



Supporting Information

for

A practical CO₂-mediated synthesis of 5,6-carboxylated silicon-rhodamines for targeted probe development

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Experimental details and characterization of compounds by NMR and ESIMS

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1. Experimental details

1.1 Materials and instrumentation

Unless explicitly stated, all reagents were purchased from commercial suppliers (Sigma Aldrich, J&K, Innochem, and Aladdin) and used without further purification. Solvents (dimethyl sulfoxide (DMSO), dimethylformamide (DMF), methanol) were purchased from J&K and used without further treatment or distillation. Silica gel (200–300 mesh) and aluminum oxide basic (200–300 mesh) were purchased from Innochem.

¹H NMR and ¹³C NMR spectra were recorded on a Bruker 400M spectrometer or Bruker 700M spectrometer with TMS as an internal standard. Chemical shifts were given in ppm and coupling constants (*J*) in Hz. High-resolution mass spectrometry data were obtained with an HP1100LC/MSD mass spectrometer and an LC/Q-TOF MS spectrometer. UV–vis absorption spectra were collected on an Agilent Cary 60 UV–vis spectrophotometer. Fluorescence measurements were performed on an Agilent CARY Eclipse fluorescence spectrophotometer.

Super-resolution images were performed by using a Nikon N-STORM/SIM 5.0 Super-Resolution Microscope System with a motorized inverted microscope ECLIPSE Ti2-E, a 100 × / NA 1.49 oil immersion TIRF objective lens (CFI HP), LU-NV series laser unit (405 nm, 488 nm, 561 nm, 647 nm), and an ORCA-Flash 4.0 sCMOS camera (Hamamatsu Photonics K.K.).

1.2 Cell culture and transfection

1.2.1 Cell culture

HeLa cells were purchased from the Cell Bank of Type Culture Collection of the Chinese Academy of Sciences. Cells were maintained in Dulbecco's modified Eagle's medium (DMEM, Gibco) supplemented with 10% fetal bovine serum (FBS, Hyclone), which were cultured in a humidified atmosphere of 5% CO₂/95% air at 37 °C. Before the imaging experiments, cells were seeded on a glass bottom cell culture dish (Nest, polystyrene, Ø 15 mm) for 1–2 days to reach 50–80% confluency. The cells were then used for further experiments.

1.2.2 Cell transfection

In a manner similar to ref [1], Halo-H₂B, Halo-actin, and Halo-TOMM20 plasmids were purchased from Addgene and transfected into cells using Lipofectamine 2000 (Invitrogen) according to the manufacturer's protocol.

For transfection, 2 µL Lipofectamine 2000 (Invitrogen) and the appropriate plasmid were first diluted in 20 µL DMEM (Dulbecco's Modified Eagle Medium), respectively. After incubation for 5 min, the diluted plasmid in 20 µL DMEM was added to the diluted Lipofectamine 2000 solution and gently mixed. The resulting mixture was further

incubated for 10 min and then added to the cell-culture dish in 1 mL DMEM. The final plasmid concentration was adjusted to 1600 ng/mL. After incubating for 4 h at 37 °C, the culture medium was changed from DMEM to DMEM with 10% FBS. The transfected cells were cultured for an additional 24–48 h prior to super-resolution imaging experiments.

1.3 Staining and imaging

Live HeLa cells were incubated with probes (150 nM or 200 nM) in DMEM for 1 h at 37 °C under 5% CO₂. Because the spirolactone-based probes exhibited low background fluorescence in the unbound state, cells were directly used for imaging without washing after incubation. For standard fluorescence imaging, excitation was at 640 nm and emission was collected at 650–750 nm. For structured illumination microscopy (SIM) and single-molecule localization microscopy (SMLM), cells were imaged immediately following the staining under the respective imaging conditions.

1.4 Localization precision analysis

The reported localization precision values (24–46 nm) represent the average of 3 to 5 independent fields of view per sample. The raw precision data for individual localized single-molecule events in each field of view were directly exported using the analysis software integrated with the Nikon N-STORM/SIM 5.0 system. The final reported range was calculated by averaging these datasets.

1.5 UV–vis absorption spectra and fluorescence spectra

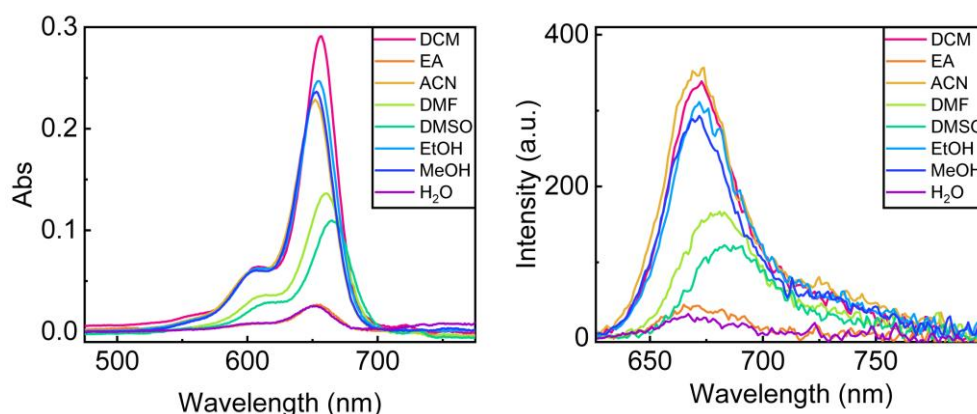


Figure S1 UV–vis absorption and fluorescence spectra of **4a** in different solvents (with 1‰ CF₃COOH).

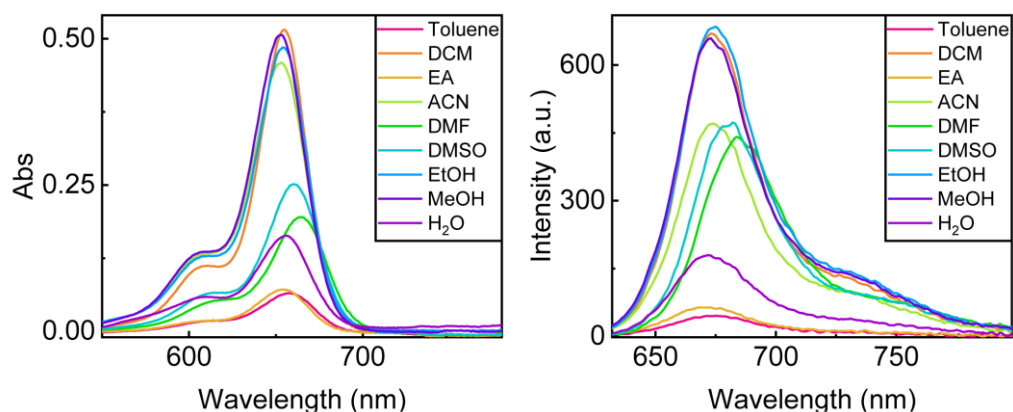


Figure S2 UV-vis absorption and fluorescence spectra of **4b** in different solvents (with 1‰ CF_3COOH).

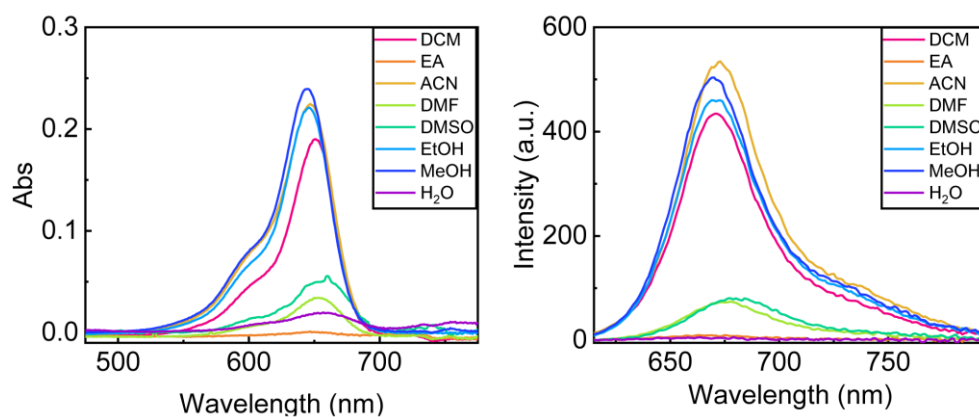


Figure S3 UV-vis absorption and fluorescence spectra of **4c** in different solvents (with 1‰ CF_3COOH).

1.6 Imaging details

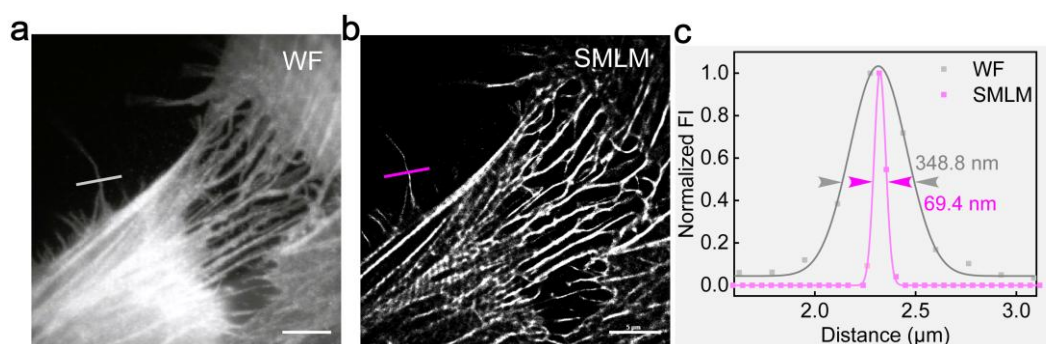


Figure S4 (a, b) Wide-field and SMLM images of actin labeled with compound **4b** in live HeLa cells. (c) Intensity profiles along the filopodia indicated by the white line in (a) and the pink lines in (b). Scale bars: 5.0 μm .

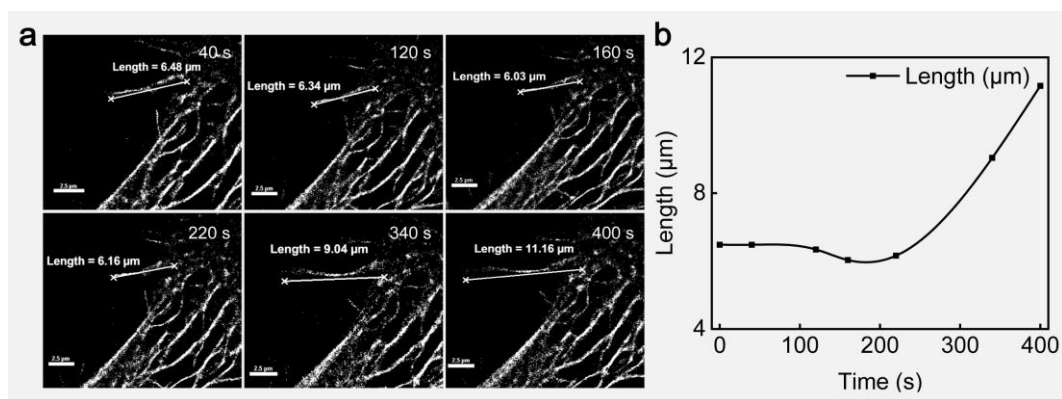


Figure S5 (a) Filopodia length at different time points in live HeLa cells labeled with compound **4b**. (b) Quantitative analysis of filopodia length in (a). Scale bars: 2.5 μm.

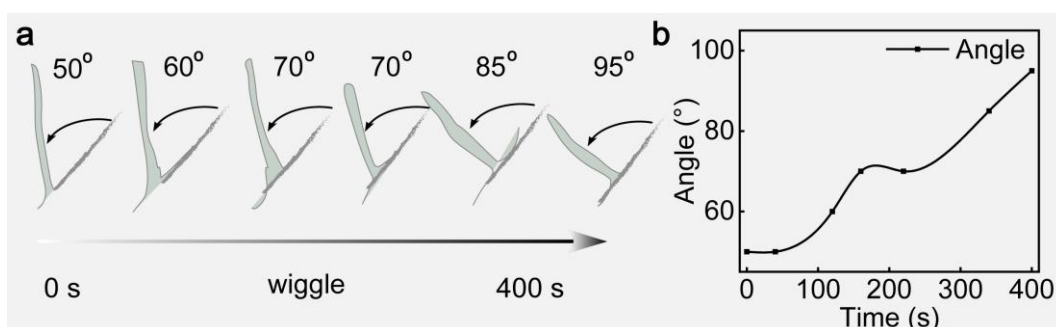
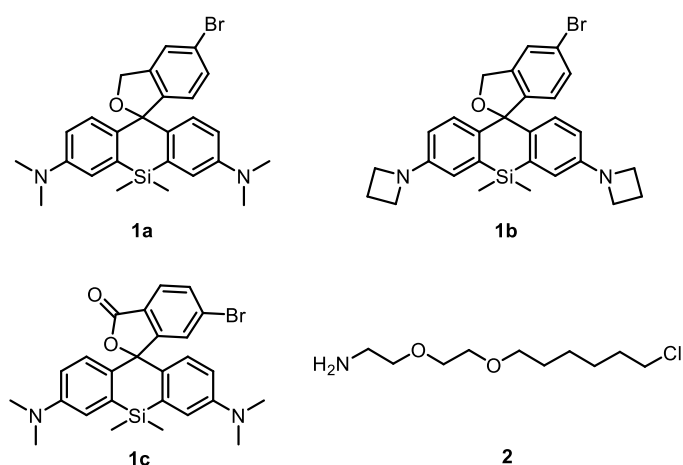


Figure S6 (a) Wiggling angles of filopodia at different time points in live HeLa cells labeled with compound **4b**. (b) Quantitative analysis of filopodia wiggling angles in (a).

2. Synthesis methods

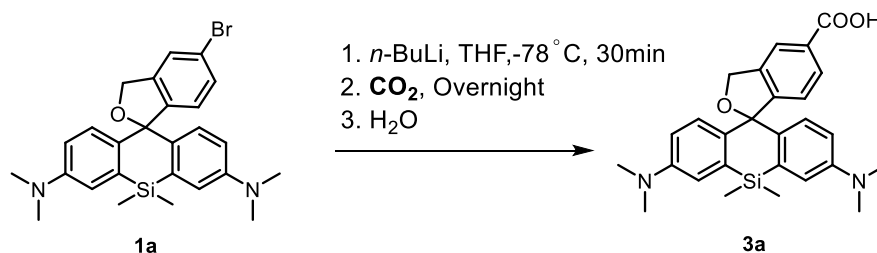
2.1 Preparation of the reaction precursors 1a–c and 2



Synthetic procedures of **1a** and **1b** from [2]. Synthetic procedure of **1c** from [3]. Synthetic procedure of **2** from [4].

2.2 Synthetic procedure for carboxy-SiR 3a–c

2.2.1 Synthesis of 3a



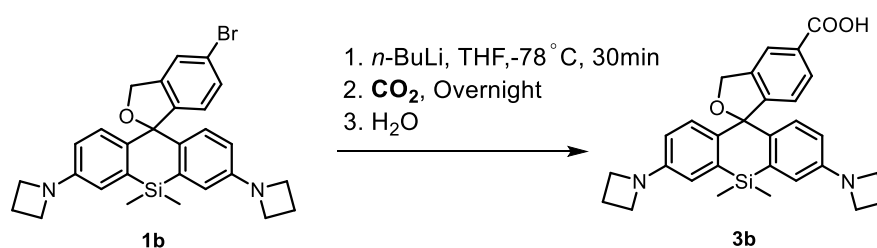
In a dry Schlenk flask, **1a** (100 mg, 0.20 mmol) was charged, sealed, and evacuated/backfilled with nitrogen (3×). Anhydrous THF (10 mL) was added, and the mixture was cooled to -78 °C. *n*-Butyllithium (2.5 M in THF, 0.2 mL) was added dropwise, and the reaction mixture was stirred at -78 °C for 30 min. CO₂ was bubbled through the solution (3×), and the mixture was allowed to warm to room temperature and stirred for 10 h. The reaction was quenched with water (10 mL), and the aqueous phase was extracted with ethyl acetate (3 × 10 mL). The combined organic layers were dried over anhydrous Na₂SO₄, filtered, and concentrated. The residue was purified by silica gel chromatography (MeOH/DCM 1:10, v/v) to afford **3a** as a blue solid in 93% yield.

¹H NMR (400 MHz, MeOD) δ 8.01 (s, 1H), 7.84 (d, *J* = 8.0 Hz, 1H), 7.07 – 6.93 (m, 4H), 6.88 (d, *J* = 8.0 Hz, 1H), 6.68 (dd, *J* = 9.0, 2.9 Hz, 2H), 5.37 (s, 2H), 2.90 (s, 12H), 0.59 (s, 3H), 0.49 (s, 3H).

¹³C NMR (400 MHz, MeOD) δ 153.96, 150.82, 140.00, 139.65, 135.77, 130.64, 130.28, 124.90, 124.39, 117.84, 115.98, 94.44, 74.00, 41.10.

HRMS (ESI) calcd for C₂₇H₃₁N₂O₃Si [M+H]⁺ 459.2099, found 459.2182.

2.2.2 Synthesis of 3b

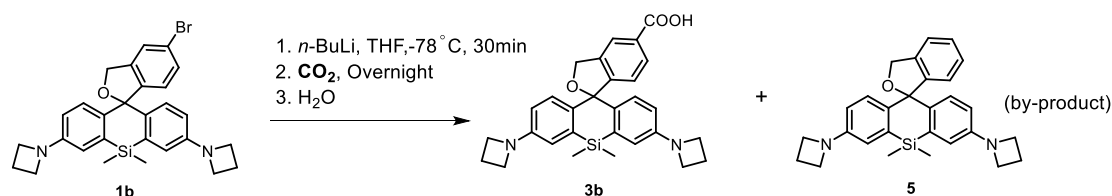


In a dry Schlenk flask, **1b** (100 mg, 0.19 mmol) was charged, sealed, and evacuated/backfilled with nitrogen (3×). Anhydrous THF (10 mL) was added, and the mixture was cooled to -78 °C. *n*-Butyllithium (2.5 M in THF, 0.19 mL) was added dropwise, and the reaction mixture was stirred at -78 °C for 30 min. CO₂ was bubbled through the solution (3×), and the mixture was allowed to warm to room temperature and stirred for 10 h. The reaction was quenched with water (10 mL), and the aqueous phase was extracted with ethyl acetate (3 × 10 mL). The combined organic layers were dried over anhydrous Na₂SO₄, filtered, and concentrated. The residue was purified by silica gel chromatography (MeOH/DCM 1:10, v/v) to afford **3b** as a blue solid in 60% yield.

^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 7.92 (s, 1H), 7.75 (s, 1H), 7.01 (d, $J = 8.2$ Hz, 2H), 6.78 (s, 1H), 6.61 (s, 2H), 6.34 (d, $J = 8.8$ Hz, 2H), 5.40 (s, 2H), 3.97 – 3.57 (m, 8H), 2.28 (s, 4H), 0.56 (s, 3H), 0.43 (s, 3H).

HRMS (ESI) calcd for $\text{C}_{29}\text{H}_{31}\text{N}_2\text{O}_3\text{Si}$ $[\text{M}+\text{H}]^+$ 483.2099, found 483.2124.

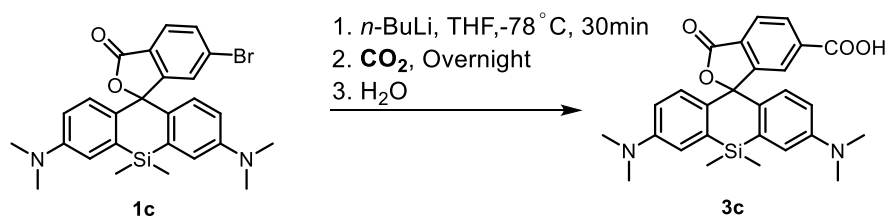
During this reaction, an uncarboxylated rhodamine byproduct (compound **5**) was also isolated and characterized.



Compound **5**: ^1H NMR (400 MHz, CDCl_3) δ 7.29 (d, $J = 4.6$ Hz, 2H), 7.25 – 7.21 (m, 1H), 7.06 (d, $J = 7.4$ Hz, 1H), 6.95 (d, $J = 8.6$ Hz, 2H), 6.66 (d, $J = 2.6$ Hz, 2H), 6.31 (dd, $J = 8.6, 2.6$ Hz, 2H), 5.21 (s, 2H), 3.86 (t, $J = 7.2$ Hz, 8H), 2.33 (p, $J = 7.2$ Hz, 4H), 0.58 (s, 3H), 0.52 (s, 3H).

HRMS (ESI) calcd for $\text{C}_{28}\text{H}_{31}\text{N}_2\text{OSi}$ $[\text{M}+\text{H}]^+$ 439.2201, found 439.2223.

2.2.3 Synthesis of **3c**



In a dry Schlenk flask, **1c** (100 mg, 0.19 mmol) was charged, sealed, and evacuated/backfilled with nitrogen (3 \times). Anhydrous THF (10 mL) was added, and the mixture was cooled to -78 $^\circ\text{C}$. *n*-Butyllithium (2.5 M in THF, 0.19 mL) was added dropwise, and the reaction mixture was stirred at -78 $^\circ\text{C}$ for 30 min. CO_2 was bubbled through the solution (3 \times), and the mixture was allowed to warm to room temperature and stirred for 10 h. The reaction was quenched with water (10 mL), and the aqueous phase was extracted with ethyl acetate (3 \times 10 mL). The combined organic layers were dried over anhydrous Na_2SO_4 , filtered, and concentrated. The residue was purified by silica gel chromatography (MeOH/DCM 1:10, v/v) to afford **3c** as a blue solid in 77% yield.

^1H NMR (400 MHz, MeOD) δ 8.22 (d, $J = 8.0$ Hz, 1H), 7.97 (d, $J = 7.9$ Hz, 1H), 7.85 (s, 1H), 7.04 (d, $J = 2.8$ Hz, 2H), 6.72 (d, $J = 8.9$ Hz, 2H), 6.62 (dd, $J = 9.0, 2.9$ Hz, 2H), 2.96 (s, 12H), 0.65 (s, 3H), 0.55 (s, 3H).

^{13}C NMR (400 MHz, MeOD) δ 173.50, 157.33, 152.49, 139.37, 133.64, 132.46, 131.41, 130.57, 127.93, 127.57, 119.24, 116.10, 50.62, 41.75, 1.55.

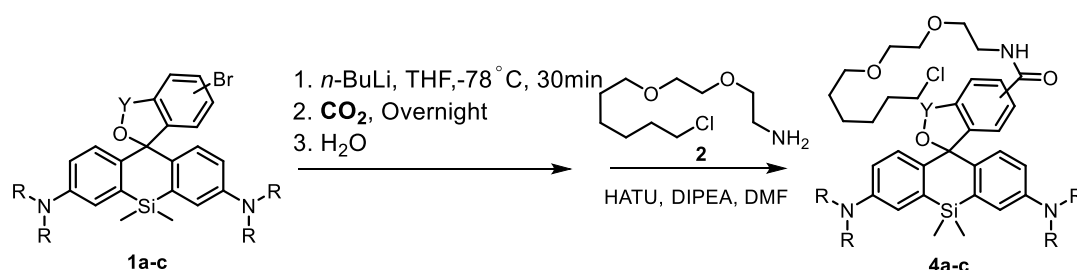
HRMS (ESI) calcd for $\text{C}_{27}\text{H}_{29}\text{N}_2\text{O}_4\text{Si}$ $[\text{M}+\text{H}]^+$ 473.1892, found 473.1894.

2.2.4 CO₂ introduction procedure

All carboxylation reactions were performed using high-purity (99.999%) CO₂ gas supplied from a 40 L cylinder. Approximately 0.5–1 L of CO₂ was first collected in a balloon and then introduced below the surface of the reaction mixture through a needle for approximately 3–5 min to ensure sufficient contact and reaction between CO₂ and the substrates. The CO₂-bubbling operation was typically repeated three times. For larger-scale reactions, additional CO₂ introduction cycles were applied as needed to ensure complete carboxylation.

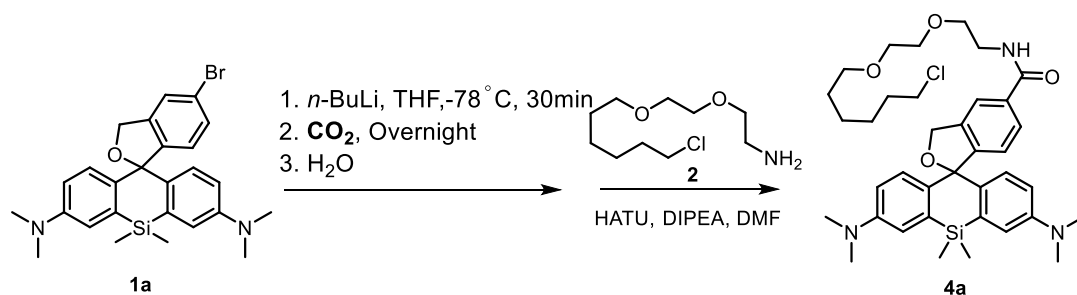
2.3 Sequential one-pot functionalization via synthetic protocol

4a–c



The carboxylation was performed as described above. The crude product was used directly without purification and dissolved in anhydrous DMF. Then, HATU and DIPEA were added, followed by compound **2**, and the mixture was stirred for 6 h. The solvent was removed under reduced pressure, and the residue was purified by silica gel chromatography (200–300 mesh).

2.3.1 Synthesis of 4a



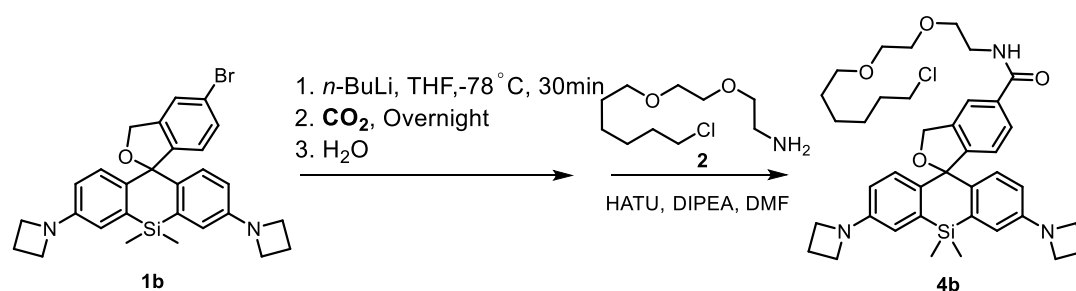
1a (50 mg, 0.10 mmol, 1 equiv) was charged in a dry Schlenk flask, sealed, and evacuated/backfilled with nitrogen (3×). Anhydrous THF (10 mL) was added, and the mixture was cooled to -78 °C. *n*-Butyllithium (2.5 M in THF, 0.1 mL, 2.5 equiv) was added dropwise, and the reaction mixture was stirred at -78 °C for 30 min. CO₂ was bubbled through (3×), then the mixture was warmed to room temperature and stirred for 10 h. After quenching with water (10 mL), the aqueous layer was extracted with ethyl acetate (3 × 10 mL), dried over Na₂SO₄, filtered, and concentrated. The crude product was dissolved in anhydrous DMF (10 mL), HATU (61 mg, 0.16 mmol, 1.6 equiv) and DIPEA (106 μL, 0.60 mmol, 6 equiv) were added, followed by **2** (22 mg, 0.10 mmol,

1.0 equiv), and the mixture was stirred for 6 h. The solvent was removed, and the residue purified by silica gel chromatography (MeOH/DCM 1:20) to give **4a** as a light green solid in 62% yield.

$^1\text{H NMR}$ (400 MHz, CDCl_3) δ 7.77 (s, 1H), 7.64 (dd, $J = 8.0, 1.7$ Hz, 1H), 7.08 (d, $J = 7.9$ Hz, 1H), 7.04 – 6.85 (m, 4H), 6.72 (s, 1H), 6.60 (dd, $J = 8.9, 2.9$ Hz, 2H), 5.26 (s, 2H), 3.67 (dd, $J = 10.6, 3.8$ Hz, 6H), 3.59 (dd, $J = 5.7, 3.0$ Hz, 2H), 3.47 (q, $J = 6.7$ Hz, 4H), 2.95 (s, 12H), 1.75 – 1.69 (m, 2H), 1.57 (d, $J = 7.2$ Hz, 2H), 1.51 – 1.44 (m, 4H), 0.62 (s, 3H), 0.54 (s, 3H).

HRMS (ESI) calcd for $\text{C}_{37}\text{H}_{51}\text{ClN}_3\text{O}_4\text{Si}$ $[\text{M}+\text{H}]^+$ 664.3332, found 664.3341.

2.3.2 Synthesis of **4b**



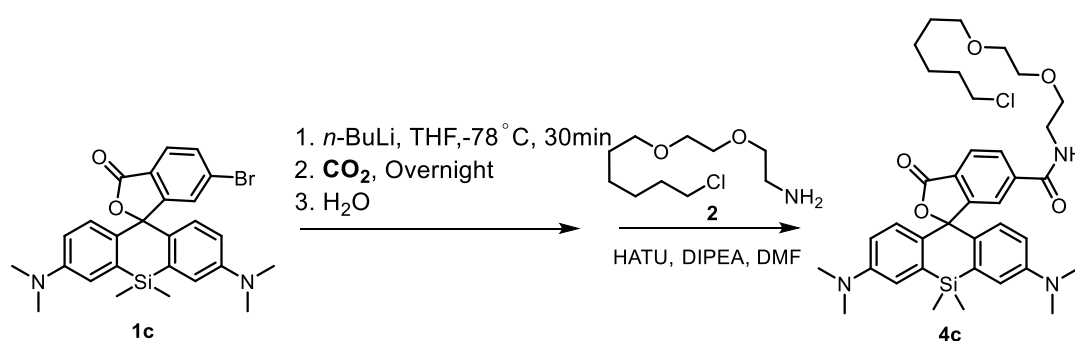
1b (30 mg, 0.05 mmol, 1 equiv) was charged in a dry Schlenk flask, sealed, and evacuated/backfilled with nitrogen (3 \times). Anhydrous THF (10 mL) was added, and the mixture was cooled to -78°C . *n*-Butyllithium (2.5 M in THF, 0.06 mL, 2.5 equiv) was added dropwise, and the reaction stirred at -78°C for 30 min. CO_2 was bubbled through (3 \times), then the mixture was warmed to room temperature and stirred for 10 h. After quenching with water (10 mL), the aqueous layer was extracted with ethyl acetate (3 \times 10 mL), dried over Na_2SO_4 , filtered, and concentrated. The crude product was dissolved in anhydrous DMF (10 mL), HATU (35 mg, 0.09 mmol, 1.6 equiv) and DIPEA (61 μL , 0.34 mmol, 6 equiv) were added, followed by **2** (13 mg, 0.05 mmol, 1 equiv), and the mixture was stirred for 6 h. The solvent was removed, and the residue purified by silica gel chromatography (MeOH/DCM 1:20) to give **4b** as a light green solid in 37% yield.

$^1\text{H NMR}$ (700 MHz, CDCl_3) δ 7.76 (s, 1H), 7.64 (d, $J = 7.9$ Hz, 1H), 7.08 (d, $J = 7.8$ Hz, 1H), 6.90 (d, $J = 8.6$ Hz, 2H), 6.75 (s, 1H), 6.66 (d, $J = 2.7$ Hz, 2H), 6.30 (dd, $J = 8.6, 2.6$ Hz, 2H), 5.24 (s, 2H), 3.88 (t, $J = 7.3$ Hz, 8H), 3.69 – 3.65 (m, 6H), 3.59 (dd, $J = 5.9, 3.3$ Hz, 2H), 3.47 (dt, $J = 15.9, 6.6$ Hz, 4H), 2.34 (p, $J = 7.2$ Hz, 4H), 1.74 – 1.71 (m, 2H), 1.58 (d, $J = 7.4$ Hz, 2H), 1.43 – 1.38 (m, 4H), 0.58 (s, 3H), 0.52 (s, 3H).

$^{13}\text{C NMR}$ (700 MHz, CDCl_3) δ 167.46, 150.41, 149.74, 140.35, 138.35, 135.39, 134.01, 128.27, 126.19, 124.63, 120.40, 115.50, 112.47, 92.62, 72.03, 71.27, 70.28, 70.02, 69.82, 52.42, 45.01, 39.76, 32.49, 29.41, 26.63, 25.38, 16.99, 1.03.

HRMS (ESI) calcd for $\text{C}_{39}\text{H}_{51}\text{ClN}_3\text{O}_4\text{Si}$ $[\text{M}+\text{H}]^+$ 688.3332, found 688.3352.

2.3.3 Synthesis of 4c



1c (50 mg, 0.09 mmol, 1 equiv) was charged in a dry Schlenk flask, sealed, and evacuated/backfilled with nitrogen (3×). Anhydrous THF (10 mL) was added, and the mixture was cooled to -78 °C. *n*-Butyllithium (2.5 M in THF, 0.1 mL, 2.5 equiv) was added dropwise, and the reaction mixture was stirred at -78 °C for 30 min. CO₂ was bubbled through (3×), then the mixture was warmed to room temperature and stirred for 10 h. After quenching with water (10 mL), the aqueous layer was extracted with ethyl acetate (3 × 10 mL), dried over Na₂SO₄, filtered, and concentrated. The crude product was dissolved in anhydrous DMF (10 mL), HATU (60 mg, 0.15 mmol, 1.6 equiv) and DIPEA (103 μL, 0.59 mmol, 6 equiv) were added, followed by **2** (22 mg, 0.09 mmol, 1 equiv), and the mixture was stirred for 6 h. The solvent was removed, and the residue purified by silica gel chromatography (MeOH/DCM 1:20) to give **4c** as a light green solid in 46% yield.

¹H NMR (400 MHz, CDCl₃) δ 7.99 (d, *J* = 7.9 Hz, 1H), 7.91 (d, *J* = 8.0 Hz, 1H), 7.67 (s, 1H), 6.96 (d, *J* = 2.9 Hz, 2H), 6.78 (d, *J* = 8.9 Hz, 2H), 6.69 (s, 1H), 6.56 (dd, *J* = 9.0, 2.8 Hz, 2H), 3.63 (d, *J* = 5.1 Hz, 6H), 3.55 (d, *J* = 5.3 Hz, 2H), 3.50 (t, *J* = 6.7 Hz, 2H), 3.39 (t, *J* = 6.6 Hz, 2H), 2.97 (s, 12H), 1.80 – 1.47 (m, 8H), 0.67 (s, 3H), 0.60 (s, 3H).

¹³C NMR (400 MHz, CDCl₃) δ 169.99, 166.21, 155.29, 149.37, 139.77, 136.66, 131.20, 129.92, 129.00, 128.10, 127.47, 125.81, 123.24, 116.58, 113.50, 92.00, 71.21, 70.24, 69.97, 69.55, 45.00, 40.25, 39.97, 32.47, 27.22, 26.62, 25.35, 1.02.

HRMS (ESI) calcd for C₃₇H₄₉ClN₃O₅Si [M+H]⁺ 678.3125, found 678.3132.

3. Compound characterizations

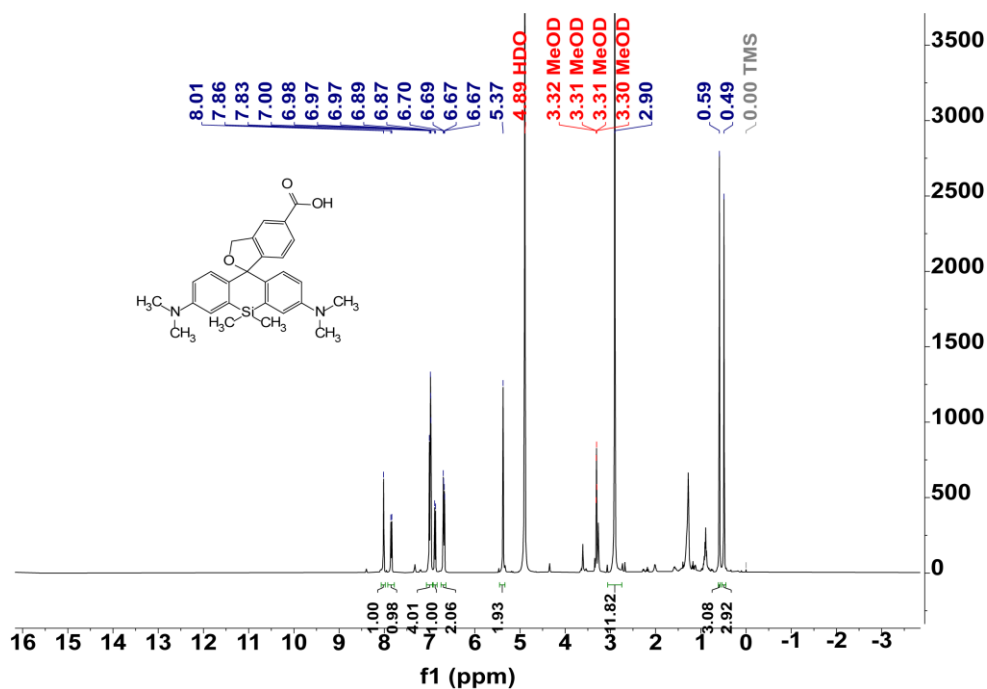


Figure S7 ^1H NMR spectrum of **3a** in MeOD

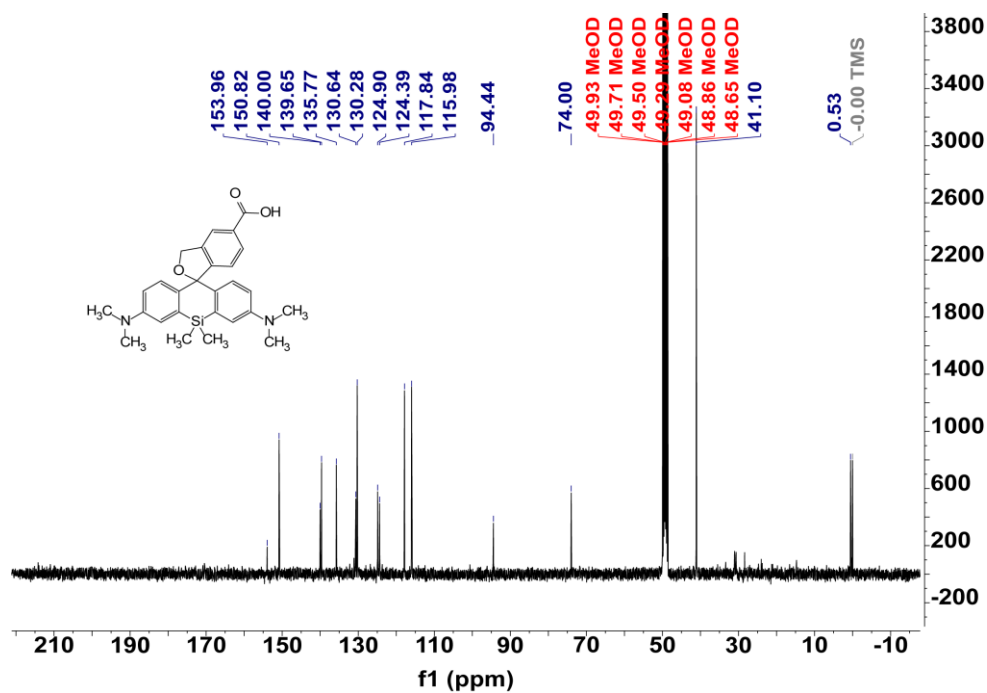


Figure S8 ^{13}C NMR spectrum of **3a** in MeOD

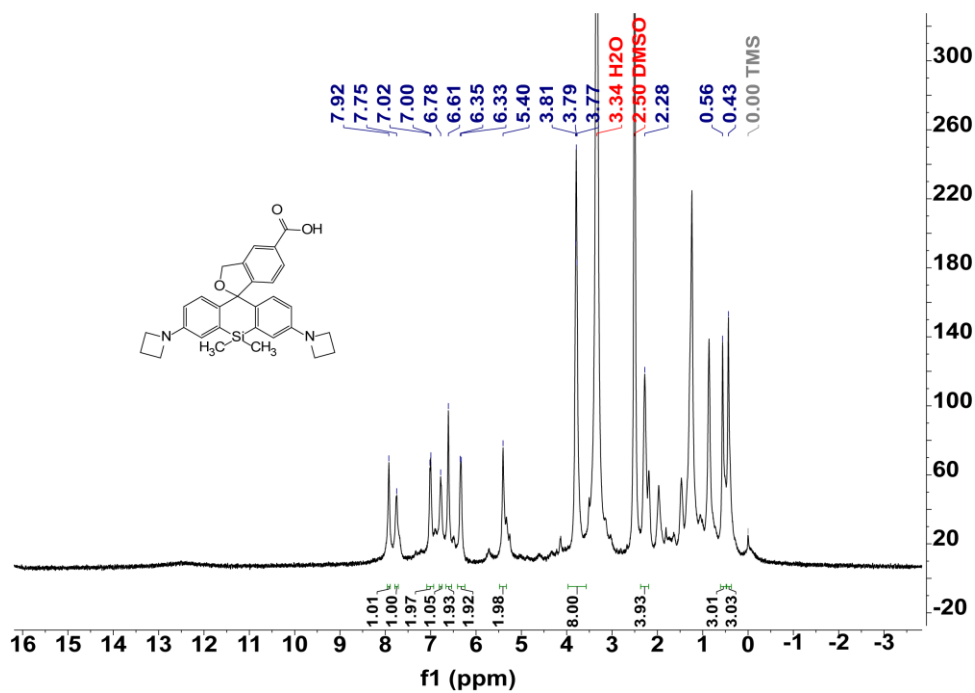


Figure S9 ^1H NMR spectrum of **3b** in $\text{DMSO-}d_6$

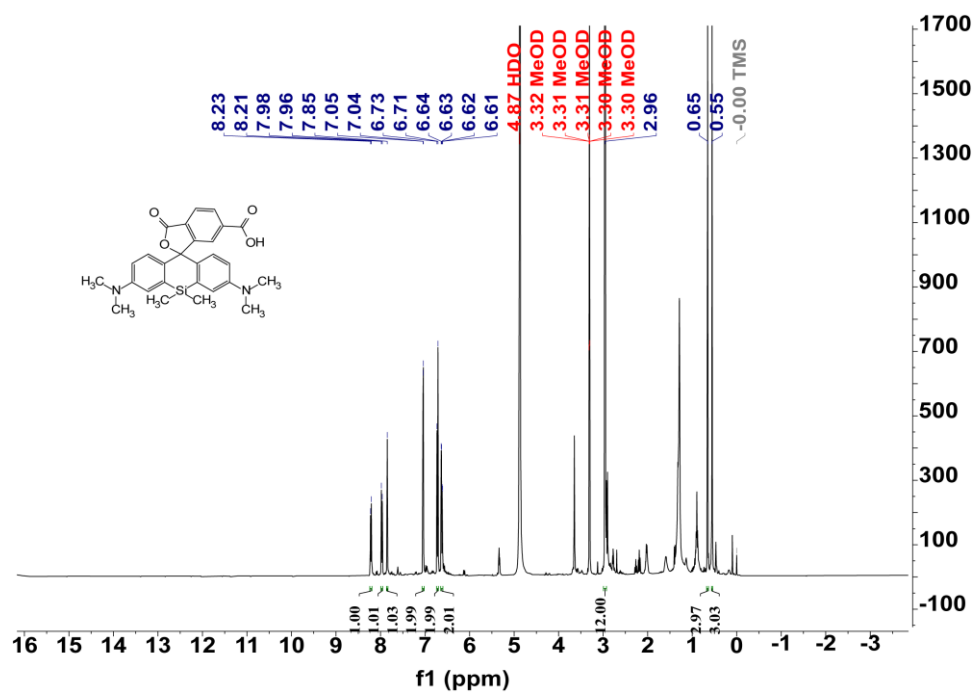


Figure S10 ^1H NMR spectrum of **3c** in MeOD

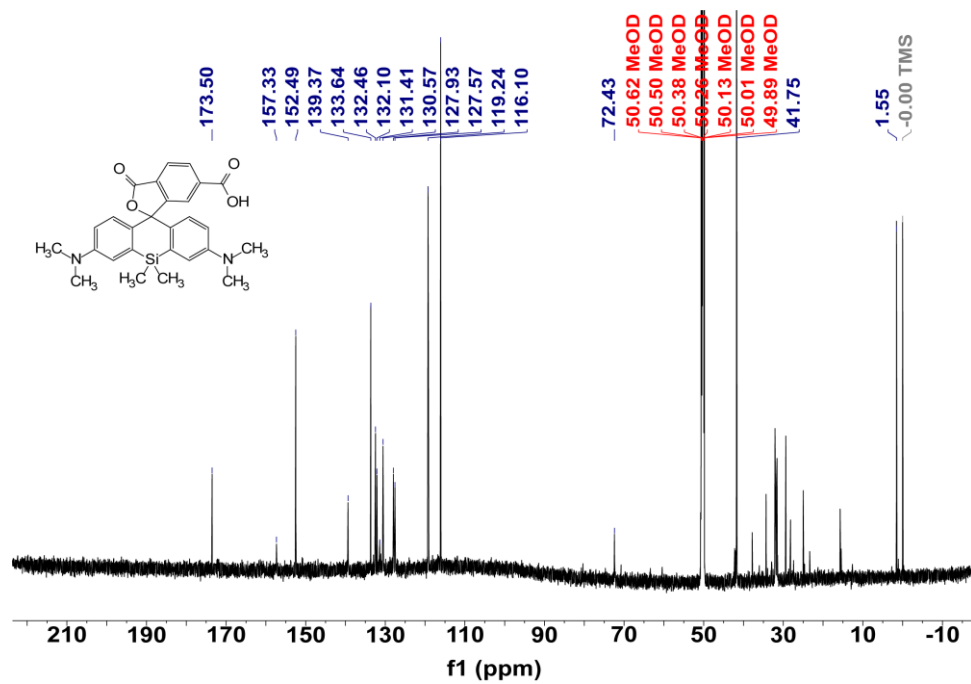


Figure S11 ¹³C NMR spectrum of 3c in MeOD

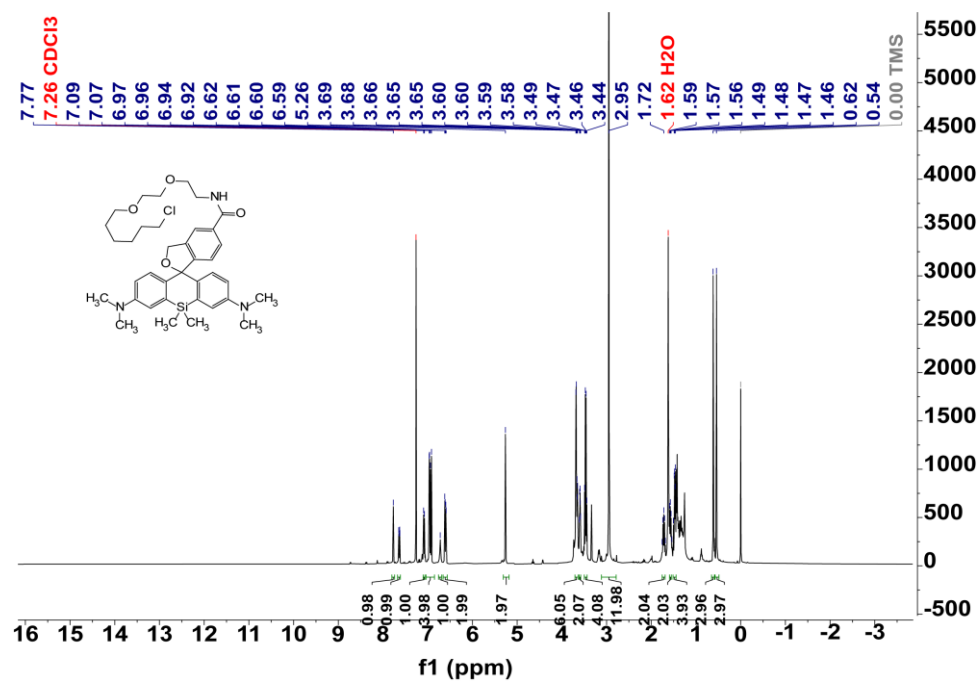


Figure S12 ¹H NMR spectrum of 4a in CDCl₃

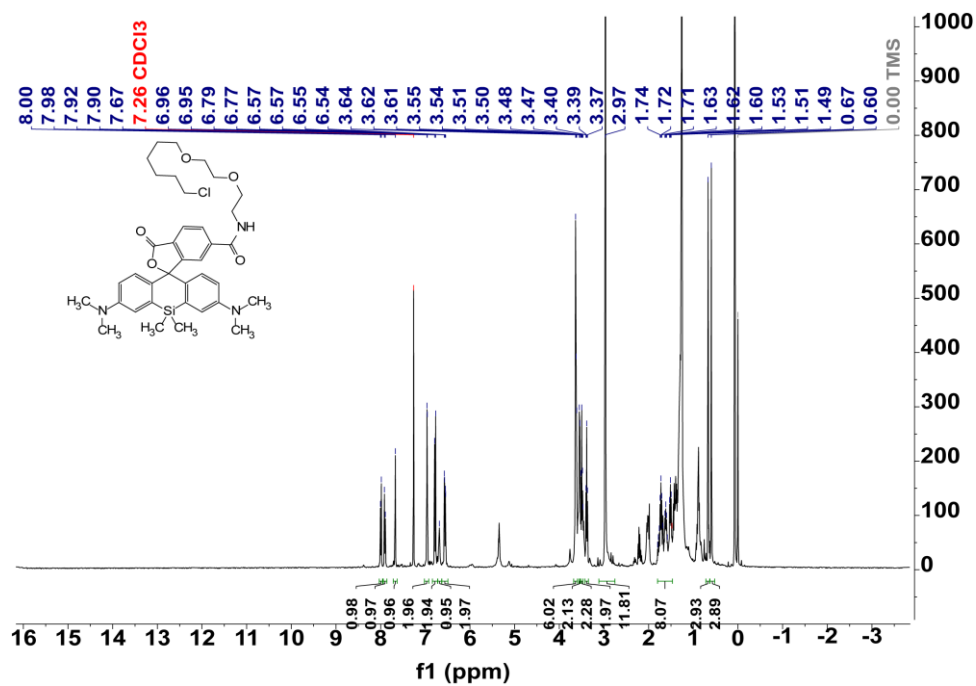


Figure S15 ^1H NMR spectrum of **4c** in CDCl₃

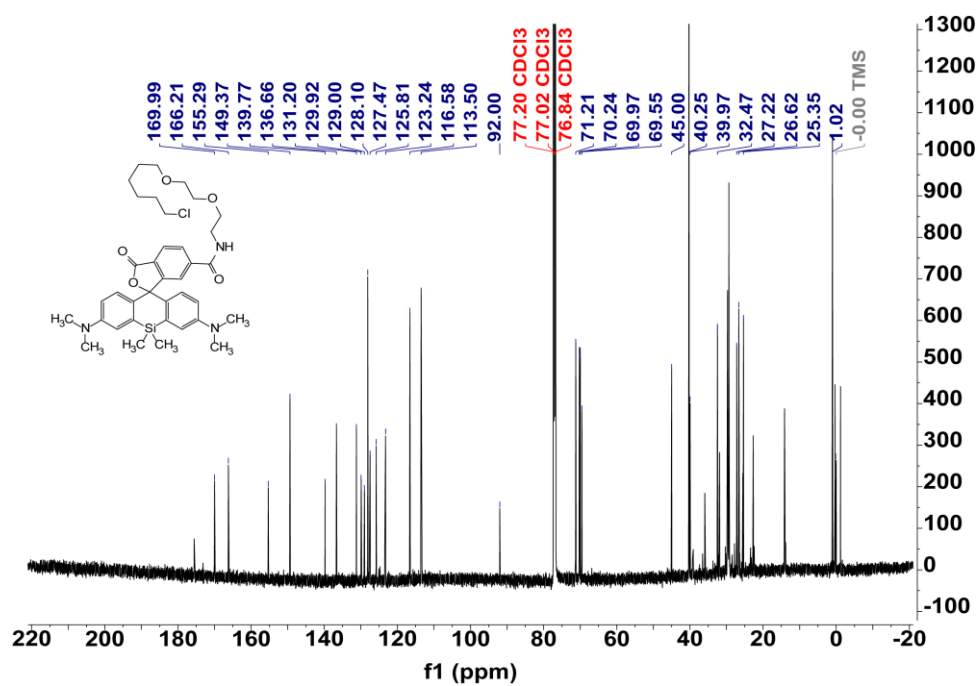


Figure S16 ^{13}C NMR spectrum of **4c** in CDCl₃

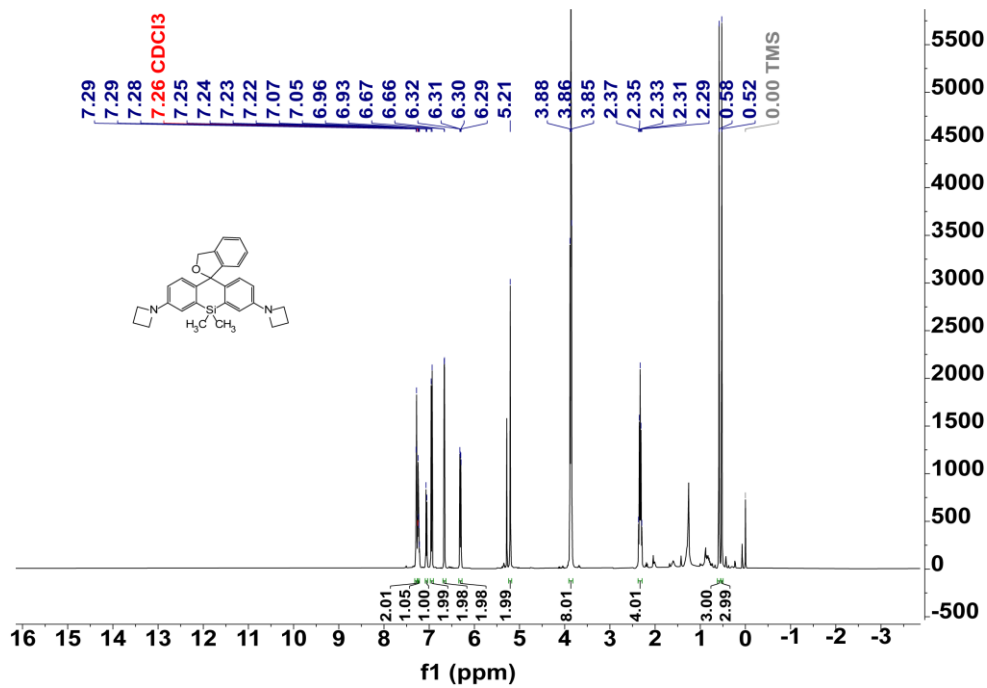


Figure S17 ¹H NMR spectrum of **5** in CDCl₃

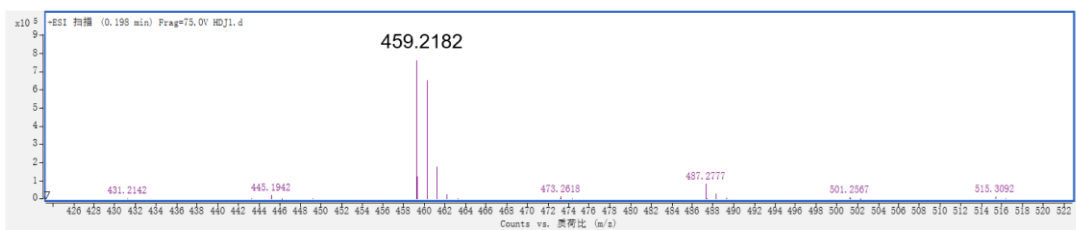


Figure S18 HRMS spectrum of **3a**

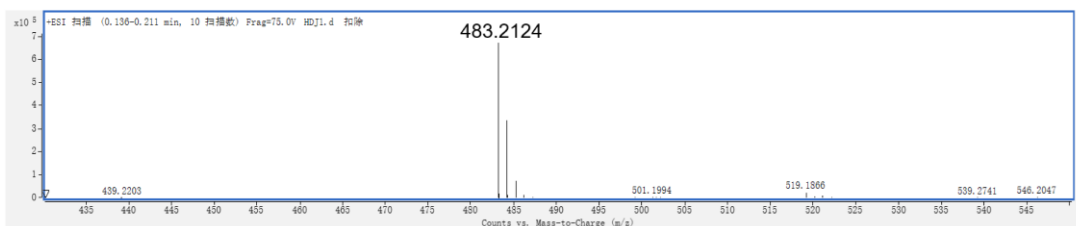


Figure S19 HRMS spectrum of **3b**

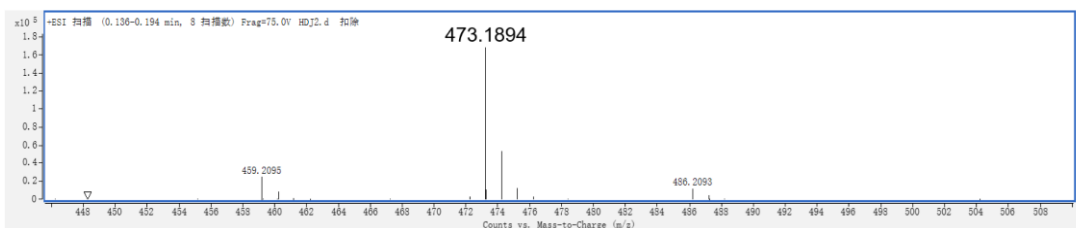


Figure S20 HRMS spectrum of **3c**

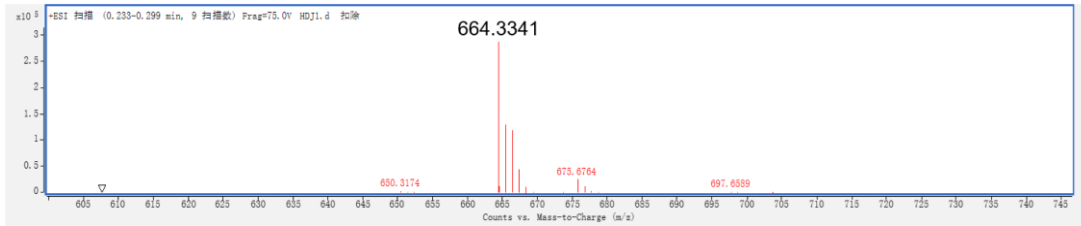


Figure S21 HRMS spectrum of **4a**

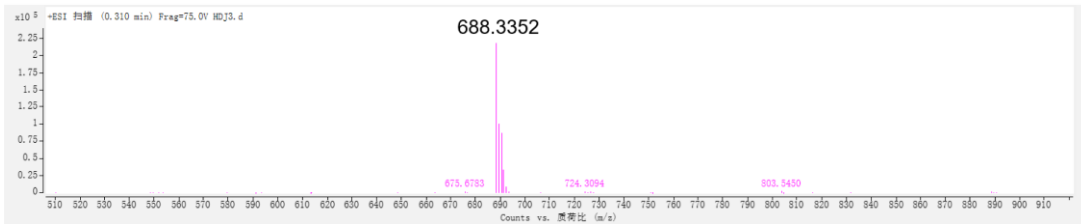


Figure S22 HRMS spectrum of **4b**

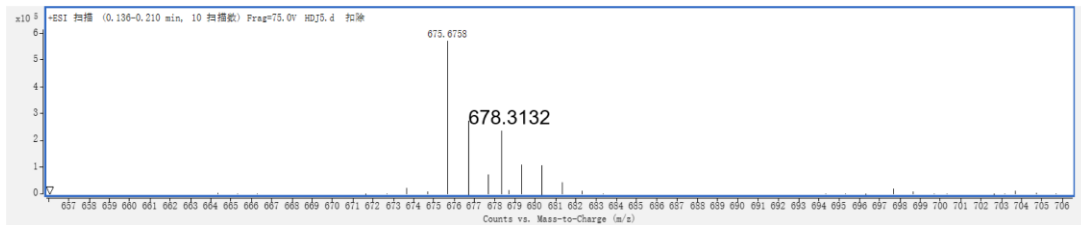


Figure S23 HRMS spectrum of **4c**

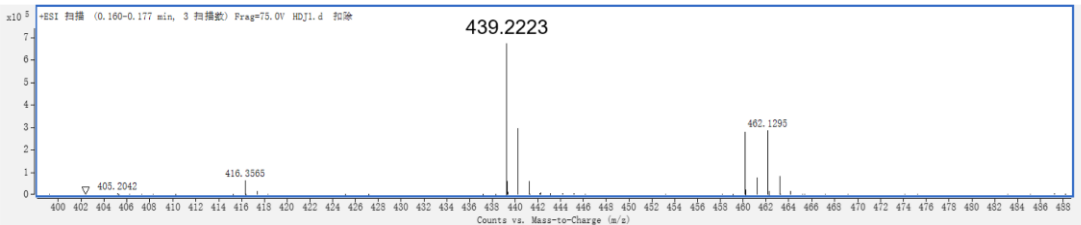


Figure S24 HRMS spectrum of **5**

4. References

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