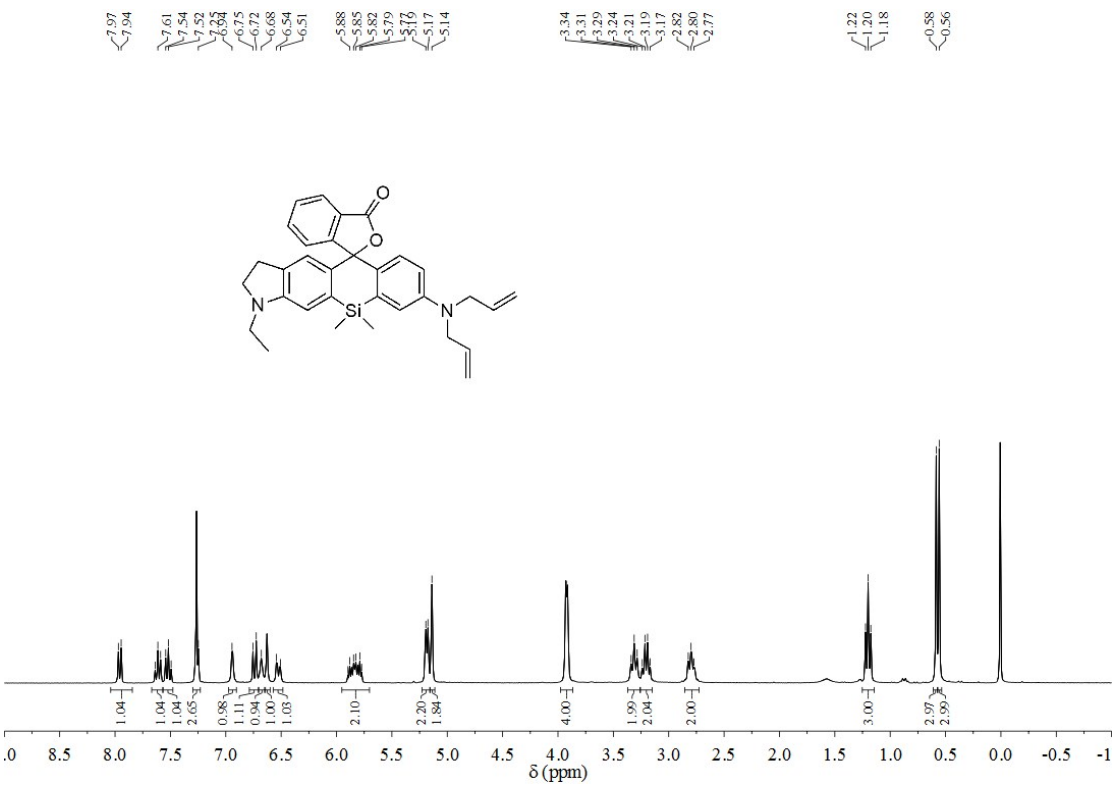
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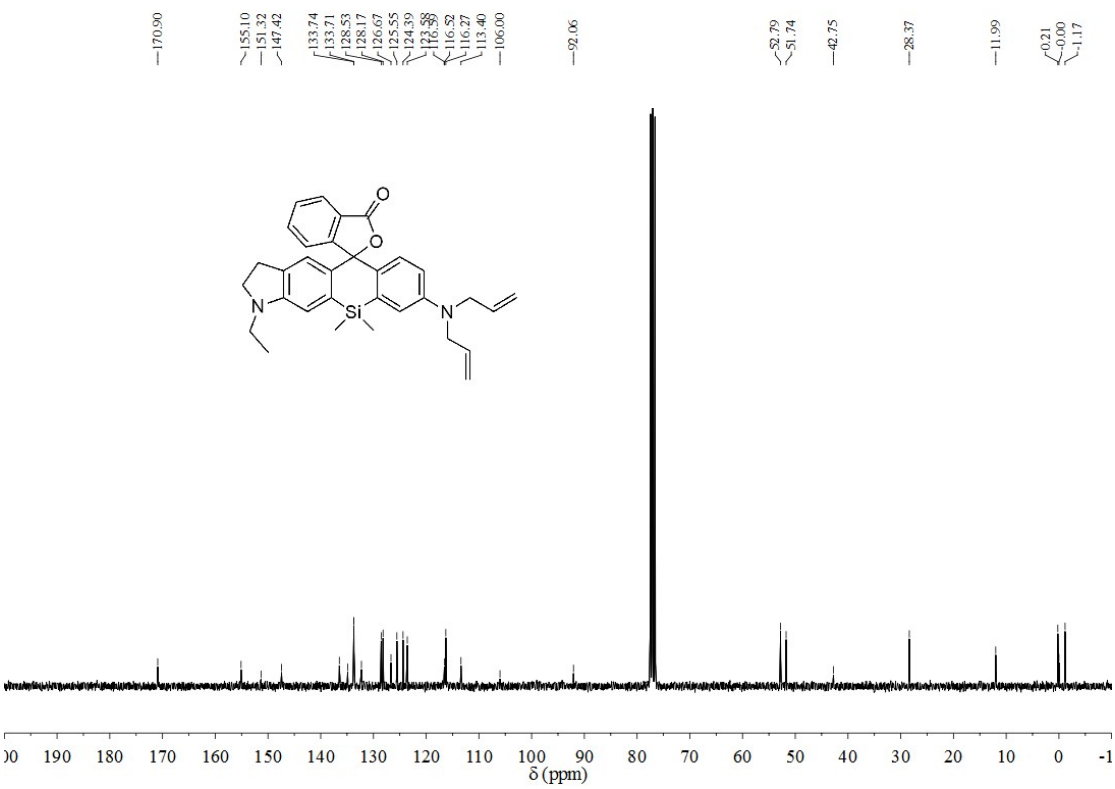
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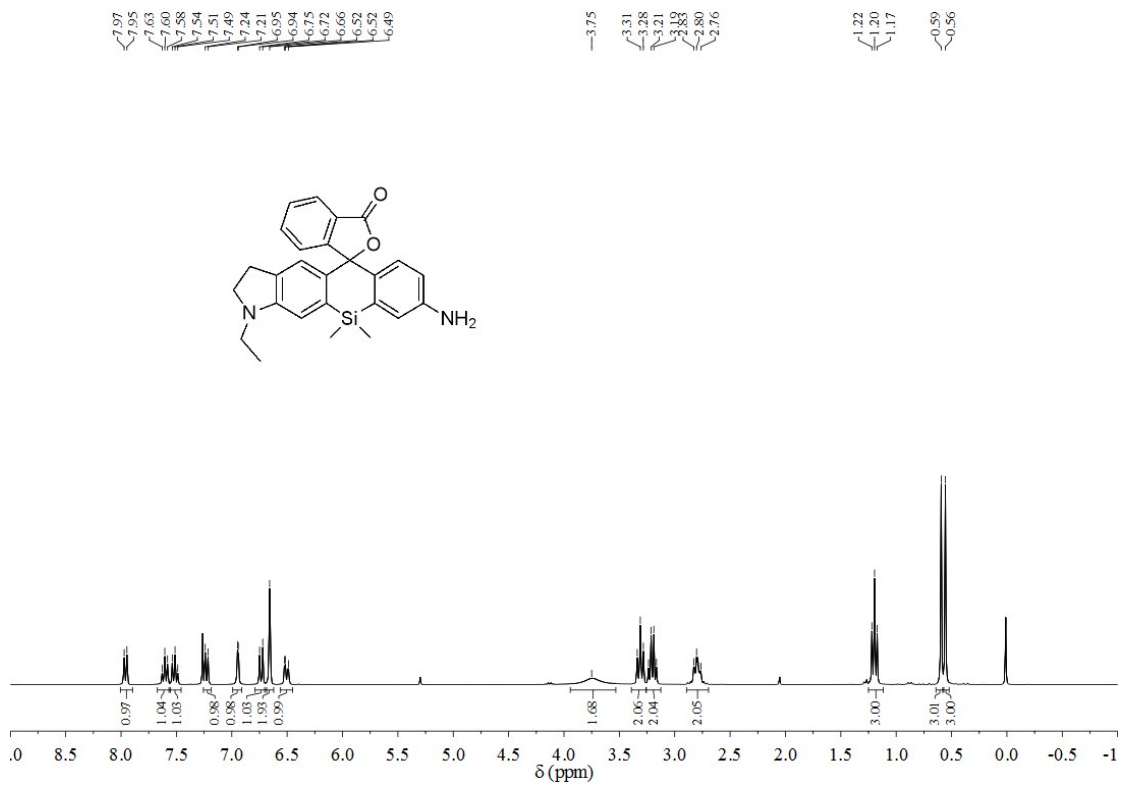
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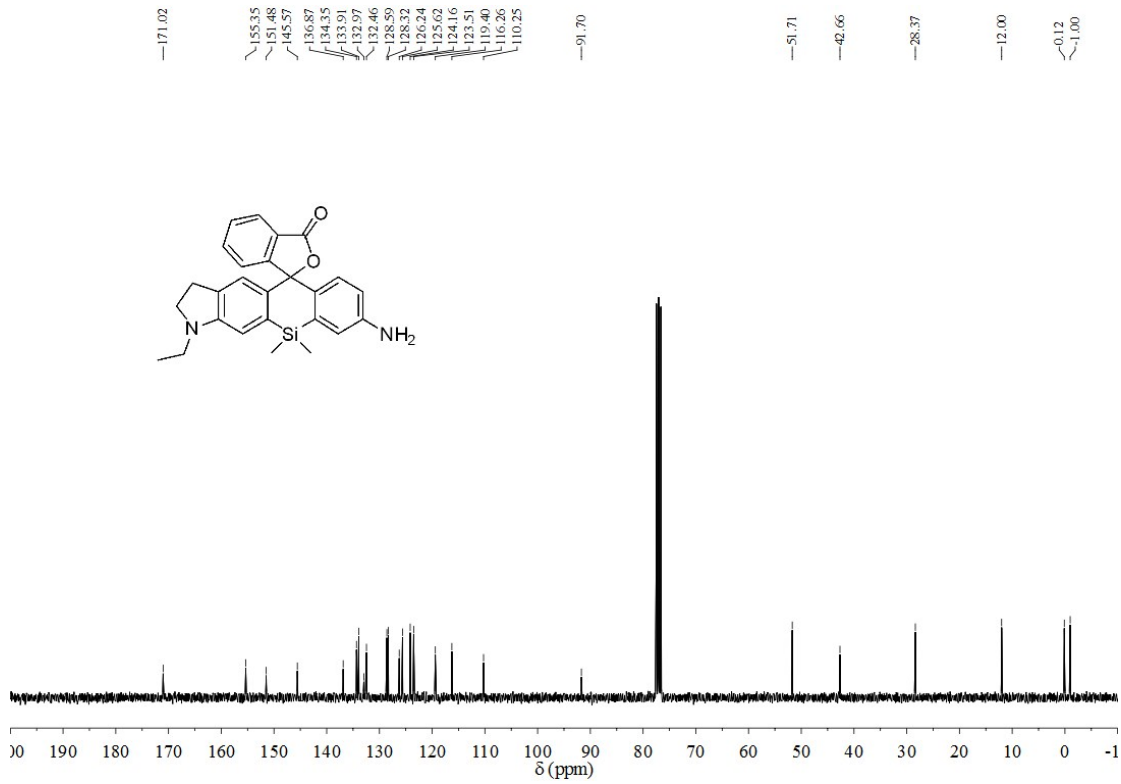
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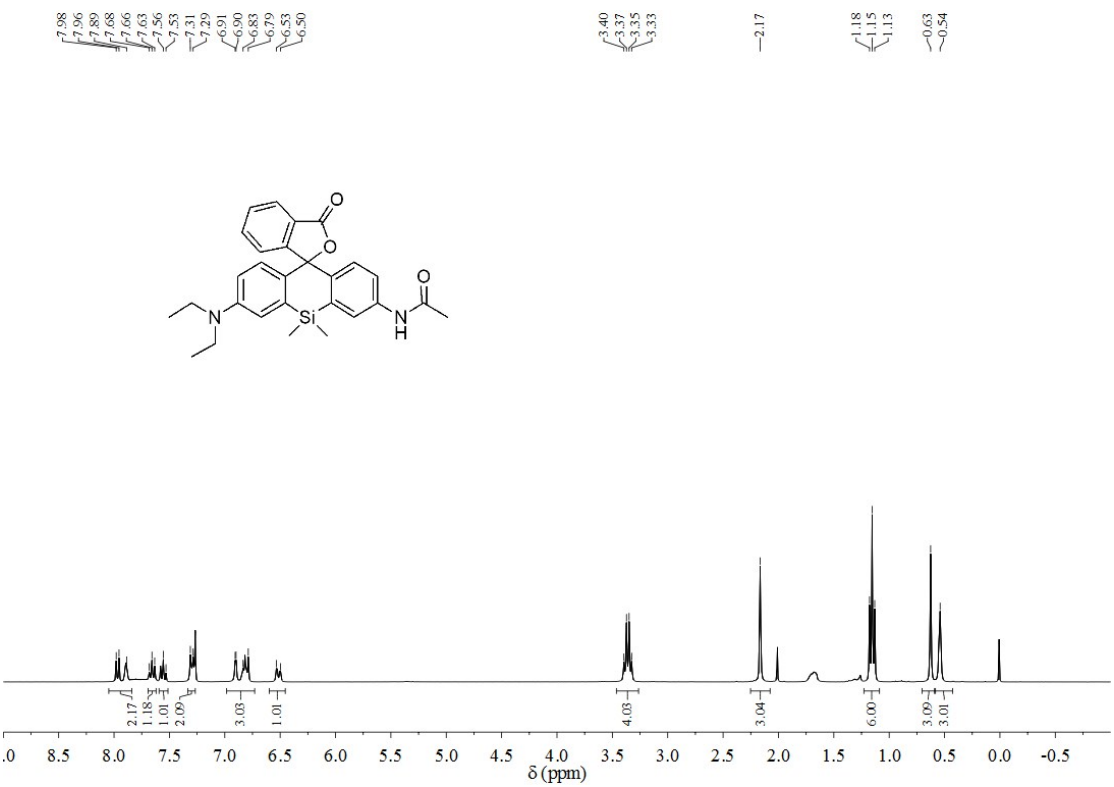
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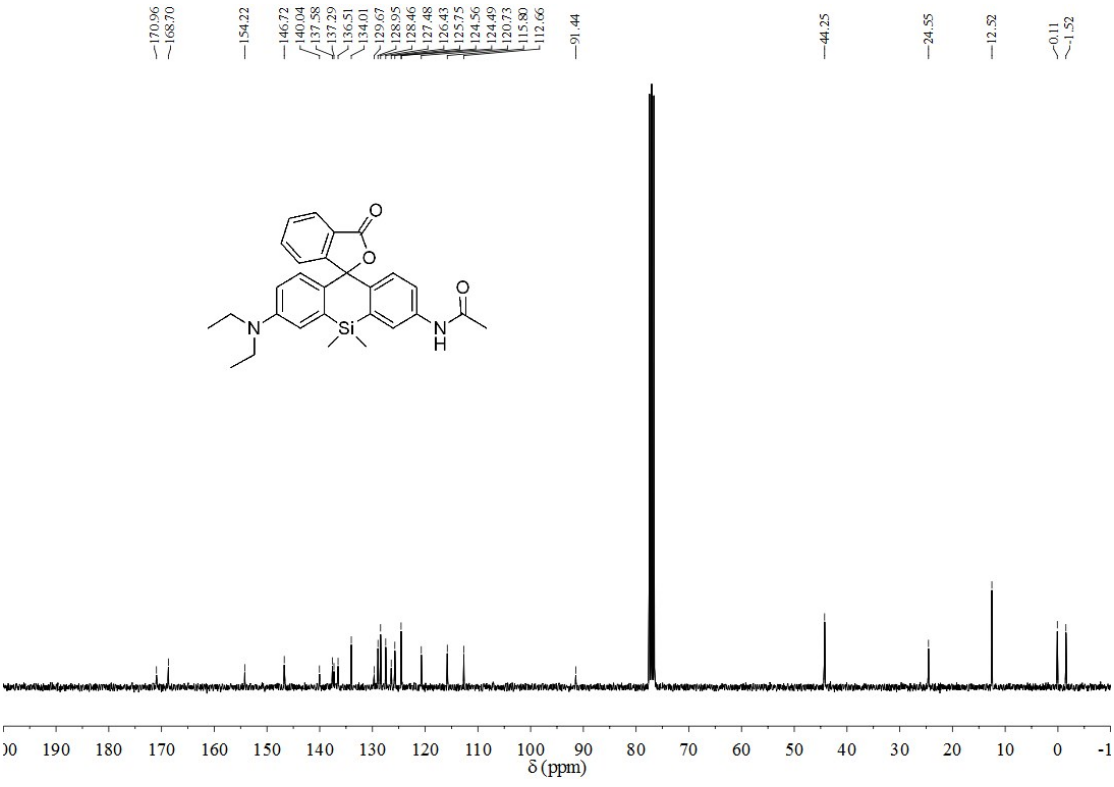
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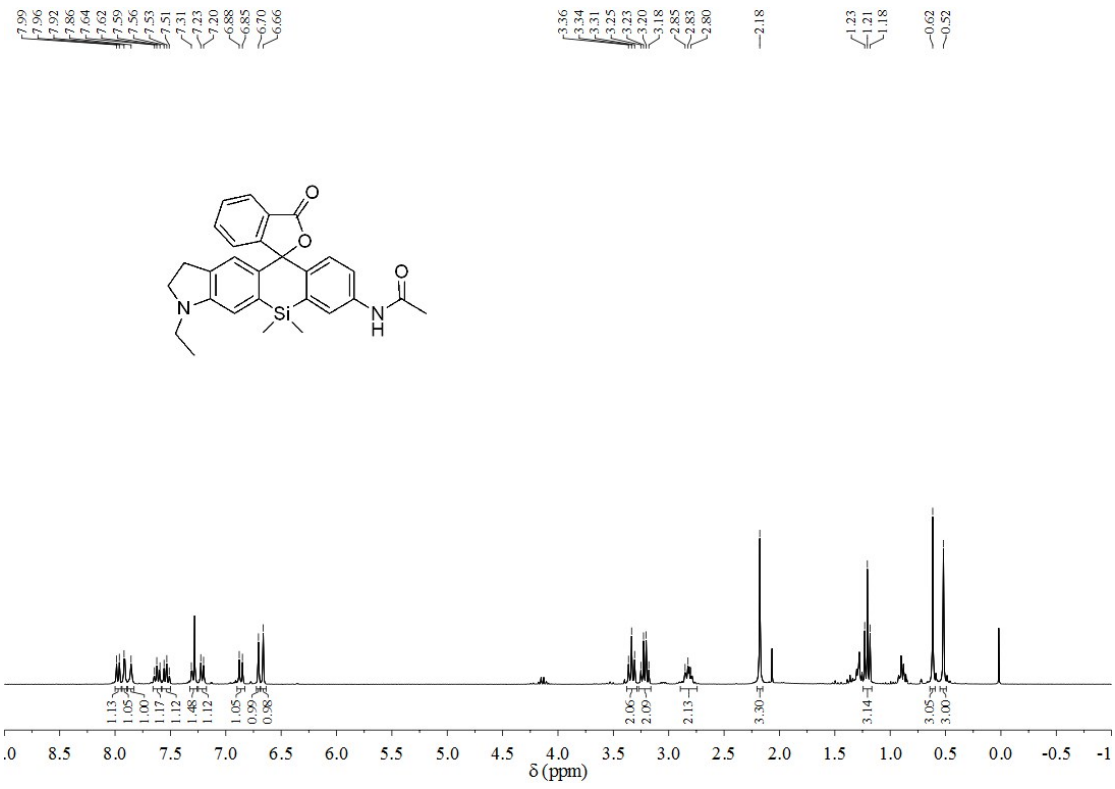
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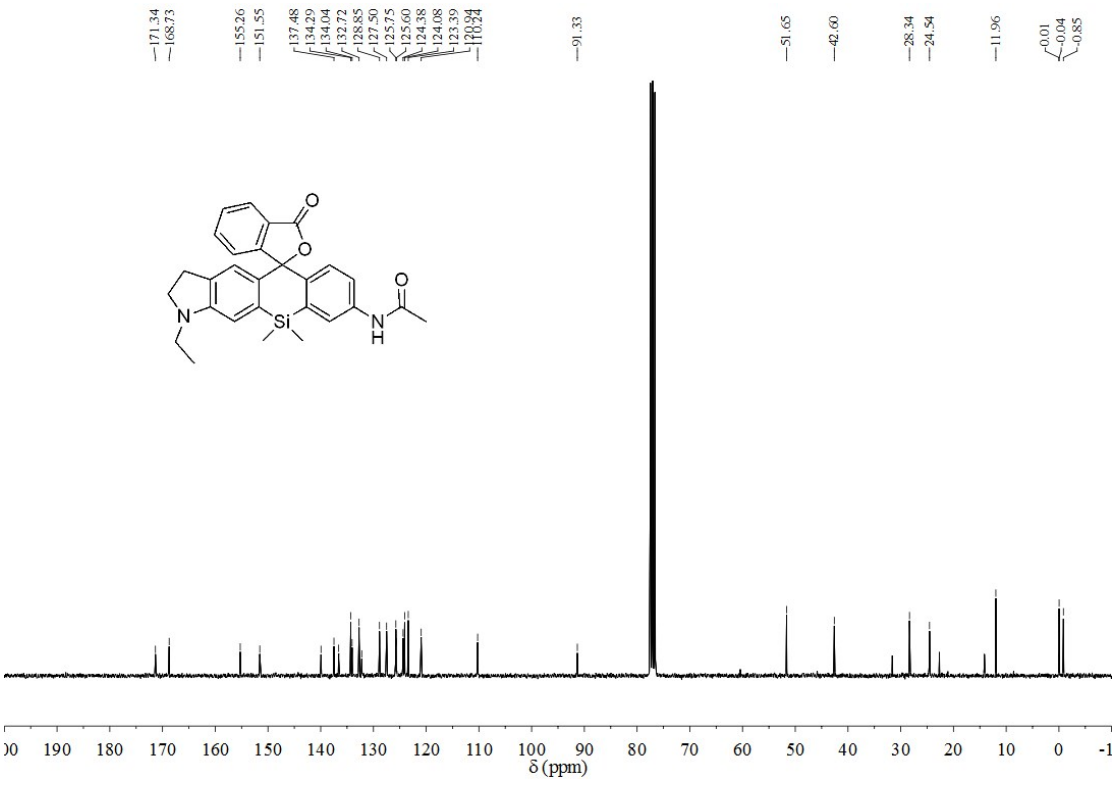
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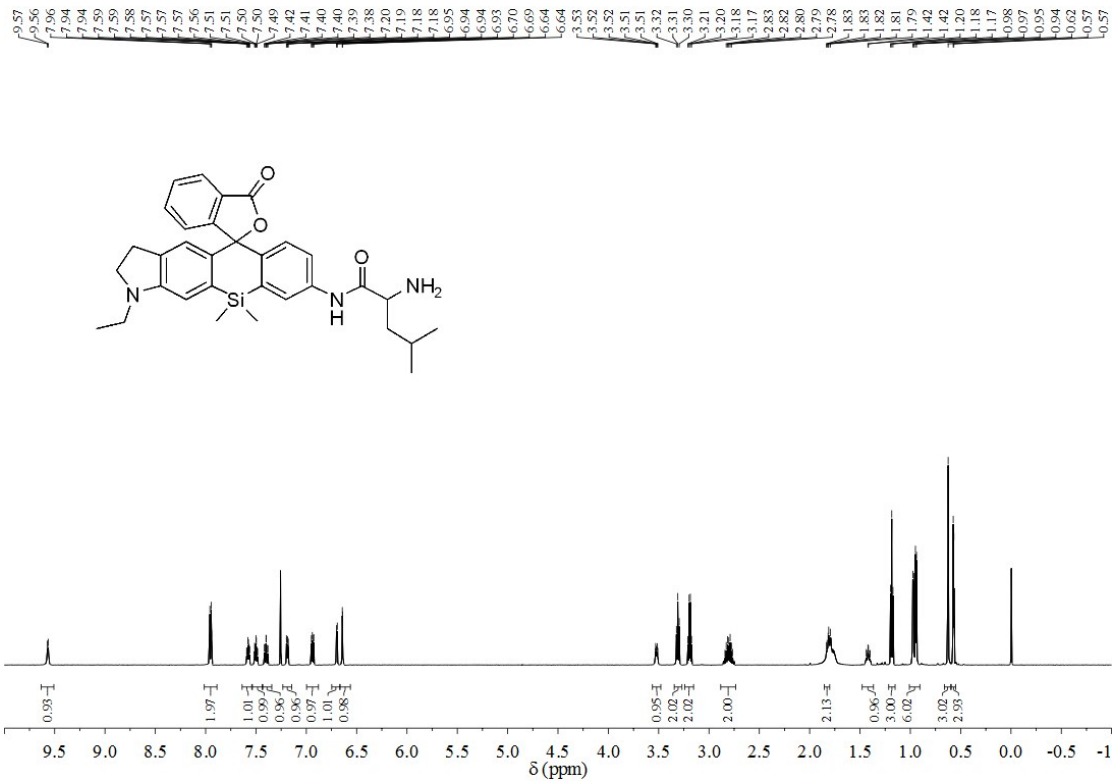
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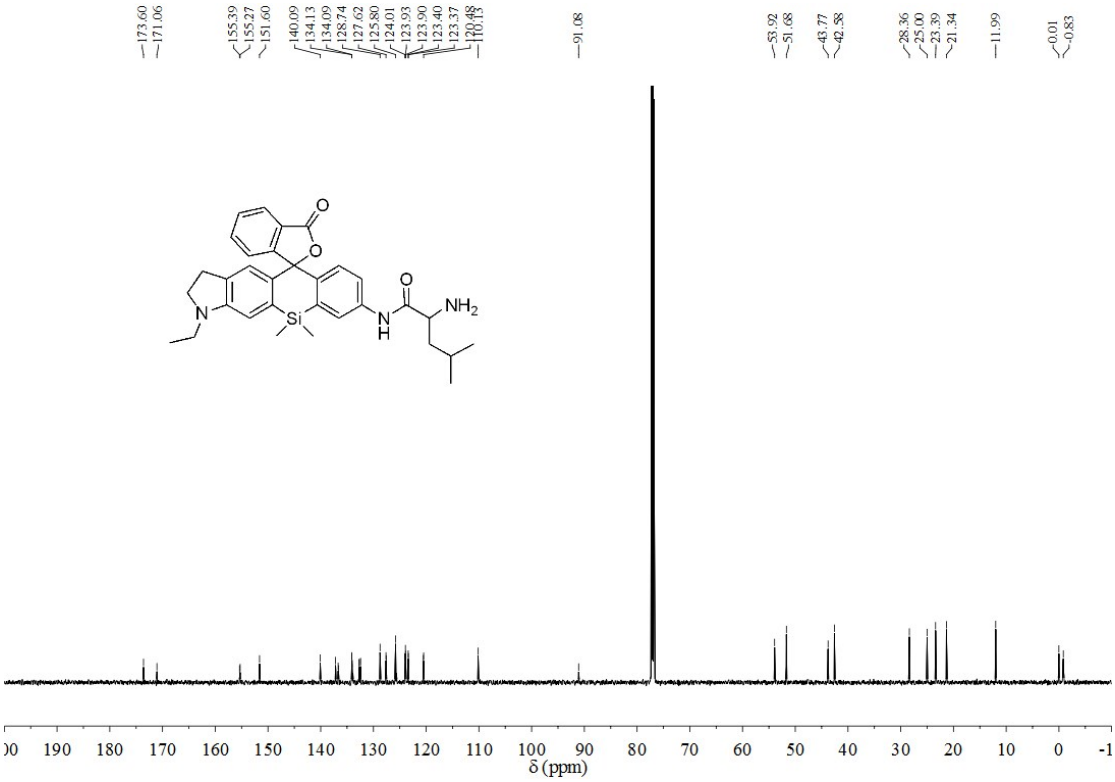
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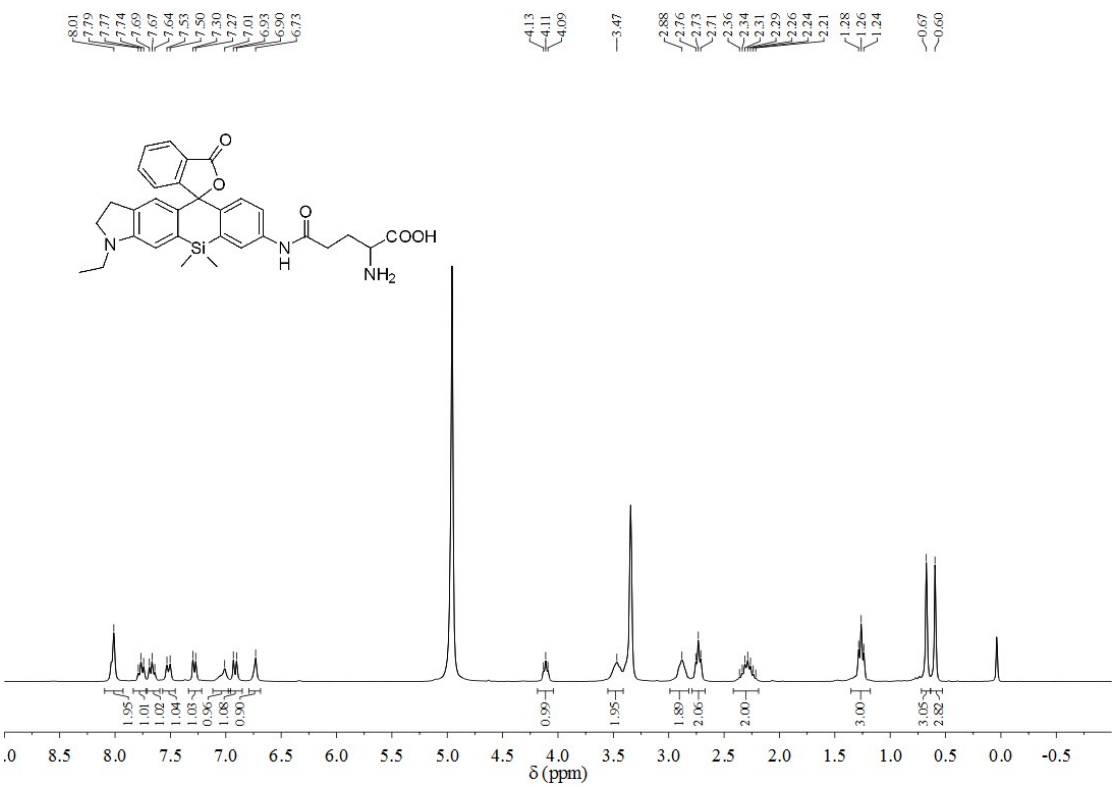
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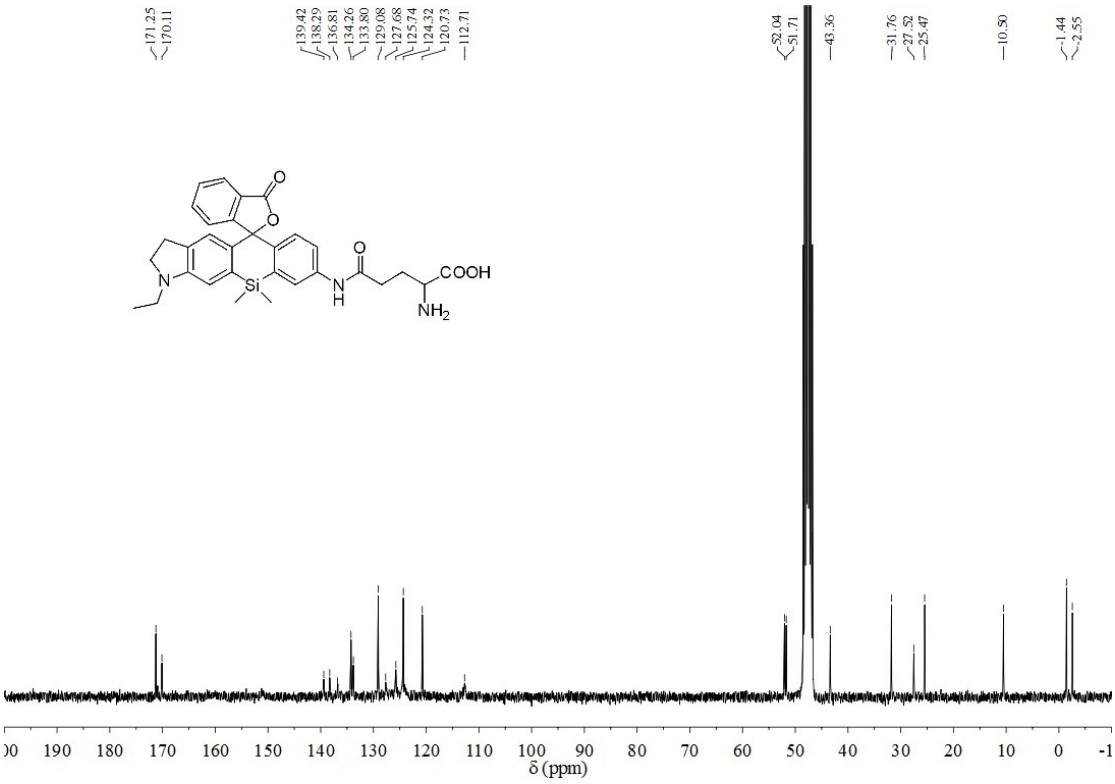
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δ (ppm)

3. Experimental Section

Spectroscopic analysis.

Stock solutions of fluorophores (1.0 mM) were obtained by dissolving the solid compounds into DMSO and further diluted with 0.2 M sodium phosphate buffer to varied pH from 2.0 to 12.0. UV-vis spectra were recorded with a Shimazu UV-2600 spectrophotometer, and fluorescence spectra were acquired with a Shimazu RF-6000 spectrophotometer.

The fluorescence spectra (λ_{ex} = 640 nm) of 2.5 μM **SiRB2-Leu** and **SiRB2-Glu** in the existence of proteases LAP and GGT at varied concentration were evaluated in PBS (pH = 7.4) at 37 °C, respectively. The kinetic of 2.5 μM **SiRB2-Leu** with LAP at varied concentrations (0 U/L, 5.0 U/L, 10 U/L and 50 U/L) were evaluated in PBS (pH = 7.4) with reaction time increased from 0 min to 60 min. The kinetic of 2.5 μM **SiRB2-Glu** with GGT at varied concentrations (0 U/L, 50 U/L, 200 U/L and 500 U/L) were evaluated in PBS (pH = 7.4) with reaction time changed from 0 min to 60 min.

To analyse the reaction rate of the probes with corresponding proteases, fluorescence intensity was monitored for the aqueous solution (containing 1% DMSO) of **SiRB2-Leu** various concentrations (0.25, 0.50, 1.0, 2.0, 5.0, 10, 15 and 20 μM) after addition of LAP. The kinetic of **SiRB2-Glu** was analyse by the similar method. Aqueous solution (containing 1% DMSO) of **SiRB2-Glu** various concentrations (0.50, 1.0, 2.0, 5.0, 10, 15, 20, 30, 50 and 100μM) after step-wise addition of GGT. The Michaelis-Menten kinetics of the cleaving reaction for probes **SiRB2-Leu** and **SiRB2-Glu** are evaluated by the equation:

$$V = V_{max}*[S] / (K_m + [S])$$

where V is reaction rate, V_{max} is maximal reaction rate, K_m (Michaelis–Menten constant) is the concentration of fluorescent probes which permits the enzyme to achieve half

V_{max} and [*S*] represents the concentration of the fluorescent probes.

MTT assay.

The cytotoxicity of **SiRB2**, **SiRB2-Leu**, **SiRB2-Glu** were evaluated using MTT assay. Hela cell lines were cultivated in 96-well plates by mixture of 90% (*V*/*V*) Dulbecco's Modified Eagle's Medium (DMEM), 10% (*V*/*V*) FBS containing probes of various concentrations: 0.1μM, 1 μM, 2 μM, 5 μM and 10 μM in a 5% CO_2/95% air incubator at 37 °C. After incubation for 24 hours, 20 μL of MTT reagent, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (5 mg/mL in PBS) was added into each well and incubated for another 4 hours in the 5% CO_2/95% air incubator at 37 °C. Then 80 μL DMSO were added into each well to dissolve the formazan crystals. Each experiment was performed for three times. Absorbance of each well was analysed at 565 nm with a Synergy^{TM} H1 multimode microplate reader (BioTek).

Cell lines and culture.

Hela (purchased from National Infrastructure of Cell Line Resource in Shanghai), HCoEpiC (purchased from KeyGEN) and LS174T (purchased from National Infrastructure of Cell Line Resource in Shanghai) cell lines were cultured in 90 % (*V*/*V*) Dulbecco's Modified Eagle's Medium (DMEM). MC38 cell lines (purchased from National Infrastructure of Cell Line Resource in Beijing) were cultured in 90 % (*V*/*V*) Roswell Park Memorial Institute (RPMI 1640). All media contained 10 % (*V*/*V*) fetal bovine serum (FBS, Corning) with 1% Antibiotic-Antimycotic (AA). The cell lines were incubated in the humidified incubator containing 5% CO_2 / 95% air at 37 °C.

In vivo fluorescence imaging.

Fluorescence imaging of SiRB2 and SiRB2-Ac in living cells. HeLa cells were incubated with **SiRB2** (2 μM) and Hoechst 33342 (1.5 μg/mL) for 30 min and washed by PBS for three times. Fluorescent imaging was performed using Leica TCS SP8 confocal laser scanning microscopy with a 63× objective. A 405 nm laser was used for fluorescent imaging of Hoechst 33342 by collecting emission from 435 nm to 500 nm (blue channel). A 633 nm laser is used for fluorescent imaging of **SiRB2** with collection wavelength ranging from 650 nm to 800 nm (red channel). The co-stain of **SiRB2-Ac** (2 μM) and Hoechst 33342 (1.5 μg/mL) followed similar procedure.

Fluorescence imaging of SiRB2-Leu in living cells. HeLa cells were incubated with **SiRB2-Leu** (2 μM), Hoechst 33342 (1.5 μg/mL) and LysoTracker™ Green DND-26 (1 μM) for 30 min and washed by PBS for three times before imaging. Blue channel was used for fluorescent imaging of Hoechst 33342 and red channel was used for the probe **SiRB2-Leu**. A 488 nm laser and collection wavelength ranging from 500 nm to 600 nm (green channel) was used for LysoTracker™ Green DND-26. For comparative experiments, we incubated HeLa cells with the leucine enzyme inhibitor (Bestatin Methyl Ester 20μM) for 24 h followed by washing with PBS for three times. Then the inhibitor-treated HeLa cells were incubated with **SiRB2-Leu** (2 μM) and Hoechst 33342 (1.5 μg/mL) for 30 min and washed by PBS for three times before imaging.

Fluorescence imaging of SiRB2-Glu in living cells. MC38, LS174T and HCoEpiC cell lines were respectively incubated with **SiRB2-Glu** (2 μM) and Hoechst 33342 (1.5 μg/mL) for 30 min, followed by PBS washing for three times. Blue channel and red channel were applied for fluorescent imaging of Hoechst 33342 and **SiRB2-Glu**, respectively. For comparative experiments, we incubated MC38 and LS174T cells with the irreversible GGT inhibitor (GGsTop 100 μM) for 4 h, followed by washing with PBS for three times, respectively. Then the inhibitor-treated MC38 and LS174T cells

were incubated with **SiRB2-Glu** (2 μM) and Hoechst 33342 (1.5 μg/mL) for 30 min and washed by PBS for three times before imaging.

Development of CAC model.

All animal experiments were performed in accordance with the ethics and regulations of animal experiments of Second Military Medical University (Shanghai, People's Republic of China). 40 female, 6-week aged BALB/c mice were randomly divided into experimental group (n = 30) and control group (n = 10). All the mice were given purified diet and drinking water. The experimental group was given a single intraperitoneal injection of the saline solution of azoxymethane (AOM, 10 mg/kg body weight). The control group was injected with the same volume (0.1 mL) of saline. After one week, 2% dextran sulphate sodium (DSS, averaged molecular weight 36000 to 50000 Da) was added into the drinking water of mice in experimental group for one week, followed by normal drinking water for another week. Mice in experimental group were treated by three DSS/water cycles.

Fluorescent imaging of the tumors *in vitro*.

Two weeks after the third DSS/water cycles, fresh colon tissues were collected by surgically excising from both AOM/DSS-treated mice in experimental group and the normal mice in control group. We longitudinally cut along the main axis to expose the mucosa layer of colon after the sacrifice. The colon was soaked in PBS solution with SiRB2-glu (50 μM) for 30 min and washed 3 times with PBS. Fluorescent imaging was obtained with the Chemiluminescence fluorescence image analysis system (PerkinElmer, IVIS Lumina III imaging system) with red laser setting (λ_{ex} = 630 nm, λ_{em} = 650 - 800 nm).

4. Spectroscopic Analysis

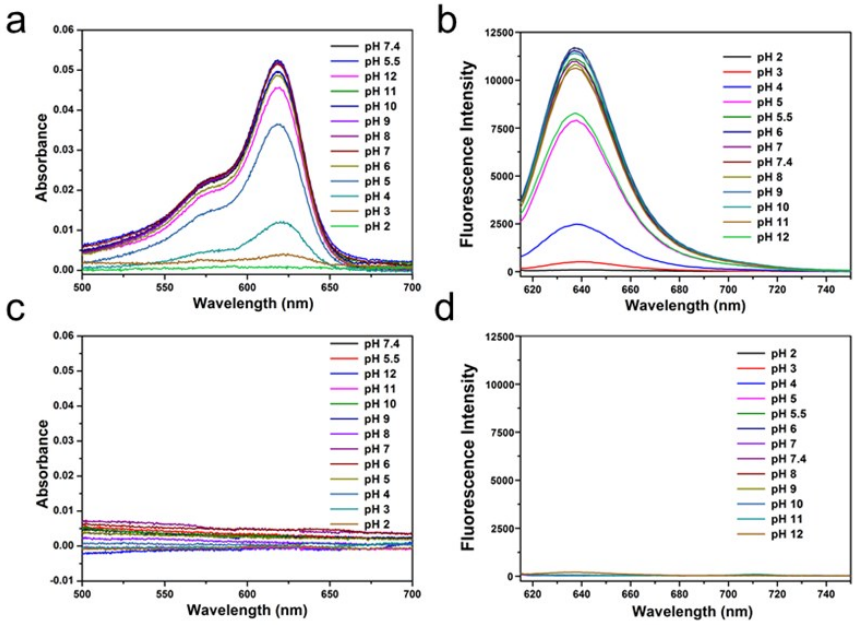

Fig. S1. (a, b) The absorption spectra (a) and fluorescence spectra (b) of **SiRB1** (2.5 μM) in the different pH. (c, d) The absorption spectra (c) and fluorescence spectra (d) of **SiRB2-Ac** (2.5 μM) in the different pH.

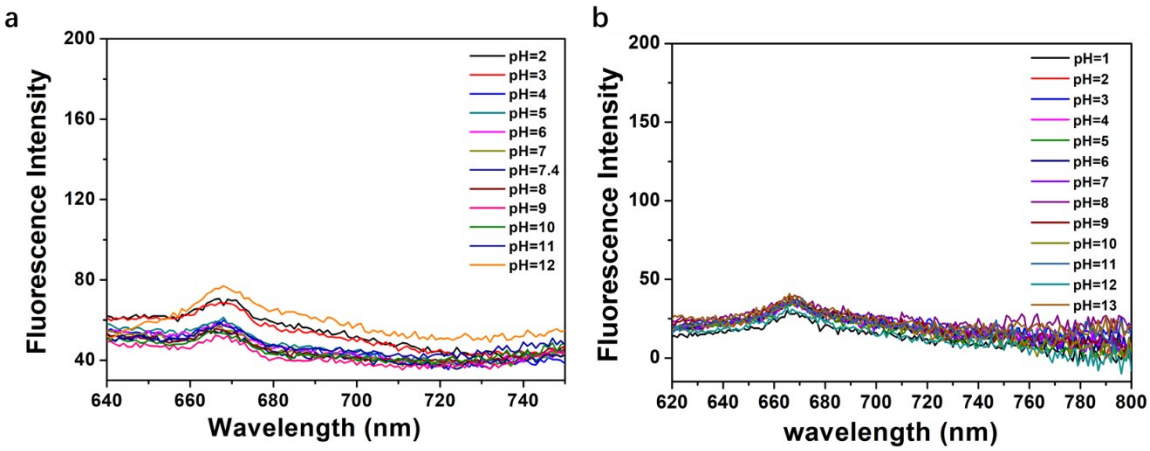

Fig. S2. The fluorescence spectra of **SiRB2-Leu** (a) and **SiRB2-Glu** (b) (both are2.5 μM) in different pH.

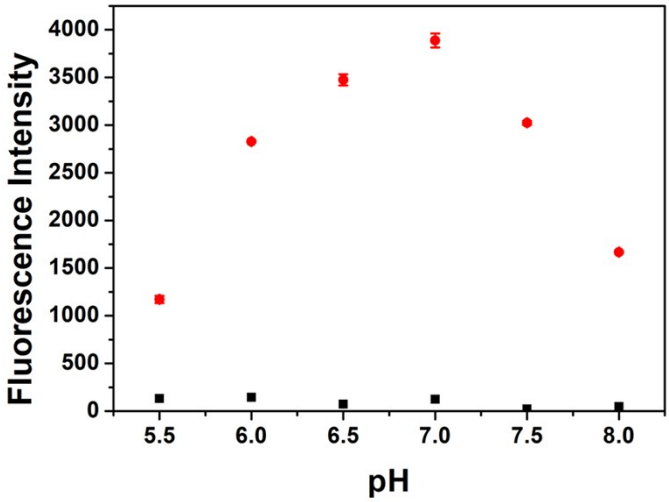

Fig. S3 The fluorescence intensity of **SiRB2-Leu** (both are 2.5 μM) in 0.2 M sodium phosphate bu□er at various pH. The red dots are fluorescence intensity of **SiRB2-Leu** with LAP and the black dots are fluorescence intensity of **SiRB2-Leu** without LAP.

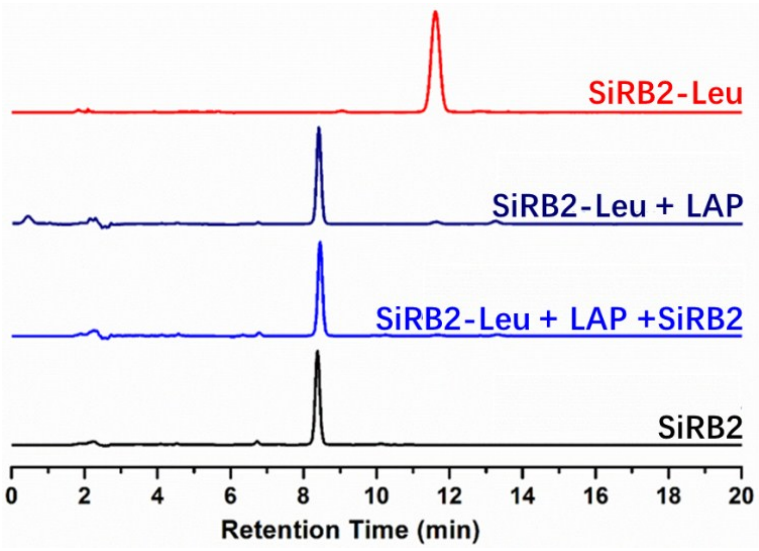

Fig. S4. The retention time of **SiRB2-Leu** (red line), **SiRB2-Leu** and LAP (purple line), **SiRB2-Leu**, LAP and **SiRB2** (blue line) and **SiRB2** (black line) by HPLC analysis.

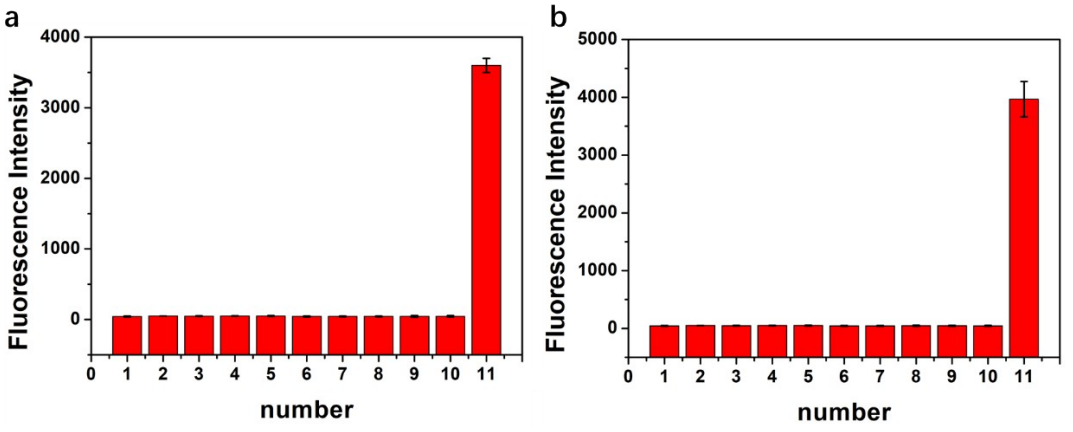

Fig. S5. The fluorescence responses of **SiRB2-Leu** (a) and **SiRB2-Glu** (b) (2.5 μM) in PBS solution (pH = 7.4) toward various species: (1) blank, (2) 200 μM of Ca^{2+}, (3) 200 μM of Fe^{2+}, (4) 200 μM of Mg^{2+}, (5) 200 μM of Zn^{2+}, (6) 200 μM of H_2O_2, (7) 200 μM of HClO, (8) 200 μM of $ONOO^-$, (9) 200 μM of OH^-, (10) 200 μM of O^{2-}, (11) is LAP and GGT, respectively.

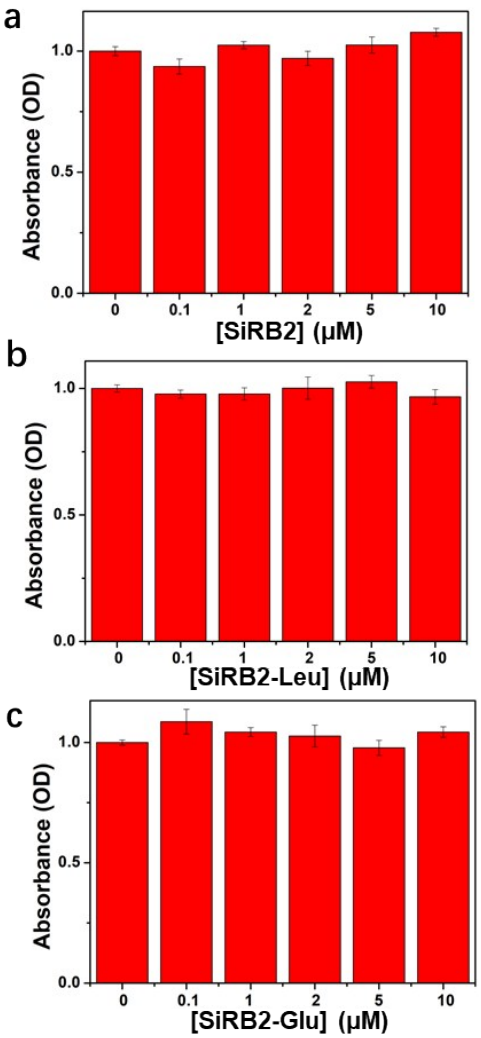

Fig. S6. Cell viability results of the HeLa cells with **SiRB2** (a), **SiRB2-Leu** (b) and **SiRB2-Glu** (c) by MTT assay. All data are presented as mean ± standard deviation (n = 3).

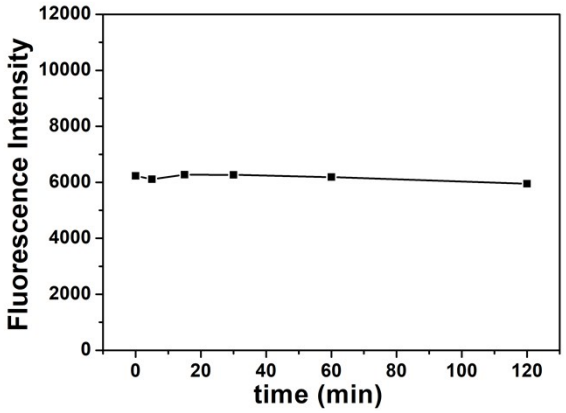

Fig. S7. Photostability of **SiRB2** at 2.5 μM.

5. Fluorescence Imaging

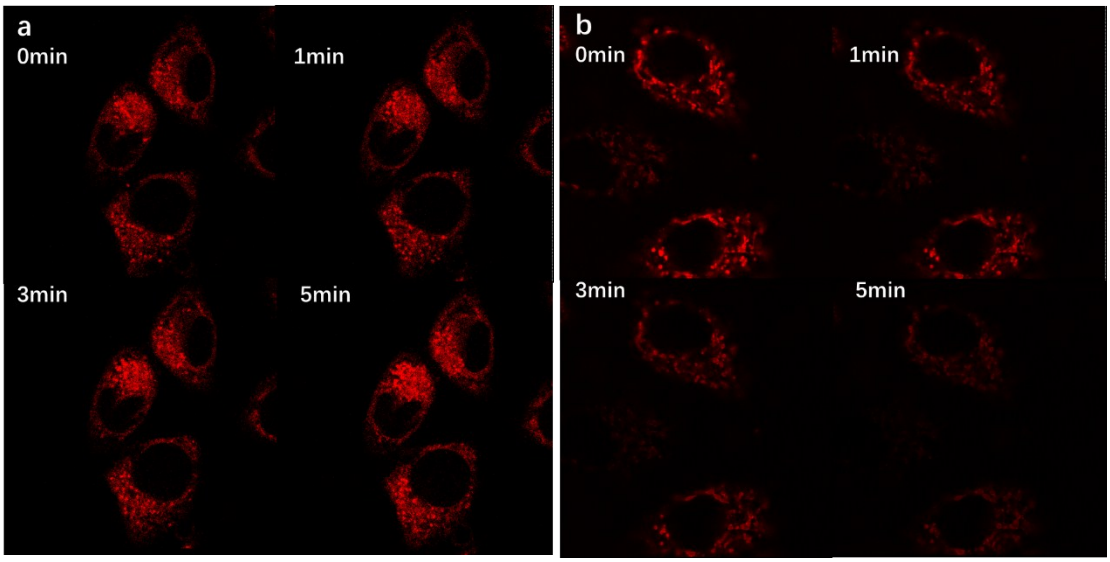

Fig. S8. The photostability of **SiRB2-Leu** *in vivo* (a). The photostability of **Cy5** *in vivo* (b).

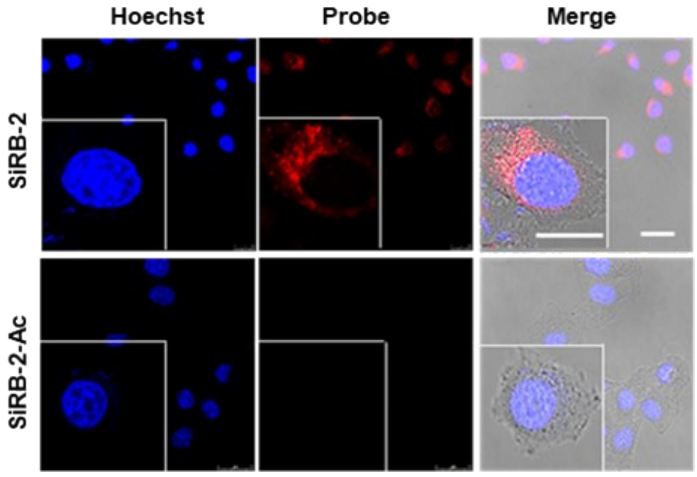

Fig. S9. Confocal microphotographs of living Hela cell lines triple-stained with Hoechst 33342 (1.5 μg/mL), and 2 μM respective **SiRB2**s: (a) **SiRB2** and (b) **SiRB2-Ac**. Confocal images in three channels were collected: blue channel for Hoechst (λ_{ex} = 405 nm, λ_{em} = 450-500 nm) and red channel for **SiRB2**s (λ_{ex} = 633 nm, λ_{em} = 650-800 nm). Enlarged images of single cells were embedded in the lower left part of each image. Scale bar represents 35 μm for the full images and represents 15 μm for the enlarged images.

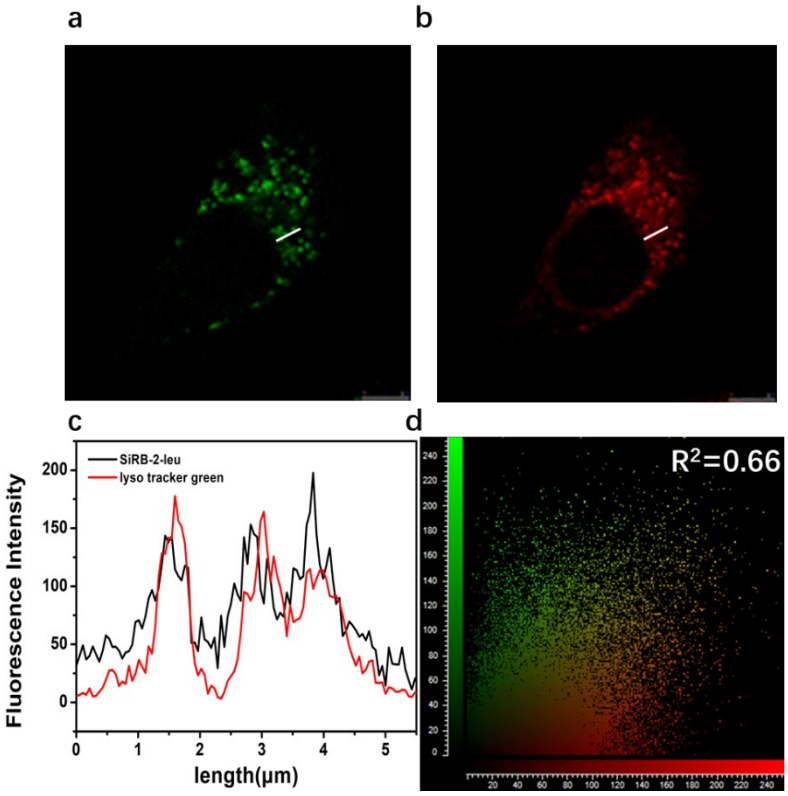

Fig. S10. Colocalization plot of LysoTracker™ Green DND-26 (a) and **SiRB2-Leu** (b) from random selected area (white line) (c). (d) is scatter plots and Pearson's R values.

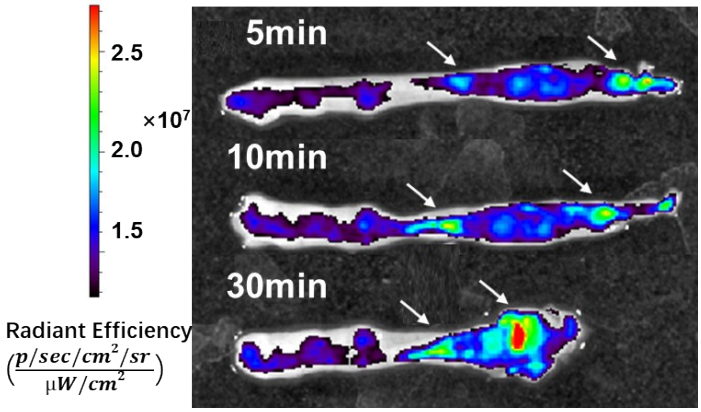

Fig. S11. The ex vivo fluorescence imaging of the cancerous colons at different time gradients (5, 10, 30 min) after incubation of 100 μM **SiRB2-Glu** in PBS.